STUDIES ON INDUCED DIABETES IN RATS WITH SPECIAL EMPHASIS ON COMBINED EFFICACY OF *GYMNEMA SYLVESTRE* AND *SWERTIA CHIRAYITA* EXTRACTS

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON INDUCED DIABETES IN RATS WITH SPECIAL EMPHASIS ON COMBINED EFFICACY OF *GYMNEMA SYLVESTRE* AND *SWERTIA CHIRAYITA* EXTRACTS"** submitted by **Mr. BHARATH KUMAR, K.,** ID No. **MHVK 1707** in partial fulfillment of the requirements for the award of **MASTER OF VETERINARY SCIENCE** 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 for the award of any degree, diploma, associate ship, fellowship or any other similar titles.

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Affectionately Dedicated to

My Parents &

My Guide

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LIST OF ABBREVIATIONS

%	Per cent
µ	Micron
µl	Microlitre
^{0}C	Degree Celsius
ADP	Adenosine diphosphate
ALT	Alanine amino transferase
APES	3-aminopropyl triethoxy-silane
AST	Aspartate amino transferase
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
bw	Body weight
CAT	Catalase
CCl_4	Carbon tetrachloride
DAB	3,3-diamine benzidine tetrahydrochloride
DC	Diabetic control
dL	Deciliter
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DPPH	α- α diphenyl β picryl hydrazyl
EDTA	Ethylene diamino tetra acetic acid
G	Glibenclamide
g	Gram
g%	Gram percentage
GLUT – 1	Glucose transporter – 1
GLUT – 2	Glucose transporter – 2

GPx	Glutathione peroxidase
GS	*Gymnema sylvestre*
GSH	Glutathione
h	Hour
H_2O_2	Hydrogen peroxide
Hb	Haemoglobin
HCl	Hydrochloric acid
HRPO	Horse raddish peroxidase
IAEC	Institutional Animal Ethics Committee
IDDM	Insulin dependent diabetes mellitus
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IU/L	International units per liter
IUPAC	International Union for Physical and Analytical Chemists
Kg	Kilogram
KOH	Potassium hydroxide
L	Liter
M	Molar
mg	Miligram
mg/dL	Milligram per deciliter
mL	Mililitre
mM	Milimolar
MW	Molecular weight
Na_2CO_3	Sodium carbonate
NAD	Nicotinamide adenine dinucleotide
NBF	Neutral buffered formalin

NC	Normal control
NIDDM	Non insulin dependant diabetes mellitus
NO	Nitric oxide
OD	Optical density
PARP	Poly ADP-ribose polymerase
PBS	Phosphate buffer saline
PCV	Packed cell volume
RBC	Red blood cell
ROS	Reactive oxygen species
rpm	Revolutions per minute
SC	*Swertia chirayita*
SE	Standard error
SOD	Superoxide dismutase
STZ	Streptozotocin
TEC	Total erythrocyte count
TLC	Total leucocyte count
TNF-α	Tumor necrosis factor alpha
UV	Ultraviolet
WBC	White Blood Cell
WHO	World Health Organisation
α	Alpha
β	Beta
μg/kg	Micro grams per kilogram
μU/ml	Micro units per millilitre

Introduction

I. INTRODUCTION

Diabetes mellitus is a complex and chronic metabolic disease which affects both animals and humans worldwide. Diabetes mellitus is characterized by a relative or absolute lack of insulin resulting in hyperglycemia. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of different organs, especially the eyes, kidney, nerve, heart and blood vessels.

The two types of diabetes reported include type I diabetes which is also called as insulin dependent diabetes mellitus (IDDM) or juvenile onset diabetes that occurs mainly due to autoimmune destruction of the pancreatic β cells leading to lack of insulin production and type II diabetes, also known as non insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes caused by development of insulin resistance coupled by failure of pancreatic β cells to compensate (American Diabetes Association, 2014).

Insulin dependent diabetes mellitus (IDDM) accounts for only 5-10% of cases among humans whereas non insulin dependent diabetes mellitus (NIDDM) has 90-95% of occurrence suggesting highest prevalence of type II diabetes. As high as 352 million people are at the risk of developing type II diabetes currently (International Diabetes Federation, 2017), mainly due to the change in lifestyle of people, obesity and stress (American Diabetes Association, 2014).

Diabetes is one of the leading causes of death in the world, raising more rapidly especially in the middle and low income countries. According to International Diabetes Federation 2017, diabetes prevalence is presumed to rise from 425 million in 2017 to 629 million by 2045. There were over 72 million cases of diabetes recorded in India in 2017 which is expected to double by 2045. Among the high income countries, 79% of global healthcare expenditure is being spent on diabetes alone, indicating that management of the diabetes is of huge economical importance (International Diabetes Federation, 2017).

Diabetes mellitus is common and most frequently diagnosed endocrine disorder of dogs and cats. Similar to humans, different types of diabetes have been identified in these animals also. Its incidence is increasing possibly due to sharing of common environment of humans with an increase in predisposing factors such as obesity, age, physical inactivity and other hormonal disorders in both the species (Hoenig, 2002).

Type I diabetes mellitus is more commonly reported in dogs, whereas, cats frequently suffer from type II diabetes. Rarely diabetes also occurs in horses, cattle and sheep. Isolated cases of diabetes are reported in monkeys, mules, ferrets, pigs, buffaloes, pigs and birds (Anderson and Low, 1990).

Conventionally, insulin-dependent diabetes mellitus is treated with exogenous insulin (Felig *et al*., 1995) and non insulin-dependent diabetes mellitus with synthetic oral hypoglycemic agents like sulphonylureas and biguanides (Rosac, 2002). However, supplementation of insulin is reported to have failed as a curative agent for complications that arise with chronic diabetes (Mukherjee and Mukherjee, 1966) and synthetic oral drugs over a period of time produce adverse health effects (Raheja, 1977).

Ancient Indian medicinal systems like Ayurveda have described many medicinal plants possessing potent hypoglycemic activity. The extracts of these herbs are used in the treatment of diabetes for lowering blood sugar level. Many useful herbs introduced into pharmacological and clinical trials have confirmed their hypoglycemic effect and repair of ß-cells of islets of Langerhans (Gupta *et al*., 2008). There is a need to introduce traditional medicine in treatment and management of diabetes mellitus to reduce the side effects and cost of conventional treatment. Among the various plants, *Gymnema sylvestre* and *Swertia chirayita* also have shown to possess potent antidiabetic property.

Gymnema sylvestre, commonly known as Gurmur, grows in the tropical regions of India and possesses potent antibiotic and antioxidant property (Pragathi *et al*., 2015). Similarly, *Swertia chirayita*, commonly called as Nelabevu is a herb grown abundantly in the Himalayan regions of India and possesses potent blood glucose lowering property and antidiabetic activity (Kavitha and Dattatri, 2013) which is also used for other aliments by tribes (Gupta *et al*., 2008). Both *Gymnema sylvestre* and *Swertia chirayita* have proven antidiabetic activity, but antidiabetic effect in combination of these two herbs has not been explored. Hence, the present study was taken up to study the combined antidiabetic efficacy of these two herbs in streptozotocin induced diabetes in Wistar albino rats. An attempt was also made to see the possibility of partial / complete replacement of oral hypoglycemic drugs like glibenclamide with these extracts for the control of diabetes mellitus.

With this background the present study was taken up with the following objectives:

1. To study pathomorphological and biochemical changes in streptozotocin induced diabetic rats.

2. To evaluate the antidiabetic effect of *Gymnema sylvestre* and *Swertia chirayita* in combination in diabetes induced rats.

3. To compare the hypoglycemic effects of *Gymnema sylvestre* and *Swertia chirayita* with an oral hypoglycemic agent glibenclamide.

Review of Literature

II. REVIEW OF LITERATURE

The available literature relevant to the present study is reviewed as here under.

2.1 Diabetes mellitus

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from the defects in insulin secretion, insulin action or both leading to disturbances in carbohydrate, fat and protein metabolism. The chronic hyperglycemia of diabetes is associated with the long term damage, dysfunction, and failure of different organs (American Diabetes Association, 2014).

Diabetes mellitus is classified mainly into two etiopathogenetic categories; type I diabetes mellitus, caused by an absolute deficiency of insulin secretion due to autoimmune destruction of pancreatic β cells and Type II diabetes mellitus caused by a combination of resistance to insulin action and inadequate compensatory insulin secretory response due to many etiological factors like obesity, physical inactivity and stress. Many other types of diabetes mellitus have also been recognized; mainly gestational diabetes, diabetes due to genetic defects in the β cells, disease of exocrine pancreas, endocrinopathies, overdose of chemicals, infections and other genetic syndromes (American Diabetes Association, 2014).

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, polyphagia and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome (Kavitha Rani, 2015).

Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations and charcot joints and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction (American Diabetes Association, 2014).

History of identification of diabetes goes back to 1000 BC by the Ayurvedic physician Sushruta in his book called *Sushruta Samhita* in which, diabetes has been described to be of two types based on clinical entities that correspond to modern classification as insulin-dependent diabetes mellitus (IDDM) and non insulin-dependent diabetes mellitus (NIDDM). During his time, diabetes was diagnosed by tasting the patient's urine for sweetness as well as other symptoms observable upon examination (Oubre *et al.,* 1997).

In ancient India, medicinal plants have been used as natural medicine since the days of Vedic glory. Many of these medicinal plants and herbs are part of our diet as spices, vegetables and fruits. Description of medicinal plants has been shown under a separate chapter in 'Ayurveda'. Sushruta and Charak compiled and classified the different herbs based on their remedial properties in their renowned treatises like 'Sushruta Samhita' and 'Charaka Samhita'. Since a long time many herbs are used in the treatment of diabetes and other complications (Gupta *et al*., 2008).

Prevalence studies on diabetes in humans worldwide have estimated that 425 million people around the world were suffering from the diabetes in 2017 and presumed to increase to 629 million by 2045. In India alone, 72 million people are suffering from diabetes. Four out of five people with diabetes live in low and middle income countries and diabetes is one of the leading causes of death in the world. Among high income countries, 79% of global healthcare expenditure is spent on diabetes (International Diabetes Federation, 2017).

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

Diabetes mellitus is one of the most frequently diagnosed endocrinopathies in cats and dogs also. The incidence of diabetes in cats ranges from 1:50 to 1:400 of population (Baral *et al.,* 2003) and it is increasing due to an increase in the frequency of predisposing factors such as obesity and physical inactivity, probably due to urbanization and indoor confinement of pets. The prevalence of the disease is 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 most common form in cats (Rand *et al.,* 2004).

2.2 Induction of diabetes

Diabetes mellitus can be induced in experimental animals by many methods, which are different for different types of diabetes. In addition to laboratory animals dog and cats also are used as diabetic animal models (King, 2012).

Type I diabetes which occurs due to damage to pancreatic beta cells, could be studied in spontaneous auto immune models, genetically induced insulin dependent models and virus induced diabetic models and also could be induced through chemical administration. For Type II diabetes, obese animal models are commonly used. Other than this high fat feeding model, non obese model of type II diabetes are also used (King, 2012).

Chemical induced type I diabetes model is through administration of a toxic chemical which leads to high percentage destruction of the beta cells and thus reduced endogenous insulin production leading to hyperglycemia and weight loss. It is relatively simple and cheap induced model of diabetes in rodents.

Streptozotocin and alloxan are the two major chemicals which are used to induce diabetes in lab animals. Both the drugs are toxic to other organs of the body also and alloxan even at light overdosing can cause general toxicity (Szkudelski, 2001).

2.2.1 Streptozotocin

Streptozotocin (STZ) is a naturally occurring compound produced by the bacterium *Streptomyces achromogenes* that exhibits broad spectrum antibacterial properties (Vavra *et al.*, 1959). The IUPAC name for streptozotocin is 2-deoxy-2-({[methyl(nitroso) amino]carbonyl}amino)-β-D-glucopyranose (Kavitha Rani, 2015).

Structurally, streptozotocin (STZ) is a N- nitrosourea derivative of D- glucosamine. It is a broad spectrum antibiotic and alkylating genotoxic agent which possesses antibacterial, tumoricidal, carcinogenic and diabetogenic properties (Lewis and Barbier, 1959).

Streptozotocin selectively accumulates in the pancreatic β cell via the low affinity GLUT 2 glucose transporter in the plasma membrane (Karunanayake *et al.*, 1976 and

Schnedl *et al.,* 1994*).* Streptozotocin acts on mitochondria, where the mitochondrial ATP generation is inhibited resulting in high concentration of intracellular ADP. The degradation of ADP generates hypoxanthine, a substrate of xanthine oxidase (XOD) whose activity is intrinsically very high in β cells. The XOD catalyzing reaction results in increased formation of uric acid and O_2 radicals that cause damage to β cells. STZ also can directly activate XOD and enhance O_2 generation resulting in O_2 radicals or hydroxyl radicals induced injury to β cells (Kawada, 1992 and Bedoya *et al.,* 1996).

Experiments also have proved that STZ induced β cell death can occur due the alkylation of DNA (Delaney *et al.,* 1995 and Elsner *et al.,* 2000). Intracellular action of STZ results in changes in DNA of pancreatic β cell by fragmentation (Yamamoto *et al.*, 1981 and Morgan *et al.,* 1994).

Streptozotocin has potent alkylating properties which is the main reason for its toxicity. However, the synergistic action of both NO and reactive oxygen species may also contribute to DNA fragmentation. DNA damage further induces activation of poly adenine diphosphate ribosylation, leading to depletion of NAD and ATP which is more important for induction of diabetes (Szkudelski, 2001).

Streptozotocin enters the β cell *via* a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, beta cells undergo destruction by necrosis (Szkudelski, 2001).

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

Raza and John (2012) stated that STZ – induced cytotoxicity in HepG2 cells is mainly mediated by increase in ROS/RNS production, oxidative stress and mitochondrial dysfunction.

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).

The study conducted by Al-Nahdi *et al*. (2019) to investigate the molecular mechanism of streptozotocin (STZ) on pancreatic beta cells, using Rin-5F cells treated with STZ revealed increased oxidative stress with increased DNA fragmentation and oxidative protein carbonylation, and the same cells treated with high glucose enhanced the oxidative stress and showed increased apoptosis and altered redox homeostasis leading to increased cytotoxicity.

