PATHOMORPHOLOGICAL STUDIES ON INDUCED DIABETES IN RATS AND EVALUATION OF ANTIDIABETIC EFFICACY OF *ALOE VERA* AND *MURRAYA KOENIGII* PLANT EXTRACTS

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CERTIFICATE

This is to certify that the thesis entitled **"PATHOMORPHOLOGICAL STUDIES ON INDUCED DIABETES IN RATS AND EVALUATION OF ANTIDIABETIC EFFICACY OF *ALOE VERA* AND *MURRAYA KOENIGII* PLANT EXTRACTS"** submitted by **Mr. MANJUNATHA, S. S., I.D. No. DVHK-1116** in partial fulfillment of the requirements for the award of **DOCTOR OF PHILOSOPHY** in **VETERINARY PATHOLOGY** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of bonafide research work carried out by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis of the award of any degree, diploma, association ship, fellowship or other similar titles.

Bengaluru
June, 2017

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Dr. N. B. SHRIDHAR

Affectionately Dedicated to

My family members

and

my guide

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LIST OF ABBREVIATIONS

%	:	Per cent
µ	:	Micron
µl	:	Microlitre/s
^{0}C	:	Degree Celsius
ADP	:	Adenosine diphosphate
ALT	:	Alanine amino transferase
ANOVA	:	Analysis of variance
APES	:	3-aminopropyltriethoxysilane
AST	:	Aspartate amino transferase
ATP	:	Adenosine triphosphate
bw	:	Body weight
BSA	:	Bovine serum albumin
CAT	:	Catalase
CCl_4	:	Carbon tetrachloride
DAB	:	3,3-diamine benzidine tetrahydrochloride
DM	:	Diabetes mellitus
DNA	:	Deoxyribonucleic acid
DPPH	:	α- α diphenyl β picryl hydrazyl
EDTA	:	Ethylene diamino tetra acetic acid
FITC	:	Fluorescein isothiocyanate
Fl	:	Femtoliters
g	:	Gram/s
GPx	:	Glutathione peroxidase
GSH	:	Glutathione
h	:	hours
H_2O_2	:	Hydrogen peroxide
Hb	:	Hemoglobin
HCl	:	Hydrochloric acid
HPLC	:	High performance liquid chromatography
HPTLC	:	High Performance Thin Layer Chromatography
HRPO	:	Horse Radish Peroxidase
IDDM	:	Insulin Dependent Diabetes Mellitus

IgG	:	Immunoglobulin G
IHC	:	Immunohistochemistry
IRMA	:	Immunoradiometric assay
IU/L	:	International units per liter
kg	:	Kilogram
KOH	:	Potassium hydroxide
L	:	Liter
M	:	Molar
mg	:	Miligram
mg/dL	:	Milligram per deciliter
ml	:	Mililitre/s
mM	:	Milimolar
Na_2CO_3	:	Sodium carbonate
NAD	:	Nicotinamide adenine dinucleotide
NBF	:	Neutral buffered formalin
NIDDM	:	Non Insulin Dependent Diabetes Mellitus
nm	:	Nanometer
PARP	:	Poly ADP-ribose polymerase
PBS	:	Phosphate Buffer Saline
pg	:	Picogram/s
pmol	:	Pico mole/s
RIA	:	Radioimmunoassay
rpm	:	Revolutions per minute
SE	:	Standard error
SOD	:	Superoxide dismutase
STZ	:	Streptozotocin
TNF-α	:	Tumor necrosis factor alpha
UV	:	Ultraviolet
WHO	:	World Health Organisation
α	:	Alpha
β	:	Beta
µg/kg	:	Micro grams per kilogram
µU/L	:	Micro units per liter

Introduction

I. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder which prevents the body to utilize glucose completely or partially. It is characterized by increased glucose concentration in the blood leading to altered carbohydrate, protein and fat metabolism. It is a group of metabolic diseases depicting high blood sugar levels, which results from defects in insulin secretion or action or both. Hyperglycemia and hyperlipidemia are two important characters of diabetes mellitus in which diabetic patients experience various vascular complications such as atherosclerosis, coronary heart disease, diabetic retinopathy, nephropathy and neuropathy (Sheetz and King, 2002).

The prevalence of diabetes is growing at an alarming rate globally and is reaching epidemic proportions. The current global prevalence of 415 million is projected to increase to 642 million by 2040 if preventive measures are not put in place. Over 60 per cent of the world's population with diabetes resides in Asia of which India and China contribute the largest (Prasanna Kumar *et al.,* 2017).

It is considered to be the complex group of disorder and two types of DM are most commonly prevalent. Complete apoptosis of insulin secreting β- pancreatic cells, resulting in the state of absolute deficiency of insulin is Type I (Juvenile onset) DM. As a matter of fact, autoimmune destruction of nearby 90% β-cells due to genetic pre-disposition becomes responsible for Type 1 DM (Ozougwu *et al.*, 2013). It is also called as insulin-dependent diabetes mellitus (IDDM) which is mainly due to less production of insulin.

Type 2 Diabetes mellitus or maturity onset diabetes mellitus accounts for 90% patients of DM caused by insufficient pancreatic insulin secretion (partial apoptosis of β-cells) or insulin resistance in peripheral tissues (Spellman, 2010). Type 2 DM is the most prevalent type of DM since 90% patients of DM are of this category **(**Mahmoud *et al.*, 2013). It is also called as non-insulin-dependent diabetes mellitus (NIDDM) which is mainly due to inability of body cells to respond to the insulin produced.

It is considered to be 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 (Hoenig, 2002). 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 in equines, bovines, ovine, swine, primates as well as birds.

In many animal species such as monkeys, cats, dogs, tree shrews, ground squifiels, foxes, dolphins, hippopotami, antelopes and most farm animals spontaneous diabetes is a common occurrence and all those occasionally become diabetic (Mordes and Rossini, 1981). Both spontaneous and experimental animal models have been used effectively to study etiology, complications, treatment and prevention of diabetes.

The first line of treatment in diabetes mellitus 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). Insulin therapy is considered to be the only satisfactory approach in diabetic mellitus, though it has several drawbacks

like insulin resistance, anorexia, brain atrophy and fatty liver in chronic treatment and episodes of hypoglycaemia (Weidmann *et al*., 1993).

Many studies have been carried out to prove the efficacy of herbal extracts in controlling hyperglycemia and in management of diabetes. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities. During the past few years, some of the new bioactive drugs isolated from hypoglycemic plants exibited antidiabetic activity with more efficacy than oral hypoglycemic agents. Several drugs such as biguanides and sulfonylureas are presently available to reduce hyperglycemia in diabetes mellitus but these drugs have side effects and thus searching for a new class of compounds is essential to overcome these problems. (Rao *et al*., 2001). The search for appropriate hypoglycemic agents has been focused on herbal products used in traditional medicine.

The use of herbs as hypoglycemic agents is a major avenue in Indian perspective particularly for treating diabetes, is required to be explored more effectively (Mukherjee and Wahile, 2006). Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (Venkatesh *et al*., 2003). Therefore, investigation on such agents from traditional medicinal plants has become more important (Suba *et al*., 2004). Many traditional plants with anti-diabetic effects have been identified but only a few 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 of enough scientific and clinical data proving their efficacy and safety.

Statistical study evidence indicated combination of allopathic and poly-herbal formulation has a better control over the individual treatment in managing diabetic complications and may be an alternative medication for T2DM. The pharmacokinetic results showed that poly-herbal formulation influenced glibenclamide when being co-administered and increased bioavailability of glibenclamide. Poly-herbal formulation could be useful for the diabetic patients on glibenclamide therapy, to obtain a better control over the blood glucose and lipid levels (Rashmi *et al.,* 2017). In the present scenario, there is a need for studies on replacement of oral allopathic antidiabetic treatment with herbal medicines by experimental research in animal models as they have no side effect and can be used for a prolonged period.

With this knowledge, the present study was carried out to evaluate the antidiabetic efficacy of the aqueous extract of *Aloe vera* and *Murraya koenigii* individually and in combination in comparison with a proven allopathic antidiabetic drug glibenclamide by hematological, biochemical, immunological, pathological and immunohistochemical methods, with the following objectives:

1. To evaluate the phytochemical properties of *Aloe vera* and *Murraya koenigii* extracts.
2. To evaluate the antidiabetic effect of *Aloe vera* and *Murraya koenigii* 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 *Aloe vera* and *Murraya koenigii* with an oral hypoglycemic agent glibenclamide.

Review of Literature

II. REVIEW OF LITERATURE

Diabetes mellitus is a complex and multifarious group of disorder characterized by hyperglycemia that has reached epidemic proportion in the present century. It is caused due to deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves. Several allopathic and herbal preparations are used for treatment and control of diabetes.

The literature related to the present study titled "Pathomorphological studies on induced diabetes in rats and evaluation of antidiabetic efficacy of *Aloe vera* and *Murraya koenigii* plant extracts" is reviewed under the following headings.

2.1 Diabetes mellitus

The pharmacopoeia of ancient India listed specific treatments for diabetes, including dietary modifications, medicinal plant remedies, and minerals. These historical accounts reveal that Non-Insulin Dependent Diabetes Mellitus (NIDDM) was well known among the ancients and that medicinal plants have been used for millennia to treat this disease (Oubre *et al.,* 1997).

King *et al.* (1998) recorded the prevalence of diabetes in human beings worldwide which was estimated to be 4.0 per cent in 1995 and likely to touch 5.4 per cent by the year 2025. Higher prevalence was reported in developed countries than in developing

countries and was recognized as a serious global health problem affecting over 150 million people and is predicted to increase to 300 million or more by the year 2025.

Rathmann and Giani (2004) found the prevalence of diabetes to be higher in men than women. The most important demographic change to diabetes prevalence across the world appeared to be the increase in the proportion of people with more than 65 years of age.

The incidence of diabetes in cats ranged from 1:50 to 1:400 of population and Diabetes mellitus was considered to be one of the most frequently diagnosed endocrinopathies in cats and dogs and the increased incidence was attributed to predisposing factors such as obesity and physical inactivity (Baral *et al.,* 2003). The prevalence of the disease was found to be more in middle-aged and older dogs while the incidence is further increasing (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 more common form in cats (Rand *et al.,* 2004).

Canine diabetes was 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 was no evidence for a canine equivalent of human type 2 diabetes and late onset and slow progression of β cell dysfunction resembles autoimmune diabetes of humans.

Fall *et al*. (2007) reported an incidence of 13 cases of type 1 diabetes mellitus for every 10000 dogs. Miniature Poodles, Dachshunds and Terriers were found to be predisposed to Type 1 Diabetes where there is β cell degeneration. T_2DM is commonly observed in obese cats due to failure of peripheral tissue to respond to insulin caused by downregulation of insulin receptors, which is initially reversible.

The frequency of diabetes mellitus for cats during the five-year study period recorded prevalence of 7.4 per 1000 cats. There was significantly higher incidence in Burmese cats (22.4 per 1000) than domestic short and long haired cats (7.6 per 1000). The mean age of first diagnosis was higher among Burmese cats (13.6 years) compared to domestic short and long haired cats (10.9 years) (Lederer *et al.,* 2009).

Hua *et al.* (2012) observed insulin resistance in patients long before the development of type 2 diabetes and stated that Prediabetes was clinically characterized by hyperinsulinemia and hyperglycemia which was due to inadequate expression and conformation of insulin receptors on the plasma membranes of various tissues.

Prevalence of diabetes in human beings worldwide was estimated to be 8.3 per cent with 387 million people currently living with diabetes and expected to rise to 592 million by 2035. One in two people with diabetes do not know that they have it and the world has recorded 4.9 million deaths due to diabetes in 2014. In South-east Asia, approximately 75 million people are diabetic and the disease is recognized as a serious global health problem affecting the world (International Diabetes Federation, 2014).

Yamada *et al*. (2015) stated that failure of β cell adaptation leading to type 2

diabetes onset with declining insulin secretion and β cell mass. β cell function then deteriorates, particularly in individuals with poor glycemic control and eventually becomes irreversible despite glucotoxicity treatments providing temporary improvements in the dysfunction to some extent.

2.2 Induction of diabetes

Rakieten *et al.* (1963) reported that streptozotocin injection leads to specific necrosis of the pancreatic β cells leading to diabetes mellitus in animals inhibiting insulin secretion thus progressing to insulin-dependent diabetes mellitus.

Diabetes was induced by injecting streptozotocin which resulted in histopathological alterations of pancreatic islets β-cell because of decrease in the β-cell nicotinamide adenine dinucleotide (NAD) concentration (Karunanayake *et al.*, 1974).

Ganda *et al.* (1976) frequently used i.v. dose of STZ to induce IDDM between 40-60 mg/kg bw and found that the range of STZ dose required to induce diabetes was not as narrow as in case of alloxan.

Streptozotocin could induce diabetes mellitus in many animal models and the induced diabetes resembled the human hyperglycaemic non-ketotic diabetes mellitus in all aspects (Weir *et al.*, 1981).

Kroncke *et al.* (1995) stated that STZ acts as a nitric oxide (NO) donor and was found to bring about the destruction of pancreatic islet cells which contributed to STZ-induced DNA damage.

Li *et al.* (2000) following multiple low dose of STZ administration in mice observed hyperglycaemia from 7th day with occurrence of insulitis and sparse islet cell area morphometrically with reduction of beta cells.

Streptozotocin or streptozocin or izostazin or zanosar (STZ) is a synthetic antineoplastic agent, an anti-tumor antibiotic and chemically is related to other nitroureas used in cancer chemotherapy. It is used to induce both IDDM and NIDDM. STZ enters the P-cell *via* a glucose transporter (GLUT-2) and causes alkylation of DNA. STZ liberates toxic amounts of nitric oxide (NO) that inhibits aconitase activity and participates in DNA damage. As a result of the STZ action, p-cells undergo destruction by necrosis **(**Szkudelski, 2001).

Wada and Yagihashi (2004) stated that the toxicity of β-cell caused by STZ involves 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.

Following the uptake of STZ into the β cells via the GLUT-2 glucose transporter, it splits into glucose and methylnitrosourea moiety. Owing to its alkylating properties, methylnitrosourea modify the biological macromolecules, fragment DNA and destroy the β cells causing a state of insulin dependent diabetes (Lenzen, 2008).

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 **(**Raza and John, 2012).

Kante and Reddy (2013) Stated that Streptozotocin selectively destroys β cells of pancreas by generating excess ROS and carbonium ion leading to DNA breaks by alkylating DNA bases. The N-nitroso-N methylurea portion of the molecule exhibits diabetogenic activity. Glucose may act as carrier for this cytotoxic group.

Shinde *et al.* (2014) used streptozotocin as a diabetogenic agent in rats and observed a gradual increase in blood glucose level from mean value of 272.67 mg/dl on day 1 in animals treated with STZ when compared to non-induced group having blood glucose level of 96 to 105 mg/dl.

2.3 Glibenclamide

Koltermann *et al.* (1984) stated that Glibenclamide acts both on insulin secretion and insulin action thus improving the level of glucose. Two main metabolites of glibenclamide (glyburide) have a hypoglycemic effect in humans by means of increased insulin secretion.

Luzi and Pozza (1997) hypothesized that glibenclamide initiates its action through binding with its receptors on the β-cell surface leading to decrease in the conductance of ATP-sensitive K^+ channels which results in more K^+ efflux and calcium influx in β-cells which eventually determine insulin secretion.

Glibenclamide impairs the recovery of glucose from insulin induced hypoglycaemia and significantly reduces blood glucose levels in normal rats. Further

hypothalamic ATP sensitive K^+ channels play significant regulatory role in peripheral glucose homeostasis (Zhang and Roane, 2001).

Serrano-Martin *et al.* (2006) stated that glibenclamide 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 resulting in stimulation of insulin release.

Prolonged exposure of rat pancreatic β-cells to the insulin secretagogue glibenclamide induces a sustained increase in basal insulin synthesis and it was proved by Wang *et al.* (2008) through *in-vitro* experimentation with cultured rat β-cells.

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.

Streptozotocin induced diabetes in rats treated with glibenclamide, showed decreased serum glucose, cholesterol, triglyceride and VLDL and improved P cell activity (Mallikarjuna, 2009; Pragathi, 2011; Nasreen, 2012; Mudasir, 2013; Manjunatha, 2013; Shesha Rao, 2013; Gurikar, 2014; Kavitha Rani, 2015).

2.4 Herbs in diabetes

Oral hypoglycemic agents such as biguanides, sulphonylureas and thiozolidinediones 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 *et al.,* 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).

The therapeutic preparations in traditional medicines contain a variety of herbal and non-herbal ingredients that are thought to act on a variety of targets by various modes and mechanisms. The beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring integrity and function of pancreatic cells, insulin releasing activity, improving glucose uptake and utilization and the antioxidant properties present in medicinal plants offer an exciting opportunity to develop them into novel therapeutics. Medicinal plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meagre. Many studies have confirmed the benefits of medicinal plants with hypoglycemic effects in the management of diabetes mellitus. The effects of these plants may delay the development of diabetic complications and correct the metabolic

abnormalities. Moreover, during the past few years some of the bioactive drugs isolated from hypoglycaemic plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy **(**Bnouham *et al.,* 2006).

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.,* 2006).

Herbal treatments for diabetes have been used in patients with insulin-dependent and non-insulin-dependent diabetes, diabetic retinopathy, diabetic peripheral neuropathy and nephropathies. Scientific validation of several Indian plant species has proved the efficacy of the botanicals in reducing the sugar level. There are several plants known for their antidiabetic activity with different mode of action and phytoconstituents. Botanicals have a major role to play in the management of diabetes, which needs further exploration for necessary development of drugs and nutraceuticals from natural resources (Mishra *et al.,* 2010)

2.4.1 *Aloe vera*

Aloe vera a member of liliaceae family *is* an ornamental and medicinal plant. It is a cactus like plant with green, dagger-shaped leaves that are fleshy, tapering, spiny, marginated and filled with a clear viscous gel. *Aloe vera (Aloe barbadensis)* is a perennial succulent xerophyte, which includes about 360 species.

Aloe vera has been used for many centuries for its curative and therapeutic properties. Although, over 75 active ingredients from the inner gel have been identified,

therapeutic effects have not been correlated well with each individual component. Many of the medicinal effects of *Aloe* leaf extracts have been attributed to the polysaccharides found in the inner leaf parenchymatous tissue, but it is believed that these biological activities should be assigned to a synergistic action of the compounds contained therein rather than a single chemical substance.

2.4.1.1 Phytochemical analysis

Tanaka *et al.* (2006) evaluated the anti-hyperglycemic effect of *Aloe vera* gel and isolated a number of compounds from the gel. On the basis of spectroscopic data, these compounds were identified as lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartenol and 24-methylene-cycloartanol. Those five phytosterols were evaluated for their anti-hyperglycemic effects in type 2 diabetic mice.

Aloe vera plant is a store house of many phytochemicals, vitamins, nutrients and anti-oxidants. The main constituent of this mucilage are D-glucose and D-mannose, tannins, steroid, enzymes, plant hormones, amino acids, vitamins and minerals. Many of the health benefits associated with *Aloe vera* have been attributed to the polysaccharides contained in the gel of the leaves (Maenthalsong, 2007; Hossain *et al.,* 2013).

Arun Kumar and Muthuselvam (2009) carried out investigation on the screening of phytochemical compounds in different extracts of *Aloe vera*. The phytochemical compound screened by qualitative method indicated presence of tannin, saponin, flavonoids and terpenoids and phlobatannins and absence of phlobotannins & steroids. In the GC-MS analysis, 26 bioactive phytochemical compounds were identified in the ethanolic extract of *Aloe vera*.

The phytochemical screening and comparative study of antimicrobial activity of *Aloe vera* extracts was carried out by Yebpella *et al.* (2011) and phytochemical screening revealed the saponins, alkaloids, glycosides, tannins, protein, and flavonoids, while cardiac glycosides and steroids were absent.

The result of the phytochemical screening of aqueous extract of *Aloe vera* showed the presence of tannins, flavonoids, terpenoids, carbohydrates, and alkaloids. *Aloe vera* plant also showed presence of saponins, glycosides, phlobatannins, antiquinones, carbohydrates and steroids been absent (Ejoba *et al.*, 2012 and Raphael, 2012).

2.4.1.2 *Aloe vera* in diabetes study

Ghannam *et al.* (1986) noticed *Aloe vera* leaf extract increasing the glucose tolerance in both normal & diabetic experiment animals. He also recorded significant hypoglycaemic effect on both clinical and experiment animals and human beings due to *Aloe vera* leaf extract.

The hypoglycemic effect of aloes and its bitter principle may be mediated through stimulating synthesis and/or release of insulin from the β-cells of Langerhans. **(**Ajabnoor, 1990**)**

Administration of the superficial layer of the *Aloe* leaf (leaf skin) to streptozotocin induced diabetic mice, significantly depressed hyperglycaemia and examination of the tissue section under the light microscope revealed less denaturation and necrosis of islets beta-cells indicated that aloe relieves the diabetic condition by direct hypoglyceration (blood glucose lowering activity) and activates beta-cells **(**Beppu *et al.*, 1993).

Effect of *Aloe vera* juice in combination with glibenclamide was investigated in diabetic patients and found that there was no response to glibenclamide alone but *Aloe vera* juice significantly reduced levels of fasting blood glucose within two weeks and of triglycerides within four weeks. It showed no effect on cholesterol levels and had no toxic effects on kidney or liver function as assessed by blood chemistry. The results support the use of *Aloe vera* in the treatment of diabetes **(**Bunyapraphatsara *et al.*, 1996).

Oral administration of *Aloe vera* might be a useful adjunct for lowering blood glucose in diabetic patients as well as for reducing blood lipid levels in patients with hyperlipidaemia **(**Vogler and Ernst, 1999).

Aloe vera leaf pulp extract showed hypoglycaemic activity on IDDM and NIDDM rats, the effectiveness being enhanced for type II diabetes in comparison with glibenclamide. On the contrary, *Aloe vera* leaf gel extract showed hyperglycaemic activity on NIDDM rats. They indicated that the pulps of *Aloe vera* leaves devoid of the gel could be useful in the treatment of non-insulin dependent diabetes mellitus **(**Okyar *et al.*, 2001).

Helal *et al.* (2003) in their study reported highly significant decrease in blood glucose and increase in liver glycogen content and serum insulin level in the diabetic group treated with aqueous extract of *Aloe vera* when compared with diabetic untreated rats. He also noticed significant increase of body weight gain and liver glycogen content and stated *Aloe vera* as antidiabetic plant.

Rajasekaran *et al.* (2004) evaluated the hypoglycemic activity in the alcoholic extract of *Aloe vera* gel. He observed effect of oral administration of *Aloe vera* extract at a concentration of 200 and 300 mg/kg of body weight on (a) normal fasted rats, (b) oral glucose-loaded rats, and (c) streptozotocin-induced diabetic rats and indicated that *Aloe vera* extract maintains the glucose homeostasis by controlling the carbohydrate metabolizing enzymes.

Oral administration of *Aloe vera* gel extract at a dose of 300 mg/kg bodyweight per day to STZ-induced diabetic rats for a period of 21 days resulted in significant reduction of fasting blood glucose, hepatic transaminases (aspartate aminotransferase and alanine aminotransferase), plasma and tissue (liver and kidney) cholesterol, triglycerides, free fatty acids and phospholipids and a significant improvement in plasma insulin. In addition, the decreased plasma levels of high-density lipoprotein-cholesterol and increased plasma levels of low-density lipoprotein-and very low-density lipoprotein-cholesterol in diabetic rats were restored to near normal levels following treatment with the extract **(**Rajasekaran *et al.*, 2006).

Tanaka *et al.* (2006) evaluated the anti-hyperglycemic effect of *Aloe vera* gel and isolated a number of compounds from the gel. On the basis of spectroscopic data, these compounds were identified as lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartenol and 24-methylene- cycloartenol and noticed that *Aloe vera* gel and phytosterols derived from *Aloe vera* gel had long-term blood glucose level control effects which were responsible for the treatment of type 2 diabetes mellitus.

Aloe vera gel has therapeutic properties such as immunostimulation, anti-inflammatory effect, wound healing, promotion of radiation damage repair, anti-bacterial, anti-viral, anti-fungal, antidiabetic and anti-neoplastic activities, stimulation of haematopoiesis and antioxidant effects **(**Reynolds and Dweck, 1999; Talmadge *et al.*, 2004**;** Ni *et al.*, 2004).

Noor *et al.* (2008) studied the beneficial effects of *Aloe vera* in streptozotocin-induced diabetic rats and diabetic induced rats fed with *Aloe vera* (300 mg/kg bw), the fasting plasma glucose levels were reduced to normal and body weight was found to be increased. In the pancreatic sections of diabetic rats fed with *Aloe vera* they noticed that the islets were comparable to normal rats.

Afaf *et al.* (2008) evaluated the antidiabetic activity of ethanolic extract of *Aloe vera* in induced hyperglycaemic and normal rats which revealed highly significant decrease in plasma glucose in the group which received 500 mg/kg body weight *Aloe vera* ethanolic extract. However, the reduction in plasma glucose level at a dose rate of 100 mg/kg body weight *Aloe vera* ethanolic extract and glibenclamide was similar.

Ayesha *et al.* (2008) studied the beneficial effects of *Aloe vera* in streptozotocin induced diabetic rats and noticed significant decrease in the fasting plasma glucose & increase in the body weight in the diabetic rats fed with *Aloe vera* (300 mg/kg bw).

Oral administration of PAG (Processed *Aloe vera* gel) for 8 weeks reduced circulating blood glucose concentrations to a normal level in DIO mice. PAG appeared to lower blood glucose levels by decreasing insulin resistance and oral administration of

PAG prevented progression of NIDDM-related symptoms in high-fat diet-fed mice suggesting that PAG could be useful for treating NIDDM **(**Kim *et al.*, 2009).

Aloe vera L. high molecular weight fractions produced significant decrease in blood glucose level which sustained for about 6 weeks and significant decrease in triglyceride 4 weeks after the treatment was observed. Oral administration of *Aloe vera* gel extract resulted in a significant decrease in serum glucose, total cholesterol and triglycerols in treated diabetic group as compared with diabetic control group **(**Mohamed, 2011).

Lanjhiyana *et al. (*2011) noticed the potential hypoglycaemic activity of *Aloe vera* in oral glucose tolerance test and normoglycemic rats and antidiabetic activity in alloxinised rats.

Gupta *et al.* (2011a) noticed significant reduction in fasting blood glucose levels and HbA1C in alloxon induced diabetic rabbits treated with *Aloe vera* leaf extract for 21 days and also a significant decrease in serum levels of triglycerides (TG), total Cholesterol (TC) and LDL Cholesterol indicating *Aloe vera* as a potent Antidiabetic drug.

Rehman *et al.* (2011) investigated the antidiabetic effect of *Aloe vera* water extract in normal and alloxon induced diabetic rats and noticed significant reduction in blood glucose level after administering *Aloe vera* water extract for 30 days orally.

Manjunath *et al.* (2016) evaluated the hypoglycemic effect of *Aloe vera* leaf dried powder and compared it with standard metformin in alloxan-induced diabetic rats. He noticed that elevated blood glucose levels in diabetic rats were reduced by the treatment

with *A. vera* leaf extract at doses of 200 mg/kg and 400 mg/kg which was comparable to 50 mg/kg of metformin with no statistically significant difference.

2.4.2 *Murraya koenigii*

The botanical classification of *Murraya koenigii* is, kingdom- Plantae, class- Eudicots, order- Sapindales, family- Rutaceae, genus- Murraya, species- *M. koenigii*.

Murraya koenigii (Curry tree) L. Spreng., is a native plant of India. Leaves of *M. koenigii* are an essential ingredient of south Indian culinary practices. Interestingly, the leaves also have medicinal properties of being rich in antidiabetic and antioxidant principles.

2.4.2.1 Phytochemical analysis

The essential oil (0.169%) obtained by steam-distillation of the curry leaves was analysed and sixty-two constituents were identified. The major constituents were found to be caryophyllene (7.3%) and terpinen-4-01 (6.1%). Oil of curry leaf was subjected to programmed screening which yielded 27 positively identified components. The most important ones responsible for the intense characteristic aroma were, B-phellandrene, B-caryophyllene, B- gurjunene, B-elemene and bthujene. Curry leaf extractives were also available in water/oil soluble and emulsified form (Verghese, 1989; Zhu and Ding, 1991).

The phytochemical investigation of genus *Murraya koenigii* carried out so far has afforded around 200 compounds with varying structures. Among these constituents, xanthonoids, terpenoids, flavonoids, iridoid and seco-iridoid glycosides and few alkaloids and miscellaneous compounds form the major classes (Brahmachari *et al.*, 2004).

The *Murraya koenigii* leaves possess 66.3% moisture; 6.1 % protein; 1.0% fat (ether extract); 16.0% carbohydrate; 64.0 % fibre; 4.2% mineral matter; 810.0 mg calcium; 600.0 mg phosphorous; 3.1 mg Iron:12600 i.u. carotene (as vitamin A): 2.3 mg nicotinic acid and 4 mg/100 g vitamin C. The leaves are devoid of thiamine and riboflavin. The leaves are fair sources of vitamin A, a rich source of calcium. Due to the presence of oxalic acid in high concentration (1.35% total oxalates; 1.15% soluble oxalates) the nutritional availability of calcium was affected. The free amino acids present in the leaves are aspargine, glycine, serine, aspartic acid, glutamic acid, threonine, alanine, proline, tyrosine, tryptophan, y – amino butyric acid, phenylalanine, leucine, isoleucine and traces of ornithine, lysine, arginine and histidine. The leaves also contain a crystalline glucoside, koenigin and a resin. The twigs and leaves contain 0.8% potash on dry mater basis (Akhila and Subramanian, 2003 and Math and Balasubramaniam, 2005a).

Leaves of *Murraya koenigii* are aromatic and contain proteins, carbohydrates, fibre, minerals, carotene, nicotinic acid and vitamin C. It is rich in vitamin A. and calcium. The leaves contain high amount of oxalic acid, leaves also contains crystalline glycosides, carbazole alkaloids, koenigin, resin, fresh leaves contain yellow colour 2.5 % volatile oil. It also contains girinimbin, iso-mahanimbin, koenine, koenigine, koenidine and koenimbine. Triterpenoid alkaloids cyclomahanimbine, tetrahydromahanmbine also presents in the leaves. Murrayastine, murrayaline, pypayafoline carbazole alkaloids and many other chemical compounds have been reported in the leaves of *Murraya koenigii* (Bonde *et al.*, 2011).

The leaves of *Murraya koenigii* contain proteins, carbohydrate, fibre, minerals, carotene, nicotinic acid, Vitamin C, Vitamin A, calcium and oxalic acid. It also contains crystalline glycosides, carbazole alkaloids, koenigin, girinimbin, iso-mahanimbin, koenine, koenidine and koenimbine. Triterpenoid alkaloids cyclomahanimbine, tetrahydromahanimbine are also present in the leaves. Murrayastine, murrayaline, pyrayafoline carbazole alkaloids and many other chemicals have been isolated from *Murrya koenigii* leaves (Bhandari, 2012).

2.4.1.2 *Murraya koenigii* in diabetes study

Narayana and Sastry (1975) reported the hypoglycemic activity of *Murraya koenigii.* The aqueous extract of the leaves of *Murraya koenigii* after oral as well as intravenous administration to normal and alloxan diabetic dogs produced the hypoglycemia.

Murraya koenigii has been mentioned in the traditional medicinal system Ayurveda. Bark, root, leaves, fruits and fruit pulp of *Murraya koenigii* are widely used in the treatment of diabetes, obesity, vomiting, constipation, indigestion, diarrhoea, dysentery, piles, nausea, to relieve kidney pain etc.

Santhakumari *et al*. (1985) reported the hypoglycemic activity of crushed leaves of *Murraya koenigii* in rabbits, human volunteers and alloxan induced diabetic rats. Iyer and Mani (1990) reported that curry leaves powder supplementation (12 g providing 2.5 g fibre) to 30 non-insulin dependent diabetes mellitus patients for a period of 1 month resulted in the transient reduction in fasting and post-prandial blood sugar levels.

Khan *et al*. (1995) reported that the hypoglycemic activity of *Murraya koenigii* was attributed to increased glycogenesis and decreased glycogen lysis and gluconeogenesis. Methanol extract of *Murraya koenigii* leaves are reported to produce hypoglycemia in human volunteers and alloxan induced rats and rabbits **(**Bhat, 1995 and Rupashree, 1999).

Yadav *et al.* (2002) reported that feeding of diet containing various doses of curry leaf powder (5, 10 and 15%) to normal rats for 7 days as well as to mild and moderate diabetic rats for 5 weeks showed varying hypoglycemic and antihyperglycemic effect.

Grover *et al.* (2003) investigated the effects of daily oral feeding of 15% of powdered leaves of *Murraya koenigii* for 60 days on serum glucose concentrations in streptozotocin diabetic rats and noticed significant improvement. Thus this plant can be used as food adjuvants in diabetic patients.

Yadav *et al*. (2004) reported that the *Murraya koenigii* supplemented diet could reduce the development of insulin resistance and diabetes. Vinuthan *et al.* (2005) reported antidiabetic activity of methanol extract of *Murraya koenigii.*

Kesari *et al*. (2005) reported the hypoglycemic effect of aqueous extract of *Murraya koenigii* and found that a single oral administration of variable dose levels (200, 300 and 400 mg/kg) of aqueous extract led to lowering of blood glucose level in normal as well as in diabetic rabbits. The maximum fall of 14.68% in normal and 27.96% in mild diabetic was observed after 4 h of oral administration of 300 mg/kg. Xie *et al*. (2006)

reported the hypoglycemic and hypolipidemic activity of *Murraya koenigii* in ob/ob mice.

Arulselvan *et al.* (2006) evaluated the antihyperglycemic efficacy of *Murraya koenigii* in STZ-induced diabetic rats and found that oral administration of ethanolic extract of *M. koenigii* at the dose of 200 mg/kg/day for a period of 30 days significantly decreased the level of blood glucose in diabetic group of animals and also had insulin stimulatory effect.

Arulselvan and Subramanian (2007) evaluated the possible protective effects of *Murraya koenigii* leaves extract against B-cell damage and antioxidant defence systems of plasma and pancreas in streptozotocin induced diabetes in rats. The changes in the cellular antioxidant defence system were assayed in pancreatic tissue homogenate. The levels of glucose, insulin, enzymatic & non-enzymatic antioxidants were altered in diabetic rats which were reverted back to near control levels after treatment of *M. koenigii* leaves extract.

Kesari *et al.* (2007) in their study examined the effect of 1 month oral administration of *Murraya koenigii* aqueous leaves extract in normal and STZ induced severe diabetic rats at the dose of 300 mg/kg bw on various biochemical parameters. In case of diabetic animals, fasting blood glucose (FBG) levels of treated animals reduced by 48.2% and a fall of 19.2 and 30.8% in TC and 22.97 and 37.1% in TG levels were also observed in treated normal as well as diabetic rats, respectively.

Carbazole alkaloids a major phytochemical constituent of MK plant, has been found to have various biological activities like anti-oxidant, antidiabetic and lipid lowering effect (Tembhurne and Sakarkar, 2010). They estimated the effect of oral administration of *Murraya Koenigii* (300 and 500 mg/kg) on the level of blood glucose, glycosylated haemoglobin, TBARS and glycogen levels in streptozotocin induced diabetic rats and noticed that ethanolic extract of *Murraya koenigii* possessed potent antioxidant properties which may be due to the presence of biological active ingredient such as carbazole alkaloids, glycoside, triterpenoids and phenolic compounds.

The bark of *M. koenigii* is dark brown and creamish brown in colour and aromatic in odor. Total ash is around 10.15%, acid insoluble ash – 2.8%, water soluble ash 3.65% Moisture content 9.42% foaming index is 111.1 Phytochemicals present in *M. koenigii* bark are alkaloids, glycosides, saponins, flavonoids, coumarin (Kaur *et al*., 2011).

Lanjhiyana *et al.* (2011b) in their study observed that the methanolic extract of *Murraya koenigii* (200 mg/kg and 400 mg/kg) showed potential hypoglycemic activity in normoglycemic and diabetic rats, and further in OGTT method. Furthermore, there was an increase in final body weight, total hemoglobin, insulin, albumin and high density lipoprotein levels, however, decrease in fluid intake, glycosylated hemoglobin, urea, creatinine, total cholesterol, triglyceride and low density lipoprotein levels. They also suggested that the methanolic extract of *Murraya koenigii* found to be potential hypoglycemic compared to aqueous extract and petroleum ether extract and would be a promising candidate for the treatment of diabetes.

Yankuzo *et al.* (2011) carried out experiment in which oral administration of variable dose levels of the aqueous extract of *Murraya koenigii* for 30 days, produced significant dose dependant decrease in serum urea and creatinine levels ($p < 0.001$), and marked increase in the levels of plasma antioxidant capacity ($p < 0.01$) in diabetic treated rats, compared to the control (non-diabetic) rats.

Bhandari (2012) stated that carbazole alkaloids which are abundantly present in the leaves, fruits, roots and bark of *Murraya koenigii* have antidiabetic, anticancer, antibacterial, anti-nociceptive and antioxidant activities and provided a detailed report of the phytochemical, pharmacological, clinical and pre-clinical works carried out on this culinary plant and enlisted the therapeutic prospects.

Significant wound healing potential was noticed in diabetic hyperlipidemic rats was noticed when aqueous extract of *Murraya koenigii* at the dose of 300 mg/kg body weight was used (Kumar *et al.,* 2012).

Dhongade *et al.* (2013) compiled the work done by various researchers on traditional uses, phytochemistry, pharmacological activities, preclinical, clinical, toxicological and recent studies on *Murraya koenigii* plant and Saha and Mazumder (2013) explored the effect of *Murraya koenigii* leaves on paraoxonase 1 activity in order to control the oxidase stress in diabetes and aqueous *M. koenigii* extract decreased the blood sugar level significantly along with reducing oxidative stress by decreasing MDA level and by increasing GSH level, SOD as well as catalase activity. They also noticed the involvement of PON1 in decreasing the oxidative stress associated with diabetes.

Ekoh *et al.* **(**2014) determined the effect of aqueous extract of *Murraya koenigii* on lipid profile in alloxon-induced diabetic rats. Fasting blood sugar, total cholesterol and triglycerol significantly reduced in the diabetic rats treated with *Murraya koenigii* aqueous extract.

Saied *et al.* (2015) evaluated the phytochemical property of curry leaves and indicated that antioxidant property of curry leaves helped in protecting from diabetes, cardiovascular diseases, high cholesterol level and many other degenerative illnesses. They indicated that bioactive components in *Murraya koenigii* and the antioxidant property of curry leaves has a role in protecting from high cholesterol level, cardiovascular diseases, diabetes and many other degenerative illnesses.

2.5 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 the role of oxidative stress in development of complications in diabetes stated that oxygen free radical can initiate peroxidation of lipids, which in turn stimulate glycation of protein, inactivation of antioxidant enzymes and play a role in the long-term complications of diabetes.

Pattnaik and Mohanty (1995) in their study on herbal therapy for diabetes stated that many plant extracts and plant products have significant antioxidant activity, which

may be an important property of plant medicines associated with the treatment of several diseases including diabetes.

The status of lipid peroxidation was investigated in rats fed with *M. koenigii*. The concentration of melondiadehyde showed a significant decrease, while hydroperoxide and conjugated dines were significantly increased in the liver and the heart. Glutathione level in liver, heart and Kidney were lowered in rats after administering this plant. Glutathione reductase, Glutathione peroxidase, Glutathione transferase, SOD and catalase activity showed a sharp increase (Khan *et al.*, 1996).

