I. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia and disturbances in carbohydrate, fat and protein metabolism. These metabolic abnormalities result either from a deficiency of the blood sugar-lowering hormone insulin or from insulin resistance, a defect in the body’s capacity to respond to insulin (Chandra et al., 2004). Thus diabetes mellitus is a complex, multi factorial disease associated with progressive deterioration of beta cell function and insulin resistance.

India, a country with a population over 1.2 billion has been reported to have currently 62.4 million people with diabetes according to Indian Council of Medical Research and expected to increase to over 100 million by 2030 (Mohan et al., 2013). Type-2 diabetes mellitus (T2DM) is the most common type of diabetes affecting majority of people with higher incidences in older population in developed countries. However in developing countries like India, diabetes incidence is higher in younger middle aged population who are in the prime of their working lives there is also increase in the prevalence of secondary complications, which is alarming. Hence there is an urgent need for the prevention and effective control of diabetes in India. More than 50 per cent of population with Type-2 DM remain undiagnosed and cost of the long term treatment is adversely affecting the economy of the country. The estimated global healthcare expenditure to treat and prevent diabetes is projected to exceed USD 490 billion by 2030 (Ramchandran et al., 2010)
Based on etiology, Diabetes mellitus is divided into two main types, Type 1 (Juvenile diabetes mellitus, insulin dependent diabetes mellitus) and Type 2 (Adult type, Non insulin dependent diabetes mellitus). The cause of diabetes depends upon the type of diabetes. Type 1 occurs mainly due to destruction of pancreatic beta cell through autoimmune mediated mechanism resulting in absolute insulin deficiency. Type 2 DM occurs due to insulin resistance or abnormal insulin secretion (Thevenod et al., 2008; Singh et al., 2011).

Diabetes is also associated with lifestyle factors and genetics (Craig et al., 2009). Chromosomal and mitochondrial DNA mutation, chemicals and drugs like pentamidine, nicotinic acid, glucocorticoids, thyroid hormone, beta adrenergic agonists, thiazides, alpha interferon can cause diabetes. In addition, abnormalities in the pancreas such as pancreatitis, pancreatectomy, neoplasia, cystic fibrosis, fibrocalculus pancreateopathy can result in diabetes. Cushing syndrome, glucagonoma, phaeochromocytoma, hyperthyroidism can also mediate diabetes.

The main diagnostic criteria of diabetes include elevated blood glucose level and symptoms such as polyuria, polydipsia, weight loss, glycosuria and ketonuria and could be confirmed by measurement of a marked elevation in the blood glucose level (Craig et al., 2009).

Diabetes is a very complex disease in people and equally so in dogs and cats. It is one of the most frequently diagnosed endocrinopathies in cats and dogs and the incidence is increasing due to an increase in the frequency of predisposing factors such as obesity and physical inactivity in these animals. Type 1 diabetes mellitus is the most common
type reported in dogs, whereas type 2 in cats especially in males. Diabetes has also been reported in many other species of animals but only rarely such as equines, bovines, ovine, swine, primates as well as birds.

To understand the pathogenesis and testing of therapeutic agents, appropriate experimental models are required. Diabetes animal models could be obtained through chemical, dietary or surgical manipulations. Recently, large numbers of new genetically modified animal models comprising transgenic, generalized knockout and tissue specific knockout mice have been extensively used for screening anti diabetic drugs (Frode et al., 2008).

Treatment of diabetes mellitus is a global problem and successful treatment is yet to be discovered. The first line of treatment includes insulin therapy and oral hypoglycemic agents but associated with several side effects and is inadequate to alter the course of diabetic complications (Venkatesh et al., 2010). At present, insulin therapy is the only satisfactory approach in diabetic mellitus, even though it has several drawbacks like insulin resistance (Piedrola et al., 2001), anorexia, brain atrophy and fatty liver in chronic treatment and episodes of hypoglycaemia (Weidmann et al., 1993).

Currently available oral treatment includes sulfonylureas, biguanides, alpha – glucosidase inhibitors and glinides which are used alone or in combination with other drugs to result in better effect. Sulphonylureas are useful in the treatment of diabetes which cannot be controlled by diet or other available therapy. Tolbutamide, chlorpropamide, glibenclamide, to lazamide etc. are some of the important sulphonylureas which are absorbed rapidly from the intestine. Biguanides control all types of diabetes
and reduce glucose absorption from the intestine and also can be used to treat mild diabetes associated with pregnancy. However, many of these oral antidiabetic agents have a number of serious side effects. Long-term treatment with sulphonylurea may desensitize the beta cells of the pancreas and high concentrations of sulphonylurea inhibit insulin biosynthesis *in vitro* and perhaps also *in vivo* (Anderson and Borg, 1980; Melander *et al.*, 1987).

The limitations of currently available oral antidiabetic agents have encouraged a concerted effort to discover new drugs to manage type 2 diabetes more efficiently. Thus discovery and development of novel therapeutic agents and alternate strategy has become prime in the recent years. Currently the focus is more on development of indigenous, inexpensive plant derived traditional antidiabetic treatment with no side effects. World health organization expert committee on diabetes has also recommended that traditional medicinal herbs be further investigated (Modak *et al.*, 2007).

Herbal medication has been used for the treatment of variety of ailments and a huge number of populations in the world are still entirely dependent upon traditional medicines. A number of medicinal plants and their formulations are being used for treating diabetes in Ayurvedic medicine system as well as in ethnomedicinal practices (Pareek *et al.* 2009). Since the time of Charaka and Sushruta indigenous remedies have been used in the treatment of diabetes. From the ethnobotanical information, more than 1200 species of plants have been screened for antidiabetic activity on the basis of ethnomedicinal uses (Singh *et al.*, 2011) and about 800 plants with antidiabetic potential have been reported (Venkatesh *et al.*, 2010; Singh *et al.*, 2011 and Patel *et al.*, 2012).
Several plants have been used as dietary adjuvant and in treating a number of diseases even without any knowledge of their proper function and constituents mainly because of their fewer side effects compared to synthetic hypoglycaemic agents and also because of their safety, effectiveness and availability. There are about 200 pure compounds from plant sources reported to reduce blood glucose level. The compounds are mainly alkaloids, carbohydrates, glycosides, flavonoids, steroids, terepenoids, peptides, aminoacids, lipids, phenolics, glycopeptides and iridoids. The families of plants with most potent hypoglycaemic effects include leguminoseae, lamiaceae, liliaceae, cucurbitaceae, asteraceae, moraceae, rosaceae, euphorbiaceae and araliaceae (Bnouham et al., 2006) which are all tested pharmacologically in alloxan induced diabetic rat model system.

Although hundreds of traditional plants with anti diabetic effects have been identified, only a small number of them have been evaluated scientifically for their efficacy. The hypoglycaemic effect of some of the herbal extracts has been confirmed in human and animal models. However, the major drawback in usage of herbal medicine in modern medical practices is the lack enough of scientific and clinical data proving their efficacy and safety. There is a need for conducting clinical research, experimental evaluation in various animal models for their efficacy and safety, pharmacological and toxicological evaluation and long term studies. Also, there is a need for studies on replacement of oral antidiabetic treatment with herbal medicines by experimental research in animal models. Hence, the present study was conducted with *Trigonella foenum graecum* and *Coccinia indica* commonly known as methi and Little gourd (kovai) respectively which are reported to possess hypoglycaemic effect (Raju et al., 2001;
Srinivasan, 2006; Khalki *et al.*, 2010) to evaluate their antidiabetic effect individually and in combination in comparison with an oral antidiabetic drug glibenclamide.

1. To evaluate the phytochemical properties of *Trigonella foenum graecum* and *Coccinia indica* extracts.

2. To evaluate the antidiabetic effect of *Trigonella foenum graecum* and *Coccinia indica* individually and in combination in induced diabetes in rats.

3. To study pathomorphological and biochemical changes in induced and treated diabetic rats.

4. To compare the hypoglycemic effects of *Trigonella foenum graecum* and *Coccinia indica* with an oral hypoglycemic agent glibenclamide.

5. To evaluate the immunomodulatory activity of *Trigonella foenum graecum* and *Coccinia indica* extracts by Flow cytometry
Review of Literature
II. REVIEW OF LITERATURE

Diabetes mellitus is a common disorder of metabolism characterized by an elevated blood glucose level and other metabolic defects resulting from defects in insulin secretion, insulin action, or both. Diabetes mellitus is not a pathogenic entity but a group of aetiologically different metabolic disorder with a potential to cause devastating chronic complications. Several traditional medicinal herbs have been investigated upon recommendations of world health organization expert committee on diabetes in prevention and control of diabetes. The available literature pertaining to antidiabetic effect of *Trigonella foenum graecum* and *Coccinia indica* herbs is reviewed as here under.

2.1 Phytochemical analysis of plant extracts

2.1.1 *Trigonella foenum graecum*

Petit *et al.* (1995) and Yoshikawa *et al.* (1997) isolated furostanol saponins called trigoneoside Ia, Ib, IIa, IIb,IIIa and IIIb, glycoside and trifoenoside A from alcoholic extract of *Trigonella foenum graecum*. They claimed that these saponins were the active compounds which owe to hypoglycaemic effects of fenugreek.

Broca *et al.* (1999) isolated an active ingredient 4 hydroxyisoleucine (4-OH-Ile) from fenugreek seeds and demonstrated that the insulinotropic and antidiabetic properties of fenugreek seeds were due to 4-OH-Ile which functions in a glucose-dependent manner by *in vitro* studies.
Yadav et al. (2005) indicated that fenugreek seeds contain sapogenins which lower serum triglycerides, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) by increasing biliary cholesterol excretion in liver.

Srinivasan (2006) identified the chemical composition of the active antidiabetic ingredient found in Trigonella and reported that antihyperglycemic effect of fenugreek was due to delayed gastric emptying which caused inhibition of glucose transporter by the pectin (50%) of fenugreek seeds. He stated that pectin forms a colloid suspension when hydrated and decreases the rate of gastric emptying and slows carbohydrate absorption.

The lipid-lowering effect of fenugreek also been attributed to its oestrogenic constituent which indirectly increase the thyroid hormone T4 (Baquer et al., 2011).

The nutrient composition of fenugreek seeds consists of approximately 30 per cent protein, 26 per cent of starch, 13 per cent natural detergent fibre, 4 per cent gum, 6 per cent lipids and 11 per cent ash. It is also rich in calcium, iron, beta-carotene and other vitamins. In comparison to other legumes, fenugreek seeds contain higher proportions of minerals including Ca, P, Fe, Zn and Mn. Trigonelline is an important alkaloid component of the seeds, which also contain some aromatic constituents such as n-alkenes, sesquiterpenes and nonalactone. Fenugreek seeds are also rich in saponins, including diosgenin, gitogenin and tigogenin. In general, fenugreek contains three important chemical constituents with medicinal value i.e., steroidal sapogenins; galactomannans and isoleucine. These constituents have placed fenugreek among the
most commonly recognized "nutraceutical" or health food products (Chauhan et al., 2011)

### 2.1.2 *Coccinia indica*

Presence of cephalandrol, tritriacontane, lupeol, b-sitosterol, cephalandrine A, cephalandrine B, stigma-7-en-3-one, taraxerone and taraxerol has been reported by Ray and Kundu (1987) and Rastogi et al. (1993). The presence of polysaccharides, xyloglucan, carotenoids and cryptoxanthin was reported by Purohit et al. (1999) and Yadav et al. (2010).

The phytochemical screening of the 50 per cent methanolic extract obtained from whole parts of *Coccinia indica* done by Chandira et al. (2010) revealed that it contained carbohydrates, glycosides, fix oils and fats, proteins and amino acids, saponins, tannins, phytosterol, alkaloids, phenolic compounds, flavonoids, gum and mucilage; methanolic extract obtained from fruits of *Coccinia indica* contained steroids, saponins, ellagic acid, lignin’s, triterpenoids, in addition to alkaloids, tannins, flavonoids, glycosides and phenols and the aqueous extract of fresh leaves of *Coccinia indica* contained anthraquinons in addition to alkaloids, carbohydrate, proteins and amino acids, tannins, saponins, flavonoids, phytosterol, triterpenes.

The aqueous extract of fresh leaves of Ivy Gourd (*Coccinia indica*) exhibited anthraquinons in addition to alkaloids, carbohydrate, proteins, amino acids, tannins, saponins, flavonoids, phytosterol and triterpenes. Presence of cephalandrol, tritriacontane, lupeol, b-sitosterol, cephalandrine A, cephalandrine B, stigma-7-en-3-one, taraxerone and taraxerol, has also been reported (Yadav et al., 2010)
Deokate and Khadabadi (2011) proved the insulin stimulatory effect of *Coccinia indica* leaves on existing beta-cells in diabetic rats. They reported that *C. indica* possess hypoglycemic, antidiabetic, hypolipidemic, hepatoprotective, larvicidal, anti-inflammatory, analgesic and antipyretic activities and consists of various phytoconstituents such as cephalandrol, tritriacontane, lupeol, b-sitosterol, cephalandrine A, cephalandrine B, stigma-7-en-3-one, taraxerone and taraxerol. They indicated that the terpenoids, saponins, flavonoids, sterols and alkaloids are responsible for antidiabetic effect of the plant.

### 2.2 Diabetes mellitus

The first mention of diabetes found in Indian literature goes as far back as 1000BC, during which time the physicians diagnosed diabetes by tasting the patient’s urine for sweetness. The *Sushruta Samhita*, an Ayurvedic textbook written between the fourth and fifth centuries B.C., also describes two types of diabetes. Thus, 2500 years ago itself Ayurvedic physicians recognized and categorized clinical entities corresponding to insulin-dependent and non insulin dependant diabetes mellitus. The pharmacopoeia of ancient India has listed specific treatments for diabetes, including dietary modifications, medicinal plant remedies, and minerals. These historical accounts reveal that NIDDM was well known among the ancients and that medicinal plants have been used for millennia to treat this disease (Oubre *et al.*, 1997).

The worldwide prevalence of diabetes in human beings was estimated to be 4 per cent in 1995 and expected to rise to 5.4 per cent by the year 2025. It is higher in developed than in developing countries and recognized as a serious global health
problem affecting over 150 million and is likely to increase to 300 million or more by the year 2025 (King et al., 1998) and to 100 million by 2030 by (Mohan et al., 2013).

Diabetes mellitus is one of the most frequently diagnosed endocrinopathies in cats and dogs. The incidence of diabetes in cats ranges from 1:50 to 1:400 of population (Baral et al., 2003) and it is escalating due to an increase in the frequency of predisposing factors such as obesity and physical inactivity. The prevalence of the disease is more in middle-aged and older dogs while the incidence is further increasing in cats (Prahl et al., 2003). Type 1 diabetes previously called insulin dependent diabetes is most common in dogs, whereas non insulin dependent or adult onset diabetes appears to be the most common form in cats (Jacquie et al., 2004).

Among humans the prevalence of diabetes is higher in men than women. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people with more than 65 years of age (Wild et al., 2004).

Canine diabetes can be classified into insulin deficiency diabetes (IDD), resulting from congenital deficiency or acquired loss of pancreatic beta cells, or insulin resistance diabetes resulting mainly from hormonal antagonism of insulin function (Catchpole et al., 2005). They also reported that there is no evidence of canine equivalent of human type 2 diabetes but resembles autoimmune diabetes of humans with a late onset and slow progression of beta cell dysfunction.
Ramachandran et al. (2010) reported that diabetes in Asian countries is disproportionately high in young to middle-aged adults. This could have long-lasting adverse effects on a Nation’s health and economy, especially for developing countries. The International Diabetes Federation (IDF) estimates the total number of people in India with diabetes to be around 50.8 million in 2010, rising to 87.0 million by 2030.

Type I is also called as insulin-dependent diabetes mellitus (IDDM) which is produced mainly due to less production of insulin and type II as non-insulin-dependent diabetes mellitus (NIDDM) which is produced mainly due to inability of body cells to respond to the insulin produced. As per WHO, August 2011 fact sheet Number 312, 346 million people worldwide have diabetes and more than 80 per cent of diabetes deaths occur in low and middle-income countries. It is also projected that death due to this will be double between 2005 and 2030 (Amita rai et al., 2012)

2.3 Induction of diabetes

Streptozotocin (STZ) is a naturally occurring compound produced by the bacterium Streptomyces achromogenes that exhibits broad spectrum antibacterial properties (Vavra et al., 1959) and most preferred for experimental induction of diabetes in animals.

Karunanayake et al. (1974) used streptozotocin for the induction of diabetes and showed that histopathological alterations in pancreatic islets β-cell were due to decrease in the β-cell nicotinamide adenine dinucleotide (NAD).
Karunanayake et al. (1975) in an experiment to show the metabolic fate and elimination of STZ in adult male Wistar albino rats observed that STZ caused destruction of β-cells of islets of Langerhans when given intravenously at 60 mg/kg body weight in about three days.

Ganda et al. (1976) reported that the frequently used single intravenous dose of STZ to induce IDDM is between 40-60 mg/kg b w and the range of STZ dose required to induce diabetes is not as narrow as it is in case of alloxan.

Wier et al. (1981) showed that streptozotocin could induce diabetes mellitus in many animal models and the induced diabetes resembled the human hyperglycaemic non-ketotic diabetes mellitus in all aspects.

Katsumata et al. (1992) reported that a single dose of STZ below 40 mg/kg may be ineffective in inducing diabetes and also mentioned that the increased blood glucose provides partial protection to the diabetogenic action of alloxan. Hence suggested that fasting animals before induction of diabetes would make them more susceptible to alloxan’s action.

Dog represents an excellent animal model for the study of diabetic retinopathy as it reproduces all but the very late changes observed in the human diabetic eye. Anderson et al. (1993) studied the chronic diabetic effects in beagle dogs by administering a mixture of alloxan (40 mg/kg) and streptozotocin (35 mg/kg) intravenously instead of either of the drugs individually. They indicated that when these drugs administered
individually produced high mortality in animals owing to severe hypoglycaemic episode and produced severe adverse effects on several organ systems.

The target and intracellular action of STZ on pancreatic beta cells results in changes in DNA of pancreatic β-cells comprising of its fragmentation (Morgan et al., 1994).

It was proposed that pancreatic islet cell destruction could be due to nitric oxide from STZ which superficially damage DNA of the cells (Kroncke et al., 1995). However Bedoya et al. (1996) reported that STZ generates reactive oxygen species which also could contribute for DNA fragmentation and evoke deleterious changes in the cells.

In an attempt to reverse the diabetes mellitus in human beings by islet cell transplantation, Rastellini et al. (1997) conducted an experiment where they created diabetic animal model by injecting anterior hypophyseal extract. With this experimentation they further proved that the diabetes could also be produced by immunological destruction of islets β-cells.

Li et al. (2000) in an investigation of Type-I diabetes following multiple low dose of STZ administration in mice observed hyperglycaemia from 7th day onwards which was coincided with occurrence of insulitis and reduction in islet cell area morphometrically. In addition they also noticed 2-3 fold increase in the number of alpha cells with a significant reduction and disappearance of beta cells.

Streptozotocin is a glucosamine-nitrosourea compound. As with other alkylating agents in the nitrosourea class, it is toxic to cells by causing damage to the DNA, though
other mechanisms may also contribute. DNA damage induces activation of poly ADP-ribosylation, which is likely more important for diabetes induction than DNA damage itself (Szkudelski, 2001). He also attributed the cytotoxic action of alloxan and STZ on β-cell of rats pancreas while inducing diabetes mellitus to reactive oxygen species and stated that the source of these reactive species are different for alloxan and STZ induced diabetes.

The toxicity of β-cells caused by STZ has been reported to involve both genetic and nongenetic mechanisms. STZ entry into β-cells results in induction of nitric oxide (NO) donation, poly ADP ribose polymerase (PARP) induction and free radical generation which are responsible for β-cell toxicity and hence diabetes. The NO and other free radicals cause non-specific molecular damage with in β-cells and the DNA damage caused by STZ induces PARP which when over expressed acts as pro death stimulus (Wada and Yagihashi, 2004).

Alloxan and streptozotocin are toxic glucose analogues that preferentially accumulate in pancreatic beta cells via the GLUT2 glucose transporter. Following its uptake into the beta cells, streptozotocin splits into its glucose and methylnitrosourea moiety. Owing to its alkylating properties, the latter modifies biological macromolecules, fragments DNA and destroys the beta cells, causing a state of insulin-dependent diabetes (Lenzen, 2008).

Mir et al. (2008) in an effort to investigate the biochemical and histomorphological changes in diabetes used streptozotocin at the rate of 65 mg/kg as a single shot intravenously upon 12 hours of fasting in rabbits. They observed an increase
in blood sugar, blood urea and serum creatinine levels biochemically in addition to histopathological alterations in pancreas, kidney, liver, heart, lungs and brain and suggested that rabbits serve as a good model to study diabetic complications.

Dhanush (2009) reported that streptozotocin at the dose rate of 45 mg/kg body weight effectively induced diabetes in adult male Wistar rats within 48 to 72 hours with mean serum glucose values above 400 mg/dL. He also reported increased levels of serum AST, ALT, triglycerides and cholesterol in diabetic rats than normal control.

Komolafe et al. (2009) assessed the changes in serum lipid profiles of experimentally-induced diabetic Wistar rats to elucidate the effects STZ induced diabetes on the serum levels of cholesterol and triglycerides. The results revealed a significant increase in the serum levels of total cholesterol, triglyceride, low-density lipoprotein cholesterol, and very low-density lipoprotein cholesterol of diabetic rats when compared with the control rats while a significant decrease in the high-density lipoprotein cholesterol was observed. The study revealed that induction of diabetes in rats using STZ results in development of hyperlipidemia.

Zafar et al. (2009a) studied the effects of streptozotocin-induced diabetes on kidney morphology, anatomy, architecture and on the activities of aminotransferases (AST and ALT), alkaline phosphatase (ALP) and pseudocholinesterase (PChE) in albino rats. Histopathology of kidney showed lesions similar to human glomerulosclerosis, glomerular membrane thickening, arteriolar hyalinization and tubular necrosis. Increased levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and pseudocholinesterase (PChE) were observed in the kidney.
Zafar et al. (2009b) studied the relationship and effects of diabetes on liver morphology, architecture and function by using streptozotocin (STZ)-induced diabetic rats as an experimental model. Histopathological examination of liver showed accumulation of lipid droplets, lymphocytic infiltration, increased fibrous content, dilatation and congestion of portal vessels and proliferation of bile ducts. Increased levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), ALP and PChE were observed in the liver.

Ebuehi et al. (2010) studied oxidative stress in alloxan-induced diabetic female and male rats and found that the catalase activities in liver and kidney of diabetic male or female rats were significantly lower than in non-diabetic male or female rats. However, the reduction in the catalase activities in liver and kidney of diabetic female rats was pronounced as compared to the diabetic male rats. The cholesterol and triglyceride concentrations of diabetic male or female rats were significantly higher than in non-diabetic male or female rats. They concluded that Alloxan induced diabetes caused an elevation of cholesterol and triglyceride concentrations, but resulted in a decrease in catalase activity in the liver and kidney and indicated that sex or gender differences do not significantly affect oxidative stress in alloxan-induced diabetes.

The effects of streptozotocin (STZ) on levels of glucose, AST, ALT and cholesterol in rat serum samples were investigated in vivo. After the treatment of STZ in rats produced change in the levels of serum glucose, AST, ALT, and cholesterol. The levels of glucose, AST and ALT increased significantly, while levels of cholesterol
decreased significantly in different doses of STZ administed intraperitoneally. Streptozotocin was well tolerated, and no side effects were observed (Cennet et al., 2010)

Streptozotocin is highly cytotoxic to the pancreatic beta-cells and therefore is commonly used to induce experimental type 1 diabetes in rodents. STZ-induced cytotoxicity in HepG2 cells is mediated by the increase in ROS/RNS production, oxidative stress and mitochondrial dysfunction (Haider and Annie, 2012).

Mallikarjun et al. (2013) observed that streptozotocin at the dose rate of 45 mg/kg body weight effectively induced diabetes in adult male Wistar rats within 48 to 72 hours with mean serum glucose values above 400 mg/dL. He also reported increased levels of serum AST, ALT, triglycerides and cholesterol in diabetic rats than normal control.

2.4 Antidiabetic drug - Glibenclamide

Glibenclamide is a second generation sulfonylurea drug along with other drugs like gliclazide and glipizide and is 100 times more potent than the first generation ones like chlorpromide and tolbutamide (Lebovitz et al., 1983).

Glibenclamide (glyburide) improves the glucose control by acting both on insulin secretion and insulin action as identified by Koltermann et al. (1984). However Pfeifer et al. (1980) were of the opinion that the drug’s predominant effect is on insulin secretion.

Guiot et al. (1994) studied the effects of glibenclamide on pancreatic β-cell proliferation in vivo in normal mice and showed that glibenclamide at 2 mg/kg body weight when given intra peritoneally for 7-30 days caused degranulation of β-cells and increased β-cell mass and concluded that glibenclamide has a β-cytotropic effect.
Glibenclamide initiates its action through binding with its receptors on the β-cell surface and subsequently causes a decrease in the conductance of ATP-sensitive K\(^+\) channels which results in more K\(^+\) efflux and calcium influx in β-cells which eventually determines insulin secretion (Luzzi et al., 1997). They further stated that the sulfonylurea drugs are generally completely absorbed from intestine but there can be delay in their absorption as it depends on level of hyperglycaemia.

Zhang et al. (2003) in an experiment showed that the hypoglycaemic effect of glibenclamide impaired the recovery of glucose from insulin induced hypoglycaemia and significantly reduced blood glucose levels in normal rats and finally concluded that hypothalamic ATP sensitive K\(^+\) channels play significant regulatory role in peripheral glucose homeostasis.

Ling et al. (2006) conducted in vitro and in vivo studies to understand the effects of glibenclamide on the functional state of beta cells of islets. They observed that glibenclamide caused more than 50 per cent decrease in the cellular insulin content owing to degranulation and an elevation in basal insulin biosynthetic activity.

Glibenclamide is one of the leading drugs from sulphonylureas category. It acts by inhibiting ATP-sensitive potassium channels in pancreatic beta cells which results in cell membrane depolarization opening voltage-dependent calcium channel. So the level of intracellular calcium in the beta cell increases and results in stimulation of insulin release (Serrano-Martin, 2006).
Wang et al. (2008) hypothesised that prolonged exposure of rat pancreatic β-cells to the insulin secretagogue glibenclamide induces a sustained increase in basal insulin synthesis and proved the same through in-vitro experimentation with cultured rat β-cells. They concluded from their results that the prolonged exposure to glibenclamide causes activation of protein translation in pancreatic β-cells through the calcium regulated mTOR, PKA and MEK signalling pathways.

Erejuwa et al. (2011) investigated the effect of honey as an adjunct to glibenclamide on glycemic control in streptozotocin-induced diabetic rats. They found that honey significantly increased insulin, decreased hyperglycemia and fructosamine. Although glibenclamide alone significantly reduced hyperglycemia, glibenclamide combined with honey produced significantly much lower blood glucose compared to glibenclamide or metformin alone.

Lal et al. (2011) reported that both glibenclamide and Trigonella seed extract possessed hypoglycemic effect which was significantly more than either of drug given alone.

2.5 Herbs in diabetes

Oral hypoglycemic agents such as biguanides, sulphonylureas and thiazolidinediones or insulin therapy are the mainstay of treatment of diabetes and are effectively used in controlling hyperglycemia, but they fail to significantly alter the course of complications and side effects caused by them (Rang and Dale, 1991). Recently, the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine. The ethnobotanical information reports about 800 plants that
may possess anti-diabetic potential (Alarcon-Aguilara et al., 1998). The problem associated with the development of herbal medicine includes presence of quite a lot of active ingredients, with fairly dissimilar pharmacological profiles, lack of quality control, lack of government regulations regarding safety and efficacy, insufficient clinical trials, and inadequate information on the adverse effects and drug-herbal interactions (Philip, 2004).

Grover and Vats (2002) reported DM as a well recognised ancient disease in India and indigenous remedies have been used in the treatment of DM since the time of Charaka and Sushrutha.

The world health organization has also recommended encouraging use of herbal extracts, especially in countries where conventional treatment of diabetes seems to be insufficient (Santhakumari et al., 2003).

The NAPRALERT database lists over 1200 species of plants representing 725 genera in 183 families extending from the marine algae and fungi with antidiabetic activity. Over half of these have been used ethnopharmacologically in traditional medicine as antidiabetics, and some 50 per cent of these traditional remedies have been studied experimentally. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (Edwin et al., 2008)
Shojaii et al. (2012) updated systematic review of the published literature to find effective antihyperglycemic herbs and herbal preparations used in clinical trials for improving glycemic control in type 2 diabetes mellitus. They performed an electronic literature search of Medline for single herbs or combination of herbs used as potential therapy for type 2 diabetes mellitus. Out of 38 trials, reviewed the evidence for glycemic control was observed in 26 trials (68.4%). They reported that several herbal medicine used in clinical trials have beneficial effects on glycemic control, but there is still insufficient evidence to draw definitive conclusion about the efficacy of herbs and herbal preparations for treatments of diabetic patients.

2.6 Herb - *Trigonella foenum graecum*

*Trigonella foenum graecum* also known as fenugreek, locally as methi, a well-known traditional medicinal herb in India, possesses diverse biological activities and pharmacological functions. *T. Foenum graecum* seeds have been used as traditional medicines not only in diabetes but also in high cholesterol, inflammation and gastrointestinal ailments (Hafeez et al., 2003)

Sauvaire et al. (1998) demonstrated by *in vitro* studies that the amino acid 4-OH-Ile in fenugreek seeds causes glucose induced insulin release in human and rat pancreatic cells. They also reported that 4-OH-Ile acts only on pancreatic beta cells, as the levels of somatostatin and glucagons were not altered.

Broca et al. (1999) suggested that the improvement of diabetic state, of streptozotocin treated rats was partly from a direct stimulating effect of 4-OH-Ile of *Trigonella foenum-graecum* on beta cell function.
The significantly increased activities of the two enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase during diabetes in liver and kidney were found to be lowered to almost control values by the use of the antidiabetic compounds *Trigonella foenum graecum* seed powder (Gupta *et al.*, 1999)

Gupta *et al.* (2001) reported that adjunct use of fenugreek seeds improved glycemic control and decreased insulin resistance in mild type-2 diabetic patients along with a favourable effect on hypertriglyceridemia.

Xue *et al.* (2001) showed that rats treated with *Trigonella foenum graecum* extract had an increase in body weight and a decrease in kidney body weight ratio \( (p \leq 0.05) \), compared with diabetic group. They also observed lowered blood glucose, glycated hemoglobin, triglycerides, total cholesterol and high density lipoprotein cholesterol in a dose dependent manner \( (p \leq 0.05) \) in treated rats.

Grover *et al.* (2002) stated that the fenugreek is traditionally used in India, especially in the Ayurveda and Unani systems and the preliminary animal and human trials have suggested the possible hypoglycaemic and anti-hyperlipedemic properties of seed powder when taken orally. They also indicated that the high fibre content (30% soluble fibres and 20% insoluble fibres) could be responsible for the slow rates of glucose absorption.

The seeds of *Trigonella foenum graecum*, commonly used as a spice in Middle Eastern countries and widely used in south Asia and Europe, are known to have anti-diabetic properties (Basch *et al.*, 2003).
Grover et al. (2005) investigated hypoglycemic and hypolipidaemic effect of the aqueous extract of the fenugreek seeds in alloxan-induced diabetic rats and found that it significantly reduced the levels of blood sugar, serum triglycerides, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) after daily oral administration of the extract for the period of 21 days.

Treatment of diabetic rats with insulin, Trigonella foenum graecum, seed powder (TSP), vanadate and a combined therapy of lower dose of vanadate with TSP revived normoglycemia and restored the altered level of Na(+)/K(+) ATPase, lipid peroxidation and membrane fluidity and also induced the redistribution of GLUT4 transporter (Siddqui et al., 2006).

Mohammad et al. (2006) showed effectiveness of the antidiabetic compounds vanadate and Trigonella in reverting the diabetes effect on the Glut-4 transporter to normal levels in experimental diabetes.

An herbal preparation prepared using cow urine and Gymnema sylvestre, Momordica charantia, Eugenia jambolana, Aegle marmelos, Cinnamomum tamala, Aloe barbadensis, and Trigonella foenu graecum was studied by Jarald et al. (2008), for antidiabetic activity in alloxan induced diabetic rats. They observed that the herbal alcoholic extract preparation significantly lowered the blood sugar level compared to the aqueous herbal extracts.

Das et al. (2008) studied the combined effect of Trigonella foenum graecum seed and Coccinia indica leaves with the commonly used drug Glimepiride in diabetic rats and
observed a significant hypoglycaemic effect in treatment group with improvement in general body condition of the animals.

Mowla et al. (2009) showed that *T. Foenum graecum* seed extract (1.5gm/kg), decreased the blood glucose levels in dose dependent manner in NIIDM rats and the effect was comparable with the oral hypoglycemic drug glimepiride. In standard drug (glimepiride) treatment, the per cent of blood sugar decrease was more as compared to their respective controls. No visible symptoms of toxicity or mortality in rats were observed after administrating very high dose (3g/kg) of *T. Foenum graecum* seed extract.

Haeri et al. (2009) noticed that the levels of glucose and liver damage marker aspartate transaminase were markedly and significantly (p≤0.001) elevated in diabetic control as compared to normal control group. Alanine transaminase was elevated slightly (18%), and all markers were restored to near control values after treatment with 4-hydroxyisoleucine at 50 mg/kg per day for 8 weeks, with the effect being significant (p≤ 0.01) for all markers. This prolonged exposure to 4-hydroxyisoleucine was well tolerated in control animals and did not alter levels of glucose or liver damage markers significantly. These findings indicated that 4-hydroxyisoleucine administration is a useful and well-tolerated treatment for insulin resistance, both directly as a hypoglycaemic and also as a protective agent for the liver.

To evaluate the hepatoprotective effects of alcoholic extract of *Momordica charantia, Syzgium cumini, Aegle marmelos, Coccinia indica,* and seeds of *Trigonella foenum graecum* in diabetic albino rats Mishra et al. (2009) estimated serum levels of SGPT, SGOT, ALP and total bilirubin. They found that the polyherbal formulation
extract controlled and restored the SGPT, SGOT, ALP and total bilirubin levels comparable to control animals and concluded that the alcoholic extracts of *Coccinia indica* and seeds of *Trigonella foenum graecum* have significant hepatoprotective activity.

Uemura *et al*. (2010) identified diosgenin, a major aglycone of saponins in fenugreek to promote adipocyte differentiation and to inhibit expressions of several molecular candidates associated with inflammation in 3T3-L1 cells. These results suggested that fenugreek ameliorated diabetes by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues and its effects were mediated by diosgenin.

Ramadan *et al*. (2010) reported that Egyptian fenugreek seed powder at high dose completely modulated the immunosuppressive activity of cyclophosphamide (CP) including leucopenia, decrease in weights and cellularity of lymphoid organs, serum γ-globulin level, delayed type of hypersensitivity response and delay in the skin-burn healing process.

Mostofa *et al*. (2010) demonstrated the hypoglycaemic action of alcoholic extract of *T. foenum graecum* seed (500 mg/kg) and *Stevia* leaves (100 mg/kg) and compared its action with known oral hypoglycaemic drug glimepramide (at 800 mg/kg) in normal and STZ induced diabetic rats. They found that the hypoglycaemic efficacy of combined group with glimepramide was significantly higher as compared to those of herbal extracts individually or in combination.
Tripathi and Chandra (2010) investigated the effect of *Trigonella foenum graecum* on antioxidant enzymes and lipid peroxidation in liver and kidney of alloxan induced diabetic rats. They observed that the administration of *Trigonella foenum graecum* (45 g seed/kg body weight/day) extract in diabetic rats progressively improved the levels of fasting blood glucose, significantly decreased lipid peroxidation and increased the activities of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST) and reduced glutathione (GSH) contents in liver and kidney tissues of diabetic rats. They stated that the anti-hyperglycemic and antioxidant properties of *Trigonella foenum graecum* were comparable with that of metformin, a standard hypoglycemic drug.

*T. foenum graecum* and *L. termis* on oral administration at a dose of 75 mg /kg body weight individually or in combination resulted in marked improvement in histological picture of pancreas, especially after 21 days when compared with diabetic rats. The administration of these plants also resulted in significant reduction in blood glucose, serum cholesterol level and triglycerides in addition, to increased glycogen storage in hepatic cells in treated diabetic rats (Eman and Elaziz, 2011).

Lal et al. (2011) in a study on combined treatment with *Trigonella foenum graecum* seed extract and glibenclamide, a standard antidiabetic drug observed a steady and significant reduction in the hyperglycaemic during the monitoring period. They also observed a significant (P≤0.05) reduction in blood glucose level in treated rats compared to diabetic control.
Baquer et al. (2011) reported that 4-hydroxyisoleucine fraction of *Trigonella foenum graecum* upregulate Glut-4 transport and activate glucose transport at the transcription level similar to that by insulin through *in vitro* studies.

Mohana et al. (2012) reported that the seeds of *T. Foenum graecum* contain amino acid 4-hydroxyisoleucine, which significantly decreases the plasma triglyceride levels. They also observed that the *Trigonella foenum graecum* extract possessed immunomodulatory, anti-inflammatory and antipyretic activities in their experimental study.

### 2.7 Herb - *Coccinia Indica*

Shibib et al. (1993) in an attempt to evaluate the hypoglycaemic activity of *Coccinia indica* and *Momordica charantia* in diabetic rats used STZ as a diabetogenic agent at 65 mg/kg intraperitonially as a single dose and selected only the rats which showed blood glucose level of 200 mg/dl and above for the study.

Biazid et al. (1993) showed that ethanolic extracts of *Coccinia indica* and *Momordica charantia* at 200 mg/kg have an anti hyperglycaemic effect in normal and STZ diabetic rats as evidenced by 23 per cent and 27 per cent decrease in blood sugar respectively. They further stated that the anti hyperglycaemic and hypoglycaemic effects were possibly due to inhibition of glucose-6-phosphatase and fructose-1, 6-biphosphatase in the liver and stimulation of red cell and hepatic glucose-6-phosphate dehydrogenase activities.
The haematological parameters were measured in streptozotocin induced diabetic rat models consisting of controls, diabetic and insulin treated diabetic rats. The platelet counts were significantly reduced (P≤0.05) in diabetic control rats whereas, treated rats showed significant improvement in platelet count (Jacksoni et al., 1996).

Venkateswaran and pari (2003) investigated the effects of long term *Coccinia indica* leaf extract (200 mg/kg body weight) treatment for 45 days on lipoprotein (HDL) cholesterol plasma thiobarbituric acid reactive substances, hydroperoxides, vitamin E and ceruloplasmin profiles in normal and streptozotocin (STZ) induced diabetic rats. The results showed that there was a significant increase in plasma thiobarbituric acid reactive substances, hydroperoxides, vitamin E and ceruloplasmin in STZ-induced diabetic rats, accompanied by a decrease in high density lipoprotein (HDL) cholesterol. The treatment of diabetic rats with *Coccinia indica* leaf extract (CLEt) (200 mg/kg body weight) for 45 days returned these levels close to normal. These results suggested that *Coccinia indica* leaf extract possesses hypolipidemic, antioxidant as well as hypoglycemic effects in the STZ-induced diabetic rat.