2.2.2 Induction of diabetes by streptozotocin (STZ)

Karunanayake *et al.* (1975) in a study on metabolic fate and elimination of STZ in adult male Wistar albino rats, observed that STZ caused destruction of β cells of islets of Langerhans when given intravenously at 60 mg/kg in about three days.

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

Weir *et al.* (1981) showed that streptozotocin could induce diabetes mellitus in many animal models and the induced diabetes resembled human hyperglycaemic nonketotic diabetes mellitus in all aspects.

Streptozotocin action on β cells was accompanied by characteristic alterations in blood insulin and glucose concentrations. Two hours after injection, the hyperglycemia was observed with a concomitant drop in blood insulin. About six hours later, hypoglycemia occured with high levels of blood insulin. Finally, hyperglycemia developed and blood insulin levels decreased (West *et al*., 1996).

Study of type I diabetes conducted by Li *et al.* (2001) with multiple low doses of STZ administration in mice resulted in hyperglycemia from 7^{th} day onwards and coincided with occurrence of insulitis and reduction in islet cell area morphometrically. In addition, they documented a 2-3 fold increase in the number of alpha cells with a significant reduction and disappearance of β cells.

Diabetes induced in the male adult Wistar albino rats weighing around 250-300 grams using streptozotocin at 60 mg/kg bw intravenously, resulted in increase in the blood glucose level, consumption of water and volume of urine excreted and decrease in body weight, serum insulin and C-peptide and enlargement of pancreas initially followed by degeneration of β cells in the islets of Langerhans (Akbarzadeh *et al.*, 2007).

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

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

Adeghate *et al*. (2010) reported that diabetes could be induced in the male Wistar rats by a single intra peritoneal injection of streptozotocin at the dose rate of 60 mg/kg bw. The number of insulin-positive cells decreased significantly ($p < 0.05$) at 24 hrs after STZ treatment with a concomitant increase in the number of glucagon-immunoreactive cells.

Electron microscopy showed coalescing of beta cell granules 18 hrs after STZ treatment and a near to complete degranulation of beta cells at 21 h after STZ administration.

Zafar *et al.* (2009) studied the relationship and effects of diabetes on liver morphology, architecture and function by using streptozotocin induced diabetic rats as an experimental model. Histopathological examination of liver showed accumulation of lipid droplets, lymphocytic infiltration, increased fibrous content, dilatation and congestion of portal vessels and proliferation of bile ducts along with increased levels of AST, ALT, ALP and PChE.

Cennet and Ebubekir (2010), in an investigation on streptozotocin induced diabetes in Sprague Dawley albino rats observed increased levels of serum glucose, ALT and AST with decreased cholesterol level after 6 h of induction.

Streptozotocin readily induced significant decrease in body weight gain, increased serum glucose level and liver enzyme levels, decreased antioxidant enzyme levels and altered morphological architecture of pancreatic islets in rats when used at 45 mg/kg (Dhanush, 2009; Pragathi, 2011; Mallikarjuna *et al.*, 2013; Manjunatha, 2013; Mudasir *et al.*, 2013; Shesha Rao, 2013; Gurikar, 2014; Rani *et al*., 2016 and Parasuraman *et al*., 2019).

Yaman *et al*. (2017) reported that streptozotocin at the dose rate of 45 mg/kg bw, single intra peritoneal injection can effectively induce diabetes in Wistar albino rats. They observed degenerative changes in beta-cells which caused decrease in the number of functioning beta-cells and insulin immunoreactivity in the pancreas. *Heracleum persicum* (HP) treatment improved the number of immunoreactive β cells significantly.

Hsiao *et al.* (2019) induced diabetes in the male Wistar albino rats by single intra peritoneal injection of streptozotocin at the dose rate of 50 mg/kg bw, and on treatment with low-energy shock wave therapy weekly for ten consecutive weeks observed significant reduction in blood glucose, HbA1c, and urine volume and improved pancreatic islets area and insulin production in the rats.

2.3 Treatment substances

2.3.1 Glibenclamide

Present therapies for diabetes include many oral hypoglycemic agents as primary alternative treatment of type II DM. The glucose lowering drugs include insulin secretagogues (sulfonylureas, meglitinides), insulin sensitizers (biguanides, metformin, thiazolidinediones), α- glucosidase inhibitors (miglitol, acarbose). The aim of these oral hypoglycaemic agents is to ameliorate the underlying metabolic disorder, related to inadequate insulin resistance, insulin secretion, and to augment hepatic gluconeogenesis (Sharma and Gupta, 2017).

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

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

Study conducted by Guiot *et al.* (1994) in mice to record the effect of glibenclamide on pancreatic β cell proliferation showed that glibenclamide at 2 mg/kg bw when given intraperitonially for 7-30 days caused degranulation of β-cells, increased β-cell mass and concluded that glibenclamide has a β-cytotropic effect.

Glibenclamide initiates its action by binding with its receptors on the β-cell surface and subsequently causes a decrease in the conductance of ATP-sensitive K^+ channels which results in more of K^+ efflux and calcium influx in β-cells which eventually determines insulin secretion (Luzi and Pozza,1997). They further stated that the sulfonylurea drugs in general are completely absorbed from intestine but there can be a delay in their absorption as it depends on level of hyperglycemia. Similar observation was also made by Serrano-Martin *et al.*, (2006).

Zhang and David (2001) in an experiment showed that the hypoglycaemic effect of glibenclamide impaired the recovery of glucose from insulin induced hypoglycemia and significantly reduced blood glucose levels in normal rats. They noticed that hypothalamic ATP sensitive K^+ channels play significant regulatory role in peripheral glucose homeostasis.

Elmali *et al.* (2004) conducted a study to see the effect of the glibenclamide at the dose rate of 5 mg/kg bw on the antioxidant status of kidney and liver in streptozotocin induced diabetic rats and observed restoration of antioxidant enzyme levels in the liver in addition to glucose lowering effect.

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

Wang *et al.* (2008) hypothesized that prolonged exposure of rat pancreatic β-cells to the insulin secretagogue glibenclamide induces a sustained increase in basal insulin synthesis and proved the same through *in-vitro* experimentation with cultured rat β-cells. They concluded from their results that the prolonged exposure to glibenclamide causes activation of protein translation in pancreatic β-cells through the calcium regulated mTOR, PKA and MEK signaling pathways.

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

Streptozotocin induced diabetes in rats treated with glibenclamide was observed to decrease hyperglycemia, serum cholesterol, triglyceride and very low density lipoprotein and improve β cell activity (Pragathi, 2011; Mudasir *et al.*, 2013; Mallikarjuna *et al.*, 2013; Manjunatha, 2013; Shesha Rao, 2013; Gurikar, 2014 and Parasuraman *et al*., 2019).

Kavitha Rani. (2015) observed that glibenclamide treated diabetic rats at the dose rate 600 µg/kg bw showed significant improvement in architecture of islet of Langerhans and liver morphology.

Zhou *et al.* (2019) stated that glibenclamide promotes insulin release and further activates autophagy through the adenosine 5′- monophosphate (AMP) activated protein kinase (AMPK) pathway in MIN-6 cells. Inhibition of autophagy with autophagy inhibitor 3-methyladenine (3-MA) potentiated insulin secretion level in MIN-6 cell, and increased the secretory function of glibenclamide.

2.3.2 Herbs in diabetes treatment

The World Health Organization (WHO) has listed 21,000 plants which are used for medicinal purpose around the world. These include 2500 plant species in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called the ‘botanical garden of the world’ (Seth and Sharma, 2004).

Indian literature like Ayurveda have mentioned about herbal remediation for a number of human ailments. Among Indian traditional medicinal plants several potential anti-diabetic plants and herbs are being used as part of diet since prehistoric time. These herbs have confirmed blood sugar lowering property and cure diabetes and its complications (Gupta *et al*., 2008).

Many useful herbs introduced in pharmacological and clinical trials have confirmed their blood sugar lowering effect and repair of ß-cells of islets of Langerhans (Gupta *et al*., 2008) and these herbal drugs are non toxic, relatively cheaper and popular compared to oral hypoglycemic drugs due to their cost and associated side effects (Momin, 1987).

2.3.2.1 *Gymnema sylvestre*

Gymnema sylvestre is a perennial woody climber plant belonging to Asclepiadeceae family, grown in tropical regions of India like forests of Central India and the Western Ghats and used as household remedy for diabetes. The plant is known as *Periploca of the woods* in English; *Meshashringi* and *Sarpadarushtrika* in Sanskrit and *Gurmar* in Hindi (Persaud *et al*., 1999 and Kar *et al.*, 2003).

Gymnema sylvestre is taxonomically classified into Kingdom Plantae, under Angiosperms - Eudicots - Asterids, Order: Gentianales, Family: Asclepiadaceae, Genus: *Gymnema*, Species: *G. sylvestre,* with a binomial nomenclature *Gymnema sylvestre* R. Br. (Anon, 2019).

A study conducted by Variyar and Gupta (1964) to evaluate the effect of parenteral administration of the alcoholic extract of leaves of *Gymnema sylvestre* on hypoglycaemic response of the diabetogenic hormones, somatotropin and corticotropin in albino rats showed that the somatotropin and corticotropin induced hyperglycemia was markedly inhibited by the extract when given at a dose of 200 mg/kg bw intramuscularly.

Shanmugasundaram *et al.* (1988) evaluated the effect of *Gymnema sylvestre*, for the control of type I diabetes induced by streptozotocin in experimental animals. The study showed the hypoglycaemic effect of *Gymnema sylvestre* with attainment of blood glucose homeostasis and increased serum insulin level, in addition to restoration of islets of Langerhans by the herbal extract.

Baskaran *et al*. (1990) documented that human patients with Type II diabetes mellitus supplemented with the *Gymnema sylvestre* herb leaf extract at 400 μg/day showed significant reduction in blood glucose, glycosylated haemoglobin, glycosylated plasma proteins and increased serum insulin level.

Okabayashi *et al.* (1990) studied the effect of *Gymnema sylvestre* on glucose homeostasis by experimental induction of hyperglycemia in rats with administration of streptozotocin and supplemented *Gymnema sylvestre* through diet for 35 days. They observed reduction in serum glucose level, an improved glucose tolerance, gain in body weight, food intake and increase in pancreas weight in *Gymnema sylvestre* treated rats after 4 weeks of supplementation.

Water soluble extract of leaves of *Gymnema sylvestre* supplemented to the streptozotocin induced diabetic rats showed control in the blood glucose homeostasis and increased serum insulin level besides doubling the islet number and β cell number in pancreas of the treatment group (Shanmugasundaram *et al.*, 1990).

Sahu *et al*. (1996) stated that gymnemic acid was the main phytochemical in *Gymnema sylvestre* which possesses hypoglycemic activity by occupying the receptor locations in the absorptive external layer of the intestines thereby preventing the absorption of sugar molecules, which results in lowered blood sugar level.

Wang *et al.* (1998) in a study on the inhibitory effect of gymnemic acid on intestinal absorption of oleic acid in rats observed that it potentially inhibited the absorption of oleic acid in intestine which was dose dependent and reversible. They also observed that the extent of inhibition and the recovery progress were extremely similar to that of glucose absorption.

Persaud *et al.* (1999) evaluated the effects of alcoholic extracts of *Gymnema sylvestre* on insulin secretion from islets of Langerhans of rats and several pancreatic β cell lines and concluded that *Gymnema sylvestre* stimulates insulin release by increased membrane permeability rather than exocytosis by regulated pathways. The herbal alcoholic extract was able to enhance insulin release from β cells and the islets of Langerhans.

Sugihara *et al.* (2000) investigated antihyperglycemic action of a crude saponin fraction and five triterpene glycosides derived from the methanol extract of leaves of *Gymnema sylvestre* in STZ diabetic mice and reported that gymnemic acid IV, isolated from the leaves of *Gymnema sylvestre* produced potent hypoglycaemic, antihyperglycemic, glucose uptake inhibitory and gut glycosidase inhibitory effects in STZ induced diabetic mice.

Shigematsu *et al.* (2001) reported that when *Gymnema sylvestre* extract was orally administered once a day to rats fed with high or normal fat diet for 3 weeks, it improved serum cholesterol and triglyceride levels through its effect on the lipid metabolism.

Kanetkar *et al*. (2007) stated that hypoglycemic effect of gymnemic acids from *Gymnema sylvestre* could be due to increase in secretion of insulin, or promotion of regeneration of islet cells, or increased utilization of glucose or inhibition of glucose absorption from intestine.

Sathya *et al.* (2008) reported that administration of *Gymnema sylvestre* to alloxan induced diabetic rats resulted in decrease in the glucose, urea, uric acid and creatinine levels with an increase in liver glycogen, serum and tissue protein concentration.

Mall *et al*. (2009) reported that aqueous extract of *Gymnema sylvestre* given to alloxan induced diabetic rats caused decrease in the fasting blood glucose, cholesterol and serum triglyceride content and elevated serum HDL and cholesterol, which indicated that *Gymnema sylvestre* had significant antidiabetic activity and hypolipidemic activity.

Ahmed *et al.* (2010) stated that gymnemic acid of *Gymnema sylvestre* leaf extract, delayed the absorption of glucose in the blood, as it competes with glucose for binding to insulin receptors by virtue of its atomic configuration which is similar to glucose. This action was found to inhibit the craving for sugars and also delayed the absorption from the intestines. Hence, the leaves have been shown to be effective in management of type II diabetes mellitus by increasing the secretion of insulin, promoting regeneration of islet cells, increasing utilization of glucose by enhancing the enzymes required for uptake of glucose, increasing the phosphorylase activity, decreasing gluconeogenic enzymatic activity and by inhibiting glucose absorption from intestine.

Vijayanand and Satish (2012) stated that *Gymnema sylvestre* possess hypoglycemic and hypolipidemic property and was capable of regenerating β-cells and hence could be used as a drug for treating diabetes mellitus.

Mallikarjuna *et al.* (2013) conducted a study using a hydroalcoholic extract of *Gymnema sylvestre* leaves on streptozotocin induced diabetic rats at the dose rate of 100 mg/kg bw and observed significant reduction in the serum levels of glucose, cholesterol, triglycerides, ALT and AST and with significant improvement in the serum insulin levels. They also stated that *Gymnema sylvestre* alleviated the damage caused by STZ morphologically in the beta cells of islets of Langerhans and hepatocytes.