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_2O_2 in diabetic tissues which inhibits Cu-Zn-SOD and 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_2O_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 **(**Telci *et al.*, 2000; Turk *et al.*, 2002).

Carbazole alkaloids isolated from *Murraya koenigii* are recognized as major sources of natural antioxidants and thus play an important role in the chemoprevention of diseases resulting from lipid peroxidation (Nakatani, 2000).

Methanol extracts (75%) of *Aegle marmelos, Momordica charantia, Trigonella foenum-graecum, Eclipta prostrata, Salacia oblonga, Coriandrum sativum, Vernonia anthelmintica* and *Murraya koenigii,* plants used extensively in the Indian system of medicine, were found to be potent inhibitors of lipid peroxide formation and scavengers of hydroxyl and superoxide radicals *in vitro.* Sabu and Kuttan (2002) indicated that oxidant induced alterations in the glucose utilizing system during diabetic manifestation is partially reversed by the administration of herbal extracts having antioxidant activity.

Baliga *et al.* (2003) reported the dose-dependent nitric oxide (NO) scavenging activity of aqueous leaf extract of *Murraya koenigii.* They suggested *Murraya koenigii* to be a potent and novel therapeutic agent for scavenging of NO and thus regulating pathological conditions caused by excessive generation of NO and its oxidation product, peroxynitrite.

It was suggested that an aryl hydroxyl substituent on the carbazole rings from the *Murraya koenigii* plays a role in stabilizing the thermal oxidation and rate of reaction against DPPH radical **(**Tachibana *et al.,* 2003).

Oral administration of ethanolic extract of *Aloe vera* at a concentration of 300 mg kg body weight for 21 days resulted in a significant reduction in fasting blood glucose, thiobarbituric acid reactive substances, hydroperoxides and alpha-tocopherol and significant improvement in ascorbic acid, reduced glutathione and insulin in the plasma of diabetic rats. Similarly, the treatment also resulted in a significant reduction in thiobarbituric acid reactive substances, hydroperoxides, superoxide dismutase, catalase and glutathione peroxidase and significant improvement in reduced glutathione in the

pancreas of STZ-induced diabetic rats when compared with untreated diabetic rats indicating ethanolic extract of *Aloe vera* more effective than glibenclamide in controlling oxidative stress and confirmed the ethnopharmacological use of *Aloe vera* in ameliorating the oxidative stress in diabetes (Rajasekaran *et al.,* 2005).

Resmi *et al.* (2006) in their study on antioxidant activity of *Albizzia lebbeck* in alloxan diabetic rats reported that there was a decline in the activities of antioxidant enzymes, such as SOD, CAT, GPX and GST 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 reduced in the liver and kidneys of diabetic rats.

Arulselvan and Subramanian (2007) studied on assessing the changes in the cellular antioxidant defence system such as the level of reduced glutathione and activities of superoxide dismutase, catalase and glutathione peroxidase in pancreatic tissue homogenate and recorded altered levels of glucose, glycosylated hemoglobin, insulin, TBARS, enzymatic and non-enzymatic antioxidants in diabetic rats. They stated that the alterations in anti-oxidant levels were reverted back to near control levels after the treatment of *M. koenigii* leaves extract.

In rats fed with *Murraya koenigii* leaf powder and *Brassica juncea* seeds, there was a decrease in the concentration of malondialdehyde, while hydroperoxides and conjugated dienes were increased in liver and heart. There was increased activity of

Superoxide dismutase and catalase in liver and heart of administered groups. Glutathione levels in liver, heart and kidney were lowered in rats administered these spices. Glutathione reductase, glutathione peroxidase and glutathione S-transferase activity showed a sharp increase in the administered group (Prasad, 2007).

Arulselvan and Subramanian (2007) assayed the changes in cellular antioxidant defence system in pancreatic tissue homogenate. The levels of glucose, insulin, enzymatic & non-enzymatic antioxidants were altered in diabetic rats which were reverted back to near control levels after treatment of *M. koenigii* leaves extract.

Mohammadi *et al.* (2009) evaluated the antioxidant properties of an anti-diabetic plant extract of stem bark of *Pterocarpus marsupium* using various *in vitro* radical scavenging assays as well as by using liver slice cultures as a model system. They found that the whole aqueous extract showed high antioxidant activity in all different assays used and also protected mitochondria against oxidative damage.

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 *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.

Dineshkumar *et al.* (2010) identified two carbazole alkaloids, mahanimbine and koenigine, from the leaves of *Murraya koenigii* and stated regarding antioxidant property of these alkaloids and also showed Koenigine having high degree of radical-scavenging properties.

Pragathi (2011) studied the hypoglycemic effect of *Gymnema sylvestre* and cow urine in diabetic rat model and observed the decreased levels of SOD, CAT and GPx in streptozotocin induced diabetic animals. However, she recorded improvement in antioxidant enzyme levels when animals were treated with *Gymnema sylvestre* and cow urine for the duration of 45 days.

The antioxidative properties of the leaf extracts of *Murraya koenigii* using different solvents were evaluated based on the oil stability index (OSI) together with their radical scavenging ability against 1, 1-diphenyl-2-picrylhydrazyl. (Bonde *et al.*, 2011)

Singh *et al.* (2012) evaluated oxidative stress in the form of levels of lipid peroxidation, non-enzymatic (GSH) 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 % to 50 % indicating coexistence of metabolic disturbances and oxidative stress in diabetic animals.

Phoboo *et al.* (2013) showed that the mixture of inflorescence and leaf aqueous extracts of *Swertia chirayita* had highest antioxidant activity by 2,2-diphenyl-1-picrylhydraazyl radical inhibition assay whereas stem extract had moderate to high antioxidant activity and the root extract had low antioxidant activity.

Gurikar (2014) in his study on antidiabetic effect of chromium picolinate on STZ induced diabetic rats, observed a significant increase in antioxidant enzymes SOD, GPx and catalase in groups treated with chromium picolinate or in combination with glibenclamide, when compared to diabetic rats.

Florence *et al.* (2014) studied on antidiabetic and antioxidant effect of aqueous extract of *Annona Muricata* (Moraceae) twigs in streptozotocin induced diabetic rats and reported that on long term administration orally there was reduction in blood glucose levels, serum creatinine, MDA, ALT, AST whereas SOD and CAT activity were retained.

Kavitha Rani (2015) studied the hypoglycemic effect of *Gymnema sylvestre* and Chromium in diabetic rat model and observed the 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 chromium for the duration of 45 days.

2.6 Pathology

2.6.1 Body weight

Body weight loss in diabetic rats has been reported by several workers. They attributed increased catabolism of carbohydrates, proteins and fats and altered uptake of glucose and glycogenesis due to hyperinsulinemia. Further, decreased protein synthesis due to lack of insulin leading to diminished transport of amino acids to the muscle was also attributed by several workers (Hakim *et al.,* 1997; Rubin and Strayer, 2008;

Mallikarjuna, 2009; Pragathi, 2011; Nasreen, 2012; Mudasir, 2013; Gurikar, 2014 and Kavitha Rani, 2015).

2.6.2 Clinical signs

Pillion *et al.* (1988) studied the effect 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 catabolism of other tissues. Concomitant with increased intestinal mass, food and water intake increased significantly in STZ diabetic animals and 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.

Hoenig (2002) described that the clinical signs in diabetic pets which were similar to those in people and are not necessarily specific to diabetes. He reported increase in blood sugar levels, increased thirst and urination. He opined that weight loss may occur and can be rapid depending on the state of the disease which occurs in spite of extreme appetite.

Warkins (2003) noticed polyuria, polydipsia and weight loss as a major sign of insulin deficiency and protein synthesis was attributed to decrease in the absence of insulin, partly because of the diminished transport of amino acid into muscle.

Tasa *et al.* (2005) found in their trial that food and fluid consumption, blood glucose, triglycerides and total cholesterol were significantly increased, whereas body weight and insulin levels were significantly decreased in STZ- induced diabetic rats in comparison with the control rats.

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 body weight and the volume of insulin decreased in the diabetic animals. They observed degeneration of β- cells in pancreas of diabetic rats.

The diabetic rats in STZ induced diabetes exhibited clinical signs like polyuria, polydipsia, polyphagia, restlessness and poor body condition (Mallikarjuna, 2009; Dhanush, 2009; Pragathi, 2011; Mudasir, 2011; Manjunath, 2013; Gurikar, 2014; Kavitha Rani, 2015).

2.6.3 Gross pathology

In the diabetic rats, the liver appeared pale, soft and friable due to STZ induced liver damage (Laguens *et al.*, 1980; Rajesh and Latha, 2004).

Spleen in the STZ induced diabetic rats appeared to be reduced in size and pale with wrinkled capsular surface (Adeghate *et al.*, 2010 and Pragathi, 2011).

Pancreas in induced diabetes appeared slightly congested and showed decrease in size from day 15 onwards which appeared as a thin gelatinous strip on day 45 (Manjunatha, 2013; Shesha Rao, 2013: Gurikar, 2014).

Kavitha Rani (2015) in her study on diabetes recorded congested and atrophic changes in the pancreas of the diabetic induced rats and in other organs such as liver, kidney, intestine, lung and heart appreciable gross lesions were not recorded.

2.6.4 Histopathology

Sharma *et al.* (2003) in their studies on alloxan-induced diabetic rabbits which were sacrificed after one month of the experiment reported mild congestion of central vein, fatty change in hepatocytes and vacuolation of the myocytes. In pancreas, partly hyalinized islets, reduced number of β-cells with cytoplasmic vacuolations in occasional cells was noticed.

The pathological changes in chronic diabetes induced by single dose of STZ at 60 mg/kg body weight in Wistar albino rats was studied by Kaneez (2004) and observed 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.

Soleimani *et al.* (2007) evaluated the effect of methanolic extracts of *Equisetrum arvense*. L. on the pancreatic beta cells in STZ induced diabetic rats and 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.

In experimentally induced diabetes in rabbits, histopathological changes in various organs was recorded by Mir *et al.* (2008) which 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 and haemorrhage and myopathy in heart.

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

The antidiabetic activity of some herbal plants in streptozotocin induced diabetic albino rats was studied by Prasad *et al*. (2009) and they observed significant architectural disarray in pancreatic islets of diabetic control group extending into surrounding exocrine tissue.

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

Mallikarjuna (2009) observed improvement in size, shape and cellularity of islets in pancreas of experimentally induced diabetes rats treated with *Momardica charantia* and *Gymnema*. He also noticed increase in the number of granulated β- cells and attainment of almost normal distribution of α and β-cells in diabetic rats treated with *Gymnema sylvestre.*

Atangwho *et al.* (2010) noticed damaged islets, markedly shrunken in mass and infiltrated with lymphocytes in untreated streptozotocin (STZ) diabetic rats which were significantly different from the non-diabetic control rats which showed (numerous) islets cell mass devoid of fibrosis, distributed widely with well stained nuclei.

Mudasir (2011) reported swelling of hepatocytes with highly vacuolated cytoplasm and obliteration of the sinusoidal spaces in liver by 15^{th} day in diabetic rats with moderate to severe congestion. On 30^{th} day the changes were more severe with mild biliary hyperplasia. On 45^{th} day there were focal areas of necrosis of hepatocytes.

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

The endocrine component of pancreas, the islets of Langerhans revealed hypocellularity with loss of normal architecture. There was loss of demarcation between

islets and the surrounding exocrine portion. There was altered distribution of α and β-cells. The β-cells were highly vacuolated with fusiform shaped condensed nucleus. The cytoplasmic granularity of β-cells was greatly reduced (Harini, 2013).

The characteristic histopathological lesions in exocrine and endocrine portion of pancreas in diabetic rats were recorded by (Gurikar, 2014; Kavitha Rani, 2015) which included reduced number of islets which were irregular and varied in size in endocrine portion of pancreas. Loss of architecture and reduced number of cells that were either necrotic or highly swollen with vacuolated cytoplasm in Islets was also recorded. The cytoplasmic granularity was reduced and some cells appeared elongated and fusiform. The normal distribution of α and β-cells was altered and showed an increase in the number of α cells. They also identified the loss of architecture and wide separation of lobules with presence of edema fluid, haemorrhage and mild infiltration of mononuclear cells in exocrine portion of pancreas.

2.7 Immunohistochemical demonstration of insulin

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

Zhou *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 noticed smaller islets with degranulation in the β cells on immunohistochemical staining of pancreatic cells from untreated diabetic (STZ) rats.

Immunohistochemical studies on the effect of *Aloe vera* on the pancreatic β-cells in neonatal streptozotocin-induced type-II diabetic rats indicated immunoreactivity of β-cells of the control diabetic group were not different from any of the treatment groups: glibenclamide, *Aloe vera* leaf gel, or pulp extracts (Bolkent *et al.*, 2005).

Adewole and Ojewole (2007) carried out experiment on insulin-induced immunohistochemical and morphological changes in pancreatic beta-cells of streptozotocin-treated diabetic rats. They observed that the pancreatic insulin contents of the insulin-treated group showed approximately 45-fold increase in immunoreactivity on 30^{th} 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 found 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.

Lemke *et al.* (2008) conducted experiment on obesity and non-insulin-dependent diabetes mellitus in Swiss-Webster mice associated with late-onset hepatocellular carcinoma. They performed insulin IHC 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.

Mudassir (2011) observed a decline in the number of insulin positive cells in the islets of diabetic rats by IHC due to STZ, which improved upon treatment with *Momordica charantia* at 200 mg/kg.

Shesha Rao (2013) observed that *Coccinia indica* and half dose of glibenclamide significantly increased the number of insulin positive cells when compared to their individual treatments. Similarly, Manjunatha (2013) noticed regeneration of β cells comparable to that of glibenclamide indicating the synergistic action of *Pterocarpus marsupium* and *Swertia chirayita* extracts in alleviating the STZ effects in pancreas.

Gurikar (2014) in an experimental study in STZ induced diabetic Wistar albino rats, demonstrated that there was pancreatic β cell regeneration in groups supplemented with chromium alone or in combination with glibenclamide when compared to diabetic rats and established a synergistic action of chromium with glibenclamide by immunohistochemistry. Similarly, Kavitha Rani (2015) noticed regeneration of β cells comparable to that of glibenclamide indicating the synergistic action of *Gymnema sylvestre e*xtract and *chromium* in alleviating the STZ effects in pancreas.

2.8 Immunoradiometric Analysis for insulin

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) noted no significant change in the serum insulin level of the diabetic animals treated with beriberine suggesting that beriberine probably exerted

antihyperglycemic activity by an extra pancreatic mechanism independent of insulin secretion.

Akbarzadeh *et al.* (2007) in their experimental study on induced diabetes mellitus in Wistar rats measured the insulin and c-peptide levels by radio-immunoassay method. They recorded the decreased levels of insulin and c-peptide in diabetic rats.

Gayathri and Kannabiran (2008) studied diabetes associated metabolic alteration in streptozotocin induced diabetic rats. They reported significant decrease in levels of plasma insulin after induction of diabetes. Oral administration of aqueous extract of *Pterocarpus marsupium* bark for the period of 12 weeks increased the levels of insulin near to normal compared with untreated control rats.

Significant reduction in serum insulin, protein and albumin levels was noticed by Rajnish and Radhey (2009) after induction of diabetes by streptozotocin. They reported significant elevation of serum insulin and albumin in comparison to diabetic rats after administration of methanolic extract of *Pterocarpus marsupium* for the period of 14 days.

Mallikarjuna (2009) observed significant reduction in insulin level upon induction of diabetes in rats and on treatment with *Momardica charantia* and *Gymnema sylvestre* extract with glibenclamide, the insulin level significantly increased.

Studies on hypoglycemic effect of aged garlic extract on experimentally induced diabetes mellitus in rats revealed low level of insulin in diabetic control rats when compared to normal control animals. However, the rats treated with aged garlic extract

showed significantly higher levels of serum insulin compared to diabetic control animals Nasreen (2012).

Gurikar (2014) in his study on antidiabetic potential of chromium picolinate in STZ diabetic rats recorded an increased activity of serum insulin levels by Immunoradiometric assay in chromium treated groups when compared to diabetic controls. Kavitha Rani (2015) in her study on pathomorphological and biochemical studies on the effect of *Gymnema sylvestre* and chromium in induced diabetes in rats estimated the serum insulin level by Immuno radiometric assay and observed that the insulin level significantly reduced on induction of diabetes in rats and on treatment with *Gymnema sylvestre* extract and chromium with glibenclamide, the insulin level significantly increased.

Materials and Methods

III. MATERIALS AND METHODS

The present study was carried out at the Department of Veterinary Pathology, Veterinary College, Hebbal, KVAFSU, Bengaluru to evaluate the antidiabetic efficacy of *Aloe vera* and *Murraya koenigii* individually as well as in combination in rats. Hypoglycemic effects of these plants were compared with a regularly used oral allopathic hypoglycemic agent glibenclamide.

3.1 Experimental animals

Genetically normal adult female *Wistar albino* rats weighing 170-200 g were procured from Central Animal Facility, Indian Institute of Science, Bengaluru for the study. They were maintained under standard laboratory conditions and offered *ad libitum* standard commercial rat feed (Rodent Feed, Indian Immunologicals, Hyderabad, India) and clean drinking water. The experiment was carried out for a period of 45 days after obtaining permission from Institutional Animal Ethics Committee, Veterinary College, Hebbal, Bengaluru.

3.2 Sources

3.2.1 Drugs and chemicals

Streptozotocin was used for inducing diabetes in rats which was procured from Sigma Chemicals, St. Louis, USA. All other chemicals and reagents used for the study were of analytical grade.

3.2.1.2 Preparation of streptozotocin solution

The working injectable STZ solution was prepared in fresh citrate buffer having a pH 3.5-4.5 and the same was maintained at 4-8 ^{o}C. The required quantity of STZ was dissolved in ice-cold citrate buffer to give a concentration of 40 mg/kg and injected intraperitonially to rats immediately to avoid degradation**.**

3.2.2 Sources of plant extracts

In the present study, antidiabetic effect of aqueous extracts of *Aloe vera* and *Murraya koenigii* were studied. *Aloe vera* belongs to the family of Aloaceae and genus *Aloe L.* and *Murraya koenigii* to the family of Rutaceae and genus *Murraya.*

3.2.2.1 *Aloe vera*

The aqueous extract of *Aloe vera* used in the present study was obtained from Natural Holistics Company, Bengaluru, India. The powdered extract was weighed according to body weight and dissolved in water to make the final concentration and administered to the experimental animals (Plate 1 & 3).

3.2.2.2 *Murraya koenigii*

The aqueous extract of *Murraya koenigii* used in the present study was obtained from Natural Holistics Company, Bengaluru, India. The powdered extract was weighed according to body weight and dissolved in water to make the final concentration and administered to the experimental animals (Plate 2 & 4).

3.3 Glibenclamide solution

Glibenclamide (Daonil®, 5 mg) an oral hypoglycaemic drug purchased from local chemist shop was dissolved in distilled water (82.33 ml) to make a concentration of 60 μg/ml solution. This was used as stock solution and administered orally at a dose of 600 μg/kg body weight (Babu and Prince, 2004).

3.4 Administration of plant extracts and glibenclamide

Throughout the period of experiment, the plant extracts and glibenclamide were administered orally to their respective groups by using clean gavaging needle attached to an appropriate disposable syringe during morning hours daily for a period of 45 days.

3.5 Experimental design

After procurement, the rats were maintained under standard laboratory conditions for a period of 15 days for acclimatization in the experimental room. Then the rats were divided into nine different groups with ten animals in each based on the body weight. Care was taken to maintain the intra group weight variation to be less than 20 g and inter-group weight variation by 30 g.

The groups and treatments used were as follows:

Group I	Normal control: Used for studying baseline values of the parameters
Group II	Diabetic control: Streptozotocin induced diabetic rats
Group III	Diabetic rats supplemented with glibenclamide at a dose of 600 μg/kg body weight
Group IV	Diabetic rats supplemented with aqueous extract of *Aloe vera* at the dose rate of 300 mg/kg body weight

Group V	Diabetic rats supplemented with aqueous extract of *Murraya koenigii* at the dose rate of 300 mg/kg body weight
Group VI	Diabetic rats supplemented with combined extract of *Aloe vera* and *Murraya koenigii* at the dose rate of 150 mg/kg body weight each
Group VII	Diabetic rats supplemented with combined extract of *Aloe vera* and glibenclamide at the dose rate of 150 mg/kg and 300 µg/kg body weight, respectively
Group VIII	Diabetic rats supplemented with combined extract of *Murraya koenigii* and glibenclamide at the dose rate of 150 mg/kg and 300 µg/kg body weight, respectively
Group IX	Diabetic rats supplemented with combined extract of *Aloe vera, Murraya koenigii* at the dose rate of 150 mg/kg each and glibenclamide at the dose of 300 µg/kg body weight, respectively

The rats of Group I and II were gavaged only with saline and the rats of all other groups with their respective treatments daily for 45 days. Throughout the experimental period the rats were observed for their health status and the clinical signs exhibited were recorded.

3.6 Experimental induction of diabetes

The rats were fasted overnight and injected with freshly prepared streptozotocin (Sigma chemicals, USA) at the dose of 40 mg/kg intraperitonially in 0.1M citrate buffer having a pH 3.5- 4.5 to induce diabetes (Babu and Prince, 2004). The control Group I received citrate buffer alone.

3.6.1 Confirmation of diabetes

The diabetic state was confirmed by estimating the serum glucose levels 72 h post STZ injection using Trivitron diagnostic kit with fully automatic biochemical analyser

(Thermo Scientific Company, Europe). The animals with serum glucose level above 200 mg/dl were considered diabetic and selected for the study.

3.7 Clinical observation

Rats of all the groups were observed daily for the feed and water intake, general behaviour, alertness, urine output, diarrhoea and also for the development of clinical symptoms.

3.8 Collection of serum samples

Blood was drawn from the rats at different time intervals such as 3^{rd}, 15^{th}, 30^{th} and 45^{th} day 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 into 2 ml Eppendorf's tube and subjected for glucose estimation immediately after collection and the remaining serum samples were stored at -20 ^{o}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 humanely under light ether anaesthesia on 15^{th} and 30^{th} and the remaining rats on 45^{th} day of experimentation. Such sacrificed animals were subjected for detailed post mortem examination and gross changes, if any were recorded in various organs. A piece of liver was collected in ice cold normal saline for estimation of antioxidant enzymes. Further, representative tissue samples from various organs like

liver, spleen, lung, kidney and heart were collected in 10 per cent neutral buffered formalin (NBF) for the pathomorphological evaluation.

3.10 Parameters analysed

3.10.1 Phytochemical analysis

Qualitative phytochemical tests were performed to notice the presence of different phytochemicals in aqueous extract of *Aloe vera* and *Murraya koenigii.*

3.10.1.1 Physical analysis

Physicochemical constants like color, odor, taste, loss on drying, water soluble extractive values and pH of the 1% W/V aqueous solution of *Aloe vera and Murraya koenigii* were carried using standard protocols (Baravalia, 2010).

3.10.1.2 Chemical (Organic) analysis

The extract obtained was subjected to qualitative chemical tests for identification of various phytoconstituents using standard protocols (Harborne, 1998; Ejoba, 2012; Hasan *et al*., 2013).

3.10.1.2.1 Test for carbohydrates

The extract (100 mg) was dissolved in five ml of distilled water and filtered. The filtrate was subjected to various tests.

Molisch's test

To two ml of filtrate, two drops of Molisch's reagent was added, the mixture was shaken and one ml of conc. H_2SO_4 was added slowly along the sides of the test tube and allowed to stand.

3.10.1.2.3 Test for proteins

Biuret test

Few mg of the residue was taken in water and one ml of 4% NaOH solution was added to it. To this, a drop of 1 per cent solution of $CuSO_4$ was added.

3.10.1.2.4 Test for saponins

Foam test

Aqueous extract (five ml) was shaken vigorously with five ml of distilled water in a test tube and warmed.

3.10.1.2.5 Test for alkaloids

A small portion from the respective extract was shaken with about three ml of 1.5% HCl and filtered. The filtrate was tested with the alkaloid reagents.

Mayer's test

Mayer's reagent was added in a test tube containing two to three ml of filtrate.

3.10.1.2.6 Test of flavonoids

Aqueous sodium hydroxide test

A fraction of the extract was treated with 1N aqueous NaOH solution.

3.10.1.2.7 Test for tannins and phenolic compounds

3.10.1.2.7.1 Ferric chloride test

About two ml of aqueous extract was stirred with two ml of distilled water and few drops of $FeCl_3$ solution (5% w/v) were added.

3.10.1.2.8 Test of fixed oils and fats

Oily spot test

One drop of aqueous extract was placed on filter paper and the solvent was allowed to evaporate.

3.10.1.2.9 Test for glycosides

The extract (0.5 g) was hydrolysed with 20 ml of 0.1 N HCl and filtered. The filtrate was used to test the presence of glycosides.

Keller-Kiliani test

To the filtrate, few drops of glacial acetic acid, one drop of 5% $FeCl_3$ and few drops of conc. H_2SO_4 was added.

3.10.1.2.10 Test for anthraquinones

Borntrager's test

A small fraction of aqueous extract was dissolved in one ml of benzene and then 0.5 ml of diluted ammonia solution was added to the benzene solution.

3.10.1.2.11 Detection of phytosterols

The aqueous extract (0.5 g) was treated with ten ml chloroform and filtered. The filtrate was used to test the presence of phytosterols (Peach and Tracey, 1955; Finar, 1959).

Leiberman-Bucharat test

To the filtrate, few drops of acetic acid and conc. H_2SO_4 were added.

3.10.2 Body weight

The rats were weighed on the day of the start of the experiment and on 3^{rd}, 15^{th}, 30^{th} and 45^{th} day of the study.

3.10.3 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 the Hemoglobin (Hb) was estimated using the auto hematology analyzer (BC-2800 vet, Mindray).

3.10.4 Serum biochemistry

The serum samples collected at various intervals were subjected to the biochemical analysis for the estimation of glucose, cholesterol, triglycerides, ALT and AST using Fully Automatic biochemical analyzer (Thermo Scientific Company, Europe) with commercial biochemical kits (Trivitron, Chennai, India). These parameters were estimated as per the procedure described by Tietz (1976).

3.10.5 Immunoradiometric assay (IRMA) for *in-vitro* determination of insulin in serum

The serum levels of insulin were quantified using the Insulin (e) 'IRMA KIT IM3210' of IMMUNOTECH, Czech Republic.

3.10.5.1 Principle of the assay

The immunoradiometric assay of insulin is a "sandwich" type assay. The mouse monoclonal antibodies directed against two different epitopes of insulin which do not compete with each other are used. The assay is employed for the measurement of immunoreactive insulin comprising of free insulin and insulin bound to anti-insulin antibodies directly in serum.

The serum samples, controls and calibrators were incubated in tubes coated with the first monoclonal antibody in the presence of the second monoclonal antibody labeled with Iodine 125. After incubation, the content of tubes was rinsed so as to remove unbound I-labeled antibody. The bound radioactivity was determined in a gamma counter.

The insulin concentrations in the samples were obtained by interpolation from the standard curve. The concentration of insulin in the samples were directly proportional to the radioactivity.

3.10.5.2 Reagents

All reagents of the kit were stored at 2-8°C. The kit contained the following reagents.

1. **Anti-insulin monoclonal antibody coated tubes: 2 x 50 tubes** (ready-to-use)
2. **^{125}I-labeled monoclonal anti-insulin antibody: one 11 mL vial** (ready-to-use)

The vial contained 640 kBq, of I^{125}-labeled immunoglobulins in buffer containing bovine serum albumin, sodium azide (< 0.1 %) and a dye.

3. **Calibrators: five 1 mL vials and one 2 mL "zero calibrator" vial** (ready-to use)

The calibrator vials contained from 0 to 300 uIU/mL of insulin in buffer with bovine serum albumin and preservatives. The calibrators were calibrated against the international standard, WHO 66/304 (1 uIU/mL = 43.3 ng/L = 7.46 pmol/L).

4. **Control sera: two vials** (lyophilized)

The vials Cl and C2 contained insulin lyophilized in human serum. The expected values were in the concentration range as indicated below. Cl = 8.10-13.5 uIU/mL C2 = 52.4- 78.9 uIU/mL

The content of the control vials marked Cl and C2 were reconstituted with 1 ml of distilled water as indicated on the label. After a period of 10 minutes, the contents were mixed gently to avoid foaming before dispensing. The vials were stored at 2-8°C.

5. Wash solution (20x): one 50 mL vial

The vial contained 50 ml of wash solution which was mixed with 950 mL of distilled water and homogenized. The diluted solution was stored at 2-8°C.

3.10.5.3 Assay procedure

All the reagents were brought to room temperature before the assay.

Determination of the standard curve

1. The calibrators (50 µl) named 0, 1, 2, 3, 4 and 5 with predetermined concentrations of insulin and 50 µl of known controls Cl and C2 were dispensed into the antibody coated tubes separately in duplicate.
2. I^{125} labeled tracer solution (100 µl)* was dispensed into these tubes and incubated at 18-25° C for two hours on an orbital shaker at 280 rpm.

   *Two additional tubes with only 100 µl of tracer solutions (for total cpm) were dispensed.
3. The contents of the tubes were carefully aspirated and washed with 2 ml of wash solution twice to remove the traces of the tracer solution.

4. The radioactivity was determined using the Scintillation Gamma Counter PRIA-I set (Para Electronics, Mumbai), (Courtesy: Department of Veterinary Pharmacology and Toxicology, Veterinary College, Bangalore) for ^{125}I for one minute.

5. The values were used to obtain a standard curve and equation of linearity, y = mx+c, where, y = radioactivity (cpm sample - cpm calibrator) and x = Insulin concentration.

6. The serum samples were run similarly and for each sample, the value (cpm sample - cpm cal^O) was located on the vertical axis and the corresponding insulin concentration on the horizontal axis.

7. Results obtained for the similarly run samples were compared against the standard equation for the determination of insulin concentration in samples assayed at the same time as the calibrators.

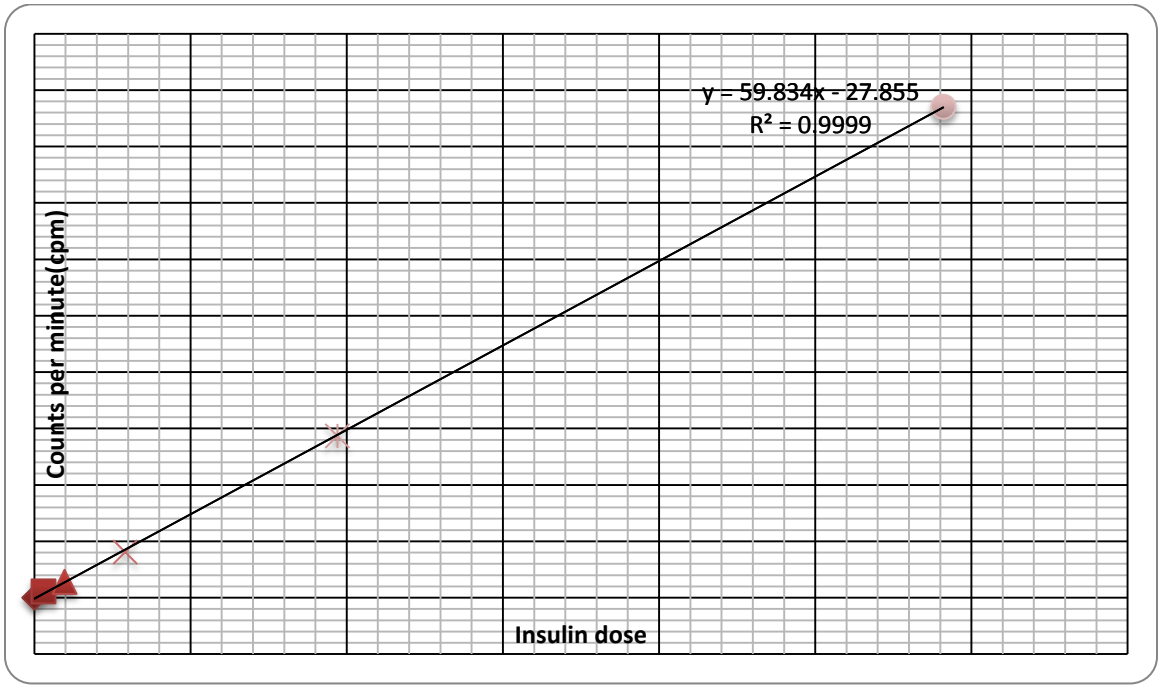
Standard curve

Total activity: 313784 cpm

Calibrators	**Insulin (µIU/mL)**	**cpm (n=2)**	B/T (%)	**Cpm cal – cpm cal^O**
0	0	753.5	0	0
1	2.91	821.5	0.07	224.5
2	9.7	1157.5	0.18	560.5
3	29.1	2214	0.52	1617
4	97	6333.5	1.83	5736.5
5	291	18002.5	5.55	17405.5

The linear equation obtained (Plate M-l) was Y= 59.83x - 27.85, with correlation co-efficient R = 0.999.

Plate M-l: Linear graph showing standard curve depicting the insulin dose determined against counts per minute and its correlation co-efficient



3.10.6 Estimation of antioxidant enzymes

3.10.6.1 Material collection

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

3.10.6.2 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 (0.5 g liver crushed in 10 ml of ice cold 0.1 mol/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.

3.10.6.3 Protein estimation

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

3.10.6.3.1 Principle

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

3.10.6.3.2 Reagents

1. BSA stock solution (l mg/ml): Standard stock solution.
2. Analytical reagents:

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

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

   Solution C: Sodium or potassium tartarate (2 g in 100 ml of 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.

3.10.6.3.3 Procedure

For standard solution

1. Different dilutions of BSA solution were prepared by mixing stock BSA solution (l mg/ml) and water as given in the table. The final volume in each of the test tubes was 5 ml. The BSA concentration range is 0.05 to l mg/ml.
2. From these different dilutions, 0.2 ml of protein solution was taken and 2 ml of analytical reagent was added to that. 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.

BSA (ml)	Water (ml)	Sample concentration (mg/ml)	Sample volume (ml)	Analytical reagent	Folin-Ciocalteau reagent (ml)
0.25	4.75	0.05	0.2	2	0.2
0.5	4.5	0.1	0.2	2	0.2
1	4	0.2	0.2	2	0.2
2	3	0.4	0.2	2	0.2
3	2	0.6	0.2	2	0.2
4	1	0.8	0.2	2	0.2
5	0	1.0	0.2	2	0.2

For samples

1. The homogenate (0.2 ml) was taken and 2 ml of analytical reagent was added to that. This solution was incubated at room temperature for 10 minutes.
2. Folin-Ciocalteau (0.2 ml) 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.

3.10.6.4 Assay of enzymatic antioxidants

3.10.6.4.1 Estimation of Superoxide dismutase (SOD)

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

3.10.6.4.1.1 Principle

Superoxide anion 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.

3.10.6.4.1.2 Homogenate preparation

To 0.5 ml of tissue homogenate, 0.25 ml of ethanol and 0.15 ml of chloroform was added and mechanically shaken for 15 minutes. Then the contents were centrifuged

at 13000 g for 15 minutes at 4 ^{0}C. The supernatant was separated and used for the test. It was expressed as units/minute/mg protein.

3.10.6.4.1.3 Procedure

To 2 ml of 0.1 M 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 2 mM pyrogallol were also added, mixed and the OD value was taken at 0, 1, 2 and 3 min intervals at 420 nm wavelength spectrophotometrically.

3.10.6.4.1.4 Calculation

SOD = 1/X value x ΔOD x Dilution factor / 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.

3.10.6.4.2 Estimation of catalase (CAT)

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

3.10.6.4.2.1 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.

3.10.6.4.2.2 Procedure

To 0.2 ml of homogenate, 1 ml of 30 mM H_2O_2 was added and the OD value was taken at 240 nm spectrophotometrically at an interval of 1 min for 3 min. Blank contained 0.2 ml of distilled water plus 1 ml of 30 mM H_2O_2.

3.10.6.4.2.3 Calculation

Catalase = 1/X value x ΔOD /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 H_2O_2 decomposed per minute per mg of protein.

3.10.6.4.3 Estimation of Glutathione peroxidase (GPx)

Glutathione peroxidase was determined by the method described by Rotruck *et al.* (1973).

3.10.6.4.3.1 Principle

GPx reacts with H_2O_2 and reduced glutathione giving rise to oxidoreductase which forms a colored complex with dithio bis-nitrobenzoic acid (DTNB). The intensity of color development is directly proportional to the amount of GPx present in the tissue.

3.10.6.4.3.2 Procedure

The reaction mixture contained 2.0 ml of 0.4 M Tris- HCl buffer, pH 7.0, and 0.01 ml of 10 mM sodium azide, 0.2 ml of tissue homogenate, 0.2 ml of 10 mM glutathione and 0.5 ml of 0.2 mM H_2O_2. The contents were incubated at 37 ^{0}C for 10

minutes followed by the termination of the reaction by the addition of 0.4 ml of 10 % (v/v) TCA, centrifuged at 5000 rpm for 5 min. The absorbance of the product was read at 430 nm and expressed as nmol/mg protein.

3.10.6.4.3.3 Calculation

Value = OD Value X Dilution factor/ Total Protein

Unit: nmol / mg protein.

3.10.7 Pathology

Two animals from each group were sacrificed humanely on day 15, 30 and the rest at the end of the study on 45^{th} day. The sacrificed animals were subjected to detailed post mortem examination and the 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 the routine paraffin embedding technique and 4 μ sections were cut and subjected to the H&E staining and Immunohistochemistry (Luna, 1968).

3.10.8 Immunohistochemical detection of insulin

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

3.10.8.1 Materials

3.10.8.1.1 Immunochemicals

1. **Primary antibody**: Ready to use Flex Polyclonal Guinea Pig Anti-Insulin shown to react with insulin antigen was procured from Dako Cytomation, Denmark. It was stored at 2 to 8^{o}C until use.
2. **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 2 to 8 ^{o}C until end.

3.10.8.1.2 Section adhesive 3-aminopropyltriethoxy-silane (APES)

Procured from Sigma chemicals, USA.

3.10.8.1.3 Hydrogen peroxide (H_2O_2) in methanol (3 %)

Three per cent H_2O_2 in methanol was prepared by adding one ml of 30 per cent H_2O_2 to 9 ml of methanol.

3.10.8.1.4 0.01 M Citrate buffer (pH-6)

1. Citric acid solution (50 ml of 0.1 M) was prepared by dissolving 1.051 g of citric acid (MW = 210.14) in 50 ml of distilled water.
2. Sodium citrate solution (100 ml of 0.1 M) was prepared by dissolving 2.941 g of sodium citrate in 100 ml of distilled water.

3. Citrate buffer (1000 ml of 0.01 M) was prepared by adding 18 ml of 0.1 M citrate solution and 82 ml of 0.1 M sodium citrate solution to 900 ml of distilled water. The pH was adjusted to 6.0 with 1 N NaOH.
4. All the solutions were prepared freshly just before the use.

3.10.8.1.5 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) to 1 ml of 0.01 M PBS to which 12 µl of 3 per cent H_2O_2 was added.