Mallick et al. (2007) studied the effect of aqueous methanolic extract of *Coccinia indica* and root of *Musa paradisiaca* in separate as well as in composite manner at a concentration of 80mg/100g body weight/day in streptozotocin induced diabetic rat. They observed a significant remedial effect on blood glucose level as well as carbohydrate metabolic enzymes and on quantity of liver and skeletal muscle glycogen. In addition serum insulin level that was diminished in streptozotocin induced diabetic rat was observed to recover significantly after the co-administration of extracts.
Singh et al. (2008) studied the effect of *Coccinia indica* leaves extract (200 mg/kg) in alloxan induced diabetic rats, on parameters related to oxidative stress (SOD, CAT, GPx, GSH and LPO) and lipid metabolism (total lipids, TG, TC, LDL, VDL, and VLDL) which showed down regulated antioxidant defence and dysregulation of lipid metabolism. After treatment with herbal extract they observed a significant increase in hepatic antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), GSH and lipid metabolism showed improvement in total lipids, TG, TC, LDL, VDL, VLDL and finally concluded that *Coccinia indica* leave extract had hepatoprotective effect at 200 mg/kg body weight.

Das et al. (2008) investigated the effect of alcoholic extracts of *Trigonella foenum graecum* seed & *Coccinia indica* leaves with the commonly used drug Glimepiride in STZ-induced diabetic rats. They observed that moderately alleviated the effects of STZ in diabetic rats and significantly improved the physiological and biochemical parameters such as body weight, haemoglobin, serum glucose, cholesterol, triglyceride, ALT, AST and serum insulin in diabetic rats. Whereas, the effect of herbal extract with glimepiride showed the maximum improvement in biochemical, haematological, insulin and lipid profiles which were comparable to normal control.

Mallick and Ghosh (2009) investigated the indices of protein metabolic disorders which deviated from normal in STZ-induced rats. They observed a significant protection after the treatment with *Coccinia indica* in diabetic rats especially in those rats treated with in a composite manner.
Rafiq et al. (2009) induced diabetes in rats using streptozotocin (45 mg/kg) and treated the diabetic rats with *Coccinia indica* and observed weight gain, decreased blood glucose, serum cholesterol and triglyceride levels. Further in their study they observed significant decrease in antioxidant activity in streptozotocin induced diabetic rats and after treatment with alcoholic *Coccinia indica* extract a significant increase in levels of SOD, CAT and GPx activities in the liver and kidneys of treated rats.

The aqueous extract of fresh leaves of Ivy Gourd (*Coccinia indica*) exhibited on phytochemical analysis the presence of anthraquinons in addition to alkaloids, carbohydrate, proteins and amino acids, tannins, saponins, flavonoids, phytosterol, triterpenes, cephalandrol, tritriacontane, lupeol, b-sitosterol, cephalandrine A, cephalandrine B, stigma-7-en-3-one and taraxerone and taraxerol, (Yadav et al., 2010)

Rajesh et al. (2010) investigated the effect of combined aqueous fruit extract of *Morinda citrifolia* and *Coccinia indica* in STZ induced diabetes in rats which showed hyperglycaemia. The diabetic rats on treatment with combined aqueous fruit extracts of *Morinda citrifolia* and *Coccinia indica* showed significant reduction in serum cholesterol, triglycerides (TG) and LDL cholesterol levels.

Saba et al. (2010) studied the haematological parameters in alloxan induced diabetes in the Wistar rats, and observed that the PCV and RBC count of the untreated diabetic animals (group) were significantly lower (P≤0.05) than those of either the normal control (group) or those given *Parquetina nigrescens* extract.
Eyong et al. (2011) evaluated the effect of co-administration of extracts of Vernonia amygdalina and Azadirachta indica on haemopoitic and immunological indices of normal and diabetic rats. White blood cells, PCV, Hb which were significantly decreased (p≤0.05) in diabetic control rats relative to the normal control, upon administration of the combined extracts of VA and AI improved in diabetic rats. The haematological parameters like packed cell volume, haemoglobin content and red blood cell count significantly (p≤0.05) increased and were comparable with those of normal control.

In an experimental study for a period of 90 days in rat with Coccinia indica extract and glibenclamide individually and in combination Amita Rai et al. (2012) observed a synergistic effect on hypoglycaemic, hypolipidemic and antioxidant effects in diabetic patient and reported that the extract could be used safely in the treatment of diabetes.

2.8 Radioimmunoassay for insulin (RIA)

Radioimmunoassay (RIA) was first developed by Yalow and Berson (1961) to determine levels of insulin anti insulin complexes in diabetics. The principle of RIA involves competitive binding of radiolabeled antigen and unlabeled antigen to a high affinity antibody.

Punitha et al. (2005) reported that no significant change was noted in the serum insulin level of the diabetic animals treated with beriberine, and stated that beriberine probably exerted antihyperglycemic activity by an extra pancreatic mechanism independent of insulin secretion.
Akbarzadeh et al. (2007) in his experimental study on induced diabetes mellitus in Wistar rats measured the insulin and c-peptide levels by radio immunoassay method. They recorded decreased levels of insulin and c-peptide in diabetic rats.

Gayathri and Kannabiran (2008) studied the diabetic associated metabolic alteration in streptozotocin induced diabetic rats. Plasma insulin concentration was detected by radioimmunoassay kit and they found a significant decrease in levels of plasma insulin after induction of diabetes. After oral administration of extract of *Pterocarpus marsupium* bark for the period of 12 weeks, they observed increased levels of insulin near to normal level compared with untreated control rats.

Rajnish and Radhey (2009) noticed significant reduction in serum insulin, protein and albumin levels after induction of diabetes by Streptozotocin. After administration of methanolic extract of *Pterocarpus marsupium* for a period of 14 days, they found a significant elevation in serum insulin and albumin levels in comparison to diabetic rats.

Dhanush (2009) in his study on *Eugenia jambolona* and *Tinospora cordifolia* in experimentally induced diabetes in rats observed decrease in serum insulin level after treatment with herbal extracts by radio immuno assay.

Nasreen (2012) conducted a study on hypoglycemic effect of aged garlic extract on experimentally induced diabetes mellitus in rats and estimated insulin level in serum by Radio Immuno Assay. She observed that the insulin level significantly reduced on induction of diabetes in rats and on treatment with aged garlic extract, the insulin values significantly increased.
Mallikarjuna *et al.* (2013) in his study on pathomorphological and biochemical evaluation of hypoglycaemic effects of *Momordica charantia* and *Gymnema sylvestre* in experimentally induced diabetes in rats and estimated insulin level in serum by Radio Immuno Assay. They observed that the insulin level significantly reduced on induction of diabetes in rats and on treatment with *Momordica charantia* and *Gymnema sylvestre* extract with glibenclamide, the insulin level significantly increased.

2.9 **Antioxidant enzymes**

In general, the effect of antioxidants in free radical injury is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule (Lachman *et al.*, 1986).

Baynes (1991) in his study on role of oxidative stress in development of complications in diabetes stated that oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of antioxidant enzymes and play a role in the long-term complications of diabetes.

Anjali and Manoj (1995) in their study on herbal therapy for diabetes stated that many plant extracts and plant products have been shown to have significant antioxidant activity, which may be an important property of plant medicines associated with the treatment of several diseases including diabetes.

Kakkar *et al.* (1998) in their investigation on oxidative stress in rat liver and pancreas during progression of diabetes, observed a decline in the activity of SOD in
liver of STZ (80 mg/kg b.w) induced diabetic rats and concluded that the decreasing trend in SOD activity could be due to accumulation of H$_2$O$_2$ in diabetic tissues which inhibits Cu-Zn-SOD. They attributed the cause to hypoinsulinaemia which increases the activity of enzyme fatty acyl-CoA oxidase that initiates β-oxidation of fatty acids resulting in increased production of H$_2$O$_2$.

High levels of oxidative stress with excessive generation of free radicals and depleted levels of free radical scavenging enzymes have been demonstrated in several studies, both in experimental animal models of diabetes and in human diabetic subjects (Rousselot et al., 2000; Telci et al., 2000 and Turk et al., 2002).

Venkateswaran and Pari (2003) in their study on antioxidant effect of methanol derived extract from *Coccinia indica* in streptozotocin induced diabetic rats reported that the level of reduced glutathione was significantly lowered in STZ induced diabetic rats, suggesting the inactivation caused by reactive oxygen species. They also observed that further treatment of diabetic rats with methanol derived extract from *Coccinia indica* induced an increase in the level of glutathione.

Prosenjit et al. (2004) studied the anticarcinogenic activity of *Swertia cheretia* and reported reduced activities of GST, GPx as well as SOD with activation of CAT in the liver of the mice when treated with crude and purified fractions.

Joshi et al. (2004) in their study on non insulin dependent diabetes mellitus in rats recorded a significant decrease in SOD and CAT levels. However, methanolic extract of
Pterocarpus marsupium heartwood and glibenclamide significantly reversed the SOD and CAT levels.

Sathishsekar and Subramanian (2005) in their experiment on study of antioxidant properties of Momordica charantia (bittergourd) seeds on streptozotocin induced diabetes in rats showed that there was reduction in activities of GPx and GST in the liver and kidney which resulted in a number of deleterious effects due to the accumulation of toxic products.

Numerous experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to an increased formation of free radicals and consequently to the onset of oxidative stress which has been implicated in diabetes associated complications (Mehta et al., 2006).

Resmi et al. (2006) in a study on antioxidant activity of Albizia lebbeck in alloxan diabetic rats reported that there was a decline in the activities of antioxidant enzymes, such as SOD, CAT, GPX etc. in diabetic rats which indicated the extent of free radical induced damage due to hyperglycemia.

Kaleem et al. (2006) in their study on antidiabetic and antioxidant activity of Annona squamosa extract in streptozotocin-induced diabetic rats reported that the SOD, CAT and GPx activities were significantly reduced in the liver and kidneys of diabetic rats.

Tripati and Chandra (2010) reported that the administration of Momordica charantia (13.33 g pulp/kg body weight/day) and Trigonella foenum graecum (9 g seeds
powder/kg body weight/day) extracts in diabetic rats remarkably improved the elevated levels of fasting blood glucose and significantly decreased lipid peroxidation ($p \leq 0.001$) and significantly increased activities of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST) and reduced glutathione (GSH) contents in heart tissue of diabetic rats.

Iwueke et al. (2010) in their experiment on alloxan induced diabetic rats observed an increase in catalase activity which is known to scavenge and detoxify $H_2O_2$ and they attributed the cause to increased concentration of $H_2O_2$ in the system, leading to utilization of the enzyme.

Singh et al. (2010) in their study on hepatoprotective activity of aqueous extract of seven plants Annona squamosa, Cassia fistula, Coccinia indica, Angifera indica, Ocimum sanctum, Lagerstroemia losflos-reginee, and Murraya koenigii reported reduction of SOD, GSH and GPx due to diabetes induced liver damage which significantly improved in dose dependent manner upon administration of combined plant extracts.

Pragathi (2011) in her study on evaluation of hypoglycemic effect of Gymnema sylvestre and cow urine in diabetic rat model observed decreased levels of SOD, CAT and GPx in streptozotocin induced diabetic animals. However, they recorded improvement in antioxidant enzyme levels when animals were treated with Gymnema sylvestre and cow urine for duration of 45 days.
Prem Kumar et al. (2012) evaluated oxidative stress in the form of levels of lipid peroxidation, non enzymatic (GSH) and enzymatic (SOD, CAT and GPx) antioxidants in liver, muscle and kidney. They showed an increase in lipid peroxidation and decrease in antioxidants ranging from 30 per cent to 50 per cent indicating coexistence of metabolic disturbances and oxidative stress in diabetic animals.

Mallikarjuna et al. (2013) in his study on pathomorphological and biochemical evaluation of hypoglycaemic effects of *Momordica charantia* and *Gymnema sylvestre* in experimentally induced diabetes in rats, observed decline in levels of SOD, CAT and GPx. However, they recorded improvement in antioxidant enzyme levels when animals were treated with *Momordica charantia* and *Gymnema sylvestre* for duration of 45 days.

### 2.10 Pathology

Pillion et al. (1988) studied the effects of streptozotocin induced diabetes mellitus in male Fisher rats and found a significant loss of total body weight associated with adipose and muscle tissue wasting. Paradoxically, intestinal mass and length were increased in STZ-treated rats despite increased catabolism in other tissues. Concomitant with increased intestinal mass, food and water intake increased significantly in STZ diabetic animals. They proposed that the adult STZ-diabetic rat responds to loss of available insulin by polyphagia, polydipsia, and catabolism of adipose and muscle tissue and that a large percentage of available synthetic fuel is devoted to the production of additional intestinal tissue.

Li et al. (2000) made a morphometric analysis of islet endocrine cells in type-I diabetic mice induced by administration of multiple low doses of STZ. They observed a
reduction in islet cell area by 35 per cent and a gradual decrease in insulin positive β-cells with number of alpha cells increased by 2-3 times per islet area.

Sharma et al. (2003) carried out histological studies on heart, liver and pancreas in alloxan-induced diabetic rabbits which were sacrificed after one month of the experiment and observed mild congestion of central vein, fatty change in hepatocytes and vacuolation of the myocytes. In pancreas, they observed partly hyalinised islets, reduced number of β-cells with cytoplasmic vacuolations in occasional cells.

STZ administration causes islet atrophy through β-cell loss, which depletes the central core of islet tissue leaving an ostensibly thickened layer of peripheral islet non-β cells (Zhang et al., 2003).

Kaneez, (2004) studied the pathological changes in chronic diabetes induced by single dose of STZ at 60 mg/kg body weight in Wistar albino rats. They observed lesions such as thickening of glomerular basement membrane, expansion of mesangium, glomerular sclerosis, interstitial and tubular fibrosis, arteriosclerosis, hyperaemia and oedema in kidneys, lipidosis and oedema in liver, extensive haemorrhage, oedema and signs of myocardial infarction in heart.

Gholamali et al. (2005) showed that alloxan caused severe necrotic changes of pancreatic islets, especially in the center of the islets nuclear changes like karyolysis, and in some places residue of destructed cell. They also observed relative reduction in the size and number of islets, especially around the large vessel, and also severe reduction in the number of beta cells.
Akbarzadeh et al. (2007) studied streptozotocin induced diabetes in male adult rats by injecting 60 mg/kg of streptozotocin intravenously. Three days after the induction of diabetes, consumption of food and water, volume of urine and glucose increased in the diabetic animals in comparison with normal animals, but the weight of body and the volume of insulin decreased in the diabetic animals. They observed degeneration of β-cells in pancreas of diabetic rats.

The effect of methanolic extracts of *Equisetrum arvense* on the pancreatic beta cells in STZ induced diabetes rats was evaluated by Soleimani et al. (2007). They observed small, atrophic, irregular shaped islets comprising of only a few small, degranulated and highly vacuolated β-cells in diabetic rats and an improvement in islet morphology with an increase in the number of islets and β-cells in plant treated group.

Mir et al. (2008) observed the histopathological changes in various organs in experimentally induced diabetes in rabbits. The observations included slight congestion and mild degree of degeneration in pancreatic acini, decreased cellularity in islets, fusiform appearance of cells of some islets, congestion and haemorrhage in alveoli and bronchioles of lungs, congestion of kidney, degeneration and congestion of liver, haemorrhage and myopathy in heart and mild neuronal damage in brain.

STZ induced β-cell toxicity associated with severe depletion of intracellular insulin stores has been attributed to rapid catabolism of nicotinamide adenine dinucleotide (NAD\(^+\)). The sub physiological levels of NAD\(^+\) are associated with severe reduction in insulin production as the low energy supply to the β-cells turns the
proapoptotic signals of PARP overactivation into necrotic processes and eventually cell death (Cardinal et al., 1999; Nugent et al., 2008).

Intravenous administration of alloxan to rats resulted in selective damage of beta cells of islets with loss of regular arrangement of alpha and beta cells reduction in the granularity, hydropic degeneration as well as clumping, pyknosis, and necrosis of beta cells (Singh et al., 2008).

Prasad et al. (2009) in their investigation on the antidiabetic activity of some herbal plants in streptozotocin induced diabetic albino rats observed that the pancreatic islets of diabetic control group revealed significant architectural disarray which also extended into surrounding exocrine tissue.

Dhanush (2009) in his study on pathomorphological and biochemical evaluation of hypoglycemic effects of Eugenia jambolona and Tinospora cordifolia in experimentally induced diabetes in rats observed reduced number of islets which were irregular in shape with swollen, highly vacuolated and necrotic β-cells on 15th day of the study.

Shah et al. (2009) demonstrated the effect of 4-hydrroxyisoluecine isolated from fenugreek on the pancreatic beta cells in STZ induced diabetic mice. They observed small, atrophic, irregular shaped islets with reduction in the number of beta cells and also noticed degranulation and cytoplasmic vacuolations in most of the beta cells. Plant extract treated group showed an improvement in the islet morphology in terms of increase in number of pancreatic islets, number of beta cells, reduction in cytoplasmic vacuolations, increase in
granularity and presence of tiny islet indicating neogenesis from pancreatic duct or progenitor cells

Atangwho et al. (2010) reported that untreated streptozotocin (STZ) diabetic rats presented damaged islets which were markedly shrunken in mass and infiltrated with lymphocytes.

Hassan et al. (2010) observed the zonaglomerulosa cells of the STZ-induced diabetic rats showing apparent increase in the cytoplasmic vacuolation and pyknotic nuclei. Electron microscopic study showed an apparent increase in the number of lipid droplets associated with mitochondrial degeneration.

Eman and Elaziz (2011) studied pathological changes in male adult rats by injecting 110 mg/kg of alloxan intravenously. Fourteen days after the induction of diabetes, pancreas showed vacuolar changes with pyknosis of some nuclei of cells in the islets of Langerhans, some areas revealed atrophy of the islets with pyknotic nuclei. Treatment of diabetic rats with T. foenum graecum which contains 4-hydroxyisoleucine showed partial restoration of normal cellular population and size of islet cells.

Mudasir (2011) in his study on pathomorphological and biochemical evaluation of hypoglycaemic effects of Momordica charantia and cow urine in experimentally induced diabetes in rats observed improvement in size, shape and cellularity of islets. There was an increase in the number of granulated β- cells and attainment of almost normal distribution of α and β-cells in diabetic rats treated with Momordica charantia, cow urine and glibenclamide.
Mohana et al. (2012) in their study on alloxan induced diabetic rats recorded extensively damaged islets of Langerhans with irregular appearance. Treatment of diabetic rats with metformin and aqueous extract of Cassia occidentalis showed moderate expansion and partial restoration of cellular population and size of the islets.

Kadagi et al. (2012) in their experiment on hypoglycemic activity of cow urine distillate in streptozotocin induced diabetic rats observed extensive damage of the islets of Langerhans with destruction, degeneration and necrosis of β-cells indicating cytotoxicity of streptozotocin on islets. Treatment of diabetic rats with glibenclamide and cow urine distillate showed partial restoration of normal cellular population and size of islet cells.

Hameed et al. (2013) investigated the changes of adrenal gland tissue for histological examination in streptozotocin induced diabetes in rats, and observed normal appearance of adrenal gland architecture in normal control, and severe fatty degenerative changes in diabetic control, whereas, moderate fatty degenerative changes were observed in cortex of adrenal gland in diabetic rat treated with green tea.

Mallikarjuna et al. (2013) in his study on pathomorphological and biochemical evaluation of hypoglycaemic effects of Momordica charantia and Gymnema sylvestre in experimentally induced diabetes in rats observed improvement in size, shape and cellularity of islets. There was an increase in the number of granulated β-cells and attainment of almost normal distribution of α and β-cells in diabetic rats treated with G.sylvestre.
2.11 Immunohistochemical demonstration of insulin

Kakkar et al. (1998) in their investigation on the increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes observed a 50-60 per cent reduction in insulin immunoreactivity during third week of the study in the pancreas of STZ induced diabetic rats.

Sheng et al. (2004) in their study on the effects of timely insulin treatment on protection of β cells in a rat model of type 2 diabetes mellitus observed that immunohistochemical staining of pancreatic cells from untreated diabetic (STZ) rats revealed smaller islets with degranulation in the β cells.

Bolkent et al. (2005) in their study on immunohistochemical studies on the effect of Aloe vera on the pancreatic β-cells in neonatal streptozotocin-induced type-II diabetic rats observed that immunoreactivity of β-cells of the control diabetic group was not different from that of glibenclamide, Aloe vera leaf gel or pulp extracts treatment groups.

Adewole and Ojewole (2007) in their experiment on insulin induced immunohistochemical and morphological changes in pancreatic beta-cells of streptozotocin treated diabetic rats observed that the pancreatic insulin contents of the insulin treated group increased approximately by 45 fold in immunoreactivity on 30th day of the study, when compared with the immunoreactivity of the same insulin treated diabetic rats on Day 10 of the 40 day study period.

Ozmen et al. (2007) in their study on the effect of insufficient insulin treatment in streptozotocin induced diabetes mellitus reported that there was a severe reduction in
insulin secreting cells in the group treated with 8 IU of short-acting insulin than that in the diabetic control as revealed by immunohistochemistry.

In an experiment on the obesity and non-insulin dependent diabetes mellitus in Swiss Webster mice associated with late-onset hepatocellular carcinoma, insulin IHC was performed to confirm the presence of islet metaplasia by demonstrating isolated insulin producing cells within the exocrine pancreas and/or foci of insulin producing cells admixed with exocrine like cells in poorly defined islet like sub lobules (Lemke et al., 2008).

Pragathi (2011) in a study on effect of *Gymnema sylvestre* in STZ induced diabetes in rats observed an appreciable increase in the number of beta cells of pancreas by immunohistochemistry on 30th and 45th day of the experiment.

Mudasir (2011) in his experiment on *Momordica charantia*, Cow urine along with glibenclamide in streptozotocin treated diabetic rats and treated groups observed an increase in immunoreactivity on 30th and 45th day of the study.

### 2.12 Special staining for beta cells

Chakravarthy *et al.* (1980) quantitated beta cell population by counting beta cells after staining with Gomori's aldehyde fuschin satin and showed that administration of flavenoid fraction extracted from bark of *Pterocarpus marsupium* 24 hours after alloxan, administration caused restoration of beta cell population.

Adeyemi *et al.* (2008) in a study on the effects of *Annona muricata* (Linn) on the morphology of pancreatic islet cells in experimentally induced diabetes in Wistar rats
observed a recovery of the β-cells, regeneration of islet cells and reduction in the vacuolations caused by administration of STZ using Gomori's chrome alum hematoxylin phloxin stain.

Khalifa et al. (2009) in their study on therapeutic role of low carbohydrate ketogenic diet in diabetes showed the presence of only a few necrotic beta cells in the islets by Gomori's chrome alum hematoxylin-phloxine staining of pancreas sections. Also they observed that both alpha and delta cells were unaffected.

Pragathi (2011) in a study on the effects of Gymnema sylvestre on the morphology of pancreatic islet cells in experimentally induced diabetes in Wistar rats observed recovery of β-cells, regeneration of islet cells and reduction in the vacuolations caused by administration of STZ using Gomori's chrome alum hematoxylin phloxin stain.

2.13 Immunomodulatory effect of medicinal plants

Neelam et al. (2001) investigated the alcoholic extract of stem bark of Mangifera indica Linn for its effect on cell mediated and humoral components of the immune system in mice and reported that administration of test extract increased in humoral antibody (HA) titre and delayed type hypersensitivity (DTH) in mice.

Bilal et al. (2003) evaluated the immunomodulatory activity of aqueous extract of Trigonella foenum graecum in male Swiss albino mice by measuring body weight, relative organ weight, cellularity of lymphoid organs, delayed type of hypersensitivity (DTH) response, plaque-forming cell (PFC) assay, haemagglutination titre (HT), quantitative haemolysis of SRBC (QHS) assay, phagocytosis, and lymphoproliferation in
various groups of animals. The results showed a stimulatory effect on immune functions in mice by *Trigonella foenum graecum*.

A novel polysaccharide α-d-glucan possessing unique immune stimulating properties was isolated and characterized from the medicinal plant *Tinospora cordifolia* and was shown to attract different subsets of the lymphocytes such as natural killer (NK) cells (331%), T cells (102%), and B cells (39%) at 100 μg/ml concentration by Raveendran Nair *et al.* (2004).

The stimulatory effect of *Andrographis paniculata* extract and andrographolide on cytotoxic T lymphocyte (CTL) production was determined in BALB/c mice by Sheeja and Kuttan (2007) who reported a significant increase in CTL production in both *in vivo* and *in vitro* models.

Hedman *et al.* (2008) in a study on identification of markers in type-1 diabetic patients observed increased percentages of CD8+CCR and CD8+ CD45RA cells in peripheral blood and low percentage of CD4+ and CD8+ cells.

Halogappa *et al.* (2010) investigated the effect of aqueous extract of *Pterocarpus marsupium* on elevated inflammatory cytokine, tumor necrosis factor (TNF)-α in type 2 diabetic rats and observed a significant (*P* ≤ 0.001) decrease in the elevated TNF-α level.

Ramadan *et al.* (2010) reported that Egyptian fenugreek seed powder (FSP) at high dose completely modulated the immunosuppressive activity of cyclophosphamide (CP) including leucopenia, decrease in weights and cellularity of lymphoid organs, serum
\( \gamma \)-globulin level, delayed type of hypersensitivity response and delay in the skin-burn healing process.

Immunomodulatory, beta-cell, and neuroprotective actions of fenugreek oil in alloxan induced diabetes were studied by Ramadan et al. (2010) and observed a fewer infiltrated inflammatory cells, near to normal value of white blood cell, mean corpuscular volume, and lymphocytes counts in pancreas.

The immunomodulatory activity of ethanolic extract from *Trigonella foenum graeceum* was studied for its effect on phagocytic activity, cell mediated and humoral immune response in mice. Immunomodulatory effect was assessed by carbon clearance test, delayed type of hypersensitivity (DTH), T cell population test, and sheep erythrocyte agglutination test (SEAT) in mice treated with extract at doses of 200 and 400 mg/kg body weight. In carbon clearance test, trigonella extract exhibited significantly high phagocytic index against control group, indicating stimulation of the reticulo-endothelial system. A significant decrease in mean difference, in the foot paw thickness in DTH indicated its anti-inflammatory activity. In addition a significant increase in antibody titer and increase in T-cell rosette formation in T-cell population test against control were also observed. These results indicated immunomodulatory activity of *Trigonella foenum graeceum* in indigenous medicine (Tripathi et al., 2010)

Studies in T1D pathogenesis for the past several decades have been mainly focused on the role of adaptive immunity. T cells are believed to be the major effector cells responsible for beta cell destruction. CD8+ T cells have been found to be critical for disease pathogenesis in both T1D patients and mouse model for spontaneous autoimmune diabetes (Gren et al., 2012).
Materials and Methods
III. MATERIALS AND METHODS

The present study was conducted at the Department of Veterinary Pathology, Veterinary College, Bangalore during the year 2011-13 to evaluate the antidiabetic effect of *Trigonella foenum graecum* and *Coccinia indica* individually as well as in combination and also to compare the hypoglycemic effects with an oral hypoglycemic agent, glibenclamide in rats.

3.1 Phytochemical analysis

Phytochemical analysis of the alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* was carried out using HPTLC technique (Wagner et al., 1984).

Analyses were performed for the following constituents:

1. Alkaloids
2. Anthracene derivatives
3. Bitter principles
4. Coumarins
5. Flavonoids
6. Glycosides
7. Saponins

3.1.1 Procedure of TLC

Pre-coated silica gel 60F 254 TLC aluminium 10x10 cm (Merck, India) type of plates were used for HPTLC. From each alcoholic extract, 5 µl samples were spotted on a TLC silica gel plate (CAMAG Linomat 5, Germany). Chromatography was performed
using solvent systems. This procedure was followed for the analysis of alkaloids, anthracene derivatives, flavonoids, bitter principles, coumarins, saponins and glycosides.

3.1.2 Alkaloids

3.1.2.1 Preparation of extracts for Thin Layer Chromatography

The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* (0.5 g each) were weighed and mixed with 10 ml of 0.5 N HCl. The contents were vortexed and pellets were discarded. To the supernatant 30 per cent Na$_2$CO$_3$ (pH 10) was added and centrifuged at 2000 rpm for 5 minutes and supernatant was discarded. The precipitate was washed with chloroform and chloroform extract was collected. Once again the residue was washed with methanol and methanol extract was collected. The chloroform and methanol extract were concentrated to one ml and used for chromatography.

3.1.2.2 Chromatography solvent

a) Toluene: Ethyl acetate: Diethylamine: (70:20:10)

b) Ethyl acetate: methanol: water: (100:13.5:10) were used as solvent systems for the detection of alkaloids in alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica*

3.1.2.3 Detection

a) Without chemical treatment

TLC plates were observed under UV-254 nm and UV-366 nm.
b) Dragendorff reagent

The ready to use reagent (SD Fine-Chem Limited, Mumbai) was used. One ml of Dragendorff reagent was diluted with four ml of acetic acid and 20 ml of water. The plate was immersed in the reagent for one second and examined under white light.

3.1.3 Anthracene derivatives

3.1.3.1 Preparation of extracts for TLC

The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* (0.5 g each) were weighed and extracted by warming for five minutes on the water bath with five ml of methanol. The clear filtrates were used directly for HPTLC.

3.1.3.2 Chromatography solvent

A mixture of ethyl acetate: methanol: water: (100:17:13) was used as solvent system for the detection of anthracene derivatives in alchoholic extracts of *Trigonella foenum graecum* and *Coccinia indica*.

3.1.3.3 Detection

a) Without chemical treatment

TLC plates were observed under UV-254 nm and UV-366 nm.

b) Natural products-polyethylene glycol

The plate was heated to 100°C for three minutes, then dipped in solution A (1 g diphenylboronic acid aminoether ester dissolved in 200 ml ethyl acetate), dried and dipped in solution B (10 g polyethylene glycol 400, dissolved in 200 ml dichloromethane).
c) Potassium hydroxide

Five per cent ethanolic KOH was prepared and the plate was immersed in the reagent for one second. The plate was observed in visible and UV-366 nm light.

3.1.4 Bitter principles

3.1.4.1 Preparation of extracts for TLC

The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* (1 g) were extracted separately for 10 minutes with 10 mL methanol at 60 °C on the water bath. The mixtures were filtered and the filtrates were evaporated to a volume of about two ml.

3.1.4.2 Chromatography solvent

Ethyl acetate: methanol: water: (77:15:08) was used as solvent system for the detection of bitter principles in alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica*.

3.1.4.3 Detection

a) Without chemical treatment

Silica gel aluminium plates were observed under UV-254 nm and UV-366 nm.

b) Vanillin-sulphuric acid

The reagent consisted of 5 per cent ethanolic sulphuric acid (Solution I) one per cent ethanolic vanillin (Solution II). The plate was sprayed vigorously with 10 ml of solution I, followed immediately by five to 10 ml of solution II after heating the TLC
plate at 100°C for five to 10 minutes. The plate was examined under white light and UV 366 nm.

3.1.5 Coumarins

3.1.5.1 Preparation of extracts for TLC

The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* (1 g) were extracted separately by shaking with 10 ml methanol for 30 min on the water bath. The clear filtrates were evaporated to about one ml and 20 µl was applied to TLC.

3.1.5.2 Chromatography solvent

Toluene: ether (1:1, saturated with 10% acetic acid), was used as solvent system for the detection of coumarins in alcoholic extracts of *Trigonella foenum graceum* and *Coccinia indica*.

3.1.5.3 Detection

a) Without chemical treatment

TLC plates were observed under UV-254 nm and UV-366 nm.

b) Potassium hydroxide

Ethanolic KOH (5%) was used as spray reagent. The plate was immersed in the reagent for one second and was observed at UV-366 nm.
3.1.6 Flavonoids

3.1.6.1 Preparation of extracts for TLC

The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* were extracted separately with 10 mL methanol for five minutes in a water bath at about 60 °C. The clear filtrates were used for chromatography.

3.1.6.2 Chromatography solvent


The ethyl acetate, formic acid and glacial acetic acid were mixed first and the water was added gradually with vigorous shaking. This was used as solvent system for the detection of flavonoids in alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica*.

3.1.6.3 Detection

a) Without chemical treatment

TLC plates were observed under UV-254 nm and UV-366 nm.

b) Natural products-polyethylene glycol

The plate was heated to 100°C for three minutes, then dipped in solution A (1 g diphenylboronic acid amino ether ester dissolved in 200 ml ethyl acetate), dried in a stream of cold air, then dipped in solution B (10 g polyethylene glycol 400, dissolved in 200 ml dichloromethane).
c) Fast blue salt B

The spray reagent was prepared by dissolving 0.5 g fast blue salt in 100 mL water. The plate was sprayed and dried. The plate was examined under white light and UV 366 nm.

3.1.7 Glycosides

3.1.7.1 Preparation of drug extracts for TLC

The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* (1 g) were extracted separately by shaking with 10 ml methanol for 30 min on the water bath. The clear filtrates were evaporated to about one ml and 20 µl was applied to TLC plates.

3.1.7.2 Chromatography solvent

Toluene: ether (1:1, saturated with 10% acetic acid), was used as solvent system for the detection of glycosides in alcoholic extracts of *Trigonella foenum graceum* and *Coccinia indica*

3.1.7.3 Detection

a) Without chemical treatment

TLC plates were observed under UV-254 nm and UV-366 nm

b) Aniline-diphenylamine phosphoric acid

The spray reagent was prepared using four grams of diphenylamine and four ml aniline which were dissolved in 160 ml acetone. To this 30 ml of O-phosphoric acid was
carefully added. The plate was immersed in the reagent for one second and then heated at 120°C. The plate was examined under white light. This method was used to detect the presence of glycosides.

3.1.8 Saponins

3.1.8.1 Preparation of extracts for TLC

The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* (2 g) were extracted separately by heating for 10 min under reflux with 10 ml of 70% ethanol and the clear filtrates were evaporated to about five ml for chromatography.

3.1.8.2 Chromatography solvent

Chloroform: methanol: water: (64:50:10) was used as solvent system for the detection of saponins in alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica*.

3.1.8.3 Detection

a) Without chemical treatment

The plates were observed UV-254 nm or UV-366 nm.

b) Blood reagent

Ten mL of 3.6% sodium citrate was added to 90 ml of fresh bovine blood. 0.2 ml of this mixture was mixed with 30 ml phosphate buffer pH 7.4. The plate was sprayed in horizontal position and the plate was observed in visible light.
c) Vanillin-sulphuric acid

The reagent consisted of 5 per cent ethanolic sulphuric acid (solution I) 1 per cent ethanolic vanillin (Solution II). The plate was sprayed vigorously with 10 ml Solution I, followed immediately by five to 10 ml of Solution II after heating the TLC plate at 100 °C for five to 10 min. The plate was examined under white light and UV 366 nm.

3.2 Experimental animals

Healthy female albino Wistar rats weighing 190±20g obtained from R.L Instrument and lab animal supplier, Yeswanthpur, Bangalore, were used for the present investigation. The animals were maintained under standard laboratory conditions, with provision of standard laboratory animal feed (Amruth Feeds, Bangalore) and clean drinking water ad libitum. The animals were acclimatized to the experimental conditions for two weeks after procurement. After acclimatization, animals were grouped and housed in polypropylene rat cages during the experimental period. The experiment was carried out for a period of 90 days. The study was carried out with a prior approval from the Institutional Animal Ethical Committee, Veterinary College, Bangalore-560024.

3.3 Sources

3.3.1 Drugs and chemicals

To induce experimental diabetes in rats, streptozotocin was used which was procured from Sigma Chemicals, St. Louis, USA. All the other chemicals used for the study were of analytical grade.
3.3.1.1 Preparation of STZ solution

Fresh 0.1 M citrate buffer having pH 4.5 was prepared and the same was maintained at 4-8°C. The STZ of required quantity was dissolved in ice-cold citrate buffer and injected intraperitoneally to rats immediately to avoid degradation.

3.3.1.2 *Trigonella foenum graecum*

The alcoholic seed extract of *Trigonella foenum graecum* used in the present study was obtained from Plantex Herbal Drug Company, Vijaywada. The powdered extract was weighed according to body weight and dissolved in distilled water to make the final concentration and given to the experimental animals.

3.3.1.4 *Coccinia indica*

The alcoholic plant extract of *Coccinia indica* used in the present study was obtained from Plantex Herbal Drug Company, Vijaywada. The powdered extract was weighed according to body weight and dissolved in distilled water to make the final concentration and given to the experimental animals.

3.3.1.5 Glibenclamide solution

Glibenclamide (Daonil®, 5 mg), an oral hypoglycaemic drug was dissolved in distilled water (82.33 ml) to give a concentration of 60 μg/ml. This was used as a stock solution and administered orally at a dose of 600 μg/ kg using clean and dry gavaging needles (Ramalingam et al., 2004).
3.4 Administration of plant extracts and glibenclamide

Throughout the period of the experiment the plant extracts and glibenclamide were administered orally for their respective groups using clean gavaging needle attached to an appropriate disposable syringe during morning hours of the day for a period of 90 days.

3.5 Experimental design

One hundred and eight female albino Wistar rats were weighed and randomly distributed into nine groups of twelve rats each. Care was taken to maintain the intra-group weight variation to be less than 25 g and inter-group weight variation by 35 g.

The groups and treatments used were as follows,

<table>
<thead>
<tr>
<th>Group I (NC)</th>
<th>Normal control:- Used for studying baseline values of the parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II (DC)</td>
<td>Diabetic control:- Streptozotocin induced diabetic rats</td>
</tr>
<tr>
<td>Group III (GC)</td>
<td>Diabetic rats supplemented with glibenclamide at a dose of 600 μg / kg</td>
</tr>
<tr>
<td>Group IV (TFG)</td>
<td>Diabetic rats supplemented with extract of <em>Trigonella foenum graecum</em> at the dose rate of 1g/kg body weight.</td>
</tr>
<tr>
<td>Group V (CI)</td>
<td>Diabetic rats supplemented with extract of <em>Coccinia indica</em> at the dose rate of 200mg /kg body weight.</td>
</tr>
<tr>
<td>Group VI (TFG + CI)</td>
<td>Diabetic rats supplemented with extract of <em>Trigonella foenum graecum</em> and <em>Coccinia indica</em> at the dose rate of 1g/kg and 200 mg/kg body weight respectively.</td>
</tr>
<tr>
<td>Group VII (TFG + G)</td>
<td>Diabetic rats supplemented with extract of <em>Trigonella foenum graecum</em> and Glibenclamide at the dose rate of 1g/kg, and 300 μg /kg body weight respectively.</td>
</tr>
<tr>
<td>Group VIII (CI + G)</td>
<td>Diabetic rats supplemented with extract of <em>Coccinia indica</em> and Glibenclamide at the dose rate of 200 mg/kg and 300 μg/kg body weight respectively</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Group IX (TFG + CI + G)</td>
<td>Diabetic rats supplemented with extract of <em>Trigonella foenum graecum</em>, <em>Coccinia indica</em> and Glibenclamide at the dose rate of 1 g/kg, 200 mg/kg and 300 μg/kg body weight respectively.</td>
</tr>
</tbody>
</table>

### 3.6 Experimental induction of diabetes

The animals were fasted overnight and diabetes was induced in Groups II to IX by a single intra peritoneal injection of a freshly prepared solution of streptozotocin (45 mg/kg body weight) in 0.1 M cold citrate buffer having a pH of 4.5 (Babu and Prince, 2004). The diabetic control animals received citrate buffer alone.

#### 3.6.1 Confirmation of diabetes

The diabetic state was confirmed by estimating the blood glucose levels after 72 hours of STZ injection using ready to use Span diagnostic kit with semi-automatic biochemical analyzer. The animals that showed the blood glucose level above 200 mg/dl were considered as diabetic. After confirmation of diabetic state, the treatment was commenced.

The rats of group I and II were gavaged only with normal saline and the rats of all other groups with their respective treatments daily for 90 days. The animals were observed daily for recording treatment effect.