Gymnema sylvestre (Asclepiadaceae) is popularly known as “gurmar” for its distinct property as sugar destroyer. The phytoconstituents responsible for sweet suppression activity includes triterpene saponins known as gymnemic acids, gymnemasaponins and a polypeptide, gurmarin. The herb exhibits a broad range of therapeutic effects as an effective natural remedy for diabetes, besides being used for arthritis, anemia, osteoporosis, hypercholesterolemia, cardiopathy, asthma, constipation, microbial infections, indigestion and as an anti-inflammatory and diuretic. *Gymnema sylvestre* has good prospects in the treatment of diabetes as it shows positive effects on blood sugar homeostasis, controls sugar cravings and promotes regeneration of pancreas and reduces blood cholesterol and triglyceride levels (Tiwari *et al*., 2014).

Study conducted by Suman *et al*. (2015) showed that the extract of *Gymnema sylvestre* leaves @ 400 mg/kg bw in streptozotocin induced diabetic rats significantly decreased the blood glucose and glycosylated haemoglobin levels besides having cardioprotective and hepatoprotective effects as indicated by significant reduction in the CPK-MB and SGPT levels.

Pragathi *et al*. (2015) in their study on the hypoglycaemic effect of *Gymnema sylvestre* in streptozotocin induced diabetic rats, observed significant difference in body weight, hemoglobin, serum glucose, cholesterol, triglycerides, AST, ALT and antioxidant enzymes in diabetic rats as compared to normal rats. *Gymnema sylvestre* in aqueous solution possessed dose dependent effect and dosage of 100 mg/kg bw was more effective and alleviated STZ induced diabetic effects, normalized the microscopic architecture of pancreas, improved the number of β cells and enhanced the insulin secretion by the β cells.

Rani *et al.* (2016) in their study compared the efficacy of *Gymnema sylvestre* leaf extract and/or trivalent chromium by oral supplementation in reducing the oxidative stress encountered in streptozotocin induced diabetes mellitus in Wistar albino rats and observed that the levels of hepatic antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase progressively increased in the group treated with *Gymnema sylvestre* and in combination with chromium than diabetic group rats.

Kumar *et al.* (2017) reported that methanolic leaf extract of *Gymnema sylvestre* and *Andrographis paniculata* showed antidiabetic and antioxidant effect from 1st week of administration itself at the dose rate of 30 mg/kg bw and 50 mg/kg bw respectively in streptozotocin induced diabetes in Sprague Dawly rats.

Laha and Paul (2019) stated that antidiabetic activity along with antioxidant potential of *Gymnema sylvestre* is due to the presence of flavonoids, phenols and various bioactive compounds like gymnemic acid, gymnemarerin and other secondary metabolites like saponins, tanins (phenolic compounds) and triterpenoids that are antioxidants but show antidiabetic activity also.

2.3.2.2 *Swertia chirayita*

Swertia, a genus in the family Gentianaceae include a large group of annual and perennial herbs, representing approximately 135 species. In India, 40 species of *Swertia* are recorded (Kirtikar and Basu, 1993), of which *Swertia chirayita* is considered the most important for its medicinal properties like bitter stomachic, febrifuge, antidiabetic, anthelmintic, antimalarial and antidiarrheal actions. *Swertia chirayita* was first described by Roxburgh under the name of *Gentiana chyrayta* in 1814 (Scartezzini and Speroni, 2000).

Swertia chirayita is a critically endangered medicinal herb that grows at high altitudes in the sub temperate regions of the Himalaya mainly from Kashmir to Bhutan. It is commonly called Chiretta, Bhunimba, Kairata and Bitter stick (Joshi and Dhawan, 2005).

Botanical classification of this genus is as follows: family: Gentianaceae, tribe: Gentianeae, subtribe: Swertiinae, genus: *Swertia*, species: *chirayita*. Binomial nomenclature: *Swertia chirayita* (Chopra *et al.,* 1956).

Mukerjee and Mukerjee (1987) reported that significant blood sugar lowering effect was observed when 95% ethanol extract of *Swertia chirayita* (Buch-Ham) was fed to healthy albino rats consisting of fed, fasted and glucose loaded models.

Sekar *et al.* (1987) tested blood sugar lowering activity in rats with 95 % ethanol extract and four fractions of *Swertia chirayita.* They found that the hexane fraction caused maximum lowering than the ethanol extract in fed, glucose-loaded and tolbutamide-pretreated animal models, but not in fasted rats.

Hexane fraction of the *Swertia chirayita* given at the dose rate of 250 mg/kg bw for 28 days lowered blood sugar of albino rats with increased glycogen content of liver and insulin release from pancreatic β cells (Chandrasekar *et al.*, 1990).

Saxena *et al.* (1991) studied the effect of swerchirin (1:8 dihydroxy 3:5 dimethoxy xanthone) isolated from hexane fraction of *Swertia chirayita* on the blood sugar level of healthy and streptozotocin treated rats at 35 and 65 mg/kg bw. They found significant hypoglycemic activity in normal and STZ diabetic rats treated at 35 mg/kg bw but not in streptozotocin diabetic rats treated at 65 mg/kg bw.

Bajpai *et al.* (1991) reported that a xanthone was isolated from the hexane fraction of the *Swertia chirayita* plant and identified as 1,8-dihydroxy-3,5-dimethoxyxanthone (swerchirin). They observed that it had a very significant blood sugar lowering effect in fasted, fed, glucose loaded and tolbutamide pretreated albino rat models.

Saxena *et al.* (1993) investigated the mechanism of blood sugar lowering by swerchirin (SWI) isolated from the hexane fraction of *Swertia chirayita*. They found that single oral administration of SWI (50 mg/kg bw) induced about 60 % fall in blood glucose by 7 h post-treatment. This was also associated with marked depletion of aldehyde-fuchsin stained beta-granules and immunostained insulin in the pancreatic islets.

The mechanism of dose-dependent hypoglycemic effect, the margin of safety and ED50 of three structurally unrelated compounds, tolbutamide (TB), centpiperalone (CP) and a swerchirin containing fraction (SWI) from the plant *Swertia chirayita* were investigated in experimental models by Saxena *et al.* (1996). The per cent blood sugar lowering, increase in immuno reactive insulin levels and beta cell degranulation were highest in CP treated normal rats and they concluded that CP was more effective than swerchirin ($P < 0.01$) and TB. Swerchirin was found to be superior blood glucose lowering agent than tolbutamide.

Kar *et al.* (2003) conducted a comparative study on 30 hypoglycemic medicinal plants and found that alcoholic extract of *Swertia chirayita* at 250 mg/kg bw once daily for two weeks exhibited hypoglycemic activity in alloxan induced diabetic rats.

The phytochemical investigation of the genus *Swertia* detected some 200 compounds with varying structural patterns. Among these constituents xanthonoids, terpenoids, flavonoids and alkaloids formed the major classes along with other compounds (Brahmachari *et al.*, 2004).

Saha *et al.* (2004) studied anticarcinogenic activity of *Swertia chirayita* by analyzing detoxification enzymes GST, GPx, SOD and CAT. They found that these enzymes were activated at different degrees following treatment with infusion crude extract and a purified amarogentin rich extract of *Swertia chirayita*.

Suryawanshi *et al.* (2006) investigated the phytochemical constituents with antidiabetic activities in methanolic extract of *Swertia chirayita* by high performance liquid chromatography/electrospray ionization tandem mass spectrometry. They isolated xanthone and secoiridoid glycosides consisting of mangiferin, amarogentin, amaroswerin, sweroside and swertiamarin.

Saxena *et al*. (2007) evaluated the blood sugar lowering activity of 95% ethanolic extract of *Swertia chirayita* in fasted, fed, glucose loaded and diabetic models using male albino rats by giving single dose of extract (250 mg/kg bw) orally, administered with 2% acacia suspension and observed a significant blood sugar lowering effect in all the models.

Phytochemical analysis was conducted by Phoboo *et al.* (2010) in crude and ethanolic extracts of different parts of *Swertia chirayita* (root, shoot and inflorescence with leaf). Three main phytochemicals mangiferin, amarogentin and swertiamarin were identified by HPLC in crude and ethanolic extracts of all plant parts and possessed anti-diabetic, anti-pyretic, anti-malarial, anti-inflammatory properties.

Study conducted by Chen *et al.* (2011) investigated the antioxidant activity of *Swertia chirayita* in *in vitro* and *in vivo* models and found that ethanolic extract of *Swertia chirayita* possessed antioxidant effects and increased the SOD, CAT, GSH levels.

Renu *et al*. (2011) proved the antidiabetic effect of ethanolic extract of *Swertia chirayita* in streptozocin – nicotinamide model of induced diabetes in Wistar albino rats which showed reduction in serum glucose, cholesterol and triglyceride levels.

Nagalekshmi *et al.* (2011) evaluated hepatoprotective activity of *Andrographis paniculata* and *Swertia chirayita* in acute hepatotoxicity induced by paracetamol (150 mg/kg bw) in Swiss albino mice. The paracetomol induced elevated levels of serum marker enzymes such as SGPT, SGOT, ALP and increased levels of lipid peroxides, reduced SOD, CAT, GSH and GPx concentrations in liver tissue were restored to the control levels with the administration of extracts at 100mg and 200mg/kg bw respectively.

Verma *et al*. (2013) evaluated the antihyperglycemic activity of ethanolic extracts of *Swertia chirayita* and *Androghis paniculata* in streptozotocin induced diabetic rats and observed favorable effects in body weight, blood glucose level, lipid profile and renal markers. The extracts also increased the number of insulin producing β–cells. They stated that ethanol extract of *Androghis paniculata* plant had better effects than *Swertia chirayita*.

Study conducted by Manjunath (2013) showed existence of synergistic effect in alleviation of damage caused by streptozotocin induced diabetic rats between combinations of *Pterocarpus marsupium* and *Swertia chirayita* and *Pterocarpus marsupium* and *Swertia chirayita along with glibenclamide.*

Kavitha and Dattatri (2013 and 2015) reported that both aqueous and methanolic extracts of *Swertia chirayita* at the dose of 200 mg/kg bw and 50 mg/kg bw respectively showed potent antidiabetic effect on streptozotocin induced diabetes in rats by lowering the blood glucose levels.

Thomson *et al.* (2014) stated that aqueous bark extract of *Swertia chirayita* exerts antidiabetic effect by stimulation of insulin secretion and enhancement of insulin action.

Rajesh *et al*. (2017) conducted a study to evaluate the antihyperglycemic effects of *Swertia chirayita* root extract on indinavir treated Swiss albino Wistar rats. They stated that indinavir was capable of producing diabetic like symptoms in rats. The group treated with *Swertia chirata* root extract (500 mg/kg bw) decreased glucose and also improved lipid levels, and insulin levels which were almost similar to the effect produced by the standard drug metformin and pioglitazone.

Bhowmik *et al.* (2018) conducted study on neonatal-streptozotocin induced type 2 diabetic model to evaluate the effect of *Swertia chirayita* and observed a significant decrease in the fasting blood glucose, serum cholesterol and triglyceride treated with *Swertia chirayita* extracts.

Aleem and Kabir (2019), stated that *Swertia chirata* medicinal plant native to temperate Himalaya constitute many phytochemicals. The main chemical ingredients are swertiamarin, amarogentin, swechirin, mangiferin, sweroside, gentianine, amaroswerin, oleanolic acid, swertanoone, ursolic acid. Phytochemical analysis revealed presence of alkaloids, flavonoids, steroids, glycosides, triterpenoids, saponins, xanthones and ascorbic acid.

2.4 Antioxidants in diabetes

Oxidative stress is a main complication of diabetes which is due to increased free radical formation and failure of the scavenging antioxidant enzyme system that leads to long term damage to the vital organs in diabetes (Gunasekaran *et al.,* 2019). In ancient times many traditional plant medicines with potent antioxidant property were used to reduce the oxidative damage caused by diabetes and also in management of long term diabetes (Gupta *et al*., 2008).

Mechanisms of action 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).

The oxidative stress during diabetes is mainly due the auto oxidation reactions of sugars, glycation of proteins and lipid peroxidation which lead to increased production of oxygen free radicals and compromised free radical inhibitory and scavenger system like antioxidant enzymes. These cause long term complications in diabetes (Baynes, 1991).

Kakkar *et al*. (1998) demonstrated that there was an increase in the lipid peroxidation product and activity of antioxidant enzymes in the liver and pancreas during initiation and progression of STZ induced diabetes and also stated that low activity of antioxidant enzymes in pancreas compared with liver increased susceptibility of pancreatic tissue to oxidative damage during development of diabetes.

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 (Bonnefont-Rousselot *et al.,* 2000; Telci *et al.*, 2000 and Turk *et al.,* 2002).

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

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

Nagalekshmi *et al.* (2011) in their study on hepatoprotective activity of *Swertia chirayita* reported reduction in SOD, GSH and GPx levels due to paracetamol induced liver damage. However, they observed that administration of *Swertia chirayita* extract significantly improved the levels of SOD, GSH and GPx in a dose dependent manner.

Chen *et al.* (2011) assayed the antioxidant effect of ethanolic extract of *Swertia chirayita* in liver and kidney of CCl_4 intoxicated animals and recorded a decrease in SOD, CAT and GSH levels. The animals treated with *Swertia chirayita* extract and vitamin E did not exhibit toxicity signs of CCl_4 indicating that ethanolic extract of *Swertia chirayita* possesses antioxidant effect comparable to that of vitamin E.

Raza and John (2012) conducted a study to demonstrate oxidative stress produced by the streptozotocin on HepG2 cells. They showed that there was a time and dose dependent increase in the production of reactive oxygen species (ROS) and NO production in the HepG2 cells contributing for oxidative stress.

Singh *et al.* (2012) evaluated oxidative stress in the form of levels of lipid peroxidation, non enzymatic (GSH) and enzymatic (SOD, CAT and GPx) antioxidants in liver, muscle and kidney. They showed an increase in lipid peroxidation and decrease in antioxidants ranging from 30 % to 50 % indicating coexistence of metabolic disturbances and oxidative stress in diabetic animals.