3.10.8.1.6 0.01M phosphate buffer saline (pH-7.2)

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

1. Sodium chloride (MW 58.44)	40 g
2. Potassium chloride (MW 74.56)	1 g
3. Disodium hydrogen orthophosphate (MW 141.96)	7.2 g
4. Potassium dihydrogen orthophosphate anhydrous (MW 136.09)	1 g
5. Distilled water	500 ml

Wash buffer of 1 X concentration was prepared using 10 X PBS by adding 25 ml of 10 X PBS to 225 ml of distilled water. To this 125 µl Tween 20 was added and the pH was adjusted to 7.2.

3.10.8.1.7 Harris haematoxylin for nuclear staining (Luna, 1968)

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

3.10.8.2 Preparation of organosilane (APES) treated slides for IHC

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

3.10.8.3 Method

1. Tissue sections were mounted on 3-aminopropyltriethoxy-silane (APES) coated slides and dried at 37 ^{o}C for three hours. Later stored at 4 ^{o}C for further processing later.
2. The paraffin tissue sections were deparaffinized using xylene and rehydrated using descending grades of ethanol.
3. Endogenous peroxidase was blocked by covering the whole section with 3 per cent of H_2O_2 in methanol (100 µl). This was incubated at room temperature for fifteen minutes and later washed with three changes of wash buffer.

4. 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 min and later washed with three changes of wash buffer.

5. 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.

6. Addition of secondary antibody: Polyclonal Rabbit Anti-Guinea Pig Immunoglobulin conjugated with HRP was added to section and incubated at room temperature in humidified chamber for 30 min. After incubation sections were washed with PBS as mentioned earlier.

7. Addition of DAB plus substrate: Freshly prepared 3, 3-diamine benzidine tetrahydrochloride (DAB) with 3 per cent H_2O_2 was poured to cover the sections. This was 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.

8. Nuclear counter staining with Harris haematoxylin was carried out for 45 sec. The sections were washed with distilled water, dehydrated with ascending grades of ethanol and cleared with xylene and cover slipped with DPX mounting media.

3.10.8.4 Percentage positivity of insulin secreting cells

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

3.11 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 one way and two-way analysis of variance (ANOVA).

Results

IV RESULTS

The present study was conducted to evaluate antidiabetic efficacy of *Aloe vera* and *Murraya koenigii* aqueous extracts in induced diabetes in rats and to compare the hypoglycemic effect with that of an oral hypoglycemic agent glibenclamide.

4.1 Phytochemical analysis

Phytochemical analysis of *Aloe vera* and *Murraya koenigii* aqueous extracts were carried out in the present investigation by physical method and chemical (organic) methods. The results are presented as here under.

4.1.1 Physical analysis

Table 1. Physical analysis of aqueous extract of *Aloe vera* and *Murraya Koenigii*

Test parameter	***Aloe vera*** **aqueous extract**	***Murraya koenigii*** **aqueous extract**
Color	White	Greenish brown
Taste	Very bitter	Bitter
Odour	Characteristic	Pleasant
Water soluble extractives	98.50 %	78.39 %
pH of 1% W/V Aq. Soln.	4.20	6.23
Loss on drying at 105^{o}C	2.50 %	2.59%

4.1.2 Chemical (Organic) analysis

4.1.2.1 Test for carbohydrate

Molisch's test:

Purple-violet color ring at the interphase was noticed when aqueous extracts of *Aloe vera* and *Murraya koenigii* were used indicating the presence of carbohydrate.

4.1.2.2 Test for protein

Biuret test:

Violet color was formed when aqueous extracts of *Aloe vera* and *Murraya koenigii* were used indicating the presence of carbohydrate.

4.1.2.3 Test for saponin

Foam test:

Stable foam was not formed when aqueous extract of *Aloe vera* was used whereas stable foam, honey comb in shape was formed when aqueous extract of *Murraya koenigii* was used in the test indicating absence and presence of saponin in the plant extracts respectively.

4.1.2.4 Test for alkaloids

Mayer's Test:

Cream colored precipitate was formed when aqueous extracts of *Aloe vera* and *Murraya koenigii* were used indicating the presence of alkaloids in both the plant extracts.

4.1.2.5 Test for flavonoids

Aqueous sodium hydroxide test

On testing both aqueous extract of *Aloe vera* and *Murraya koenigii*, intense yellow color was observed which became colorless on addition of few drops of diluted HCl indicating presence of flavonoids in both.

4.1.2.6 Test for tannins

Ferric chloride test

Greenish precipitate was formed when both the plant extracts were used in the test indicating the presence of tannins.

4.1.2.7 Test for fixed oils and fats

Oily spot test

There was formation of oily stain on filter paper when aqueous extract of both *Aloe vera* and *Murraya koenigii* were used indicating the presence of fixed oils and fat.

4.1.2.8 Test for glycosides

Keller-kiliani test

At the junction of two liquid layers reddish brown color was formed along with the change in the upper layer color to bluish green when *Murraya koenigii* extract was used in the test indicating the presence of glycosides whereas negative result was noticed in *Aloe vera* aqueous extract.

4.1.2.9 Test for anthraquinones

Borntrager's test

The reddish pink color was noticed in lower phase indicating the presence of anthraquinones in *Murraya koenigii* extract but when *Aloe vera* extract was used no color was formed indicating the absence of anthraquinones in aqueous extract of *Aloe vera*.

4.1.2.10 Test for phytosterols

Leiberman-Buchart test

Deep red color ring was formed at the junction of two liquid layers indicating the presence of phytosterols in both *Aloe vera* and *Murraya koenigii* aqueous extracts.

Table 2. Organic (Chemical) analysis of aqueous extract of *Aloe vera* and *Murraya koenigii*

Variables tested	*Aloe vera* aqueous extract	*Murraya koenigii* aqueous extract
Carbohydrate	+	+
Protein	+	+
Saponin	-	+
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Fixed oils & fat	+	+
Glycosides	-	+
Anthraquinone	-	+
Phytosterols	+	+

4.2 Induction of diabetes

To induce diabetes, rats were administered with streptozotocin at the dose rate of 40 mg/kg in citrate buffer (pH 3.5-4.5) intraperitonially. Blood samples were collected 72 hr after STZ dosing and the diabetes status was confirmed by the estimation of fasting blood glucose levels using fully automatic biochemical analyser.

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 *Aloe vera* (Group IV), *Murraya koenigii* (Group V), combination of *Aloe vera* and *Murraya koenigii* (Group VI), combination of *Aloe vera* and glibenclamide (Group VII), combination of *Murraya koenigii* and glibenclamide (Group VIII) and combination of *Aloe vera*, *Murraya koenigii* and glibenclamide (Group IX).

The rats belonging to various groups (Group I to IX) were analysed by subjecting them for evaluation of various parameters and the results are presented as here under.

In the present study, the normal control rats remained healthy as evaluated by various parameters throughout the experimental period.

All the rats from the Group II to IX became diabetic on 3rd day and the mean serum glucose levels ranged from 418.80 ± 23.90 mg/dl to 463.90 ± 16.31 mg/dl against 87.80 ± 5.39 mg/dl of control rats of Group I.

4.3 Body weight

The mean (± SE) body weights of Group I (Normal control) rats showed a gradual increase from day 3 to day 45 and were 194.40 ± 2.19, 203.8 ± 2.42, 211.25 ± 2.51 and 219.67 ± 2.78 g on day 3, 15, 30 and 45 post-treatment, respectively.

The mean (± SE) body weight of Group II (diabetic control) rats were 173.28 ± 1.28, 164.4 ± 1.80, 158.13 ± 1.62, 150.17 ± 1.44 g on 3^{rd}, 15^{th}, 30^{th} and 45^{th} day, respectively and showed progressive decrease from day 3 to day 45 and were significantly lower compared to normal control animals with $P \leq 0.001$ on day 3, 15, 30 and day 45 of the study.

The mean (± SE) body weight of Group III (rats treated with glibenclamide) were 182.50 ± 3.52, 193.4 ± 3.70, 199.75 ± 3.74 and 204.67 ± 4.86 g on 3^{rd}, 15^{th}, 30^{th} and 45^{th} days of the study, respectively. There was a significant improvement in the body weight with $P \leq 0.001$ on day 15, 30 and 45 of the study compared to diabetic control and the improvement was statistically comparable ($P \geq 0.05$) to that of normal control animals on day 30.

The mean (± SE) body weight of Group IV (rats treated with *Aloe vera)* were 174.50 ± 2.03, 180.8 ± 2.37, 186.5 ± 1.52, and 190.33 ± 2.09 on 3^{rd}, 15^{th}, 30^{th} and 45^{th} days, respectively. The values were found to be significantly increased ($P \leq 0.001$) on 15^{th}, 30^{th} and 45^{th} day of the study when compared to diabetic control, however values were lesser in comparison to normal control and glibenclamide treated groups and the improvement was statistically comparable at $P \geq 0.05$.

The mean (± SE) body weight of Group V (rats treated with *Murraya koenigii)* rats were 185.60 ± 4.29, 193.9 ± 4.88, 198.75 ± 4.40, and 203.33 ± 4.39 on 3rd, 15th, 30th and 45th days, respectively. The values were found to be significantly improved with P≤ 0.05 on day 3 and P≤ 001 on days 15, 30 and 45 of the study when compared to diabetic control. The mean body weight values were comparable (P≥ 0.05) to normal control and glibenclamide treated animals.

The rats of Group VI, VII, VIII and IX also showed improvement with values of 198.80 ± 2.91, 172.30 ± 1.07, 192.56 ± 3.64 and 189.50 ± 4.16 on 3rd day to 213.17 ± 2.18, 187.17 ± 0.79, 215.83 ± 2.39 and 212.33 ± 6.69 on 45th day post treatment, respectively. The value of mean (± SE) body weights were higher compared to diabetic control group, statistically higher (P≤ 0.001) value was noticed only on 30th and 45th day. However, the values were lesser in comparison to normal control and glibenclamide treated groups and the improvement was statistically comparable at P≥ 0.05 (Table 3 and Fig. 1).

4.4 Serum biochemistry

4.4.1 Serum glucose

The mean (± SE) serum glucose level in the normal control animals was observed to be 87.80 ± 5.39, 82.50 ± 5.01, 84.88 ± 4.71 and 86.66 ± 4.78 on 3rd, 15th, 30th and 45th day, respectively. Throughout the experimental period significant (P≥ 0.05) variation in values was not found.

The mean (± SE) serum glucose levels in the diabetic animals were observed to be 418.80 ± 23.90, 327.50 ± 22.44, 303.25 ± 20.42 and 311.83 ± 27.03 on 3rd, 15th, 30th and 45th day, respectively. In comparison with normal control animals the serum glucose values were significantly higher (P≤ 0.001) on all the intervals of study.

The mean (± SE) serum glucose levels in the Group III animals showed a gradual and significant reduction (P≤ 0.001) from 15th to 45th day with values of 219.80 ± 5.92, 206.12 ± 5.76 and 181.66 ± 7.60 mg/dl when compared to diabetic control animals. However, the serum glucose values were significantly higher (P≤ 0.001) in comparison with those of normal control rats except at 45th day.

In the present study, the mean (±SE) serum glucose levels reduced gradually from 3rd to 45th day with values of 463.90 ± 16.31, 258 ± 12.93, 238.75 ± 13.43 and 197.33 ± 10.07 mg/dl, respectively in *Aloe vera* treated rats (Group IV). The mean glucose values were significantly lesser (P≤ 0.05) on day 3, (P≤ 0.01) on day 30 and (P≤ 0.001) on day 15 and 45, when compared to those of diabetic control rats and significantly higher in comparison with those of normal control rats with P≤ 0.001 on 3rd, 15th, 30th and 45th day.

The mean (± SE) serum glucose values of *Murraya koenigii* treatment group were 439.40 ± 17.71, 251.7 ± 13.90, 220.62 ± 9.23 and 184.33 ± 7.01 mg/dl on 3rd, 15th, 30th and 45th days, respectively. It revealed a progressive decrease from 3rd day to 45th day post-treatment. The mean values were significantly lesser at P≤ 0.05 on 3rd day and at P≤ 0.001 on 15th, 30th and 45th in comparison to those of diabetic control rats. However, the mean serum glucose values were observed to be significantly higher in comparison with those of normal control rats with P≤ 0.001 on 3rd, 15th, 30th and 45th day.

There was a gradual decrease in the mean (± SE) serum glucose values in the combined treatment groups. Group VI rats showed decrease from 436.10 ± 24.30 to 194.5 ± 6.40, Group VII rats from 440.40 ± 15.74 to 187.83 ± 5.61, Group VIII rats from 444.80 ± 14.71 to 182.16 ± 4.20 and Group IX rats from 445.10 ± 13,11 to 178.83 ± 1.93 mg/dl on 3rd and 45th day post-treatment, respectively. The serum glucose values were significantly lower ($P \leq 0.001$) when compared to diabetic control on 15th, 30th and 45th day post-treatment. Though the values appeared declined, they were not comparable to those of normal control rats of the study and differed significantly higher ($P \leq 0.001$) at 3rd, 15th, 30th and 45th day. However, the decline in the mean serum glucose values of these groups were comparable to those of glibenclamide and individual treatment groups of *Aloe vera* and *Murraya koenigii* (Table 4 and Fig. 2).

4.4.2 Serum cholesterol

The mean (± SE) serum cholesterol level in the normal control animals was observed to be 83.34 ± 1.78, 84.8 ± 1.88, 87.2 ± 2 and 84.62 ±1.99 on 3rd, 15th, 30th and 45th day, respectively. There was no significant ($P \geq 0.05$) variation in values throughout the experiment.

The diabetic rats of Group II revealed a significant increase ($P \leq 0.001$) in the serum cholesterol level from day 3 to day 45 of experiment in comparison with the normal control. The mean (± SE) serum cholesterol values were 121.54 ± 3.59, 128.78 ± 2.47, 136.8 ± 2.94 and 141.5 ± 2.39 mg/dl on 3rd, 15th, 30th and 45th day post-treatment, respectively.

The serum cholesterol values in glibenclamide treatment group were significantly reduced ($P \leq 0.001$) from 15th day when compared to diabetic control rats. The decrease was consistently observed from 15rd day (115.06 ± 1.79 mg/dl) to 45th day post-treatment (103.2 ± 1.83 mg/dl). When compared to normal control rats the mean values were significantly higher at all the intervals.

There was a decrease in the mean (± SE) serum cholesterol values in *Aloe vera* treated rats from 3rd day (122.29 ± 2.03 mg/dl) to 45th day (104.3 ± 1.4 mg/dl). These values were significantly lower ($P \leq 0.001$) compared to diabetic control values on 15th, 30th and 45th day post-treatment. Though the values showed a declining trend, the mean values were significantly higher compared to normal control group. In comparison with glibenclamide, the mean cholesterol values did not vary significantly throughout the study period.

There was a gradual decrease in the mean (± SE) serum cholesterol values in *Murraya koenigii treated* rats with values of 114.50 ± 2.58, 107.41 ± 2.28, 101.9 ± 1.43 and 95.82 ± 1.48 on 3rd, 15th, 30th and 45th day post-treatment, respectively. The mean values were lower in relation to those of diabetic rats and significantly differed ($P \leq 0.001$) from 15th day post-treatment. However, the mean serum cholesterol values were observed to be significantly higher in comparison with those of normal control rats. In comparison with glibenclamide, the mean cholesterol values did not vary significantly ($P \geq 0.05$) throughout the study period.

There was a decrease in the mean (± SE) serum cholesterol values in the combined treatment groups. Group VI rats showed decrease from 120.07 ± 2.03 to 102.3

± 1.69, Group VII rats from 117.53 ± 1.99 to 98.63 ± 1.68, Group VIII rats from 116.4 ± 2.76 to 97.3 ± 2.92 and Group IX rats from 120.19 ± 2.59 to 99.28 ± 2.32 mg/dl on 3rd and 45th day post-treatment, respectively. The serum cholesterol values were significantly lower (P≤ 0.001) when compared to diabetic control on 15th, 30th and 45th day post-treatment. Though the values appeared declined, they were not comparable to those of normal control rats of the study and differed significantly (P≤ 0.001) higher in all the groups except for 45th day in Group VIII which showed significant variation at P≤ 0.01. However, the declines in the mean serum glucose values of all the groups were comparable to those of glibenclamide and individual treatment groups of *Aloe vera* and *Murraya koenigii* (Table 5 and Fig. 3).

4.4.3 Serum triglycerides

The mean (± SE) serum triglycerides level in the normal control animals was observed to be 86.09 ± 1.98, 90.27 ± 1.9, 91.23 ± 0.98 and 94.26 ± 1.54 on 3rd, 15th, 30th and 45th day respectively. There was no significant (P≥ 0.05) variation in values throughout the experiment.

In the present study, the diabetic rats of Group II revealed a significant increase (P≤ 0.001) in the serum triglyceride levels when compared to those of normal control animals. The mean (± SE) values ranged from 152.52 ± 4.13, 170.46 ± 3.41, 182.16 ± 3.32 and 188.7 ± 3.25 on 3rd, 15th, 30th and 45th day post-treatment, respectively.

The mean (± SE) serum triglyceride values of glibenclamide treatment group also showed similar trend of cholesterol values. The mean (± SE) values reduced to 98.96 ± 1.76 mg/dl on 45th day from that of 147.54 ± 1.62 mg/dl on 3rd day post-treatment. The

mean values were significantly reduced ($P \leq 0.001$) compared to that of diabetic control rats on 15^{th}, 30^{th} and 45^{th} days of treatment and were comparable to that of normal control values on 45^{th} day post-treatment.

The mean (± SE) values of serum triglyceride in *Aloe vera* treated rats showed a progressive decline with values of 141.74 ± 3.19, 126.65 ± 2.28, 111.03 ± 1.46 and 99.81 ± 1.94 mg/dl on 3^{rd}, 15^{th}, 30^{th} and 45^{th} day, respectively. The decline in the mean values was significant ($P \leq 0.001$) compared to those of diabetic control rats on 15^{th}, 30^{th} and 45^{th} day. These values however, remained significantly higher in relation to those of normal control rats on 3^{rd}, 15^{th}, 30^{th} days and were comparable to that of normal control values on 45^{th} day post-treatment. The mean values were comparable to those of glibenclamide group in all intervals of the study.

Gradual decrease in the mean (± SE) serum values of triglyceride was observed in rats of *Murraya koenigii treatment* group from 3^{rd} day onwards with values of 139.36 ± 3.3, 120.49 ± 2.71, 101.86 ± 1.73 and 89.05 ± 1.82 mg/dl on 3^{rd}, 15^{th}, 30^{th} and 45^{th} days, post-treatment respectively. The mean values were significantly lower ($P \leq 0.001$) when compared to those of diabetic rats on 15^{th}, 30^{th} and 45^{th} day post-treatment and significantly higher in relation to those of normal control rats on all the days of observation except at 45^{th} day which was comparable to normal animals. The mean values were comparable to those of glibenclamide group in all intervals of the study.

There was a decrease in the mean values of serum triglyceride in the combined treatment groups with values of 140.73 ± 4.95, 142.25 ± 3.49, 141.98 ± 3.66 and 143.32 ± 4.73 mg/dl on 3^{rd} day to 93.85 ± 1.79, 97.45 ± 2.51, 93.36 ± 2.51 and 95.28 ± 3.17

mg/dl on 45th day post-treatment in group VI, VII, VIII and IX respectively. The decline in serum triglyceride values coordinated with those of serum cholesterol levels. There was a significant variation ($P \leq 0.001$) when the mean values were compared with those of diabetic control rats which showed a consistent increase from 3rd to 45th day.

The improvement in triglyceride levels was comparable to that of normal control rats only on 45th day and differed significantly on 3rd, 15th and 30th day post-treatment. In comparison with glibenclamide and individual treatment groups of *Aloe vera and Murraya koenigii* the mean serum values did not differed significantly at any intervals of the study (Table 6 and Fig. 4).

4.4.4 Serum alanine aminotransferase (ALT)

The mean (± SE) serum aspartate aminotransferase (AST) levels in the normal control animals were observed to be 56.41 ± 2.67, 58.64 ± 2.67, 60.3 ± 2.57 and 61.13 ± 2.09 IU/L on 3rd, 15th, 30th and 45th days, respectively. There was no significant change ($P \geq 0.05$) in ALT values throughout the experiment.

The diabetic control rats showed a significantly higher ($P \leq 0.001$) mean (± SE) serum ALT values throughout the study period as compared to normal control animals. The mean (± SE) values on 3rd, 15th, 30th and 45th day post-treatment of the study was 122.65 ± 3.23, 128.51 ± 3.23, 141.18 ± 1.87 and 202.28 ± 3.28 IU/L, respectively.

The serum alanine aminotransferase values in glibenclamide treated group showed a gradual decline from 119.51±4.08 IU/L on day 3 to 76.78±2.23 IU/L on 45th day. The decrease was significant ($P \leq 0.001$) from day 15 till the end of study period in

comparison with diabetic control (Group II) animals. However, the values were significantly higher when compared to those of normal control rats with P≤ 0.001 on 3rd, 15th, 30th and 45th day.

The mean (± SE) serum ALT values in *Aloe vera* treatment group revealed a gradual decrease with values of 123.75 ± 1.76, 109.09 ± 1.76, 92.31 ± 2.31 and 84.03 ± 2.44 IU/L on 3rd, 15th, 30th and 45th days respectively. These values were significantly lesser (P≤ 0.001) when compared to those of diabetic control rats. Though the mean (± SE) values showed a decreasing trend, when compared to normal rats they were significantly higher (P≤ 0.001) at 3rd, 15th, 30th and 45th day. The decrease in the ALT values in the present study was very much similar to that of glibenclamide treated groups and did not significantly vary on all the days of observation.

There was a decrease in the mean (± SE) values of serum ALT in *Murraya koenigii* treatment rats from 3rd day onwards with mean values of 118.77 ± 3.16 on 3rd day to 76.5 ± 1.09 IU/L on 45th day post-treatment. The reduction in mean values was significant (P≤ 0.001) in comparison with that of diabetic control rats on 15th, 30th and 45th day post-treatment. However, the values were significantly higher in comparison with those of normal control rats with P≤ 0.001 at 3rd, 15th, 30th and 45th day. In comparison with glibenclamide treatment group, there was no significant difference.

The decline in the mean values of ALT was also observed in combined treatment (Groups VI, VII, VIII and IX) on all the intervals of study. The decrease was from 121.05 ± 1.55, 120.65 ± 3.91, 124.22 ± 2.63 and 119.65 ± 2.38 IU/L on 3rd day to 87.65 ± 2.07, 80.93 ± 1.27, 82.9 ± 2.63 and 75.42 ± 1.8 IU/L on 45th day post-treatment in Groups VII

and VIII, respectively. The mean ALT values were significantly lower ($P \leq 0.001$) when compared to those of diabetic control animals on 15th, 30th and 45th day post-treatment and were significantly higher ($P \leq 0.001$) in comparison with normal control group on 3rd, 15th, 30th days. It was observed that the mean values of ALT did not significantly vary from those of glibenclamide group except Group V on 30th day and Group VI (*Aloe vera* + *Murraya koenigii*) on 45th day which showed variation ($P \leq 0.05$) and ($P \leq 0.01$) respectively. Group VI showed moderately higher ($P \leq 0.05$) values compared to Group V *(Murraya koenigii + Glibenclamide)* group (Table 7 and Fig. 5).

4.4.5 Serum aspartate aminotransferase (AST)

The mean (± SE) serum aspartate aminotransferase (AST) levels in the normal control animals were observed to be 81.64 ± 1.91, 83.39 ± 1.78, 86.35 ± 2.01 and 85.83 ± 1.79 IU/L on 3rd, 15th, 30th and 45th days, respectively. There was no significant change ($P \geq 0.05$) in AST values throughout the experiment.

A significantly higher ($P \leq 0.001$) mean (± SE) serum AST values were observed in the diabetic control rats during entire period of the study with the mean values of 252.22 ± 14.99, 260.69 ± 13.13, 270.05 ± 11.05 and 289.38 ± 6.62 IU/L on 3rd, 15th, 30th and 45th day post-treatment, respectively compared to normal control rats.

The AST values in glibenclamide treated animals revealed similar decreasing trend as that of ALT which was from 255.65 ± 15.23 IU/L on 3rd day comparable to that of diabetic control rats to 146.83 ± 5.05 IU/L on 45th day. The decrease in AST values were significant ($P \leq 0.001$) in comparison with those of diabetic control on 15th, 30th and 45th day post-treatment. Though there was an appreciable decrease in the mean values,

they were not comparable to those of normal control rats and remained significantly higher at $P \leq 0.001$ on 3rd, 15th, 30th day and 45th day.

A decline in the mean (± SE) values of serum AST was observed in *Aloe vera* treatment group which were 242.17 ± 8.35, 207.99 ± 4.72, 187.85 ± 4.19, 148.33 ± 3.12 IU/L on 3rd, 15th, 30th and 45th day post-treatment, respectively. The mean values were significantly lesser ($P \leq 0.001$) in comparison with those of diabetic control group on 15th day post-treatment onwards and were significantly higher ($P \leq 0.001$) in relation to normal control rats. In comparison with glibenclamide treatment group, there was no difference.

The mean (± SE) AST values in the rats of *Murraya koenigii* treatment group showed a progressive decrease from 3rd day onwards to 45th day with values of 245.57 ± 11.26, 205.5 ± 4.15, 179.17 ± 2.89 and 141.3 ± 3.64 IU/L, respectively. The decrease in the serum values was found to be highly significant ($P \leq 0.001$) when compared to those of diabetic control on 15th, 30th and 45th days of post-treatment. Though there was a decreasing trend in the values, they were significantly higher with $P \leq 0.001$ on 3rd, 15th 30th and 45th day. There was no significant difference between glibenclamide treatment group in all intervals of the study.

The gradual decline in the mean values of AST observed in combined treatment groups of rats appeared similar to the decline of mean ALT values of individual treatment on all the intervals of study. The decrease was from 246.61 ± 8.6 IU/L on 3rd day to 156.167 ± 6.1 IU/L on 45th day post-treatment in Group VI animals. The decrease in values in Groups VII, VIII, and IX were 242.44 ± 9.86, 241.01 ± 11.26, 249.09 ± 11.2

IU/L on 3rd day to 145.56 ± 4.92, 128.55 ± 1.84, 135.51 ± 4.47 IU/L on 45th day post-treatment, respectively. The mean AST values were significantly lower (P≤ 0.001) when compared to those of diabetic control animals on 15th, 30th and 45th day post-treatment and were significantly higher (P≤ 0.001) in comparison with normal control group on 3rd, 15th, 30th and 45th day. It was observed that the mean values of AST of combined groups did not significantly vary from those of glibenclamide. Group VI showed moderately higher (P≤ 0.05) and (P≤ 01) values respectively on 30th and 45th day respectively when compared to Group V *(Murraya koenigii + Glibenclamide)* group (Table 8 and Fig. 6).

4.4.6 Serum insulin

The mean (± SE) serum insulin levels in the normal control animals were observed to be 4.51 ± 0.05, 4.53 ± 0.04, 4.52 ± 0.05 and 4.73 ± 0.06 µU/L on 3rd, 15th, 30th and 45th days, respectively. There was no significant change (P≥ 0.05) in insulin values throughout the experiment.

The mean (± SE) insulin values in the diabetic control animals were 3.72±0.02, 3.67 ± 0.02, 3.69 ± 0.02 and 3.74 ± 0.02 µU/L on 3rd, 15th, 30th and 45th day, respectively. The mean insulin values showed a significant decrease (P≤ 0.001) in diabetic control group when compared to normal control animals at all the intervals of study.

In the present study, the glibenclamide treated animals showed increase in the mean serum insulin values which were 3.61 ± 0.09, 3.72 ± 0.10, 4.13 ± 0.11, 4.46 ± 0.06 µU/L on 3rd, 15th, 30th and 45th day of treatment respectively. These mean (± SE) serum insulin values were significantly increased on 30th and 45th day with P≤ 0.01 and P≤ 0.001, respectively in glibenclamide treated group when compared to diabetic control

values. However, they were not comparable to those of normal control animals and remained significantly lower ($P \leq 0.001$) at 3rd and 15th day whereas ($P \leq 0.001$) at 45th day but was comparable on 30th day.

The serum insulin levels in *Aloe vera* treated group showed a gradual progressive improvement from 3rd day (3.57 ± 0.09 µU/L) to 45th day (4.37 ± 0.08 µU/L) post-treatment. The values were found to be significantly increased on 45th day with $P \leq 0.001$ compared to diabetic control. Though there was an increase in the mean value, they failed to reach close to the normal control values and were significantly lower ($P \leq 0.001$) at day 3, 15 & 45 and on 30th day it was found to be a $P \leq 0.001$. In comparison with glibenclamide treated group, the values were comparable and did not differ significantly.

There was progressive increase in the mean (± SE) values of serum insulin in *Murraya koenigii* treated rats from 3rd day (3.64 ± 0.09 µU/L) to 45th day (4.50 ± 0.04 µU/L) post-treatment. The mean insulin values were significantly increased compared to diabetic control rats only on 30th & 45th day with $P \leq 0.01$ and $P \leq 0.001$ respectively. However, failed to reach the normal range of control rats at any interval of study and remained significantly lower ($P \leq 0.001$ to $P \leq 0.01$). In comparison with the glibenclamide treatment group, the mean insulin values were comparable with no significant difference.

The mean (± SE) serum insulin values in combined treatment groups showed a consistent improvement from 3.62 ± 0.08, 3.53 ± 0.11, 3.58 ± 0.10 and 3.55 ± 0.05 µU/L to 4.44 ± 0.03, 4.37 ± 0.01, 4.44 ± 0.03 and 4.45 ± 0.05 on 3rd to 45th day in Groups VI, VII, VIII and IX, respectively. The mean serum insulin values appeared significantly

higher than those of diabetic control rats on 45th day and failed to reach the values of normal control rats on all the days of observation and were significantly lower ($P \leq 0.001$ to $P \leq 0.01$). In comparison with glibenclamide treated group and individual groups of *Aloe vera* and *Murraya koenigii*, the values were comparable and did not differ significantly in any of the combination groups (Table 9 and Fig. 7).

4.5 Haematology

4.5.1 Haemoglobin

The mean (± SE) haemoglobin levels in the normal control animals were observed to be 15.58 ± 0.27, 15.71 ± 0.39, 15.64 ± 0.3 and 15.62 ± 0.39 g % on 3rd, 15th, 30th and 45th days, respectively. There was no significant change ($P \geq 0.05$) in hemoglobin values throughout the experiment.

The mean (± SE) haemoglobin values were 13.25 ± 0.24, 12.79 ± 0.34, 11.75 ± 0.29 and 10.82 ± 0.17 g % on 3rd, 15th, 30th and 45th day of experiment, respectively in diabetic control rats. The decrease was progressive in nature and highly significant ($P \leq 0.001$) in comparison with that of normal control group.

The rats of glibenclamide treatment group showed a consistent increase in the mean (± SE) Hb values from day 3 to day 45. The mean Hb values were 13.01±0.46, 14.04 ± 0.26, 14.75 ± 0.21 and 15.65 ± 0.09 g % on day 3, 15, 30 and 45 post-treatment, respectively. The increase in the mean Hb values was significantly higher ($P \leq 0.01$) at 15th day and ($P \leq 0.001$) at 30th and 45th day in comparison with that of diabetic control

and when compared to normal control animals there was no significant variation except on the 3rd day & 15th day which showed significantly lower values at P≤ 0.001.

The mean (± SE) values of hemoglobin in *Aloe vera* treated rats were 13.16 ± 0.27, 13.81 ± 0.25, 14.63 ± 0.25 and 15.62 ± 0.22 on 3rd, 15th, 30th and 45th day post treatment. Similar to glibenclamide treatment group the values were significantly higher (P≤ 0.001) compared to diabetic control group at 30th and 45th day of observation. Further, the mean values were comparable with that of normal control at 30th and 45th day. However, there was no difference in the values when compared to glibenclamide and were comparable at all intervals of the study.

The mean (± SE) Hb values of *Murraya koenigii* treated rats were 13.17 ± 0.4, 13.94 ± 0.32, 14.74 ± 0.3 and 15.72 ± 0.1 on 3rd, 15th, 30th and 45th day post treatment, respectively. They also showed improvement and were significantly higher (P≤ 0.001) compared to diabetic control group at 30th and 45th day of observation and were comparable with normal control on 30th and 45th day post treatment. There was no significant difference in the values when compared to glibenclamide and were comparable at all intervals of the study.

The mean (± SE) Hb levels of combination treatment groups also showed improvement from 3rd to 45th day post treatment with values of 13.07 ± 0.31, 12.97 ± 0.5, 13.27 ± 0.4 and 13.39 ± 0.25 on 3rd day to 15.57 ± 0.26, 15.57 ± 0.38, 15.88 ± 0.19 and 15.97 ± 0.24 on 45th day of Group VI, VII, VIII and IX, respectively. The values did not differ from those of control rats on 15th, 30th and 45th day post-treatment. When compared to diabetic control all the groups showed significant improvement (P≤ 0.001) on 15th, 30th

and 45th day post treatment. The mean (± SE) Hb values were comparable with glibenclamide treatment group at all intervals of the study with no significant variation. There was no significance difference between rats of treatment groups fed individually with *Aloe vera* and *Murraya koenigii* (Table 10 and Fig. 8).

4.6 Antioxidants

4.6.1 Super oxide dismutase (SOD)

The mean (± SE) SOD activity in the normal control animals were observed to be 39.45±3.76, 40.30±3.93 and 40.72±3.62 U/min/mg protein on 15th, 30th and 45th day, respectively. There was no significant change in the mean SOD values throughout the experiment.

The mean (± SE) levels of SOD activity in Group II (diabetic control) rats were 26.98±1.66, 26.88±1.57 and 27.23±1.31 U/min/mg protein on 15th, 30th and 45th day, respectively and showed progressive decrease from day 15 to day 45 and were significantly lower compared to normal control animals.

The mean (± SE) SOD activity of Group III (rats treated with glibenclamide) were 27.31±1.1, 37.81±2.45 and 40.38±3.12 U/min/mg protein on 15th, 30th and 45th days of the study, respectively. There was a significant improvement in the SOD with $P \leq 0.05$ on day 45 of the study compared to diabetic control and the improvement was statistically not comparable to that of normal control animals in 30th and 45th day.

The mean (± SE) SOD activity of Group IV (rats treated with *Aloe vera)* were 27.38 ± 0.83, 35.02 ± 2.34 and 38.46 ± 1.9 U/min/mg protein on 15th, 30th and 45th days,

respectively. The values were found to be significantly improved with P≤ 0.05 only on day 45 of the study when compared to diabetic control. Also, the values were not comparable with normal control on day 30 & 45 whereas there was significant rise (P≤ 0.05) on day 15. In addition, the results were comparable with those of glibenclamide treated rats throughout the experiment (P≥ 0.05).

The mean (± SE) SOD activity of Group V rats were 27.85 ± 0.49, 36.14 ± 2.50 and 39.85 ± 1.53 U/min/mg protein on 15^{th}, 30^{th} and 45^{th} days, respectively. The values were found to be significantly improved with P≤ 0.5 only on day 15 of the study when compared to diabetic control. The values were not comparable with normal control on all the days of study and were comparable with those of glibenclamide treated rats throughout the experiment (P≥ 0.05).

The rats of Group VI, VII, VIII and IX also showed improvement with SOD activity of 27.77 ± 1.46, 27.72 ± 0.61, 28.06 ± 0.75 and 28.44 ± 1.55 on 15^{th} day to 38.22 ± 1.01, 39.25 ± 0.28, 40.76 ± 1.55 and 41.46 ± 1.80 U/min/mg protein on 45^{th} day post treatment, respectively. The value of mean (± SE) SOD activity were higher compared to diabetic control group, statistically higher (P≤ 0.001) was noticed only on 45^{th} day. However, values were lesser in comparison to normal control and were not comparable at any intervals of study and with glibenclamide treated groups improvement was statistically comparable at P> 0.05 (Table 11 and Fig. 9).

4.6.2 Catalase (CAT)

The mean (± SE) CAT activity in the normal control animals were observed to be 12.89 ± 0.56, 12.97 ± 0.62 and 13.07 ± 0.72 µ moles of H_2O_2/min/mg protein on 15^{th},

30th and 45th day, respectively. There was no significant change in the mean CAT values throughout the experiment.

The mean (± SE) CAT activity values of diabetic control rats progressively decreased and the values are 5.81 ± 0.12, 6.40 ± 0.11 and 7.53 ± 0.15 µ moles of H_2O_2/min/mg protein on 15th, 30th and 45th day of experiment and the decrease was highly significant ($P \leq 0.001$) in comparison with those of normal control group.

The rats of glibenclamide treated group showed a consistent increase in the mean (± SE) CAT activity from day 15 to day 45. The mean CAT values were 7.59 ± 0.25, 12.40 ± 0.04 and 13.92 ± 0.06 µ moles of H_2O_2/min/mg protein on 15, 30 and 45 post-treatment, respectively. There was significant improvement in the mean CAT levels when compared to diabetic control with $P \leq 0.001$ on 45th day. The improvement was not comparable to normal control group with significantly lesser values ($P \leq 0.001$) throughout the study period.

The mean (± SE) CAT activity of the rats treated with *Aloe vera* showed an improvement from 15th to 45th day with values of 8.40 ± 0.04 on 15th day to 10.71 ± 0.08 µ moles of H_2O_2/min/mg protein on 45th day, respectively. The values of CAT activity were higher compared to diabetic control animals and statistically significant variation was seen on 30th and 45th day post treatment ($P \leq 0.01$ and $P \leq 0.001$). Also, the values were not comparable with normal control on all the days of study with significantly lesser values ($P \leq 0.001$). However, the results were comparable with those of glibenclamide treated rats throughout the experiment.

The mean (± SE) values of CAT activity in rats of *Murraya koenigii* treated were 8.38 ± 0.25, 9.63 ± 0.09 and 11.57 ± 0.19 µ moles of H_2O_2/min/mg protein on 15th, 30th and 45th day post treatment, respectively. They also showed improvement and were significantly higher ($P \leq 0.05$ and $P \leq 0.001$) compared to diabetic control group at 30th and 45th days of observation and were not comparable with normal control with significantly lesser values. However, there was no significant difference in the values when compared to glibenclamide and were comparable in all intervals of the study.

The rats of Group VI, VII, VIII and IX also showed improvement with CAT activity of 8.50 ± 0.14, 8.66 ± 0.03, 8.63 ± 0.31 and 8.53 ± 0.26 on 15th day to 11.40 ± 0.05, 11.73 ± 0.04, 11.9 ± 0.04 and 11.92 ± 0.04 µ moles of H_2O_2/min/mg protein on 45th day post treatment, respectively. The value of mean CAT activity were statistically higher ($P \leq 0.001$) compared to diabetic control group on 30th and 45th day in all groups and on 15th day moderately higher ($P \leq 0.05$) values seen in Group VI and IX and significantly higher ($P \leq 0.001$) was observed in Group VII and VIII. However, in comparison to normal control animals the values were significantly less on 30th and 45th day. With glibenclamide group, the values were comparable at 15th day and on 30th and 45th day Group VI to IX showed improvement with statistical significant variation at $P \leq 0.001$ and $P \leq 0.01$, respectively (Table 12 and Fig. 10).

4.6.3 Glutathione peroxidase (GPx)

The mean (± SE) value of GPx activity in the normal control animals were observed to be 162.35 ± 5.85, 165.25 ± 8.15 and 170.15 ± 4.35 µM of glutathione utilized/min/mg protein on 15th, 30th and 45th day, respectively. There was no significant change in the mean GPx activity throughout the experiment.