### 3.7 Clinical observation

Rats of all the groups were observed for feed and water intake, general behaviour, alertness, urine output, diarrhoea and any other clinical signs.
3.8 Collection of serum samples

To evaluate the biochemical parameters, serum was collected for which blood was drawn from the retro-orbital plexus under light ether anesthesia at different time intervals on Day 3, 15, 30, 45 and 90 post STZ injection of the study.

About 2 ml of blood from each animal of all groups was collected separately in clean test tubes, allowed to clot for 30 min and then centrifuged at 3000 rpm for 10 min. The separated serum was collected and subjected for glucose estimation immediately after collection and the remaining serum samples were stored at -20°C for further analysis.

3.9 Sacrifice of animals

To study the progressive effects of the treatments given to different groups, two rats from each group were sacrificed under light ether anaesthesia on Day 15 and 30, 6 animals on 45th day and remaining two rats on Day 90 of the experiment. Sacrificed animals were subjected for detailed post mortem examination and gross changes, if any were recorded. A piece of liver was collected in ice cold normal saline for estimation of antioxidant enzymes. Further, representative tissue samples from pancreas, liver, kidney, spleen, lungs, heart and intestine were collected in 10 per cent neutral buffered formalin (NBF) for histopathology and immunohistochemistry.
3.10 Parameters analysed

3.10.1 Body weight

The rats were weighed on the day of the commencement of the experiment and on Day 3, 15, 30, 45 and 90 of the study to evaluate the effect of various treatments on body weight.

3.10.2 Serum biochemistry

The serum samples collected at various intervals were subjected to biochemical estimation of serum concentration of glucose, cholesterol, triglycerides, ALT and AST using Semi-Automatic Biochemical Analyzer with commercial biochemical kits (Span diagnostics, Bangalore). These parameters were estimated from serum samples as per the procedure described by Tietz, (1976).

3.10.3 Radio-immunoassay

For the estimation of serum insulin concentrations, radio-immunoassay was performed using iodine labelled insulin assay kit (RIAK-1) obtained from Board of Radiation and Isotope Technology (BRIT), BARC, Mumbai, India. (Courtesy: Department of Pharmacology and Toxicology, Veterinary College, Bangalore-24).

3.10.3.1 Principle of the Assay

The radioimmunoassay method is based upon the competition of unlabeled insulin in the standard and radio iodinated (I-125) insulin for the limited binding sites on a specific antibody. At the end of incubation, the antibody bound and free insulin are separated by the second antibody polyethylene glycol (PEG) aided separation method.
Insulin concentration of samples is quantitated by measuring the radioactivity associated with the bound fraction of sample and standards.

3.10.3.2 The insulin kit contained the following reagents

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Kit reagent</th>
<th>Color</th>
<th>No. of vials</th>
<th>Reconstitution of one vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$^{123}$I-Insulin</td>
<td>Red</td>
<td>2</td>
<td>Added 6 ml assay buffer</td>
</tr>
<tr>
<td>2</td>
<td>Insulin standard (1 mIU/vial)</td>
<td>White</td>
<td>1</td>
<td>Added 5 ml assay buffer</td>
</tr>
<tr>
<td>3</td>
<td>Insulin antiserum (Guinea pig)</td>
<td>Green</td>
<td>1</td>
<td>Added 10 ml assay buffer</td>
</tr>
<tr>
<td>4</td>
<td>Insulin free serum</td>
<td>-</td>
<td>1</td>
<td>Added 2 ml double distilled water</td>
</tr>
<tr>
<td>5</td>
<td>Second antibody (Anti-Guinea pig IgG)</td>
<td>Blue</td>
<td>1</td>
<td>Ready to use solution</td>
</tr>
<tr>
<td>6</td>
<td>Polyethylene glycol</td>
<td>-</td>
<td>1</td>
<td>Ready to use solution</td>
</tr>
<tr>
<td>7</td>
<td>Assay buffer</td>
<td>-</td>
<td>1</td>
<td>Ready to use solution</td>
</tr>
<tr>
<td>8</td>
<td>Insulin controls A &amp; B</td>
<td>-</td>
<td>2</td>
<td>Added 0.5 ml double distilled water</td>
</tr>
</tbody>
</table>

The insulin concentration in the reconstituted standard (A) was 200 µU/ml. Five more standard dilutions were prepared as follows:

<table>
<thead>
<tr>
<th>Insulin standard</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard-A (ml)</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Assay buffer (ml)</td>
<td>1.0</td>
<td>1.5</td>
<td>2.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Insulin concentration (µU/ml)</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

After the reagents were reconstituted as shown in the above tables, the assay was carried out as shown in the assay flow chart below:
### 3.10.3.3 ASSAY FLOW CHART

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Assay buffer (mL)</th>
<th>Insulin Standard (mL)</th>
<th>Serum Sample (mL)</th>
<th>Insulin free serum (mL)</th>
<th>Insulin antiserum (mL)</th>
<th>$^{125}$I-Insulin (mL)</th>
<th>Second antibody (mL)</th>
<th>Polyethylene glycol (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,4</td>
<td>0.4</td>
<td></td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>5,6</td>
<td>0.3</td>
<td></td>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>7,8</td>
<td>0.2</td>
<td>0.1E</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>9,10,</td>
<td>0.2</td>
<td>0.1D</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>11,12</td>
<td>0.2</td>
<td>0.1C</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>13,14</td>
<td>0.2</td>
<td>0.1B</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>15,16</td>
<td>0.2</td>
<td>0.1A</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>17,18</td>
<td>0.3</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>19,20</td>
<td>0.3</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td></td>
<td>0.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Mix gently. Incubate all tubes at +2 to +4°C overnight.

Mix gently. Incubate all the tubes for 3 hours at room temp.

Centrifuge the tubes at 1500g for 20 minutes.

Vortex and keep all the tubes at room temp. For 20 minutes.
All the contents in tubes were mixed properly and kept at room temperature for 20 minutes. Then the tubes were centrifuged at 1500 G for 20 minutes. After centrifugation the tubes were decanted and radioactivity in the precipitate was counted with the help gamma scintillation counter. The counts were taken by keeping the tubes for 60 seconds in the gamma counter.

The insulin values (µU/ml) for the samples were calculated with the help of software SAPRICAL PC based RIA counter model PRIA-I (para electronics, Mumbai). Intra and inter assay variations were 8 per cent & 12 per cent respectively.

3.10.4 Estimation of antioxidant enzymes

3.10.4.1 Material collection

Immediately after sacrificing the animals, liver samples were collected. Sample of liver was excised into ice cold normal saline and then blotted dry and stored at -20° C for further analysis.

3.10.4.2 Homogenate preparation

Liver tissue was homogenized with ice cold 0.1 M Tris-HCl buffer of pH 7.4 to make 30% homogenate w/v (0.5g liver crushed in 10ml of ice cold 0.1mol/l Tris-HCl buffer). This homogenate was centrifuged at 3000 rpm for 10 min. The supernatant was collected and used for estimation of total protein, superoxide dismutase, catalase and glutathione peroxidase.
3.10.4.3 Protein estimation

Protein content of the tissues was estimated by the method described by Lowry et al. (1951).

3.10.4.3.1 Principle

The phenolic groups of tyrosine and tryptophan residues in a protein will produce a blue purple complex with maximum absorption at 660 nm wavelength with Folin-Ciocalteau reagent which consists of sodium tungstate molybdate and phosphate, thus intensity of colour depends on the amount of these aromatic amino acids present and will thus vary for different proteins.

3.10.4.3.2 Reagents

1. BSA stock solution (1 mg/ml): Standard stock solution.

2. Analytical reagents:
   
   Solution A: Sodium carbonate 20g in 1000 ml of 0.1 N Sodium hydroxide.

   Solution B: Copper sulphate (1g in 100 ml distilled water).

   Solution C: Sodium or potassium tartarate (2g in 100 ml distilled water).

   Solution D: 1 ml each of solution B and solution C was mixed.

   Later 50 ml of solution A was mixed with 1ml of solution D.

3. Folin-Ciocalteau reagent solution: Folin-Ciocalteau reagent was diluted with distilled water in the ratio of 1:2 just before use.
3.10.4.3.3 Procedure

For standard solution

1. Different dilutions of BSA solutions were prepared by mixing stock BSA solution (1 mg/ml) and water as given in the table. The final volume in each of the test tubes was 5ml. The BSA concentration range is 0.05 to 1 mg/ml.

2. From these different dilutions, 0.2 ml of protein solution was taken and 2ml of analytical reagent was added. This solution was incubated at room temperature for 10 minutes.

3. Then 0.2 ml of Folin-Ciocalteau reagent was added to each test tube and incubated for 30 minutes.

4. The optical density reading was taken by measuring the absorbance at 660 nm.

5. A standard curve was plotted for OD values against known protein concentration.

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<th>BSA (ml)</th>
<th>Water (ml)</th>
<th>Sample concentration (mg/ml)</th>
<th>Sample volume (ml)</th>
<th>Analytical reagent</th>
<th>Folin-ciocalteau reagent (ml)</th>
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</tbody>
</table>
For samples

1. 0.2 ml of homogenate was taken and 2ml of analytical reagent was added. This solution was incubated at room temperature for 10 minutes.

2. 0.2 ml of Folin-Ciocalteau reagent was added to each test tube and incubated for 30 minutes.

3. The optical density reading was taken by measuring the absorbance at 660 nm against blank containing distilled water instead of homogenate.

4. The protein concentration was determined by comparing OD value obtained with the standard curve plotted.

3.10.4.4 Assay of enzymatic antioxidants

3.10.4.4.1 Estimation of Superoxide Dismutase (SOD)

Superoxide dismutase activity was determined by the method described by Marklund and Marklund, (1974).

3.10.4.4.1.1 Principle

Superoxide an ion is an intermediate in the auto oxidation of pyrogallol which occurs at pH 8.2. The ability of SOD to inhibit the auto oxidation of pyrogallol at pH 8.2 provides the basis for enzyme activity.

3.10.4.4.1.2 Homogenate preparation

To 0.5ml of tissue homogenate, 0.25 ml of ethanol and 0.15ml of chloroform was added and mechanically shaken for 15 minutes. Then the contents were centrifuged at
13000g for 15 minutes at 4°C. The supernatant was separated and used for the test. It was expressed as units/minute/ mg protein.

3.10.4.4.1.3 Procedure

To 2ml of 0.1M tris HCl (pH 8.2), 0.5 ml of homogenate was added. To this, 1.5 ml of distilled water and 0.5 ml of 2mM pyrogallol were also added, mixed and the OD value was taken at 0, 1, 2 and 3 minute intervals at 420 nm wavelength spectrophotometrically.

3.10.4.4.1.4 Calculation

\[ \text{SOD} = \frac{1}{X} \times \Delta \text{OD} \times \text{Dilution factor} / \text{Total protein} \] where X corresponds to the number of intervals for which the OD value was taken.

**Unit of activity**: The enzyme activity was expressed in terms of units per minute per mg of protein. One unit of SOD was defined as the amount of enzyme required to inhibit pyrogallol auto-oxidation reaction by 50 per cent.

3.10.4.4.2 Estimation of catalase (CAT)

Catalase was estimated by the method described by Caliborne, (1985).

3.10.4.4.2.1 Principle

Catalase activity was determined by monitoring the decrease in absorbance spectrophotometrically at 240 nm due to decomposition of hydrogen peroxide. The difference in extinction coefficient per unit time was measured as catalase activity.
3.10.4.4.2 Procedure

To 0.2 ml of homogenate, 1 ml of 30 mM \( \text{H}_2\text{O}_2 \) was added and the OD value was taken at 240 nm spectrophotometrically at an interval of 1 minute for 3 minutes. Blank contained 0.2 ml of distilled water plus 1 ml of 30 mM \( \text{H}_2\text{O}_2 \).

3.10.4.4.3 Calculation

\[
\text{Catalase} = \frac{1}{\text{X value}} \times \Delta \text{OD} / \text{Total protein where X corresponds to the number of intervals for which the OD value was taken.}
\]

Unit of activity: Enzyme activity was expressed as μmol of \( \text{H}_2\text{O}_2 \) decomposed per minute per mg of protein.

3.10.4.4.3 Estimation of Glutathione peroxidase (GPx)

Glutathione peroxidase was determined by the method described by Rotruck \textit{et al.} (1973).

3.10.4.4.3.1 Principle

GPx reacts with \( \text{H}_2\text{O}_2 \) and reduced glutathione giving rise to oxidoreductase which forms a coloured complex with dithio bis-nitrobenzoic acid (DTNB). The intensity of colour development is directly proportional to amount of GPx present in the tissue.

3.10.4.4.3.2 Procedure

The reaction mixture contained 2.0 ml of 0.4M Tris- HCl buffer, pH 7.0, and 0.01 ml of 10mM sodium azide, 0.2 ml of tissue homogenate, 0.2 ml of 10mM glutathione and 0.5 ml of 0.2mM \( \text{H}_2\text{O}_2 \). The contents were incubated at 37\(^\circ\)C for 10 minutes followed by the termination of the reaction by the addition of 0.4 ml 10\% (v/v) TCA, centrifuged at
5000 rpm for 5 minutes. The absorbance of the product was read at 430nm and expressed as nmol/mg protein.

3.10.4.4.3 Calculation

Value = OD Value X Dilution factor/ TOTAL Protein

Unit: nmol / mg protein.

3.10.5 Hematological evaluation

On the scheduled days of blood collection, first few drops of blood were collected in a vial containing Ethylene diamine tetra acetic acid (EDTA) as an anticoagulant and subjected for estimation of blood parameters which included total RBC count, total WBC count, Hemoglobin (Hb), platelet count using auto hematology analyzer (BC-2800 Vet, Mindray).

3.11 Pathology

Two animals from each group were sacrificed humanely on day 15, 30, 45 and rest at the end of the study on 90\textsuperscript{th} day. The sacrificed animals were subjected to detailed post mortem examination.

Gross lesions if any in various organs were recorded. The representative tissue samples of 3-5 mm thickness were collected in 10 per cent NBF for histopathological examination. The tissues were processed by routine paraffin embedding technique and 4\textmu sections were cut and subjected for routine H & E staining (Luna, 1968), immunohistochemistry and special staining (Gomori’s chrome alum hematoxylin and phloxine stain).
3.12 Immunohistochemical detection of insulin in the pancreatic islets

Sections of pancreas were subjected for immunohistochemistry to demonstrate insulin in the β-cells of islets of Langerhans using polyclonal antibody raised against insulin antigen.

3.12.1 Materials

3.12.1.1 Immunochemicals

- **Primary antibody**: Ready to use Flex Polyclonal Guinea Pig Anti-Insulin (Code No. IS002) shown to react with insulin antigen was procured from Dako Cytomation, Denmark. It was stored at -20°C until used.

- **Secondary antibody**: Polyclonal Rabbit Anti-Guinea Pig Immunoglobulins conjugated with HRP (Horse Raddish Peroxidase) known to detect guinea pig immunoglobulins bound to antigen in tissue sections was procured from Dako Cytomation, Denmark and was used at a dilution of 1:75. It was stored at -20°C until end.

3.12.1.2 Section adhesive 3-aminopropyltriethoxy-silane (APES)

Procured from Sigma chemicals, USA.

3.12.1.3 Hydrogen peroxide (H₂O₂) in methanol (3%)

Three per cent H₂O₂ in methanol was prepared by adding one ml of 30 per cent H₂O₂ in 9 ml of methanol.
3.12.1.4 0.01M Citrate buffer (pH-6)

- 50 ml of 0.1M citric acid solution was prepared by dissolving 1.051 g of citric acid (MW = 210.14) in 50 ml of distilled water.

- 100 ml of 0.1M sodium citrate solution was prepared by dissolving 2.941 g of sodium citrate in 100 ml of distilled water.

- 1000 ml of 0.01M citrate buffer was prepared by adding 18 ml of 0.1M citrate solution and 82 ml of 0.1M sodium citrate solution to 900 ml of distilled water. The pH was adjusted to 6.0 with 1N NaOH.

- All the solutions were prepared freshly just before the use.

3.12.1.5 DAB plus substrate

3,3-diamine benzidine tetrahydrochloride substrate was prepared freshly at the time of use by addition of 1 mg of 3,3-diamine benzidine tetrahydrochloride (Sigma Chemicals, USA) in 1 ml of 0.01 M PBS to which 12 μl of 3 per cent H₂O₂ was added.

3.12.1.6 0.01M phosphate buffer saline (pH-7.2)

10X concentration of 500 ml PBS was prepared by adding the following chemicals

- Sodium chloride (MW 58.44) 40 g
- Potassium Chloride (MW 74.56) 1 g
- Disodium hydrogen orthophosphate (MW 141.96) 7.2 g
- Potassium dihydrogen orthophosphate anhydrous (MW 136.09) 1 g
- Distilled water 500 g
1X concentration of wash buffer was prepared using 10X PBS by adding 25ml of 10X PBS to 225ml of distilled water. To this 125μl of Tween 20 was added and the pH was adjusted to 7.2.

3.12.1.7 **Harris haematoxylin for nuclear staining (Luna, 1968)**

Harris haematoxylin was used for nuclear staining. Counter staining was carried out for 45 seconds.

3.12.2 **Preparation of organosilane (Apes) treated slides for IHC**

- The slides were placed on racks, washed thoroughly in soap water, rinsed in tap water and finally rinsed in distilled water.
- The slides were allowed to dry completely.
- A 2% solution of 3-aminopropyltriethoxy-silane (APES) in acetone in a dry staining dish was prepared.
- The slides were immersed in the APES solution for 5-15 minutes.
- The slides were rinsed in acetone and then rinsed in two changes of distilled water.
- Slides were allowed to dry at 37°C for two hours and then stored at room temperature until used.

3.12.3 **Method**

- Tissue sections were mounted on 3-aminopropyltriethoxy-silane (APES) coated slides and dried at 37°C for three hours. Later stored at 40°C until its usage.
- The paraffin tissue sections were deparaffinized using xylene and rehydrated using descending grades of ethanol.

- Endogenous peroxidise was blocked by covering the whole section with 3 per cent of \( \text{H}_2\text{O}_2 \) in methanol (100 \( \mu \text{l} \)) and incubated at room temperature for fifteen minutes. Later washed in three changes of wash buffer.

- Heat induced epitope retrieval (HIER) was carried out by immersing tissue sections in a cooker containing citrate buffer (pH 6.0) and was cooked for 6 minutes after maximum pressure was attained. Sections were allowed to cool down to room temperature for approximately 30 minutes. Later washed in three changes of wash buffer.

- **Addition of primary antibody:** - Ready to use Flex Polyclonal Guinea Pig Anti-Insulin was added to cover the sections. Subsequently the sections were incubated at room temperature in humidified chamber for one hour and washed with wash buffer as mentioned earlier.

- **Addition of secondary antibody (Polyclonal Rabbit Anti-Guinea Pig Immunoglobulins conjugated with HRP):** - The whole section was covered with secondary antibody and incubated at room temperature in humidified chamber for 30 minutes. After incubation sections were washed with PBS as mentioned earlier.

- **Addition of DAB plus substrate:** - Freshly prepared 3,3-diamine benzidine tetrahydrochloride (DAB) with 3 per cent \( \text{H}_2\text{O}_2 \) was poured to cover the section and incubated for 15-20 minutes or until the desired stain intensity was achieved. Later the sections were washed again with three changes of distilled water.
• Nuclear counter staining with Harris haematoxylin was carried out for 45 seconds. The sections were washed in distilled water, dehydrated with ascending grades of ethanol and cleared with xylene and cover slipped with DPX mounting media.

• Pancreas of non-diabetic control rats was used as a positive control for standardization of the technique.

3.12.3 Percentage positivity of insulin secreting cells

To determine the percentage positivity for insulin production, the number of insulin positive cells immunohistochemically in 1000 β-cells (approximately 10-12 islets) were counted under high magnification and was expressed in percentage.

3.13 Special staining for β-cells

Pancreatic sections were stained by Gomori’s chrome alum hematoxylin and phloxine stain for demonstration of β-cells (Gomori, 1941).

3.13.1 Solutions

Bouin’s solution

• Picric acid, saturated aqueous solution 750.0 ml
• Formalin (37-40%) 250.0 ml
• Glacial acetic acid 50.0 ml
• Potassium permanganate solution
• Potassium permanganate 0.3 g
• Distilled water 100 ml
• Sulphuric acid concentrated 0.3 ml
5% Sodium bisulphite solution

- Sodium bisulphite 5.0 g
- Distilled water 100.0 ml
- Chromium Hematoxylin solution
- Hematoxylin, 1% aqueous solution 50.0 ml
- Chromium potassium sulphate, 3% aqueous solution 50.0 ml

To 100 ml of chromium hematoxylin solution 0.1 g of potassium iodate was added and boiled until deep blue. The solution was filtered before use.

1% Acid alcohol solution

- Alcohol, 70% 1000.0 ml
- Hydrochloric acid, concentrated 10.0 ml

0.5% Phloxine B stain

- Phloxine B 0.5 g
- Distilled water 100.0 ml

5% Phosphotungstic acid solution

- Phosphotungstic acid 5.0 g
- Distilled water 100.0 ml

3.13.2 Staining procedure

- The sections were deparaffinised and hydrated to distilled water.
- Mordanted in Bouin’s solution for 16 hours.
Washed in tap water to remove picric acid, for 15 minutes.

Kept in potassium permanganate solution for 1 minute.

Differentiated in sodium bisulfite solution.

Washed well in tap water.

Kept in chromium hematoxylin solution for 10 minutes or less. Checked under microscope and stained until beta cells stood out deep blue.

Differentiated in acid alcohol solution for 1 minute.

Washed in tap water until the section was clear blue.

Stained with Phloxine B solution for 5 minutes.

Rinsed in distilled water.

Kept in phosphotungstic acid solution for 1 minute.

Washed in tap water for 5 minutes till the section regained its red colour.

Differentiated in 95% alcohol. Rinsed in 80% alcohol for 15 to 20 seconds.

Dehydrated in absolute alcohol, then cleared in xylene, two changes each.

Mounted with DPX

### 3.14 FLOW CYTOMETRY

#### 3.14.1 Materials

1. The monoclonal anti-mouse CD4 antibodies conjugated with Fluorescence isothyocynite (FITC) and anti-mouse CD8 antibodies conjugated with Phylloerythrin cyan-7 (PE-cy7) were procured from eBioscience, USA.

2. Flow cytometry tubes

3. Sheath fluid (BD Biosciences, USA)
4. Lysis buffer – Prepared 10 x lysis buffers by adding 9ml of distilled water to one ml of lysis buffer.

5. Sterile distilled water

6. Sterile phosphate buffer saline (PBS) – pH 7.4

7. Micro pipette and micro tips (10 µl, 50 µl and 1000 µl)

### 3.14.2 Procedure

1. Taken 50 µl of dipotassium EDTA added blood in four flow cytometry tubes

2. Added 10 µl anti-mouse CD4 antibody in first tube, 10 µl anti-mouse CD8 antibody in second and 10 µl of both anti-mouse CD4 and CD8 in third tube and the fourth tube served as unstained control.

3. The above tubes were used for setting up the flow cytometry for taking cell counts of test samples.

4. In the test sample tubes added 10 µl of anti-mouse CD4 and 10 µl of anti-mouse CD8 in each tube.

5. Incubated the tubes in dark at room temperature for 30 minutes.

6. Added 2 ml of 10X lysis buffer to each tube and mixed properly.

7. Incubated the tubes for 10 minutes at room temperature.

8. After incubation, centrifuged the tubes at 1200 rpm for 10 minutes.

9. Discarded the supernatant solution.

10. Added 2 ml of sterile PBS to each tube.
11. Centrifuged the tubes at 1200 rpm for 10 minutes.

12. Suspended the pellet in 400 µl of sheath fluid and recorded the cell counts in flow cytometry.

13. Data acquisition and post acquisition analysis was conducted using FACS Diva software version 6.1.3 (BD Biosciences, USA).

14. The percentages of CD4+ and CD8+ T-lymphocytes were calculated after counting 10,000 events or cells per sample by flow cytometry.

15. The CD4/CD8 ratio was calculated by dividing the percentage of CD4+ cells by percentage of CD8+ cells.

3.15 Statistical analysis

Statistical analysis was performed using the statistical software GraphPad Prism, version 5 for windows. Mean values and standard error were calculated and all values were expressed as Mean (±SE). The data were analysed by two way analysis of variance (ANOVA).
Results
IV. RESULTS

The present study was conducted to evaluate the antidiabetic efficacy of *Trigonella foenum graecum* and *Coccinia indica* extracts in STZ induced diabetes in rats and to compare the hypoglycemic effects with an oral hypoglycemic agent glibenclamide.

To induce diabetes, rats were administered with streptozotocin at the dose rate of 45 mg/kg body weight in citrate buffer (pH 3.5-4.5) intra peritonially. Blood samples were collected 72 hr after STZ dosing and the diabetes status was confirmed by the estimation of fasting blood glucose levels using semi-autoanalyzer.

The various groups in this study included normal control (Group-I), diabetic control (Group-II), diabetic rats treated with glibenclamide at 600 μg /kg body weight (Group-III), diabetic rats treated with *Trigonella foenum graecum* at the dose rate of 1g/kg body weight (Group-IV), diabetic rats treated with *Coccinia indica* at 200 mg/kg body weight (Group-V), diabetic rats treated with *Trigonella foenum graecum* at 1g/kg and *Coccinia indica* at 200 mg/kg body weight (Group-VI), diabetic rats treated with *Trigonella foenum graecum* at 1g/kg and glibenclamide at 300 μg /kg body weight (Group-VII), diabetic rats treated with *Coccinia indica* at 200mg/kg and glibenclamide at 300 μg /kg body weight (Group-VIII) and diabetic rats treated with *Trigonella foenum graecum, Coccinia indica* and Glibenclamide at the dose rate of 1g/kg, 200mg/kg and 300 μg /kg body weight respectively (Group-IX).

The animals were examined for body weight gain, serum biochemistry for glucose, ALT, AST, cholesterol ,triglyceride, serum insulin estimation, haematology,
antioxidant estimation, estimation of CD4 and CD8 cells by flow cytometry, clinical symptoms, gross and histopathology, immunohistochemistry for insulin and special stain (Gomori’s chrome alum hematoxylin and phloxine stain) for beta cells. In addition the phytochemical analysis of the *Trigonella foenum graecum* and *Coccinia indica* extracts was also carried out. The results of the study are presented as hereunder.

### 4.1 Phytochemical analysis

Phytochemical analysis of *Trigonella foenum graecum* and *Coccinia indica* alcoholic extracts of seed and leaves powder was carried out using High Performance Thin Layer Chromatography Technique (Plates 1-4).

HPTLC profile of alcoholic extract was generated in solvent systems of different polarities in order to ascertain the total number of chemical moieties which help in designing the method of isolation and characterization of bioactive compounds.

#### 4.1.1 Alkaloids

**a) Without chemical treatment**

There was a pronounced quenching of fluorescence on TLC plates at UV-254 nm, intense blue fluorescence at UV-366 nm for *Trigonella foenum graecum* and *Coccinia indica* extracts on TLC plates at UV-254 nm and UV-366 nm (Plate 5).

**b) Dragendorff reagent**

There were blue or brown bands on TLC plates at UV-254 nm and on TLC plates at UV-366 nm for *Trigonella foenum graecum* extract (Plate 5).
Intense blue bands appeared on TLC plates for *Coccinia indica* extract at any UV wave lengths (Plate 5).

**Inference:** The alcoholic *Trigonella foenum graecum* seed extract and *Coccinia indica* leaves extracts are positive for the presence of alkaloids.

### 4.1.2 Anthracene derivatives

**a) Without chemical treatment**

There was pronounced quenching on TLC plates at UV-254 nm and development of blue bands on at UV-366 nm with brown and light yellow bands at visible light for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extracts (Plate-6).

**b) Natural products – polyethylene glycol**

Bluish brown bands appeared for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plates in visible light and UV - 366 nm.

**Inference:** The alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract were positive for the presence of anthracene derivatives.

### 4.1.3 Bitter principles

**a) Without chemical treatment**

There was pronounced quenching of fluorescence for alcoholic *Trigonella foenum graecum* seed extract but there was no pronounced quenching for alcoholic *Coccinia indica* leaves extract on TLC plates at UV-254 nm and UV- 366 nm (Plate 7).
b) Vanillin-sulphuric acid

Greenish fluorescence appeared for alcoholic *Trigonella foenum graecum* seed extract but there was no fluorescence appeared for alcoholic *Coccinia indica* leaves extract on TLC plates at UV-366 nm (Plate 7).

**Inference:** The alcoholic *Trigonella foenum graecum* seed extract consists of Bitter principles, whereas alcoholic *Coccinia indica* leaves extract is negative for the presence of Bitter principles.

4.1.4 Coumarins

a) Without chemical treatment

There was distinct fluorescence quenching for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plate at UV-254 nm and blue fluorescence appeared on TLC plate at UV-366 nm (Plate 8).

b) Potassium hydroxide

There was appearance of pronounced blue fluorescence for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plates at UV- 366 nm.

**Inference:** The alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract are positive for the presence of coumarins.
4.1.5 Flavonoids

a) Without chemical treatment

Fluorescence quenching for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plates at UV-254 nm was observed and light blue fluorescence appeared on TLC plates at UV-366 nm.

b) Natural products – polyethylene glycol

Intense blue fluorescence appeared for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plate at UV-366 nm (Plate 3A) and brown bands appeared in visible light (Plate 9).

c) Fast blue salt B

Blue fluorescence appeared for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plates at UV-366 nm.

**Inference:** The alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract contain flavonoids.

4.1.6 Glycosides

a) Without chemical treatment

There was pronounced fluorescence quenching for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plate at UV-254 nm (Plate 10).
b) Aniline-diphenylamine phosphoric acid

Dark blue bands appeared for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plate at UV-366 nm.

**Inference:** The alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract are positive for presence of glycosides.

4.1.7 Saponins

a) Without chemical treatment

Saponins for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract were detectable by exposure to UV-254 nm or UV-366 nm.

b) Blood reagent

White zones was observed for alcoholic *Trigonella foenum graecum* seed extract whereas, no white zone appeared for alcoholic *Coccinia indica* leaves extract on TLC plate in the visible light (Plate 11).

c) Vanillin-sulphuric acid

Blue zones were observed for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plate at UV-366 nm (Plate 11).

**Inference:** The alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract are positive for presence of saponins.
4.2 Induction of diabetes

In the present study, rats with fasting serum glucose concentration above 200 mg/dL were considered as diabetic and included in the present study. Streptozotocin effectively induced diabetes in all the rats in the present study which exhibited clinical signs like polyuria, polydipsia, weight loss, and decreased physical activities.

On Day 3, the blood glucose levels ranged from 418.83±5.57 to 499.40±15.62 and the mean (± SE) blood glucose levels of Groups-II, III, IV, V, VI, VII, VIII and IX were 458.58±40.52, 418.83±5.57, 464.83±19.78, 475.69±18.05, 493.32±11.19, 495.53±13.92, 491.69±15.28 and 499.40±15.62mg/dl respectively, which were significantly (P ≤ 0.001) higher compared to normal control animals.

Normal control rats of Group-I remained healthy as evaluated by various parameters throughout the experimental period.

4.2.1 Body weight

The mean (± SE) body weight of diabetic control rats showed a progressive decrease from Day 3 to Day 90 and were 157.41±2.44, 162.91±2.00, 155.60±2.40, 151.12±5.43 and 137.00±9.00 on 3rd, 15th, 30th, 45th and 90th day post-treatment respectively (Table 1 and Figure 1). The mean body weight values were significantly (P≤0.001) higher in control group on 3rd to 90th day of the study which were 182.00±1.53, 186.50±1.94, 195.80±1.62, 203.62±1.48 and 228.00±3.00 respectively (Table 1; Figure 1).
The mean (± SE) body weights of Group-III animals treated with glibenclamide showed a progressive increase which were 164.91±2.39, 169.75±3.97, 194.10±3.21, 201.50±2 and 212.00±3.00 g on 3rd, 15th, 30th, 45th and 90th day of the study respectively. The mean body weights were significantly higher (P≤0.001) when compared to diabetic control animals on 30th, 45th and 90th day of the study and were comparable with those of normal control on the same intervals of the study (Table 1 and Figure 1).

Similarly, the mean (± SE) body weight of Groups-IV, V and VI treated with Trigonella foenum graecum and Coccinia indica individually and in combination respectively showed a significant improvement (P≤0.001) in body weight from 30th day onwards compared to diabetic control and the values were comparable with those of negative control group.

The improvement in mean (± SE) body weight of rats of Groups VII, VIII and IX treated with Trigonella foenum graecum with glibenclamide half dose, Coccinia indica with glibenclamide half dose and Trigonella foenum-graecum, Coccinia indica with glibenclamide at half dose was significant (P≤0.001) compared to diabetic control, from 30th day onwards and were comparable with those of control group on the same days of examination. In addition glibenclamide with plant extract groups (VII, VIII and IX) did not differ in the improvement in body weight on any day of examination with those groups (IV, V and VI) treated with plant extracts individually or in combination without glibenclamide.
4.3 Serum biochemistry

4.3.1 Serum Glucose

The mean (± SE) serum glucose values in control rats were 89.25±2.86, 86.00±4.21, 84.20±5.67, 85.87±4.22 and 77.50±3.50 on 3rd, 15th, 30th, 45th, and 90th day of experiment respectively.

The mean (± SE) serum glucose value in the diabetic control animals was observed to be drastically increased to 458.58±40.52 mg/dL on 3rd day and was 572.50±28.50 mg/dL on 90th day with 526.00±21.74 mg/dL, 483.80±48.80 mg/dL and 565.37±45.65 mg/dL on 15th, 30th and 45th day of the treatment respectively. The serum glucose values were significantly higher (P≤0.001) compared to all other treatment and control groups (Table 2; Figure 2).

In the glibenclamide treatment group there was a progressive reduction in mean (± SE) serum glucose values from 418.83±5.57 mg/dL on 3rd day to 157.90±11.60 mg/dL on 90th day. The values were significantly lower (P≤0.001) from those of diabetic control rats. However, the serum glucose values were significantly higher (P≤0.001) in comparison with those of normal control (Group-I) rats throughout the experiment (Table 2; Figure 2).

The mean (± SE) serum glucose values in Groups IV, V and VI treated with *Trigonella foenum graecum*, *Coccinia indica* and in their combination respectively revealed a progressive reduction from Day 3 from 463.83±19.78, 475.69±18.05 and 493.32±11.19 mg/dL to 237.75±19.05, 230.10±15.410 and 215.40±3.20 mg/dL
respectively on Day 90 of treatment. The reduction was statistically significant (P≤0.001) when compared to diabetic control animals (Group-II). Though the mean glucose values were observed to be reduced, they were significantly higher (P≤0.001) compared to normal control animals and glibenclamide group and were comparable with those of Group VII, VIII and IX on 90th day of the treatment (Table 2; Figure 2).

There was a gradual reduction in mean (± SE) serum glucose values in the rats of Groups VII, VIII and IX treated with *Trigonella foenum graecum*, *Coccinia indica* and in their combination with glibenclamide at half dose respectively from 495.53±13.92, 491.69±13.92 and 499.40±15.62 mg/dL on 3rd day to 204.55±5.95, 221.40±5.00 and 174.10±5.40 mg/dL on 90th day respectively. The values were significantly lower (P≤0.001) from those of diabetic control rats from 15th day onwards of the study. However, the serum glucose values were significantly higher (P≤0.001) in comparison with those of normal control (Group-I) rats throughout the experiment and were comparable between the groups as well as compared to Groups III to VI on 90th day of the treatment (Table 2; Figure 2).

### 4.3.2 Serum cholesterol

The mean (± SE) serum cholesterol values in control rats were 80.88±3.58, 81.15±3.63, 84.65±5.52, 81.68±3.07 and 79.70±2.39 mg/dL on 3rd, 15th, 30th, 45th, and 90th day of experiment respectively.

In the present investigation, the diabetic rats of Group-II revealed a significant increase (P≤0.001) in the mean (± SE) serum cholesterol level from 3rd to 90th day of experiment in comparison with those of normal control (Group-I) with mean serum
cholesterol values of 136.21±7.61, 155.96±5.28, 178.29±6.69, 194.67±6.64 and 217.80±12.40 mg/dL on 3rd, 15th, 30th, 45th and 90th day post-treatment respectively (Table 3; Figure 3).

The mean (± SE) serum cholesterol values in glibenclamide treated group (Group-III) were significantly reduced (P≤0.001) when compared to diabetic control rats (Group-II). A consistent decrease was observed from 15th day onwards with a value of 125.74±5.36 to 90th day of treatment with 90.20±8.00 mg/dL of cholesterol. However, the serum cholesterol values were higher in comparison with those of normal control (Group-I) rats throughout the experiment and became comparable on 90th day of the treatment (Table 3; Figure 3).

The other treatment groups (Group-IV to IX) revealed a progressive improvement in the mean cholesterol values from 15th day onwards and were significantly lesser (P≤0.001) compared to diabetic control on all the days of observation. However, remained significantly higher in comparison with normal control group (Group-I) on 3rd, 15th, 30th and 45th day but became comparable on 90th day.

In addition between the groups, the improvement in mean values of cholesterol was comparable throughout the study period (Table 3; Figure 3).

4.3.3 Serum triglyceride

The mean (± SE) serum triglyceride values in control rats (Group-I) were 95.95±0.99, 96.70±1.07, 96.03±1.13, 96.20±1.23 and 97.95±1.45 mg/dL on 3rd, 15th, 30th, 45th, and 90th day of experiment respectively.
The diabetic rats of Group-II revealed a significant increase (P≤0.001) in the mean (± SE) serum triglyceride levels when compared with those of normal control animals (Group-I). The mean values ranged from 207.18±1.34 mg/dL on 3rd to 364.20±0.10 mg/dL 90th day of the study (Table 4; Figure 4).

The mean (± SE) serum triglyceride values of the glibenclamide treated group (Group-III) also showed a progressive decrease to 122.10±6.50mg/dL on 90th day from that of 215.82±0.93mg/dL on 3rd day post-treatment. The mean values were significantly improved (P≤0.001) compared to those of diabetic control rats on 15th, 30th, 45th and 90th days of treatment but in comparison with normal control rats, the values were significantly higher (P≤0.001) throughout the experiment (Table 4; Figure 4).

There was a progressive decrease in the mean (± SE) serum triglyceride values in Groups IV, V and VI treated with Trigonella foenum graecum, Coccinia indica individually and in their combination respectively from 3rd day (209.26±1.65, 215.27±1.05 and 216.18±1.36 mg/dL) to 90th day (149.55±2.85, 155.40±0.90 and 125.80±2.60 mg/dL) of treatment. The observed decrease was significant (P≤0.001) when compared to diabetic control group throughout the course of the experiment. However, the values were significantly higher (P≤0.001) compared to normal control animals. In addition, the values were comparable between the groups as well as with that of Group III on 90th day (Table 4; Figure 4).

The mean (± SE) serum triglyceride values of the Groups VII, VIII and IX rats treated with Trigonella foenum graecum, Coccinia indica individually and in their combination with glibenclamide at half dose respectively were found to be progressively
decreasing from 211.93±1.66, 215.24± 1.06, 211.89±1.02 mg/dL on 3\textsuperscript{rd} day to 116.85±7.75, 114.45±1.95 and 111.50±5.10 mg/dL on 90\textsuperscript{th} day of treatment.