Manjunath (2013) observed a significant decrease in liver antioxidant enzyme activities of SOD, CAT and GPx in diabetic rats and on treatment with combined herbal extract of *Pterocarpus marsupium, Swertia chirayita* along with glibenclamide noted improvement in the liver antioxidant enzyme activity.

Pragathi *et al.* (2015) in their study on effect of *Gymnema sylvestre* in streptozotocin induced diabetic model showed a significant reduction in the SOD, CAT, GPx in liver of diabetic rats.

Rani *et al.* (2016) suggested that *Gymnema sylvestre* herbal extract and trace element chromium can effectively reduce the oxidative stress caused by streptozotocin induced diabetes by significantly increasing antioxidant enzyme levels and could be used in management of type II diabetes mellitus.

Al-Nahdi *et al.* (2019) stated that Rin-5F pancreatic beta cells are extremely susceptible to oxidative stress due to excessive production of endogenous and exogenous reactive oxygen/nitrogen species (ROS/RNS) and low antioxidant defences, particularly, associated with GSH metabolism.

Gunasekaran *et al.* (2019), conducted a study to evaluate antioxidant activity of methanolic extract *Gymnema* leaves by three different methods such as DPPH free radical quenching activity, reduced power assay and hydroxyl free radical quenching activity. They observed significant antioxidant activity of the extract in all the three methods.

2.5 Pathology

Pillion *et al.* (1988) studied the effects of streptozotocin-induced diabetes mellitus in male Fisher rats and found loss of total body weight associated with adipose and muscle tissue wasting. They reported that the adult STZ-diabetic rat responds to loss of available insulin by polyphagia, polydipsia and catabolism of adipose and muscle tissue.

In a morphometric analysis of islet endocrine cells in type-I diabetes induced by administration of multiple low doses of STZ, Li *et al.* (2001) observed a reduction in islet cell area by 35 % and a gradual decrease in insulin positive β-cells with an increase in the number of alpha cells by 2-3 times per islet area.

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

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 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.

Study conducted to observe histopathological changes in various organs in diabetes mellitus rabbits induced by streptozotocin, showed slight congestion and mild degree of degeneration in pancreatic acini, decreased cellularity in islets, fusiform appearance of cells of some islets, congestion and haemorrhage in alveoli and bronchioles of lungs, congestion of kidney, degeneration and congestion of liver, haemorrhage and myopathy in heart and mild neuronal damage in brain (Mir *et al.*, 2008).

Dhanush (2009) experimentally induced diabetes in rats and observed reduced number of islets which were irregular with β-cells that were either necrotic or highly swollen with vacuolated cytoplasm. Some cells appeared elongated and fusiform. The normal distribution of β and α cells was altered and showed an increase in the number of α cells. By the end of the study he observed mild to moderate fibrotic change in the islet with infiltration of few inflammatory cells and centrilobular vacuolar degeneration along with focal areas of necrosis, loss of normal architecture and infiltration of inflammatory cells in liver of some diabetic rats.

Atangwho *et al.* (2010) reported that in untreated streptozotocin (STZ) diabetic rats islets were damaged which were markedly reduced in mass and infiltrated with lymphocytes, in contrast to those in non-diabetic control rats that were well preserved, devoid of fibrosis and distributed widely with well stained nuclei.

Erejuwa *et al*. (2011) in their study on antioxidant effects of honey, glibenclamide, metformin and their combination on the kidneys of STZ induced diabetic rats observed changes such as necrosis of the epithelium, thickening of the glomerular basement membrane and mesangial matrix expansion.

Vijayanand and Satish (2012) conducted a study to see the antidiabetic effect of *Gymnema sylvestre* extract on streptozotocin induced diabetic rats and observed severe congestion with decrease in number of islets of Langerhans and β cell in diabetic rats. Upon treatment with *Gymnema sylvestre* they observed increase in the β cell concentration in diabetic rats.

In STZ induced diabetic rats, Verma *et al*. (2013) observed a decrease in the pancreatic islet number and size, atrophy and vacuolation and connective tissue proliferation in the parenchyma of pancreas islets. On treatment with plant extracts of *Andrographis paniculata* and *Swertia chirayita*, they observed a decrease in all abnormal histological changes compared to diabetic rat.

Manjunath (2013) in his study observed that in diabetic rats, both exocrine and endocrine pancreas were affected. Exocrine pancreas revealed loss of achitecture 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 beta cells in a progressively increasing manner with fibrosis of few islets at the end of the study. These diabetic rats treated with combination of *Pterocarpus mersupium* and *Swertia chirayita* and along with glibenclamide showed better improvement in reducing abnormal histological alteration.

In streptozotocin induced diabetic rats, islets of Langerhans showed loss of normal architecture and appeared irregular in shape and loss of normal distribution of α and β cells in comparison with islets of normal control rats. The β-cells were either highly swollen with vacuolated cytoplasm or elongated and fusiform with condensed nucleus. The cytoplasmic granularity of β-cells was reduced. Exocrine portion also revealed presence of bluish tinged amorphous material consisting of scattered zymogen granules extruded from degenerating and necrotic cells both intra and interlobularly. Hepatocyte were swollen with highly vacuolated cytoplasm. Heart muscle fibres appeared atrophied, and renal tubular cells were degenerated. In spleen revealed drastic depletion of lymphocytes from the periarteriolar sheath as well as from the follicles was observed at the end of the experiment (Pragathi *et al*., 2015).

Yaman *et al*. (2017) in their study on streptozotocin induced diabetic rats observed degenerative and necrotic changes in the islet cells of Langerhans, with significant cell loss and hydropic degeneration and degranulation in β cell. In liver large or small, irregularly-edged, partially rounded vacuoles were observed in the cytoplasm of degenerated hepatocytes. Mild fibrosis, bile duct proliferation and inflammatory cell infiltration were also detected in portal areas.

Muzumbukilwa *et al*. (2019) reported that streptozotocin induced diabetic rats exhibited significant weight loss, polydipsia, impaired glucose tolerance, fasting hypoinsulinemia and impaired liver function tests compared to controls. Hepatic damage characterized by degeneration and necrosis of hepatocytes with infiltration of inflammatory cells infiltrates and fragmentation of the nucleus and cell lysis were also observed.

2.6 Immunohistochemical demonstration of insulin

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

Yuvuz *et al*. (2003) studied the protective effects of melatonin against β-cell damage in streptozotocin-induced diabetes in rats and observed degeneration of islet cells and weak immunohistochemical staining of insulin in diabetic rats, where as the melatonin treated group showed increased staining of insulin and preservation of islet cells.

Zhou *et al.* (2004) in his study on the effect of insulin on β cells in a rat model of type 2 diabetes mellitus observed smaller islets with degranulation in the β cells, in the untreated diabetic rats with insulin.

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

Pragathi (2011) in STZ diabetic rats observed that *Gymnema sylvestre* plant extract at 50 and 100 mg/kg bw induced only a marginal increase in the number of insulin immune reactive β cells. Similarly, Mudasir *et al.* (2013) 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.

Manjunath (2013) in his study on immunohistochemistry of combined treatment groups observed better regeneration of β cell population which was comparable to that of glibenclamide control indicating the synergistic action of *Pterocarpus marsopium* and *Swertia chirayita* with the glibenclamide.

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 in improvement in β cell number.

Rani *et al*. (2016) observed decrease in the expression of the insulin receptor in muscle, adipose tissue and liver in the streptozotocin induced diabetic rats compared to the normal control animals, characterized by absence or negligible presence of granular brownish coloured immune reaction in the cytoplasm as well as the cell membrane of the cells.

Yaman *et al*. (2017) conducted a study to investigate the antioxidant properties and protective effects of *Heracleum persicum* (HP) extract in streptozotocin (STZ)-induced diabetic rats and observed an increased immune reactivity to glutathione peroxidase 1 (GPx-1) in liver and kidney and insulin in the beta cells of pancreas was increased in *Heracleum persicum* (HP) treated diabetic rats.

El-Sherbiny *et al*. (2019) conducted a study to investigate the therapeutic response of human umbilical cord blood mesenchymal stem cells (HUCBMSCs) to streptozotocin induced diabetes in albino rats. In rats which were implanted with HUCBMS cells they observed presence of cell clusters with blue cytoplasm in liver parenchyma and around central veins with immune positivity to the anti-human insulin immunostaining indicating that these cells were active and secreting insulin.

Materials and Methods

III. MATERIALS AND METHODS

The present experiment was carried out during 2017-19 at the Department of Veterinary Pathology, Veterinary College, Hebbal, KVAFSU, Bangalore, to evaluate the combined antidiabetic effect of *Gymnema sylvestre* and *Swertia chirayita* in diabetes induced rats. Also, to compare the hypoglycemic effects of *Gymnema sylvestre* and *Swertia chirayita* with an oral hypoglycemic agent glibenclamide.

3.1 Experimental animals

Fifty healthy adult male *Wistar albino* rats weighing 180-200 g were procured from Raghavendra Enterprises, Bangalore for the study. The experiment was carried out for a period of 45 days after obtaining permission from Institutional Animal Ethics Committee (IAEC). Rats were maintained under standard laboratory conditions in polypropylene rat cages with sterilized corncob bedding material procured from Sparconn life sciences, Bangalore and were offered *ad libitum* of standard commercial rat feed and clean drinking water throughout the experimental period.

3.2 Sources

3.2.1 Drugs and chemicals

To induce experimental diabetes in rats, streptozotocin was used which was procured from Sigma Aldrich Corporation, 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 of 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 45 mg/kg and injected intraperitonially to rats immediately to avoid degradation**.**

3.2.2 Sources of plant extracts

3.2.2.1 *Gymnema sylvestre*

The hydroalcoholic extract of *Gymnema sylvestre* (Plate 1) used in the present study was obtained from Chemiloids Life Sciences Private Limited, Vijayawada, Andra Pradesh. The powdered extract was used at the dose rate of 200 mg/kg body weight, weighed according to body weight and dissolved in distilled water to make the final concentration and administered to the experimental animals.

3.2.2.2 *Swertia chirayita*

The aqueous extract of *Swertia chirayita* (Plate 2) used in the present study was obtained from Chemiloids Life Sciences Private Limited, Vijayawada, Andra Pradesh. The powdered extract was used at the dose rate of 200 mg/kg body weight, weighed according to body weight and dissolved in distilled water to make the final concentration and administered to the experimental animals.

3.3 Glibenclamide solution

Glibenclamide (Glinil®, 5 mg) an oral hypoglycaemic drug purchased from a local chemist shop was dissolved in distilled water (41.6 mL) to make a concentration of 0.12

mg/mL solution. This was used as stock solution and administered orally at a dose of 600 μg/kg (Lakshmi, 2015).

3.4 Administration of plant extracts and glibenclamide

The plant extracts and glibenclamide were administered orally to their respective groups by using clean gavaging rat feeding needle attached to an appropriate disposable syringe during morning hours daily for a period of 45 days throughout the study

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 with *ad libitum* provision of food and clean water. The groups and treatments used were as follows

Group I (NC)	**Normal control**: normal rats administered orally with saline
Group II (DC)	**Diabetic control**: streptozotocin induced diabetic rats
Group III (G)	**Glibenclamide treatment group**: diabetic rats supplemented with glibenclamide at a dose of 600 μg/kg body weight (full dose)
Group IV (GS+SC)	**Combined treatment group (full dose)**: diabetic rats treated with *Gymnema sylvestre* + *Swertia chirayita* extracts at 200 mg/kg bw each
Group V (GS+SC+G)	**Combined treatment group with glibenclamide (half dose):** diabetic rats treated with *Gymnema sylvestre* + *Swertia chirayita* extracts at 100 mg/kg bw each along with glibenclamide at 300 μg/kg bw.

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.

3.6 Experimental induction of diabetes

To induce diabetes, the rats were fasted overnight and injected with freshly prepared streptozotocin (Sigma chemicals, USA) at the dose of 45 mg/kg intraperitonially in 0.1M citrate buffer having a pH of 3.5- 4.5 (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 blood glucose level at 72 hours post STZ injection using glucometer (Accu-check). The animals with blood 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

To evaluate the biochemical parameters, blood was drawn from the retro-orbital plexus of the rats under light ether anaesthesia 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 Eppendorff 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 ketamine and xylazine anaesthesia on 15^{th} and 30^{th} day 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 pancreas, liver, heart, lung, kidney and intestine were collected in 10 per cent neutral buffered formalin (NBF) for histopathology for the pathomorphological evaluation.

3.10 Parameters analysed

3.10.1 Body weight

The animals were weighed individually at the beginning of the study and on 3^{rd}, 15^{th}, 30^{th} and 45^{th}day of the experiment to evaluate the effect of various treatments on body weight.

3.10.2 Haematological parameters

The blood samples collected in EDTA vials from the animals at 15^{th}, 30^{th} and 45^{th} day of the experiment were subjected for haemoglobin, total erythrocyte count, total leucocyte count, platelet count estimation.

3.10.3 Biochemical parameters

The serum samples collected from the animals at 15th, 30th and 45th day of the experiment were subjected for biochemical estimation of serum levels of glucose, cholesterol, triglycerides, ALT and AST using semi-automatic biochemical analyser with Erba biochemical kits.

3.10.4 Estimation of antioxidant enzymes

3.10.4.1 Material collection

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

3.10.4.2 Homogenate preparation

Liver tissue was homogenized with ice cold 0.1 M Tris-HCl buffer of pH 7.4 to make 30 % homogenate w/v (0.5 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.

3.10.4.3 Protein estimation

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

3.10.4.3.1 Principle

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

3.10.4.3.2 Reagents

BSA stock solution (l mg/mL): Standard stock solution.

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.

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

3.10.4.3.3 Procedure

For standard solution

1. Different dilutions of BSA 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 was 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.

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

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

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

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

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

3.10.4.4 Assay of enzymatic antioxidants

3.10.4.4.1 Estimation of superoxide dismutase (SOD)

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

3.10.4.4.1.1 Principle

Superoxide 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.

3.10.4.4.1.2 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 ºC. The supernatant was separated and used for the test. It was expressed as units/minute/mg of protein.