The mean (± SE) value of GPx activity of diabetic control rats gradually decreased and the values were 125.78 ± 10.5, 134.9 ± 8.7 and 138.75 ± 3.55 µM of glutathione utilized/min/mg protein on 15th and 45th day of experiment and the decrease was significant ($P \leq 0.01$) in comparison with those of normal control group.

The rats of glibenclamide treated group showed a consistent increase in the mean (± SE) GPx activity from day 15 to day 45. The mean values GPx activity were 136.85 ± 4.45, 144.05 ± 4.75 and 147.2 ± 4.1 µM of glutathione utilized/min/mg protein on 15, 30 and 45 days post-treatment, respectively. There was moderate improvement in the mean GPx activity when compared to diabetic control and normal control group.

The mean (± SE) GPx activity of the rats treated with *Aloe vera* showed an improvement from 15th to 45th day with values of 136.4 ± 3.2 on 15th day to 159.95 ± 1.35 µM of glutathione utilized/min/mg protein on 45th day, respectively. The values of GPx activity were higher compared to diabetic control animals. The results were comparable with those of glibenclamide treated rats throughout the experiment.

The mean (± SE) GPx activity of rats of *Murraya koenigii* treated rats were 133.85 ± 4.45, 163.55 ± 3.35 and 169.2 ± 0.6 µM of glutathione utilized/min/mg protein on 15th, 30th and 45th day post treatment, respectively. They showed improvement and were significantly higher ($P \leq 0.05$) compared to diabetic control group 45th days of observation and were comparable with normal control. There was no significant difference in the values when compared to glibenclamide and were comparable at all intervals of the study.

The rats of Group VI, VII, VIII and IX also showed improvement with GPx activity of 135.5 ± 0.8, 133.4 ± 1.1, 133.55 ± 6.65 and 131.6 ± 3 on 15th day to 168.1 ± 5.4, 166.05 ± 7.15, 170.05 ± 3.75 and 169.1 ± 5.5 µM of glutathione utilized/min/mg protein on 45th day post treatment, respectively. The value of mean GPx were higher compared to diabetic control group and statistically higher ($P \leq 0.05$) value was noticed only on 45th day. However, in comparison to normal control animals the values were significantly less and with glibenclamide group, the values were comparable without significant variation at different intervals of the study (Table 13 and Fig. 11).

4.7 Pathology

4.7.1 Clinical signs

In the present study, Normal control rats (Group I) remained healthy and were active throughout the period of experiment (Plate 5).

Polyuria, polydypsia, polyphagia, restlessness and poor body condition were recorded by 48 to 72 h post STZ injection in diabetic control rats (Group II) and the signs persisted till 45th day of the study in a relatively severe degree (Plate 6 and 7).

The animals of Glibenclamide treated rats (Group III) manifested clinical signs similar to that of diabetic control rats during initial period of study but the intensity of clinical signs decreased gradually from 3rd to 45th day post treatment (Plate 8).

The animals of all other treatment groups (Group IV to IX) exhibited clinical signs similar to those of diabetic control rats which comprised of polyuria, polydipsia, weakness, ruffled hair coat by 48 to 72 hr post STZ injection. The intensity of various

clinical signs exhibited were gradually reduced from 3rd to 45th day post treatment in all the groups with remarkable improvement in the body condition of the rats after treatment.

4.7.2 Gross pathology

The Normal Control rats of Group I did not reveal any gross abnormalities during the experimental study (Plate 9 & 11).

In the diabetic control rats (Group II), there was gradual decrease in the size of the pancreas which was noticeable from day 15 of the study. The atrophic changes in the pancreas was a distinct feature observed in the diabetic control rats (Plate 10 & 12). From day 30 of the experimental study, the liver was pale, soft and friable. The spleen appeared pale and reduced in size. The other organs such as kidneys, intestine, lungs and heart did not reveal any gross lesions throughout the study period.

The pancreas of glibenclamide treatment group on various intervals of experimental period showed reduction in size initially but progressive improvement was observed from day 15. The liver showed increase in size and paleness in color on day 15 but the color and consistency of the liver reverted to normalcy during the 30th to 45th day of post-treatment. The other organs viz, kidney, heart, lung and intestine did not reveal any distinct gross changes at any interval of the study.

Grossly, the pancreas in other treatment groups (IV to IX) also appeared reduced in size on day 15, but gradually improved with slight variation between different treatment groups. A better improvement was observed in rats of combination groups (VI, VII, VIII and IX) than individual groups (IV and V). No recordable macroscopic changes were observed in other organs in all the groups except in liver (Plate 13, 14, 15 and 16).

4.7.3 Histopathology

4.7.3.1 Group I

Pancreas of Normal control rats (Group I) examined during different period of experiment (day 15, 30 and 45) revealed normal appearance of exocrine and endocrine portion. The acini were normal and Islets of Langerhans were more in number, round to oval in shape and of larger size (Plate 17, 18 and 23). The other organs viz, liver, kidney, heart, lung, and spleen also exhibited normal appearance on histopathological examination (Plate 33 and 34).

4.7.3.2 Group II

In Diabetic control rats of Group II, the pancreas showed numerous lobules with loss of architecture on 15th day post STZ injection. Vacuolated, degenerating and necrotic cells were noticed in the acini. However, in some lobules hyperplastic acinar cells were also observed. The lobules which were intact exhibited large number of necrotic cells. There number of Islets of Langerhans per lobule was drastically reduced indicating loss of normal architecture. The normal distribution of alpha and beta cells appeared altered with considerable reduction in the number of cells.

On 30th day of experimental study, further reduction in the number of islets which appeared shrunken and showed increase in severity in those lesions comparatively to the lesions at 15th day. The reduction in beta cells was clearly enmarked in immunihistochemical study.

On 45th day of the study, small sized islets were noticed predominantly which were difficult to identify. The islets revealed depletion of beta cells and increase in the

number of alpha cells. The persisting beta cells were characterised by the loss of cytoplasmic granularity, cytoplasmic vacuolations and necrosis. The microscopic picture of islets in diabetic rats indicated progressive destruction of beta cells from day 15 to day 45 of the experimental study (Plate 25 to 28).

The histopathological changes in liver included, swelling of hepatocytes with increase in granularity, vacuolations, obliteration of sinusoidal spaces and areas of individual hepatocyte necrosis. Moderate to severe degree of congestion and infiltration of inflammatory cells were also noticed (Plate 35 and 36).

Microscopically, spleen revealed depletion of lymphoid cells from periarteriolar sheath as well as from the follicles. The red pulp was hypocellular with hemosiderosis. These changes were prominent by 45^{th} day post STZ treatment (Plate 39).

Kidneys of diabetic rats (Group II) did not show consistent microscopic lesions throughout the study period but some animals showed mild swelling of tubular epithelium and a slight degree of vacuolar degeneration and congestive changes. Similarly, histopathological examination of heart, lungs and intestine did not show distinct lesions throughout the study period.

4.7.3.3 Group III

In glibenclamide treatment group there was a declining trend in the severity of the lesions in the pancreas. With the advancement of time a progressive improvement towards attainment of normal architecture of acini and Islets of Langerhans were observed from 15^{th} to 45^{th} day of experiment. The pancreatic acini revealed vacuolated,

degenerating and necrotic cells on 15th day. However, on 30th and 45th day attainment of normal architecture with few lobules showing hyperplastic cells were seen.

On 15th day, the islets revealed presence of a few small islets comprising of vacuolated beta cells and there was improvement in the architecture of pancreas. By day 30 post-treatment, there was improvement in number, shape and size of islets which revealed compactness in the arrangement. The cells consisted of round to oval nucleus and scanty cytoplasm indicative of alpha cells. However, a few vacuolated beta cells were also seen.

By 45th day post-treatment, the number of islets appeared increased which were either round or oval shaped with compact arrangement of beta cells at the core and alpha cells at the periphery exhibiting increase in cellularity. The beta cells appeared round with increased cytoplasmic granularity. However, in occasional islets mild to moderate vacuolar changes persisted (Plate 41 and 42).

The liver showed improvement in the architecture from day 15 to day 45 post-treatment. On 15th day post-treatment, the liver lobules revealed cell swelling and centrilobular vacuolar degeneration. The blood vessels were mildly congested and the sinusoidal spaces were obliterated.

On 30th and 45th day of post-treatment there was an improvement in liver morphology especially in the form of reduction in hepatic vacuolar degenerative changes and attainment of almost normal architecture. Spleen revealed improvement in lymphocyte population compared to diabetic control animals.

Histopathologically kidney, heart, lungs and intestine did not reveal any appreciable changes throughout the period of experimentation.

4.7.3.4 Groups IV, V, VI, VII, VIII and IX

In the present study, there was progressive improvement in the architecture of acini and islets in pancreas in all the treatment groups from 15^{th} to 45^{th} day of the experimental study.

The exocrine portion of acini revealed improvements which were comparable to glibenclamide treatment. On 15^{th} day, vacuolated, degenerative and necrotic cells were seen in many lobules. Few lobules also showed large number of apoptotic cells. Neovascularisation and formation of new blood vessels was evident on 15^{th} day. By 30^{th} day many of the lobules showed hyperplastic acinar cells. On 45^{th} day post treatment most of the acini showed normal architecture with few of the lobules containing hyperplastic acinar cells.

The microscopic changes observed in the endocrine pancreas on 45^{th} day post-treatment included presence of only few islets which were of small to moderate size with irregular shape.

Compared to individual herbal treatment groups (*Aloe vera* or *Murraya koenigii*) treatment groups the combination groups (VI, VII, VIII and IX) revealed more number of islets per lobule and the islets were of bigger size and had more compact arrangement. Tendency to form new islets near the blood vessels was a consistent feature seen in many

of the lobules. Many of the precursor cells appeared to have derived from the ductal epithelial cells (Plate 45, 49 and 53).

On 30th day the islets which were considerably larger compared to 15th day and revealed hypercellularity with more number of alpha cells and few normally appearing beta cells. Compared to individual treatment groups (Group IV and V), combination treatment groups revealed islets of larger size and were more in number with compact arrangement of cells.

On 45th day, the islets showed almost normal architecture, increased cellularity with more number of islets per lobule compared to 15th and 30th day of experimental study. However, normal α: β ratio was not attained compared to normal control group. Most of the beta cells occupied core and alpha cells at the periphery (Plate 46, 50, 54, 57, 58 and 61).

The microscopic lesions in the liver of diabetic animals treated with *Aloe vera or Murraya koenigii or* in combination, showed an improvement in the architecture of the organ compared to diabetic control animals with reduction of lesions like cell swelling, vacuolations and granularity of the cytoplasm. By 30th day further improvement in the architecture was observed. On day 45, cellular swelling, granularity and vacuolations of cytoplasm reduced significantly and they were of mild nature (Plate 37 and 38).

The spleen of the combined treatment groups revealed increase in lymphocyte population compared to diabetic control animals with the advancement of time. By 45th day normal architecture of spleen was observed in all the treatment groups. Few animals

in treatment group showed hypercellularity with secondary follicle formation (Plate 40) Characteristic microscopic changes were not noticed in the kidneys, lungs, intestine and heart in the combined treatment groups of animals throughout the study period.

4.7.4 Immunohistochemistry

In the present study, immunohistochemical demonstration of insulin was carried out to identify and enumerate β-cells in various treatment groups using polyclonal insulin antibody. 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 of islets were evaluated. Swollen cells with irregular borders consisting of scattered slightly stained cytoplasm were considered as degenerating cells and the cells which appeared round with compact arrangement of densely stained cytoplasm were considered as regenerated β-cells after damage induced by streptozotocin.

In the Normal control group (Group I) most of the islets revealed intensely stained positive cells in large number. The α-cells and acini of exocrine pancreas were negative and the IHC positive β-cells revealed densely stained cytoplasmic granules that were compactly arranged and limited to the regular membrane (Plate 19, 20, 21, 22 and 24). The mean percentages of insulin secretory cells were 75.42 ± 2.79, 77.09 ± 2.75 and 76.06 ± 2.59 on 15^{th}, 30^{th} and 45^{th} day, respectively.

The pancreas islets in Diabetic control animals (Group II) revealed a sudden decrease in the number of insulin positive β cells. The mean percentages of insulin secretory cells were 4.58 ± 1.03, 3.91 ± 0.51 and 4.34 ± 0.38 on 15^{th}, 30^{th} and 45^{th} day

respectively. Most of the IHC positive cells appeared swollen and irregular with granular material in the cytoplasm indicating degenerating cells (Plate 29 to 32).

In Group III, glibenclamide treated diabetic rats, progressive increase in number of insulin positive cells was noticed. The mean (± SE) percentage of positive cells showed an increase in values from 14.28 ± 2.06 on 15^{th} day to 65.35 ± 2.97 on 45^{th} day. The numbers of degenerating cells were less compared to the diabetic control group and β-cells with more compact insulin positive granular material in the cytoplasm increased as the treatment progressed. The improvement was significantly higher ($P \leq 0.001$) compared to diabetic control group at 30^{th} and 45^{th} days of observation and were not comparable with normal control with significantly lesser values (Plate 43 and 44).

All other treatment groups (Group IV to IX) also showed progressive improvement in the insulin positive cells at regular intervals of post treatment (Plate 47, 48, 51, 52, 55, 56, 59, 60 and 62 to 64). The mean (± SE) percentage positive cells showed increase in values from 12.45 ± 1.23, 13.35 ± 1.17, 12.73 ± 1.39, 12.87 ± 1.48, 13.91 ± 0.66 and 14.67 ± 0.54 on 15^{th} day to 52.98 ± 1.34, 61.86 ± 3.45, 56.65 ± 2.69, 58.51 ± 2.88, 63.29 ± 2.07 and 64.48 ± 2.63 on 45^{th} day of Groups IV, V, VI, VII, VIII and IX, respectively. The values were significantly higher ($P \leq 0.001$) compared to diabetic control group on 30^{th} and 45^{th} day. However, in comparison to normal control animals the values were non-significant in Group VIII & IX. In comparison with glibenclamide group, the values were similar on 15^{th} and 30^{th} day without significant variation, but on 45^{th} day only Group V showed significant variation (Table 14 and Fig. 12).

Table 3. The Mean (± SE) animal body weight (g) values of various groups at different intervals of time

Groups	Days Post Treatment			
	3	**15**	**30**	**45**
Group I (NC)	194.40±2.19^{ab}	203.8±2.42^{a}	211.25±2.51^{a}	219.67±2.78^{a}
Group II (DC)	173.28±1.28^{e}	164.4±1.80^{d}	158.13±1.62^{e}	150.17±1.44^{d}
Group III (GC)	182.50±3.52^{cd}	193.4±3.70^{b}	199.75±3.74^{bc}	204.67±4.86^{b}
Group IV (AV)	174.50±2.03^{de}	180.8±2.37^{c}	186.5±1.52^{d}	190.33±2.09^{c}
Group V (MK)	185.60±4.29^{bc}	193.9±4.88^{b}	198.75±4.40^{c}	203.33±4.39^{b}
Group VI (AV+MK)	198.80±2.9^{a}	204.3±2.81^{a}	209.38±1.89^{a}	213.17±2.18^{ab}
Group VII (AV+G)	172.30±1.07^{e}	178.1±1.31^{c}	181.5±0.85^{d}	187.17±0.79^{c}
Group VIII (MK+G)	192.56±3.64^{ab}	202.6±3.95^{ab}	208.5±3.13^{ab}	215.83±2.39^{a}
Group IX (AV+MK+G)	189.50±4.16^{bc}	196.3±5.41^{ab}	204.5±5.45^{abc}	212.33±6.69^{ab}

Mean values with different superscript differ significantly

^{a}Comparison with Group I, ^{b}Comparison with Group II, ^{c}Comparison with Group III

Values are statistically significant at $P \leq 0.05$

Fig. 1: Mean (± SE) animal body weights (g) values of various groups at different intervals of time

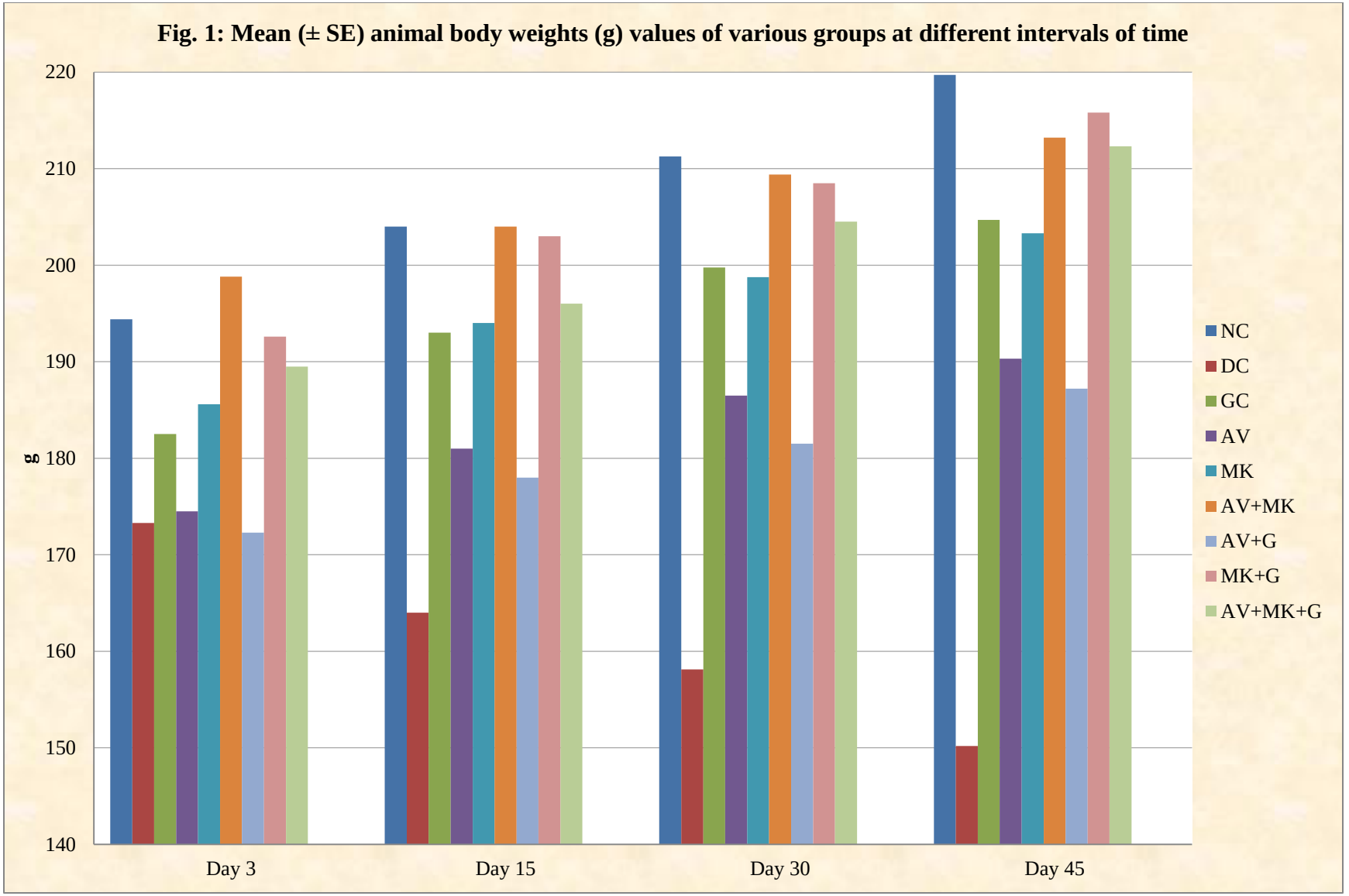


Table 4. The Mean (± SE) serum glucose (mg/dL) values of various groups at different intervals of time

Groups	Days Post Treatment			
	3	**15**	**30**	**45**
Group I (NC)	87.80 ± 5.39^{b}	82.5 ± 5.01^{e}	84.88 ± 4.71^{e}	86.66 ± 4.78^{c}
Group II (DC)	418.80 ± 23.90^{a}	327.5 ± 22.44^{a}	303.25 ± 20.42^{a}	311.83 ± 27.03^{a}
Group III (GC)	438.00 ± 12.81^{a}	219.8 ± 5.92^{cd}	206.12 ± 5.76^{cd}	181.66 ± 7.60^{b}
Group IV (AV)	463.90 ± 16.31^{a}	258 ± 12.93^{b}	238.75 ± 13.43^{b}	197.33 ± 10.07^{b}
Group V (MK)	439.40 ± 17.71^{a}	251.7 ± 13.90^{b}	220.62 ± 9.23^{bc}	184.33 ± 7.01^{b}
Group VI (AV+MK)	436.10 ± 24.30^{a}	229.8 ± 10.23^{bc}	223.5 ± 11.16^{bc}	194.5 ± 6.40^{b}
Group VII (AV+G)	440.40 ± 15.74^{a}	215.5 ± 6.58^{cd}	202.5 ± 5.34^{cd}	187.83 ± 5.61^{b}
Group VIII (MK+G)	444.80 ± 14.71^{a}	213.9 ± 4.46^{cd}	197.12 ± 4.85^{cd}	182.16 ± 4.20^{b}
Group IX (AV+MK+G)	445.10 ± 13.11^{a}	198.5 ± 2.56^{d}	188.75 ± 1.68^{d}	178.83 ± 1.93^{b}

Mean values with different superscript differ significantly

^{a}Comparison with Group I, ^{b}Comparison with Group II, ^{c}Comparison with Group III

Values are statistically significant at $P \leq 0.05$

Fig. 2: Mean (± SE) serum glucose (mg/dL) values of various groups at different intervals of time

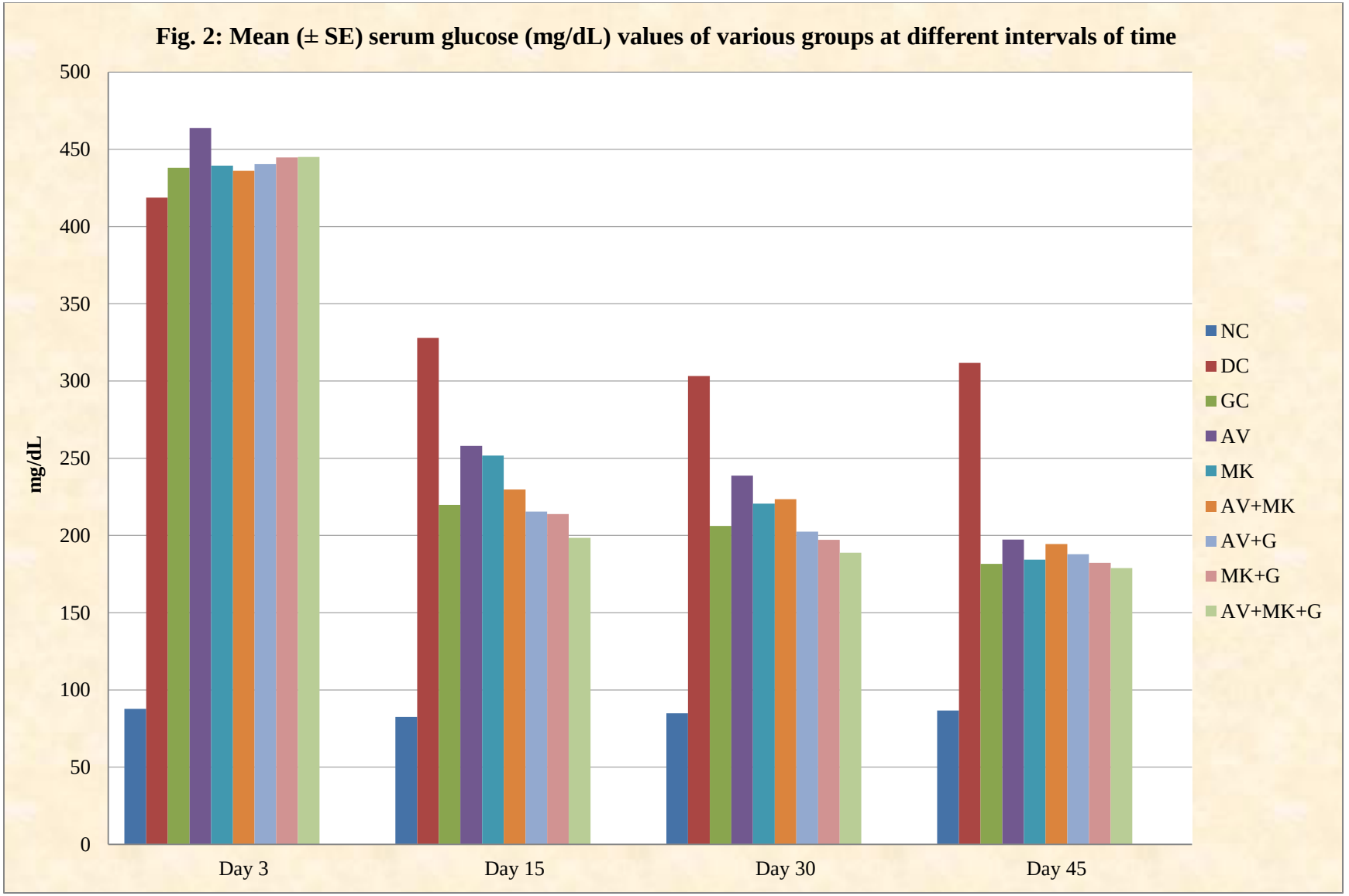


Table 5. The Mean (± SE) serum cholesterol (mg/dL) values of various groups at different intervals of time

Groups	Days Post Treatment			
	3	**15**	**30**	**45**
Group I (NC)	83.34±1.78^{b}	84.8±1.88^{d}	87.20±2.00^{e}	84.62±1.99^{e}
Group II (DC)	121.54±3.59^{a}	128.78±2.47^{a}	136.8±2.94^{a}	141.5±2.39^{a}
Group III (GC)	119.81±1.82^{a}	115.06±1.79^{b}	108.5±1.43^{bc}	103.2±1.83^{bc}
Group IV (AV)	122.29±2.03^{a}	115.31±1.77^{b}	109.4±1.27^{b}	104.3±1.4^{b}
Group V (MK)	114.50±2.58^{a}	107.41±2.28^{c}	101.86±1.43^{d}	95.82±1.48^{d}
Group VI (AV+MK)	120.07±2.03^{a}	114.18±1.79^{b}	107.4±2.08^{bcd}	102.3±1.69^{bc}
Group VII (AV+G)	117.53±1.99^{a}	109.77±2.12^{bc}	103.1±1.47^{bcd}	98.63±1.68^{bcd}
Group VIII (MK+G)	116.4±2.76^{a}	109.77±2.23^{bc}	102.4±1.94^{cd}	97.3±2.92^{cd}
Group IX (AV+MK+G)	120.19±2.59^{a}	106.53±2.26^{c}	101.46±1.77^{d}	99.28±2.32^{bcd}

Mean values with different superscript differ significantly

^{a}Comparison with Group I, ^{b}Comparison with Group II, ^{c}Comparison with Group III

Values are statistically significant at $P \leq 0.05$

Fig. 3: Mean (± SE) serum cholesterol (mg/dL) values of various groups at different intervals of time

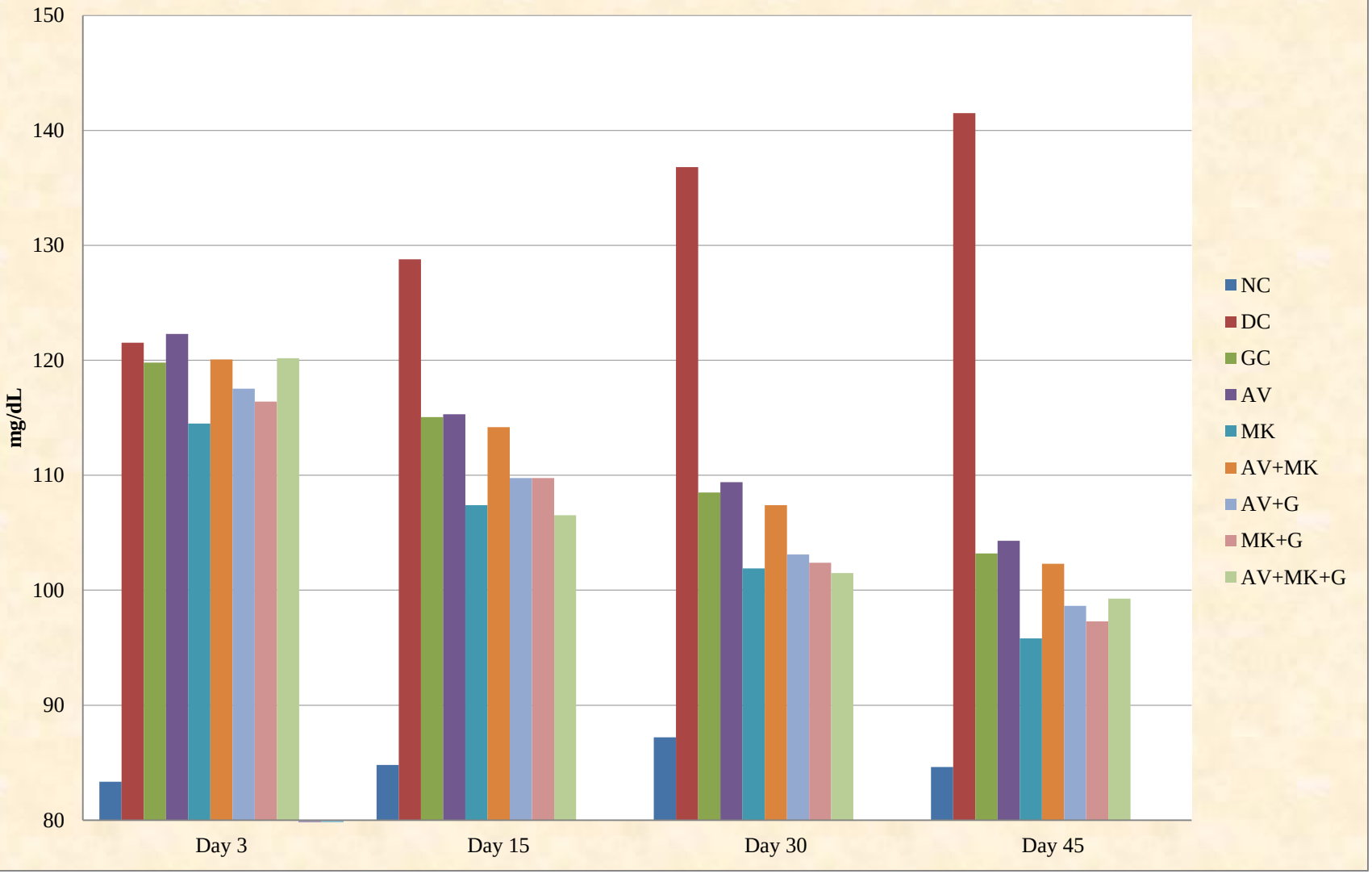


Table 6. The Mean (± SE) serum triglyceride (TG) (mg/dL) values of various groups at different intervals of time

Groups	Days Post Treatment			
	3	**15**	**30**	**45**
Group I (NC)	86.09±1.98^{c}	90.27±1.9^{c}	91.23±0.98^{d}	94.267±1.54^{bc}
Group II (DC)	152.52±4.13^{a}	170.46±3.41^{a}	182.16±3.32^{a}	188.7±3.25^{a}
Group III (GC)	147.54±1.62^{ab}	128.68±1.83^{b}	111.71±2.09^{b}	98.967±1.76^{b}
Group IV (AV)	141.74±3.19^{ab}	126.65±2.28^{b}	111.03±1.46^{b}	99.817±1.94^{b}
Group V (MK)	139.36±3.3^{b}	120.49±2.71^{b}	101.86±1.73^{c}	89.05±1.82^{c}
Group VI (AV+MK)	140.73±4.95^{b}	126.95±3.35^{b}	112.91±2.13^{b}	93.85±1.79^{bc}
Group VII (AV+G)	142.25±3.49^{ab}	126.56±2.79^{b}	113.54±2.44^{b}	97.45±2.51^{b}
Group VIII (MK+G)	141.98±3.66^{ab}	124.43±2.76^{b}	107.45±1.6^{bc}	93.367±2.51^{bc}
Group IX (AV+MK+G)	143.32±4.73^{ab}	125.45±2.63^{b}	108.34±1.55^{bc}	95.283±3.17^{bc}

Mean values with different superscript differ significantly

^{a}Comparison with Group I, ^{b}Comparison with Group II, ^{c}Comparison with Group III

Values are statistically significant at $P \leq 0.05$

Fig. 4: Mean (± SE) serum triglyceride (mg/dL) values of various groups at different intervals of time

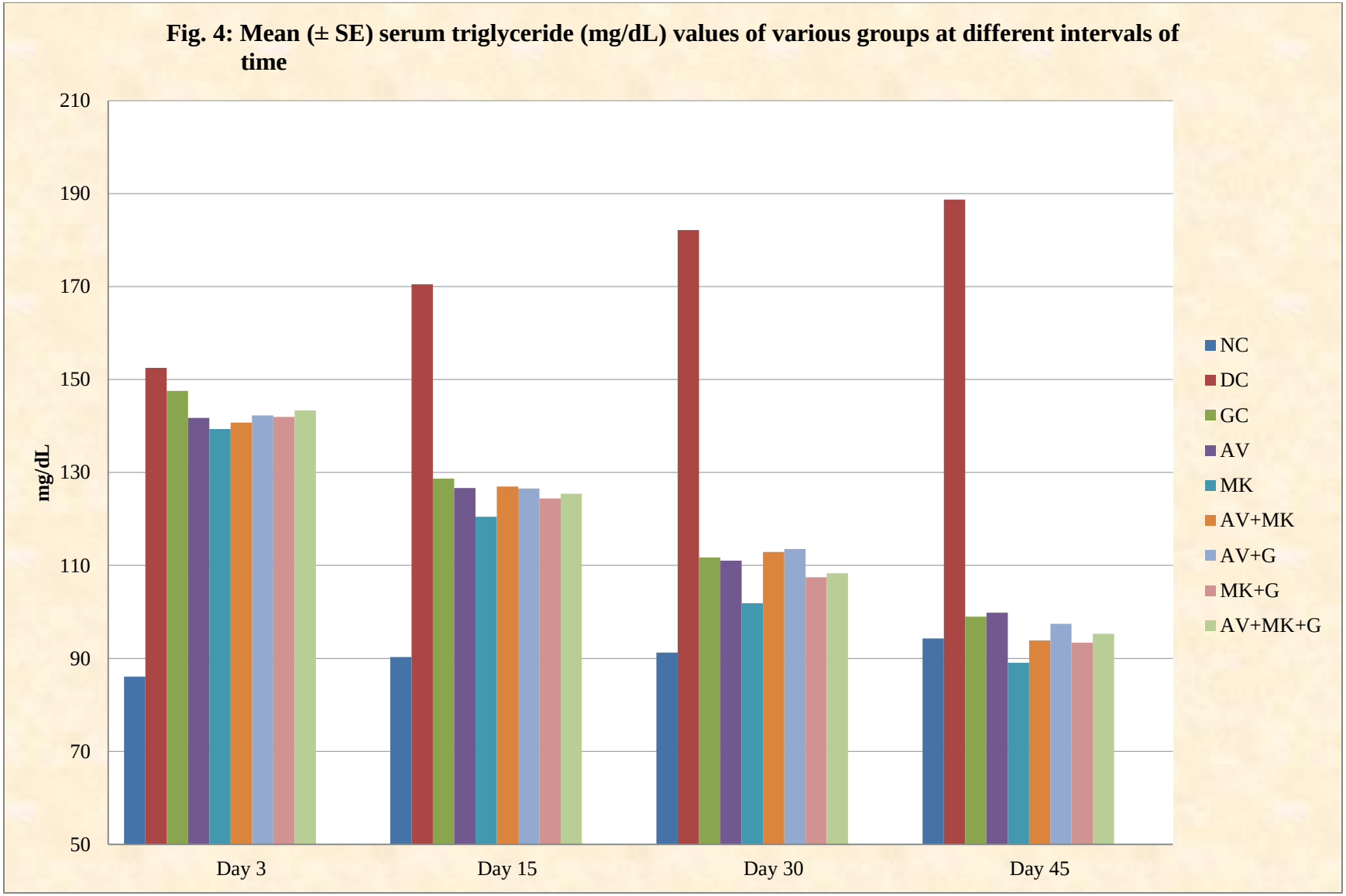


Table 7. The Mean (± SE) serum alanine aminotransferase (ALT) (IU/L) values of various groups at different intervals of time

Groups	Days Post Treatment			
	3	**15**	**30**	**45**
Group I (NC)	56.41±2.67^{b}	58.64±2.67^{d}	60.3±2.57^{f}	61.13±2.09^{e}
Group II (DC)	122.65±3.23^{a}	128.51±3.23^{a}	141.18±1.87^{a}	202.28±3.28^{a}
Group III (GC)	119.51±4.08^{a}	108.2±4.08^{b}	96.43±3.49^{bc}	76.78±2.23^{cd}
Group IV (AV)	123.75±1.76^{a}	109.09±1.76^{b}	92.31±2.31^{bcd}	84.03±2.44^{b}
Group V (MK)	118.77±3.16^{a}	101.74±3.16^{c}	82.99±2.07^{e}	76.5±1.09^{cd}
Group VI (AV+MK)	121.05±1.55^{a}	111.93±1.55^{b}	97.56±2.14$^{\mathbf{b}}$	87.65±2.07^{b}
Group VII (AV+G)	120.65±3.91^{a}	106.18±3.91^{bc}	89.92±2.82^{cde}	80.93±1.27^{bcd}
Group VIII (MK+G)	124.22±2.64^{a}	109.17±2.6347^{b}	91.61±3.57^{bcd}	82.9±2.63^{bc}
Group IX (AV+MK+G)	119.65±2.38^{a}	107.21±2.3809^{b}	87.99±3.02^{de}	75.42±1.8^{d}

Mean values with different superscript differ significantly

^{a}Comparison with Group I, ^{b}Comparison with Group II, ^{c}Comparison with Group III

Values are statistically significant at $P \leq 0.05$

Fig. 5: Mean (± SE) serum alanine aminotransferase (ALT) (IU/L) values of various groups at different intervals of time