The values were significantly lower (P≤0.001) compared to diabetic control rats on 15\textsuperscript{th}, 30\textsuperscript{th} 45\textsuperscript{th} and 90\textsuperscript{th} day of treatment. However, the mean triglyceride (± SE) values were significantly higher (P≤0.001) compared to normal control animals throughout the study except on 90\textsuperscript{th} day of treatment. In addition the values were comparable between the groups (VII to IX) and with those of Groups III and VI on 90\textsuperscript{th} day (Table 4; Figure 4)

4.3.4 Serum alanine aminotransferase (ALT)

In the present investigation, Group-II rats showed a significantly higher (P≤0.001) mean (± SE) serum ALT values throughout the study period in comparison with those of normal control animals. The mean values on 3\textsuperscript{rd}, 15\textsuperscript{th}, 30\textsuperscript{th}, 45\textsuperscript{th} and 90\textsuperscript{th} day of the study were 130.33±7.34, 163.02±8.74, 206.58±13.60, 228.80±21.51 and 228.05±6.44 IU/L respectively as compared to normal control animal values of 53.07±1.64, 51.81±1.70, 51.52±1.9052.61±1.83 and 56.40±2.10 IU/L on the respective days (Table 5; Figure 5).

The mean (± SE) serum alanine aminotransferase values in glibenclamide treated group showed a gradual improvement from 120.84±2.00 IU/L on 15\textsuperscript{th} day to 95.40±3.00 on 90\textsuperscript{th} day. The decrease was significant (P≤0.001) from Day 15 onwards till the end of the study period in comparison with diabetic control (Group-II) animals. However, the values were significantly higher (P≤0.001) when compared with normal control rats (Group-I) throughout the study (Table 5; Figure 5).
The mean (± SE) serum ALT values of the Groups IV, V and VI treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination respectively decreased progressively from 137.96±2.00, 139.47±1.59 and 141.85±1.93 IU/L on 3rd day to 89.65±1.84, 94.45±1.95 and 76.39±1.79 IU/L on 90th day post-treatment. The values were significantly lesser (P≤0.001) when compared to diabetic control rats on 15th to 90th day post-treatment. However, the values were significantly higher (P≤0.001) than those of normal control animals throughout the experiment. The ALT values were comparable with that of glibenclamide group and did not significantly vary between the groups (IV, V and VI) on 15th, 30th and 45th day but was significantly different (P≤ 0.001) on 90th (Table 5; Figure 5).

The mean (± SE) serum alanine aminotransferase values of Groups VII, VIII and IX rats treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination with glibenclamide at half dose respectively showed a gradual improvement from 141.18±1.48, 142.09±3.58 and 138.98±1.40 on 15th day to 71.55±0.90, 70.15±1.25 and 66.05±0.65 respectively on 90th day. The decrease was significant (P≤0.001) from Day 15 onwards till the end of the study period in comparison with diabetic control (Group-II) animals. However, the values were significantly higher (P≤0.001) when compared with normal control rats (Group-I) on 15th and 30th day but comparable on 45th and 90th day. In addition, when compared to glibenclamide treated group the mean ALT values were comparable on 15th, 30th and 45th but significantly (P≤0.001) lower on 90th day of the study. The mean values were also significantly lower (P≤0.001) when compared to Groups IV and V but comparable to that of Group VII on 90th day (Table 5; Figure 5)
4.3.5 Serum aspartate aminotransferase (AST)

Group-II rats showed a significantly higher (P≤0.001) mean (± SE) serum AST values at different intervals of the study in comparison with those of normal control and other treatment groups which were 170.10±10.20, 208.01±9.23, 247.82±21.23, 286.87±23.35 and 301.15±5.35 IU/L on 3\textsuperscript{rd}, 15\textsuperscript{th}, 30\textsuperscript{th}, 45\textsuperscript{th} and 90\textsuperscript{th} day respectively (Table 6; Figure 6).

The mean (± SE) AST values in glibenclamide treatment group revealed similar decreasing trend as that of ALT which was from 180.48±3.05 IU/L on 3\textsuperscript{rd} day, comparable to that of diabetic control rats, to 94.15±2.25 IU/L on 90\textsuperscript{th} day. The decrease in AST values was significant (P≤0.001) in comparison with that of diabetic control on all the intervals of observation. Though there was appreciable decrease in the mean values, they were not comparable to those of normal control rats and remained significantly higher on 15\textsuperscript{th}, 30\textsuperscript{th} and 90\textsuperscript{th} day but comparable on 45\textsuperscript{th} day (Table 6; Figure 6).

The mean (± SE) serum AST values in the rats of Groups IV, V and VI treated with *Trigonella foenum graecum*, *Coccinia indica* individually and in their combination respectively showed a significant decrease (P≤0.001) from 180.05±2.31, 182.17±2.87 and 177.25±3.19 on 3\textsuperscript{rd} day to 88.50±3080, 90.73±2.48 and 83.75±1.95 IU/L on 90\textsuperscript{th} day of the treatment. The decrease in the values was significant (P≤0.001) when compared with diabetic control group on 15\textsuperscript{th}, 30\textsuperscript{th} 45\textsuperscript{th} and 90\textsuperscript{th} day post-treatment. The values were significantly higher (P≤0.001) compared to normal control animals on 15\textsuperscript{th} and 30\textsuperscript{th} day but comparable on 45\textsuperscript{th} and 90\textsuperscript{th} day. In addition, the serum AST values were comparable
with those of glibenclamide treatment group on all days of observation. Between the groups the AST value did not vary significantly (P>0.001) (Table 6; Figure 6).

The mean (± SE) serum AST values in the Groups VII, VIII and IX rats treated with Trigonella foenum graecum, Coccinia indica individually and in their combination with glibenclamide at half dose respectively decreased from 182.65±2.01, 181.36±2.54, 181.94±2.56 IU/L on 3rd day to 80.80±1.40, 88.80±2.40 and 70.85±3.45 IU/L respectively on 90th day of the treatment. The decrease in the serum AST values was found to be significant (P≤0.001) when compared with diabetic control group on all the intervals of the study and the values were significantly higher (P≤0.001) compared to normal control animals on 15th and 30th day but comparable on 45th and 90th day. There was no significant difference in the mean AST values between the groups on all the days of observation (Table 6; Figure 6).

4.3.6 Serum Insulin

The mean (± SE) serum insulin levels in the normal control animals were observed to be 53.85±3.21, 52.85±3.16, 54.15±2.78, 52.08±5.35 and 53.95±4.51 μU/ml on 3rd, 15th, 30th, 45th and 90th day respectively and there was no significant difference (P>0.01) in values throughout the experiment (Table 7; Figure 7).

The mean (± SE) serum insulin values of diabetic control rats showed a progressive decrease and were 17.58±1.70, 15.66±0.87, 13.61±1.37, 14.05±1.81 and 12.76±0.94 μU/ml on 3rd, 15th, 30th, 45th and 90th day of the treatment respectively. The serum insulin values were significantly (P≤0.001) lower compared to normal control
animals and other treatment groups (Group III to Group IX) throughout the study (Table 7; Figure 7).

In Group-III animals, treated with glibenclamide the mean (± SE) serum insulin values progressively increased and were 15.42±2.06, 18.44±2.41, 25.73±2.07, 31.01±2.91 and 35.19±5.63 μU/ml on 3rd, 15th, 30th, 45th and 90th day of the study respectively. The values were significantly higher (P≤0.001) on 30th 45th and 90th day post-treatment when compared to diabetic control animals but remained significantly lower (P≤0.001) compared to normal control animals throughout the study (Table 7; Figure 7).

The mean (± SE) serum insulin levels of Groups IV, V and VI treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination respectively significantly improved (P≤0.001) from 30th day onwards compared to diabetic control rats which were 14.97±1.03, 17.96±1.36 and 18.76±1.06 on 3rd day to 37.47±8.87, 32.03±9.15 and 46.09±5.90 μU/ml on 90th day of the treatment. However, the values were significantly lesser (P≤0.001) on all the days of treatment except on 90th day in combined treatment Group VI in comparison to that of control group. With respect to glibenclamide treatment group the improvement in insulin level did not vary significantly (P≤0.001) and also between the groups no significant difference was observed though numerically the values were higher in combined group on 90th day (Table 7; Figure 7).

The mean (± SE) serum insulin levels of Groups VII, VIII and IX rats treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination with
glibenclamide at half dose showed a progressive increase from 17.87±0.83, 17.17±1.34 and 16.89±2.11 μU/ml /ml on 3rd day to 39.75±8.36, 48.10±7.29 and 51.60±5.22 μU/ml on 90th day of the treatment. The increase in the serum value was found to be significantly higher (P≤0.001) when compared with diabetic control group from 15th day onwards. Though there was an increasing trend in the values, they were still significantly lower (P≤0.001) compared to normal control animals throughout the study except on 90th day. In addition, when compared with glibenclamide, trigonella, coccinia and combined treatment groups the mean insulin values of Groups VII, VIII and IX were comparable throughout the experimental period (Table 7; Figure 7).

4.4 Estmation of Antioxidant enzyme levels

4.4.1 Superoxide dismutase (SOD)

The normal control rats of Group-I exhibited normal activity of superoxide dismutase (SOD), in the liver tissue throughout the course of the experiment. The mean (± SE) superoxide dismutase (SOD) activity in the normal control animals was observed to be 19.23±1.10, 20.45±0.66, 19.17±0.64 and 21.14±1.01 U/min/mg of protein on 15th, 30th, 45th and 90th day respectively. There was no significant variation in the values through the experiment between different intervals (Table 8; Figure 8).

The mean (± SE) superoxide dismutase (SOD) activity in the diabetic control animals drastically decreased to 5.66±0.27 U/min/mg of protein on 15th day to 3.95±0.06 U/min/mg of protein on 90th day of the treatment. The superoxide dismutase (SOD) activity was significantly lower (P≤0.001) at different intervals of study in comparision with those of normal control animals (Table 8; Figure 8).
The mean (± SE) superoxide dismutase (SOD) activity in the Group-III (diabetic rats treated with glibenclamide @ 600 /kg b w) showed a gradual and significant increase (P≤0.001) from Day 15 to Day 90 with values of 5.49±0.37, 7.36±0.24, 8.29±0.51 and 9.84±0.50 U/min/mg of protein respectively when compared to diabetic control animals. However the values were significantly lower (P≤0.001) when compared to normal control animals throughout the experiment.

The mean (± SE) superoxide dismutase (SOD) activity in Groups IV, V and VI treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination respectively revealed a gradual increase from 6.94±0.60, 6.12± 0.25 and 6.05 ±0.07 U/min/mg of protein on 15th day to 12.06±0.24, 11.77±0.44 and 13.00±0.20 U/min/mg of protein on 90th day respectively.

The increase in values was significant (P≤0.001) from those of diabetic control rats from 15th day onwards of the study. However, the superoxide dismutase (SOD) activity was significantly lesser (P≤0.001) in comparison with those of normal control (Group-I) rats throughout the experiment. There was significant difference (P≥0.05) between the values at different intervals compared to glibenclamide group and also the values did not vary between the groups significantly on 90th day (Table 8; Figure 8).

The mean (± SE) superoxide dismutase (SOD) activity of Groups VII, VIII and IX treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination with glibenclamide at half dose respectively were observed to be progressively improved from 5.98±0.11, 6.02 ± 0.04 and 6.05±0.16 on 15th day to
12.51±0.95, 12.30±0.40 and 15.23±0.67 U/min/mg of protein respectively on 90\textsuperscript{th} day. The values were significantly higher (P≤0.001) compared to diabetic control rats on 30\textsuperscript{th}, 45\textsuperscript{th} and 90\textsuperscript{th} post-treatment. However, the mean superoxide dismutase (SOD) activity failed to reach those values of normal control rats on any day of the observation and differed significantly (P≤0.001). In addition, when compared with glibenclamide group the values on 90\textsuperscript{th} day were significantly higher (P≤0.001). Among the combined groups Group, IX showed a significant increase in the mean values compared to Group VII and VIII on 30\textsuperscript{th}, 45\textsuperscript{th} and 90\textsuperscript{th} day (Table 8; Figure 8).

4.4.2 Glutathione peroxidase (GPx)

The normal control rats of Group-I remained healthy and exhibited normal activity of glutathione peroxidase in the liver tissue throughout the course of the experiment. The mean (± SE) glutathione peroxidase activity in the normal control animals was observed to be 48.38±1.06, 46.12±0.34, 45.07±1.51 and 46.80±1.54 μmoles of glutathione utilized/min/mg protein, on 15\textsuperscript{th}, 30\textsuperscript{th}, 45\textsuperscript{th} and 90\textsuperscript{th} day respectively (Table 9; Figure 9).

The mean (± SE) glutathione peroxidase activity in the diabetic control animals were drastically decreased to 19.73±1.38 μmoles of glutathione utilized/min/mg protein on 15\textsuperscript{th} day and to 11.70±1.85 μmoles of glutathione utilized/min/mg protein on 90\textsuperscript{th} day of the treatment. The glutathione peroxidase activity was significantly lower (P≤0.001) at different intervals of study as compared with those of normal control and other treatment groups (Table 9; Figure 9).
The mean (± SE) glutathione peroxidase activity in the Group-III (diabetic rats treated with glibenclamide animals showed a gradual and significant increase (P<0.001) from Day 15 to Day 90 with values of 20.30±0.52, 23.85±0.71, 25.95±1.64 and 27.23±0.11 μmoles of glutathione utilized/min/mg protein when compared to diabetic control animals. The values were significantly lower (P≤0.001) when compared to normal control animals throughout the experiment.

The mean (± SE) glutathione peroxidase activity in the Groups IV, V and VI treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination respectively revealed a gradual increase from 20.13±0.68, 19.93±0.18 and 21.34±0.10 μmoles of glutathione utilized/min/mg protein respectively on 15th day to 30.99±0.46, 29.02±0.90 and 32.79±0.65 μmoles of glutathione utilized/min/mg protein on 90th day.

The increase in mean (± SE) values was significant (P≤0.001) compared to those of diabetic control rats from 30th day onwards of the study. However, the glutathione peroxidase activity was significantly lower (P≤0.001) in comparison with those of normal control (Group-I) rats throughout the experiment. In addition, when compared to glibenclamide group the improvement in mean value was significant (P≤0.001) on 90th day in Group IV and VI. Between the groups (IV to VI) the Group VI showed a significant increase ((P≤0.001) on 90th day (Table 10; Figure 10).

The mean (± SE) glutathione peroxidase activity of Groups VII, VIII and IX treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination with glibenclamide at half dose respectively showed a progressive increase
from 21.43±0.23, 19.86±0.32 and 21.86±0.54 μmoles of glutathione utilized/min/mg protein on 15th day to 29.80±1.42, 31.08±2. 37 and 37.28±1.16 μmoles of glutathione utilized/min/mg protein on 90th day of treatment respectively.

The values were significantly higher (P≤0.001) compared to diabetic control rats on 15th, 30th, 45th and 90th day of treatment. The mean glutathione peroxidase activity failed to reach those values of normal control rats on any day of the observation and differed significantly (P≤0.001). In addition, when compared with Groups III, IV, V the values were comparable on 90th day. Among the groups, the Group IX showed a significant increase compared to Group VII and VIII on all the days of observation (Table 9; Figure 9).

4.4.3 Catalase (CAT)

The mean (± SE) catalase values in control rats (Group-I) were 76.86±1.33, 76.66±0.54, 75.80±1.93, and 77.23±1.89 μmoles of H2O2 /min/mg of protein on 15th, 30th, 45th, and 90th day of experiment respectively.

The diabetic rats of Group-II revealed a significant decrease (P≤0.001) in the mean (± SE) serum catalase levels when compared with those of normal control animals (Group-I). The mean values ranged from 26.23±1.11. μmoles of H2O2 /min/mg of protein on 15th to 19.25±0.91 μmoles of H2O2 /min/mg of protein on 90th day of the study (Table 10; Figure 10).

The mean (± SE) catalase values of the glibenclamide treated group (Group-III) showed a progressive increase to 45.67±3.54 μmoles of H2O2 /min/mg of protein on 90th
day from that of 27.73±1.61 μmoles of H₂O₂ /min/mg of protein on 15th day of treatment. The mean values were significantly improved (P≤0.001) compared to those of diabetic control rats on 15th, 30th, 45th and 90th day of treatment but in comparison to normal control rats, the values were significantly lower (P≤0.001) throughout the experiment (Table 10; Figure 10).

There was a progressive increase in the mean (± SE) catalase values in *Trigonella* treated group (Group-IV), Coccinia treated group(Group-V) and combined treatment group (Group VI) which was significant (P≤0.001) compared to diabetic control group (group II) but did not significantly vary (P≤0.001) between the groups. However the mean values were numerically higher in Group VI throughout the study. The values failed to reach those of negative control on any of the interval of observations of the study.

The mean catalase (CAT) values of Group-VII and VIII were significantly improved (P≤0.001) compared to diabetic control on all the interval of study but were lesser compared to negative control. Between the groups the values were comparable throughout the study. The mean values of Groups VII and VIII were also comparable with those of Groups IV, V and VI on all days except on 90th day for group VI where in the mean enzyme value was significantly higher (P≤0.001).

The group IX rats treated with combination of *Trigonella* with *Coccinia* extract along with glibenclamide half dose showed gradual and significant improvement (P≤0.001) in the mean catalase values on all the intervals of examination which were 26.63±0.85, 42.56 ±0.93, 50.0±2.20 and 58.73 ±0.39 on 15th, 30th, 45th and 90th day of the
treatment. The mean values were significantly higher compared to those Group III, Group IV, Group V, Group VII and Group VIII on all the days of observation. However the values were comparable with those of Group VI treated with *Trigonella and Coccinia* in combination on 45th and 90th day. The values were however, significantly lesser compared to normal control group (group I).

### 4.5 Haematology

#### 4.5.1 Haemoglobin (Hb)

The mean (± SE) haemoglobin values of diabetic control rats progressively decreased from 9.76±0.39 g/dL on 3rd day to 5.80±0.39g/dL on 90th day of experiment and the decrease was highly significant (P≤0.001) in comparison with those of normal control group as well as treatment groups (Groups III to IX) (Table 11; Figure 11).

The rats of glibenclamide treatment group (Group-III) showed a consistent increase in the mean (± SE) Hb value from Day 3 to Day 90. The mean Hb values were 9.75±0.36, 11.79±0.27, 12.98±0.26, 13.26±0.27 and 15.25±0.65 g/dL on Day 3, 15, 30, 45 and 90 post-treatment respectively. The improvement in the mean Hb was significant (P≤0.001) in comparison with those of diabetic control rats (Group-II) and were comparable to those of all other treatment groups (Groups-IV to IX) on 90th day of experiment (Table 11; Figure 11).

The mean (± SE) Hb values of the Groups IV, V and VI treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination respectively
showed an improvement and the values were 10.20±0.34, 10.00±0.31 and 9.92±0.20 g/dL on 3rd day to 14.15±0.55, 13.80±0.40 and 14.60±0.30 g/dL on 90th day of treatment.

The values were significantly higher compared to diabetic control animals (P≤0.001) throughout the study and were significantly lower (P≤0.001) compared to normal control animals on all the days of the study. The values were comparable with those of Group-III and did not significantly vary between the groups on 45th and 90th day of the experiment (Table 11; Figure 11).

The mean (± SE) values of Hb of Groups VII, VIII and IX rats treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination with glibenclamide at half dose were 9.95±0.24, 9.75±0.21 and 9.75±0.22 g/dL on 3rd day and 13.92±0.31, 14.70±0.10 and 15.00±0.10 g/dL on 90th day respectively.

The improvement in Hb values was significant (P≤0.001) compared with diabetic control animals from 15th day onwards. However, the values were significantly lesser compared to normal control animals, comparable with that of glibenclamide treated group and did not vary between the groups (VII, VIII and IX) on 45th and 90th day of the study. The improvement in Hb value of Group IX was comparable to that of control group on 90th day (Table 11; Figure 11).

### 4.5.2. Total red blood cell count (TRC)

The mean (± SE) Total red blood cell counts levels in the normal control animals were observed to be 9.01±0.65, 9.08±0.76, 9.96±1.06 and 11.05±0.55 x10¹²/L on 15th, 30th, 45th and 90th day respectively.
The mean (± SE) TRC values of diabetic control rats progressively decreased from 6.33±0.58 cu mm on 3rd day to 4.30±0.50 cu mm on 90th day of experiment and the decrease was highly significant (P≤0.001) in comparison with those of normal control group (Table 12; Figure 12).

The mean (± SE) TRC values of the Group-III (diabetic rats treated with glibenclamide @ 600 mg/kg b w) showed an improvement and the values were 5.35±0.12, 6.36±0.13, 7.34±0.10 8.30±0.23 and 9.60±0.20 x10^{12}/L on 3rd, 15th, 30th, 45th and 90th day post-treatment.

The values were significantly higher compared to diabetic control animals (P≤0.001) from 30th day onwards whereas significantly lower (P≤0.001) compared to normal control animals on all the days of study.

The rats of Groups IV, V and VI treated with *Trigonella foenum graecum*, *Coccinia indica* individually and in their combination respectively showed a progressive increase in the mean (± SE) TRC values from 30th day onwards. The mean TRC values were 5.71±0.16, 5.11±0.13 and 5.71±0.16 cu mm on 3rd day and 7.55±0.50, 7.60±0.30 and 7.55±0.50 x10^{12}/L on 90th day respectively. The mean values of TRC were significantly improved (P≤0.001) from 30th day onwards in comparison with those of diabetic control (Group-II) animals. However, in comparison to normal control animals the mean TRC values were significantly lower (P≤0.001) on all the days of the study. In addition the improvement in the values was comparable to that of Group III and did not vary between the groups on 90th day (Table 12; Figure 12).
The rats of Groups VII, VIII and IX rats treated with *Trigonella foenum graecum*, *Coccinia indica* individually and in their combination with glibenclamide at half dose respectively showed a consistent increase in the mean (± SE) TRC value from Day 3 to Day 90. The mean TRC values were 5.71±0.16, 5.70±0.10 and 5.76±0.19 x10^12/L on Day 3 and 8.75± 0.15, 9.30±0.10 and 9.50± 0.10 x10^12/L on Day 90 of treatment respectively. The improvement in the mean TRC values was significant (P≤0.001) from 30th day onwards in comparison with those of diabetic control rats (Group- II) and were significantly lower (P≤0.001) compared to normal control (Group-I) rats on all the days of study. The values were comparable to glibenclamide treated group and did not significantly differ (P>0.05) between the groups (Table 12; Figure 12).

**4.5.3 Total leucocyte count (TLC)**

The mean (± SE) Tlc values in the normal control animals were observed to be 8.51±0.30, 8.07±0.43, 8.30±0.39, 8.66±0.48 and 10.05±0.45 X 10^3 cells /µL on 3rd, 15th, 30th, 45th and 90th day respectively and there was no significant difference (P>0.01) in values throughout the experiment (Table 13; Figure 13).

In the present study, the diabetic rats of Group-II revealed a significant decrease (P≤0.001) in the mean (± SE) TLC levels from 15th to 90th day of experiment in comparison with that of normal control (Group-I). The mean TLC values were 6.01±0.15, 5.70±0.13, 4.75±0.12, 3.76±0.14 and 3.05±0.15 x 10^3/µL on 3rd, 15th, 30th, 45th and 90th day of treatment respectively (Table 13; Figure 13).

The mean (± SE) TLC values of Group-III rats treated with glibenclamide were observed to be progressively increased from 6.49±0.16 x 10^3/µL on 15th day to 8.75±0.15
x $10^3/\mu$L on 90th day post-treatment. The values were significantly higher (P≤0.001) compared to diabetic control rats on 15th, 30th, 45th and 90th day of treatment and were comparable with that of normal control (Group I) on 45th and 90th day of observations (Table 13; Figure 13).

The mean (± SE) values of TLC in Groups IV, V and VI treated with *Trigonella foenum graecum, Coccinia indica* and in their combination respectively were significantly increased (P≤0.001) when compared to diabetic control rats from Day 15 to Day 90 of the experiment. The mean (± SE) values were 5.60±0.24, 5.15±0.11 and 5.27±0.16 on 3rd day and increased to 7.30±0.90, 6.85±0.25 and 7.90±0.40 x $10^3/\mu$L on 90th day of the study respectively. However, the mean values were significantly lower (P≤0.001) compared to normal control on all the days of observations but comparable to glibenclamide group (III) on 90th day. Between the groups the mean TLC values did not differ significantly (Table 13; Figure 13).

There was a progressive increase in TLC towards normalcy in rats of Groups VII, VIII and IX treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination with glibenclamide at half dose respectively from 3rd day to 90th day of the experiment. The mean values were significantly improved (P≤0.001) compared to diabetic control rats from 30th day onwards. However, with respect to normal control rats the mean values were significantly lesser (P≤0.001) throughout the experiment except on 90th day. The mean TLC values of combined Group (IX) were comparable to those of control group on 45th and 90th day of the treatment (Table 13; Figure 13).
4.5.4 Platelet count

The mean (± SE) total platelet count in the normal control animals were observed to be 442.58±23.18, 436.50±23.26, 440.70±22.89, 467.12±27.50 and 453.50±24.50 x $10^9$/L on 3rd, 15th, 30th, 45th and 90th day respectively, where there was no significant difference (P>0.01) in values throughout the experiment (Table 1; Figure 14).

The mean (± SE) total platelet count of diabetic control rats progressively decreased 274.16±22.68 x $10^9$/L on 15th day and 277.50±29.50 x $10^9$/L on 90th day of experiment and the decrease was highly significant (P≤0.001) in comparison with those of normal control group on all the days of observation (Table 1; Figure 14).

The mean (± SE) values of total platelet count of Group-III rats treated with glibenclamide progressively increased and the values were 296.83±23.64, 333.30±17.03, 361.12±10.23 and 404.50±20.50 x $10^9$/L on Day 15, 30, 45 and 90 respectively.

The mean total platelet count was significantly higher (P≤0.001) than that of diabetic control and was comparable to that of glibenclamide group only on 90th day of treatment.

The other treatment groups (Group IV to IX) showed limited trend of improvement in the mean total platelet count throughout the study period and were comparable and did not significantly (P>0.001) differ between them. However the mean values were significantly higher (P≤0.001) compared to diabetic control on 45th and 90th day and comparable with normal control group (Group I) on 90th day.
4.6 Pathology

4.6.1 Clinical signs

In the present study, Group –I rats remained healthy and active throughout the period of experiment (plate 12).

Group-II diabetic rats exhibited clinical signs such as polyuria, polydipsia, polyphagia, restlessness and mild diarrhoea by 48-72 hour post STZ injection and the signs persisted till the end of the study in a relatively more severe form as compared to the normal healthy control animals throughout the experimental period (plate 13).

The animals of Group III, IV, V, VI, VII, VIII and IX manifested clinical signs similar to those of diabetic control rats which comprised of polyuria, polydipsia, weakness, ruffled hair coat on 15th day of treatment. However, the intensity of various clinical signs gradually decreased by 90th day of treatment in all the groups with appreciable improvement in body condition of Group IX rats.

4.6.2 Gross pathology

The diabetic control rats appeared grossly emaciated. The pancreas showed congestion and a progressive decrease in size which became appreciable from 15th day of the present study. On 45th and 90th day the pancreas was atrophied and appeared as a thin gelatinous strip and was difficult to collect from the duodenal loop. The spleen was atrophied and the adrenal gland was comparatively enlarged than the normal control rats. The other organs such as liver, kidney, intestine, lung brain and heart did not show any appreciable gross lesions throughout the study period (plate 19).
Grossly, the pancreas of glibenclamide treatment group appeared reduced in size on 3rd day but showed improvement from 15th day onwards. Liver, kidney, heart, lungs, spleen, intestine, adrenal gland and brain did not reveal any appreciable macroscopic changes on any interval of examination.

The gross changes in the pancreas observed in other groups (IV to IX) were similar to those of diabetic group on 3rd day but gradually improved with slight variation between the different treatments. A better improvement was observed in rats of combined treatment with *Trigonella foenum graecum*, *Coccinia indica* and glibenclamide half the dose treatment (Group IX) compared to others and was comparable to that of glibenclamide treatment group. No appreciable macroscopic changes were observed in other organs in all the groups.

**4.6.3 Histopathology**

**4.6.3.1 Group-II (Diabetic control)**

In the present study, on 15th day of post STZ injection, affection of both exocrine and endocrine components of pancreas was observed. The exocrine portion revealed loss of normal lobular architecture characterized by reduction in the lobular size and widely separated out lobules. The widened interlobular space revealed presence of edema fluid, haemorrhage and mild infiltration of mononuclear infiltration perivascularly. The blood vessels were severely congested. The acinar epithelial cells were highly vacuolated, degenerated and necrotic with loss of zymogen granules. In some lobules complete loss of acinar cells was observed (plate 34 and 36).
In the endocrine component, pancreas revealed reduced number of islets per lobule with loss of normal architecture. There was loss of demarcation between islets and the surrounding exocrine portion and the islet cells were observed to be infiltrating into exocrine portion. The islets were hypo cellular with altered number and distribution of α and β-cells. The β-cells were swollen and highly vacuolated with fusiform shaped condensed nucleus. A variable number of beta cells per islet were also necrotic. The cytoplasmic granularity of β-cells was highly reduced. There was also presence of apoptotic cells in the islets. The α-cells at the periphery were unaffected and the number of α-cells was more comparatively (Plate 37-39).

On 30th day, the lesions were similar to those of 15th day with disorganized exocrine component and islets. The β cells were highly vacuolated and necrotic and there was mild α cell hyperplasia in some of the islets. In addition mild ductular hyperplasia was also observed (Plate 38).

On 45th day, damage to the exocrine portion persisted with slight improvement in the number and size of islets. Some of the islets were hyper cellular with increase in the number of α-cells. The islets showed a ‘star fish’ appearance with infiltration of the islet cells into the surrounding exocrine portion in different directions. There was absence of cells with normal β-cell morphology and showed persistence of occasional highly vacuolated β-cells. Apoptotic cells were observed both in endocrine and exocrine portion of the pancreas. The histopathology of islets of Langerhans in diabetic rats indicated progressive destruction of β-cells from 15th to 45th day of investigation (Plate 35).
On 90\textsuperscript{th} day of the experiment, there was an improvement in the architecture of exocrine portion with regeneration of acinar cells. There was complete absence of cellular damage and revealed formation of compact lobules comprising of hyperplastic exocrine tissue characterized by densely packed exocrine glands with hyperchromatic nucleus, basophilic scanty cytoplasm and absence of zymogen granule formation. Only the centrally placed exocrine glands in the lobules revealed varying amount of zymogen granule formation (Plate 39).

The morphological appearance of islets was also improved and appeared larger in size as well as increased in number. The islets were irregular, compact and hyper cellular. The cells lacked granularity of beta cells and resembled alpha cells. Persistence of occasional highly vacuolated beta cells and apoptotic cells were also observed in a few islets. In addition there was mild to moderate degree of hyperplasia of ductular epithelial cells traversing into the exocrine portion with formation of small cluster of cells and ducts. Small sized newly formed islets were also observed adjacent to the hyperplastic ducts.

Microscopically in diabetic control rats, liver showed highly swollen and vacuolated hepatocytes and obliteration of the sinusoidal spaces by 15\textsuperscript{th} day along with moderate to severe congestion which persisted on 30\textsuperscript{th}, 45\textsuperscript{th} and 90\textsuperscript{th} day of the study also. On 45\textsuperscript{th} and 90\textsuperscript{th} day occasional focal areas of necrosis with infiltration of mononuclear cells was observed and the severity of vacuolar degeneration reduced (plate 45).

Spleen revealed central necrosis of lymphoid follicles of occasional splenic corpuscles with lymphocytolysis. Heart revealed degeneration of muscle fibers with loss
of sarcoplasm. Microscopically other visceral organs such as kidneys, lungs, intestine did not reveal any appreciable changes throughout the study. However, in adrenal gland the zona glomerulosa and fasciculata revealed highly swollen and vacuolated cells with loss of normal arrangement of cords and obliteration of sinusoids. The vessels were highly congested both in cortex and medulla. These lesions persisted from 15th day to 90th day with mild variation in the severity between the intervals (Plate 47-50).

4.6.3.2 Group-III (Glibenclamide treatment group)

In glibenclamide treatment group, on 15th day the microscopic changes were similar to those observed in diabetic control group. There was affection of both exocrine and endocrine portion. The exocrine portion revealed loss of normal architecture of pancreatic lobules with necrosis, severe vacuolations of acinar cells and wide separation of lobular tissue by edema and haemorrhage. The islets were reduced in number per lobules, irregular and smaller in size and revealed typical STZ induced lesions characterized by presence of swollen and highly vacuolated and necrotic beta cells (Plate 51).

By 30th day post treatment, there was an appreciable improvement in the shape, number and size of the islets which were well demarcated from exocrine portion. Small sized newly formed islets were observed in the midst of exocrine portion adjacent to intra lobular pancreatic ducts. The islets were compact and showed hyper cellularity with alpha cells however several round cells with cytoplasmic granularity resembling beta cells were also present. Occasional highly swollen and vacuolated cells indicating STZ effect were also observed in a few islets.
By 45th day of treatment the microscopic picture was similar to that of 30th day which also showed ductular hyperplasia extending into the exocrine portion as individual or small clusters of cells and presence of newly formed beta cells in small number adjacent. Some of the beta-cells in the islets revealed well formed cytoplasmic granularity. However, there was persistence of STZ effect in a few cells.

On 90th day of treatment in addition to the changes observed at 45th day, there was more progressive ductular epithelial hyperplasia comprising endocrine cells either within or in adjacent area. The exocrine portion was compact and the islets revealed normal architectural details with cord like arrangement of the cells (Plate 52).

Microscopically, liver revealed an improvement in the architecture towards normalcy from 15th day to 90th day of the treatment. On Day 15 hepatocytes revealed cell swelling and mild granular to vacuolar degeneration with obliteration of sinusoidal space. By 30th day there was an improvement in liver morphology with respect to vacuolar degeneration and cell swelling. On 45th and 90th day liver revealed almost normal architecture (plate 59).

Histopathologically, kidney, heart, lungs, spleen and intestines did not reveal any appreciable changes throughout the period of experimentation. Adrenals also appeared normal except for mild vacuolations in zona fasciculata cells on 15th day (plate 60-62).

5.6.3.3 Group IV, V and VI – diabetic rats treated with *Trigonella foenum graecum*, *Coccinia indica* and their combination.

In the present study in Groups IV, V and VI treated with *Trigonella foenum graecum*, *Coccinia indica* and their combination showed microscopically a progressive
and significant improvement in morphology of pancreatic islets from 15\textsuperscript{th} to 90\textsuperscript{th} day of the experiment (Plate 63-92).

The exocrine portion revealed vacuolar and necrotic changes similar to that of diabetic control group in the acinar cells on 15\textsuperscript{th} day of treatment and the architecture improved and was well maintained on 30\textsuperscript{th}, 45\textsuperscript{th} and 90\textsuperscript{th} day of the study.

In the endocrine portion of pancreas on 15\textsuperscript{th} day, the lesions were similar to those of diabetic control however there was an increase in the number of small sized islets. The islets were irregular and showed STZ effect in the form of cell swelling, cytoplasmic vacuolations and less cellularity. On 30\textsuperscript{th} day, there was an improvement in the size and number of the islets. The islets were comparatively larger, compact, hyper cellular and extended into the surrounding exocrine tissue. There was an increase in the number of α-cells and also presence of cells with β-cell morphology. The persistence of STZ effect in occasional cells was observed. There was ductular hyperplasia and comprised beta cells in occasional areas within as well as in the adjacent place.

On 45\textsuperscript{th} day, more number of islets that had attained normal architecture with compact arrangement of cells was observed. Some of the islets were hypercellular but irregularly shaped. The number of α-cells was more which were distributed throughout the islets with loss of their normal distribution and few polyhedral cells with pale granular cytoplasm indicative of β-cells.

Ductal proliferation of moderate degree similar to that of 30\textsuperscript{th} day persisted with appearance of newly formed small islets adjacent to them.
On 90\textsuperscript{th} day, hyperplastic change involving the exocrine portion was observed. Hyperplasia of ductular epithelium was prominent and was observed in most of the lobules either as individual cells, small clusters of cells or as well formed ducts with stratification of lining cells. In addition such ductular structures also encompassed a few cells with the morphology of beta cells. Newly formed small and large islets were also observed adjacent to the ducts. The morphological appearance of islets was similar to those of 45\textsuperscript{th} day of the experiment.

The reconstruction of islet was better in combined group compared to individual treatment groups.

Microscopically, liver showed mild granular to vacuolar change on 15\textsuperscript{th} day and normal architecture by 30\textsuperscript{th} day which persisted on 45\textsuperscript{th} and 90\textsuperscript{th} day of experimentation (Plate 91).

Other organs like kidney, lung, heart, muscle, brain, spleen and intestine did not show any appreciable change throughout the study and maintained their normal structure.

Adrenal gland showed mild vacuolar change in zona glomerulosa and zona fasciculata cells on 15\textsuperscript{th} and 30\textsuperscript{th} day but appeared normal microscopically on 45\textsuperscript{th} and 90\textsuperscript{th} day (Plate 92).

\textbf{5.6.3.4 Group VII, VIII and IX – diabetic rats treated with Trigonella foenum graecum, Coccinia indica individually and in their combination along with Glibenclamide half dose}

Microscopically Group VII, VIII and IX rats treated with Trigonella foenum graecum, Coccinia indica individually and in their combination along with
Glibenclamide half dose showed a progressive and significant improvement in morphology of pancreatic islets from 15\textsuperscript{th} to 90\textsuperscript{th} day of the experiment (plate 93-124).

The exocrine component of pancreas appeared normal throughout the study. On Day 15, the islets were more in number and were hyper cellular with more number of alpha cells and comprised occasional highly vacuolated and swollen cells. The exocrine component was compact.

On Day 30, 45 and 90 the islets were more in number and comparatively larger than those of the 15\textsuperscript{th} day. The islet morphology was normal with normal distribution of alpha cells at the periphery and beta cells at the centre of the islet. There was a progressive increase in the number of beta cells from 30\textsuperscript{th} day to 45\textsuperscript{th} day and remained comparable to 45\textsuperscript{th} day on 90\textsuperscript{th} day. There was also hyperplastic change involving duct epithelium on 30\textsuperscript{th}, 45\textsuperscript{th} and 90\textsuperscript{th} day.

The improvement in group VIII and IX was better than that of Group VII in pancreas microscopically.

Microscopically liver showed swelling and mild granular change on 15\textsuperscript{th} day and attained normal morphological architecture by 30\textsuperscript{th} day of the study.

No appreciable microscopic changes were observed in any of the other organs examined including adrenal gland (plate 111-114).
4.7 Immunohistochemical evaluation of Insulin secretion by beta- cells

In the present study, immunohistochemical demonstration of insulin was carried out to evaluate insulin secretory function of β-cells in various treatment groups using polyclonal anti-insulin antibody. Appearance of dark brown granular staining of cytoplasm of β-cells was considered as positive reaction and based on the level of expression and percentage of cells showing positivity, the functional status was evaluated (Table 15; Figure 15).

4.7.1 Group-I (Normal control group)

In the normal animals, all the islets revealed intensely positive cells in large number which showed granular brown staining restricted to cytoplasm. The granules were compactly arranged, limited by a regular membrane. The nucleus was unstained and appeared lightly basophilic. The α-cells and exocrine component were negative for immunostaining. The mean percentage value of insulin secretory cells was found to be 79.50± 2.05, 81.50± 2.50, 79.85±1.18 and 79.00±1.00 on 15th, 30th, 45th and 90th day of the present study respectively (Table 15; Figure 15) and (Plate 27).