3.10.4.4.1.3 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 minute intervals at 420 nm wavelength spectrophotometrically.

3.10.4.4.1.4 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.

3.10.4.4.2 Estimation of catalase (CAT)

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

3.10.4.4.2.1 Principle

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

3.10.4.4.2.2 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 minute for 3 minutes. Blank contained 0.2 mL of distilled water plus 1 mL of 30 mM H_2O_2.

3.10.4.4.2.3 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.

3.10.4.4.3 Estimation of glutathione peroxidase (GPx)

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

3.10.4.4.3.1 Principle

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

3.10.4.4.3.2 Procedure

The reaction mixture contained 2.0 mL of 0.4 M Tris- HCl buffer, pH 7.0, 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 °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 minutes. The absorbance of the product was read at 430 nm and expressed as nmol/mg of protein.

3.10.4.4.3.3 Calculation

Value = OD Value X Dilution factor/ Total Protein

Unit: nmol / mg of protein.

3.10.5 Pathology

Two animals from each group were sacrificed humanely on Day 15, 30 and the rest at the end of the study on 45th 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 H & E staining (Luna, 1968),

3.10.6 Immunohistochemistry (IHC) for demonstration of insulin secreting pancreatic β cells

Immunohistochemical staining of pancreas for demonstration of insulin in pancreatic islets and β cells was done at different intervals of study using rabbit monoclonal anti-insulin antibody.

3.10.6.1 Materials

3.10.6.1.1 Immunochemicals

Primary antibody: Monoclonal rabbit anti-insulin antibody (Clone–EP 125 and catalogue no. –PR048-6 mL RTU): Pathnsitu, USA was procured and stored at 2-8^{0}C.

PolyExcel HRP/DAB detection system: Secondary kit containing PolyExcel H_2O_2, target binder, PolyHRP, Stunn DAB Substrate buffer along with DAB substrate chromogen were procured from Pathnsitu, USA and stored at 2 -8^{0}C.

3.10.6.1.2 Section adhesive 3-aminopropyl triethoxy-silane (APES)

Procured from HiMedia Laboratories Pvt. Ltd., Mumbai and stored at room temperature.

3.10.6.2 Epitope retrieval solution

3.10.6.2.1 Tris –EDTA buffer (10 mM Tris base, 1 mM EDTA solution and pH 9.0) for insulin

1000 mL of Tris –EDTA buffer was prepared by dissolving 1.21 g of Tris base and 0.37 g of EDTA and finally the pH was adjusted to 9.0 with 1 N NAOH.

3.10.6.2.2 Phosphate buffer saline (pH -7.4)

1 X concentration of 1000 mL of PBS was prepared by adding following chemicals.

Sodium chloride (MW 58.44)	40 g
Potassium chloride (MW 74.56)	1 g
Disodium hydrogen orthophosphate (MW 141.96)	7.2 g
Potassium dihydrogen orthophosphate anhydrous (MW 136.09)	1 g
Distilled water	500 mL

Wash buffer of 1 X concentration was prepared using 10 X PBS by adding 25 mLof 10 X PBS to 225 mL of distilled water, to which 125 μL of Tween 20 was added and pH was adjusted to 7.2.

3.10.6.2.3 Harris haematoxylin for nuclear staining (Luna, 1968)

Harris haematoxylin (50 %) solution was prepared by adding equal amount of Harris haematoxylin and distilled water and counter staining was carried out for 45 seconds.

3.10.6.2.4 Preparation of organosilane (APES) treated slides for IHC

The slides were washed thoroughly in two changes of 1 % acid alcohol (1 mL HCL in 99 mL isopropyl alcohol). The slides were washed in running tap water for 10 minutes. A 4 % solution of 3-aminopropyltriethoxy-silane (APES) in acetone in a dry staining dish was prepared. The slides were immersed in the APES solution for 30 seconds to one minute.

The slides were rinsed in distilled water, then rinsed in acetone and finally rinsed in distilled water (10 dips each). The slides were allowed to dry at 37^0C for two hours and then stored at room temperature until use.

3.10.6.2.4.1 Staining method

- Tissue sections were mounted on 3-aminopropyltriethoxy-silane (APES) coated slides and dried at 37^0C for three hours. Later stored at 4^0C until use.
- The paraffin tissue sections were deparaffinized using xylene and rehydrated using descending grades of ethanol.
- Heat induced epitope retrieval (HIER) was carried out by immersing tissue sections in a cooker containing Tris–EDTA buffer (pH 9.0)
- Sections were allowed to cool down to room temperature for approximately 30 minutes. Later washed in two changes of distilled water and then washed in (PBS) wash buffer (pH 7.4).
- Whole sections were covered with polyH_2O_2 to reduce the nonspecific background staining caused by endogenous peroxidase. This was incubated at room temperature for ten minutes and later washed in two changes of wash buffer.
- Required amount of primary antibody was added to cover the sections and were incubated at room temperature in humidified chamber for one hour and washed with two changes of wash buffer.

- Then Sections were covered by the target binder which attaches to the primary antibody present on the section, after period of 15 minutes washed with two changes of wash buffer.
- The secondary antibody PolyHRP was added to cover the whole section and incubated at room temperature in humidified chamber for 45 minutes and washed with two changes of wash buffer.
- The DAB working solution was poured to cover the sections and incubated at room temperature for five minutes than washed twice with the distill water.
- Nuclear counter staining with Harris haematoxylin was carried out for 45 seconds.
- The sections were washed in distilled water, dehydrated with ascending grades of ethanol and cleared with xylene and cover slipped with DPX mounting media.

Results

IV. RESULTS

The present study was conducted to evaluate the biochemical and pathomorphological changes in streptozotocin induced diabetes in rats and also to determine the efficacy of combined herbal extract of *Gymnema sylvestre* and *Swertia chirayita* with or without glibenclamide in amelioration of streptozotocin induced diabetic changes.

The study included five treatment groups comprising 10 rats each, randomized with minimum variation in initial body weights. The groups were normal control (Group I (NC)), diabetic control (Group II (DC)), diabetic rats treated with glibenclamide at 600 μg/kg bw (Group III (G)), diabetic rats treated with combined herbal extracts *Gymnema sylvestre* and *Swertia chirayita* at 100% dosage at 200 mg/kg bw each (Group IV (GS+SC)) and diabetic rats treated with combined herbal extracts *Gymnema sylvestre* and *Swertia chirayita* at 50% dosage at 100 mg/kg bw each and along with glibenclamide at 300 μg/kg bw (Group V (GS+SC+G)). The results of the study are presented under the following subheadings.

4.1 Induction of diabetes

In the present study, diabetes was induced by a single intra-peritoneal injection of streptozotocin at 45 mg/kg body weight in Groups II to V. Rats with fasting serum glucose level above 200 mg/dL were considered as diabetic and included in the present study. Streptozotocin effectively induced diabetes in the present study in the rats which exhibited clinical signs such as polyuria, polydipsia, weight loss and decreased physical activities, with hyperglycemia of >200 mg/dL.

On Day 3, the mean (±SE) blood glucose values of Groups II, III, IV and V were estimated and the values were 471.14 ± 1.75, 480.20 ± 2.53, 475.68 ± 3.38 and 472.85 ± 0.64 mg/dL respectively, which were significantly ($P < 0.05$) higher compared to that of normal control animals.

4.2 General observations

In the present study normal control rats (Group I) were active throughout the period of experiment.

The diabetic rats (Group II) exhibited clinical signs such as polyuria, polydipsia, polyphagia, restlessness, mild diarrhoea and deteriorated body condition by 48-72 hour post STZ injection and these signs persisted till the end of the study in a relatively severe degree.

The animals of Group III manifested clinical signs similar to that of diabetic control rats during initial period of study. The intensity of various clinical signs decreased gradually from 3rd to 45th day post treatment with glibenclamide.

The animals of Group IV and V manifested clinical signs similar to those of diabetic control rats which comprised of polyuria, polydipsia, weakness, ruffled hair coat by 48 to 72 hour post STZ injection. However, the intensity of various clinical signs gradually decreased from 3rd to 45th day post treatment in both the groups with noticeable improvement in body condition.

4.2.1 Body weight

The mean body weights in grams, with standard error of mean at different time intervals of 3^{rd}, 15^{th}, 30^{th} and 45^{th} days of the experiment have been presented in Table 1 and Figure 1.

The mean body weight values of Group II diabetic rats were observed to be progressively decreasing from Day 3 to Day 45 and the values were 198.33 ± 1.66, 186.66 ± 3.33, 158.50 ± 0.85 and 147.50 ± 0.64 g on 3^{rd}, 15^{th}, 30^{th} and 45^{th} day post STZ treatment respectively. The mean values were significantly ($P < 0.05$) lesser compared to normal control Group I as well as other treatment groups (Group III, IV & V) on all the days of observation.

There was a significant ($P < 0.05$) progressive improvement in the mean body weight of Group III diabetic rats treated with glibenclamide compared to diabetic control (Group II) and the values were 206.16 ± 1.29 g on 3^{rd} day and 247.50 ± 0.64 g on 45^{th} day. However, the mean body weight was significantly lesser ($P < 0.05$) in comparison with that of normal control (Group I) on all the days of examination.

The Group IV diabetic rats treated with *Gymnema sylvestre* and *Swertia chirayita* extracts at 200 mg/kg bw and Group V diabetic rats treated with combined extracts at 100 mg/kg bw along with half dose rate of glibenclamide at 300 µg/kg bw also showed significant ($P < 0.05$) progressive improvement in the mean body weight in comparison with that of diabetic control group (Group II) with the values of 190.83 ± 2.71 and 194.16 ± 3.27 g on 3^{rd} day and 230.00 ± 1.29 and 242.50 ± 0.64 g on 45^{th} day respectively.

However, the mean body weight values remained significantly ($P < 0.05$) lesser compared to normal control and glibenclamide treated group on all the days of the study.

Among the treatment groups (Group IV and V), a significant ($P < 0.05$) improvement in the mean body weight was observed in the Group V rats from 15^{th} day onwards compared to Group IV rats.

4.3 Serum biochemistry

4.3.1 Serum glucose

The mean values of serum glucose (mg/dL) with standard error of mean at different time intervals of 3^{rd}, 15^{th}, 30^{th} and 45^{th} day of the experiment have been presented in Table 2 and Figure 2.

The mean serum glucose levels of Group II diabetic control rats were observed to be progressively increasing from Day 3 to Day 45 and the values were 471.14 ± 1.75 and 528.50 ± 3.09 mg/dL respectively. The mean values were significantly ($P < 0.05$) higher compared to those of normal control (Group I) rats with values of 88.48±0.78, 110.5±1.162, 100.50±1.936 and 73.85±1.39 mg/dL on 3^{rd}, 15^{th}, 30^{th} and 45^{th} day of the study respectively.

There was a significant ($P < 0.05$) progressive improvement in the mean glucose levels of Group III diabetic rats treated with glibenclamide compared to diabetic control (Group II) observed from 15^{th} day of the study and the values were 480.20 ± 2.5 on 3^{rd} day and 235.11 ± 1.23 mg/dL on 45^{th} day. However, the mean glucose level was significantly

(P < 0.05) higher in comparison with that of normal control (Group I) on all the days of examination.

The Group IV and Group V rats showed a significant (P < 0.05) progressive improvement in the mean serum blood glucose values in comparison with that of Group II diabetic control rats and the values were 475.68 ± 3.38 and 472.85 ± 0.64 mg/dL on 3rd day and 323.40 ± 2.85 and 306.13 ± 0.80 mg/dL on 45th day respectively. However, the mean serum glucose values remained significantly (P < 0.05) higher compared to the normal control and glibenclamide treatment group on all the days of examination.

Among the treatment groups (Group IV and V), a significant (P < 0.05) decrease in the mean glucose value was observed in the Group V rats treated with combined herbal extract along with glibenclamide, than Group IV rats from 15th day to 45th day.

4.3.2 Serum alanine aminotransferase (ALT)

The mean values of serum alanine aminotransferase (IU/L) with standard error of mean at different time intervals of 15th, 30th and 45th days of the experiment have been presented in Table 3 and Figure 3.

The diabetic control rats showed a progressive increase in the mean serum ALT values during the study period which were significantly (P < 0.05) higher as compared to that of normal control rats with the values of 191.16 ± 2.41 IU/L on 15th day and 243.42 ± 0.95 IU/L on 45th day post treatment.

There was a significant (P < 0.05) progressive decrease in the mean serum ALT values of Group III rats when compared to the diabetic control (Group II) with the values were 140.83 ± 0.75 IU/L on 15th day and 96.28±0.89 IU/L on 45th day. However, the mean values remained significantly ($P < 0.05$) higher compared to normal control on all the days of the observation.

There was also a significant ($P < 0.05$) progressive decrease in the mean serum ALT values of Group IV and V in comparison with that of diabetic control rats and the values were 177.70 ± 0.59 and 163.10 ± 0.74 IU/L on 15th day and 121.45 ± 0.27 and 106.77 ± 1.05 IU/L on 45th day respectively. However, the mean ALT values remained significantly ($P < 0.05$) higher compared to normal control rats and glibenclamide treatment group. Among treatment groups mean serum ALT values of Group V rats showed significant ($P < 0.05$) improvement in comparison with Group IV rats.

4.3.3 Serum aspartate aminotransferase (AST)

The mean values of serum aspartate aminotransferase (IU/L) with standard error of mean at different time intervals of 15th, 30th and 45th day of the experiment have been presented in Table 4 and Figure 4.

The mean serum AST values of diabetic control rats progressively increased from 262.88 ± 0.66 IU/L on 15th day to 278.20 ± 2.11 IU/L on 45th day, and the values were significantly ($P < 0.05$) higher when compared to the normal control rats on the all days of the experiment.

There was a significant ($P < 0.05$) progressive decrease in the mean serum AST values of Group III rats from 15th day onwards and were significantly lesser ($P < 0.05$) compared to those of diabetic control (Group II) with the values of 179.67±0.81 IU/L on

15th day and 102.59±1.60 IU/L on 45th day. However, the mean serum AST values were significantly ($P < 0.05$) higher compared to the normal control on all the days of observation.