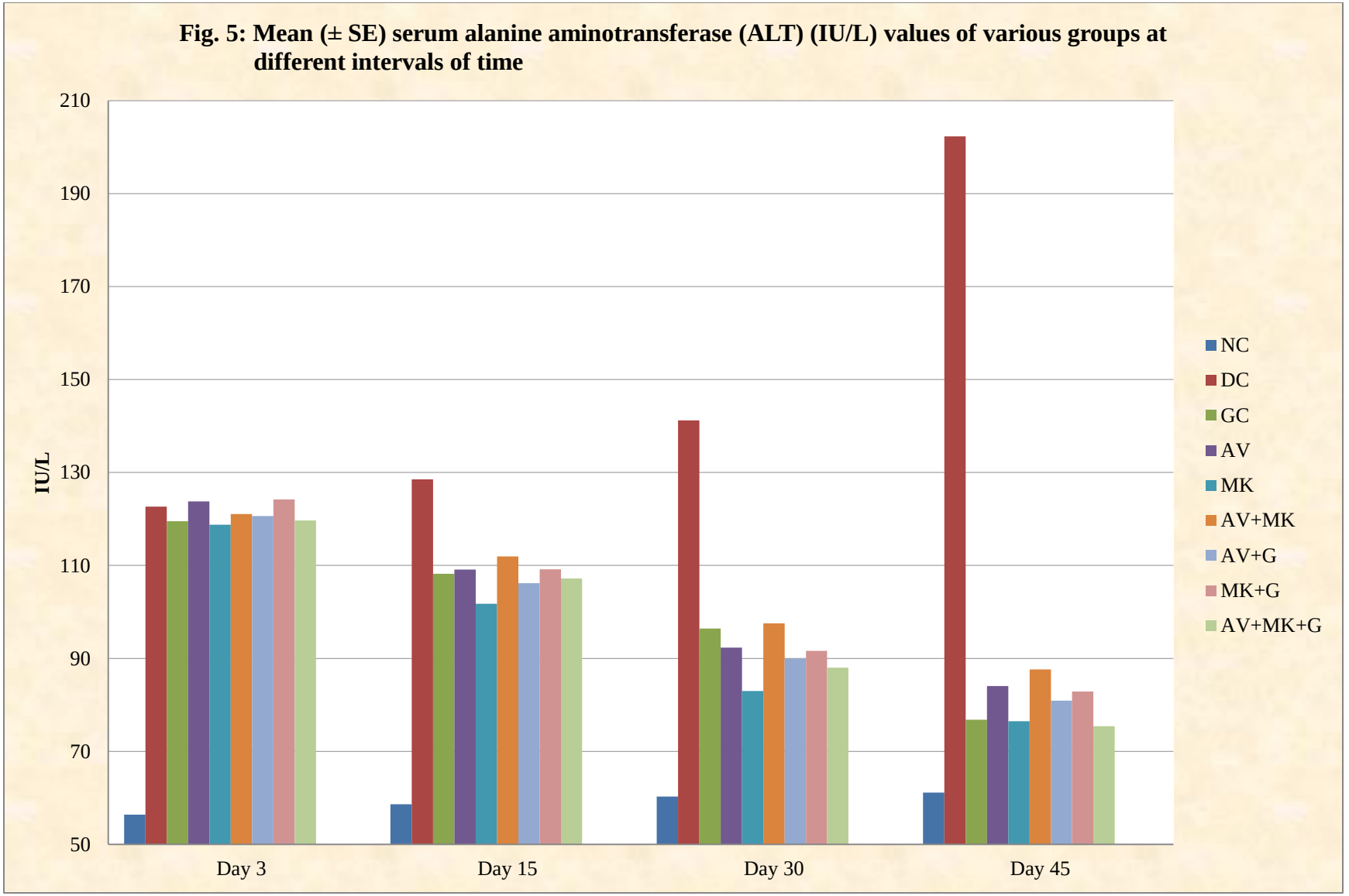


Table 8. The Mean (± SE) serum aspartate aminotransferase (AST) (IU/L) values of various groups at different intervals of time

Groups	Days Post Treatment			
	3	**15**	**30**	**45**
Group I (NC)	81.64±1.91[b]	83.39±1.78[c]	86.35±2.01[f]	85.83±1.79[e]
Group II (DC)	252.22±14.99[a]	260.69±13.13[a]	270.05±11.05[a]	289.38±6.62[a]
Group III (GC)	255.65±15.23[a]	203.43±7.65[b]	189.61±2.58[bc]	146.83±5.05[bc]
Group IV (AV)	242.17±8.35[a]	207.99±4.72[b]	187.85±4.19[bcd]	148.33±3.12[bc]
Group V (MK)	245.57±11.26[a]	205.5±4.15[b]	179.17±2.89[cde]	141.3±3.64[cd]
Group VI (AV+MK)	246.61±8.6[a]	208.72±2.9[b]	192.5±3.73[b]	156.16±6.1[b]
Group VII (AV+G)	242.44±9.86[a]	203.1±3.79[b]	181.66±2.86[bcde]	145.56±4.92[bc]
Group VIII (MK+G)	241.01±11.26[a]	199.51±6.65[b]	171.42±3.96[e]	128.55±1.84[d]
Group IX (AV+MK+G)	249.09±11.2[a]	205.68±4.44[b]	175.76±2.64[de]	135.51±4.47[cd]

Mean values with different superscript differ significantly

[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III

Values are statistically significant at $P \leq 0.05$

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

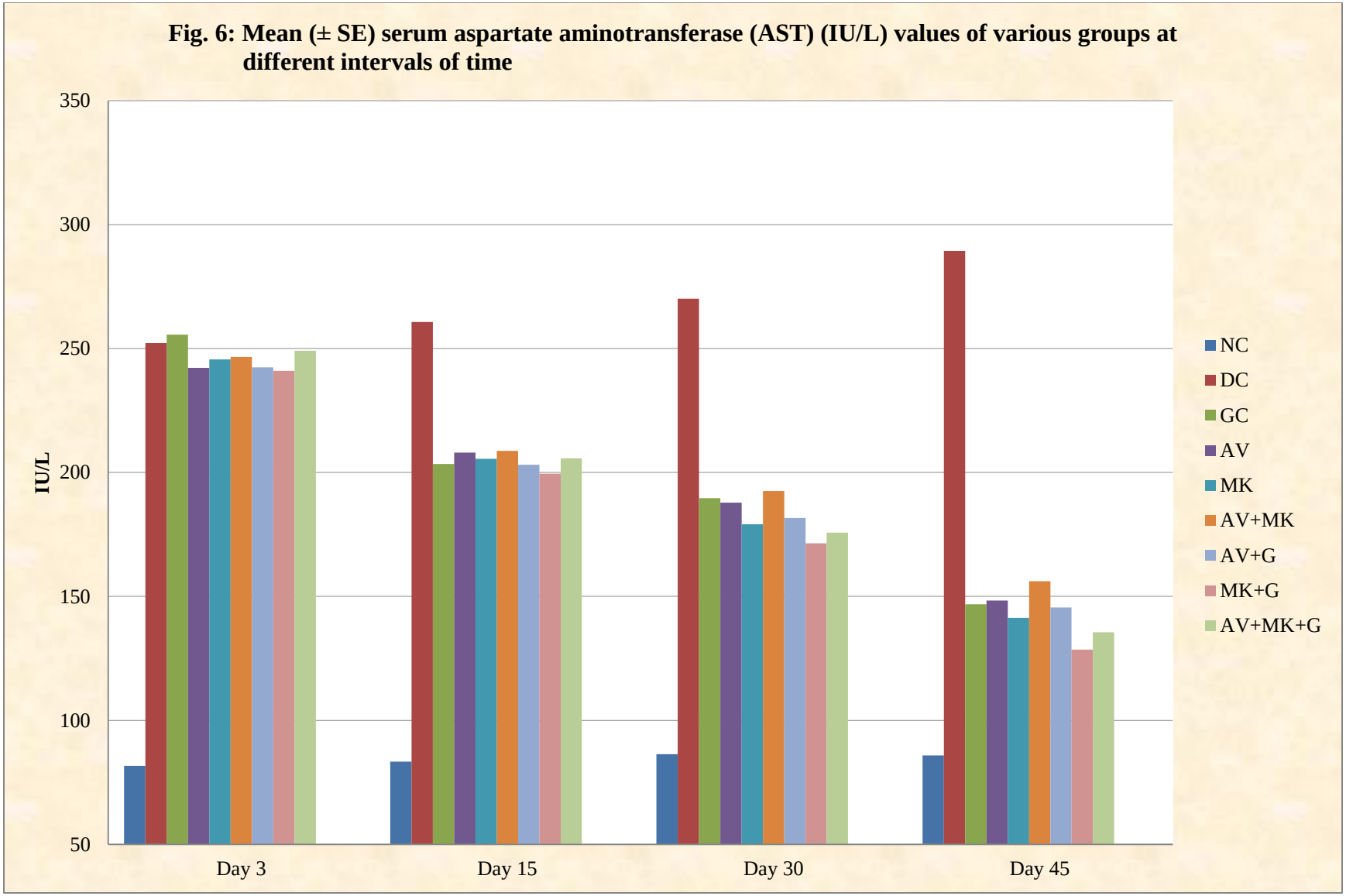


Table 9. The Mean (± SE) serum insulin (µU/L) values of various groups at different intervals of time

Groups	Days Post Treatment			
	3	**15**	**30**	**45**
Group I (NC)	4.51±0.05[a]	4.53±0.04[a]	4.52±0.05[a]	4.73±0.06[a]
Group II (DC)	3.72±0.02 [b]	3.67±0.02 [b]	3.69±0.02[c]	3.74±0.02[c]
Group III (GC)	3.61±0.09 [b]	3.72±0.10 [b]	4.13±0.11[b]	4.46±0.06 [b]
Group IV (AV)	3.57±0.09 [b]	3.74±0.05 [b]	4.04±0.06[b]	4.37±0.08 [b]
Group V (MK)	3.64±0.09 [b]	3.73±0.11 [b]	4.11±0.09[b]	4.50±0.04 [b]
Group VI (AV+MK)	3.62±0.08 [b]	3.70±0.05 [b]	4.01±0.07[b]	4.44±0.03 [b]
Group VII (AV+G)	3.53±0.11 [b]	3.635±0.12 [b]	3.96±0.08[b]	4.37±0.01 [b]
Group VIII (MK+G)	3.58±0.10 [b]	3.69±0.10 [b]	4.05±0.06[b]	4.44±0.03 [b]
Group IX (AV+MK+G)	3.55±0.05 [b]	3.68±0.04 [b]	4.09±0.05[b]	4.45±0.05 [b]

Mean values with different superscript differ significantly

[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III

Values are statistically significant at $P \leq 0.05$

Fig. 7: Mean (± SE) serum insulin (µU/L) values of various groups at different intervals of time

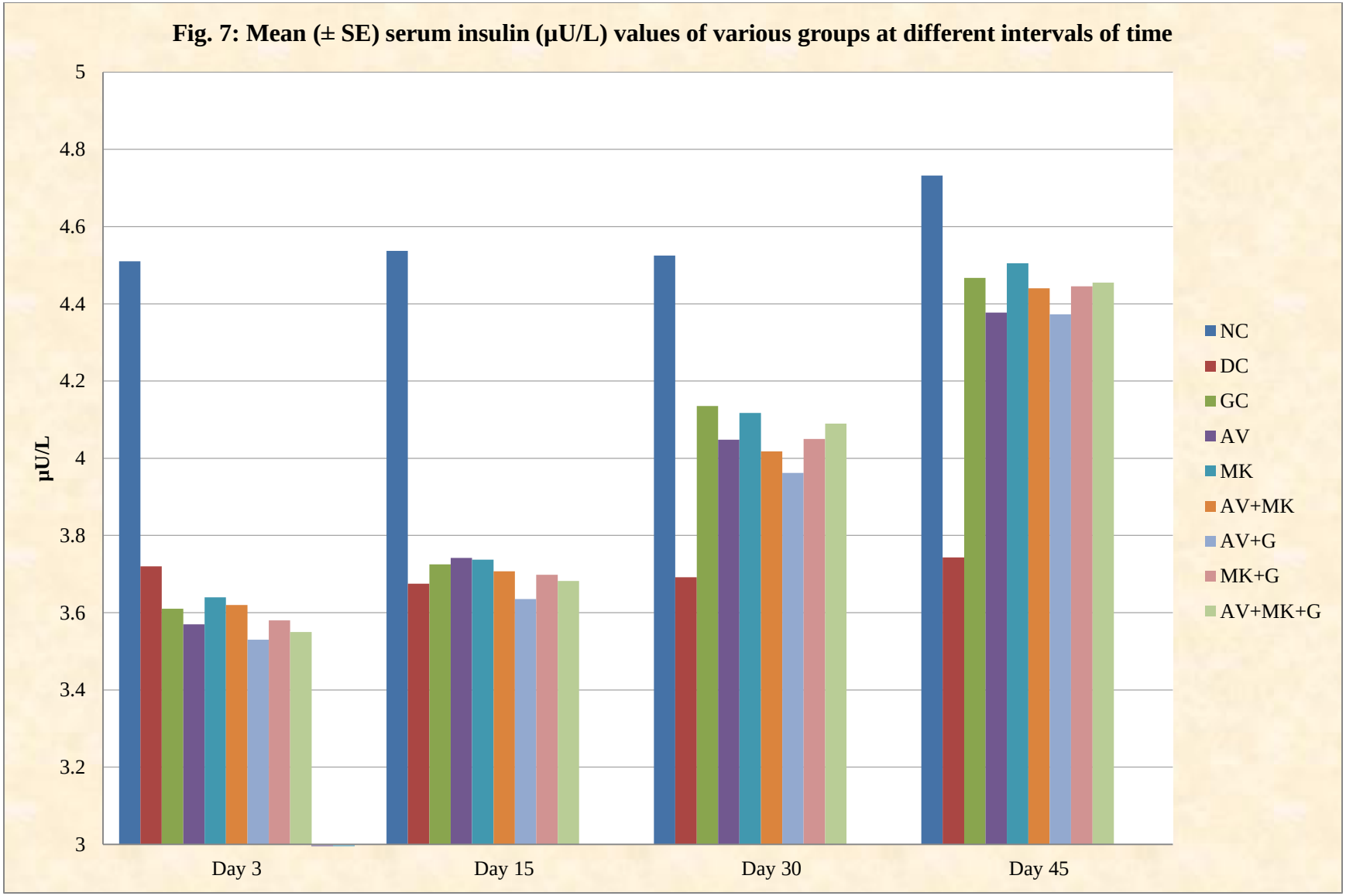


Table 10. The Mean (± SE) haemoglobin (g/dL) values of various groups at different intervals of time

Groups	Days Post Treatment			
	3	**15**	**30**	**45**
Group I (NC)	15.58±0.27[a]	15.71±0.39[a]	15.64±0.3[a]	15.62±0.39 [a]
Group II (DC)	13.25±0.24[b]	12.79±0.34[c]	11.75±0.29[c]	10.82±0.17 [b]
Group III (GC)	13.01±0.46 [b]	14.04±0.26[b]	14.75±0.21[b]	15.65±0.09 [a]
Group IV (AV)	13.16±0.27 [b]	13.81±0.25[b]	14.63±0.25[b]	15.62±0.22 [a]
Group V (MK)	13.17±0.4 [b]	13.94±0.32[b]	14.74±0.3[b]	15.72±0.1 [a]
Group VI (AV+MK)	13.07±0.31 [b]	13.7±0.24[b]	14.51±0.28[b]	15.57±0.26 [a]
Group VII (AV+G)	12.97±0.5 [b]	13.69±0.49[b]	14.54±0.41[b]	15.57±0.38 [a]
Group VIII (MK+G)	13.27±0.4 [b]	14.07±0.4[b]	14.86±0.48[ab]	15.88±0.19 [a]
Group IX (AV+MK+G)	13.39±0.25 [b]	14.22±0.2[b]	15.04±0.19[ab]	15.97±0.24 [a]

Mean values with different superscript differ significantly

[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III

Values are statistically significant at $P \leq 0.05$

Fig. 8: Mean (± SE) hemoglobin (g/dL) values of various groups at different intervals of time

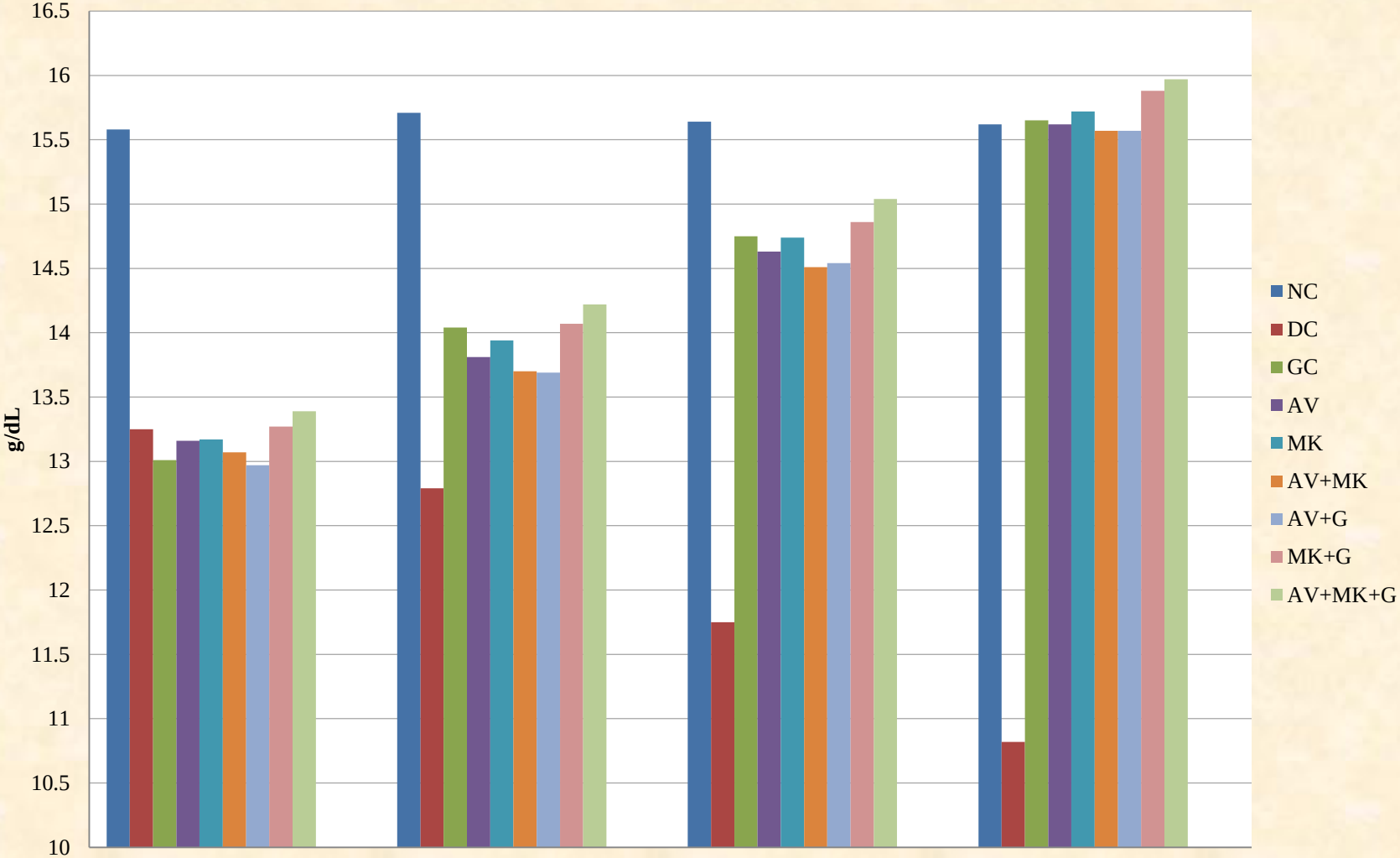


Table 11. The Mean (± SE) values of activities of SOD (U/min/mg protein) in the liver of various groups at different intervals of time

Groups	Days Post Treatment		
	15	**30**	**45**
Group I (NC)	39.45±3.76[a]	40.30±3.93 [a]	40.72±3.62 [a]
Group II (DC)	26.98±1.66[b]	26.88±1.57 [b]	27.23±1.31 [b]
Group III (GC)	27.31±1.1[b]	37.81±2.45 [a]	40.38±3.12 [a]
Group IV (AV)	27.38±0.83[b]	35.02±2.34 [a]	38.46±1.9 [a]
Group V (MK)	27.85±0.49[b]	36.14±2.50 [a]	39.85±1.53 [a]
Group VI (AV+MK)	27.77±1.46[b]	35.95±0.26 [a]	38.22±1.01 [a]
Group VII (AV+G)	27.72±0.61[b]	39.29±2.07 [a]	39.25±0.28 [a]
Group VIII (MK+G)	28.06±0.75[b]	36.57±1.06 [a]	40.76±1.55 [a]
Group IX (AV+MK+G)	28.44±1.55[b]	36.68±1.96 [a]	41.46±1.80 [a]

Mean values with different superscript differ significantly

[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III

Values are statistically significant at $P \leq 0.05$

Fig. 9: The Mean (± SE) values of activities of SOD (U/min/mg protein) in the liver of various groups at different intervals of time

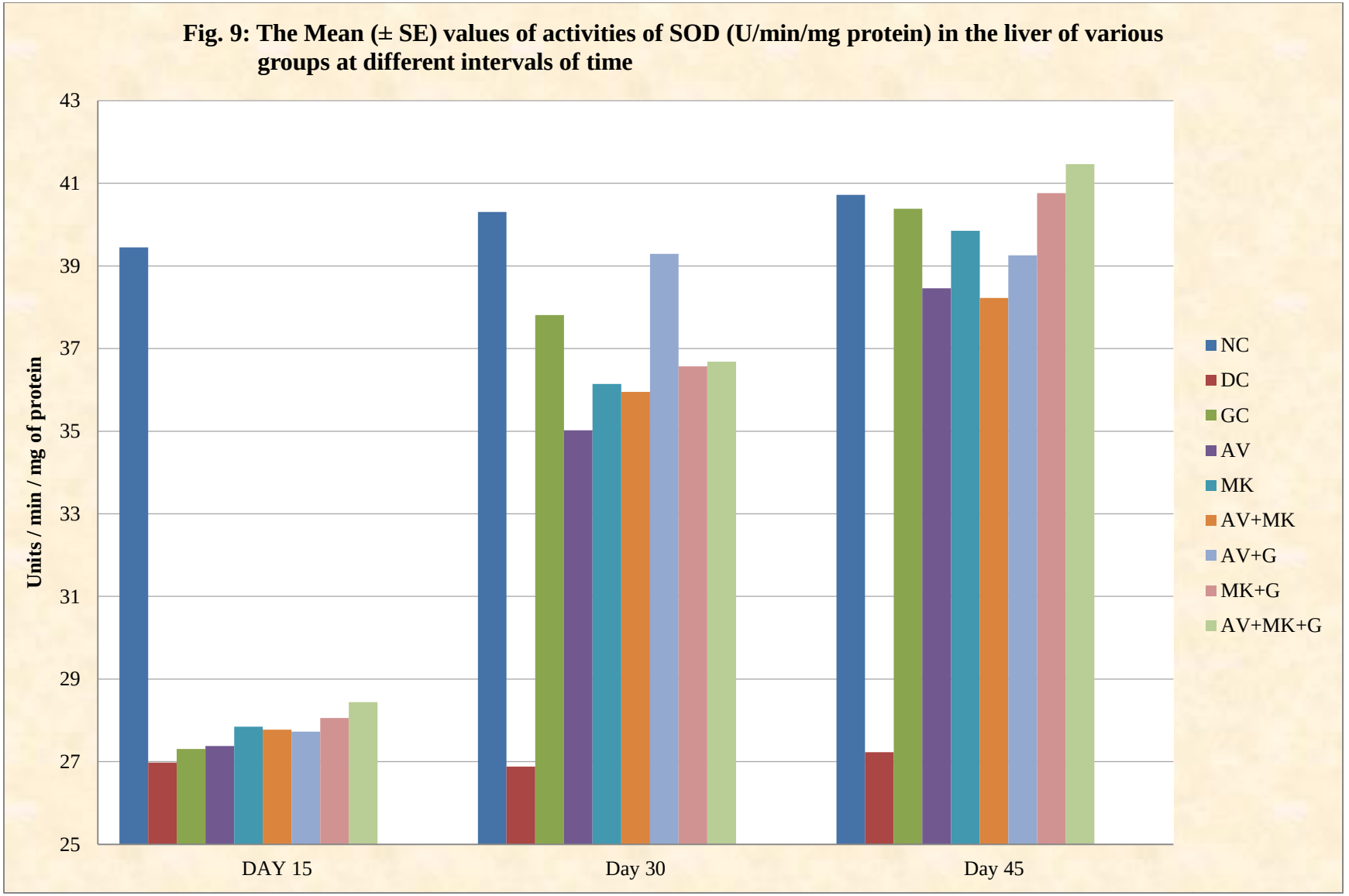


Table 12. The Mean (± SE) mean values of activities of CAT (µ moles of H_2O_2/min/mg protein) in the liver of various groups at different intervals of time

Groups	Days Post Treatment		
	15	**30**	**45**
Group I (NC)	12.89±0.56 [a]	12.97±0.62 [a]	13.07±0.72 [b]
Group II (DC)	5.81±0.12 [d]	6.40±0.11 [c]	7.53±0.15 [e]
Group III (GC)	7.59±0.25 [c]	12.40±0.04 [a]	13.92±0.06 [a]
Group IV (AV)	8.40±0.04 [bc]	9.53±0.08 [b]	10.71±0.08 [d]
Group V (MK)	8.38±0.25 [bc]	9.63±0.09 [b]	11.57±0.19 [cd]
Group VI (AV+MK)	8.51±0.14 [bc]	9.68±0.16 [b]	11.40±0.05 [cd]
Group VII (AV+G)	8.66±0.03 [b]	9.83±0.03 [b]	11.73±0.04 [c]
Group VIII (MK+G)	8.63±0.31 [b]	9.87±0.06 [b]	11.9±0.04 [c]
Group IX (AV+MK+G)	8.53±0.26 [b]	9.72±0.09 [b]	11.92±0.04 [c]

Mean values with different superscript differ significantly

[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III

Values are statistically significant at $P \leq 0.05$

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

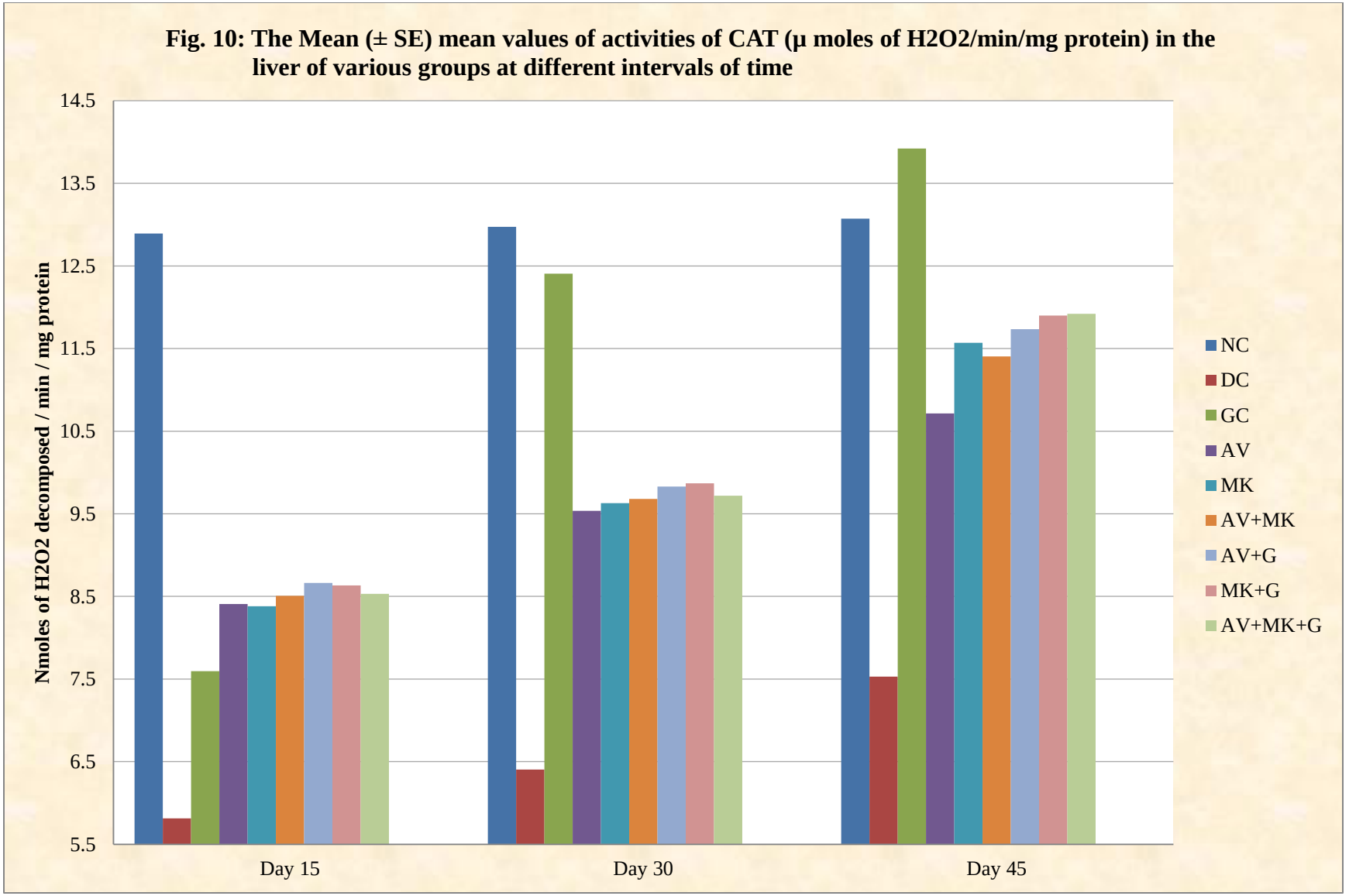


Table 13. The Mean (± SE) values of activities of GPx (µM of glutathione utilized/min/mg protein) in the liver of various groups at different intervals of time

Groups	Days Post Treatment		
	15	**30**	**45**
Group I (NC)	162.35±5.85[a]	165.25±8.15[a]	170.15±4.35[a]
Group II (DC)	125.78±10.5[b]	134.9±8.70[b]	138.75±3.55[c]
Group III (GC)	136.85±4.45[b]	144.05±4.75[ab]	147.2±4.10[bc]
Group IV (AV)	136.4±3.2[b]	155.45±3.85[ab]	159.95±1.35[ab]
Group V (MK)	133.85±4.45[b]	163.55±3.35[a]	169.20±0.60[a]
Group VI (AV+MK)	135.5±0.8[b]	163.15±6.95[a]	168.10±5.40[a]
Group VII (AV+G)	133.4±1.1[b]	160.6±9.3[a]	166.05±7.15[a]
Group VIII (MK+G)	133.55±6.65[b]	164.25±4.95[a]	170.05±3.75[a]
Group IX (AV+MK+G)	131.6±3.00[b]	166.9±5.40[a]	169.1±5.50[a]

Mean values with different superscript differ significantly

[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III

Values are statistically significant at $P \leq 0.05$

Fig. 11: The Mean (± SE) values of activities of GPx (µM of glutathione utilized/min/mg protein) in the liver of various groups at different intervals of time

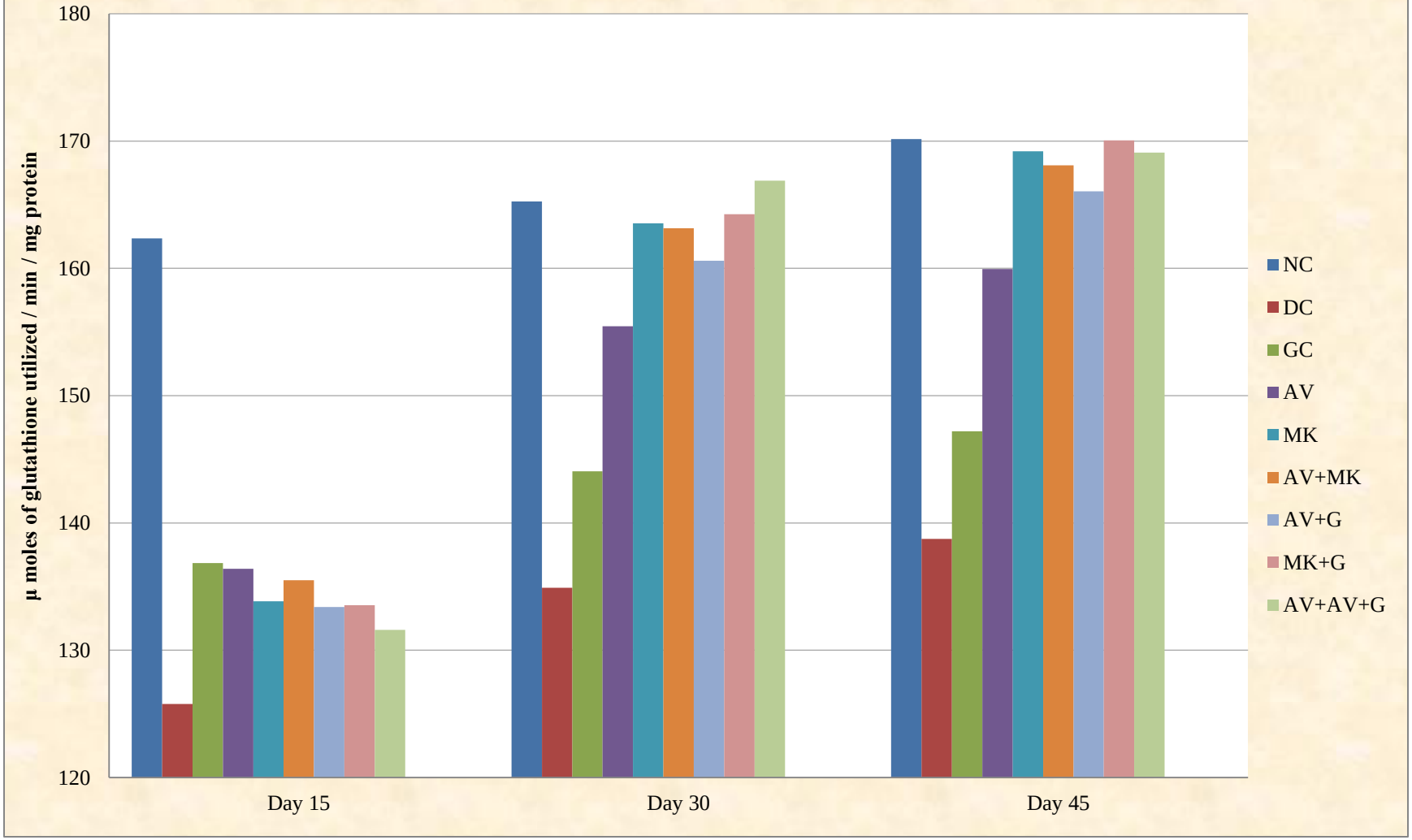


Table 14. The Mean (± SE) percentage positivity of insulin secreting cells of various groups at different intervals of time

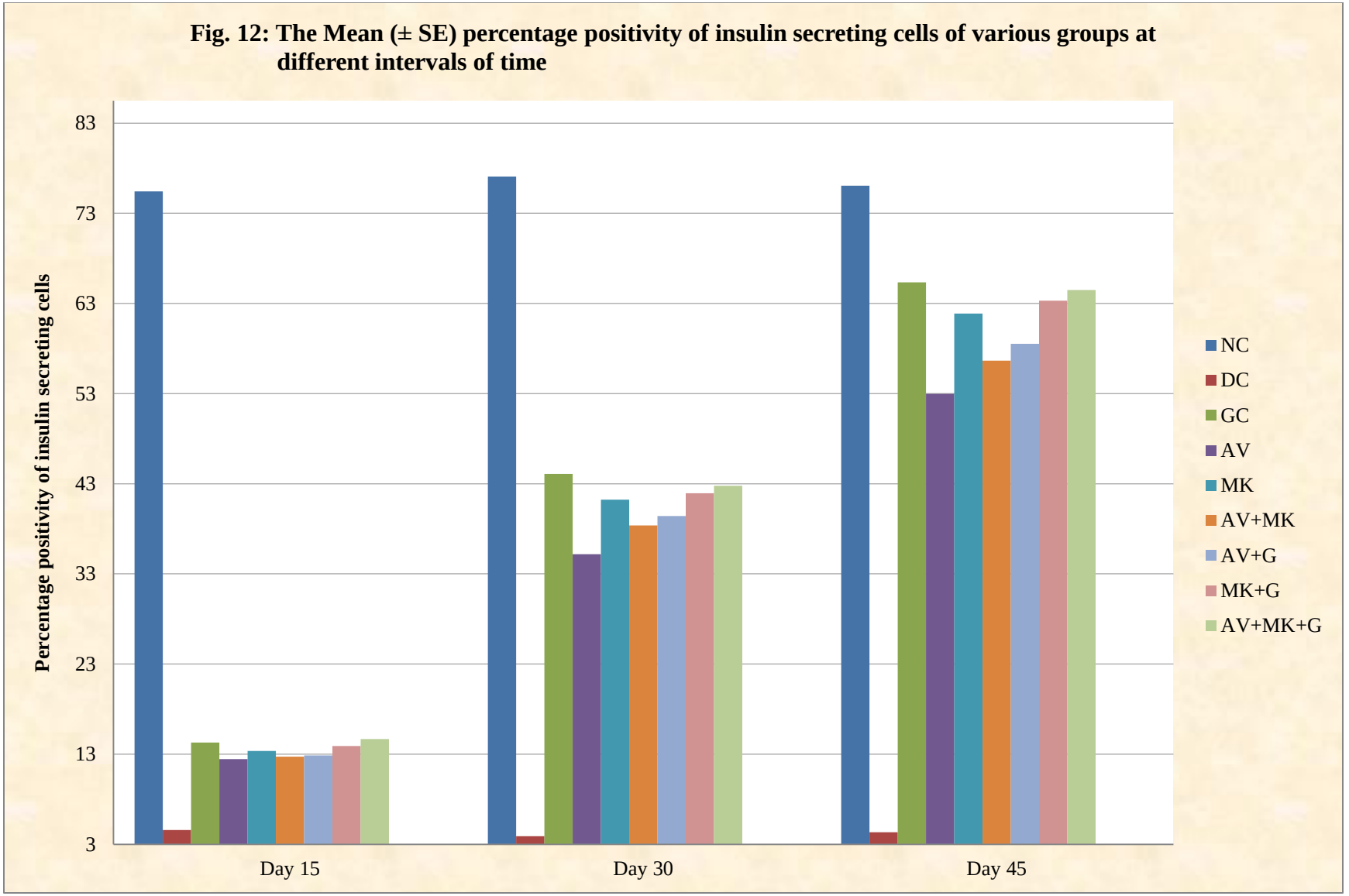
Groups	Days Post Treatment		
	15	**30**	**45**
Group I (NC)	75.42±2.79[a]	77.09±2.75[a]	76.06±2.59[a]
Group II (DC)	4.58±1.03[c]	3.915±0.51[c]	4.34±0.38[d]
Group III (GC)	14.28±2.06[b]	44.10±2.22[b]	65.35±2.97[b]
Group IV (AV)	12.45±1.23[b]	35.17±3.04[b]	52.98±1.34[c]
Group V (MK)	13.35±1.17[b]	41.24±1.96[b]	61.86±3.45[b]
Group VI (AV+MK)	12.73±1.39[b]	38.36±3.03[b]	56.65±2.69[bc]
Group VII (AV+G)	12.87±1.48[b]	39.43±3.83[b]	58.51±2.88[bc]
Group VIII (MK+G)	13.91±0.66[b]	41.93±1.70[b]	63.29±2.07[b]
Group IX (AV+MK+G)	14.67±0.54[b]	42.78±3.43[b]	64.48±2.63[b]

Mean values with different superscript differ significantly

[a]Comparison with Group I, [b]Comparison with Group II, [c]Comparison with Group III

Values are statistically significant at $P \leq 0.05$

Fig. 12: The Mean (± SE) percentage positivity of insulin secreting cells of various groups at different intervals of time