4.7.2 Group-II (Diabetic control group)

The islets of Langerhans in pancreas of diabetic animals revealed a drastic reduction in the number of insulin positive cells. The insulin positive cells appeared irregular with light coloured scattered of granular material in the cytoplasm. The intensity of colouration of the granules was light (Plate 40). Some of islets with complete absence of insulin immune reaction were also observed.
The mean percentage positivity of insulin secretory cells at 15th, 30th, 45th and 90th day of the experiment were 3.50 ± 0.50, 2.50 ± 0.50, 2.83 ± 0.31 and 3.00 ± 0.00 respectively which were significantly lesser (P≤0.001) in comparison with that of normal control group on all the days of observation (Table 15; Figure 15) and (Plate 40-42).

4.7.3 Group-III (Diabetic rats treated with glibenclamide)

In the glibenclamide treated diabetic rats there was a progressive increase in the number of insulin positive cells from 15th to 90th day of treatment. On 15th day the number of insulin positive cells was less and was comparable to that of diabetic control rats. On 30th day of the experiment, a drastic increase in the number of insulin positive cells per islet was observed which were comparatively larger, compact and darkly stained. However a few cells revealed dispersion of granules which were lightly stained. On 45th and 90th day a further increase in the number of beta cells was observed. However, the values were significantly lesser compared to normal control rats. In some beta cells the insulin granules appeared to be marginated to the cell membrane (Plate53). From 30th day onwards small clusters or individual insulin positive cells were found within the ducts, adjacent to ducts as well as within exocrine portion of pancreas. The mean percentage of insulin positive cells was 7.50±0.50, 27.00±1.00, 42.33±1.68 and 46.50±1.50 on 15th, 30th, 45th and 90th day of experiment respectively which were significantly higher (P≤0.001) compared to diabetic rats but significantly lesser (P≤0.001) in comparison with that of normal control rats (Table 15; Figure 15) and (Plate 54-56).
4.7.4 Groups IV, V and VI – diabetic rats treated with *Trigonella foenum graecum*, *Coccinia indica* and their combination.

The immunohistochemical examination in diabetic rats treated with *Trigonella foenum graecum*, *Coccinia indica* and their combination revealed progressive improvement in the number of insulin positive cells from 15\textsuperscript{th} day to 90\textsuperscript{th} day (Plate 69, 81, and 90).

The insulin immune positive cells on 15\textsuperscript{th} day were lightly stained and significantly more (P<0.01) than that of diabetic group. The number of insulin positive beta cells increased on 30\textsuperscript{th}, 45\textsuperscript{th} and 90\textsuperscript{th} day. The beta cells were large, polyhedral and consisted darkly stained granular cytoplasm resembling normal beta cells. The cells were concentrated more at the centre of the islet. However, a few cells were also observed at the periphery. Insulin immune positive cells were also observed in the exocrine portion as well as within and adjacent to the ductal structures.

The mean percentage number of insulin positive cells were 6.50±0.50, 24.00±1.00, 37.50±1.25 and 38.00±2.00 in Group IV, 12.50±1.50, 31.50±1.50, 35.83±1.37 and 39.50±1.50 in Group V and 11.00±1.00, 33.50±0.50, 35.18±2.75c and 47.00±2.00 in Group VI on 15\textsuperscript{th}, 30\textsuperscript{th}, 45\textsuperscript{th} and 90\textsuperscript{th} day respectively which were significantly higher (P≤0.001) in comparison with that of diabetic control group but comparable to that glibenclamide treated group on 90\textsuperscript{th} day (Table 15; Figure 15).
5.7.5 Group VII, VIII and IX – diabetic rats treated with *Trigonella foenum graecum*, *Coccinia indica* and their combination with Glibenclamide half dose

The diabetic rats treated with *Trigonella foenum graecum*, *Coccinia indica* individually and in combination with half dose of glibenclamide showed a progressive increase in the number of insulin positive cells from 15\(^{th}\) to 90\(^{th}\) day of the experiment. Insulin immune positive cells were also observed in the exocrine portion as well as within and adjacent to the ductal structures which were intensely stained (Plate 108, 121).

The percentage of insulin positive cells was found to be 12.00±2.00, 22.50±3.50, 38.16±1.53 and 40.50±1.50 for Group VII 15.50±1.50, 37.50±3.5, 70.33±1.60 and 75.50±0.50 in Group VIII and 17.50±1.50, 52.00±1.00, 73.33±4.43 and 76.00±1.00 in Group IX on 15\(^{th}\), 30\(^{th}\), 45\(^{th}\) and 90\(^{th}\) day respectively (Table 15; Figure 15).

In comparison with diabetic control the percentage of insulin positive cells was significantly (P≤0.001) higher on all the days of observation and the diabetic rats of Group VIII and IX treated with *Coccinia indica* individually with half dose of glibenclamide and *Coccinia indica* and *Trigonella foenum graecum* in combination with glibenclamide at half dose showed a significant increase in the number of insulin positive cells from 15\(^{th}\) to 90\(^{th}\) day of the experiment. The number of insulin positive beta cells drastically increased from 30\(^{th}\) to 90\(^{th}\) day. The beta cells were large, polyhedral and consisted of varyingly stained granular cytoplasm in intensity with a morphology and distribution similar to that of normal beta cells. The number of insulin positive cells was comparable to that of normal control. The cells were concentrated more at the centre of
the islet. Insulin immune positive cells were also observed in the exocrine portion as well as within and adjacent to the ductal structures which were intensely stained.

The percentage of insulin positive cells was found to be 15.50±1.50, 37.50±3.5, 70.33±1.60 and 75.50±0.50 in group VIII and 17.50±1.50, 52.00±1.00, 73.33±4.43 and 76.00±1.00 in Group IX on 15th, 30th, 45th and 90th day of the experiment. The percentage of insulin positive cells was significantly (P≤0.001) higher in comparison with diabetic control and other treatment groups (III, IV, V, VI, VII) and comparable with normal control at 45th and 90th day (Table 15; Figure 15).

4.8 Special staining for beta cells

In the present study, special staining was done for demonstration of beta cells using Gomori’s chrome alum hematoxylinphloxine stain. In the normal group, pancreas revealed islets with large number of β-cells which occupied the core and α-cells at the periphery. The β-cells were polygonal in shape with deep blue granular cytoplasm and α-cells with reddish pink cytoplasm (Plate 28).

There was severe depletion of β-cells in the islets of STZ induced diabetic rats with progressive increase in α-cells.

Pancreas of all the treatment groups revealed hypercellularity with more number of α-cells with occasional β-cells which failed to attain normal morphology. However, among all the groups, the islets of Group- VIII and IX revealed better islet morphology in terms of number and distribution of β-cells (Plate 124).
4.9 Immunomodulatory effect of *Trigonella foenum graecum* and *Coccinia indica*

**Flow cytometry**

Blood samples collected at Day 45 of the sacrifice were subjected for CD4⁺ and CD8⁺ T lymphocytes analysis by flow cytometry to know the immunostimulatory and immunomodulatory effects of the extracts.

4.9.1 Normal control group (I)

The mean (± SE) values of CD4⁺ and CD8⁺ of Group-I (normal control) rats were 65.36±1.17 and 27.71±3.12 per cent on 45th day post-treatment respectively. The ratio of CD4⁺:CD8⁺ was 2.02±0.08 (Table 16; Figure 16-21).

4.9.2 Diabetic control group (II)

The mean (± SE) CD4⁺ and CD8⁺ values of group-II (Diabetic control) rats were 37.30±1.26 and 52.36±0.57 per cent on 45th day respectively and showed significantly lower (P≤ 0.001) CD4⁺ and significantly higher (P≤0.001) CD8⁺ values compared to normal control animals on day 45 of the study. The ratio of CD4⁺:CD8⁺ was 0.71±0.03 which was significantly lower (P≤ 0.001) compared to normal control (Table 16; Figure 16-21).

4.9.3 Glibenclamide Group (III)

The mean (± SE) CD4⁺ and CD8⁺ values of group-III (rats treated with glibenclamide) were 38.75±1.54 and 50.01±0.93 per cent on 45th day of the study respectively. There was no significant (P≤ 0.001) improvement in the CD4⁺ values and CD8⁺ values when compared to diabetic control and the improvement was not
comparable to normal control group. The ratio of CD4+:CD8+ was 0.76±0.05 and showed no significantly improvement (\( P \leq 0.001 \)) compared to diabetic control and was not comparable with that of normal control groups (Table16 Figure 16-21).

4.9.4 Combined groups (IV, V, and VI)

The mean (±SE) values of CD4+ in Group-IV, V and VI, treated with *Trigonella foenum graecum*, *Coccinia indica* individually and both *trigonella foenum graecum* and *coccinia indica* together respectively were 43.98±3.78, 41.34±2.92 and 55.83±0.53. The combined treatment Group VI showed significant increase in the mean (±SE) CD4+ values compared to diabetic and other treatment group except Group-IX treated with *Trigonella foenum graecum*, *Coccinia indica* and Glibenclamide half dose (Table 16; Figure 16-21).

The mean (±SE) CD8+ values in Groups-IV, V and VI was 48.97±4.12, 48.22±1.09 and 37.18±1.66 respectively. The combined treatment Group-VI showed a significant decrease in mean CD8+ values compared to diabetic and other treatment groups.

The mean ratio between CD4+ and CD8+ was 0.93±0.15, 0.86±0.07 and 1.51±0.07 respectively for Groups IV, V and VI. The mean (±SE) CD4+/CD8+ ratio was significantly higher in Group-IV compared to diabetic control and other treatment groups except Group-IX on 45\(^{th}\) day of the experiment.
4.9.5 Combined extract treatment with glibenclamide (Group-VII, VIII and IX)

The mean (±SE) values of CD4+ cells in group VII, VIII and IX on 45th day of treatment were 43.48±4.17, 32.31±4.87 and 54.31±4.66 and the CD8+ values were 50.05±3.90, 61.72±4.57 and 41.12±4.11 respectively. It was observed that both mean (±SE) CD4+ and CD8+ values were significantly improved in Group-IX compared to diabetic control and was comparable with that of Group-VI treated with both plant extracts without glibenclamide

The CD4+/ CD8+ mean ratio was 0.91±0.17, 0.40±0.20 and 1.38±0.22 for Groups, VII, VIII and IX respectively. The mean ratio was significantly increased in Group-IX compared to diabetic control and other treatment groups except Group VI where in the values were comparable (Table 16; Figure 16-21).
Table 1. The Mean (± SE) animal body weight (g) values of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Group I</td>
<td>182.00±1.53\textsuperscript{a}</td>
</tr>
<tr>
<td>Group II</td>
<td>157.41±2.44\textsuperscript{b}</td>
</tr>
<tr>
<td>Group III</td>
<td>164.91±2.39\textsuperscript{b}</td>
</tr>
<tr>
<td>Group IV</td>
<td>158.91±2.76\textsuperscript{b}</td>
</tr>
<tr>
<td>Group V</td>
<td>159.83±2.50\textsuperscript{b}</td>
</tr>
<tr>
<td>Group VI</td>
<td>159.16±1.55\textsuperscript{b}</td>
</tr>
<tr>
<td>Group VII</td>
<td>158.00±1.73\textsuperscript{b}</td>
</tr>
<tr>
<td>Group VIII</td>
<td>156.66±2.10\textsuperscript{b}</td>
</tr>
<tr>
<td>Group IX</td>
<td>158.83±1.85\textsuperscript{b}</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).

Table 2. The Mean (± SE) serum glucose (mg/dL) values of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Group I</td>
<td>89.25±2.86\textsuperscript{c}</td>
</tr>
<tr>
<td>Group II</td>
<td>458.58±40.52\textsuperscript{ab}</td>
</tr>
<tr>
<td>Group III</td>
<td>418.83±5.57\textsuperscript{b}</td>
</tr>
<tr>
<td>Group IV</td>
<td>464.83±19.78\textsuperscript{ab}</td>
</tr>
<tr>
<td>Group V</td>
<td>475.69±18.05\textsuperscript{ab}</td>
</tr>
<tr>
<td>Group VI</td>
<td>493.32±11.19\textsuperscript{a}</td>
</tr>
<tr>
<td>Group VII</td>
<td>495.53±13.92\textsuperscript{a}</td>
</tr>
<tr>
<td>Group VIII</td>
<td>491.69±15.28\textsuperscript{a}</td>
</tr>
<tr>
<td>Group IX</td>
<td>499.40±15.62\textsuperscript{a}</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).
Fig. 1. The Mean (± SE) animal body weight (g) values of different groups at different intervals of time

Fig. 2. The Mean (± SE) serum glucose (mg/dL) values of different groups at different intervals of time
Table 3. The Mean (± SE) serum cholesterol (mg/dL) values of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Group I</td>
<td>80.88±3.58b</td>
</tr>
<tr>
<td>Group II</td>
<td>136.21±7.61a</td>
</tr>
<tr>
<td>Group III</td>
<td>136.16±7.65a</td>
</tr>
<tr>
<td>Group IV</td>
<td>143.40±8.19a</td>
</tr>
<tr>
<td>Group V</td>
<td>133.43±4.81a</td>
</tr>
<tr>
<td>Group VI</td>
<td>136.53±6.35a</td>
</tr>
<tr>
<td>Group VII</td>
<td>133.01±5.84a</td>
</tr>
<tr>
<td>Group VIII</td>
<td>138.86±7.19a</td>
</tr>
<tr>
<td>Group IX</td>
<td>125.68±4.64a</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).

Table 4. The Mean (± SE) serum triglyceride (mg/dL) values of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Group I</td>
<td>95.95±0.99c</td>
</tr>
<tr>
<td>Group II</td>
<td>207.18±1.34b</td>
</tr>
<tr>
<td>Group III</td>
<td>215.82±0.93a</td>
</tr>
<tr>
<td>Group IV</td>
<td>209.26±1.65b</td>
</tr>
<tr>
<td>Group V</td>
<td>215.27±1.05a</td>
</tr>
<tr>
<td>Group VI</td>
<td>216.18±1.36d</td>
</tr>
<tr>
<td>Group VII</td>
<td>211.93±1.66ab</td>
</tr>
<tr>
<td>Group VIII</td>
<td>215.25±1.06ab</td>
</tr>
<tr>
<td>Group IX</td>
<td>211.89±1.02ab</td>
</tr>
</tbody>
</table>

The means have at least one common superscript not significantly different (P≤0.001).
Fig. 3. The Mean (± SE) serum cholesterol (mg/dL) values of different groups at different intervals of time

Fig. 4. The Mean (± SE) serum triglyceride (mg/dL) values of different groups at different intervals of time
Table 5. The Mean (± SE) serum alanine aminotransferase (ALT) (IU/L) values of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
<th>3</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td>53.07±1.64</td>
<td>51.81±1.70</td>
<td>51.52±1.90</td>
<td>52.61±1.83</td>
<td>56.40±2.10</td>
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<tr>
<td>Group II</td>
<td></td>
<td>130.33±7.34</td>
<td>163.02±8.74</td>
<td>206.58±13.60</td>
<td>228.80±21.51</td>
<td>228.05±6.44</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td>140.83±3.55</td>
<td>120.84±2.00</td>
<td>108.84±3.54</td>
<td>90.11±4.22</td>
<td>95.40±3.00</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td>137.96±2.00</td>
<td>118.70±1.24</td>
<td>107.43±2.17</td>
<td>101.17±2.16</td>
<td>89.65±1.84</td>
</tr>
<tr>
<td>Group V</td>
<td></td>
<td>139.47±1.59</td>
<td>126.64±8.57</td>
<td>109.15±1.20</td>
<td>100.60±2.82</td>
<td>94.45±1.95</td>
</tr>
<tr>
<td>Group VI</td>
<td></td>
<td>141.85±1.93</td>
<td>106.89±1.76</td>
<td>94.95±1.90</td>
<td>86.36±1.53</td>
<td>76.29±1.79</td>
</tr>
<tr>
<td>Group VII</td>
<td></td>
<td>141.18±1.48</td>
<td>106.22±1.46</td>
<td>93.18±1.20</td>
<td>79.20±1.55</td>
<td>71.55±0.90</td>
</tr>
<tr>
<td>Group VIII</td>
<td></td>
<td>142.09±3.58</td>
<td>119.75±1.93</td>
<td>99.11±1.53</td>
<td>80.62±2.67</td>
<td>70.15±1.25</td>
</tr>
<tr>
<td>Group IX</td>
<td></td>
<td>138.98±1.40</td>
<td>108.85±1.87</td>
<td>87.43±0.79</td>
<td>70.55±1.89</td>
<td>66.05±0.65</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).

Table 6. The Mean (± SE) serum aspartate aminotransferase (AST) (IU/L) values of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
<th>3</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td>67.06±2.24</td>
<td>67.91±2.60</td>
<td>67.53±1.80</td>
<td>69.78±2.22</td>
<td>71.70±3.10</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td>170.10±10.20</td>
<td>208.01±9.23</td>
<td>247.82±21.23</td>
<td>286.87±23.35</td>
<td>301.15±5.35</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td>180.48±3.05</td>
<td>152.89±2.13</td>
<td>120.15±2.24</td>
<td>94.73±2.22</td>
<td>94.15±2.25</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td>180.05±2.31</td>
<td>158.05±2.56</td>
<td>137.83±1.37</td>
<td>104.67±2.47</td>
<td>88.50±3.80</td>
</tr>
<tr>
<td>Group V</td>
<td></td>
<td>182.17±2.87</td>
<td>160.32±2.15</td>
<td>136.56±1.68</td>
<td>125.27±2.48</td>
<td>90.73±2.48</td>
</tr>
<tr>
<td>Group VI</td>
<td></td>
<td>177.25±3.19</td>
<td>150.11±2.02</td>
<td>124.32±1.40</td>
<td>93.91±2.05</td>
<td>83.75±1.95</td>
</tr>
<tr>
<td>Group VII</td>
<td></td>
<td>182.65±2.01</td>
<td>150.90±2.50</td>
<td>121.88±1.45</td>
<td>84.05±1.60</td>
<td>80.80±1.40</td>
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<tr>
<td>Group VIII</td>
<td></td>
<td>181.36±2.54</td>
<td>157.81±1.86</td>
<td>122.95±1.58</td>
<td>87.60±2.37</td>
<td>88.80±2.40</td>
</tr>
<tr>
<td>Group IX</td>
<td></td>
<td>181.94±2.56</td>
<td>143.89±1.45</td>
<td>115.29±1.13</td>
<td>65.42±1.52</td>
<td>70.85±3.45</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).
Fig. 5. The Mean (± SE) serum alanine aminotransferase (ALT) (IU/L) values of different groups at different intervals of time

Fig. 6. The Mean (± SE) serum aspartate aminotransferase (AST) (IU/L) values of different groups at different intervals of time
Table 7. The Mean (± SE) serum insulin (µU/ml) values of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Group I</td>
<td>53.85±3.21^a</td>
</tr>
<tr>
<td>Group II</td>
<td>17.58±1.70^b</td>
</tr>
<tr>
<td>Group III</td>
<td>15.42±2.06^b</td>
</tr>
<tr>
<td>Group IV</td>
<td>14.79±1.03^b</td>
</tr>
<tr>
<td>Group V</td>
<td>17.96±1.36^b</td>
</tr>
<tr>
<td>Group VI</td>
<td>18.76±1.06^b</td>
</tr>
<tr>
<td>Group VII</td>
<td>17.87±0.83^b</td>
</tr>
<tr>
<td>Group VIII</td>
<td>17.17±1.34^b</td>
</tr>
<tr>
<td>Group IX</td>
<td>16.89±2.11^b</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).

Table 8. The Mean (± SE) values of activities of SOD (U/min/mg protein) in the liver of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Group I</td>
<td>19.23±1.10^a</td>
</tr>
<tr>
<td>Group II</td>
<td>5.66±0.27^b</td>
</tr>
<tr>
<td>Group III</td>
<td>5.49±0.37^b</td>
</tr>
<tr>
<td>Group IV</td>
<td>6.94±0.60^b</td>
</tr>
<tr>
<td>Group V</td>
<td>6.12±0.25^b</td>
</tr>
<tr>
<td>Group VI</td>
<td>6.05±0.07^b</td>
</tr>
<tr>
<td>Group VII</td>
<td>5.98±0.11^b</td>
</tr>
<tr>
<td>Group VIII</td>
<td>6.02±0.04^b</td>
</tr>
<tr>
<td>Group IX</td>
<td>6.05±0.16^b</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).
Fig. 7. The Mean (± SE) serum insulin (µU/ml) values of different groups at different intervals of time.

Fig. 8. The Mean (± SE) values of activities of SOD (U/min/mg protein) in the liver of different groups at different intervals of time.
Table 9. The Mean (± SE) values of activities of GPx (µM of glutathione utilized/min/mg protein) in the liver of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Group I</td>
<td>48.38±1.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>19.73±1.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>20.30±0.52&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>20.13±0.68&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>19.93±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VI</td>
<td>21.34±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VII</td>
<td>21.43±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VIII</td>
<td>19.86±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IX</td>
<td>21.86±0.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).

Table 10. The Mean (± SE) mean values of activities of CAT (µ moles of H<sub>2</sub>O<sub>2</sub>/min/mg protein) in the liver of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Group I</td>
<td>76.86±1.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>26.23±1.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>27.73±1.61&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>26.55±1.80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>29.37±0.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VI</td>
<td>27.77±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Group VII</td>
<td>27.36±2.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VIII</td>
<td>27.25±1.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IX</td>
<td>26.63±0.85&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).
Fig. 9. The Mean (± SE) values of activities of GPx (µM of glutathione utilized/ min/mg protein) in the liver of different groups at different intervals of time.

Fig. 10. The Mean (± SE) mean values of activities of CAT (µ moles of H₂O₂/min/mg protein) in the liver of different groups at different intervals of time.
Table 11. The Mean (± SE) Haemoglobin (g/dL) values of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Group I</td>
<td>14.16±0.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>9.76±0.39&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>9.75±0.36&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>10.20±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>10.00±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VI</td>
<td>9.92±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VII</td>
<td>9.95±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VIII</td>
<td>9.75±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IX</td>
<td>9.75±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).

Table 12. The Mean (± SE) TRC count (x10<sup>12</sup>/L) values of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Group I</td>
<td>9.00±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>6.33±0.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>5.35±0.12&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>5.71±0.16&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>5.11±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VI</td>
<td>5.65±0.15&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VII</td>
<td>5.71±0.16&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VIII</td>
<td>5.70±0.10&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IX</td>
<td>5.76±0.19&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).
Fig. 11. The Mean (± SE) Haemoglobin (g/dL) values of different groups at different intervals of time

Fig. 12. The Mean (± SE) TRC count (x10^12/L) values of different groups at different intervals of time
Table 13. The Mean (± SE) TLC count (x10^3/µL) values of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
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<td></td>
<td>3</td>
</tr>
<tr>
<td>Group I</td>
<td>8.51±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>6.01±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>4.97±0.15&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>5.60±0.24&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>5.15±0.11&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VI</td>
<td>5.27±0.16&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VII</td>
<td>4.70±0.14&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VIII</td>
<td>4.79±0.16&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IX</td>
<td>4.91±0.20&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).

Table 14. The Mean (± SE) Platelet count (x10⁹/L) values of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Group I</td>
<td>442.58±23.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>281.08±16.61&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>280.16±18.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>291.41±13.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>290.41±14.81&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VI</td>
<td>286.00±16.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VII</td>
<td>283.41±14.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VIII</td>
<td>287.58±14.82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IX</td>
<td>302.83±14.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).
Fig. 13. The Mean (± SE) TLC count (x10³/µL) values of different groups at different intervals of time

Fig. 14. The Mean (± SE) Platelet count (x10⁹/L) values of different groups at different intervals of time
Table 15. The Mean (± SE) percentage positivity of insulin secreting cells of different groups at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
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<tr>
<td>Group I</td>
<td>79.50±2050&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>3.50±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Group III</td>
<td>7.50±0.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>6.50±0.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>12.50±1.50&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VI</td>
<td>11.00±1.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VII</td>
<td>12.00±2.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VIII</td>
<td>15.50±1.50&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IX</td>
<td>17.50±1.50&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The Means have at least one common superscript indicate not significantly different.

Table 16: The mean (±SE) values of CD4+, CD8+ and Ratio CD4+/CD8+ cells (percentage) of different groups at day 45 of the study at different intervals of time

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD4+</td>
</tr>
<tr>
<td>Group I</td>
<td>68.36±1.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>37.30±1.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>38.75±1.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>43.98±3.78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>41.34±2.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VI</td>
<td>55.83±0.53&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VII</td>
<td>43.48±4.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VIII</td>
<td>32.31±4.87&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IX</td>
<td>54.31±4.66&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The means with at least one common superscript are not significantly different (P≤0.001).
Fig. 15. The Mean (± SE) percentage positivity of insulin secreting cells of different groups at different intervals of time.

Fig. 16. The mean (±SE) values of CD4+ cells (percentage) of different groups at day 45 of the study at different intervals of time.
Fig.17. The mean (±SE) values of CD8+ cells (percentage) of different groups at day 45 of the study at different intervals of time.

CD8+

Fig.18. The mean (±SE) CD4+/CD8+ ratio percentage (%) values of different treatment groups at different intervals of time.

CD4/CD8
Fig. 19. Flow cytometry results of normal control animal

Fig. 20. Flow cytometry results of diabetic control animal

Fig. 21. Flow cytometry results of diabetic animal treated with combination of *Trigonella foenum graecum*, *Coccinia indica* and glibenclamide (Group IX)
Plate 1  *Coccinia indica* leaves, along with fruits.

Plate 2  Alcoholic extract of *Coccinia indica* leaves.

Plate 3  *Trigonella foenum graecum* seeds.

Plate 4  Alcoholic extract of *Trigonella foenum graecum* seeds.

Plate 5  Phytochemical analysis of *Trigonella foenum graecum* and *Coccinia indica* for alkaloids showing pronounced quenching of fluorescence on TLC plates at UV-254 nm and intense blue fluorescence at UV-366 nm.

Plate 6  Phytochemical analysis *Trigonella foenum graecum* and *Coccinia indica* for anthracene derivatives showing pronounced quenching on TLC plates at UV-254 nm and development of blue bands at UV-366 nm with brown and yellow bands at visible light.

Plate 7  Phytochemical analysis *Trigonella foenum graecum* and *Coccinia indica* for bitter principles showing pronounced quenching on TLC plates at UV-254 nm and blueish fluorescence at UV-366 nm with brown bands at visible light.

Plate 8  Phytochemical analysis *Trigonella foenum graecum* and *Coccinia indica* for coumarins showing pronounced quenching on TLC plates at UV-254 nm and blue fluorescence at UV-366 nm.
Plate 9  Phytochemical analysis *Trigonella foenum graecum* and *Coccinia indica* for flavonoids showing pronounced quenching on TLC plates at UV-254 nm and intense blue fluorescence at UV-366 nm.

Plate 10  Phytochemical analysis *Trigonella foenum graecum* and *Coccinia indica* for glycosides showing pronounced quenching on TLC plates at UV-254 nm and UV-366 nm with dark brown bands.

Plate 11  Phytochemical analysis *Trigonella foenum graecum* and *Coccinia indica* for saponin showing pronounced quenching on TLC plates at UV-254 nm and intense blue fluorescence at UV-366 nm with brownish yellow zones at visible light.

Plate 12  Normal control animal showing good body condition and shiny hair coat on Day 90 of the study.

Plate 13  Diabetic animal showing poor body condition, dehydration and ruffled soiled hair coat on 15th day of the experiment.

Plate 14  Diabetic animal compared with glibenclamide treated group rat on 15th day of the experiment. Note the variation in body size, condition and hair coat.

Plate 15  Rat treated with *Trigonella foenum graecum and Coccinia indica* (Group-VI) showing improvement in body condition, hair coat on 30th day of the experiment.

Plate 16  Rat treated with *Trigonella foenum graecum, Coccinia indica* and glibenclamide half dose showing improvement in body condition, hair coat in comparison with normal control on 45th day of experiment.
Plate 17  *Trigonella foenum graecum, Coccinia indica* and glibenclamide combination treatment showing improvement in body condition, hair coat of a rat in comparison with normal control on 90th day of experiment.

Plate 18  Pancreas and other visceral organs of normal control animal showing normal architecture on 90th day of the experiment.

Plate 19  Pancreas of diabetic rat appearing as pale, thin gelatinous mass on 90th day of the experiment.

Plate 20  Pancreas and visceral organs of rat treated with glibenclamide (group III) showing improvement in the gross appearance of pancreas. Organ showing clear lobulations on Day 30.

Plate 21  Pancreas and Visceral organs of animal treated with combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide (group IX) showing improvement in size on 90th day of the experiment.

Plate 22  Pancreas of animal treated with combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide (group IX) showing improvement in size and appearance on 90th day of the experiment.

Plate 23  Section of pancreas of normal control showing a normal islet with round to oval shape and compact arrangement of beta cells at the centre and alpha cells at the periphery.

H&E X 200

Plate 24  Section of pancreas showing normal islets of Langerhans with compact arrangement of beta cells at the centre and alpha cells at the periphery.

H&E X 100
Plate 25  Islet of Langerhans from a normal control animal showing intensely stained insulin positive beta cells in large number.

IHC X 200

Plate 26  Islet from normal control animal showing dark brown granular staining of cytoplasm of beta cells.

IHC X 1000

Plate 27  Islet of Langerhans from the normal control animal showing intensely stained insulin positive cells in large number.

IHC X 200

Plate 28  Pancreas from normal control animal showing large sized islet with more number of beta cells and compact arrangement of beta cells at the centre and alpha cells at the periphery on day 45 of the experiment.

Gomori’s X 200

Plate 29  Section of liver from control rat on 45th day showing central vein and well formed hepatic cords with normal appearing hepatocytes.

H&E X 200

Plate 30  Section of normal heart showing compact arrangement of cardiac myocytes.

H&E X 200

Plate 31  Section of spleen from normal control animal showing compact arrangement of splenic corpuscle.

H&E X 200

Plate 32  Section of kidney from normal control animal showing normal architecture on 45th day.

H&E X 200
Plate 33  Section of adrenal gland from the normal control animal showing normal architecture.
          H&E X 200

Plate 34  Pancreas of diabetic control animal showing loss of normal architecture and degeneration and necrosis of acinar cells on Day 15 of the experiment.
          H&E X 40

Plate 35  Pancreas of diabetic animal showing loss of normal architecture, atropic and vacuolated acinar cells with vacuolated and degenerating Islet cells on 15th day of the experiment.
          H&E X 200

Plate 36  Pancreas of diabetic control animal showing loss of normal architecture, degeneration and necrosis of islet cells.
          H&E X 200

Plate 37  Islet of Langerhans showing presence of extensive vacuolation and necrosis of beta cells on Day 15.
          H&E X 1000

Plate 38  Pancreas of diabetic control animal showing complete loss of normal architecture with shrunken and vacuolated Islet cells on Day 30 with increase in alpha cells.
          H&E X 200

Plate 39  Islet of Langerhans from diabetic animal showing loss of normal arrangement, vacuolated and degenerating beta cells with increase in number of alpha cells on Day 90.
          H&E X 200

Plate 40  Islet from diabetic control animal showing mild immunoreactivity for insulin at Day 15 of the experiment.
          IHC X 200
Plate 41  Islet of Langerhans from the diabetic control animal showing few insulin positive cells on Day 30 of the study.

IHC X 200

Plate 42  Islet of Langerhans from the diabetic control animal showing lightly stained insulin positive cells indicating degenerative cells on Day 45 of the study.

IHC X 200

Plate 43  Pancreas from diabetic animal showing irregular shaped islet with loss of beta cells and increase in alpha cells on Day 15 of the study.

Gomori’s X 200

Plate 44  Islet from the diabetic animal showing improvement in architecture of islet with more number of pink stained alpha cells than blue stained beta cells on 45th day of the experiment.

Gomori’s X 200

Plate 45  Section of liver from diabetic control animal showing highly swollen hepatocytes with cytoplasmic vacuolation on Day 15.

H&E X 200

Plate 46  Section of liver from diabetic control animal showing highly swollen hepatocytes with cytoplasmic vacuolations on Day 15.

H&E X 1000

Plate 47  Section of spleen from diabetic control animal on 15th day showing lymphocytolysis.

H&E X 200

Plate 48  Section of kidney from diabetic control animal on 15th day showing normal architecture with mild congestion.

H&E X 200
Plate 49  Section of heart from diabetic control animal on 15\textsuperscript{th} day showing degeneration of muscle fibres.  
H&E X 200

Plate 50  Section of adrenal gland from diabetic control animal on 15\textsuperscript{th} day showing vacuolation in zona fasciculata cells.  
H&E X 200

Plate 51  Islet of Langerhans from diabetic animal treated with glibenclamide showing slight improvement in architecture and more number of alpha cells on Day 15.  
H&E X 200

Plate 52  Section of pancreas from glibenclamide treated animal showing compact arrangement of islet with increase in cellularity on Day 90 of the study.  
H&E X 200

Plate 53  Islet of Langerhans from a diabetic control animal treated with glibenclamide showing a few insulin positive cells on Day 15 of the study.  
IHC X 200

Plate 54  Islet from a glibenclamide treated animal showing increase in number of beta cells in the islet on Day 45 of the study.  
IHC X 200

Plate 55  Islet of Langerhans from a diabetic control animal treated with glibenclamide showing comparatively more number of insulin positive cells on Day 90 of the study.  
IHC X 200

Plate 56  Islet of Langerhans from a diabetic control animal treated with glibenclamide showing comparatively more number of insulin positive cells on 90\textsuperscript{th} day of the study.  
IHC X 200
Plate 57  Section of pancreas from diabetic rat treated with glibenclamide showing improvement with increase in beta cell population on Day 15. Note the pink stained alpha cells at the periphery of the islet.

Gomori’s X 200

Plate 58  Section of pancreas from diabetic rat treated with glibenclamide showing improvement with increase in beta cell population on Day 90. Note the blue stained beta cells at the centre of the islet.

Gomori’s X 200

Plate 59  Section of liver from a diabetic rat treated with glibenclamide on Day 45 of the study showing improved architecture.

H&E X 200

Plate 60  Muscle from glibenclamide treatment group on Day 90 showing well formed normal appearing muscle bundles with compact arrangement of fibres.

H&E X 200

Plate 61  Spleen sections from glibenclamide treatment group on Day 90 showing normal appearing corpuscles with compact arrangement.

H&E X 200

Plate 62  Section of adrenal gland from glibenclamide treatment group on Day 90 showing normal architecture and few vacuolated cells.

H&E X 200

Plate 63  Pancreas from a diabetic rat treated with *Trigonella foenum graecum* showing persistence of STZ effect in exocrine portion at Day 15 of treatment.

H&E X 100

Plate 64  Pancreas from a diabetic rat treated with *Trigonella foenum graecum* on 30th day post-treatment showing improvement in the architecture of islet with hypercellularity.

H&E X 200
Plate 65  Pancreas from a diabetic rat treated with *Trigonella foenum graecum* on 45th day post-treatment showing improvement in the architecture of islet with hypercellularity however with occasional swollen and highly vacuolated beta cells.

H&E X 100

Plate 66  Pancreas from a diabetic rat treated with *Trigonella foenum graecum* showing proliferation of ductal epithelium on 90th day of treatment.

H&E X 100

Plate 67  Islet of Langerhans of animal treated with *Trigonella foenum graecum* showing only few insulin positive cells on Day 15 of the treatment.

IHC X 200

Plate 68  Islet from diabetic animal treated with *Trigonella foenum graecum* showing an improvement in the number of insulin positive cells on Day 30.

IHC X 200

Plate 69  Islet from diabetic animal treated with *Trigonella foenum graecum* showing more number of insulin positive cells on Day 45 of the experiment.

IHC X 200

Plate 70  Islet from diabetic animal treated with *Trigonella foenum graecum* showing more number of insulin positive cells with compact arrangement of granules in the cytoplasm on Day 90 of the experiment.

IHC X 200

Plate 71  Islet from diabetic animal treated with *Trigonella foenum graecum* showing insulin positive cells with compact arrangement of granules in the cytoplasm on Day 90 of the experiment.

IHC X 1000

Plate 72  Islet from diabetic rat treated with *Trigonella foenum graecum* showing regeneration of islet with improvement in alpha and beta cell population on Day 30.

Gomori’s X 200
Plate 73  Section of liver from a diabetic rat treated with *Trigonella foenum graecum* showing improvement in architecture on Day 90.

H&E X 200

Plate 74  Section of muscle from a diabetic rat treated with *Trigonella foenum graecum* appearing normal on 90th day.

H&E X 200

Plate 75  Section of adrenal gland from a diabetic rat treated with *Trigonella foenum graecum* with normal architecture on 90th day.

H&E X 200

Plate 76  Section of pancreas on Day 15 from a rat treated with *Coccinia indica* showing altered shape of islet with vacuolated cells.

H&E X 100

Plate 77  On Day 45, diabetic rat treated with *Coccinia indica* showing improvement in the overall architecture of islet of Langerhans. Note the arrangement of alpha and beta cells, granularity as well as amount of cytoplasm.

H&E X 200

Plate 78  On Day 90 diabetic rat treated with *Coccinia indica* showing improvement in the overall architecture of islet of Langerhans however, with persistence of occasional beta cells with STZ effect.

H&E X 200

Plate 79  Pancreas from a diabetic rat treated with *Coccinia indica* showing scanty immunoreactivity on Day 15 of the study.

IHC X 200

Plate 80  Islet from the animal treated with *Coccinia indica* showing improvement in immunoreactive cells on Day 30.

IHC X 200
Plate 81 Pancreas from diabetic animal treated with *Coccinia indica* showing more number of insulin positive cells with compact arrangement of granules in the cytoplasm on Day 90 of the treatment.

IHC X 200

Plate 82 Islet from the animal treated with *Coccinia indica* showing improvement in the number of immunoreactive cells on Day 90.

IHC X 1000

Plate 83 Section of pancreas from diabetic rat treated with combination of *Trigonella foenum graecum* and *Coccinia indica* on Day 15 showing an improvement in architecture of islet with hypercellularity.

H&E X 200

Plate 84 Section of pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum* and *Coccinia indica* on Day 30 showing an improvement in architecture of the islet.

H&E X 200

Plate 85 On Day 45 pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum* and *Coccinia indica* showing a large sized islet with hypercellularity.

H&E X 200

Plate 86 Section of pancreas on Day 90 from a diabetic rat treated with combination of *Trigonella foenum graecum* and *Coccinia indica* showing well formed large islet of Langerhans. Note more number of cells with beta cells morphology.

H&E X 100

Plate 87 Section of pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum* and *Coccinia indica* showing a few insulin immunopositive beta cells at 15th day of treatment.

IHC X 200

Plate 88 Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum* and *Coccinia indica* showing increase in number of immunoreactive cells at 30th day of treatment.

IHC X 200
Plate 89  Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum* and *Coccinia indica* showing increase in number of immunoreactive cells placed at the centre of the islet at 45th day of treatment.

IHC X 200

Plate 90  Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum* and *Coccinia indica* showing of immunoreactive cells distributed at the core of the islet on 45th day of treatment.

IHC X 200

Plate 91  Improved architecture of liver on Day 45 post-treatment from diabetic rat treated with of *Trigonella foenum graecum* and *Coccinia indica*.

H&E X 200

Plate 92  Improved architecture of adrenal on Day 45 post-treatment from diabetic rat treated with of *Trigonella foenum graecum* and *Coccinia indica*.

H&E X 200

Plate 93  Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum* and glibenclamide showing a large and well formed islet on Day 45 of the treatment.

H&E X 200

Plate 94  Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum* and glibenclamide showing large and well formed islet on Day 90 of the treatment. However note a few apoptotic cells.

H&E X 200

Plate 95  Pancreas from a diabetic rat treated with *Trigonella foenum graecum* and glibenclamide showing a occasional insulin positive cells on Day 15 of the treatment.