The mean AST values of Group IV and V also showed significant ($P < 0.05$) decrease from 15th day onwards and were significantly ($P < 0.05$) lesser compared to those of diabetic control with the values of 247.86 ± 0.14 and 227.65 ± 0.29 IU/L on 15th day and 192.15 ± 0.68 and 163.00 ± 0.72 IU/L on 45th day respectively. However, the mean serum AST value of both the groups remained significantly ($P < 0.05$) higher compared to normal control and Group III rats. Among the treatment groups a significant ($P < 0.05$) improvement was observed in the Group V rats compared to Group IV rats.

4.3.4 Serum triglycerides

The mean values of serum triglyceride (mg/dL) with standard error of mean at different time intervals of 15th, 30th and 45th day of the experiment have been presented in Table 5 and Figure 5.

The diabetic control (Group II) rats showed a significant ($P < 0.05$) progressive increase in the mean serum triglyceride values in comparison to normal control rats on all the days of study with values of 234.97 ± 1.82 mg/dL on 15thday and 303.34 ± 0.68 mg/dL on 45th day of the experiment.

In glibenclamide treated (Group III) rats a significant ($P < 0.05$) decrease in the mean serum triglyceride values was observed when compared to diabetic control rats. The values were 200.50 ± 0.52 mg/dL on 15th day and 107.71±3.03 mg/dL on 45th day of the study. However, the mean serum triglyceride values were significantly ($P < 0.05$) higher in comparison with normal control (Group I) on 15^{th} and 30^{th} day but comparable on the 45^{th} day post treatment.

The treatment groups IV and V also revealed progressive significant ($P < 0.05$) decrease in the mean serum triglyceride values in comparison with diabetic control with the values of 212.17 ± 0.50 and 185.75 ± 0.15 mg/dL on 15^{th} and 131.19 ± 1.21 and 111.24 ± 0.28 mg/dL on 45^{th} day respectively. However, the mean serum triglyceride values remained significantly ($P < 0.05$) higher compared to normal control and glibenclamide control groups on all the days of observation.

Among the treatment groups (Group IV and V), a significant ($P < 0.05$) improvement in the mean serum triglyceride was observed in the Group V rats from 15^{th} day onwards compared to Group IV rats.

4.3.5 Serum cholesterol

The mean values of serum cholesterol (mg/dL) with standard error of mean at different time intervals of 15^{th}, 30^{th} and 45^{th} day of the experiment have been presented in Table 6 and Figure 6.

In the present investigation, the mean serum cholesterol level of Group II diabetic control rats was observed to be progressively increasing from Day 15 to Day 45 with the values from 161.31 ± 0.53 to 212.5 ± 1.61 mg/dL respectively. The mean serum cholesterol values were significantly ($P < 0.05$) lesser compared to the normal control Group I rats on all the days of examination.

There was a significant ($P < 0.05$) progressive improvement in the mean serum cholesterol level of Group III rats compared to diabetic control rats (Group II) from 15th day of the study and the values were 126.50 ± 1.42 mg/dL on 15th day and 108.5 ± 0.68 mg/dL on 45th day. However, the mean serum cholesterol level was significantly ($P < 0.05$) higher in comparison with the normal control (Group I) on all the days of study.

The mean serum cholesterol level of treatment groups (Group IV and Group V) showed significant ($P < 0.05$) progressive improvement in comparison with that of Group II diabetic control rats with the values of 147.50 ± 1.67 and 138.00 ± 2.84 mg/dL on 15th day to 117.50 ± 3.74 and 106.50±0.38 mg/dL on 45th day respectively. However, the mean serum cholesterol values were significantly ($P < 0.05$) higher compared to normal control rats on all days of examination. The mean serum cholesterol level of both the groups remained significantly ($P < 0.05$) higher when compared to Group III rats except on 45th day of the study, where the values were comparable. Among the treatment groups the values did not differ significantly ($P \geq 0.05$) except on the 30th day, but numerically a better improvement was observed in the Group V rats treated with combined herbal extract along with glibenclamide combination.

4.4 Haematology

4.4.1 Haemoglobin (Hb)

The mean values of haemoglobin in g%, with standard error of mean at different time intervals of 15th, 30th and 45th day of the experiment have been presented in Table 7 and Figure 7.

In diabetic control group (II), there was a progressive decrease in the mean haemoglobin values from 9.41 ± 0.29 on 15th day to 7.21 ± 0.25 g % on 45th day. The mean values were significantly (P <0.05) lesser compared to those of normal control rats.

There was a significant (P <0.05) progressive improvement in the mean Hb values of Group III rats compared to the diabetic control rats with the values of 10.93 ± 0.22 on 15th day and 14.56 ± 0.20 g % on 45th day. However, the mean Hb values were significantly (P <0.05) lesser compared to that of normal control on all the intervals of observation.

In Group IV and V rats the mean Hb values were observed to be progressively increasing and significantly (P <0.05) higher compared to that of diabetic control rats (Group II) on all the days of observation with the values of 11.01 ± 0.05 and 11.03 ± 0.19 g % on 15th day and 13.65 ± 0.27 and 13.85 ± 0.09 g % on 45th day of the study respectively. However, the mean Hb values were significantly (P <0.05) lesser compared to that of normal control rats. In addition the mean Hb values of both the treatment groups were comparable to that of Group III rats on all the days of observation.

Among the treatment groups (Group IV and V) the mean Hb values did not differ significantly (P ≥ 0.05) on any day of the study, but numerically the values were higher in Group V rats compared to Group IV rats from 30th day of the study.

4.4.2 Total erythrocyte count (TEC)

The mean values of total erythrocyte count in 10^6 /µL with standard error of mean at different time intervals of 15th, 30th and 45th days of the experiment have been presented in Table 8 and Figure 8.

The diabetic control rats (Group II) showed a progressive decrease in the mean TEC values which were significantly ($P < 0.05$) lesser compared to normal control rats (Group I) on all the intervals of study with the values of 5.72 ± 0.08 x 10^6 /µL on 15^{th} day and 5.12 ± 0.06x 10^6 /µL on 45^{th} day.

There was a significant ($P < 0.05$) progressive increase in the mean TEC values in Group III rats compared to diabetic control rats with the values of 7.21 ± 0.08 on 15^{th} day and 9.77 ± 0.03 x 10^6 /µL on 45^{th} day respectively. However, the mean TEC value was comparable to that of normal control (Group I) on 45^{th} day of the study.

There was also a significant ($P < 0.05$) progressive increase in the mean TEC values in both Group IV and V compared to the diabetic control with the values of 6.95 ± 0.01 and 7.06 ± 0.04 x 10^6 /µL on 15^{th} day and 7.92 ± 0.02 and 8.11 ± 0.03 x 10^6 /µL on 45^{th} day respectively. However, the mean TEC values remained significantly ($P < 0.05$) lesser compared to normal control rats on all the days of examination.

The mean TEC values of both the treatment groups (Group IV and V) were significantly ($P < 0.05$) lesser compared to Group III rats on 30^{th} day and 45^{th} day of the study but comparable on 15^{th} day. In addition Group IV and Group V rats did not differ significantly except on 45^{th} day, where Group V rats showed significantly ($P < 0.05$) higher mean TEC value compared to Group IV rats.

4.4.3 Total leukocyte count (TLC)

The mean values of total leukocyte count in 10^3 /µL with standard error of mean at different time intervals of 15th, 30th and 45th day of the experiment have been presented in Table 9 and Figure 9.

The mean total leucocyte count values in diabetic control group (Group II) showed a progressive increase which were significantly (P <0.05) higher compared to normal control rats on all the days of examination with the values of 12.27 ± 0.20 on 15th day and 14.09 ± 0.05 x 10^3/µL on 45th day. There was a significant (P <0.05) progressive decrease in the mean TLC values in Group III rats compared to the diabetic control rats (Group II) and the values were 10.45 ± 0.36 and 8.69 ± 0.05 x 10^3/µL on 15th and 45th day of experiment respectively. However, the mean TLC values remained significantly (P <0.05) higher compared to normal control group but comparable on 45th day of the study.

A progressive decrease in the mean TLC value was observed in both the treatment groups (Group IV and V) which was significantly (P <0.05) lesser compared to the diabetic control and the values were 10.86 ± 0.08 and 10.26 ± 0.16 x 10^3/µL on 15th day and 9.77 ± 0.20 and 7.87 ± 0.19 x 10^3/µL on 45th day, respectively. However, the mean TLC value of both the groups remained significantly (P <0.05) higher than normal control rats but were comparable to that of Group III rats on all the days of the observation. Among the treatment groups (Group IV and V) the Group V rats showed a significant (P <0.05) improvement compared to Group IV rats on 45th day post treatment.

4.4.4 Total platelets count

The mean values of total platelet count in 10^3 /µL with standard error of mean at different time intervals of 15th, 30th and 45th day of the experiment have been presented in Table 10 and Figure 10.

The mean (±SE) platelet values in diabetic control group (Group II) showed progressive decreses which were significantly (P <0.05) lesser compared to normal control rats with the values of 295.16 ± 5.70 on 15th day and 266.83 ± 4.48 x 10^3/µL on 45th day.

The Group III rats showed a significant (P <0.05) progressive increase in the mean platelet values compared to those of diabetic control rats and the values were 327.47±7.26 and 404.45 ± 3.76 x 10^3/µL on Day 15 and 45 post-treatment respectively. However, the mean platelet values remained significantly (P <0.05) lesser compared to normal control on all the days of experiment.

There was also a progressive increase in the mean platelet values of both treatment groups (Group IV and V) which were significantly (P <0.05) higher compared to that of diabetic control rats with the values of 321.34 ± 1.26 and 325.92 ± 5.12 x 10^3/µL on 15th day and 412.24 ± 1.02 and 428.13 ± 2.10 x 10^3/µL on 45th day post treatment respectively. However, the mean platelet values of Group IV and V were significantly (P <0.05) lesser compared to normal control group on all the days of observation.

The mean platelet values of Group IV and Group V rats were comparable to that of Group III rats on all the days of observation. Among the treatment groups, Group V rats showed significant (P <0.05) increase in the total platelet value compared to Group IV rats on 30th and 45th day of the study but on the 15th day the values were comparable.

4.5 Tissue antioxidant enzyme assays

4.5.1 Hepatic superoxide dismutase (SOD)

The mean values of hepatic superoxide dismutase (units/min/mg protein) with standard error of mean at different time intervals of 15th, 30th and 45th day of the experiment have been presented in Table 11 and Figure 11.

The mean liver SOD values in diabetic control group (II) were progressively decreasing and the values were significantly (P<0.05) lesser in comparison with control groups from 15th day onwards with values of 4.70 ± 0.07, 3.76 ± 0.05 and 3.65 ± 0.12 U/min/mg protein on 15th, 30th, and on 45th day respectively. The normal control (Group I) rats exhibited normal activity of SOD in the liver tissue throughout the experimental period and the mean SOD values were 24.87 ± 0.45, 23.78 ± 0.77 and 27.26 ± 0.82 U/min/mg protein on 15th, 30th, and on 45th day of the study respectively.

There was a significant (P<0.05) progressive increase in the mean SOD values of the Group III rats compared to the diabetic control rats with the values of 6.71 ± 0.07 on 15th day and 10.41 ± 0.10 on 45th day. However, the mean values were significantly (P<0.05) lesser compared to the normal control group.

The mean SOD values of both the treatment groups (Group IV and V) showed progressive improvement and values were significantly (P<0.05) higher compared to the diabetic control on all the days of observation with values of 5.04 ± 0.01 and 5.22 ± 0.03

U/min/mg protein on 15th day and 7.32 ± 0.02 and 7.72 ± 0.02 U/min/mg of protein on 45th day respectively. However, the mean values were significantly ($P<0.05$) lesser compared to that of normal control and glibenclamide control (Group III) rats.

Among the treatment groups, Group V rats showed significant ($P<0.05$) improvement in the mean SOD values compared to the Group IV rats on all the days of examination.

4.5.2 Hepatic catalase (CAT)

The mean values of hepatic catalase (µ moles of H_2O_2 decomposed/min/mg protein) with standard error of mean at different time intervals of 15th, 30th and 45th day of the experiment have been presented in Table 12 and Figure 12.

The diabetic control rats showed a significant ($P<0.05$) decrease in the mean CAT values compared to the normal control rats and the values were 23.66 ± 0.58 on 15th day and 18.16 ± 0.20 µ moles of H_2O_2/min/mg protein on 45th day respectively. The mean CAT values of normal control rats were 70.72 ± 0.48, 72.82 ± 0.78 and 76.86 ± 0.34 µ moles of H_2O_2/min/mg protein on 15th, 30th and 45th day of the study respectively.

The rats of glibenclamide treated group showed a significant ($P<0.05$) consistent increase in the mean CAT activity from Day 15 to Day 45 with values were 28.44 ± 0.12 on 15th day and 40.03 ± 0.17 µ moles of H_2O_2/min/mg protein on 45th day respectively. However, the mean CAT values were significantly ($P<0.05$) lesser compared to the normal control rats.

The Group IV and V rats showed a significant ($P<0.05$) increase in the mean CAT activity compared to the diabetic control rats from 30th day onwards with the values of 24.84 ± 0.10 and 27.94 ± 0.17 to 29.35 ± 0.31 and 31.78 ± 0.08 μ moles of H_2O_2/min/mg protein on 30th to 45th day respectively and the values were comparable on 15th day of the study. However the mean values were significantly ($P<0.05$) lesser compared to the normal control rats and glibenclamide control (Group III) rats.

Among the treatment groups a significant ($P<0.05$) improvement was observed in the Group V rats compared to Group IV from 30th day onwards, but on 15th day the values were comparable.

4.5.3 Hepatic glutathione peroxidase (GPx)

The mean values of hepatic glutathione peroxidase (μ moles of glutathione utilized / min/mg protein) with standard error of mean at different time intervals of 15th, 30th and 45th day of the experiment have been presented in Table 13 and Figure 13.

The mean values of GPx activity of diabetic control rats were significantly ($P<0.05$) decreased compared to the normal control rat and the values were 16.61 ± 0.11, 15.27 ± 0.27 and 13.03 ± 0.17 μM of glutathione utilized/min/mg protein on the 15th, 30th and 45th day respectively. The mean GPx activity of normal control were 38.16 ± 0.46, 38.24 ± 0.99 and 43.06 ± 0.56 μM of glutathione utilized/min/mg protein on 15th, 30th and 45th day of study respectively.