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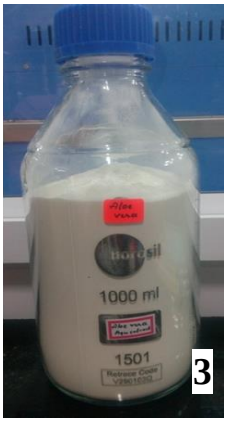
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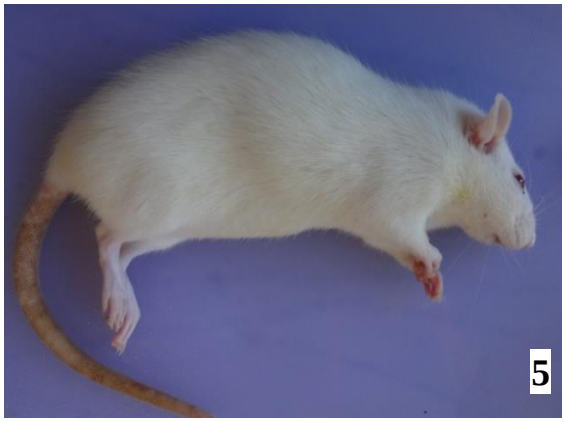
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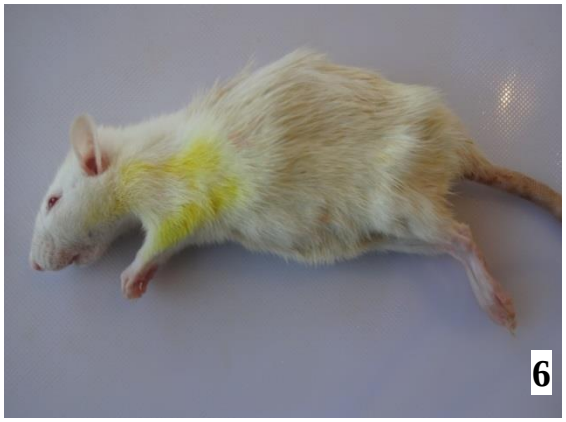
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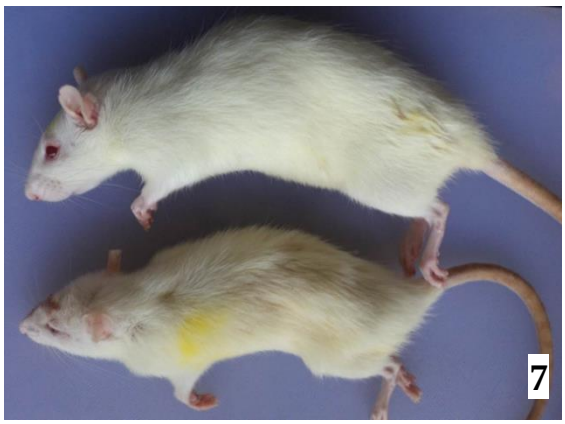
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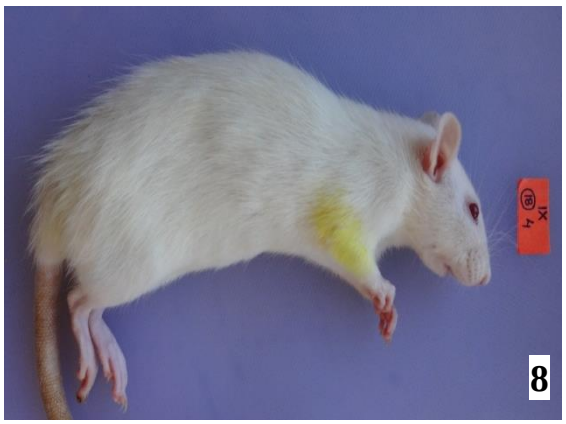
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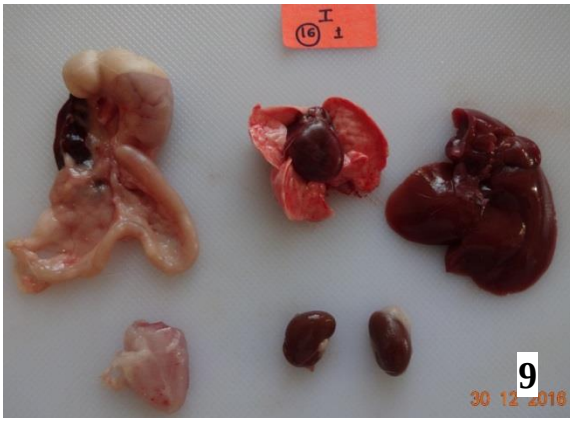
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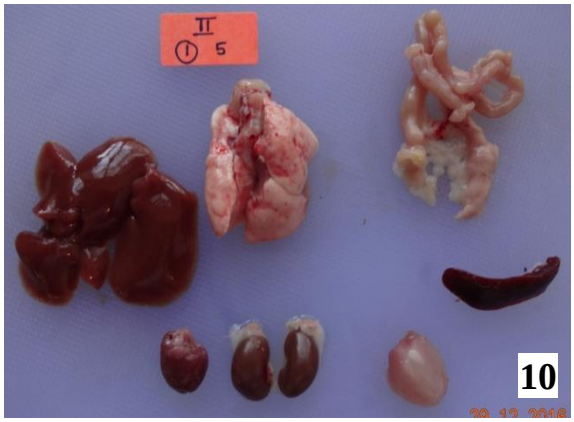
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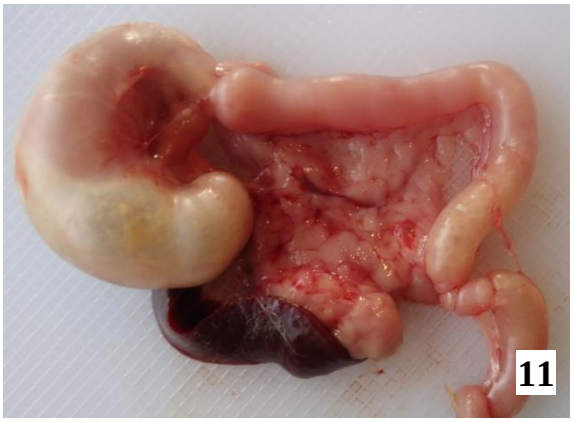
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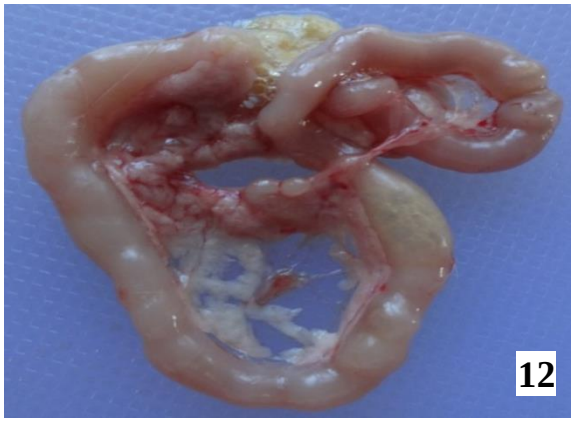
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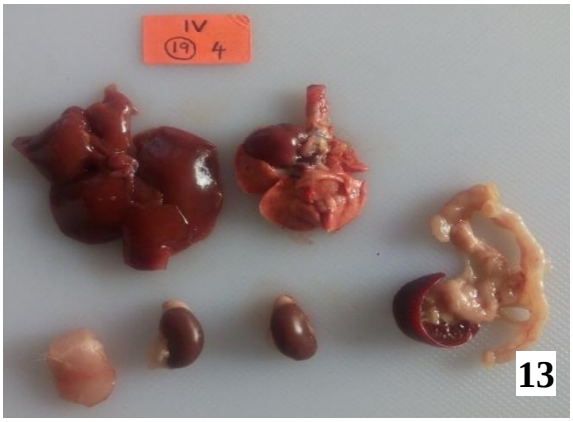
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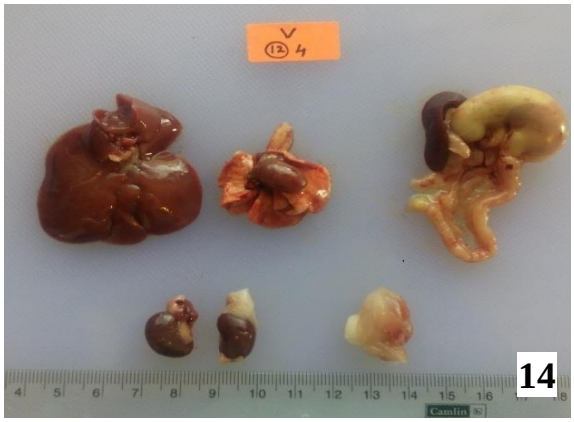
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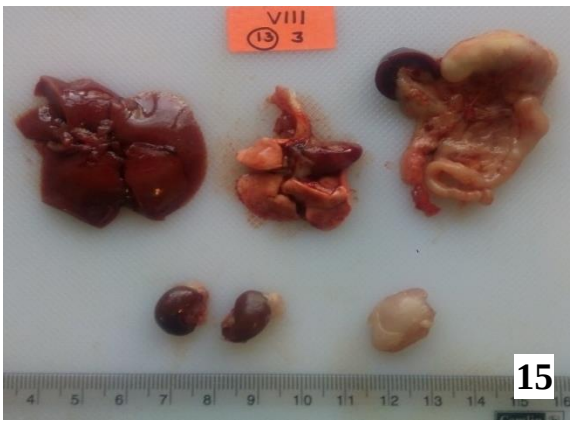
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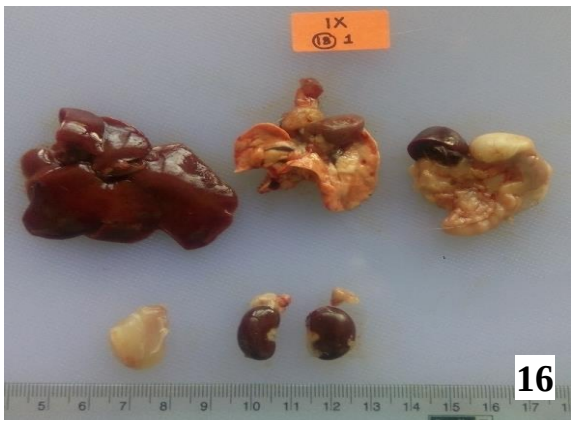
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Plate 17 Section of pancreas of Normal control animal showing well-formed islet, round in shape with compact arrangement of alpha and beta cells.

H&E X 200

Plate 18 Section of pancreas of Normal control animal showing normal islets of Langerhans and the intact exocrine portion.

H&E X 100

Plate 19 Section of pancreas of Normal control animal showing Islet of Langerhans with compact arrangement of insulin positive beta cells at the centre and alpha cells at the periphery.

IHC X 100

Plate 20 Section of pancreas of Normal control animal showing two islets of Langerhans with compact arrangement of beta cells.

IHC X 100

Plate 21 Section of pancreas of Normal control animal with islet of Langerhans showing intensely stained insulin positive cells in large number.

IHC X 200

Plate 22 Section of pancreas of Normal control animal showing beta cells at the centre and alpha cells at the periphery in islet of Langerhans.

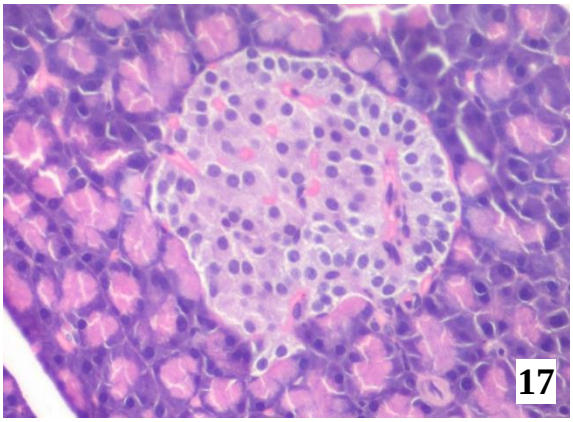
IHC X 1000

Plate 23 Section of pancreas of Normal control animal showing well-formed islet of Langerhans, oval in shape with more number of cells.

H&E X 200

Plate 24 Section of pancreas of Normal control animal showing islet of Langerhans with numerous insulin positive beta cells having dark brown granular cytoplasm.

IHC X 1000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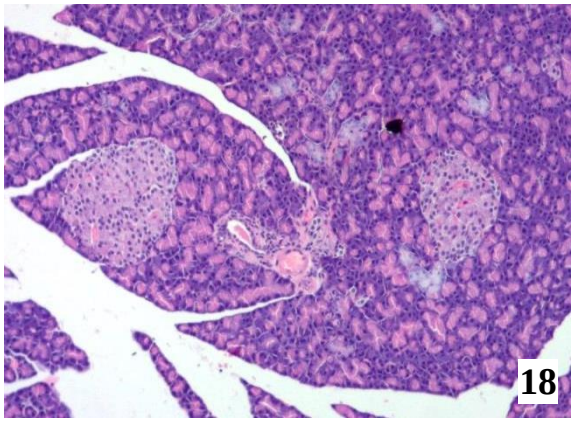
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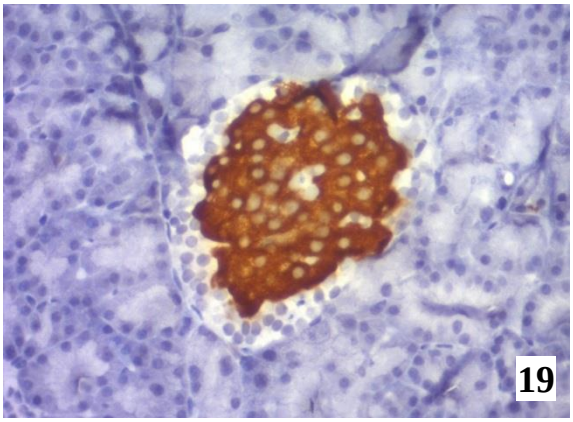
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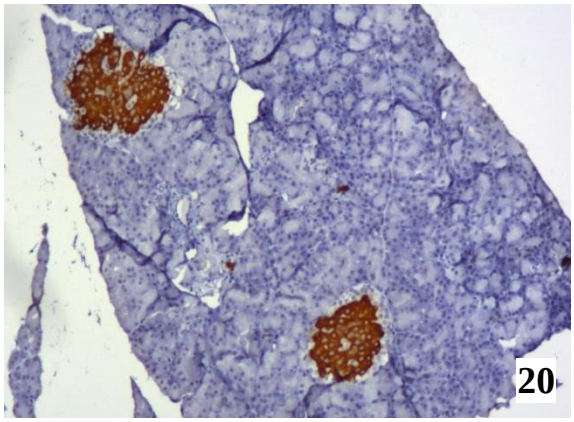
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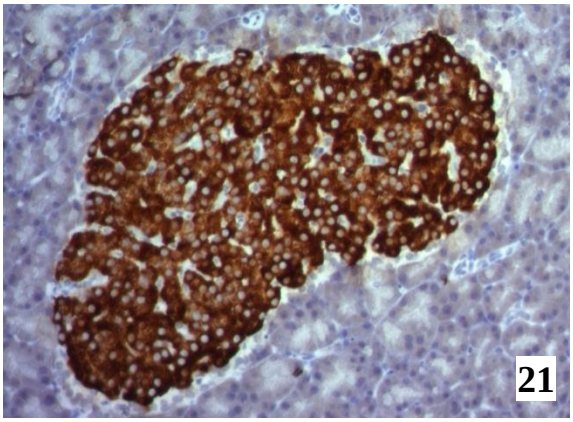
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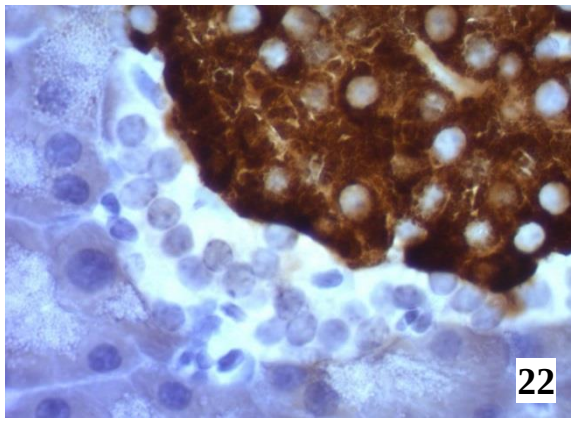
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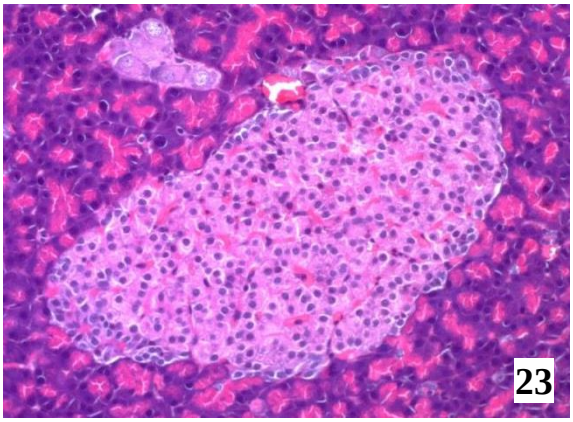
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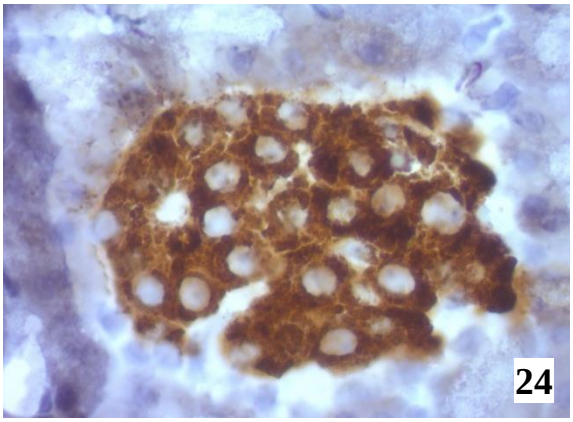
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Plate 25 Section of pancreas of Diabetic control animal showing loss of normal architecture and degeneration and necrosis of acinar cells on 15th day of the experiment.

H&E X 100

Plate 26 Section of pancreas of Diabetic control animal showing loss of normal architecture, atrophic, vacuolated and degenerating Islet cells on 15th day of experiment.

H&E X 200

Plate 27 Section of pancreas of Diabetic control animal showing loss of architecture of exocrine portion with congestive and necrotic changes on 30th day.

H&E X 100

Plate 28 Section of Pancreas from Diabetic control animal showing degenerative and necrotic changes in the Islets with loss of demarcation between endocrine and exocrine portion on 30th day.

H&E X 200

Plate 29 Section of Pancreas from Diabetic control animal showing islet with least immunoreactivity for insulin at 15th day of the experiment.

IHC X 200

Plate 30 Section of Pancreas from Diabetic control animal showing islet with mild immunoreactivity for insulin at 30th day of the experiment.

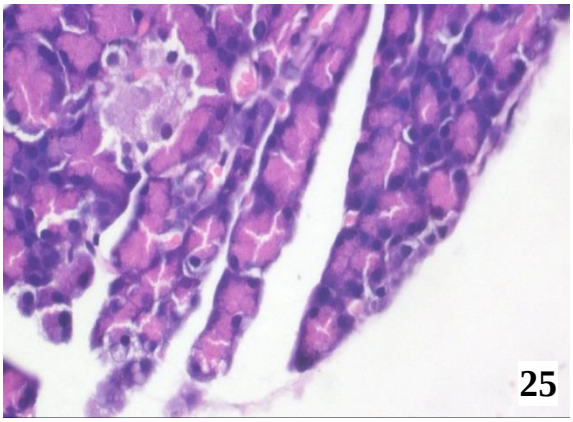
IHC X 200

Plate 31 Section of Pancreas from the Diabetic control animal showing islet with few insulin positive cells on 30th day.

IHC X 200

Plate 32 Section of Pancreas from the Diabetic control animal showing islet with lightly stained insulin positive cells on 45th day.

IHC X 200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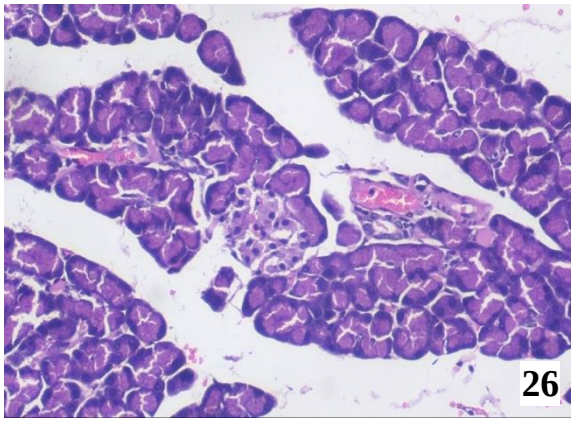
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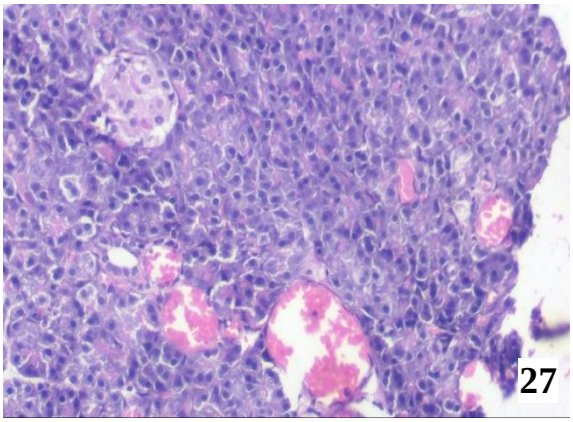
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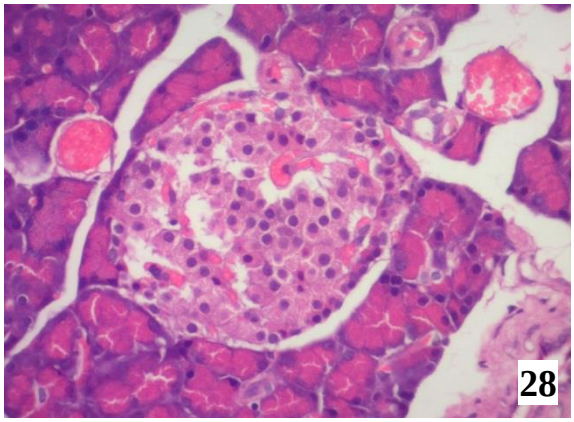
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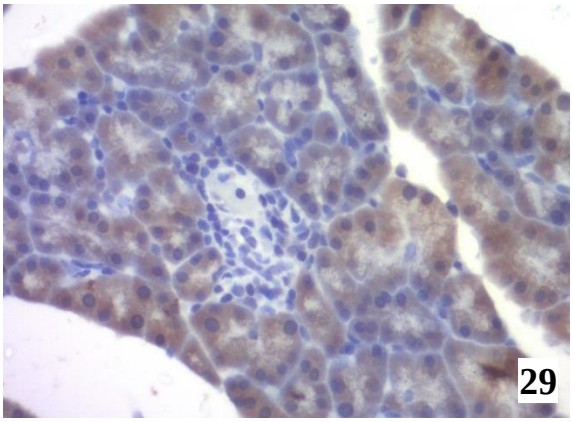
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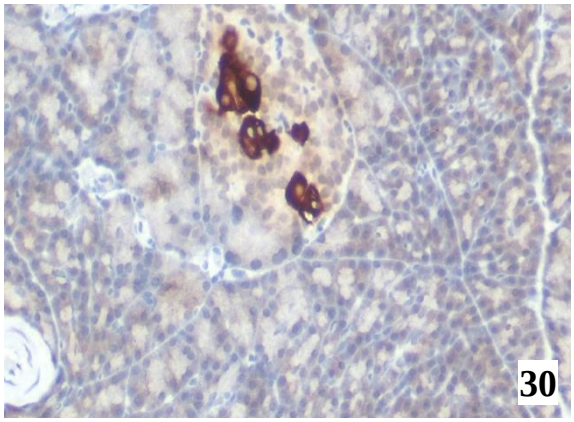
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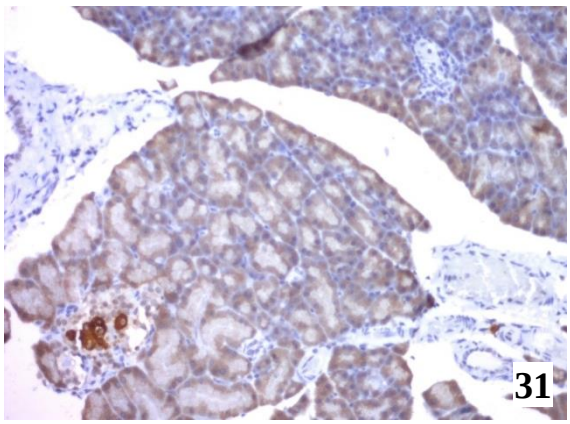
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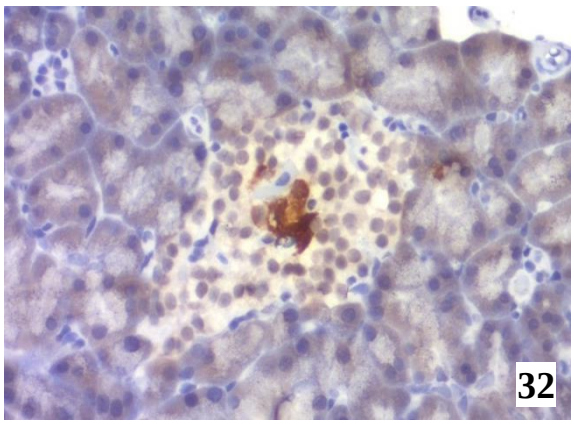
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Plate 33 Section of liver from Normal control animal showing central vein and well-formed hepatic cords with normal hepatocytes on 15th day.

H&E X 200

Plate 34 Section of spleen from Normal control animal showing compact arrangement of splenic corpuscles on 15th day.

H&E X 100

Plate 35 Section of liver from Diabetic control animal showing highly swollen hepatocytes with cytoplasmic vacuolations on 15th day.

H&E X 200

Plate 36 Section of liver from Diabetic control animal showing congestive changes and obliteration of sinusoidal space on 45th day.

H&E X 200

Plate 37 Section of liver from *Aloe vera* treated diabetic animal showing improvement in hepatocyte morphology on 45th day.

H&E X 200

Plate 38 Section of liver from *Murraya koenigii* treated animal showing improvement in hepatocyte morphology on 45th day.

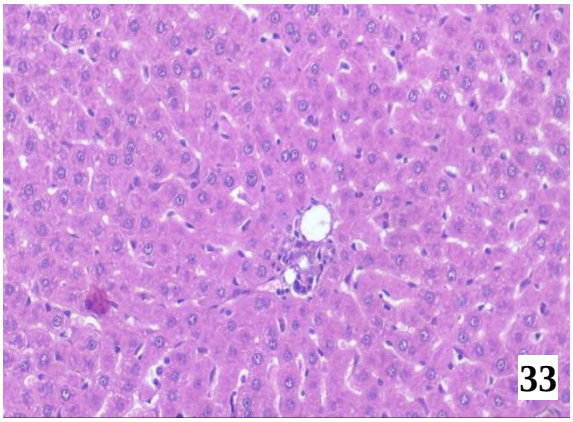
H&E X 200

Plate 39 Section of spleen from Diabetic control animal showing depletion of lymphoid cells from periarteiolar sheath and follicles on 15th day.

H&E X 100

Plate 40 Section of spleen from diabetic rats treated with *Aloe vera* and *Murraya koenigii* showing improvement in lymphoid cell proliferation on 45th day.

H&E X 100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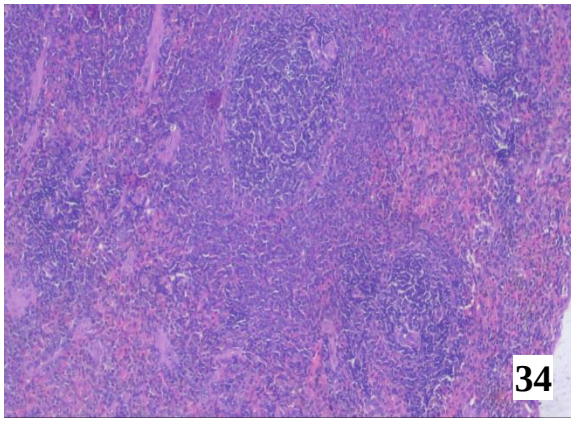
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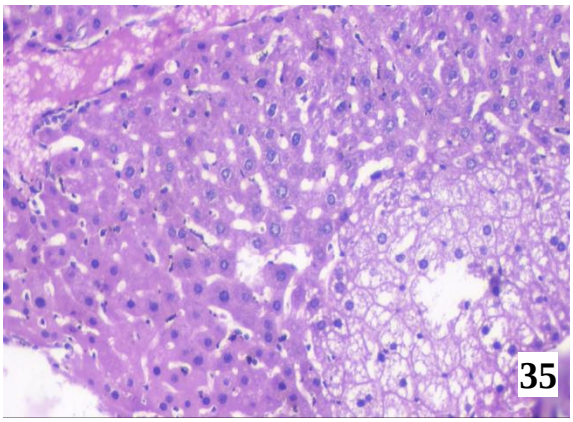
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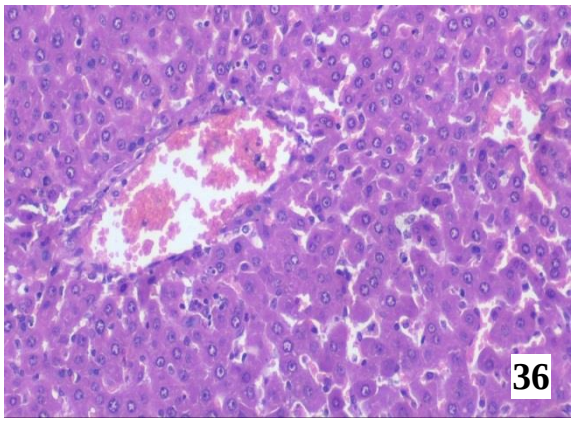
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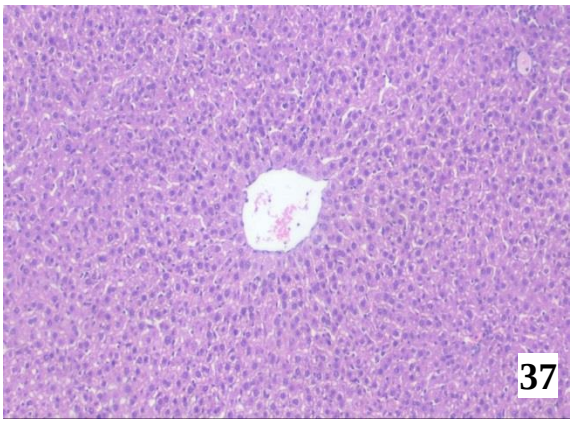
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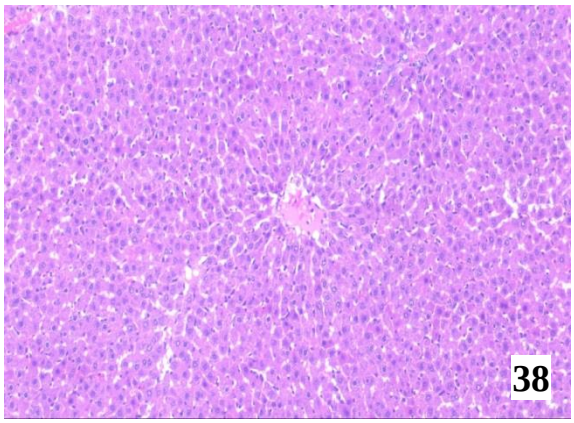
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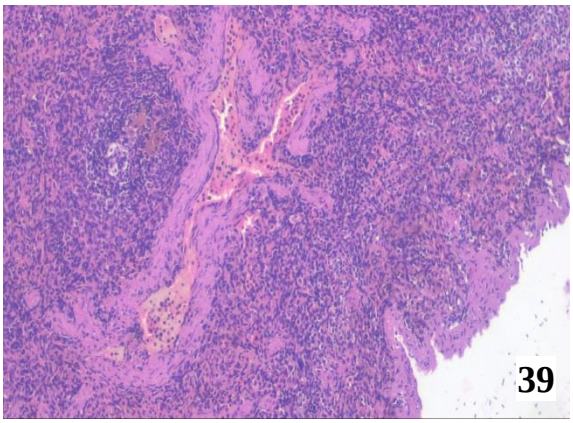
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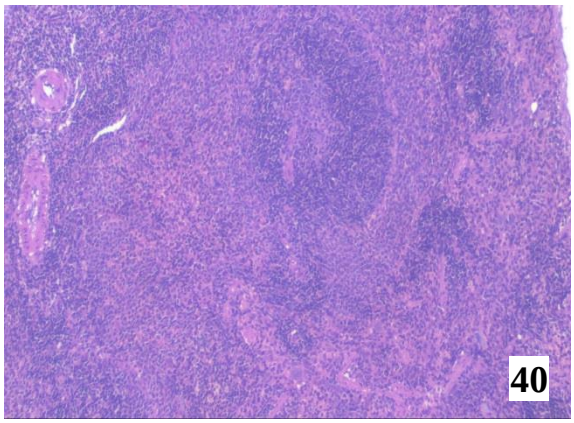
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Plate 41 Section of Pancreas from diabetic animal treated with glibenclamide showing improvement in architecture and repopulation of cells on 15th day in islets of Langrhans.

H&E X 200

Plate 42 Section of Pancreas from diabetic animal treated with glibenclamide showing islet of Langerhans with improvement and compact arrangement of cells and increase in cellularity on 45th day.

H&E X 200

Plate 43 Section of Pancreas from diabetic animal treated with glibenclamide showing islet with insulin positive cells on 15th day.

IHC X 200

Plate 44 Section of Pancreas from diabetic animal treated with glibenclamide showing islet with appreciable improvement and increase in beta cells on 45th day.

IHC X 400

Plate 45 Section of Pancreas from diabetic animal treated with *Aloe vera* showing regeneration of the Islet of Langerhans on 15th day.

H&E X 200

Plate 46 Section of Pancreas from diabetic animal treated with *Aloe vera* showing compact islet with improvement in morphology on 45th day.

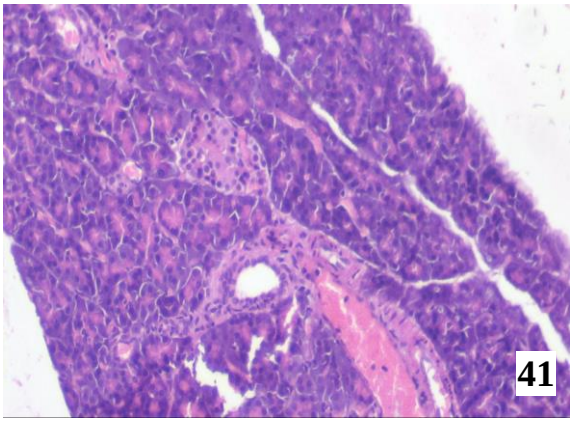
H&E X 200

Plate 47 Section of Pancreas from diabetic animal treated with *Aloe vera* showing islet with minimum number of insulin positive cells on 15th day.

IHC X 200

Plate 48 Section of Pancreas from diabetic animal treated with *Aloe vera* showing islets with more number of beta cells exhibiting increased granularity on 45th day.

IHC X 200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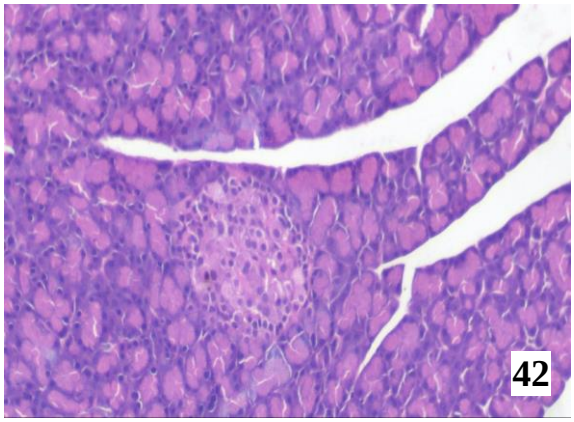
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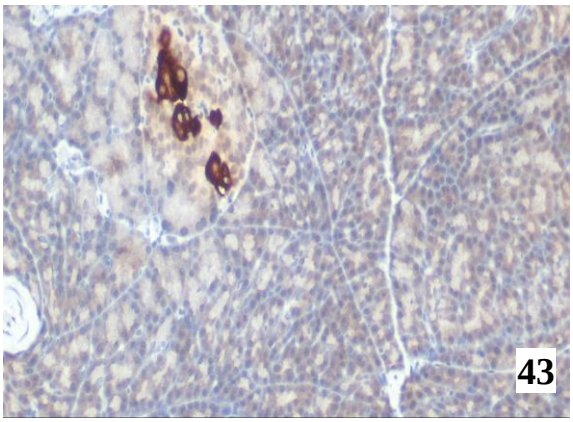
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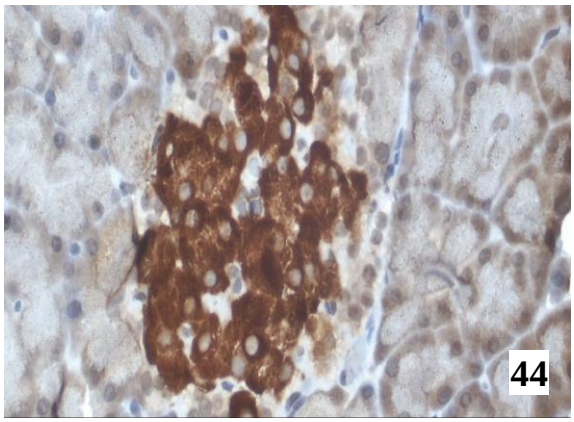
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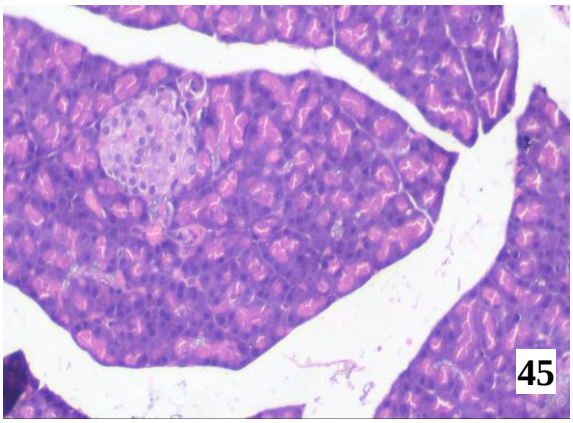
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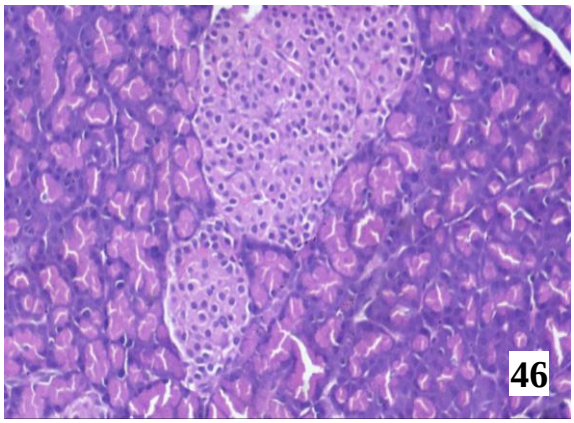
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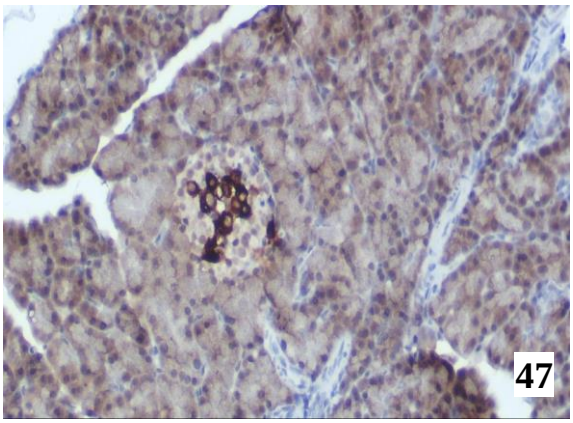
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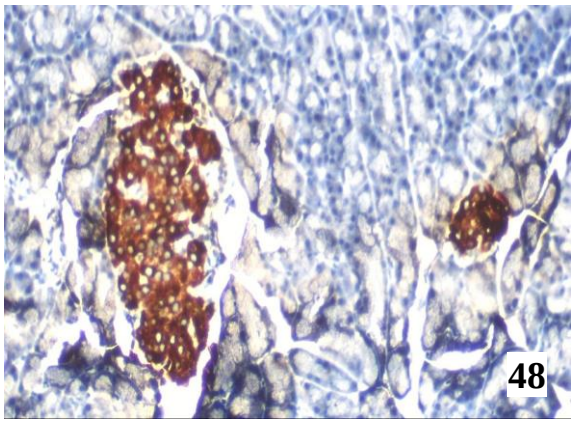
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Plate 49 Section of pancreas from diabetic animal treated with *Murraya koenigii* showing regenerating islet of Langerhans on 15th day.