IHC X 200

Plate 96  Islets from diabetic animal treated with *Trigonella foenum graecum* and glibenclamide showing improvement in number of insulin positive cells on Day 30 distributed throughout the islet.

IHC X 200
Plate 97  Pancreas from a diabetic rat treated with *Trigonella foenum graecum* and glibenclamide showing increase in the number of insulin positive cells with compact arrangement of granules in the cytoplasm on Day 45 of the treatment.  
IHC X 200

Plate 98  Pancreas from a diabetic rat treated with *Trigonella foenum graecum* and glibenclamide showing more number of insulin positive cells with compact arrangement of granules on Day 90 of the treatment. Note distribution of beta cells throughout the islet.  
IHC X 200

Plate 99  Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum* and glibenclamide showing improvement in the architecture of islet with more number of beta cells at the centre on Day 45 of the treatment.  
Gomori’s X 200

Plate 100  Section of liver from a diabetic rat treated with the combination of *Trigonella foenum graecum* and glibenclamide showing almost normal architecture on 90th Day of treatment.  
H&E X 200

Plate 101  Section of adrenal from a diabetic rat treated with the combination of *Trigonella foenum graecum* and glibenclamide on 90th day post treatment showing normal architecture.  
H&E X 200

Plate 102  Pancreatic islet from a diabetic rat treated with *Coccinia indica* and glibenclamide on 15th day of treatment showing improvement in the architecture with increase in cellularity. Note persistence of STZ induced changes in occasional cells.  
H&E X 100

Plate 103  Pancreas from a diabetic rat treated with combination of *Coccinia indica* and glibenclamide showing large and well formed islet on Day 45 of treatment with increase in the number of cells with beta cells morphology.  
H&E X 200

Plate 104  Pancreas from a diabetic rat treated with combination of *Coccinia indica* and glibenclamide showing large and well formed islet on Day 90 of the treatment.  
H&E X 200
Plate 105  Section of pancreas from a diabetic rat treated with *Coccinia* and glibenclamide showing a few insulin positive cells on Day 15 of the treatment.
IHC X 200

Plate 106  Pancreas from a diabetic rat treated with *Coccinia* and glibenclamide showing improvement in number of insulin positive cells on Day 30 distributed throughout the islet.
IHC X 200

Plate 107  Islet from diabetic rat treated with *Coccinia indica* and glibenclamide showing more number of insulin positive cells on Day 45 of the treatment distributed at the centre.
IHC X 200

Plate 108  Pancreas from a diabetic rat treated with *Coccinia indica* and glibenclamide showing increase in the number of insulin positive cells with cord like arrangement similar to that of normal islet on Day 90 of the treatment.
IHC X 200

Plate 109  Immunopositive beta cells from *Coccinia indica* and glibenclamide treated diabetic rat showing brown coloured granules in the cytoplasm on 90th day of the treatment.
IHC X 1000

Plate 110  Pancreas from a diabetic rat treated with combination of *Coccinia indica* and glibenclamide showing increase in number of beta cells on Day 90 of the study. Note distribution of beta cells at the centre.
Gomori’s X 200

Plate 111  Normal appearing heart section from a diabetic rat treated with the combination of *Coccinia* and glibenclamide on 90th day of treatment.
H&E X 100

Plate 112  Normal appearance of liver from a diabetic rat treated with the combination of *Coccinia indica* and glibenclamide on 90th day of treatment
H&E X 200
Plate 113  Normal appearance of spleen from a diabetic rat treated with the combination of *Coccinia indica* and glibenclamide on 90\textsuperscript{th} day of treatment.

H&E X 200

Plate 114  Normal appearance of adrenal gland from a diabetic rat treated with the combination of *Coccinia* and glibenclamide on 90\textsuperscript{th} day of treatment.

H&E X 200

Plate 115  Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing large and irregular islet on Day 15 of the treatment

H&E X 200

Plate 116  Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing large and well formed islets on Day 45 of the treatment.

H&E X 200

Plate 117  Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing hyperplasia of ductal epithelium of exocrine component on Day 90 of the treatment.

H&E X 200

Plate 118  Pancreas from diabetic rat treated with *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing a few immunoreactive cells on Day 15 of the study.

IHC X 200

Plate 119  Pancreas from a diabetic rat treated with *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing improvement in the number of immunoreactive cells on Day 30.

IHC X 200

Plate 120  Pancreas from a diabetic rat treated with *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing increase in the number of insulin positive cells with distribution of cells at the centre of the islet on Day 15 of the treatment.

IHC X 200
Plate 121 Pancreas from a diabetic rat treated with *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing increase in the number of insulin positive cells with morphology comparable to that of normal islet on Day 90 of the treatment.  
IHC X 200

Plate 122 Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing insulin positive cells in the midst of ductal epithelium cells on 90th day of treatment.  
IHC X 1000

Plate 123 Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing newly formed small islets adjacent to the ducts at 90th day of treatment.  
IHC X 1000

Plate 124 Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing a large islet with increase in number of beta cells on Day 45 of the treatment.  
Gomori’s X 200

Plate 125 Liver from a diabetic rat treated with combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing well maintained architecture at Day 90 of the treatment.  
H&E X 100

Plate 126 Spleen from a diabetic rat treated with combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing improvement in density of lymphoid cells. Also note the secondary follicle formation.  
H&E X 200

Plate 127 Section of kidney from a diabetic rat treated with the combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide on Day 90 of treatment showing normal architecture.  
H&E X 100

Plate 128 Section of heart from a diabetic rat treated with the combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide showing normal architecture on Day 45.  
H&E X 100
V. DISCUSSION

Diabetes mellitus is a chronic, systemic group of metabolic disorders affecting a large sector of population in the world. It is a disorder of carbohydrate, fat and protein metabolism that results from absolute or relative deficiency of insulin secretion or insulin resistance which constitute type 1 (Insulin dependent Diabetes mellitus) and type 2 (Non insulin dependent diabetes mellitus) diabetes respectively (Chandra et al., 2004; Thevenod et al., 2008; Singh et al., 2010 and Patel et al., 2012). Type 1 occurs mainly due to destruction of pancreatic beta cell islets through autoimmune mediated mechanism resulting in absolute insulin deficiency. Type 2 DM occurs due to insulin resistance or abnormal insulin secretion. Diabetes is characterized by increased thirst, increased urinary output, hyperglycaemia, ketonemia and ketonuria mainly due to the abnormalities in carbohydrate, protein and fat metabolism. The rapid increasing trend in occurrence of diabetes in human population is mainly attributed to the sedentary life style, urbanization and ageing.

The high cost of modern treatment of diabetes stresses upon an urgent need for the development of alternate strategies for the prevention and treatment of diabetes. Currently, the focus is on using traditional methods of treatment and prevention procedures by use of herbal preparations. Herbs for diabetes treatment are not new. Since ancient times, plants and plant extracts are used to combat diabetes. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, approximately 150 species of plants are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and
is called as botanical garden of the world. Although hundreds of plants have been identified to possess antidiabetic effect, not many have been scientifically tested to prove their efficacy and safety. In this regard the current study was carried out to evaluate the efficacy of two plant extracts *Trigonella foenum graecum* and *Coccinia indica* individually and in combination in comparison with an oral antidiabetic drug glibenclamide and in addition to know the performance and synergistic effects of these plants in combination with glibenclamide individually and in combination in the treatment of experimentally induced diabetes mellitus in rats as experimental model.

In the present study, the hypoglycaemic effect of *Trigonella foenum graecum* extract and *Coccinia indica* in diabetic rat models, was evaluated in comparision with glibenclamide, a reference antidiabetic drug. The antidiabetic effect of *Trigonella foenum graecum* and *Coccinia indica* was confirmed with the help of phytochemistry, serum biochemical analysis, histopathology, immunohistochemistry, special staining and flow cytometry of various treatment groups and the results of the study are discussed as here under.

### 5.1 Phytochemical analysis

Phytochemical analysis of the *Trigonella foenum graecum* and *Coccinia indica* was carried out using High Performance Thin Layer Chromatography (HPTLC) Technique in the present study before conduct of the experiment.

Medicinal plants are extensively used for management of health since a long time and are becoming popular not only in village folk but also in urban people. The medicinal value of plants mainly depends upon their active ingredients or the bioactive
substances such as alkaloids, flavanoid, saponins, glycosides and other related active
metabolites which are extensively used in the drug and pharmaceutical industry. Thus the
preliminary phytochemical tests are helpful in finding chemical constituents of a plant
material to which the biological effect obtained could be correlated effectively (Chauhan
et al., 2011 and Kalaiselvi et al., 2012).

In the present study, the alcoholic extract of both *Trigonella foenum graecum* and
*Coccinia indica* were found positive for alkoloid, flavonoid, bitter principle, coumarin,
anthracene, saponin and glycoside derivatives.

Several earlier workers have subjected *Trigonella foenum graecum* for their
phytochemical analysis (Mowla et al., 2009; Ahirwar et al., 2010; Yadav et al., 2010;
Dande et al., 2012; Sumayya et al., 2012; and Sheikh et al., 2012) and have observed
presence of alkaloid, saponin, flavonoids, bitter principles, coumarins, anthracence
derivatives and glycosides similar to the results of the present study. Ahirwar et al.
(2010) while subjecting petroleum extract, ethalonolic extract and aqueous extract for
phytochemical analysis obtained steroids in trigonella seeds only in petroleum extract and
absent in aqueous and alcoholic extracts. Dande et al. (2012) on phytochemical analysis
observed that the steroidal saponins were in large quantity in trigonella seed extract.
Mowla et al. (2009) subjected crude ethanol extract of *T. foenum graecum* seeds for
phytochemical analysis to check the presence of alkaloid, steroid, flavonoid,
carbohydrate, glycoside and glucosides in it and observed the presence of alkaloid,
steroid and carbohydrate but no flavonoid, glycoside and glucosides in the crude seed
extract. Yadav et al. (2010) showed the presence of alkaloid, flavonoids, amino acid,
tannins, protein, starch, mucilage and saponins in the methonolic and aqueous extracts of Trigonella.

Amino acids like isoleucine, 4-hydroxyisoleucine, histidine, leucine, lysine, L-tryptophan, arginine; saponins like graecunins, fenugrin B, fenugreekine, trigofoenosides A-G; Steroidal sapinogens like yamogenin, diosgenin, smilagenin, sarsasapogenin, tigogenin, neotigogenin, gitogenin, neogitogenin, yuccagenin, saponaretin; Fibers like gum, neutral detergent fiber and others like coumarin, lipids, vitamins, minerals, 28 per cent mucilage; 22 per cent proteins; 5 per cent of a stronger-swelling, bitter fixed oil components were reported by (Yadav et al., 2010).

Sheikh et al. (2013) also observed presence of glucosides, phenol, flavonols, amino acid, alkaloides, steroids, tannin, polysaccharide, pectin and hemicelluloses, fats volatile oil in the ethanolic extract of fenugreek. Sumayya et al. (2012) indicated that fenugreek (Trigonella foenum graecum) helps in balancing cholesterol, lowering sugar level, curing skin inflammation (wounds, rashes, boils), treating arthritis, asthma, sore throat, due to the phytoconstituents such as flavonoids, alkaloids, terpenoids, steroids, saponins, anthocyanin, tannin etc.

Glucose-lowering and antidiabetic effects of fenugreek have been attributed to the galactomannan rich soluble fiber fraction of fenugreek. Insulinotrophic and antidiabetic properties also have been associated with the amino acid 4-hydroxyisoleucine that occurs in fenugreek at a concentration of about 0.55 per cent. In vitro studies have indicated that this amino acid causes direct pancreatic β-cell stimulation. Delayed gastric emptying and inhibition of glucose transport also have been postulated as possible mechanisms. A
study of alloxan-induced diabetes in mice has shown that the hypoglycemic activity of dialysed fenugreek seed extract was comparable to that of insulin. Fenugreek seed extract also has been shown to improve intraperitoneal glucose tolerance in normal mice.

Flavonoids are the natural substances with phenolic structures present in fruits, vegetables, grains, bark, roots, flowers, tea, and wine. Flavonoids have been reported to have antiatherosclerotic effects, anti-inflammatory effects, anticancerous effects, antithrombogenic effect, antiviral effect and anti osteoporotic effects. The main mechanism by which the flavonoids function is through their antioxidant activity which combines with free radicals and make then unavailable for their action.

The alcoholic leaf extract of *Coccinia indica* was reported (Tamilselvan et al., 2011) to possess tannins, saponins, alkaloids, flavonoids, carbohydrates, triterpenoids, glycosides etc on phytochemical analysis. Similar observations have also been reported by Deokate and Khadabadi, 2011; Sivaraj et al., 2011 and Kumar et al., 2012. They have reported varying effects of the extract and attributed to antioxidant activity and alkaloids to be responsible for antidiabetic property (Yadav et al., 2010 and Deokate and Khadabadi, 2011).

**5.2 Induction of diabetes**

Induction of experimental diabetes is indeed the first step in evaluation of antidiabetic effect of any agent claimed to possess hypoglycaemic effect. There are several methods of induction of experimental diabetes in laboratory animal models such as surgical removal of pancreas, by chemicals like alloxan or streptozotocin and by injection of the anterior hypophysis extract (Akbarzadeh et al., 2007). However,
induction of diabetes by chemicals which selectively destroy pancreatic beta cells is simple and more convenient to use (Szkudelski 2001).

Streptozotocin has been extensively used to induce diabetes for various diabetes studies in laboratory animals by many workers (Ganda et al., 1976; Weir et al., 1981; Shabib et al., 1993; Li et al., 2000; Babu et al. 2004; Akbrazadeh et al., 2007; Dhanush, 2009; Pragathi, 2011; Mudasir, 2011; Nasreen, 2012 and Mallikarjuna et al., 2013). STZ induces dose-dependent hyperglycaemia when administered either intra venously or intra peritonially (Babu et al., 2004).

In the present study diabetes was induced in rats (Group II to IX) by administration of streptozotocin at the dose rate of 45 mg/kg intra peritonially. All the rats from groups-II to IX became diabetic and showed hyperglycaemia with increase in mean serum glucose levels ranging from 418.58±40.52 mg/dl to 499.40±15.62 mg/dl by 72 hrs after STZ administration.

Streptozotocin (STZ) is structurally a N-nitrosourea derivative of D-glucosamine with molecular weight of 265 and empirical formula of C14 H27 N5 O12. It is a naturally occurring, cytotoxic agent produced by the bacterium Streptomyces achromogenes that is particularly toxic to the pancreatic insulin producing beta cells in mammals (LeDoux et al., 1986; Saini et al., 1996). It is a broad spectrum antibiotic and alkylating genotoxic agent which possesses antibacterial, tumoricidal, carcinogenic and diabetogenic properties.
The selective destruction of insulin producing beta cells by STZ has been attributed mainly to induction of diabetes (Karunanayake, 1975; Tjalve, 1983; Li et al., 2001; Babu and Prince, 2004; Selvan et al., 2008; Dhanush, 2009; Mudasir, 2011; Pragathi, 2011 and Mallikarjuna et al., 2013). The diabetogenic action of STZ is the direct result of irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin (Zafar et al., 2009).

Beta-cell toxicity and diabetogenic properties of STZ are mediated through diverse mechanisms including targeted uptake of STZ in beta cells by GLUT2 receptors (Hosokawa et al., 2001) and increased oxidative stress due to NO release and ROS production (Friederich et al., 2009). Streptozotocin has a glucose moiety in its chemical structure which helps in its entry into pancreatic beta cells via low affinity GLUT2 glucose transporter in the plasma membrane (Tjalve, 1983 and Elsner et al., 2000). A reduced expression of GLUT2 has been found to reduce diabetogenic action of STZ (Thulesen et al., 1997 and Schmidt et al., 2002). Thus insulin producing cells that do not express GLUT2 are resistant to STZ. STZ induced damage can also be seen in kidney and liver as these organs also express GLUT2 (Rerup, 1970).

STZ induces beta-cell dysfunction and apoptosis at lower doses while causes beta-cell necrosis at higher doses. Insulin-secretory cells also develop resistance on repeated exposures to STZ through a wide spectrum of toxin tolerance mechanisms (Elsner et al., 2000). The cytotoxic effect of STZ has been attributed to its entry into beta cells through glucose transporter and alkylation of DNA leading to DNA damage. Such damage has been reported to induce activation of poly ADP ribosylation which leads to
depletion of cellular NAD, ATP and formation of free radicals (hydrogen peroxide, super oxide and hydroxyl radicals) and liberation of nitric oxide which induce further DNA damage ultimately causing loss of beta cells (Szkudelski, 2001 and Mir et al., 2008).

Induction of diabetes with hyperglycaemia by STZ has been attributed to increase in mitochondrial oxidative stress in ischemia and hypoxia, depletion of GSH and mitochondrial DNA, increased production of ROS and NO have all been linked to increased oxidative arbonylation of mitochondrial proteins. (Haider and Anne, 2012)

Streptozotocin (STZ) as an antibiotic is also often used in the treatment of different types of cancers (Weiss et al., 1982 and Dolan et al., 1997). Even therapeutic doses (up to 15 mM) of STZ induce pancreatic beta-cell death by inducing apoptosis (Harel et al., 2002).

5.2.1 Normal control group (Group-I)

The animals belonging to control group remained healthy throughout the experimental period. All the values of various parameters analysed were within the normal range and indicated their healthy status.

5.2.2 Diabetic control group (Group-II)

The rats belonging to diabetic control group (Group-II) remained hyperglycaemic throughout the study period and revealed various biochemical and pathological changes indicative of diabetes.
In the diabetic control animals a significant decrease in the mean body weight was observed. The decrease was statistically significant \((P \leq 0.001)\) from day 3 of post STZ injection. A number of earlier workers also have encountered weight loss in diabetic animals after induction of diabetes with STZ (Neera Singh et al., 1989; Ananthan et al., 2003; Mahdi et al., 2003; Chhanda et al., 2006; Nafisa et al., 2007; Soleimani et al., 2007; Akbrazadeh et al., 2007; Dhanush, 2009; Pragathi, 2011; Oyedemi et al., 2011; Nasreen, 2012 and Mallikarjun et al., 2013).

The weight loss in diabetic rats could be attributed to hypoinsulinism that occurs in diabetes; decreased protein synthesis in the absence of insulin which is partly due to diminished transport of amino acids to the muscle (Warkins, 2003); loss of fluids leading to dehydration through glycosuric polyuria and altered uptake of glucose and glycogenesis by the target cells (Rubin and Strayer 2008). Insulin, being an anabolic hormone which is functionally antidiabetic in nature, causes increased catabolism of carbohydrates, proteins and fat in its hypoinsulinaemic state (Rubin and Strayer 2008) contributing for weight loss.

In addition gastrointestinal neuropathy associated with disordered gastrointestinal motor and sensory function which frequently occurs in diabetes, may lead to reduction in the food intake and coupled with degradation of structural proteins and muscle wasting with consequent weight loss (Schmidt, 2002; Rotimi et al., 2011 and Oyedemi et al., 2011).

The mean serum glucose levels in the diabetic control rats were observed to be drastically increased compared to normal control group rats from Day 3 till the end of the
experiment indicating hyperglycaemia. Diabetes, in general is characterised by hyperglycaemia either due to decreased insulin level or resistance to insulin at cellular level or both. Insulin, a hormone produced by the beta cells of pancreas in general enables the cells to absorb glucose from the blood and also helps in the utilization of the glucose in the cells by glycolysis, tricarboxylic acid cycle, hexose monophosphate shunt, and glycogenesis processes. Deficiency or resistance to insulin leads to decreased glucose transport across the cells and its utilization, decreased glycolysis, increased glycogenolysis, elevated hepatic glucose production and increased breakdown of fat contributing for hyperglycaemic state in diabetes (Karunanayake et al., 1974; Ganda et al., 1976; Shibib et al., 1993; Ali et al., 1993; Sarkar et al., 1996; Li et al., 2000; Rao et al., 2001; Ahmed et al., 2001; Mahdi et al., 2003; Akbarzadeh et al., 2007; Dhanush, 2009; Pragathi, 2011 Mudasir 2011 and Mallikarjuna et al., 2013).

Serum cholesterol and triglyceride levels in the diabetic control group were found to be significantly higher compared to normal control group in the present study throughout the experimental period, indicating hyperlipidemia as also reported by Platel et al., 1993; Platel and Srinivasa 1995; Ahmed et al., 2001; Shigematsu et al., 2001; Mahdi et al., 2003; Chaturvedi et al., 2004; Nafisa et al., 2007 and Mudasir, 2011. Hyperlipidemia is regarded as a consistent feature in experimental STZ and alloxan induced diabetes in rats which is characterized by hypercholesterolemia and hypertriglycerideridemia.

In diabetes insulin deficiency increases excessive breakdown of adipose store by stimulation of lipoprotein lipase leading to increased mobilisation of fatty acids for
energy purpose and their excessive accumulation in liver on excess esterification to triglycerides and conversion to cholesterol (Ahmed et al., 2001; Fernandes et al., 2007; Mudasir, 2011 and Mallikarjuna et al., 2013). In addition deficiency of insulin also causes diminished levels of LDL receptors leading to increased LDL cholesterol values in diabetes (Ahmed et al., 2001 and Nafisa et al., 2007) contributing for hyperlipidemia.

In the present study a progressive increase in the mean serum values of ALT and AST mean values was observed in diabetic rats which were significantly higher compared to normal control animals throughout the study period, a finding also reported by many workers (Karunanayake et al., 1975; Shabib et al., 1993; Szkudelski, 2001; Asli et al., 2007; Dandagi et al., 2008; Chaudhari et al., 2009; Dhanush, 2009; Pragathi, 2011; Nasreen, 2012 and Mallikarjun et al., 2013).

ALT and AST are the leakage enzymes present in the cytosol and organelles of hepatocytes and released into the circulation when injury to organelles such as mitochondria occurs. An elevation in serum levels of ALT and AST indicate active hepatic damage and loss of functional integrity of the cell membrane (Rajesh and Latha, 2004 and Muhammad et al., 2008).

Liver damage in experimentally induced diabetes with STZ is due to the toxic effect of STZ itself. Hepatocytes like beta cells of pancreas also express GLUT2 receptors through which streptozotocin enters the cells and induce both plasma membrane and organellar membrane damage especially that of rough endoplasmic reticulum and mitochondria by inducing oxidative stress with NO release and ROS production (Laguens et al., 1980). Experimental studies have shown that subtle
membrane changes are sufficient to allow passage of intracellular enzymes to the extracellular space (Garella, 1997). Usually a very high concentration gradient exists between the hepatocytes and the sinusoidal space for enzymes. Cell damage increases permeability causing cytosolic isoenzymes to spill into the sinusoids and from there into the peripheral blood. In the present study, histopathological observations clearly substantiated the hepatic damage induced by streptozotocin. Similar findings have also been reported by many workers (Hearse, 1979; Virdi et al., 2003; Zafar et al., 2009; Dhanush, 2009; Abdollahi et al., 2010 and Mallikarjuna et al., 2013).

The serum insulin values in the present study were estimated by RIA and the mean insulin values were significantly (P ≤ 0.001) lowered in diabetic control groups compared to normal control animals, a finding also reported by many earlier workers in STZ induced diabetes in rats (Sarkar et al., 1996; Ahmed et al., 2001; Rao et al., 2001; Ananthan et al., 2003; Nafisa et al., 2007; Dhanush, 2009; Pragathi, 2011; Nasreen, 2012 and Mallikarjun et al., 2013).

Insulin is a major hormone secreted by β-cells of islets of Langerhans of pancreas. Insulin is released in response to nutrient inflow from the gut and to gastrointestinal secretagogues. Streptozotocin, a diabetogenic substance induces diabetes in animals by its cytotoxic effect specific for β-cells of islets, accounting for hypoinsulinaemia which was also observed in the present study and substantially supported by microscopical and immunohistochemical evidence of degeneration and destruction of beta cells of islets and scanty immunoreactivity for insulin (Sarkar et al., 1996; Ahmed et al., 2000; Rao et al., 2001; Hassan et al., 2010; Mudasir, 2011 and Mallikarjuna et al., 2013).
Altered activities of respiratory enzymes and aerobic O2 utilization are critical processes for ROS production under physiological conditions. Production of ROS is markedly enhanced in many pathological conditions in which the respiratory chain is impaired. High blood glucose level induces overproduction of ROS by mitochondrial electron transport chain during respiration. Increasing evidences suggest that there is a close link between hyperglycemia, oxidative stress, and diabetic complications (Raza et al., 2012).

Oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus as evidenced in both experimental and clinical studies. Free radicals are formed disproportionately in diabetes by glucose oxidation, non enzymatic glycation of proteins, and by subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus. The antioxidants play an important role in protection against damage caused by reactive oxygen species (ROS). The endogenous antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), Glutathione-s-transferase (GST) and glutathione peroxidise (GPx) that are responsible for the detoxification of deleterious oxygen radicals.

Superoxide dismutase (SOD) has been postulated as one of the most important enzymes in the enzymatic antioxidant defence system which catalyses the dismutation of superoxide radicals to produce H2O2 and molecular oxygen. Catalase (CAT) is a
hemeprotein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals. Glutathione peroxidase (GPx), an enzyme with selenium and Glutathione-S-transferase (GST) work together with glutathione in the decomposition of H$_2$O$_2$ or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione.

The oxidative stress in diabetes is indicated by subsequent changes in oxidative stress biomarkers, such as superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, vitamins which drastically reduce and lipid peroxidation, nitrite concentration, non enzymatic glycosylated proteins which enormously increase along with hyperglycemia (Baynes and Thorpe 1999; Maritim et al., 2003 and Traverso et al., 2004).

In the current study the SOD, CAT and GPx activities were significantly reduced in the liver of diabetic rats from 15th to 90th day. Similar type of findings has also been reported in alloxan and STZ induced diabetic rat models (Prem kumar singh et al., 2012 and Pragathi, 2011). Compromised antioxidant system, denoted by increased lipid peroxidation and decreased levels of both non-enzymatic and enzymatic antioxidants, is a feature of diabetes and such changes have been noticed in alloxan or STZ induced diabetes (Bonnefont-Rousselot et al., 2000 and Maritim et al., 2003). Persistent hyperglycemia during diabetes leads to production of free radicals or impaired antioxidant defenses via several mechanisms (Saxena et al., 1993; Maritim et al., 2003 and Valko et al., 2007). Decline in the activities of antioxidant enzymes in the liver of
diabetic animals indicate the extent of free radical induced damage due to STZ induced hyperglycemia (Resmi et al., 2006 and Kaleem et al., 2006).

The mean haemoglobin percentage in diabetic control rats was observed to decline drastically in the present study to 5.80±0.40 on 90th day of experiment from 9.76±0.39 of 3rd day. In hyperglycaemia advanced glycation products (AGEs) are formed due to binding of glucose with various proteins such as haemoglobin, albumin, collagen, LDL or crystalline proteins to form labile Schiff bases which later undergo further modification to form Amadori products (Singh et al., 2008) and the rate of glycation is proportional to the blood glucose concentration. Glycosylation of haemoglobin leading to its reduction in blood has also been reported by Pari et al., 2003; Dhanush, 2009; Pragathi, 2011 and Mallikarjuna et al., 2013.

In the present study analysis of haematological parameters in STZ induced diabetic rats revealed a reduction in the total erythrocyte count indicating an anaemic situation, decreased total leucocyte count and reduced total platelet count. Reactive oxygen species have been implicated in the mechanism of red cells damage (Rao et al., 2001 and Mohammed et al., 2009). In diabetes mellitus increased red blood cell aggregation, decreased deformability, decreased life-span and membrane ATPases activity occur which are thought to contribute for clinical abnormalities of microcirculation (Thornalley, 1988 and Stanescu et al., 2002).

In diabetes mellitus production of excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death. The anaemic condition that occurs in diabetes mellitus has been reported to be due to the
increased non-enzymatic glycosylation of red blood cell membrane proteins and increased production of lipid peroxides, which consequently have toxic effects on cells (Halliwell and Gutteridge, 1985; Tsai et al., 1994 and Kawamura et al., 1994), that lead to haemolysis of red blood cell (Arun and Ramesh, 2002; Oyedemi et al., 2011 and Mohammed et al., 2012). Thus anemia could be attributed to destruction of RBCs and reduced rate of its release from the bone marrow to blood (Helal, 2000 and Xie et al., 2003). However, Stanescu et al. (2002) showed through in vitro studies that blood cells when incubated with high concentration of glucose show an increase in passive transmembrane lipid movement and express phosphatidylserine on plasma membrane and indicted that this may increase procoagulant activity and contribute significantly to vascular occlusion (Thornalley, 1988). Also stated that externalized erythrocyte phosphatidylserine may trigger the removal of erythrocytes by macrophages, by the similar process involved in the programmed cell death.

Streptozotocin is a well known chemical reported to suppress the immune system by destroying white blood cells and certain organs in the body (Oyedemi et al., 2011). The reduction in total TRC count could be attributed to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection, thus may have consequential effects on the immune system and phagocytic activity of the animals (Afolayan and Yakubu, 2009; Oyedemi et al., 2011; Saba et al., 2010 and Mohammed et al., 2012).

In diabetes there is an increased platelet activation and subsequent aggregation favouring thrombosis (Colwell and Nesto, 2003 and Brand et al., 1998) and also
depression of bone marrow stem cells which could be contributory for a reduced platelet count observed in the present study. The decrease in circulating platelet number may also mean an increase in aggregated platelet typical of atherosclerotic disorders of diabetes mellitus.

The clinical signs in STZ induced diabetic group in the present study comprised polyuria, polydipsia, polyphagia, restlessness and weight loss. Similar findings have also been reported by many workers (Pillion et al., 1988; Warkins, 2003; Babu and Prince, 2004; Akbarzadeh et al., 2007; Dhanush, 2009; Pragathi, 2011; Mudasir 2011 and Mallikarjuna et al., 2013). The clinical manifestations in diabetes are a result of metabolic derangement associated with hyperglycaemia due to hypoinsulinism or increased insulin resistance. When hyperglycaemia exceeds the renal threshold for reabsorption, glycosuria occurs which further induces an osmotic diuresis leading to polyuria in diabetes along with a profound loss of water and electrolytes. Increased level of glucose in blood depletes intracellular water and triggers osmoreceptor of the thirst centres in the brain leading to polydipsia. In addition, the catabolism of protein and fats due to insulin deficiency induces a negative energy balance which leads to polyphagia. Despite the increased appetite, catabolic effects persist resulting in weight loss and muscle weakness (Kumar et al., 2008) a feature also observed in the present study.

In the present study, the diabetic rats appeared emaciated which could be attributed to the drastic weight loss that occurred in response to hypoinsulinism with increased catabolism of carbohydrate, proteins and fat. Grossly pancreas appeared slightly congested and showed progressive decrease in size from day 15 onwards which
appeared as a thin gelatinous strip by 45th day and persisted up to day 90. The selective
cytotoxic effect of streptozotocin on β-cells of islets of Langerhans and free radical injury
to exocrine portion could be attributed to the progressive decrease in the size of pancreas
in the present study (Szkudelski, 2001; Mir et al., 2008 and Mudasir, 2011)

In the diabetic rats of group II, the organ liver grossly appeared pale, soft and
friable which could be directly attributed to the STZ induced liver damage as hepatocytes
also express GLUT2 receptors (Laguens et al., 1980; Rajesh and Latha, 2004;
Muhammad et al., 2008; Pragathi, 2011; Nasreen, 2012 and Mallikarjuna et al., 2013).

The other organs such as kidney, lung, intestine, stomach, brain and heart did not
reveal any gross lesions in the present study contrary to the observation of several
workers who observed damage also to kidney in STZ induced diabetes (Morfologia et al.,
2009). The adrenal glands appeared enlarged in the diabetic rats compared to control rats.

The diabetic control rats in the present study microscopically revealed affection of
both exocrine and endocrine components of pancreas. The exocrine portion showed loss
of normal lobular architecture characterized by reduction in the lobular size and wide
separation of lobules with presence of edema fluid, haemorrhage and mild infiltration of
mononuclear cells. The blood vessels were severely congested. The acinar epithelial cells
were highly vacuolated, degenerated and necrotic with loss of zymogen granules.

The cellular damage in the exocrine portion in the present study could be due to
the oxidative stress injury secondary to induced diabetes with STZ. The increased release
of ROS and NO and decreased antioxidant levels during the process of hyperglycaemia
may be accounted for the exocrine injury. In addition the released pancreatic enzymes from the acinar cells following degeneration and necrosis could be contributory for exocrine damage.

In the endocrine component, there was reduction in the number and size of the islets per lobule which were irregular in shape with loss of demarcation between islets and the surrounding exocrine portion. The islets were hypo cellular with altered number and distribution of α and β-cells. The β-cells were swollen and highly vacuolated with fusiform shaped condensed nucleus. A variable number of beta cells per islet were also necrotic. The cytoplasmic granularity of β-cells was highly reduced. There was also presence of apoptotic cells in the islets. The α-cells at the periphery were unaffected and were more in number comparatively. There was persistence of exocrine and endocrine damage till 45th day of experiment however with slight improvement in endocrine portion with alpha cell hyper cellularity. An appreciable improvement in the appearance of exocrine component and size and number of islets was observed by 90th day of the study during which the islets were hyper cellular however with very few granulated beta cells which was also shown immunohistochemically with only occasional cells with insulin immunoreactivity in the present study.

Injury to the endocrine component of pancreas in the present study could be attributed to STZ which causes direct irreversible damage to β-cells of pancreatic islets of Langerhans, resulting in degranulation and loss of insulin secretion, as also reported by Li et al., 2000; Babu and Prince, 2004; Jelodar et al., 2005; Mir et al., 2008; Selvan et al., 2008; Dhanush, 2009; Atangwho et al., 2010 and Mudasir, 2011.
The decrease in the number of islets and cellularity in the islets could be attributed to selective destruction of beta cells by STZ when given as a single adequate dose which produces long lasting hyperglycaemia and insulin deficiency. The STZ enters the beta cells through a glucose transporter (GLUT-2) and causes alkylation of DNA. Further, STZ also liberates toxic amounts of nitric oxide that inhibits aconitase activity, cause DNA damage and destroys β-cells by necrosis (Szkudelski, 2001; Bennett and Peg 1981 and Mir et al., 2008). The change in the shape of the β-cells could be due to partial injury to the cells by STZ (Mir et al., 2008) as cells with less expression of GLUT2 are only partially injured and also as shown by Raza et al. (2012) low doses of STZ cause apoptosis rather than necrosis of beta cells.

Beta cell regeneration under stressed conditions occurs due to proliferation of surviving beta cells, but the factors that trigger and control this response are unclear. In addition chronic changes in beta cell glucose metabolism, also serve as the positive regulator of compensatory beta cell proliferation as glucose induces beta cell replication via metabolism by glucokinase, the first step of glycolysis, followed by closure of KATP channels and membrane depolarization (Porat et al., 2011).

Liver parenchyma in the present study in diabetic control rats showed centrilobular vacuolar degeneration along with focal areas of necrosis, loss of normal architecture and infiltration of inflammatory cells. Streptozotocin, in addition to pancreatic β-cells also damages the hepatocytes leading vacuolar fatty change and necrosis as GLUT2 receptors are also exhibited by the hepatocytes (Mir et al., 2008; Muhammad et al., 2008; Pragathi, 2011; Nasreen, 2012 and Mallikarjuna et al., 2013).
In the present study, kidneys did not show any consistent microscopical changes throughout the experimental period except for mild swelling and vacuolar change in the tubules in a few animals. Bennett et al. (1981) also, in a morphological study of kidneys in STZ induced diabetic animals did not encounter any alteration and reported that STZ does not possess any significant nephrotoxic potential. However, in contrary several workers have encountered tubular changes associated with increased urea and creatinine levels in STZ induced diabetes (Mir et al., 2008 and Morfologia et al., 2009).

Microscopically in the present study, heart did not reveal any significant appreciable changes except with mild vacuolar changes for in some of the fibres. These findings could be attributed to the hyperglycaemia observed in STZ induced with formation of free radicals (Oberley, 1998 and Mir et al., 2008).

5.2.3 Glibenclamide treatment group (Group-III)

The oral antidiabetic agents have been reported to exert their effects by various mechanisms such as stimulation of beta cells to produce more insulin (sulphonylureas and meglitinides); increase the sensitivity of muscles and other tissues to insulin (thiazolidinediones); decrease gluconeogensis by the liver (biguanides) and delay the absorption of carbohydrates from GIT (alpha glucosidase inhibitors). All these agents have their own drawbacks ranging from development of resistance to lack of responsiveness. Sulphonylureas lose effectiveness in 44 per cent of patients with in six years, may worsen heart disease, lower the glucose below the normal range and increase the body weight gain.
Glibenclamide is a popular second generation sulphonylurea drug used as a cardinal drug in the treatment of type-II diabetes mellitus. Glibenclamide improves glucose control by acting both on insulin secretion and insulin action. The hypoglycaemic effect of glibenclamide is mainly due to stimulation of insulin release from functioning beta cells and sensitization of the peripheral tissues to insulin (Sweetman, 2002). The extra pancreatic glucose reducing effects include inhibition of gluconeogenesis, ketogenesis, stimulation of glucose transport, glycogen synthase activity and glycerol-3-P-acyltransferase activity. In addition it also has antioxidant and hypolipidemic actions (Rabbani et al., 2010 and Kakadiya et al., 2010).

Glibenclamide induces its antidiabetic effect by inhibiting the sulfonylurea receptor 1 (SUR1), the regulatory subunit of the ATP-sensitive potassium channels (K$_{ATP}$) in pancreatic beta cells. The initiation of insulin release from the beta cells occurs when glibenclamide binds to the surface receptors of beta cell membrane inhibiting ATP-sensitive potassium channels and causing depolarization of cell membrane. Depolarization leads to opening of voltage-dependent potassium channels which enables extracellular calcium to enter the cell. Increased intracellular calcium concentration enhances the binding of calcium to the transport protein calmodulin which leads to microfilament contraction and release of insulin from insulin containing granules. Increased insulin causes subsequent reduction in serum glucose levels which improves beta cell sensitivity to glucose and potentiates insulin secretion (Luzi and Possa, 1997; Ling et al., 2006; Pragathi, 2011; Nasreen, 2012 and Mallikarjuna et al., 2013).
In the present study the group-III rats with glibenclamide treatment from 3rd day onwards revealed an improvement in their body weight which was comparable to that of control rats. The improvement could be attributed to the effect of glibenclamide in enhancing insulin secretion by beta cells of pancreas and also increasing sensitization of the peripheral tissues to insulin.

The serum glucose levels in the glibenclamide treatment group were significantly (P≤0.001) lowered compared to diabetic control rats in the present study. The improvement in glucose level in blood as indicated earlier could be attributed to the insulin secretion by the beta cells in response to glibenclamide treatment. In addition extra pancreatic glucose reducing effects of glibenclamide like inhibition of gluconeogenesis, stimulation of glucose transport, glycogen synthase activity could also be contributory for improvement of blood glucose level (Luzi and Pozza, 1997; Ling et al., 2006; Pragathi, 2011; Nasreen, 2012; Haritha et al., 2013 and Mallikarjuna et al., 2013). Glibenclamide has also been reported to increase synthesis of GLUT2 protein which improves glycogen deposition in liver and reduce endogenous glucose production (Hribal et al., 2001). However, it was noticed in the present study that the serum glucose levels failed to reach the normal levels inspite of treatment with glibenclamide, as also observed by several workers (King and Loeken, 2004; Giacco and Brownlee, 2010 and Erejuwa et al., 2011). This could probably be due to failure of complete recovery of beta cells population in response to the glibenclamide treatment as evidenced microscopically and by immunohistochemistry for insulin in the present study. Inspite of reduced hyperglycaemia, oxidative stress has been reported to persist in glibenclamide treated
diabetic rats in which pancreas was still susceptible to ROS and NO (Erejuwa et al., 2011) which was also an observation in the present study.