A significant ($P<0.05$) progressive improvement was observed in the mean GPx activity of glibenclamide treated (Group III) rats compared to diabetic control with the values of 22.56 ± 0.37 on 15th day and 28.24 ± 0.14 µM of glutathione utilized/min/mg protein on 45th day. However, the mean values were significantly ($P<0.05$) lesser compared to the normal control.

A progressive improvement was also observed in mean GPx activity of Group IV and V rats which were significantly ($P<0.05$) higher compared to the diabetic control from 30th day of study and the mean values were 17.95 ± 0.17 and 18.17 ± 0.02 on 30th day and 19.63 ±0.13 and 21.53 ± 0.23 on 45th day respectively. However, the mean values were significantly ($P<0.05$) lesser compared to the normal control and glibenclamide treated (Group III) rats.

The treatment groups (Group IV and V) did not differ significantly except on 45th day and the mean GPx activity of Group V rats showed significant ($P<0.05$) improvement over Group IV rats.

4.6 Pathology

4.6.1 Clinical signs

In the present study rats belonging to control groups (Group I) remained healthy and active throughout the period of experiment.

The diabetic rats (Group II) exhibited clinical signs such as polyuria, polydipsia, polyphagia, ruffled haircoat, restlessness and mild diarrhoea by 48-72 h post STZ injection, and deterioration in the body condition . These signs persisted till the end of the study in a relatively severe degree.

The diabetic rats treated with glibenclamide (Group III) manifested clinical signs similar to that of diabetic control rats during initial period of study. The intensity of various clinical signs decreased gradually from 15^{th} to 45^{th} day post treatment with glibenclamide.

The diabetic rats treated with combined extracts of *Gymnema sylvestre* and *Swertia chirayita* at full dose (Group IV) and at half dose along with glibenclamide (Group V) manifested clinical signs similar to those of diabetic control rats by 48 to 72 h post STZ injection. However, the intensity of various clinical signs gradually decreased from 15^{th} to 45^{th} day post treatment in both the groups with noticeable improvement in body condition (Plate 3,4,5).

4.6.2 Gross pathology

The control rats (Group I) examined at 15^{th}, 30^{th} and 45^{th} day of experiment did not reveal any gross abnormalities (Plate 6).

In the diabetic control rats, the pancreas showed congestion and progressive reduction in the size which was appreciable from 15^{th} and 30^{th} day of the study. On 45^{th} day, the pancreas was atrophied and appeared as a thin gelatinous strip (Plate 7). Liver appeared slightly pale and enlarged on all days of the study (Plate 12). Other organs such as kidney, intestine, lung and heart did not show any appreciable gross lesions throughout the study period.

Grossly, the pancreas of glibenclamide treatment group (Group III) also revealed reduction in size on 15^{th} day compared to that of normal healthy rat with a gradual improvement from 30^{th} day to 45^{th} day (Plate 8). The liver appeared enlarged and pale on day 15 and improved in appearance, colour and consistency on 30^{th} and 45^{th} day of post-treatment. The other organs brain, kidney, heart, lung, spleen, lymph nodes and intestine did not reveal any appreciable macroscopic changes on any interval of examination.

Grossly, the pancreas in treatment groups (Group IV and V) also appeared reduced in size on 15^{th} day but gradually improved with slight variation between the treatment groups. A better improvement was observed in rats of Group V than the Group IV (Plate 9,10). No appreciable macroscopic changes were observed in any other organs in both the groups (Plate 13).

4.6.3 Histopathology

4.6.3.1 Group I (NC)

In normal control group (Group I), the architecture of the pancreas was well maintained. Endocrine pancreas appeared normal with well formed islets, comprising majority of beta (β) cells occupying the core and alpha (α) cells at the periphery (Plate 14). The β cells showed a cord like arrangement separated by capillary blood vessels. The β cells were polygonal in shape with centrally placed nucleus and lightly granular eosinophilic cytoplasm. The α cells were small, cuboidal and consisted of scanty cytoplasm (Plate 15). Exocrine acinar cells showed normal architecture with basaly placed nucleus, presence of cytoplasmic zymogen granules, and well formed ductular structures.

Microscopically the architecture of the liver, kidney, spleen, and heart was apparently normal (Plate 16, 17, 18, 19).

4.6.3.2 Group II (DC)

In the present study, on 15th day post STZ injection, both exocrine and endocrine components of pancreas were affected. The exocrine portion revealed loss of normal lobular architecture characterized by reduction in the lobular size which were widely separated out. The acini were lined by highly vacuolated, degenerating and necrotic cells with loss of zymogen granules (Plate 20). In some lobules complete loss of acinar cells was observed. Exocrine portion also revealed presence of large number of apoptotic cells with condensed and eccentrically placed nucleus, and some acini also showed vacuolated lining cells. There was also moderate congestion of vessels and haemorrhage in exocrine portion along with the infiltration of mononuclear cells (Plate 23).

The endocrine component of pancreas revealed reduced number and size of islets per lobule with loss of normal architecture. The islets were hypocellular with altered distribution of α and β cells. The β cells were degenerative and necrotic with the α cells at the periphery unaffected (Plate 21). The cytoplasmic granularity of β cells was greatly reduced. The number of α cells was comparatively more and there was also presence of apoptotic cells in the islets which appeared shrunken with condensed nucleus and highly eosinophilic cytoplasm (Plate 22).

On 30th day post STZ injection, there was further decrease in the number of islets with progressive damage to the islets. The lesions were similar to those of 15th day, however, with increase in severity and in addition ductular hyperplasia was also observed. There were also a few islets which showed hypercellularity with α cells (Plate 24).

On 45^{th} day post STZ injection, the islets revealed persistence of STZ induced damage which was characterized by hypocellularity with occasional apoptotic cells and cells with cytoplasmic granularity. The islets were fewer in number and were difficult to locate. In some occasional islets hypercellularity was observed with an increase in the number of α cells (Plate 25). The architecture of exocrine portion appeared slightly improved with reduced vascular changes. However ductular hyperplasia (Plate 26) was also observed along with perivascular infiltration of mononuclear cells.

Liver on 15^{th} day showed swelling of hepatocytes with highly vacuolated cytoplasm and obliteration of the sinusoidal spaces with moderate congestion (Plate 27). Multifocal necrotic areas with the infiltration of the mononuclear cells (Plate 29) were also observed along with increased kupffer cell number. There was also presence of preapoptotic and apoptotic cells scattered in the liver parenchyma (Plate 28). The preapoptotic cells appeared angular with highly eosinophilic cytoplasm and condensed or normal appearing nucleus. The preapoptotic cells were predominantly observed around portal triad and also scattered in the lobule parenchyma. The apoptotic cells appeared shrunken with condensed nucleus and eosinophilic cytoplasm. On 30^{th} day there was persistence of changes with mild biliary hyperplasia. On 45^{th} day also there was persistence of mild vacuolar changes along with presence of necrotic and apoptotic cells with the mononuclear cell infiltration. However, an improvement in the architecture was observed (Plate 33).

Microscopically, spleen revealed depletion of lymphoid cells from periarteriolar sheath as well as from the follicles (Plate 32). The red pulp was congested with hemosiderosis and showed lymphoid hypocellularity. These changes were observed from

15th day. However on 45th day an improvement in the lymphoid cellularity was observed in the some of the follicles and sheaths.

On 15th day kidneys of diabetic rats showed severe congestion and haemorrhages. The tubular epithelial cells were swollen with granular and vacuolar degeneration (Plate 30). Heart showed congestion and haemorrhages along with degeneration and necrosis of cardiac fibers and infiltration of inflammatory cells at multifocal areas (Plate 31, 35). Other organs such as lung and intestine, did not revealed any appreciable microscopical changes. On 45th day an improvement in the architecture of kidney and heart was observed (Plate 34).

4.6.3.3 Group III (G)

In glibenclamide treatment group, there was a progressive improvement in the architecture of the pancreas compared to STZ diabetic control group (Group II) and attainment of almost normal architecture of islets of Langerhans was observed at 45th day post treatment.

On Day 15 both exocrine and endocrine portions revealed persistence of STZ induced changes like vacuolar degeneration and necrosis along with presence of apoptotic cells. The islets were reduced in number, islets comprising highly vacuolated β cells, depletion of β cells and moderate increase in the number of α cells and occasional cells with β cells morphology (Plate 36).

By 30th day of treatment, an improvement in the number, size and architecture of islets was observed comprising more number of cells with β cell morphology (Plate 37).

Some of the islets were irregular in shape and hypercellular with more number of peripherally placed α cells. However, a few cells appeared vacuolated and apoptotic. Occasional newly formed islets were also observed along with ductular epithelial hyperplasia with in the exocrine parenchyma between acinar structure comprising elongated nuclei arranged in stratification.

By 45th day post-treatment, there was further increase in the number of islets which appeared more regular with increase in the number of β cells that were compactly arranged with α cells at the periphery (Plate 38). The β cells appeared round to polygonal with centrally placed nucleus and granulated cytoplasm. However, occasional cells were vacuolated and apoptotic. Ductal hyperplasia was more pronounced with many newly formed islets varying in size (Plate 39). The exocrine portion of pancreas also appeared normal with absence of any STZ induced changes.

Microscopically, liver revealed progressive improvement in the architecture from 15th day to 45th day post treatment. On 15th day the changes observed were very similar to those of diabetic group but reduced in severity. The various changes observed were mild congestion, swelling of hepatocytes with granular to vacuolar degeneration (Plate 40), occasional focal necrosis with infiltration of inflammatory cells, presence of occasional preapoptotic and apoptotic cells and mildly increased kupffer cell activity. An appreciable improvement in the microscopic changes was observed during 30th day and by 45th day attainment of almost normal architecture was observed with well formed hepatic lobular structures (Plate 41).

Other organs such as kidney, heart, and spleen did not reveal any appreciable changes throughout the period of experimentation.

4.6.3.4 Group IV (GS+SC)

Pancreas of Group IV rats treated with combination of extracts of *Gymnema sylvestre* and *Swertia chirayita* at 200 mg/kg bw each revealed microscopical changes which were very similar to that of diabetic control group (Group II) on 15th day post treatment involving both exocrine and endocrine portions. The lesions included congestion, haemorrhage, and oedema interlobularly, degeneration and necrosis of exocrine acinar structures, mononuclear cell infiltration (Plate 44), mild increase in ductular epithelial hyperplasia, reduction in the number of islets with loss of normal architecture, depletion of β cells and β cells with necrotic and apoptotic changes (Plate 42). Some of the islets revealed presence of cystic empty spaces (Plate 43).

On 30th day post treatment, similar lesions of 15th day persisted but in reduced severity. There was an improvement in the number of islets with an increase in the cellularity comprising more number of α cells (Plate 45). Occasional cells with β cell morphology were also evident in some of the islets. An improvement in the exocrine portion architecture was also observed with well-formed acinar structure.

On 45th day post treatment, further improvement in the number, size, shape and cellularity of the islets was observed with increase in the number of cells with β cell morphology (Plate 46). The exocrine portion of the pancreas also improved further in architecture and hyperplastic change involving ductular epithelium was observed with in the lobules traversing between the acinar structures. Occasional, newly formed islets comprising countable number of cells were also appreciable adjacent to hyperplastic ductular cells.

Microscopically, liver on 15th day post treatment revealed persistence of STZ induced pathological changes which comprised mild congestion, vacuolar degeneration of hepatocytes (Plate 47), multifocal areas of necrosis of hepatocytes with infiltration of mononuclear cells, preapoptotic cells scattered in the parenchyma as well as around the portal triad, appreciable number of apoptotic cells, increased kupffer cells activity and mild biliary hyperplasia.

By 30th day, there was an improvement in the architecture of hepatic cords. However, there was also persistence of multifocal necrotic areas with mononuclear cells infiltration and also cystic spaces with total absence of cells.

By 45th day, further improvement in the architecture of hepatocytes was observed with well formed lobular structures (Plate 48). However, mutifocal areas of cystic spaces were observed with total loss of hepatocytes. In addition occasional preapoptotic and apoptotic cells were also observed.

Among other organs on 15th day kidney and heart revealed mild to moderate degree of congestion. Spleen showed depletion of lymphoid cells from the follicles and the sheath. By 30th day, all the organs appeared normal with total absence of STZ induced changes.

4.6.3.5 Group V (GS+SC+G)

The pancreas of Group V rats treated with combination of extracts *Gymnema sylvestre* and *Swertia chirayita* at 100 mg/kg bw each along with half recommended dose of glibenclamide at 300 µg/kg bw showed similar microscopical changes as observed in Group IV rats involving exocrine and endocrine portion at 15th day post treatment (Plate 49, 50).

By 30th day, there was an improvement in the number, size, shape and cellularity of the β cells with many cells resembling β cell architecture (Plate 51). However, there was persistence of STZ induced changes in the cells also.

By 45th day there was further improvement in the architecture of islets consisting of many cells with β cell morphology (Plate 52). Persistence of STZ induced changes was still appreciable in some of the cells.

Microscopically, liver on 15th day showed all the changes that were observed in Group IV rats (Plate 53). By 30th day, there was an improvement in the lobular architecture with occasional STZ induced mild changes.

By 45th day post treatment, there was further improvement in the architecture of liver (Plate 54). However, occasional changes such as focal necrosis, along with biliary hyperplasia were also present.

Other organs such as heart, kidney and spleen did not reveal any appreciable changes on all the intervals of observation.

4.7 Immunohistochemical evaluation of insulin secretion by pancreatic β-cells

In the present study, immunohistochemical demonstration of insulin was carried out to evaluate insulin secretory function of pancreatic β cells in various treatment groups using monoclonal anti-insulin antibody. Appearance of dark brown granular staining of cytoplasm of β cells was considered as positive reaction and based on the level of expression and percentage of cells showing positivity, the functional status of β cells was evaluated. Swollen cells with irregular borders consisting of scattered lightly stained cytoplasm was considered as degenerating cells and the cells that appeared round with compact arrangement of densely stained cytoplasm as regenerated β-cells after damage induced by STZ.