H&E X 200

Plate 50 Section of Pancreas from diabetic animal treated with *Murraya koenigii* showing increased cellularity in the islets on 45th day.

H&E X 200

Plate 51 Section of Pancreas from diabetic animal treated with *Murraya koenigii* showing occasional cells positive for insulin on 15th day.

IHC X 200

Plate 52 Section of Pancreas from diabetic animal treated with *Murraya koenigii* showing increase in number of beta cells at the centre and presence of alpha cells at the periphery of islet on 45th day.

IHC X 400

Plate 53 Section of Pancreas from a diabetic animal treated with *Aloe vera* and *Murraya koenigii* showing an improvement in the islet architecture on 15th day.

H&E X 200

Plate 54 Section of Pancreas from diabetic animal treated with *Aloe vera* and *Murraya koenigii* showing large and well-formed islet on 45th day.

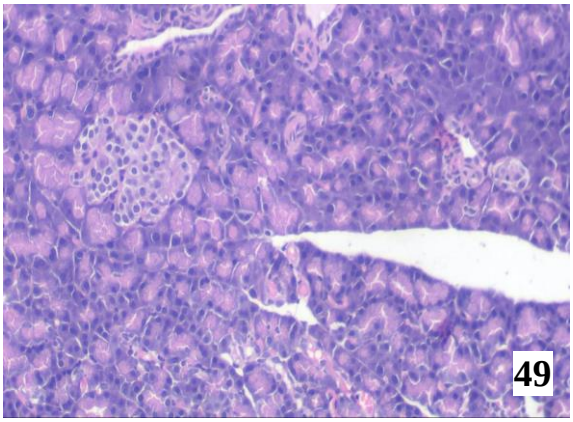
H&E X 200

Plate 55 Section of pancreas of diabetic animal treated with *Aloe vera* and *Murraya koenigii* showing moderate number of insulin positive cells in islet of Langerhans on 45th day.

IHC X 200

Plate 56 Section of pancreas from diabetic animal treated with *Aloe vera* and *Murraya koenigii* showing increased number of beta cells with better distribution in islet on 45th day.

IHC X 200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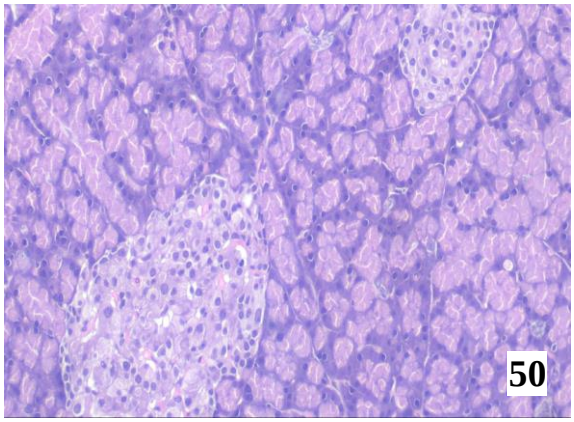
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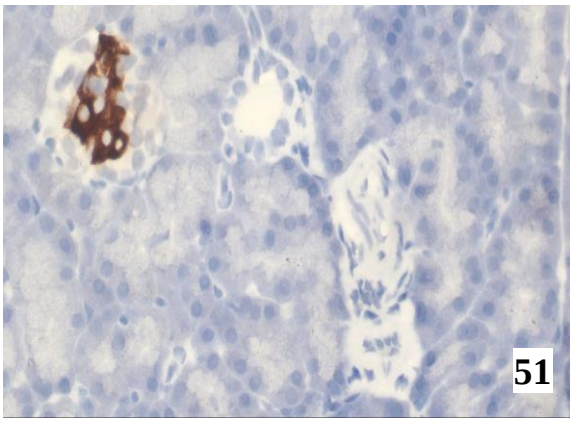
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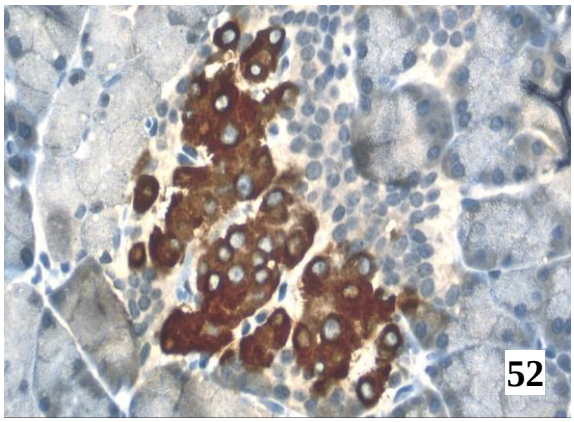
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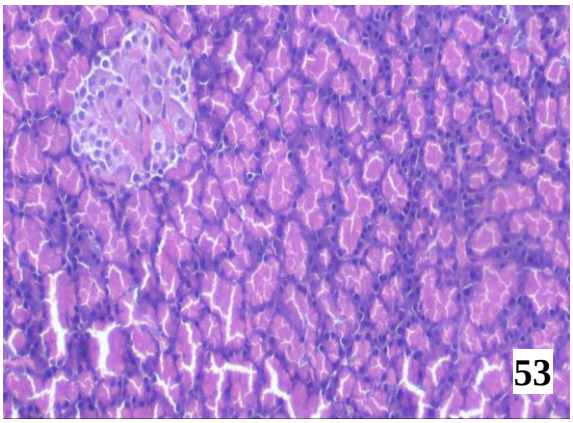
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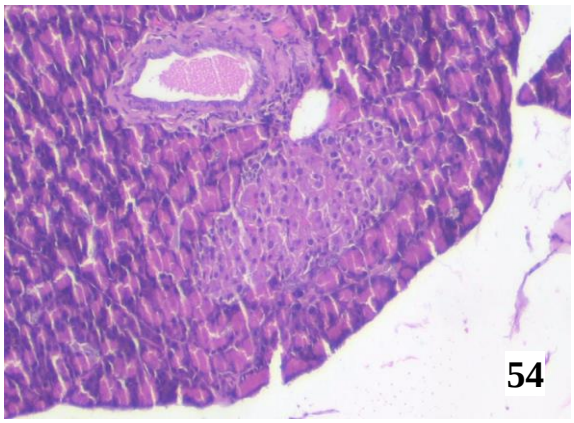
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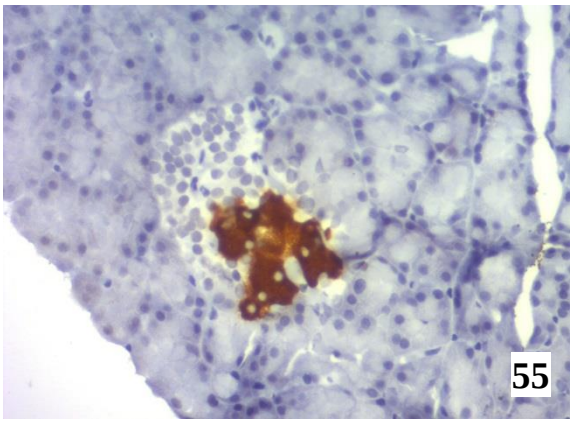
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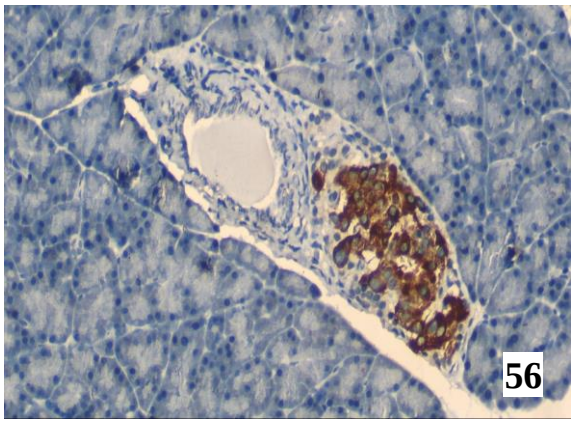
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Plate 57 Section of pancreas from diabetic animal treated with *Aloe vera* and glibenclamide in combination on 45th day post-treatment showing improvement in the architecture of islet with hypercellularity.

H&E X 200

Plate 58 Section of pancreas from diabetic animal treated with *Murraya koenigii* and glibenclamide in combination showing improvement with increased compactness of islet on 45th day.

H&E X 200

Plate 59 Section of pancreas from a diabetic animal treated with *Aloe vera* and glibenclamide in combination on 45th day post-treatment showing improvement in number of beta cells in islet.

IHC X 400

Plate 60 Section of pancreas from diabetic animal treated with *Murraya koenigii* and glibenclamide in combination showing improvement in the immune reaction with beta cell on 45th day.

IHC X 200

Plate 61 Section of pancreas from diabetic animal treated with *Aloe vera*, *Murraya koenigii* and glibenclamide in combination showing large islet with increased cellularity on 45th day.

H&E X 200

Plate 62 Section of pancreas from diabetic animal treated with *Aloe vera*, *Murraya koenigii* and glibenclamide in combination showing improvement with increase in beta cell population in islets on 45th day.

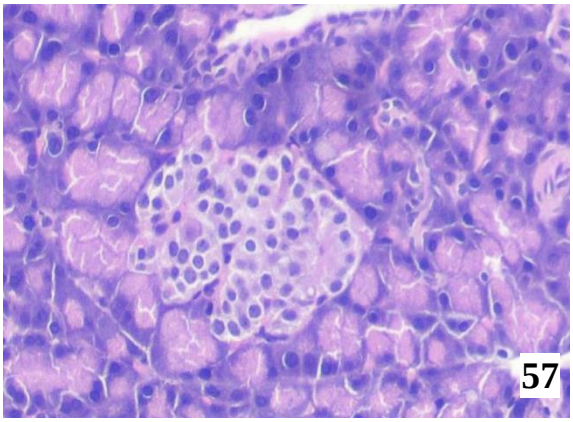
IHC X 200

Plate 63 Section of pancreas from diabetic animal treated with combination of herbal drugs and glibenclamide showing beta cells with granularity in islet of Langerhans and numerous alpha cells on 30th day.

IHC X 1000

Plate 64 Section of pancreas from diabetic animal showing new islet formation near the duct post treatment with combination of herbal drugs and glibenclamide on 30th day.

IHC X 200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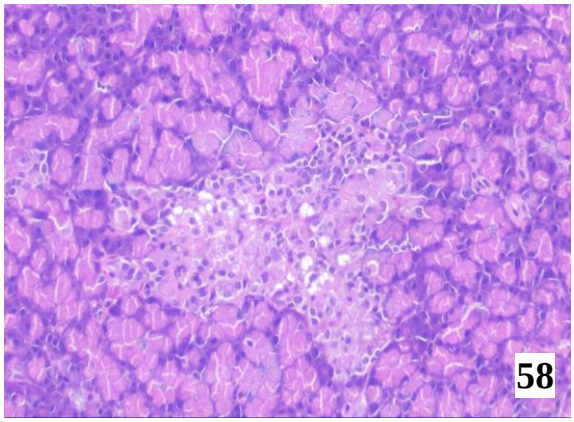
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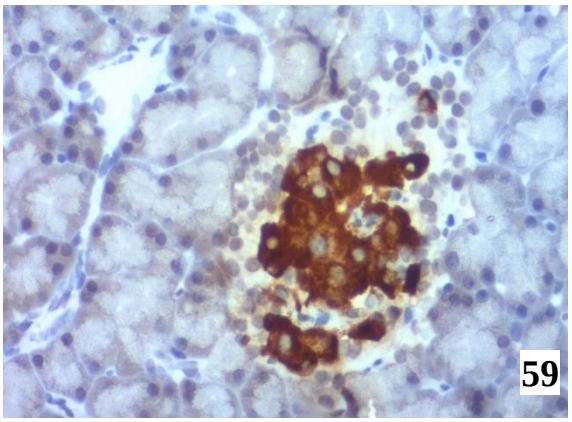
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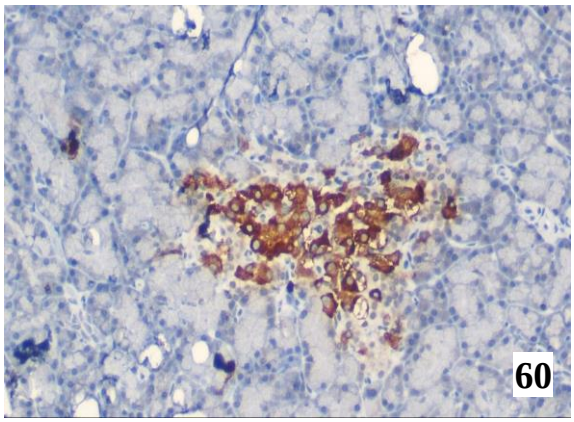
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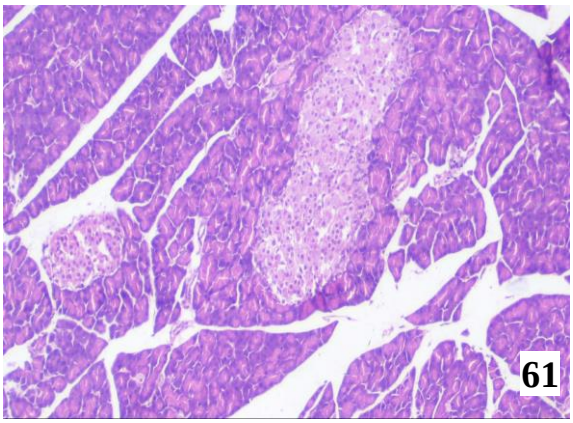
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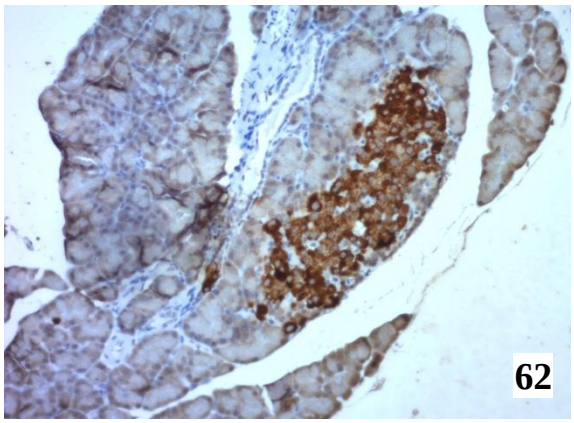
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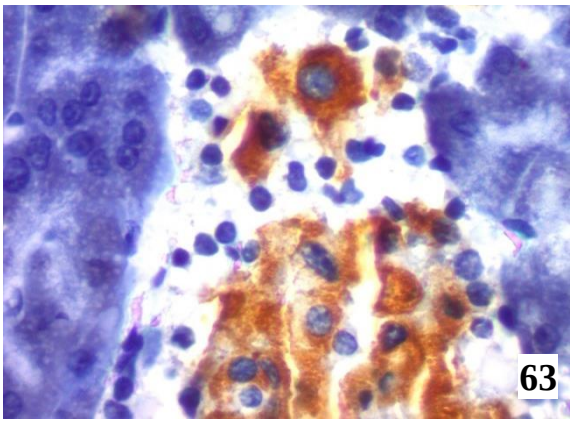
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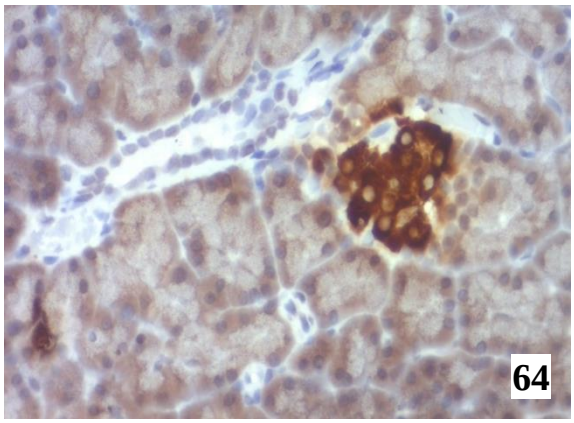
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Discussion

V. DISCUSSION

Diabetes mellitus is a chronic disorder resulting from insulin deficiency and hyperglycemia and has a high risk of development of complications for the eyes, kidneys, peripheral nerves, heart and blood vessels. Early diagnosis and prevention are critical for the control of the disease.

In the present study, the antidiabetic effect of aqueous extract of *Aloe vera* and *Murraya koenigii* in diabetic rat was evaluated in comparision with Glibenclamide, a regularly used standard antidiabetic drug. The antidiabetic effect of *Aloe vera* and *Murraya koenigii* was confirmed with the help of phytochemical analysis, serum biochemical analysis, haematological analysis, immunoradiometric assay, histopathology and immunohistochemistry of various treatment groups.

The results of the present study on pathomorphological and biochemical evaluation of antidiabetic effect of *Aloe vera* and *Murraya koenigii* in experimentally induced diabetic rats are discussed as hereunder.

5.1 Induction of diabetes

In the present study, diabetes was induced in rats of Group II to Group IX by administration of streptozotocin at the dose rate of 40 mg/kg intraperitonially. All the rats from Group II to IX became diabetic and showed hyperglycaemia with increase in mean serum glucose levels ranging from 418.80 ± 23.90 mg/dl to 463.90 ± 16.31 mg/dl by 72 hrs after STZ administration.

Streptozotocin or streptozocin or izostazin or zanosar (STZ) is a synthetic antineoplastic agent, an anti-tumor antibiotic, and chemically is related to other nitrosureas used in cancer chemotherapy. It is used to induce both IDDM and NIDDM. STZ enters the P-cell *via* a glucose transporter (GLUT-2) and causes alkylation of DNA. STZ liberates toxic amounts of nitric oxide (NO) that inhibits aconitase activity and participates in DNA damage. As a result of the STZ action, p-cells undergo destruction by necrosis (Szkudelski, 2001).

Frequently used i.v. dose of STZ to induce IDDM was between 40-60 mg/kg b w and found that the range of STZ dose required to induce diabetes was not as narrow as in case of alloxan (Ganda *et al.*, 1976). Streptozotocin could induce diabetes mellitus in many animal models and the induced diabetes resembled the human hyperglycaemic non-ketotic diabetes mellitus in all aspects and stated that STZ which acts as a nitric oxide (NO) donor was found to bring about the destruction of pancreatic islet cells contributing to STZ-induced DNA damage (Wier *et al.*, 1981; Kroncke *et al.*, 1995).

Wada and Yagihashi (2004) observed the toxicity of β-cell caused by STZ 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 leading to diabetes.

Following the uptake of STZ into the β cells via the GLUT-2 glucose transporter, it splits into glucose and methylnitrosourea moiety and owing to its alkylating properties, methylnitrosourea modify the biological macromolecules, fragment DNA and destroy the β cells, causing a state of insulin dependent diabetes (Lenzen, 2008). Streptozotocin is

highly cytotoxic to the pancreatic beta-cells and 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 (Raza and John, 2012).

Streptozotocin selectively destroys β cells of pancreas by generating excess ROS and carbonium ion leading to DNA breaks by alkylating DNA bases. The N-nitroso-N methylurea portion of the molecule exhibits diabetogenic activity. Glucose may act as carrier for this cytotoxic group (Kante and Reddy, 2013).

Selective destruction of β cells of pancreas by generating excess ROS and carbonium ion leading to DNA breaks by alkylating DNA bases may be the prime reason for induction of diabetes. In the present study destruction of β cells in the histological sections of pancreas was clearly evident and amply supported this observation.

5.2 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.

5.3 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 pathomorphological changes indicative of diabetes.

Significant decrease in the mean body weight was noticed in the diabetic control animals. The decrease was statistically significant ($P \leq 0.001$) from day 15 post STZ injection.

The weight loss in diabetic rats could be attributed to several factors such as increased catabolism of carbohydrates, proteins and fats and altered uptake of glucose and glycogenesis due to hypoinsulinemia, decreased protein synthesis due to lack of insulin leading to diminished transport of amino acids to the muscle, hyperglycemia induced polyuric loss of body fluids adversely affecting fluid transport regulation due to imbalance in K^+ and Na^+ contents of the pancreas and loss of fluids leading to dehydration through glycosuric polyuria and altered uptake of glucose and glycogenesis by the target cell (Hakim *et al.,* 1997; Rubin and Strayer, 2008; Mallikarjuna, 2009; Pragathi, 2011; Mudassir, 2011; Nasreen, 2012; Gurikar, 2014 and Kavitha Rani, 2015).

The above view points are in line with the present findings as the body weights were gradually restored back in the various treatment groups.

The mean serum glucose levels in the diabetic control rats were significantly higher ($P \leq 0.001$) on all the intervals of study in comparison with those of normal control group from day 3 to day 45 of the experiment indicating hyperglycaemia. A hyperglycemic state in diabetes could be attributed to loss of glucose homeostasis because of specific destruction of insulin producing β-cells by STZ as it acts on β-cells resulting in alteration of blood insulin and glucose concentrations.

For glucose uptake by cells, glucose utilization for glycolysis, TCA cycle, hexosemonophosphate shunt and glycogenesis insulin hormone, is highly essential. The reduction in Insulin or resistance to insulin leads to decreased glucose transport across the cells contributing to its nonutilization and elevation in blood. In addition, elevated hepatic glucose production and increased breakdown of fat also contribute for hyperglycaemic state in diabetes (Ganda *et al.,* 1976; Shibib *et al.,* 1993; Li *et al.,* 2000; Akbarzadeh *et al.,* 2007; Mallikarjuna, 2009; Kavitha Rani, 2015).

The mean (± SE) values of 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. The increase in triglyceride may be due to insulin deficiency which results in deranged metabolic process with decreased glucose utilization for energy purpose. This energy starvation induces mobilization of fatty acids from adipose tissue to liver, wherein their conversion to triglyceride and cholesterol occurs in excess contributing for hypertriglyceridaemia and hypercholesterolaemia, respectively. In experimental STZ and alloxan induced diabetes hyperlipidemia is one of the regular finding. In addition, diminished expression of LDL receptors due to chronic insulin deficiency might also be contributing to increased LDL-cholesterol value in diabetes and insulin deficiency increases excessive breakdown of adipose store by stimulation of 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 (Markku, 1995; Mendez and Balderas, 2001; Nafisa *et al.,* 2007; Shesha Rao, 2013).

In the present study, serum ALT and AST mean values were significantly higher compared to normal control animals throughout the study period. vSimilar findings were also reported by many workers (Shibib *et al.,* 1993; Szkudelski, 2001; Dandagi *et al.,* 2008; Dhanush, 2009; Mallikarjuna, 2009; Pragathi, 2011; Shesha Rao, 2013).

The elevated serum levels of liver enzymes (ALT & AST) are indicative of cellular leakage and loss of functional integrity of the cell membrane and indicate active hepatic damage. STZ has been reported to induce both plasma membrane and organellar membrane damage especially that of RER and mitochondria. Experimental studies have shown that subtle membrane changes are sufficient to allow passage of intracellular enzymes to the extracellular space. Increased levels of ALT and AST under insulin deficiency have been related to increased gluconeogenesis and ketogenesis during diabetes and alteration in the metabolism of glucose, protein and lipid could lead to alteration in the function of liver and kidney (Fleig *et al.,* 1970; Laguens *et al.,* 1980; Dhanabal *et al.,* 2006; Gayathri and Kannabiran, 2008; Rajnish and Radhey, 2009 and Singh *et al.,* 2010). In the present study, histopathological observations clearly indicated the hepatic damage caused by streptozotocin.

The serum insulin values in the present study were estimated by IRMA (Immunoradiometric analysis) method and the mean insulin values were significantly lowered in diabetic control groups compared to the normal control animals. After the administration of STZ, initially it abolishes the β-cell response to the glucose followed by a temporary return of responsiveness and then leading to the permanent damage to β-cells with a resultant fall in blood insulin levels (West *et al.,* 1996). Similar findings were also

reported by many earlier workers in STZ induced diabetes in rats (Nafisa *et al.,* 2007; Gayathri and Kannabiran, 2008; Rajnish and Radhey, 2009; Gurikar, 2014; Kavitha Rani, 2015).

Insulin is one of the important hormones secreted by β-cells of islets of Langerhans of pancreas and released in response to nutrient inflow from the gut and to gastrointestinal secretagogues. Streptozotocin, by its specific cytotoxic effect for β-cells of islets, has been reported to induce diabetes in animals accounting for hypoinsulinemia which was also observed in the present study and supported by microscopical evidence of degeneration and destruction of beta cells of islets (Bolaffi *et al.,* 1987; Bedoya *et al.,* 1996 and West *et al.,* 1996).

The mean haemoglobin percentage in diabetic control rats were observed to decline drastically from 13.25±0.24 of 3rd day to 10.82±0.17 on 45th day of experiment. Decreased haemoglobin content noticed in diabetic rats could be due to increased formation of glycosylated haemoglobin. It is reported that during diabetes mellitus the excess of glucose present in the blood reacts with haemoglobin to form glycated haemoglobin (HbA_1c) and elevated levels of HbA_1c there by reduces the levels of haemoglobin in diabetic animals (Koening *et al.,* 1976; Pari *et al*., 2001; Kaleem *et al.,* 2006 and Manjunatha, 2013).

In the present study, there was significant reduction in the concentration of SOD, CAT and GPx in the liver of diabetic rats from day 15 to day 45 of experimental study. Similar findings have been recorded in alloxan and STZ induced diabetic rat models by

several workers (Singh *et al.,* 2010; Pragathi, 2011; Gurikar, 2014 and Kavitha Rani, 2015).

Persistent hyperglycemia during diabetes conditions leads to production of free radicals or impaired antioxidant defenses via several mechanisms (Saxena *et al.,* 1993; Maritim *et al.,* 2003; Valko *et al.,* 2007). Decline in the activities of antioxidant enzymes in the liver of diabetic animals indicated the extent of free radical induced damage due to STZ induced hyperglycemia (Resmi *et al.,* 2006 and Kaleem *et al.,* 2006). During diabetes, free radical generation is associated with auto-oxidation of glucose, impaired glutathione metabolism, alterations in the antioxidant enzymes and formation of lipid peroxides (Chis *et al.,* 2009).

The clinical signs in STZ induced diabetes in Group II rats comprised polyuria, polydipsia, polyphagia, restlessness and poor body condition. These findings were in accordance with those of earlier workers (Babu and Prince, 2004; Akbarzadeh *et al.,* 2007; Mallikarjuna, 2009; Dhanush, 2009; Pragathi, 2011; Mudassir, 2011; Manjunath, 2013 and Gurikar, 2014).

The clinical signs in the diabetes are due to metabolic derangement associated with hyperglycaemia leading to decreased insulin level. When hyperglycaemia exceeds the renal threshold for reabsorption, glycosuria occurs which further induces an osmotic diuresis leading to polyuria in diabetes. Polydipsia in diabetes is due to hyperglycemia progressing to depletion of intracellular water and triggering osmoreceptor of the thirst centres in the brain. In addition, insulin deficiency induces a negative energy balance due to catabolism of protein and fats which leads to polyphagia. Despite the increased

appetite, catabolic effects persist resulting in weight loss and muscle weakness a feature also observed in the present study (Kumar *et al.,* 2008; Mallikarjuna, 2009; Dhanush, 2009; Pragathi, 2011; Mudassir, 2011 and Shesha Rao, 2013).

Grossly pancreas appeared slightly congested and showed progressive decrease in size from day 15 onwards which appeared as a thin gelatinous strip on day 45. 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 which was also noticed in the present study (Szkudelski, 2001 and Mir *et al.*, 2008).

In the diabetic rats, the liver appeared pale, soft and friable from 30^{th} day of treatment 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; Zafar *et al.,* 2009 and Shesha Rao, 2013).

Spleen in the STZ induced diabetic rats appeared to be reduced in size and pale with wrinkled capsular surface. This could be due to lymphocytolytic activity of STZ (Adeghate *et al.,* 2010 and Pragathi, 2011).

The other organs such as kidney, lung, intestine, stomach, brain and heart did not reveal any distinct gross lesions in the present study.

The present study revealed characteristic microscopic lesions in exocrine and endocrine portion of pancreas in diabetic rats. The endocrine portion revealed reduced number of islets which were irregular and varied in size. Islets showed loss of

architecture and reduced number of cells that were either necrotic or highly swollen with vacuolated cytoplasm. The cytoplasmic granularity was reduced and some cells appeared elongated and fusiform. The normal distribution of α and β-cells was altered and showed an increase in the number of α cells. The exocrine portion revealed loss of architecture and wide separation of lobules with presence of edema fluid, haemorrhage and mild infiltration of mononuclear cells. Pancreatic acini were lined by vacuolated, degenerating and necrotic cells. Some of the lobules which were intact revealed large number of necrotic cells.

The decrease in the number of islets and cellularity in the islets could be attributed to the cytotoxic effect of streptozotocin which is specific for β-cells of islets as indicated by several workers. The STZ enters the beta cells through a glucose transporter (GLUT-2) and causes alkylation of DNA. Further, STZ liberates toxic amounts of nitric oxide which inhibits aconitase activity, cause DNA damage and destroys β-cells by necrosis (West *et al.,* 1996; Szkudelski, 2001; Akbarzadeh *et al.,* 2007; Zafar *et al.,* 2009). The change in the shape of the β-cells could be due to partial injury to the cells by STZ (Mir *et al*., 2008). The damage to the exocrine acinar cells of pancreas in the present study could be due to secondary response to excessive free radical liberation and antioxidant depletion in STZ cytotoxicity. 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. (Manjunatha, 2013; Shesha Rao, 2013).

Similar types of findings were observed by previous workers in STZ induced diabetic studies (Akbarzadeh *et al.,* 2007; Mallikarjuna 2009; Pragathi, 2011; Nasreen 2012).

In the present study, microscopic examination of liver revealed vacuolar degeneration, obliteration of sinusoidal spaces with individual hepatocyte necrosis and infiltration of inflammatory cells. Streptozotocin damages the hepatocytes leading to vacuolar fatty change and necrosis (Mir *et al.,* 2008, Muhammad *et al.,* 2008, Zafar *et al.,* 2009 and Manjunath, 2013). Disturbances in carbohydrate, lipid and protein metabolism together with oxidative stress leading to insulin deficiency affect hepatic function leading to increased gluconeogenesis and ketogenesis (Singh *et al.,* 2010). Further, the damage to the hepatocytes could be attributed to increased concentration of lipid peroxides and hydroperoxides consequent to generation of free radicals with resultant antioxidant depletion (Kaleem *et al.,* 2006).

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. This could be due to negative nitrogen balance with enhanced tissue proteolysis and decreased protein synthesis which can contribute to increased serum urea and creatine levels, indicating impaired renal function in diabetic animals (Jensen *et al.,* 1986; Mir *et al.,* 2008).

Microscopically, spleen revealed drastic depletion of lymphocytes from the periarteriolar sheath as well as from the follicles. This could be due to lymphocytolytic effect of STZ (Adeghate *et al.,* 2010; Pragathi, 2011; Manjunatha, 2013).

Histopathological examination of heart, lungs and intestine did not show any lesions throughout the study period.

5.4 Glibenclamide treatment group (Group III)

Glibenclamide is a potent second generation sulphonylurea drug used as a standard allopathic drug in the treatment of Type-II diabetes mellitus. Glibenclamide improves glucose control by acting both on insulin secretion and insulin action.

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. 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; Shesha Rao, 2013). Glibenclamide has been shown to bind to the surface receptors of β-cell membrane inhibiting ATP-sensitive K+ channels and cause depolarization of cell membrane. Depolarization leads to opening of K+ channels which enables extracellular Ca2+ to enter the cell. Increased intracellular Ca2+ concentration enhances the binding of Ca2+ to the transport protein calmodulin which leads to microfilament contraction and release of insulin containing granules. Increased insulin causes subsequent reduction in serum glucose levels which improves β-cell sensitivity to glucose and potentiates insulin secretion (Luzi and Pozza, 1997; Ling *et al*., 2006).

In the present study, Group III diabetic rats were treated daily with glibenclamide at a dose rate of 60 µg/kg from 3rd day onwards. The animals showed improvement in their body weight which was comparable to that of control rats from 15th day post-treatment. The improvement could be attributed to the effect of glibenclamide in enhancing insulin secretion by regenerating/repairing beta cells of pancreas which further improves uptake of glucose by the cells (Dhanush, 2009; Pragathi, 2011; Manjunatha, 2013; Kavitha Rani, 2015).

In the present study, the serum glucose levels were significantly lowered in the glibenclamide treatment group compared to diabetic control rats. Insulin secretion by the beta cells in response to glibenclamide treatment could be responsible for the reduction in serum glucose levels which further improves sensitivity of beta cells to glucose and potentiates insulin secretion (Luzi and Pozza, 1997; Ling *et al.*, 2006). The improvement in glucose level in blood 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; Mallikarjuna, 2009; Pragathi, 2011; Nasreen, 2012). Prolonged exposure of β-cells to the insulin secretagogue glibenclamide results into glibenclamide-induced recruitment of β-cell subpopulation into elevated functional state of insulin synthesis and release (Ling *et al.*, 2006).

In the present study, the serum glucose levels failed to reach the normal levels inspite of treatment with glibenclamide in Group III, as also observed by several workers

(Erejuwa *et al*., 2011). This could be due to failure of β-cells population to attain optimum secretory functional activity in response to the glibenclamide treatment as evidenced microscopically and by immunohistochemistry for beta cells in the present study.

In the present study, the serum cholesterol and serum triglyceride levels were significantly ($P \leq 0.001$) reduced in the glibenclamide treated animals compared to diabetic control rats which clearly indicated that the treatment with glibenclamide improved hyperlipidemia in diabetes. 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 acute reduction in triglyceride of intestinal origin and not with the VLD lipoprotein sub fraction levels.

In the present study, the ALT and AST values in glibenclamide treatment group showed an improvement in the values from 15^{th} day post treatment onwards in comparison with that of diabetic control group. However, it was observed that the values were significantly higher compared to normal control group which clearly indicated that glibenclamide treatment did not reverse liver damage caused by STZ completely. This was supported by the persistence of mild degree of hepatic vascular changes till the end of the study. 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 30th day post treatment till the end of the study which could be attributed to the glibenclamide effect on beta cells. Glibenclamide which is a novel antidiabetic drug has been reported to activate pancreatic beta cells to secrete insulin on prolonged treatment. Glibenclamide has been shown to activate protein translation in pancreatic beta cells through the calcium regulated m TOR, PKA and MEK signalling pathway resulting in insulin synthesis (Luzi and Pozza, 1997 and Wang *et al.*, 2008). It was also reported that glibenclamide treatment recruits beta cells subpopulation and causes elevated and sustained basal insulin synthetic activity (Ling *et al*., 2006). 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.

In the present study, there was significant improvement in the mean (±SE) haemoglobin values when compared with the diabetic control group. Improvement in the Hb concentration could be attributed to antihyperglycaemic effect of glibenclamide and improvement of insulin level, thus reducing non-enzymatic glycosylation of hemoglobin (Rubin and Strayer, 2008).

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; Shesha Rao, 2013). 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 (Elmali *et al.,* 2004).

The clinical symptoms exhibited by diabetic animals from Group III 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; Rubin and Strayer, 2008; Manjunatha, 2013).

Gross pathological changes observed in the present group reduced progressively with advancement in time on treatment with glibenclamide. This could be attributed to alleviation of diabetes complications by the increased levels of insulin.

In glibenclamide treated group, the rats revealed a progressive improvement in the microscopic pathology and attainment of normal architecture of acini and islets of Langerhans from Day 15 to Day 45 post treatment. This could be attributed to the increased proliferation as well as recruitment of subpopulation of β-cells and thereby

increase in the beta cell mass upon treatment with glibenclamide (Guiot *et al.*,1994; Ling et al., 2006; Wang *et al.*,2008). Chronic changes in β-cell glucose metabolism, rather than blood glucose levels are the main positive regulator of basal and compensatory β-cell proliferation *in vivo*. Both glycolysis and membrane depolarization are necessary for the mitogenic effect of glucose metabolism (Porat *et al.*, 2011). This was in accordance with previous reports on glibenclamide induced β-cell proliferation (Guiot *et al.,* 1994; Ling *et al.,* 2006).

Histopathological observations of liver revealed progressive improvement in the architecture from STZ induced liver damage from 15th day onwards in glibenclamide treatment group. The results were in accordance with those of Luzi and Pozza (1997). Studies using [^{3}H]-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 GLUT2 rather than GLUT4 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's microscopic architecture (Luzi and Pozza, 1997).

Histologically, spleen revealed persistence of lymphocytic depletion on 15th day with improvement of cellular masses on 30th and 45th day compared to diabetic animals. Improvement in hyperglycaemic state and insulin level could be contributing for improvement in defence system by reducing the free radical injury (Elmali *et al.,* 2004).

Other organs like kidney, lungs, intestine and heart were normal throughout the study.

5.5 *Aloe vera* treatment group (Group IV)

5.5.1 Phytochemical analysis

In the present study, the aqueous extract of *Aloe vera* was found to be positive for presence of carbohydrate, protein, alkaloids, flavonoids, tannins, fixed oil & fat and phytosterols but negative for saponins, glycosides and anthraquinone. The presence of the above mentioned bioactive compounds in the aqueous extract of *Aloe vera* was also recorded by several workers (Hossain *et al.,* 2004; Maenthalsong, 2007; Arun Kumar *et al.*, 2009; Yebpella *et al.,* 2011; Ejoba *et al.,* 2012; Raphael *et al.,* 2012)

5.5.2 *Aloe vera* in diabetes

In the present study, the mean body weight in *Aloe vera* treated rats showed an improvement from 30^{th} day onwards in comparison with the mean weights of diabetic control rats (Group II). It was also observed that the mean body weights of *Aloe vera* treated rats were comparable with those of normal control, glibenclamide treated groups on 15^{th}, 30^{th} and 45^{th} day post-treatment.