In the present study both serum cholesterol and serum triglyceride levels were significantly (P≤0.001) reduced in the glibenclamide treated animals compared to diabetic control rats. This clearly indicated that the treatment with glibenclamide along with its hypoglycaemic effect also improved hyperlipidaemia in diabetes (Virdi et al., 2003; Fernandes et al., 2007; Mudasir, 2011 and Mallikarjuna et al., 2013). Skrapari et al. (2001) who observed improvement in triglyceride levels in type-2 diabetes with glibenclamide treatment reported that alleviation of hypertriglyceridemia was due to an acute reduction in triglyceride of intestinal origin and not with the VLD lipoprotein sub fraction levels. However, Juhavekkilainen et al. (2002) failed to notice any effect of glibenclamide on hyperlipemia in diabetic patients.

In the present study the ALT and AST values in glibenclamide treatment group showed an improvement in the values in comparison with that of diabetic control group. However, it was observed that the values were significantly higher compared to normal control group and clearly indicated that glibenclamide treatment did not reverse completely the liver damage caused by STZ in the present study. This was substantially supported by the persistence of mild degree of hepatic vascular changes till the end of the study, histopathologically. Erejuwa et al. (2011) observed persistence of oxidative stress in glibenclamide treated diabetic animals though hypoglycaemic effect was observed which was also indicated by less antioxidant activity in the present study. This clearly
indicated that there was a low degree sustained free radical injury to the liver elevating the liver enzyme levels.

Serum insulin levels in the glibenclamide treatment group appeared significantly improved in comparison with diabetic control group from 15\textsuperscript{th} day post treatment till the end of the study which could be attributed to the glibenclamide effect on beta cells as indicated earlier. The improvement in insulin level was also well substantiated by the improvement in the architecture of the islets and immunoreactivity to insulin and an increase in the number of insulin producing beta cells observed in the present study. However, the values failed to reach the normal range at any period of observation.

The mean values of SOD, CAT and GPx activities in the liver of glibenclamide treated rats were improved compared to diabetic rats. However, the values were lesser compared to the other groups treated with plant extracts individually or in combination as plants have been the rich source of several antioxidants. Similar observations were also recorded by many earlier workers (Elmali et al., 2005; Sathishsekar and Subramanian, 2005 and Upadhyay et al., 2010). Glibenclamide inspite of hypoglycaemic in nature cannot effectively alleviate the oxidative stress completely as also reported by Erejuwa et al. (2011). The level of antioxidants is indirectly proportional to free radical concentration. The sustained oxidative stress in induced diabetes in the present study could be responsible for the low concentration of antioxidants compared to other treatment groups. However the improvement observed in antioxidant level could be attributed to the hypoglycemic effect of glibenclamide by increasing the release of insulin
thereby reversing the diabetes induced changes and increase liver antioxidant enzymes (Elamli et al., 2005).

Similarly in glibenclamide treated group, there was a significant improvement in Hb concentration in comparison with the diabetic control group both on 30th and 45th day of the study. Improvement in the Hb concentration could be attributed to antihyperglycaemic effect of glibenclamide and improvement in insulin level, thus reducing non-enzymatic glycosylation of haemoglobin (Rubin and Strayer, 2008). Group – III rats also showed a significant improvement in the blood parameters such as TRC, TLC, and platelets when compared to diabetic control animals. This could be due to amelioration of diabetic symptoms by glibenclamide.

The intensity of various clinical symptoms decreased gradually with glibenclamide treatment. The reduction in the severity of clinical symptoms could be attributed to the effect of glibenclamide on improving basal glucose levels by increasing insulin secretion, insulin action and β-cell proliferation (Koltermann et al., 1984; Ling et al., 2006 and Rubin and Strayer, 2008).

Gross pathological changes observed in the glibenclamide treated rats reduced progressively from Day 15 to 90 post treatment. The results were in agreement with the findings of Ananthan et al. (2003) who attributed the improvement to glibenclamide effect.

In the present study, there was a progressive improvement in the architecture of pancreas. On 15th day of treatment the microscopical picture was similar to that of
diabetic control group. From 30\textsuperscript{th} day onwards an appreciable improvement in the shape, size and number of the islets was observed with formation of newly formed small sized islets in the midst of exocrine portion and adjacent to intra lobular pancreatic ducts. The islets were more compact with hyper cellularity comprising more of alpha cells by 45\textsuperscript{th} and 90\textsuperscript{th} day and beta cells with well formed cytoplasmic granularity in an increased number.

Additionally, progressive ductular hyperplasia extending into the exocrine portion as individual or small clusters of cells with presence of newly formed beta cells in small number in adjacent area was also observed. Though an improvement in the pancreatic architecture was observed there was persistence of STZ effect in a few cells which appeared highly swollen, vacuolated with condensed fusiform nucleus. The exocrine portion appeared normal by the end of the experiment.

Guiot \textit{et al.} (1994) and Wang \textit{et al.} (2008) attributed the improvement to increased proliferation as well as recruitment of subpopulation of $\beta$ cells and thereby increase in the $\beta$ cell mass upon treatment with glibenclamide. This could be possibly due to insulin induced regeneration of endocrine pancreas, resulting in improved histological appearance, size and number of islets (Adewole and Ojewole, 2007). In addition, as indicated by Paris \textit{et al.} (2004), the improved $\beta$-cell mass could be on an account of $\beta$-cell neogenesis from ductal epithelial cells. This was well substantiated by ductal hyperplasia and presence of newly formed small clusters of $\beta$-cells or individual cells adjacent to ductal hyperplastic tissue with insulin synthesis observed microscopically and histochemically in the present study.
Liver revealed progressive improvement in the architecture from 15\textsuperscript{th} day onwards in the glibenclamide treated rats. The results are in accordance with those of Luzi and Pozza, (1997). Studies using glibenclamide boluses have suggested that hepatocytes possess specific binding sites that may be relevant in mediating the action of the drug on the liver. Additional studies have shown that the drug has a positive action on glycogen deposition with direct action on the synthesis of GLUT-2 rather than GLUT-4 proteins and at the glycogen phosphorylase level. The effect of glibenclamide on the insulin levels and on the altered metabolism of various macromolecules may improve the liver microscopic architecture (Luzi and Pozza, 1997). Microscopically, liver revealed an improvement in the architecture though mild changes persisted till 90\textsuperscript{th} day of the treatment. On Day 15 liver lobules revealed cell swelling and granular to vacuolar degeneration with obliteration of sinusoidal space. By 30\textsuperscript{th} day there was an improvement in liver morphology with respect to vacuolar degeneration and cell swelling. On 45\textsuperscript{th} and 90\textsuperscript{th} day liver revealed mild cytoplasmic granularity.

Histopathologically, kidney, heart, lungs and intestines did not reveal any appreciable changes throughout the period of experimentation. Spleen revealed normal architecture however with varying number of megakaryocytes from 15\textsuperscript{th} day to 90\textsuperscript{th} day of the experiment. Adrenals also appeared normal except for mild vacuolations in zona fasciculata cells on 15\textsuperscript{th} day.

5.2.4 \textit{Trigonella foenum graecum} (Group IV) and \textit{Trigonella foenum graecum} with Glibenclamide treatment groups (Group-VII)

In the present study the efficacy of \textit{Trigonella foenum graecum} was evaluated in STZ induced diabetes in rats individually as well as in combination with the half dose of
glibenclamide as diabetic patients very commonly consume herb with antidiabetic effect along with antidiabetic drugs. The prime purpose of evaluation of combined effect was to know whether there is any synergistic or antagonistic effect between Trigonella and glibenclamide and also to check whether Trigonella could be used as an adjunct in diabetic therapy with oral antidiabetic agents. Hence the results of Group IV and VII with regard to biochemical, haematological, pathomorphological, immunohistochemical and immunomodulatory effects are discussed together.

Fenugreek (Trigonella foenum graecum) is an annual crop from the family Fabaceae, extensively cultivated as a food crop in India, the Mediterranean region, Yemen and North Africa. It is an important culinary and therapeutic plant in many cultures. As a spice, it is a component of many curry preparations and is often used to flavour food and stimulate appetite. Fenugreek seeds are used in India as a condiment and its green leaves are widely consumed in India as a rich source of calcium, iron, beta-carotene and other vitamins. It has been used for centuries in folk medicine to heal ailments ranging from indigestion to baldness (Petropoulos, 2002 and Chauhan et al., 2011).

The leaves of Trigonella foenum graecum are employed as an herbal medicine in many parts of the world for their cooling properties and its seeds for their carminative, tonic and aphrodisiac effects. Fenugreek seeds, which are described in the Greek and Latin Pharmacopoeias, are widely studied for their reputed antidiabetic, hypcholesterolaemic, antifertility and hypolipidemic effects. In addition, fenugreek has been reported to possess a curative gastric anti-ulcer action, anti-bacterial, anti-helminthic, anti cancerous and anti-nociceptive effects. Trigonella foenum graecum, has
health potential with the ability to lower blood glucose and cholesterol levels, and hence in the prevention and treatment of diabetes and coronary heart diseases. Fenugreek seeds are serving as natural antibacterial compounds (Lust, 1986 and Alkofahi et al., 1986).

Fenugreek consists of three important chemical constituents with medicinal value which are steroidal sapogenin, galactomannans and isoleucine. These constituents have placed fenugreek among the most commonly recognized "nutraceutical" or health food products. Trigonelline is an important alkaloid component of the seeds and the seeds also contain some aromatic constituents such as n-alkenes, sesquiterpenes and nonalactone. Fenugreek seeds are also rich in saponins, including diosgenin, gitogenin and tigogenin (Dawidar et al., 1973 and Gupta et al., 1999).

In the present study the mean body weights in *Trigonella foenum graecum* (Group IV) and *Trigonella foenum graecum* with half dose of glibenclamide (Group VII) treated rats significantly improved from 15\textsuperscript{th} day onwards in comparison with the mean weights of diabetic control rats (Group-II). The mean body weights of rats of these two groups were comparable with those of other groups (Group III, V, VI, VIII and IX) on 45\textsuperscript{th} and 90\textsuperscript{th} day post-treatment.

Similar finding was also observed by Siddiqui et al., 2006; Xue et al., 2007 and Rafiq et al., 2009 who reported that progressive increase in weight could be due to attenuation of the toxicity of STZ by fenugreek seeds particularly at high dose and also a better digestibility and utilization of nutrients in the diet because of their high fibre content. 4-hydroxy isoleucine of fenugreek which increases adipogenesis as shown both in *in vitro* and *in vivo* studies by Shah et al. (2009) and reduction in catabolic activity
such as glycogenolysis, lipolysis, and gluconeogenesis during treatment with trigonella also could be attributed to the increase in the mean body weight gain (Nirmala et al., 2009).

A progressive significant reduction in the mean blood glucose values was observed in diabetic rats treated with Trigonella alone as well as in combination with glibenclamide. The improvement in the glucose level was appreciably more in combination group and the values were higher compared to glibenclamide alone treated group (Group III). The beneficial effect of fenugreek could be attributed to several bioactive compounds present in it. Amino acid 4-hydroxyisoleucine (4-OH-Ile) of fenugreek has been shown to have insulinotropic activity and to stimulate insulin secretion (Broca et al., 2000; Hannan et al., 2007; Xue et al., 2007; Baquer et al., 2011 and Geetha et al., 2011). It was also shown to exert hypoglycaemic effect by stimulating glucose dependent insulin secretion from beta cells as well as by inhibiting the activities of alpha amylase and sucrase (Amin et al., 1987 and Sauvaire et al., 1998). Glucose transport in muscles occurs mainly through glucose transporter-4 (GLUT-4) and in diabetic state, because of deficiency of insulin, GLUT-4 translocation does not take place efficiently and remain inside, where they are not functional resulting in elevated blood glucose levels. Insulin stimulates cellular glucose uptake in muscle and adipose tissues by inducing the translocation of GLUT-4 from an intracellular pool to the plasma membrane. Trigonella has been shown to reverse the diabetes effect on the GLUT-4 transporter, thus improving the glucose concentration (Mohammad et al., 2006).
Fenugreek also acts by delaying glucose absorption due to its high soluble dietary fibre containing steroidal saponins and proteins (Valette et al., 1984; Petit et al., 1995; Basch et al., 2003) by enhancing its utilization by peripheral tissue (Raghuram et al., 1994) and by its antioxidant effect (Ravikumar and Anuradha, 1999 and Baquer et al., 2011). Saponin compounds like diosgenin, alkaloid trigonelline of Trigonella seeds are also known to inhibit intestinal glucose uptake (Al-Habori et al., 2001).

In a study on interaction of extract of Trigonella and glibenclamide in a STZ diabetic model by Lal et al. (2011) showed that the seed extract with high dose of glibenclamide was superior in eliciting better hypoglycaemic effect than with the low dose. They also observed that the combined effect was better than the glibenclamide alone treatment which was comparable to that of control rats which indicated a possible synergistic effect. They concluded that caution should be taken while supplementing antidiabetic herbal extracts with antidiabetic drugs, as the synergistic effect may cause severe hypoglycaemia leading to coma and death.

In the group VII rats with combined treatment, a better hypoglycaemic effect was observed compared to Trigonella alone treatment which indicated that Trigonella and glibenclamide half dose have a synergistic effect. However, the hypoglycaemic effect was not on par with that of the glibenclamide complete dose. The possible reason for a reduced hypoglycaemic effect in combination group compared to glibenclamide alone could be the low dose of glibenclamide used which could have failed to elicit the desired antidiabetic effect in the present study.
The treatment with Trigonella alone (Group IV) in diabetic rats reduced both cholesterol and triglyceride levels progressively and was significantly lesser compared to that of diabetic rats in the present study. In diabetes the increase in lipid profile may be a result of increased breakdown of lipids and mobilization of free fatty acids from the peripheral deposits. The controlled mobilization of serum triglycerides, cholesterol and phospholipids by *T. foenum graecum* in diabetic animals could be due to controlled tissue metabolism and improvement in the levels of insulin secretion and action.

Raju *et al.* (2001) showed that fenugreek stimulates lipogenesis and inhibit lipolysis in adipose tissue. The decrease in serum triglycerides, total cholesterol and low density lipoprotein cholesterol by fenugreek has been attributed to sapogenins which increase biliary cholesterol excretion in liver (Yadav *et al.*, 2005). The lipid lowering effect is also attributed to the oestrogenic constituent which indirectly increases the thyroid hormone T4 thereby improving the basal metabolism.

*Trigonella foenum graecum* has been shown to stimulate hepatic lipogenic enzymes similar to insulin. Insulin acts by increasing the phosphorylation of ATP-citrate lyase by c AMP dependent protein kinase and trigonella is reported to mimic insulin action and also inhibit Na+K+ATPase (Siddiqui *et al.*, 2006), thereby making more ATP available for phosphorylation (Yadav *et al.*, 2005). In insulin deficient subjects, non activation of this enzyme causes hypertriglyceridemia. The strong anti-hyperlipidemic effect of *T. foenum graecum* seed could also be through its control on hyperglycaemia, as this is a major determinant of total and very low density lipoprotein (LDL) and triglyceride concentration. Insulin inhibits adipose tissue hormone-sensitive lipase and,
therefore, reduces lipolysis and mobilization of peripheral depots causing low levels of serum lipids (Rajesh et al., 2010).

In the present study, the combined treatment with Trigonella and glibenclamide half dose revealed a synergistic effect in lowering cholesterol and triglyceride levels which were better than those of both Trigonella and glibenclamine alone treatment groups.

Trigonella alone and in combination with glibenclamide half dose treatment showed a significant reduction in serum AST and ALT levels in the diabetic rats in the present study. The improvement in AST and ALT levels in Trigonella alone group was better than glibenclamide alone group and the combination group (Group VII) revealed a synergistic effect in lowering the serum enzyme levels which was better than Trigonella alone and glibenclamide alone groups.

Liver is an important insulin dependent tissue that plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes. The hepatocellular damage elevates ALT and AST liver enzyme activity (Wehash et al., 2012). Fenugreek normalizes the liver enzyme levels either alone or in combination with glibenclamide.

The results of the present study clearly indicated that *T.foenum graecum* extract has hepatoprotective effect and thereby reduced serum levels of ALT and AST enzymes. The hepatoprotective effect of *T.foenum graecum* has been reported by many workers who have indicated that the hepatoprotection is due to the presence of flavonoids and ascorbic acid in the extract with antioxidant effect which prevent lipid peroxidation by
scavenging superoxide and hydroxyl radicals, thereby liver damage (Haeri et al., 2009 and Mishra et al., 2009).

It was also observed in the present study that the hepatoprotective effect of *T.foenum graecum* was comparatively better compared to any other treatments adopted in the present study.

The estimation of serum insulin level RIA in *T. foenum graecum* alone and in combination with glibenclamide half dose showed a significant increase in comparison with diabetic control group from 30th day onwards. In addition, it was also noticed that the improvement in insulin level did not significantly vary between Trigonella alone, Trigonella with glibenclamide and glibenclamide alone groups on 90th day of the study which indicated that the efficacy of Trigonella in diabetic rats in elevating insulin concentration was similar to that of glibenclamide treatment.

The results of the present study indicated that *T.foenum graecum* treatment had positive effect in improving the insulin level in diabetic animals, a finding well supported by those of (Hamden et al., 2009 and Rajesh et al., 2010) who attributed the improved insulin level to regeneration or repair of damaged beta cells. Attainment of normal architecture of islets of Langerhans in the present study, a finding observed microscopically also strengthens the positive effect of *T. foenum graecum* on insulin levels.

The 4-hydroxyisoleucine of fenugreek is reported to be insulinotropic and powerfully stimulate insulin secretion at all levels of cellular organization and increase
the number of insulin receptor sites to burn cellular glucose at high fibre diet (Broca et al., 2000; Sauvaire et al., 1998 and Mowla et al., 2009). They also suggested that the antidiabetic effect of fenugreek seeds in part is from direct stimulation of pancreatic beta cells, which was also evidenced in microscopical and immunohistochemistry for insulin in the present study. The mechanism of action of insulin secretion by beta cells by Trigonella is similar to that of sulphonylurea (glibenclamide) which acts by closure of $K^+$ ATP channels, membrane depolarization and stimulation of $Ca^{2+}$ influx, an initial key step in insulin secretion (Fuhlendorff et al., 1998).

In the present study antioxidant effect of *Trigonella foenum graecum* extract at 1gm/ body weight dosage was assessed by estimation of levels of antioxidant enzymes such as SOD, CAT and GPx. The mean values of SOD, CAT and GPx activities in the liver of *Trigonella foenum graecum* treated rats were significantly improved compared to diabetic control treated rats and were comparable to that of glibenclamide and other treatment groups. *Trigonella foenum graecum* has been reported to have antioxidant and free radical scavenging activity (Baquer et al. 2011). The antioxidant activity of *Trigonella foenum graecum* could be attributed to the phytochemicals compounds which include steroids, flavonoids and vitamins such as C and A (Grover and Yadav, 2005). In addition the novel amino acid 4-hydroxy isoleucine and trigonelline isolated from the seeds of *Trigonella foenum graecum* have been demonstrated to have antioxidant activity (Mawla et al., 2009 and Baquer et al., 2011).

The estimation of Haemoglobin in trigonella alone and trigonella with glibenclamide half dose revealed a progressive increase from 15th day onwards and was
significantly higher ($P \leq 0.001$) compared to diabetic control. In hyperglycaemia increased glycosylation of Hb is attributed for a drastic decrease in the circulating Hb (Shirwaikar et al., 2004 and Saravanan et al., 2009). *Trigonella foenum graecum* by their biological active ingradient, 4-hydroxyisoleucine which is insulinotropic and functions in glucose dose dependent manner is known to manage hyperglycaemic condition, thereby reduce further glycosylation of Hb and other proteins. In the present study there was no appreciable difference in the improvement of Hb concentration between Trigonella alone, glibenclamide alone and Trigonella and glibenclamine in combination as confirmed by the statistical insignificant difference.

The analysis of blood parameters revealed a significant improvement in total TRC count, TLC count and platelet count in Group IV and group VII in the present study compared to diabetic control. It was also observed that there was no statistical difference in the improvement in the blood cell values between the groups though numerically the combined group had better values of TRC and platelets. This could be attributed to the antioxidants of Trigonella which quench the ROS produced during induction of diabetes with STZ and thereby improving the hemopoietic tissue. The findings of the study support the earlier reports by Sumiyoshi (1993), that *T.foenum graecum* has immunomodulatory effect and stimulate immune functions by activating the natural killer cells, the function of T-lymphocytes and the level of interleukin-2 (Tang et al.,1997).

The significant improvement in the levels of circulating platelets in blood in Groups IV and VII could be attributed to inhibitory activity of certain constituents of *T. foenum graecum* on platelet aggregation as shown in previous studies in humans and
animals (Bordia, 1978; Srivastava, 1986; Legnani et al., 1993; Apitz-castro et al., 1988 and Lawson et al., 2005).

In both the groups (IV and VII) there was a gradual decrease in the severity of clinical signs such as polyuria, polydypsia, polyphagia and weight loss in diabetic animals treated with the alcoholic extract of T.foenum graecum alone as well as in combination with glibenclamide. Earlier workers also have reported the similar kind of improvement in condition in the diabetic animals treated with extract of the T.foenum graecum and the improvement in the condition could be attributed to the hypoglycaemic, hypolipidaemic, hepatoprotective and insulin secretagogue effects of the plant extract in diabetic subjects (Zafar et al., 2009).

Gross pathological changes observed in the Groups IV and VII reduced progressively with advancement in time on treatment with T.foenum graecum alone as well in combination with glibenclamide half dose.

Histopathologically, in pancreas there was a progressive reconstruction of normal architecture of islets from Day 15 to Day 90 post treatment in T.foenum graecum alone as well as in combination with glibenclamide half dose in the present study. In comparison, the improvement in the pancreatic architecture was better in combination group suggesting a synergistic effect between Trigonella and glibenclamide in modifying the tissue architecture.

During 15th day observation, there was persistence of STZ induced damage in the form of highly vacuolated to necrotic beta cells with affection of exocrine pancreatic
component also. However, in both the groups an improvement in endocrine and exocrine component was observed in the form of increase in the number of islets, increase in the number both beta and alpha cells in the islets and improvement in the shape of the islet from 30\textsuperscript{th} day onwards. By 90\textsuperscript{th} day the islets were large sized with compact arrangement of cells. The increase in both alpha and beta cells from 30\textsuperscript{th} day was confirmed by special staining technique as well as by immunohistochemistry in the present study. There was also ductal epithelial hyperplasia which was progressive in nature with formation of new small islets within the duct or in the adjacent area which were positive for insulin immunohistochemically. New formation of insulin positive islets cells indicated beta cell neogenesis and possible role of biological component of Trigonella seeds in islet cell regeneration during treatment of diabetes. The results of the present study were in accordance with those of Zafar et al. (2009) and Eman and Elaziz (2011) who also observed restoration of histological architecture of islets of Langerhans on treatment with \textit{T.foenum graecum} extract in diabetic rats. They attributed the restoration of islets to regeneration and repair of damaged beta cells by the stimulating effect of 4-hydroxyisoleucine of \textit{T.foenum graecum} extract.

In a study on treatment of alloxan induced diabetes in mice by Shah et al. (2009) with 4-hydroxyisoleucine extracted from \textit{Trigonella foenum graecum} showed formation of many small islets indicating islet neogenesis from pancreatic duct/ progenitor cells. This observation was further strengthened by \textit{in vitro} islet neogenesis and adipogenesis observed by them after treatment of pancreatic ductal stem cells with 4-hydroxyisoleucine. They also indicated that reduction in glycosylated Hb levels in 4-HI treated rats is suggestive of regeneration of pancreatic beta cells and induction of
regeneration of beta cells is the mechanism of hypoglycaemic effect in treatment with *Trigonella foenum graecum*. An improvement in serum insulin level and increase in the number of insulin positive cells observed by immunohistochemistry in the present study further support the regenerative potential of *Trigonella foenum graecum* in diabetes treatment.

In the present study, hyperplastic change involving the exocrine portion was also observed with formation of acini lined by cells with hyperchromatic nuclei and basophilic cytoplasm in which the zymogen granule formation was sparse or nil which could also be attributed to the biological effect of 4 HI of *Trigonella foenum graecum*.

A slightly better improvement in the morphology of the pancreas with respect to endocrine and exocrine component in treatment group with Trigonella and glibenclamide half dose indicate a synergistic effect in improving the architecture with the mechanisms specific for them.

Microscopically in the present study, the liver revealed progressive improvement in the architecture from STZ induced liver damage from 15th day onwards in both *Trigonella foenum graecum* alone and combination group with glibenclamide. *Trigonella foenum graecum* has been reported to incite hepatoprotective effect in liver damage due to various causes like CCl₄, alloxan, STZ etc (Zafar *et al.*, 2009). The hepatoprotective effect of *Trigonella foenum graecum* has been attributed to the presence of tannin and flavonoids by Yadav *et al.* (2010) and to free radical scavenging activity by antioxidants by Ebuehi *et al.* (2010). Other organs like kidney, lung, intestine, brain, heart etc were normal throughout the study in both the groups (Group IV and VII).
Based on the observations of the present study it could be indicated that fenugreek seeds alone could be used for the treatment of diabetes or as an adjunct to antidiabetic drug in reduced dosage. The result also suggested that fenugreek and glibenclamide half dose have synergestic effect in ellivation of certain biochemical parameters.

5.2.5 *Coccinia indica* (Group-V) and *Coccinia indica* with glibenclamide half dose (Group VIII)

The efficacy of *Coccina indica* was evaluated in STZ induced diabetes in rats individually as well as in combination with half dose of Glibenclamide similar to that of *Trigonella foenum graecum* in the present study to evaluate individual and combined effect, to know whether there is any synergistic or antagonistic effect between *Coccinia indica* and glibenclamide and also to check whether Coccinia could be used as an adjunct in diabetic therapy with oral antidiabetic agents. Hence the results of Groups V and VIII are discussed together with regard to biochemical, haematological, pathomorphological, immunohistochemical and immunomodulatory effects.

*Coccinia indica* (Synonym: *Coccinia grandis*, *Coccinia cordifolia*) belongs to family Cucurbitaceae and commonly called as little gourd, ivy gourd or Bimba in Sanskrit and Kandutikibel in Hindi. *C. indica* is a creeper that grows abundantly all over India, Tropical Africa, Australia, Fiji and throughout the oriental countries. The plant has been used extensively in Ayurvedic and Unani practice in the Indian subcontinent since ancient time (Azad et al. 1979). It has long tuberous fleshy roots and smooth and green fruits. The fruits, leaves, roots are used for medicinal purpose by folklore like fresh juice of roots to treat diabetes, tincture of leaves to treat gonorrhoea and the paste of leaves to
the skin diseases. Dried bark has been reported to be a good cathartic, leaves and stem as antispasmodic and expectorant and green fruits to cure sores on the tongue (Chandra sekar et al., 1990).

Scientific investigation of *Coccinia indica* showed that the crude extract has hepatoprotective, antioxidant, anti-inflammatory and anti-nociceptive, anti-diabetic, hypolipidemic, anti-bacterial and antitussive activities. Though the plant has been reported for many biological activities, no scientific data is available to attribute these activities to any specific principle component of the plant extract (Yadav et al., 2010).

The whole parts of the plant has been reported to contain carbohydrates, glycosides, fix oils and fats, proteins and amino acids, saponins, tannins, phytosterol, alkaloids, phenolic compounds, flavinoids, gum and mucilage. The fruits contain steroids, saponins, ellagic acid, lignins, triterpenoids, alkaloids, tannins, flavonoids, glycosides, phenols and fresh leaves contain anthraquinones, alkaloids, carbohydrates, proteins and aminoacids, tannins, saponins, flavonoids, phytosterol, triterpenes (Chandira et al., 2010)

*Coccinia indica* has been studied for its hypoglycemic, antidiabetic potential with different animal models since 1953 (Shakya et al., 2008). *C. indica* leaves depress the activity of the enzyme glucose-6-phosphatase and possess an antioxidant activity, which may be attributed to its protective action on lipid peroxidation and to the enhancing effect on cellular antioxidant defence contributing to the protection against oxidative damage in streptozotocin diabetes. Many clinical trial studies also have proven effectiveness and safety of this plant parts and derived formulations for antidiabetic effect. Anti-
inflammatory, analgesic and antipyretic activity of fruit and leaves were studied and found to be significant. Plant has been reported to contain saponins, flavonoids, sterols and alkaloids which are found to be responsible for antidiabetic activity (Deokate and khadabadi, 2011).

The Group V and Group VIII rats in the present study following induction of diabetes with STZ and treatment with Coccinia indica alone and in combination with gibenclamide revealed a significant increase in the body weight compared to diabetic rats from 15\textsuperscript{th} day onwards and on 90\textsuperscript{th} day it was comparable to that of control rats and did not differ significantly from those of gibenclamide alone and other treatment groups. The improvement in the body weight by feeding Coccinia indica also has been reported by many earlier workers (Venkateswaran et al., 2003; Kuriyan et al., 2008 and Rafiq et al., 2009). The increase in the body weight could be attributed to the alleviation of diabetic symptoms and hyperglycemia by insulinomimetic effect of triterpene of Coccinia indica (Rafiq et al., 2009 and Jose and Usha, 2010) and also to insulin stimulated glucose transport and anabolic effects of insulin (Xie et al., 2003 and Kanetkar et al., 2007).

In the present study the mean serum glucose values of Groups V and Group VIII reduced significantly and progressively in comparison with diabetic group from 15\textsuperscript{th} day onwards. On 90\textsuperscript{th} day the mean glucose values were comparable between Group V and VIII but were significantly higher compared to control group and gibenclamide alone group.
Presence of alkaloids, tannin, saponins, flavonoids and pectin of *Coccinia indica* has been shown to improve secretion of insulin by β-cells by promoting their regeneration and there by controlling hyperglycaemia (Ramakrishnan et al., 2011).

*Coccinia indica* has been rated ‘A’ while assessing the quality of the herbs for glycemic control. A maximum decline in the blood glucose level in STZ induced diabetes has been recorded by Mishra et al. (2009). The hypoglycaemic activity of leaf extract of *C.indica* which could be attributed to the insulinomimetic effect of triterpene of the plant which act like insulin by decreasing the elevated enzymes, glucose 6-phosphatase and lactate dehydrogenase in the glycolytic pathway and restoring the lipoprotein lipase activity in the lipolytic pathway with the control of hyperglycaemia in diabetes (Kuriyan et al., 2008). The insulin like activity of *C.indica* stimulate hepatic glucose uptake and also inhibit gluconeogenesis and glycogenolysis in liver (Jose and Usha, 2010). The hypoglycaemic effect may also be due to increase in the levels of glucose transporters and stimulation of uptake in peripheral tissues. The triterpenes of *Coccinia indica* reduce the beta cell damage and therefore potentially increase insulin secretion. The triterpene compound dehydrotrametenolic acid reduces glucose levels and also act as an insulin sensitizer possibly through its role in the activation of peroxisome proliferator activated receptor – gamma (Sato et al., 2002). Kuriyan et al. (2008) suggested that Coccinia could act through a variety of mechanisms including actions mimetic of those of sulfonylureas. In the present study the improvement in glucose level between Group V and Group VIII did not differ significantly and indicated that there was no synergistic effect in hypoglycaemic effect between *C.indica* and glibenclamide although numerically the values of Group VIII were better.
The serum cholesterol and triglyceride levels showed a significant (P≤0.001) decreasing trend from 15\textsuperscript{th} day onwards in the present study in *Coccinia indica* alone (Group V) and *Coccinia indica* in combination with glibenclamide half dose (Group VIII) groups in comparison with diabetic control group throughout the experiment. On 90\textsuperscript{th} day the mean serum cholesterol level was comparable with that of control group and did not differ between Group V and Group VIII though numerically the values were lesser in combined group than Coccinia alone group. This indicated that there was no synergistic effect between *Coccinia indica* and glibenclamide in alleviating the hypercholesterolaemia in induced diabetes. The triglyceride level in combined group on 90\textsuperscript{th} day was significantly lesser compared to *Coccinia indica* alone group which indicated that combination of glibenclamide with herbal extract has beneficial effect in controlling the triglyceride level in diabetes and Coccinia and glibenclamide have synergistic effect which could be attributed to glibenclamide induced alleviation of hypertriglyceridemia by the acute reduction in triglyceride of intestinal origin (Yadav et al., 2010) in addition to the hypolidaemic effect of *Coccina indica*.

The decrease in the cholesterol and triglyceride levels in serum by *Coccina indica* could be attributed to the triterpenes which suppress lipolysis and mobilize free fatty acids from the fat depots by decreasing the activities of lipogenic enzymes and hexokinases (Ramakrishnan et al., 2011) and to the antioxidant property of *Coccinia indica* which scavenge the free radicals produced by oxidative stress during STZ diabetes induction, thereby prevent beta cell damage and stimulate the secretion of insulin from beta cells. Insulin influences the level of enzyme lipoprotein lipase which hydrolyzes the triglyceride and increases excretion of lipids (Venkateswaran et al., 2003;
Jose and Usha, 2010 and Singh et al., 2010)) and inhibits intestinal absorption of oleic acid (Wang et al., 2008).

The serum alanine aminotransferase (ALT) and serum aspartate amino transferase (AST) levels in Group V and Group VIII were significantly improved in comparison with that of diabetic control. On 90th day the AST values did not differ between groups V and VIII and were comparable to that of glibenclamide group. However the values were significantly higher compared to those of normal control rats. The ALT values on 90th day in group V was comparable with that of glibenclamide group but the mean value in group VIII was significantly reduced which indicated synergistic effect and was statistically comparable with that of control group. The improvement could be attributed to the combined effect of coccinia indica with glibenclamide for their hypoglycaemic, insulin secretogogue and antioxidant activities.

Serum insulin values in the present investigation showed a progressive increase in Coccinia indica alone and in combination with glibenclamide treated animals from 15th day post treatment onwards in comparison with those of diabetic control rats. The mean insulin value on 90th day was significantly higher in Group VIII compared to Group V and group III. This indicated a synergistic effect in elevating the insulin level between Coccinia indica and glibenclamide. Both Coccinia indica and glibenclamide alone elevated the insulin level only moderately but in combination elevated the insulin level significantly which was comparable to that of control group.

In Coccinia indica treated group, the moderate improvement in insulin level could be attributed either to the insulinomimetic activity of triterpenes or to the stimulation of
insulin release from β cells or due to combination of both the effects (Venkateswaran and Pari, 2003 and Mallick et al., 2007). In Group VIII, the elevated insulin level could be attributed to the similar mechanism of action of both *Coccina indica* and glibenclamide. The combined effect of stimulation of insulin release by both glibenclamide and *Coccina indica* which inturn stimate beta cell regeneration could be responsible for a significant increase in the insulin level in the present study. Niedzielski, 2001, also reported from his observations that *C.indica* has a very similar ability of tolbutamide, a reference antidiabetic drug and Saklani *et al.* (2012) indicated that *Coccinia indica* has an effect in potentiating the insulin from the existing beta cells.

An another effect of *Coccinia indica* is that it preserves the cells of islets of Langerhans of beta cell function which results in a significant increase in insulin activity (Kamiya *et al.*, 2008 and Prakash *et al.*, 2005). The antioxidant property of *Coccinia indica* which scavenge the free radicals produced by oxidative stress during STZ diabetes also could prevent beta cell damage and stimulate the secretion of insulin from beta cells (Venkateswaran and Pari, 2003 and Jose and Usha, 2010). The improvement in insulin level in the present study is well supported by an increase in the number of beta cells microscopically and insulin demonstration by immunohistochemistry.

The activity of SOD, CAT and GPx in liver showed significant improvement in *Coccinia Indica* alone and in combination with glibenclamide treatment groups compared to diabetic control from 15<sup>th</sup> day onwards. On 90<sup>th</sup> day there was no significant difference in the antioxidant enzyme levels between group V and Group VIII and the values were comparable to that of group III. Similar type of finding in improvement of
the levels of SOD, GPx and GSH was recorded by administration of *Coccinia indica* by Venkateswaran and Pari, (2003). This could be due to free radical scavenging activity and hypoglycaemic effect of the extract. The antioxidant activity of Coccinia is attributed to alkaloid content of the plant. Alkaloid compounds are considered as one of the most important and widely found antioxidant sources in food and medicinal plants. There is a significant correlation between alkaloid content and antioxidant activity in different parts of the plant. *Coccinia indica* extracts contain many naturally occurring alkaloid and flavonoids that have been associated with a wide range of biological and pharmacological activities including antioxidant and antidiabetic activities (Singh *et al.*, 2010).

In the present study there was a significant improvement in the haemoglobin values from 15th day onwards in both *Coccinia indica* alone and in combination with glibenclamide groups in comparison with diabetic control group. The mean values on 90th day did not significantly vary between these groups and also when compared with glibenclamide alone complete dose group. However the values failed to reach that of control group. The findings indicated that *Coccinia indica* alone and in combination with glibenclamide half dose has effective effect in alleviating the diabetic effect on Hb values and is equivalent to the glibenclamide complete dose. However, no synergistic effect was observed in the combined group.

As reported earlier, the hypoglycaemic effect of triterpene of *Coccinia indica* could be attributed to the decreased glycation of Hb and increase in the Hb value in the circulation as it is insulinomimetic and secretagogue in function which there by improve glucose level and decrease Hb glycosylation. In addition the antioxidant effect in
alleviating the free radical injury to the islets and other organs such as liver and kidney could be contributory for decreased Hb glycosylation (Ramakrishnan et al., 2011)

The hematological values of TRC, TLC, and platelets which were significantly reduced in diabetic control rats showed significant improvement in Coccinia indica alone and in combination with glibenclamide half dose groups. There was no significant difference in the values of TLC and platelets among these treatment groups at different time intervals. However, total TRC values in the combined group had better effect compared to Coccinia indica alone group and was comparable to that of glibenclamide complete dose group. The values of TRC and TLC remained significantly lesser compared to control group.

The improvement in blood parameters in Coccinia indica treated groups could be attributed to the antioxidants of Coccinia indica which quench the ROS produced during induction of diabetes with STZ and thereby alleviating the damage in the hemopoietic tissue. In addition Coccinia indica has immunomodulatory effect and stimulate immune functions by activating the immune cells.

Clinically the rats belonging to Coccinia indica alone group and combination group with glibenclamide half dose revealed decrease in the severity of the diabetic manifestation from 15th day of treatment onwards which could be directly attributed to the alleviation of STZ effect by the Coccinia extract as well also by glibenclamide in combination group. The improvement in the clinical signs could be attributed to the antioxidant property which reduce the free radical injury and also to the triterpene of
*Coccinia indica* with insulinomimetic and hypoglycaemic activities which improve the hypoinsulinaemia in diabetes.

Grossly, a significantly appreciable improvement was observed in the appearance of pancreas and liver in both V and VIII groups which could be attributed to the alleviation of the diabetic effect by improvement in the insulin level by *Coccinia indica* as well as by glibenclamide.

Microscopically, *Coccinia indica* treatment for a period of 90 days showed an improvement in the architecture of pancreas, liver and other organs.

In pancreas there was an improvement in the number, size and shape of the islets, increase in the number of granulated β-cells and attainment of almost normal distribution of alpha and beta cells. These changes were progressive in nature from 15\textsuperscript{th} day to 90 day post treatment.