The findings of the present study are similar to those reported by Vats *et al.* (2004). The increased body weight could be attributed to the better utilization of nutrients, glucose, amino acids, fatty acids and other macromolecular components due to improved insulin release by the beta cells on *Aloe vera*. In addition, the hepatoprotective property of the plant and induction of various metabolic enzymes also could be

responsible which improved uptake of nutrients and their metabolism (Devipriya *et al.*, 2007).

It was observed in the present study that the mean serum glucose levels gradually reduced upon *Aloe vera* aqueous extract treatment and were significantly lower from 15th day onwards compared to those of diabetic control group. The decrease in glucose values was comparable to those of glibenclamide on all the days of observation. A number of workers have observed and reported the hypoglycaemic effect of the *Aloe vera* extract in the experimentally induced diabetic animals. However, the glucose values failed to reach the normal range as observed in the normal control group rats. This clearly indicated that, though *Aloe vera* was capable of declining the glucose level, the hypoglycaemic effect was not total and never attained the normal range.

The hypoglycemic effect of *Aloes* and its bitter principle may be mediated through stimulating the synthesis and/or release of insulin from the β-cells of Langerhans (Ajabnoor, 1990). Administration of the superficial layer of the *Aloe leaf* (leaf skin) to streptozotocin induced diabetic mice, significantly depressed hyperglycaemia and indicated that aloe relieves the diabetic condition by direct hypoglyceration (blood glucose lowering activity) and activates beta-cells (Beppu *et al.*, 1993).

Effect of *Aloe vera* juice in combination with glibenclamide was investigated in diabetic patients and found that there was no response to glibenclamide alone but *Aloe vera* juice significantly reduced levels of fasting blood glucose within two weeks and of triglycerides within four weeks (Bunyapraphatsara, 1996). *Aloe vera* extract maintains the glucose homeostasis by controlling the carbohydrate metabolizing enzymes.

Aloe vera gel and phytosterols derived from *Aloe vera* gel had long-term blood glucose level control effects which were responsible for the treatment of type 2 diabetes mellitus (Rajasekaran *et al.,* 2006; Tanaka *et al.,* 2006). Beneficial effects of *Aloe vera* in streptozotocin-induced diabetic rats was studied and noticed significant decrease in the fasting plasma glucose & increase in the body weight in the diabetic rats when fed with *A. vera* at the dose of 300 mg/kg body weight (Ayesha *et al.,* 2008). Processed *Aloe vera* Gel appeared to lower blood glucose levels by decreasing insulin resistance (Kim *et al.,* 2009). Investigation on the antidiabetic effect of *Aloe vera* water extract in normal and alloxon induced diabetic rats revealed significant reduction in blood glucose level after administering *Aloe vera* water extract for 30 days orally (Rehman *et al.,* 2011).

There are many reports on hypoglycaemic effect of the *Aloe vera* in the experimentally induced diabetic animals. Helal *et al.* (2003) in his study revealed highly significant decrease in blood glucose in diabetic group treated with aqueous extract of *Aloe vera* when compared with diabetic untreated rats. Oral administration of *Aloe vera* gel extract at a dose of 300 mg/kg bodyweight per day to STZ-induced diabetic rats for a period of 21 days resulted in a significant reduction in fasting blood glucose and a significant improvement in plasma insulin (Rajasekaran *et al.,* 2006). Hypoglycemic effect of *Aloe vera* leaf dried powder was evaluated and compared with standard Metformin in alloxan-induced diabetic rats by (Manjunath *et al.,* 2016) and noticed that elevated blood glucose levels in diabetic rats were reduced by the treatment with *Aloe vera* leaf extract at doses of 200 mg/kg and 400 mg/kg which was comparable to 50 mg/kg of metformin with no statistically significant difference.

The mean serum cholesterol and triglycerides values of *Aloe vera* treated animals revealed decreasing trend compared to diabetic control animals from day 15th onwards. Though the values showed a declining trend, the mean values were significantly higher compared to normal control group. In comparison with glibenclamide, the mean cholesterol values did not vary significantly throughout the study period. Similar observations have also been made by many earlier workers and have shown a better lipid lowering effect of *Aloe vera* or its constituent fraction. (Rajasekaran *et al.,* 2006; Kim *et al.,* 2009). Significant reduction in fasting blood glucose levels and HbA1C was noticed in alloxon induced diabetic rabbits treated with *Aloe vera* leaf extract for 21 days and also there was significant decrease in serum levels of triglycerides (TG), total Cholesterol (TC) and LDL Cholesterol indicating *Aloe vera* as a potent antidiabetic drug (Gupta *et al.*, 2011a).

The increase in liver cholesterol in diabetic rats could be due to increased cholesterogenesis. The present study revealed decrease in serum cholesterol and triglycerides in diabetic rats treated with aqueous extract of *Aloe vera*. The reduction may be attributed to increased clearance and decreased production of the major transporters of endogenously synthesised cholesterol and triglycerides.

The mean serum ALT and AST values in *Aloe vera* treated group revealed a significant decrease when compared to diabetic control rats (Group II). The values were comparable with the normal control animals at 45th day and when compared to glibenclamide group, there was no significant difference.

The results of the present study clearly indicated that *Aloe vera* extract has hepatoprotective effect and there by reduced serum levels of ALT and AST enzymes. The increased ALT and AST levels indicating an altered liver function, which was a consistent feature in STZ induced diabetes in animals (Dhanabal *et al.,* 2006; Gayathri and Kannabiran, 2008; Rajnish and Radhey, 2009; Singh *et al.,* 2010). The change in serum enzymes is directly related to changes in metabolism in which these enzymes are involved. The improvement noticed in the level of enzymes is consequence of an improvement in the carbohydrate, fat and protein metabolism (Dhanabal *et al.,* 2006 and Singh *et al.,* 2010). The reversal of AST and ALT activity in *Aloe vera* treated diabetic rats towards near normalcy is evidence of the prevention of cellular and tissue damage under diabetic conditions, which may further strengthen the optimized lipid metabolism in the liver of diabetic rats (Rajasekaran *et al.,* 2006). Validation of the hepatoprotective potential of *Aloe barbadensis* Mill against carbon tetrachloride induced hepatotoxicity and hepatoprotective activity was evident by restoration of serum transaminases, alkaline phosphatase, bilirubin and triglycerides. Restoration of lipid peroxidation and glutathione towards normal was also noticed (Chauhan *et al.,* 2012).

The serum insulin levels in *Aloe vera* treatment group showed a gradual and progressive improvement from day 15 post treatment compared to diabetic control (Group II). Though there was an increase in the mean values, they failed to reach the normal values and were significantly lower when compared to those of normal treatment group.

Improved insulin level in *Aloe vera* treatment could be due to regeneration or repair of damaged beta cells by *Aloe vera*. A similar observation has also been made by earlier workers who noticed increase in serum insulin after treatment with *Aloe vera* (Gayathri and Kannabiran, 2008; Rajnish and Radhey, 2009). It could be because of increase in insulin release through stimulation of β-cells regeneration. Improvement of architecture of islets of Langerhans in the present study, a finding observed microscopically also strengthens the insulinotropic role of *Aloe vera* by regenerating insulin producing cells.

There was significant improvement in the mean activities of antioxidant enzymes in liver of *Aloe vera* treated rats compared to diabetic animals and were comparable with glibenclamide control animals throughout the study period. However, the values were not comparable with normal control on all the days of study with significantly lesser values. The antioxidant activity of this plant is documented by many earlier workers (Singh *et al.,* 2010). The effect of the *Aloe vera* gel extract on tissue antioxidants is due to reduction in blood glucose level in diabetic rats, which prevents excessive formation of free radicals through various biochemical pathways and also reduces the potential glycation of the enzymes (Rajasekaran *et al.,* 2005). There are several reports regarding the decrease in activity of antioxidant enzymes (SOD, CAT, GPx and GST) in the liver and kidney of diabetic rats (Anuradha and Selvam, 1993).

There was a significant improvement in the haemoglobin values in the present study in the *Aloe vera* treated rats compared to diabetic control group. The values were comparable with glibenclamide group and normal control rats indicating the beneficiary

effect of treatment with *Aloe vera aqueous* extract. The improvement in Hb values could be attributed to the improvement in blood glucose levels thereby reducing the metabolic changes like excess glucose reacting with haemoglobin resulting in glycosylation of haemoglobin. Treatment with Aloe vera revealed significant decrease in the glycosylated haemoglobin levels in the diabetic rats which could be due to an improvement in glycemic status (Jain *et al.,* 1989).

There was a gradual decrease in the severity of clinical signs in the animals treated with the aqueous extract of *Aloe vera.* The improvement in the condition could be attributed to the *Aloe vera* treatment which proved to be highly effective in managing hyperglycemia thereby reducing the alteration in metabolism of carbohydrate, lipid and protein (Rajnish and Radhey, 2009).

Gross pathological changes also observed in the present group gradually reduced with advancement in time on treatment with *Aloe vera aqueous* extract.

Histopathological findings in the pancreas revealed progressive reconstruction of normal architecture of acini and islets from day 15 to day 45 post treatment in *Aloe vera* treated rats in the present study. The islets exhibited hypercellularity with advancement of time on treatment. Initially more number of α-cells and few β-cells were seen and with the advancement of time the percentage of β-cells increased from 12.45 ± 1.23 to 52.98 ± 1.34 on day 15 to day 45 which was well demonstrated by immunohistochemistry.

The hypoglycemic effect of aloes and its bitter principle may be mediated through stimulating synthesis and/or release of insulin from the β-cells of Langerhans (Ajabnoor,

1990). Treatment of diabetic rats with aqueous extract of *Aloe vera* resulted in the activation of β-cells and granulation returning to normal, showing insulinogenic effect. The antihyperglycemic activity of *A. vera* gel was associated with an increase in plasma insulin suggesting that the antihyperglycemic activity of *A. vera* gel could be due to an insulinogenic activity of the gel extract.

The increased levels of insulin observed in the present study indicate that *A. vera* aqueous extract stimulates insulin secretion from the remnant β-cells or from regenerated β-cells. Hence antihyperglycemic and an insulin-releasing stimulatory effect have a role in managing diabetes in diabetic rats treated with *Aloe vera.*

Microscopically, the liver showed progressive improvement in the architecture from STZ induced liver damage from 15^{th} day onwards in *Aloe vera* treated group. This could be due to better control of blood glucose thereby reducing the disturbances in carbohydrate, lipid and protein metabolisms together with oxidative stress which are likely to affect hepatic function (Singh *et al.,* 2010). Many earlier authors have been reported to incite hepatoprotective effect of *Aloe vera* in carbon tetrachloride induced liver damage (Chandan *et al.,* 2007). Hepatoprotective potential was confirmed by the restoration of ALT, AST, SOD, Catalase and Glutathione peroxidase towards near normal. Histopathology of the liver tissue also supported the biochemical findings confirming the hepatoprotective potential of aqueous extract of *Aloe vera*.

5.6 *Murraya koenigii* treatment (Group V)

5.6.1 Phytochemical analysis

In the present study, the aqueous extract of *Murraya koenigii* was found to be positive for carbohydrate, protein, saponin, alkaloids, flavonoids, tannins, fixed oil & fat, glycosides, anthraquinone and phytosterols. Similar findings were also recorded by several workers (Brahmachari *et al.*, 2004; Akhila *et al*., 2003; Math and Balasubramaniam, 2005b; Bonde *et al.,* 2011; Bhandari *et al.*, 2012).

5.6.2 *Murraya koenigii* in diabetes

In the present study, the *Murraya koenigii* treated group revealed significant improvement in the body weight progressively from 15^{th} day onwards compared to diabetic control rats and the values were comparable to those of normal control and glibenclamide treated rats. The improvement in the body weight could be attributed to the alleviation of diabetic symptoms and hyperglycaemia by increase in insulin level by insulinomimetic action of *Murraya koenigii* and also due to insulin stimulated glucose transport and anabolic effects of insulin.

The mean (± SE) serum glucose values of *Murraya koenigii* treatment group were reduced significantly from 15^{th} day to 45^{th} day in comparison to those of diabetic control rats in the present study.

Murraya koenigii leaves have been attributed with antidiabetic activity and many studies were conducted on *Murraya koenigii* have reported hypoglycemic and

antidiabetic activity which can be attributed to multiple bioactive molecules present in the extracts.

The decrease in the Serum glucose may be attributed to many bioactive phytochemicals present in the aqueous extract of *Murraya koenigii*. The phytochemical analysis of aqueous extract of *Murraya koenigii* in the present study revealed presence of alkaloids, flavonoids, tannins, saponins, carbohydrates, protein, fat, anthraquinone, glycoside and phytosterols. The hypoglycemic activity of aqueous extract of the leaves of *Murraya koenigii* after oral as well as intravenous administration to normal and alloxan diabetic dogs reported by Narayana and Sastry (1975). The hypoglycemic activity of *Murraya koenigii* was attributed to increased glycogenesis and decreased glycogenolysis and gluconeogenesis (Khan *et al.,* 1995) or due to induction of receptors and/or expression of gene/s required for insulin synthesis and release in the regenerated b-cells of pancreas (Bhat, 1995) or due to increased glucose uptake and its utilization by cells (Rupashree, 1999).

In the present study, there was significant increase in the serum insulin upon administration of aqueous extract. Cold hexane extract of *Murraya koenigii* reported to have alpha amylase inhibitory activity and inhibits the conversion of carbohydrates in to simple sugars in the gut and there by inhibits the entry of glucose in to circulation (Bowden *et al.,* 2002). *Murraya koenigii* supplemented diet was shown to inhibit the development of insulin resistance and diabetes (Yadav *et al*., 2004). Antidiabetic activity of methanol extracts of *Murraya koenigii* was reported by Vinuthan *et al.* (2005) in short term experiment in diabetic rats. Hypoglycaemic effect of single dose administration of

aqueous extract of *Murraya koenigii* was reported by Kesari *et al*., (2005). Recently Xie *et al*. (2006) reported the hypoglycemic and hypolipidemic activity of *Murraya koenigii* in ob/ob mice. Arulselvan *et al.,* (2006) and Narendhirakannan *et al*., (2006) reported the antidiabetic affect of ethanol extract of *Murraya koenigii* in STZ induced diabetic rats. The results from present study confirm the earlier reported findings.

Bioactive molecules in aqueous extracts of *Murraya koenigii* might influence the cellular and molecular changes. The increased insulin levels may be due to enhanced regeneration and neogenesis of pancreatic beta cells, which was evident in the histology of rat pancreas from the groups treated with aqueous extracts of *Murraya koenigii*. Roberto *et al.,* (2004) reported regeneration and neogenesis of pancreatic beta cells from either ductal or acinar cells in rats. The other possible mechanism of antidiabetic activity of *Murraya koenigii* is due to increased glucose uptake and its utilization by cells by increasing GLUT2 expression and glucokinase activity. Alloxan concentration-dependently reduced the mRNA expression of Glucose transporter 2 (GLUT2) and Glucokinase (GK) and the effect on GLUT2 was more marked (Gai, 2004). Glucokinase is an important glucose sensor in pancreatic beta cell that mediates the production of insulin by pancreatic beta cells. GLUT2 helps in the glucose uptake and utilization from cells.

Ethanol extract of *Murraya koenigii* reported to increase insulin and C-peptide levels and glucose tolerance. It also induces the restoration of activities of carbohydrate-metabolising enzymes, such as hexokinase, glucose-6-phosphate dehydrogenase and glycogen synthase, in diabetic rats towards near normal (Khan, 1995; Yadav, 2004;

Narendhirakannan *et al*., 2006). *Murraya koenigii* leaves extract may bring about its anti-hyperglycemic effect through insulin secretion from the remnant β-cells and from regenerated β-cells (Arulselvan *et al.*, 2007). Carbozole alkaloids a major phytochemical constituent of MK plant, has been found to have various biological activities like anti-oxidant, antidiabetic and lipid lowering effect and antioxidant property of curry leaves helped in protecting from diabetes, cardiovascular diseases, high cholesterol level and many other degenerative illnesses (Tembhurne and Sakarkar, 2010 and Naz *et al.*, 2015).

In the present study, the serum cholesterol and serum triglyceride levels showed a decreasing trend from 15th day onwards in *Murraya koenigii* treated group in comparison with diabetic control group and was comparable to that of normal control rats on 45th day of experiment.

Aqueous extracts of *Murraya koenigii* have insulinotropic action. Insulin is found to cause an increase in storage and decrease in release of lipids into circulation (Zammit, 1996). Insulin elicits a remarkable array of biological responses. The important target tissues for regulation of glucose homeostasis by insulin are liver, muscle, and fat, but insulin exerts potent regulatory effects on other cell types as well. Insulin is the primary hormone responsible for controlling the uptake, utilization, and storage of cellular nutrients. Insulin's anabolic actions include the stimulation of intracellular utilization and storage of glucose, amino acids, and fatty acids, while it inhibits catabolic processes, such as the breakdown of glycogen, fat, and protein (Granner, 1991).

Decrease in the serum lipids and triglycerides could be due to a low lysine:arginine ratio in plants. Vitamin-C and niacin in aqueous extract and vitamin E and

niacin in methanolic extract of *Murraya koenigii* might have major role in decreasing serum lipids and triglycerides. Vitamin E, vitamin C and niacin have been ascribed to be hypotriglyceridemic (Sen and Mukherjee, 1997; Satyanarayana, 1999). Ingredients of the volatile oil of *Murraya koenigii* were found to have a hypotriglyceridemic effect (Bamosa *et al*., 2002).

Murraya koenigii supplementation to atherogenic diet was found to decrease plasma triglyceride, plasma phospholipid in male albino rats (Khan *et al*., 1998). *Murraya koenigii* extract was reported to produce hypolipidemic activity in ob/ob mice (Xie *et al.* 2006). Fasting blood sugar, total cholesterol and triglycerol were significantly reduced in the diabetic rats treated with *M. koenigii* aqueous extract. The hypolipidemic effect of *Murraya koenigii* is mainly due to carbazole alkaloids, particularly the mahanimbine and antioxidant property helped in protecting from diabetes, cardiovascular diseases, high cholesterol level and many other degenerative illness (Birari *et al.* 2010).

Significant decrease in serum cholesterol and triglycerides concentration due to feeding of aqueous extract of *Murraya koenigii* in the present study could be attributed to presence of tryptophan, vitamin C, vitamin E, lower lysine:arginine ratio, niacin and β-carotene in *Murraya koenigii* extracts. The decrease in plasma total cholesterol could also be due to insulin secretogague activity and antioxidant property of aqueous extract of *Murraya koenigii*.

There was a decrease in the levels of serum ALT and AST in *Murraya koenigii* treatment rats from 3rd day onwards in comparison with those of diabetic control and were almost comparable to those of glibenclamide treated group.

The improvement noticed in the level of enzymes may be due to improvement in the carbohydrate, fat and protein metabolism as *Murraya koenigii* is considered to be hepatoprotective. Administration of aqueous *M. koenigii* leaves improved the liver function by decreasing the serum ALT, ALT and Alkaline Phosphatase level in both normal and diabetic rats (Kesari *et al.*, 2007).

Serum insulin values in the present investigation showed a progressive increase in *Murraya koenigii* treated animals from 15th day post treatment onwards in comparison with those of diabetic control rats. The insulin values in the present study, though showed an improvement in concentration, failed to reach those of normal control rats.

Bioactive molecules in aqueous extracts of *Murraya koenigii* might influence the cellular and molecular changes. The increased insulin levels may be due to enhanced regeneration and neogenesis of pancreatic beta cells, which was evident in the histology of rat pancreas from the groups treated with aqueous extracts of *Murraya koegnii* in the present study by Immunohistochemistry. Roberto *et al.* (2004) reported regeneration and neogenesis of pancreatic beta cells from either ductal or acinar cells in rats. The other possible mechanism of antidiabetic activity of *Murraya koenigii* is due to increased glucose uptake and its utilization by cells by increasing GLUT2 expression and glucokinase activity. Alloxan concentration-dependently reduced the mRNA expression of Glucose transporter 2 (GLUT2) and Glucokinase (GK) and the effect on GLUT2 was more marked (Gai, 2004). Glucokinase is an important glucose sensor in pancreatic beta cell that mediates the production of insulin by pancreatic beta cells. GLUT2 helps in the glucose uptake and utilization from cells.

Ethanol extract of *Murraya koenigii* reported to increase insulin and C-peptide levels and glucose tolerance. It also induces the restoration of activities of carbohydrate-metabolising enzymes, such as hexokinase, glucose-6-phosphate dehydrogenase and glycogen synthase, in diabetic rats towards near normal (Khan, 1995; Yadav, 2004; Narendhirakannan *et al*., 2006). Methanolic extract of *Murraya koenigii* can prevent the rise in plasma lipid concentrations which occur as a secondary complication in diabetic individuals (Vinuthan *et al.*, 2007).

The activity of SOD, CAT and GPx in liver showed significant improvement in rats compared to diabetic control treated with *Murraya koenigii* extract and were comparable with glibenclamide control on all days of the study.

Similar type of finding in improvement of the levels of SOD, GPx and GSH in the dose dependent manner was recorded by administration of *Murraya koenigii* extract by several workers. This could be due to free radical scavenging activity and hypoglycaemic effect of the extract. The antioxidant activity is attributed to phenolic content of the plant. Phenolic compounds are considered as one of the most important and widely found antioxidant sources in food and medicinal plants. There is significant correlation between phenolic content and antioxidant activity in different parts of the plant (Phoboo *et al.*, 2013). *Murraya koenigii* extracts contain many naturally occurring carbazole alkaloids and flavonoids that have been associated with wide range of biological and pharmacological activities including antioxidant and antidiabetic activities (Suryawanshi *et al.*, 2006).

The ethanolic extract of *M. koenigii* exhibits a significant antihyperglycemic as well as antioxidant activity in experimental rats. The presence of carbazole alkaloids, further strengthens the efficacy of *M. koenigii* leaves in protecting the tissue defence system against oxidative damage in streptozotocin-induced diabetes.

Preliminary phytochemical investigations in the present study also revealed the presence of biologically active ingredients such as alkaloids, glycosides, flavonoids, phenolics, etc. Thus, a sufficient supply of antioxidants may prevent or delay β-cell dysfunction in diabetes by providing protection against glucose toxicity. *Murraya koenigii* might be a potent therapeutic agent for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO and its oxidation product, peroxynitrite (Baliga *et al*., 2003)

Carbazole alkaloids isolated from *Murraya koenigii are* recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases resulting from lipid peroxidation (Nakathani, 2000). The observed antioxidant potential of *M. koenigii* may partially be responsible for its antidiabetogenic properties (Arulselvan *et al.,* 2007).

In the present study, aqueous extract of *Murraya koenigii* was found to have a better role in maintaining the liver enzyme activities such as SOD, CAT and GPx to the level of normalcy in diabetic rats.

The haemoglobin content in *Murraya koenigii* treated rats in the present investigation showed improvement from 30^{th} day onwards compared to diabetic control

rats and was comparable with normal control animals from 15th onwards. The increase in the level of haemoglobin in treated animals may be due to the decreased level of blood glucose levels.

Hyperglycaemia of diabetes has been reported to cause glycosylation of many proteins including haemoglobin (Rubin and Strayer, 2008). *Murraya koenigii* has been shown to improve secretion of insulin by β-cells and lower blood sugar levels thereby controlling hyperglycaemia and glycosylation of haemoglobin.

Clinically, *Murraya koenigii* treatment reduced the severity of the diabetic manifestation from 15th day post treatment onwards which could be directly attributed to the increased level of sugar and lowered insulin levels.

Grossly, appreciable improvement was observed in the appearance of pancreas. Microscopically, *Murraya koenigii* treatment for a period of 45 days showed an improvement in the architecture of exocrine and endocrine pancreas. In pancreas, there was an increase in the number, size and shape of the islets, increase in the β-cells percentage from 13.35 ± 1.17 to 61.86 ± 3.45 on 15th to 45th day, respectively as demonstrated by immunohistochemistry.

The improvement in architecture of pancreatic islets could be attributed to the effect of *Murraya koenigii* on pancreas resulting in promotion of regeneration of β-cell subsets or repair of damaged cells. The findings in the present study suggested the ability of *Murraya koenigii* in regeneration of pancreas. The protective effect of Murraya koenigii leaves extract on STZ-diabetic rats was evident by a moderate increase in

secretory vesicles with granules of β cell of pancreas and also by the apparently normal architecture of nucleus (Arulselvan *et al.,* 2007). Beta cell replication is controlled by the rate of intracellular glucose metabolism. Short pulse of glucose metabolism, as would happen after a meal, will trigger secretion of insulin by β-cells but not replication, while more persistent activation of the pathway will trigger replication indicating an organism's need for more β-cells (Porat *et al.,* 2011). Probably the more persistent activation of *Murraya koenigii* by insulinomimetric property may be responsible for β-cells regeneration.

Microscopically, liver revealed progressive improvement in the architecture from STZ induced liver damage. This could be due to hepatoprotective activity of *Murraya koenigii* extract by its free radical scavenging activity. Many earlier workers have screened the hepatoprotective activity of *Murraya* species against carbon tetrachloride induced liver damage (Bhandari *et al.,* 2012). The hepatoprotective activity could be attributed to tannins and carbazole alkaloids present in aqueous extract of *Murraya koenigii.*

Microscopically, spleen revealed improvement of cellular masses in terms of lymphocyte population compared to diabetic animals. The improvement in cell mass could be due to proliferative response of bone marrow cells by the carbazole alkaloids present in the *Murraya koenigii* extract.

5.7 Combined treatment group (Group VI, VII, VIII and IX)

To determine the synergistic antidiabetic effect, in the present study, Group VI rats were fed with extracts of *Aloe vera* at 150 mg/Kg body weight and *Murraya koenigii*

at 150 mg/Kg body weight, Group VII rats were fed with *Aloe vera* at 150 mg/Kg body weight and glibenclamide 300 µg/Kg, Group VIII rats were fed with *Murraya koenigii* at 150 mg/Kg body weight and glibenclamide at 300 µg/Kg body weight and Group IX rats were fed with *Aloe vera & Murraya koenigii* each at 150 mg/Kg body weight and glibenclamide at 300 µg/Kg body weight orally in combination.

The mean body weights in the combined treatment group were higher compared to diabetic control group. However, the values were lesser in comparison to normal control and glibenclamide treated groups and the improvement was statistically comparable.

The mean serum glucose level also remained in the range with no significant variation with those of individual treatment groups and glibenclamide group. But, when compared to normal control group the combined treatment groups showed better improvement on 45^{th} day and also when compared to individual treatment group, especially Group IX showed no significant variation just like glibenclamide group when compared to normal control. By this, it can be concluded that blood glucose lowering effect was better in combined treatment groups when compared to individual treatment groups. In combined treatment groups Group IX and glibenclamide combination groups (Group VII & Group VIII) had better hypoglycemic effect compared to other groups.

Similarly, mean serum cholesterol and triglyceride levels were comparable between the combined treatment and the individual treatments with *Aloe vera, Murraya koenigii* and glibenclamide control throughout the study. Groups VI and IX revealed

some amount of synergetic effect in controlling the hyperlipidemic state in induced diabetes mellitus in rats.

The ALT and AST of combined treatment groups were also comparable between the combined treatment and the individual treatments with *Aloe vera*, *Murraya koenigii* and glibenclamide control throughout the study except for moderately higher values in Group VIII (*Murraya koenigii* + glibenclamide) compared to *Murraya koenigii* (Group IV) Group. When compared to normal control on 30^{th} and 45^{th} day the ALT and AST values of Group IX showed values comparable with individual treatment group of *Aloe vera* and *Murraya koenigii* groups. So, in maintaining the ALT and AST values in induced diabetes mellitus in rats Group IX (*Aloe vera* + *Murraya koenigii* + Glibenclamide) was comparable with individual treatment groups than glibenclamide and other treatment groups.

Serum insulin levels in combined treatment groups were comparable with glibenclamide control and individual treatment groups of *Aloe vera* and *Murraya koenigii* with no significant variation. When compared to normal control, animals on 45^{th} day showed moderate variation in Group VI and IX indicating moderate synergetic effect of these groups compared to other treatment groups and Glibenclamide control.

The mean values of SOD, CAT and GPx were better in combined treatment groups compared to diabetic control animals and statistically significant improvement was seen on 30^{th} and 45^{th} day. In comparison to normal control animals the values were significantly less and with Glibenclamide group, the values were comparable at 15^{th} and 30^{th} days and on 45^{th} day Groups VIII and IX showed improvement with significant

variation indicating that Groups VIII and IX have better antioxidant activity compared to Glibenclamide control. Also, compared to individual treatment groups of *Aloe vera and Murraya koenigii*, Group IX animals showed better improvement.

The haemoglobin percentage of combined treatment groups did not differ from that of rats of treatment groups fed individually with *Aloe vera* and *Murraya koenigii*. They were comparable with Glibenclamide in all days of the study and with normal control from 15^{th} day post treatment.

The clinical symptoms and gross pathology of various organs were same as those of individual treatment groups and Glibenclamide control group throughout the study. Microscopically, the liver and spleen showed improvement with progression of treatment and improvement was comparable with Glibenclamide and individual treatment groups of *Aloe vera* and *Murraya koenigii* groups.

Histopathology of pancreas of combined treatment groups revealed 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 45^{th} day, the combined treatment groups revealed almost normal architecture with bigger islets and more number of islets per lobule, but the normal α:β ratio was not attained as in normal control group.

The immunohistochemistry of combined treatment groups revealed that Group IX showed better regeneration of β-cell population which was comparable to the Glibenclamide control indicating the synergistic action of herbal and allopathic drug

when compared to individual treatment groups of *Aloe vera* and *Murraya koenigii* groups.

Based on the above, it may be concluded that β-cell regeneration capacity of combined treatment groups is better than individual treatment groups of *Aloe vera* and *Murraya koenigii*. Among the combined treatment groups, Group VIII and IX showed better improvements which were comparable with Glibenclamide control.

The combination of two different herbal extract and herbal extract with glibenclamide has shown synergistic action and given a better result in alleviating diabetic complications (Dhanush, 2009; Mallikarjuna, 2009; Manjunatha, 2013; Shesha Rao, 2013). Similar findings were recorded in the present study.

Interactions between herbs and allopathic drugs may alter the pharmacological or toxicological effects of either component. Synergistic therapeutic effects may complicate the dosing of long-term medications. Herbs, traditionally used to decrease glucose concentrations in diabetes could theoretically precipitate hypoglycaemia if taken in combination with conventional drugs (Bailey and Day, 1989). However, in diabetes, several other herbal combinations, herbs and glibenclamide interactions have been used with good alleviation of STZ induced diabetic effects (Bunyapraphatsara *et al.,* 1996; Babu and Prince, 2004; Mutalik *et al.*, 2005; Singh *et al.*, 2010; Lal *et al.,* 2011 and Eliza and Usha, 2011).

In the present study, the combination of *Aloe vera* and *Murraya koenigii* extracts with Glibenclamide showed better results compared to *Aloe vera* and *Murraya koenigii*

extracts alone but statistically no significant synergistic effect was seen. However, Group VIII (*Murraya koenigii* + Glibenclamide) and Group IX (*Aloe vera* + *Murraya koenigii* + Glibenclamide) showed significant synergistic effect in alleviating the STZ induced diabetes and its complications.

The results of the present study indicated that combining *Aloe vera* and *Murraya koenigii* with Glibenclamide could provide an opportunity to reduce the dose of Glibenclamide, as these extracts were effective in alleviating streptozotocin induced diabetes and were comparable with glibenclamide. They help in minimizing the adverse effect of Glibenclamide and improve the therapeutic efficiency in diabetes when used in combination.

Summary

VI. SUMMARY

The present study was carried out for evaluating antidiabetic effect of *Aloe vera* and *Murraya koenigii* individually and in combination in STZ induced diabetes in rats. The efficacy of these herbal extracts was also compared with that of Glibenclamide, a standard antidiabetic drug individually and in combination. Phytochemical properties of *Aloe vera* and *Murraya koenigii* extracts were also evaluated.

The aqueous extract of *Aloe vera* was found positive for presence of carbohydrate, protein, alkaloids, flavonoids, tannins, fat and phytosterols but negative for saponins, glycosides and anthraquinone whereas the aqueous extract of *Murraya koenigii* was found positive for carbohydrate, protein, saponin, alkaloids, flavonoids, tannins, fixed oil & fat, glycosides, anthrquinone and phytosterols.

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 *Aloe vera* (Group IV), *Murraya koenigii* (Group V), combination of *Aloe vera* and *Murraya koenigii* (Group VI), combination of *Aloe vera* and Glibenclamide (Group VII), combination of *Murraya koenigii* and Glibenclamide (Group VIII) and combination of *Aloe vera*, *Murraya koenigii* and Glibenclamide (Group IX).

Diabetes was induced in rats of all the groups except normal control by intraperitoneal administration of STZ at the dose rate of 40 mg/kg.

All groups of rats were subjected to evaluation of physiological, biochemical and hematological parameters such as body weight, serum glucose, serum cholesterol, serum triglycerides, serum AST, serum ALT, insulin hormone, antioxidant enzymes, and hemoglobin. The above findings were correlated 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 treatment groups revealed an improvement in their body weight on all the intervals of the study.

There was a significant increase 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 reducing serum glucose levels. However, blood glucose lowering effect was better in combined treatment groups when compared to individual treatment groups, specially Group IX and Glibenclamide groups had better hypoglycemic effect compared to other groups.

The serum cholesterol and triglyceride levels in diabetic control rats were significantly higher as compared to normal control at all the intervals of study. Treating diabetic rats with Glibenclamide produced a significant decrease in the serum cholesterol and triglyceride levels. The various treatment groups also showed improvement in the serum cholesterol and triglyceride levels. But, Groups VII and VIII showed better

improvement comparable to Glibenclamide control indicating synergistic effect of these groups in controlling the hyperlipidemic state in induced diabetes mellitus in rats.

The serum ALT and AST values in diabetic control rats were progressively increased throughout the experiment compared to normal control. All the treatment groups (Group III to IX) showed a significant reduction in mean serum ALT and AST levels compared to diabetic control. However, the ALT and AST values in Groups V, VIII and IX showed better progress 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 (Group III to IX) showed improvement, the values were found to be lower compared to normal control and significantly higher compared to diabetic control and the increase was gradual. Among the treatment groups, Groups VI and IX showed better improvement compared to other treatment groups and Glibenclamide treatment group.

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 a significant improvement in liver antioxidant enzyme activities when compared with diabetic control animals. However, Groups V and IX showed better antioxidant activity compared to Glibenclamide control. When compared to individual treatment groups combined treatment group (Group VII, VIII and IX) animals showed better improvement.

The diabetic control rats showed a decrease in mean haemoglobin values as compared to normal control. There was improvement in Hb values in all treatment groups both individually and in combinations compared to that of Glibenclamide treated group.

Clinical signs of STZ induced diabetic rats comprised of polyurea, polydypsia, polyphagia, weight loss and poor body condition. The severity of the above signs was progressive with the advancement of time. Treatment groups (Group III to IX) showed a decrease in the severity and extent of the above-mentioned signs.

Grossly, in the diabetic control rats, pancreas was slightly congested, reduced in size and appeared as thin gelatinous strip. Similar lesions were observed grossly in treatment groups also but the extent and severity of the lesions were gradually reduced as the treatment advanced. Other organs such as liver and spleen exhibited moderate pathological lesions. The kidney, lungs, intestines, stomach and heart did not reveal any distinct gross lesions throughout the experiment.

Microscopically, in diabetic rats, both exocrine and endocrine pancreas was affected. Exocrine pancreas revealed loss of architecture with vacuolated, degenerating and necrotic cells in the acini. In endocrine pancreas, there was decrease in the number of islets, vacuolar degeneration, loss of granularity, apoptosis and necrosis of cells in a progressively increasing manner with fibrosis of few islets at the end of the study.

There was a progressive reconstruction of normal architecture of acini and islets in pancreas in all the treatment groups in the present study. Among the treatment groups, the combination groups (VI, VII, VIII and IX) revealed better improvement with islets of

larger size and more compact arrangement. Tendency to form new islets near the blood vessels was seen in many of the lobules. Many of the precursor cells were derived from the ductal epithelial cells.

Immunohistochemical demonstration of insulin showed drastic reduction in the number of insulin positive cells in the diabetic group. There was improvement in the number of insulin positive cells in various treatment groups. However, Group VIII and IX showed better regeneration of β-cell population which were comparable to the Glibenclamide control indicating the synergistic action of these groups compared to individual treatment groups of *Aloe vera* and *Murraya koenigii.*

Histopathological examination of liver in diabetic animals revealed vacuolar degeneration, increased cytoplasmic granularity, obliteration of sinusoidal space and mild infiltration of mononuclear cells. The lesions in the liver of treatment group 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 depletion of lymphocytes from the periarteriolar sheath as well as from the follicles. There was a progressive improvement in the lymphoid mass of the spleen in treatment groups. Characteristic microscopic lesions were not observed in kidney, heart, lungs, intestine and spleen in any of the control and treatment groups.

Conclusion

- Diabetes mellitus can be effectively induced by using STZ at the dose rate of 40 mg/kg body weight intraperitonially in laboratory rats.
- Both *Aloe vera* and *Murraya koenigii* aqueous extracts exhibited potent antidiabetic effect comparable to Glibenclamide in streptozotocin induced diabetic rats.
- The combination of *Aloe vera* & Glibenclamide (Group VII) and *Murraya koenigii* & Glibenclamide (Group VIII) were found to be more effective in alleviating the diabetic effect when compared to combination of *Aloe vera* with *Murraya koenigii* (Group VI).
- Synergistic effect was noticed when herbal and allopathic drug was used in combination.
- Combination of *Aloe vera*, *Murraya koenigii* and Glibenclamide (Group IX) showed better synergetic action compared to other combination.
- Both *Aloe vera* and *Murraya koenigii* can be used as potential therapeutic agents for managing diabetes and can be used in combination with Glibenclamide so as to minimize secondary complication of using allopathic drug for longer period.

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Abstract

VIII. ABSTRACT

The present study was undertaken to evaluate the antidiabetic efficacy of *Aloe vera* and *Murraya koenigii* individually and in combination with Glibenclamide in streptozotocin induced diabetic rats for a period of 45 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 *Aloe vera* (Group IV), *Murraya koenigii* (Group V), combination of *Aloe vera* and *Murraya koenigii* (Group VI), combination of *Aloe vera* and Glibenclamide (Group VII), combination of *Murraya koenigii* and Glibenclamide (Group VIII) and combination of *Aloe vera*, *Murraya koenigii* and Glibenclamide (Group IX). The phytochemical analysis of aqueous extract of *Aloe vera* and *Murraya koenigii* revealed several bioactive compounds, which were helpful in reverting hyperglycemia and hyperlipidemia near to normalcy and were helpful in bringing down the diabetic complications. There was significant variation in biochemical and pathomorphological parameters (Antioxidant assay, Immunoradiometric assay, Histopathology and Immunohistochemistry) in diabetic rats when compared to normal control rats. The alleviation of the diabetes and its complications induced by streptozotocin was observed in all the treatment groups with variable degrees of improvement. *Aloe vera* and *Murraya koenigii* extracts were effective in alleviating streptozotocin induced diabetes and were comparable with Glibenclamide. Combination of *Aloe vera* with Glibenclamide and *Murraya koenigii* with glibenclamide showed better improvement compared to individual extracts alone. Synergistic effect was observed in combination of *Aloe vera* and *Murraya koenigii* with Glibenclamide and in combination of *Aloe vera* and *Murraya koenigii* along with Glibenclamide.