The improvement in architecture of pancreatic islets could be attributed to the effect of antioxidant property of the *Coccinia indica* which prevented damage to the beta cells and resulted in promotion of regeneration of β-cell subsets or repair of damaged cells, therby restored insulin concentration to almost normal basal level (Singh et al., 2010 and Ramakrishnan et al., 2011). In the present study also a progressive improvement in the insulin producing cells was observed by immunohistochemistry for insulin.

The improvement in the architecture of the pancreas in combination group with *Coccinia indica* and glibenclamide was better than that of *Coccinia indica* alone group
which could be attributed to the combined effect in regenerating beta cells and repairing the damaged cells by the antioxidant effect. This result was well substantiated by the increase in the number of insulin positive beta cells in the combined group.

Microscopically the STZ induced liver damage in the form of necrosis and vacuolar degeneration persisted in a subtle manner on Day 15 in the present study, but the liver attained almost normal architecture which was comparable to that of control liver by 90\textsuperscript{th} day post-treatment in both group V and group VIII.

The perusal of literature did not provide much information on the effect of \textit{coccinia indica} in reversing the liver damage. However leaf extract of \textit{coccinia indica} has been reported to revert alloxan induced liver damage to the normal homeostasis by its hypoglycaemic action and stimulatory insulin release by the \(\beta\)-cells (Mallick \textit{et al.}, 2007)

Other organs such as kidneys, heart, brain, lungs, spleen, lymphnodes, adrenal and intestine showed normal architecture comparable to those of control. It is pertinent to mention here that in the present study STZ failed to induce any of the architectural changes in these organs.

\textbf{5.2.6 Combined treatment groups (Group- VI and IX)}

To determine the possible combined synergistic antidiabetic effect, in the present study, group VI rats were fed with \textit{Trigonella foenum graecum} at 1gm/Kg body weight and \textit{Coccinia indica} at 200mg/Kg body weight and group IX rats with \textit{Trigonella foenum graecum} at 1gm/Kg body weight, \textit{Coccinia indica} at 200mg/Kg body weight and glibenclamide 300 \(\mu\)g/Kg (half dosage) orally in combination.
In the present study the evaluation of antidiabetic effect of combined herbal extract treatment revealed a significant increase in the mean body weight values in both group VI and group IX from 15\textsuperscript{th} day onwards in comparison with diabetic control group. It was observed that the weight gain was better numerically in both the groups compared to other treatment groups and was comparable to that of control group. Between Group IV and Group IX, the weight gain was more in Group IX which indicated that combined treatment has better alleviating effect on diabetes induced changes. As discussed under respective individual herbal extract treatment groups, the weight gain could be attributed to the combined effect of active biological components of the Trigonella and Coccinia which improved insulin level and also increased glucose absorption at the periphery (Das \textit{et al.}, 2008 and Mishra \textit{et al.}, 2009). There was a synergetic effect observed in increasing the mean body weight in combined treatment groups.

The mean serum glucose level in the groups VI and IX significantly and progressively reduced from 15\textsuperscript{th} day onwards compared to diabetic control group and on 90\textsuperscript{th} day the mean glucose values were significantly not different between groups VI and IX in comparison with individual treatment groups III, IV and V and combined treatment groups VII and VIII. However, the values were significantly higher compared to that of control group. The mean glucose values in group IX though not statistically different was numerically better than all other groups. The results of the present study indicated that combined herbal treatment with glibenclamide has better hypoglycaemic effect compared to individual herbal and glibenclamide treatments (Das \textit{et al.}, 2008, and Mishra \textit{et al.}, 2009). The hypoglycaemic effect of combined treatment could be attributed to the
respective bioactive compounds of herbal extracts as discussed under individual herbal antidiabetic effect.

The mean serum cholesterol and triglyceride values in the present study in group VI and IX significantly improved compared to diabetic control from 15\textsuperscript{th} day onwards and on 90\textsuperscript{th} day the values were statistically did not differ between the groups and were comparable to glibenclamide treatment group. However, the values were significantly higher compared to control group. The mean values though significantly did not differ but the values were numerically lesser compared to individual herbal and glibenclamide treatment. The significant reduction in the mean cholesterol and triglyceride values in group VI indicated a synergistic effect and was found to be better in alleviating the increase in the cholesterol and triglyceride levels when a combination of herbal extracts was used along with antidiabetic drug (Mishra et al., 2009).

The ALT and AST mean values in combined treatment groups (Groups VI and IX) were significantly improved from 15\textsuperscript{th} day onwards in comparison with diabetic control group. On 90\textsuperscript{th} day the mean ALT and AST values between the groups VI and IX were comparable and did not differ significantly. The mean values were comparable with that of control group and better compared to individual herbal extract and glibenclamide treatment groups. The mean value in Group IX was numerically lesser compared to any other group. The results of the present study indicated that there was a synergistic effect between \textit{Trigonella foenum graecum} and \textit{Coccinia indica} combined treatment and was comparatively better when used along with glibenclamide half dose (Mishra et al., 2009). Decreased ALT and AST values indicate directly the hepatoprotective effect of
Trigonella and Coccinia plant extracts which could be due to the antioxidants of the plants in prevention of ROS and NO induced lipid peroxidation as discussed under individual plant extract treatment groups.

The mean serum insulin values in combined treatment groups (Groups VI and IX) were significantly higher on all the intervals of observation compared to diabetic control group. On 90th day of observation the mean insulin values were higher and comparable to that of control group. The mean insulin values of both the groups were significantly higher than individual plant extract (IV, V) and glibenclamide (III) groups. This clearly indicated that *Trigonella foenum graecum* and *Coccinia indica* have synergistic effect in improving the insulin level with their respective bioactive components and also the effect was much better when the plant extracts were used along with glibenclamide (Mishra et al., 2009). As also evidenced in microscopical examination a significant increase in the number of beta cells with insulin production observed in combined treatment groups (VI and IX) was responsible for an improved insulin level comparable to that of control group in the present study (Mallick et al., 2007).

The mean values of SOD, CAT and GPx in the combined treatment groups (VI and IX) were observed to be significantly higher compared to diabetic control on all the days of observation. On 90th day the mean SOD, CAT and GPx values between the groups VI and IX were comparable and did not differ significantly. However, the combined treatment group (IX) with plant extracts along with glibenclamide half dose was observed to be superior with high antioxidant activity compared to any other treatment groups. This indicated that there was a synergistic effect between plant extracts
and glibenclamide in alleviating free radical induced cell injury. Though a significant increase was observed the values failed to reach those of control group. Numerically the mean values were higher compared to all the other treatment groups with Group IX being the best. As discussed under individual plant extract treatment groups, both *Trigonella* and *Coccinia indica* were shown to possess alkaloids, flavonoids, vitamins with antioxidant activity responsible for hypoglycaemic effect, protection of beta cells from oxidation by inhibiting peroxidation chain reaction and regeneration of beta cells (Rang and Dale, 1991 and Singh *et al.*, 2010).

The haemoglobin percentage of combined treatment groups (VI and IX) was significantly higher than diabetic control group from 15th day onwards. The mean Hb values between Groups VI and IX did not differ on 90th day though the value of Hb in group IX was numerically higher compared to any other treatment groups. This observation indicated that the combined treatment with plant extracts and glibenclamide has better hypoglycaemic effect by which the glycosylation of Hb was reduced and there by improved mean Hb values. The individual plant extracts have been reported to contribute for hypoglycaemia by their respective bioactive compounds and antioxidant property as discussed under respective plant extract treatment groups.

There was an improvement in all the blood cells counts (TRC, TLC and platelets) of the combined treatment groups (VI and IX) compared to diabetic animals. On 90th day of the experiment no statistical difference was observed between Groups VI and IX and did not differ from other treatment groups. Numerically the values were higher in combined treatment group IX which indicated that the antidiabetic drug along with plant
extracts with antidiabetic effect has beneficial effect in counteracting the diabetes induced changes in the body. Though there was an increase in the cell counts, the values never reached values of control group at any interval of observation.

There was a gradual decrease in severity of clinical signs such as polyuria, polydypsia, polyphagia and weight loss in diabetic animals treated with combination of \textit{Trigonella foenum graecum} and \textit{Coccinia indica} (Group IV) and \textit{Trigonella foenum graecum}, \textit{Coccinia indica} and glibenclamide (Group IX) treated rats. The improvement in the body condition could be attributed to the synergistic effect on hypoglycaemic, hypolipidemic, hepatoprotective, insulinomimetic and insulin secretagogue action of the plant extracts and glibenclamide in the diabetic subjects.

Gross pathological changes observed in various organs in both the groups reduced progressively from Day 15 to Day 90 of the study. An appreciable improvement in the morphological appearance of the pancreas was observed wherein there was an increase in the mass of the pancreas in the treatment groups but was more significant in combined treatment group with plant extracts and glibenclamide. A similar observation was also made in other organs such as liver, adrenals, kidney, etc.

Histopathologically, there was a progressive improvement in the architecture of pancreas, liver, kidney and spleen from Day 15 to 90 of the study. Pancreas of combined treatment groups showed better improvement in terms of architecture of endocrine and exocrine pancreas compared to individual treatment groups. The islets of Langerhans in combined treatment groups showed better cellularity as the treatment progressed. At 90th day, the combined treatment groups revealed almost normal architecture with bigger
islets and more number of islets per lobule, with a morphological architecture similar to that of normal islets. There was an increase in cellularity with more number of cells with beta cell morphology with granular cytoplasm which were comparable with that of normal control. In addition an improvement in the exocrine portion of pancreas was also observed. The improvement in Group IX was higher compared to individual plant extract and glibenclamide groups. Between groups VI and IX the improvement was better in group IX which indicated a synergistic effect between plant extracts and the glibenclamide at half dose in alleviating hypoinsulinaemia in diabetes. This was well substantiated by the immunohistochemistry which revealed better regeneration of β-cell population compared to any other treatment groups indicating the synergistic action compared to individual treatment groups. Based on the observation it could be concluded that β-cell regeneration capacity of combined treatment groups is better than individual treatment groups of *Trigonella foenum graecum* and *Coccinia indica* and a synergism exists between plant extracts and glibenclamide half dose in alleviation of diabetic effect.

5.3 Immunohistochemistry

5.3.1 Control Group-I

In the present study, immunohistochemical staining of pancreas for insulin revealed polyhedral shaped beta cells with dark brown coloured granules in the cytoplasm with unstained round to oval shaped nucleus. The functional beta cells revealed a large number of granules densely occupying the cytoplasm which varied in their size. Occasional beta cells with sparsely scattered granules were also observed. In control rats the mean percentage value of beta cells with insulin was 78.74 ±0.92. In a
normal individual the beta cells are the most abundant cells found in the islets constituting approximately 80 per cent of the endocrine cells with 15 per cent of alpha cells, 4 per cent of delta cells and 1 per cent of pancreatic polypeptide cells.

5.3.2 Diabetic control group-II

In the present study, there was a drastic decline in the number of insulin positive cells in the islets of diabetic rats due to the specific destruction of beta cells by STZ. However, a small number of cells with mean percentage value of $3.50 \pm 0.33$ showing immunopositivity were observed which indicated survival of a few cells with retained insulin secretory function even after STZ treatment (Bolkent et al., 2005; Adewole and Ojewole, 2007 and Adeghate et al., 2010). This was proved by Adeghate et al. (2010) ultrastructurally, who demonstrated intact cytoplasmic organelles such as RER with little or no secretory granules in a few $\beta$-cells which indicated that not all cells after STZ treatment become necrotic. In addition the immature $\beta$-cells which do not express GLUT2, escape STZ cytotoxicity which is the route of entry of STZ into the cells (Schnedl et al., 1994 and Thulesen et al., 1997).

5.3.3 Glibenclamide control group-III

In the glibenclamide treatment group, there was a progressive increase in the number of immune positive cells with a mean percentage value of $42.33 \pm 0.137$ on 45th day and $46.50 \pm 1.50$ on 90th day of the study. The findings indicated that glibenclamide improved the number of beta cells in STZ diabetic rats on treatment by 30th day itself and reached maximum by 90th day. However the increase in the number of insulin positive beta cells did not reach the number of normal control rats. The possible reason for the
increase in the number could be sulphonylurea induced insulin release from surviving cells which in turn stimulate and enhance beta cell proliferation, maturation and functional activity of cells, as experimentally proved by Ling et al. (2006) and Adewole and Ojewole (2007).

4.3.4 Group-IV (Diabetic rats treated with Trigonella foenum graecum)

The immunohistochemical examination in diabetic rats treated with Trigonella foenum graecum revealed progressive improvement in the number of insulin positive cells from 15\textsuperscript{th} day to 90\textsuperscript{th} day. The improvement in immunostaining of β-cells coincided with an improvement in the serum insulin level.

The mean percentage number of insulin positive cells was significantly higher (P≤0.001) in comparison with that of diabetic control group and the percentage values were comparable to those of groups III, V and VII but lesser than the combined groups VI, VIII and IX. The increase in the number of beta cells could be attributed to the beta cell regeneration or repair induced by 4-hydroxyisoleucine and antioxidant effect of T.foenum graecum extract (Shah et al., 2009; Zafar et al., 2009 and Eman and Elaziz et al, 2011).

4.3.5 Group-V (Diabetic rats treated with Coccinia indica)

In Group V there was a progressive increase in the number of insulin positive cells from 15\textsuperscript{th} to 90\textsuperscript{th} day of the experiment. The number of insulin positive beta cells drastically increased on 30\textsuperscript{th} and only slightly increased on 45\textsuperscript{th} and 90\textsuperscript{th} days. The beta cells were large, polyhedral and consisted of darkly stained granular cytoplasm
resembling the appearance of normal beta cells. Insulin immune positive cells were also observed in the exocrine portion as well as within and adjacent to the ductal structures. In comparison with diabetic control, the percentage of insulin positive cells were significantly (P≤0.001) higher but significantly lesser (P≤0.001) compared to normal control and combined groups VII and IX on 90th day. The mean percentage number was comparable to those of glibenclamide and trigonella groups on 90th day. The increase in the immunoreactivity could be attributed to the effect of antioxidant property of the *Coccinia indica* and the triterpene of the plant which might have prevented damage to the beta cells and resulted in promotion of regeneration of β-cell subsets or repaired damaged cells, thereby increasing insulin concentration (Ramakrishnan *et al.*, 2009 and Singh *et al.*, 2010).

**5.3.6 Group-VI (Diabetic rats treated with *Trigonella foenum graecum* and *Coccinia indica*)**

The mean percentage number of insulin positive cells in *Trigonella foenum graecum* and *Coccinia indica* showed a progressive increase in the number of insulin positive cells from 15th to 90th day of the experiment. The number of insulin positive beta cells increased from 30th to 90th day. The cells were concentrated more at the centre of the islet however, a few cells were also observed at the periphery. Insulin immune positive cells were also observed in the exocrine portion as well as within and adjacent to the ductal structures. In comparison with diabetic control the mean percentage of insulin positive cells was significantly (P≤0.001) higher but significantly lesser (P≤0.001) when compared with normal control and groups VIII and IX. The mean percentage number was comparable to those of glibenclamide and Trigonella groups on 90th day.
The increase in the percentage of beta cells in the present study could be attributed to the combined effect of the plant extracts with their respective bioactive components and antioxidant effect in either regeneration or repair of the beta cells. The study indicated that β-cell regeneration capacity of combined treatment groups was better than individual treatment groups of *Trigonella foenum graecum* and *Coccinia indica* which was well substantiated by the serum insulin levels which revealed similar pattern of improvement (Das *et al.*, 2008). However no synergistic effect could be appreciated in improving the number of beta cells as it was lesser than those of Groups VIII and IX which was also substantiated by the serum insulin level which was comparable to those of individual plant extract treatment groups.

5.3.7 Group-VII (Diabetic rats treated with *Trigonella foenum graecum* and Glibenclamide half the dose)

The diabetic rats treated with *Trigonella foenum graecum* along with half dose of glibenclamide showed a progressive increase in the number of insulin positive cells from 15th to 90th day of the experiment. The beta cells were large, polyhedral and consisted of darkly stained and compactly arranged granules in the cytoplasm similar to that of normal beta cells. The cells were concentrated more at the centre of the islet and a few at the periphery. The increase in the percentage of beta cells coincided with the increase in the serum insulin level in the present study. Insulin immune positive cells were also observed in the exocrine portion as well as within and adjacent to the ductal structures which were intensely stained. This clearly indicated that there was beta cell neogenesis from ductal epithelial cells or acinar cells either in response to hyperglycaemic situation or due to the
stimulatory effect of the bioactive substance 4-hydroxyisoleucine of the plant (Shah et al., 2009; Zafar et al., 2009a and Eman and Elaziz, 2011).

In comparison with diabetic control the percentage of insulin positive cells was significantly (P≤0.001) higher and comparable with individual herbal extract groups and Glibenclamide. However, the values were lesser compared to that of control group and combined treatment groups VIII and IX. This observation indicated that there was no synergism between Trigonella and glibenclamide in improving the number of beta cells in diabetic rats.

5.3.8 Group-VIII (Diabetic rats treated with Coccinia indica and glibenclamide half the dose)

The diabetic rats treated with Coccinia indica along with half dose of glibenclamide showed comparatively a higher increase in the number of insulin positive cells from 15th to 90th day of the experiment. The mean percentage number was significantly higher compared to those of glibenclamide, individual Trigonella and Coccinia extract treated groups, and Trigonella with glibenclamide group. However it was comparable with those of normal control and all combined treatment group IX. The beta cells were large, polyhedral and consisted of varying stained granular cytoplasm in intensity with a morphology and distribution of beta cells similar to that of normal islets. Insulin immune positive cells were also observed in the exocrine portion as well as within and adjacent to the ductal structures which were intensely stained. The observation of the present study indicated that there was a synergistic effect in improving the morphology of the islets and insulin secretion by increasing the percentage of beta cells in the islets.
either through regeneration or repair of the cells by their insulinomimetic and secretogogue effects. The present results were also well substantiated by an increase in the serum insulin value which was almost equivalent to that of normal control and suggested that combination of *Coccinia indica* with half dose of glibenclamide has potential antidiabetic effect compared to Coccinia and glibenclamide individually. This also fulfils the purpose of present study of reducing the dosage of antidiabetic drug in treatment of diabetes which has several side effects in long lasting treatment. The study also suggested that *Coccinia indica* serves as a good adjunct in treatment of diabetes.

4.3.9 Group-IX (Diabetic rats treated with *Trigonella foenum graecum, Coccinia indica* and glibenclamide)

The diabetic rats treated with combination of *Trigonella foenum graecum*, *Coccinia indica* along with half dose of glibenclamide showed a significant increase in the number of insulin positive cells from 15th to 90th day of the experiment compared to any other treatment groups of the present study except group VIII treated with Coccinia and glibenclamide half dose and normal control group. The morphology and distribution of beta cells was similar to that of normal islets which showed typical cord like arrangement. The cells were concentrated at the centre of the islet. Insulin positive cells were also observed in the exocrine portion as well as within and adjacent to the ductal structures which were intensely stained. The observation of the present study indicated that there was a synergistic effect in improving the morphology of the islets and insulin secretion by the combined effect of plant extracts and the glibenclamide. However the present beta cell number increase could be attributed mainly to Coccinia and glibenclamide as the group VIII treated with these two revealed a very similar effect and
the combination of trigonella and Coccinia and Trigonella with glibenclamide failed to
elicit a similar effect. There may be a mechanism of inciting regeneration of beta cells
when Coccinia was used along with glibenclamide which needs further studies.

5.4 Special staining for alpha and beta cells

In the present study, special staining was done for the demonstration of alpha and
beta cells in the pancreatic islets using Gomori’s chrome alum hematoxylin phloxine
stain. In the normal control rats special staining revealed polyhedral beta cells with deep
blue granular cytoplasm occupying the core and alpha cells with reddish pink cytoplasm
at the periphery.

In diabetic control rats islets revealed loss of normal architecture with loss of
normal distribution of alpha and beta cells. The beta cells were swollen with highly
vacuolated cytoplasm which could be due to cytotoxic effect of STZ of islets of
Langerhans leading to its degeneration and necrosis (Szkudelski et al., 2001; Mir et al.,
2008; Pragathi, 2011 and Mudasir, 2011)

Islets of other treatment groups revealed hypercellularity with more number of
alpha cells and a few normal appearing beta cells. Among all the treatment groups
Trigonella foenum graecum, Coccinia indica and glibenclamide showed better response
in terms of increase in the number and distribution of beta cells however, alpha cells were
still more than beta cells. The possible reason could be to stimulate insulin synthesis by
increasing glucagon level as presumed by (Adeghate et al., 2010).
5.5 Flow cytometry

5.5.1 Normal control Group-I

In the present study to evaluate the immunomodulatory effect of *Trigonella foenum graecum* and *Coccinia indica*, the blood samples collected at 45\textsuperscript{th} day of the study were subjected for enumeration of CD4+ and CD8+ cells and CD4+ to CD8+ ratio by flow cytometry. The Flow cytometry analysis of control rats was 65.36 + 1.17 and 27.71+ 3.12 per cent for CD4+ and CD8+ cells respectively and the CD4+:CD8+ ratio was 2.02+ 0.08.

Normally the percentage of CD4+ T cell population of the immune system is higher compared to CD8+ cells. It is estimated to be approximately 65 per cent and 30 per cent for CD4+ and CD8+ cells respectively and 5 per cent of cells express neither CD4+ nor CD8+ molecules and said to be double negative. Normally, an elevated CD4+ count implies increased lymphocytic reactivity as helper cells predominate and a high CD8+ level implies depressed lymphocyte reactivity (Tizard, 2008).

5.5.2 Diabetic control Group-II

In group-II (Diabetic control) rats on 45\textsuperscript{th} day of study there was a significant decrease (P≤ 0.001) in CD4+ cell percentage and significantly increased (P≤0.001) CD8+ values compared to normal control. The ratio of CD4+:CD8+ was 0.65±0.04 which was significantly lesser (P≤ 0.001) compared to normal control.

Alterations in lymphocytes with decreased CD4+ count and increase in CD8+ cells are a common finding in both type I and type II diabetes (Hedman *et al.*, 2008).
Since activation of T lymphocytes plays a pivotal role in initiating immune response and cell-mediated cytotoxic activity, inhibition of lymphocyte activation by diabetic state could evoke a clinically relevant immunosuppressive effect. In DM altered functions of different types of circulating immune cells, T lymphocytes and B lymphocytes have been reported (Yu et al., 2006).

The decrease in CD4+ cells in diabetes could be attributed to several reasons such as oxidative stress, a common finding in diabetes; glucolipotoxicity due to chronic exposure to higher glucose; hypoinsulinism, depressed IL2 level and decreased response of lymphocytes to mitogen. White blood cells including lymphocytes and their functions are shown to be globally affected by ambient glucose concentrations (Alberti, 1977). It has been reported that the potential increase in basal levels of intracellular calcium in the lymphocytes of diabetic patients can lead to decreased release of IL2 and decreased response to mitogenic stimuli (Tripathi, 2010). In addition stress during diabetes has been shown to induce apoptosis of lymphocytes and Insulin to reduce apoptosis of the lymphocytes. Kitabchi et al. (1995) reported that prevention of lymphocytic apoptotic cell death from various stimuli could be due to activation of phosphatidylinositol 3-kinase-Akt pathway (Hotchkiss and Karl, 2003). Experimental evidence suggests that antioxidant supplementation reduces oxidative stress in diabetics and improve insulin concentration and thus modulate immune cells. Venkatesha et al. (2011) also reported that CD4+ CD25+ cell therapy delayed occurrence of several immunological diseases including diabetes in rat models.
Elevated CD8+ T cell count in diabetes in conjunction with significantly low levels of CD4+ T cells would indicate acute critical illness with consequently an overload of the immune response. In such a situation, there is stimulation of suppressor CD8+ T cells and limitation of antigen induced lymphocyte proliferative responses by the production of suppressor factors. Additionally, the elevation of CD8+ T cells may be as a result of sequestration of CD4+ T cells to the pancreas in T2DM involving anti-islet T cell mediated pathogenic mechanisms (Yu et al., 2006). The chronic activation of lymphocytes due to glucose toxicity could increase CD8+ cells. Long standing hyperglycemia has been reported to result in chronic activation of lymphocytes and platelets due to activation of immune system and chronic systemic inflammation (Papatheodorou et al., 2006).

5.5.3 Glibenclamide group (III)

Flow cytometry analysis of rats treated with glibenclamide did not show any significant improvement in the CD4+ and CD8+ values and in their ratio compared to diabetic control. This finding indicated that treatment with oral antidiabetic drug though improves diabetic state with improvement in glucose, insulin, and other biochemical parameters, fails to improve the immune status and lacks immunomodulatory effect.

5.5.4 Treatment Groups IV, V, VI

Herbal drugs are known to possess immunomodulatory properties and generally act by stimulating both specific and nonspecific immunity. Many plants used in traditional medicine have immunomodulating activities (Wagner and Proksh, 1985). Modulation of immune responses to alleviate the diseases has been of interest for many
years and the concept of ‘Rasayana’ in Ayurveda is based on related principles. Immunomodulation could be immunostimulation in a drug-induced immunosuppression model and immunosuppression in an experimental hyperreactivity model by the same preparation (Harsh et al., 1969 and Butler et al., 2004). Apart from being specifically stimulatory or suppressive, certain agents have been shown to possess activity to normalize or modulate pathophysiological processes and are hence called immunomodulatory agents. A number of medicinal plants as rasayanas, have been claimed to possess immunomodulatory activity (Veerapur et al., 2004). *Trigonella foenum graecum* and *Coccinia indica* are two such plants used in herbal medicine.

In the present study the combined treatment with *Trigonella foenum graecum* and *Coccinia indica* showed a significant improvement in the CD4+ and CD8+ cell counts compared to diabetic, glibenclamide and individual treatment with Trigonella and Coccinia extract groups. The results indicated that there is a synergistic effect in improving the T cell profile between Trigonella and Coccinia in the present study as individually they failed to increase the number of CD4+ cells significantly. The fenugreek extract has been shown to increase in T-cell immune response significantly with activation of the CD4+ and CD8+ cells. In diabetes an elevated circulating inflammatory cytokines such as TNFα, IL-1β and IL-6 are observed in patients with hyperglycemia (Spranger et al., 2003 and Manning et al., 2008). The cytokine TNFα has been reported to down regulate the peroxisome proliferator activator receptors PPAR-γ expression which modulates important metabolic events of the cell (Berger et al., 2005).
The aqueous extract of *Trigonella foenum graecum* is known to decrease the levels of elevated TNFα in type-2 diabetic rats (Halagappa et al., 2010). Trigonella seeds contain steroid, saponins compounds diasgenin, alkaloids and trigonelline compounds which upregulate PPAR-γ expression thereby modulate inflammatory cytokine TNFα (Vishwakarma et al., 2005 and Halagappa et al., 2010). The immunomodulatory effect of *Trigonella foenum graecum* could also be due to the antioxidant activity, hypoglycaemic effect and insulinotropic effect of its bioactive compounds (Smriti et al., 2012).

*Coccinia indica* has also been proved to be an immunomodulator due to its effect on haemopoietic tissue and its anti-inflammatory activity (Yadav et al., 2010). Anti-inflammatory activity of *Coccinia indica* has been specifically attributed to the cephalandrol, tritriacontane, lupeol, b-sitosterol, cephalandrine A, cephalandrine B, stigma-7-en-3-one, taraxerone and taraxerol, terpenoids, saponins, flavonoids, sterols present in the plant (Mandal et al., 1992). In addition the insulinomimetic action of the triterpenes of Coccinia may also be contributory for the immunomodulation.

**5.5.5 Combined treatment groups with glibenclamide (VII, VIII and IX)**

Among the combined treatment groups (VII, VIII, IX) the improvement in CD4+ and CD8+ cells and their ratio was significantly higher in groups IX rats treated with Trigonella, Coccinia along with glibenclamide half dose compared to Group VII and VIII. The improvement in T cell profile of group IX was comparable to that of Group VI treated with Trigonella and Coccinia combinely. The improvement in T cell profile could be attributed to the combined synergistic effect of plant extracts and not due to glibenclamide as the glibenclamide alone failed to improve the CD4+ cell number.
Thus in the present study the antioxidants present in the herbal preparation may be responsible in bringing about immune modulation. Antioxidants could modulate the functions of the neutrophils, their opsonising capacity and T and B cell proliferation response. Herbal preparations are said to affect the immune reactions through their anti-inflammatory actions. In most cases the therapeutic efficiency of these plants may, in part, be mediated via their influence on the immune response. This could be attributed to decrease in the number of lymphocytes in circulation due to lymphocytolytic effect of STZ. Adeghate et al. (2010) and Pragathi, (2011) observed lymphocytolysis in the spleen of the diabetic control animals due to STZ.
VI. SUMMARY

The present study was focussed on evaluation of antidiabetic effect of *Trigonella foenum graecum* and *Coccinia indica* individually and in combination in induced diabetes in rats. The efficacy of these herbal extracts was also compared with that of glibenclamide, a novel cardinal antidiabetic drug individually and in combination. Also phytochemical properties of *Trigonella foenum graecum* and *Coccinia indica* extracts were evaluated.

The alcholic extracts *Trigonella foenum graecum* and *Coccinia indica* were found to be positive for flavonoids, saponins, alkaloid, bitter principles, coumarines, anthracene and glycosides, and *Coccinia indica* was negative for the bitter principle.

The study was conducted in Wistar albino rats which were divided into nine different groups. The various groups in this study included normal control (Group-I), diabetic control (Group-II), diabetic rats treated with glibenclamide (Group-III), diabetic rats treated with *Trigonella foenum graecum* (Group-IV), *Coccinia indica* (Group-V), combination of *Trigonella foenum graecum* and *Coccinia indica* (Group-VI), combination of *Trigonella foenum graecum* and glibenclamide half dose (Group-VII), combination of *Coccinia indica* and glibenclamide half dose (Group-VIII) and combination of *Trigonella foenum graecum, Coccinia indica* and glibenclamide half dose (Group-IX).

Diabetes was induced in rats of all the groups except normal control by intraperitoneal administration of STZ at the dose rate of 45mg/kg b.w. Diabetes status
was confirmed by estimating serum glucose levels and it was observed that all the rats
became diabetic with hyperglycaemia.

All groups of rats were subjected to evaluation of physiological, biochemical and
hematological parameters such as body weight, serum glucose, serum cholesterol, serum
triglyceride, serum AST, serum ALT, insulin level, antioxidant enzymes, hemoglobin,
TRC, TLC, platelets and the findings were correlated and confirmed with clinical signs,
histopathology and immunohistochemistry.

The rats of group-I remained healthy throughout the experiment as evaluated by
various biochemical and pathomorphological parameters.

The rats belonging to diabetic control group (Group-II) showed significant
decrease in the mean body weight throughout the experiment. The animals of all the
treatment groups (Groups III to IX) revealed an improvement in their body weight. The
mean body weight values in the combined treatment group were comparable with those
of glibenclamide, *Trigonella foenum graecum* and *Coccinia indica* treatment groups on
all the intervals of the study. There was a synergetic effect with increase in the mean
body weight in combined treatment groups.

There was a significant increase \((P \leq 0.001)\) in serum glucose values of diabetic
control animals from Day 3 post STZ injection and progressively increased till the end of
the study. All the treatments used in the present study were effective in improving serum
glucose levels. However, blood glucose lowering effect was better in combined treatment
groups when compared to individual treatment groups, especially groups IX and
in addition combined glibenclamide groups also had better hypoglycemic effect compared to other groups.

The serum cholesterol and triglyceride levels in diabetic control rats were significantly higher (P≤0.001) as compared to normal control at all the intervals of time. Treating diabetic rats with glibenclamide produced a significant decrease in the serum cholesterol and triglyceride levels. The various treatment groups used in the present study were also effective in improving the serum cholesterol and triglyceride levels. The groups VI and IX showed synergetic effect in controlling the hyperlipidemic state in induced diabetes mellitus in rats and the values were comparable to those of control and glibenclamide treatment group.

The serum ALT and AST values in diabetic control rats were progressively increased throughout the experiment compared to normal control and the increase was statistically significant (P≤0.001). All the treatment groups (groups-III to IX) showed a significant reduction in mean serum ALT and AST levels compared to diabetic control. However, in maintaining the ALT and AST values, groups VI and IX showed better effect than glibenclamide and other treatment groups.

A gradual and significant reduction in serum insulin was noticed in the diabetic control group at all the time intervals compared to normal control. Though, the mean serum insulin values of all the treatment groups (Groups-III to IX) were lower compared to normal control, they were significantly higher compared to diabetic control and the increase was gradual. When compared to normal control animals on 90th day a moderate
variation in groups VI and IX indicating moderate synergetic effect of these groups compared to other treatment groups and glibenclamide treatment group was found.

There was a significant decrease in the mean liver antioxidant enzyme activities of SOD, CAT and GPx of diabetic rats compared to normal control animals. All the treatment groups showed significant improvement in liver antioxidant enzyme activities in comparison with diabetic control animals. However, groups VI and IX showed better antioxidant activity compared to glibenclamide control. Also compared to individual treatment groups of Trigonella foenum graecum and Coccinia indica, group IX animals showed better improvement.

The diabetic control rats showed a decrease in mean haemoglobin percentage values as compared to normal control. Animals of all other groups showed a significant increase in Hb values as compared to diabetic control rats. The effect of herbal extracts both individually and in combinations was comparable to that of glibenclamide treatment group in improving Hb values.

The diabetic rats recorded a moderate decrease in TRC, TLC and platelet count compared to normal control animals. There was a improvement in blood cells counts of the treatment groups (III to IX) compared to diabetic animals. They were comparable with those of normal and glibenclamide control animals. Combined treatment groups showed better improvement in blood cell counts compared to the individual treatment groups of Trigonella foenum graecum and Coccinia indica extracts.
The Flow cytometry analysis of group-II (Diabetic control) rats showed significantly lower CD4+ and significantly higher CD8+ values compared to normal control. The rats of treatment groups (III to IX) showed improvement in values of CD4+ and CD8+ when compared to diabetic control animals. Compared to other treatment groups, among these, groups IV, VI and IX showed better improvement.

Clinical signs of STZ induced diabetic rats were comprised of polyurea, polydypsia, polyphagia, weight loss and poor body condition. The severity of the signs was progressive as the time advanced. Treatment groups (groups-III to IX) revealed a decrease in the severity and extent of the above mentioned signs from 15th to 90th day of treatment.

Grossly, in the diabetic control rats, pancreas was slightly congested, reduced in size and appeared as thin gelatinous strip. Other organs such as liver, spleen, kidney, lungs, intestines, stomach, brain and heart did not show any gross lesions throughout the experiment. Similar lesions were observed grossly in treatment groups also but the extent and severity of the lesions reduced, progressively on treatment.

Microscopically, in diabetic rats, both exocrine and endocrine pancreas was affected. Exocrine pancreas revealed loss of architecture with vacuolated, degenerated and necrotic cells in the acini. In endocrine pancreas there was a decrease in the number of islets, vacuolar degenerations, and loss of granularity, apoptosis and necrosis of cells in a progressively increasing manner.
There was a progressive reconstruction of normal architecture of acini and islets in pancreas in all the treatment groups in the present study. The exocrine portion of acini revealed improvement which was comparable to glibenclamide treatment. In endocrine pancreas compared to glibenclamide, Groups VIII and IX treatment groups revealed better improvement with islets of bigger size and more compact arrangement. Tendency to form new islets near the blood vessels were constant feature seen in many of the lobules in all treatment groups.

Immunohistochemical demonstration of insulin showed drastic reduction in the number of insulin positive cells in the diabetic group. There was a improvement in number of insulin positive cells in all treatment groups. However, Groups VIII and IX showed better regeneration of β-cell population which was comparable to that of control group (I) indicating the synergistic action of plant extracts and half dose of glibenclamide compared to individual treatment groups of *Trigonella foenum graecum*, *Coccinia indica* and glibenclamide.

Special staining for β-cells revealed complete destruction of β-cells with relative increase in α-cells in diabetic animals. Islets of various treatment groups revealed hypercellularity with improvement in α and β-cell population. However, normal α: β ratio was altered as compared to normal control group.

Histopathological examination of liver in diabetic animals revealed vacuolar degenerations, increased cytoplasmic granularity, and obliteration of sinusoidal space and mild infiltration of mononuclear cells. The lesions in the liver of treatment animals showed an improvement in the architecture of the organ compared to diabetic animals.
with reduction of lesions like cell swelling, vacuolations and granularity of the cytoplasm.

Microscopically spleen revealed mild depletion of lymphocytes from the follicles. There was a progressive improvement in the lymphoid mass of the spleen in treatment groups.

There were no detectable microscopic lesions observed in kidney, heart, lungs, intestine, stomach, brain and spleen in any of the control and treatment groups.

**Conclusion**

- Diabetes mellitus could be induced effectively by using STZ at the dose of 45 mg/kg intraperitonially in laboratory rats

- The *Trigonella foenum graecum* and *Coccinia indica* extracts have a good antioxidant effect compared to glibenclamide and have a synergistic effect in alleviating the free radical injury induced by STZ.

- There is a synergistic effect between the two herbal extracts in improving liver enzymes levels, hyperlipidaemic state, antioxidants and in immunomodulation.

- Both *Trigonella foenum graecum* and *Coccinia indica* extracts have potential antidiabetic activity but cannot be used as sole replacement therapeutic agents for conventional antidiabetic drugs. However can be used as adjunct to antidiabetic drugs.
• *Coccinia indica* along with half dose of glibenclamide has a synergistic effect in improving insulin level and number of beta cells in bringing about regeneration of beta cells and in increasing the insulin production and secretion.

• *Coccinia indica* in combination group is better than *Trigonella foenum graecum* in providing hypoglycaemic effect and gives an opportunity to reduce the dose of glibenclamide, and may help in minimizing the adverse effect of glibenclamide as well as in achieving enhanced therapeutic effect.

• Lastly the present study high lights that the indigenous medicinal plants can be used successfully as an alternative treatment in the management of diabetes with or without antidiabetic drugs.
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VIII. ABSTRACT

The present study was taken up to evaluate the efficacy of *Trigonella foenum graecum* and *Coccinia indica* individually and in combination along with glibenclamide in streptozotocin induced diabetic rats for a period of 90 days. The various groups in this study included normal control (Group-I), diabetic control (Group-II), diabetic rats treated with glibenclamide (Group-III), diabetic rats treated with *Trigonella foenum graecum* (Group-IV), diabetic rats treated with *Coccinia indica* (Group-V), diabetic rats treated with *Trigonella foenum graecum and Coccinia indica* (Group-VI), diabetic rats treated with *Trigonella foenum graecum* and glibenclamide (Group-VII), diabetic rats treated with *Coccinia indica* and glibenclamide (Group-VIII). Diabetic rats treated with *Trigonella foenum graecum, Coccinia indica* and glibenclamide (Group-IX) respectively.

There was significant variation in biochemical and pathomorphological parameters of diabetic rats when compared to normal control rats. The alleviation of the diabetic and its complications induced by streptozotocin was observed in all the treatment groups with variable degree of improvement. *Trigonella foenum graecum* and *Coccinia indica* extracts were effective in alleviating streptozotocin induced diabetes and were comparable with glibenclamide. Combination of *Trigonella foenum graecum* with glibenclamide and *Coccinia indica* with glibenclamide showed better improvement compared to individual extracts alone and improvement was statistically significant. However the combined treatment of *Coccinia indica* with glibenclamide half dose and *Trigonella foenum graecum* and *Coccinia indica* with glibenclamide half dose revealed a very good antidiabetic effect with reference to improvement in insulin level and beta cell number which indicated a synergistic effect between *Coccinia indica* and glibenclamide half dose.

**Key words:** Diabetes, *Trigonella foenum gaecum, Coccinia indica*, glibenclamide, streptozotocin