The mean percentage values of insulin positive cells with standard error of mean at different time intervals of 15^{th}, 30^{th} and 45^{th} day of the experiment have been presented in the Table 14 and Figure 14.

4.7.1 Group I (NC)

In the normal control animals, all the islets revealed insulin positive cells in large number which showed an intense granular brown staining in the β cells restricted to the cytoplasm. The granules were compactly arranged in the cytoplasm, limited by a regular membrane. The insulin positive β cells occupied the core of the islets and were arranged in the form of cords separated by blood vessels (Plate 55, 56). The α cells and exocrine component were negative for immune reaction. The mean per cent of insulin secretory cells were found to be 80.66 ± 0.71 on 45^{th} day of the experiment

4.7.2 Group II (DC)

The islets of Langerhans in pancreas of diabetic animals on 15^{th} day were irregular, small and indistinct and revealed a drastic reduction in the number of insulin positive cells.

The insulin positive cells appeared swollen, vacuolated, irregular with scattered granular material in the cytoplasm (Plate 57). The intensity of coloration of the granules varied from light brown to dark brown and were dispersed within the cytoplasm and also intercellularly.

On 30th and 45th day a few islets revealed hypercellularity with one or two cells immunopositive (Plate 58) and the mean percentage positivity of insulin secretory cells was 2.83 ± 0.16, 3.00 ± 0.25 and 3.66 ± 0.49 on 15th, 30th and 45th day respectively, which was significantly lesser ($P \leq 0.05$) in comparison with those of normal control group.

4.7.3 Group III (G)

In glibenclamide treated diabetic rats there was a progressive increase in the number of insulin positive cells. The mean percentage values of insulin positive β cells were 10.66 ± 0.42, 28.83 ± 0.83 and 54.16 ± 2.34 on 15th, 30th and 45th day of experiment respectively.

The diabetic rats treated with glibenclamide revealed a progressive improvement in the number of immune positive β cells from 15th day onwards with a highly appreciable improvement on 45th day. The mean percentage values of β cells were significantly higher ($P<0.05$) compared to diabetic control and treatment groups (Group IV & V) on all the days of the study but significantly lesser than normal control. On 15th day the islets were small in size and few in number with occasional cells positive for insulin (Plate 59). There were dispersed granules with an elongated and irregular appearance of β cells. However, on 30th day the number of insulin positive cells increased meagrely and by 45th day many islets with almost compact arrangement of β cells was observed (Plate 60). Also small and newly formed islets were observed in multiple areas (Plate 61). In addition, individual and small cluster of insulin positive cells were also observed scattered in the parenchyma as well as adjacent to ducts.

4.7.4 Group IV (GS+SC) and Group V (GS+SC+G)

There was a progressive increase in the insulin positive cells immunohistochemically in both Group IV and V treated with combination of herbal extracts alone and along with glibenclamide respectively from 15th to 45th day of the study.

The mean percentage of insulin positive cells were 7.00 ± 0.36, 12.16 ± 0.47 and 21.00 ± 0.36 in Group IV and 8.50 ± 0.22, 15.66 ± 0.55 and 26.00 ± 1.15 in Group V on 15th, 30th and 45th day respectively. A significant improvement was observed in Group V rats over Group IV rats in the mean percentage of immune positive cells. They were significantly ($P<0.05$) higher compared to diabetic control but significantly ($P<0.05$) lesser compared to normal and glibenclamide control.

On 15th day the islets were observed to be similar to that of diabetic control rats with depleted β cells and occasional insulin positive cells with reduced intensity (Plate 62, 65). On 30th and 45th day there was an increase in the number of islets with an improvement in the architecture and a significant increase in the number of cells with insulin positivity compared to 15th day (Plate 63, 66). Also small and newly formed islets were observed in multiple areas (Plate 64, 67).

Table 1. The mean (±SE) animal body weight (g) values of different groups at different intervals of time

Groups	Days Post Treatment			
	3	15	30	45
Group I (NC)	219.50 ± 0.95^{aw}	248.16 ± 1.52^{ax}	277.66 ± 2.32^{ay}	304.50 ± 3.42^{az}
Group II (DC)	198.33 ± 1.66^{bw}	186.66 ± 3.33^{bx}	158.50 ± 0.85^{by}	147.50 ± 0.64^{bz}
Group III (G)	206.16 ± 1.29^{bw}	216.66 ± 0.69^{cx}	233.50 ± 0.78^{cy}	247.50 ± 0.64^{cz}
Group IV (GS+SC)	190.83 ± 2.71^{bcw}	207.50 ± 0.64^{dx}	215.00 ± 1.29^{dy}	230.00 ± 1.29^{dz}
Group V (GS+SC+G)	194.16 ± 3.27^{bw}	213.00 ± 3.27^{cx}	230.83 ± 0.83^{cy}	242.50 ± 0.64^{ez}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

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

Groups	Days Post Treatment			
	3	15	30	45
Group I (NC)	88.48 ± 0.78^{aw}	110 ± 0.50^{ax}	100.50 ± 1.93^{ay}	73.85 ± 1.39^{az}
Group II (DC)	471.14 ± 1.75^{bw}	487.25 ± 2.84^{bx}	501.98 ± 2.27^{by}	528.50 ± 3.09^{bz}
Group III (G)	480.20 ± 2.53^{bw}	384.33 ± 1.78^{cx}	371.50 ± 2.19^{cy}	235.11 ± 1.23^{cz}
Group IV (GS+SC)	475.68 ± 3.38^{bw}	447.27 ± 0.21^{dx}	391.50 ± 2.71^{dy}	323.40 ± 2.85^{dz}
Group V (GS+SC+G)	472.85 ± 0.64^{bw}	428.05 ± 0.24^{ex}	352.21 ± 1.06^{ey}	306.13 ± 0.80^{ez}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

Fig. 1: The mean (± SE) animal body weight (g) values of different groups at different intervals of time

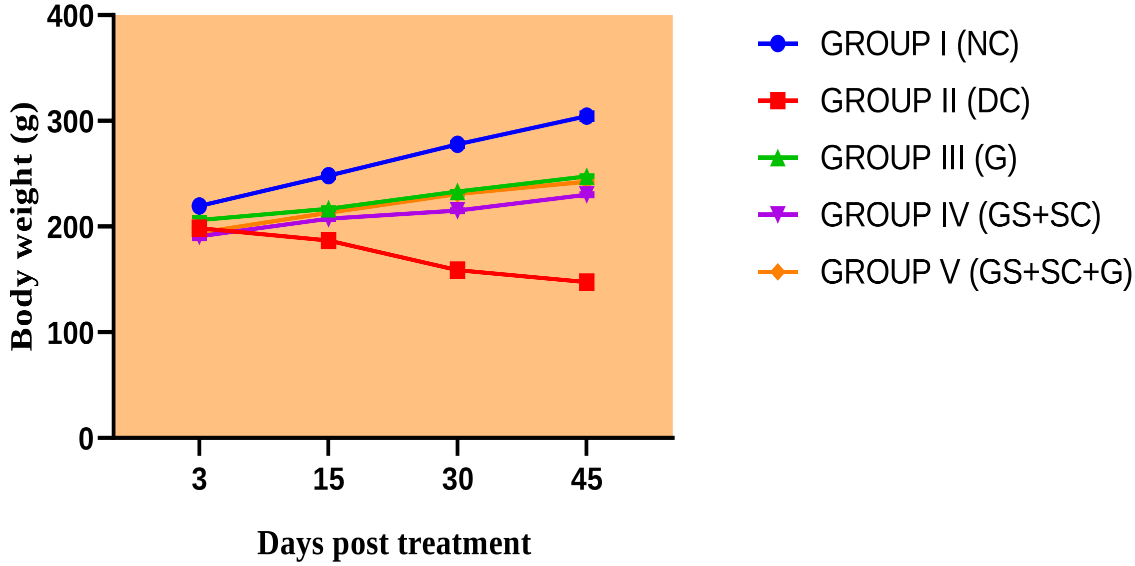


Fig. 2: The mean (± SE) serum glucose (mg/dL) values of different groups at different intervals of time

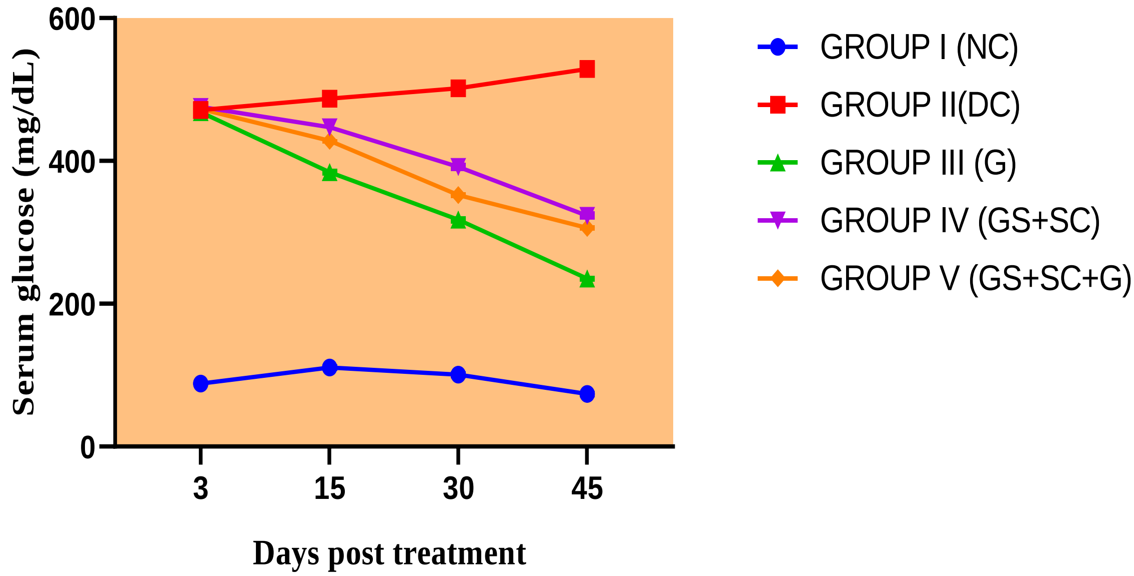


Table 3. The mean (±SE) serum alanine aminotransferase (ALT) (IU/L) values of different groups at different intervals of time

Groups	Days Post Treatment		
	15	30	45
Group I (NC)	59.50 ± 2.19^{ax}	63.78 ± 1.43^{ay}	60.07 ± 0.14^{az}
Group II (DC)	191.16 ± 2.41^{bx}	232.52 ± 0.47^{by}	243.42 ± 0.95^{bz}
Group III (G)	140.83 ± 0.75^{cx}	114.90 ± 0.82^{cy}	96.28 ± 0.89^{cz}
Group IV (GS+SC)	177.70 ± 0.59^{dx}	158.70 ± 0.49^{dy}	121.45 ± 0.27^{dz}
Group V (GS+SC+G)	163.10 ± 0.74^{ex}	135.40 ± 0.80^{ey}	106.77 ± 1.05^{ez}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

Table 4. The mean (±SE) serum aspartate aminotransferase (AST) (IU/L) values of different groups at different intervals of time

Groups	Days Post Treatment		
	15	30	45
Group I (NC)	73.90 ± 0.09^{ax}	68.54 ± 1.9^{axy}	68.18 ± 0.24^{ay}
Group II (DC)	262.88 ± 0.66^{bx}	270.66 ± 0.70^{by}	278.20 ± 2.11^{by}
Group III (G)	179.67 ± 0.81^{cx}	133.99 ± 1.46^{cy}	102.59 ± 1.60^{cz}
Group IV (GS+SC)	247.86 ± 0.14^{dx}	208.24 ± 0.48^{dy}	192.15 ± 0.68^{dz}
Group V (GS+SC+G)	227.65 ± 0.29^{ex}	188.37 ± 1.99^{ey}	163.00 ± 0.72^{ez}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

Fig. 3: The mean (± SE) serum alanine aminotransferase (ALT) (IU/L) values of different groups at different intervals of time

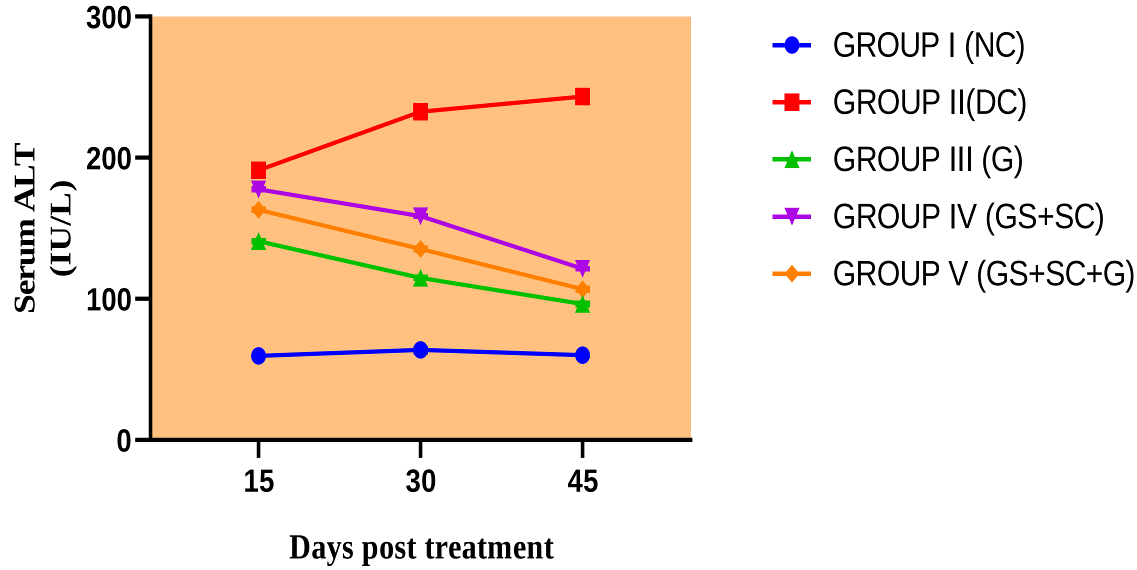


Fig. 4: The mean (± SE) serum aspartate aminotransferase (AST) (IU/L) values of different groups at different intervals of time

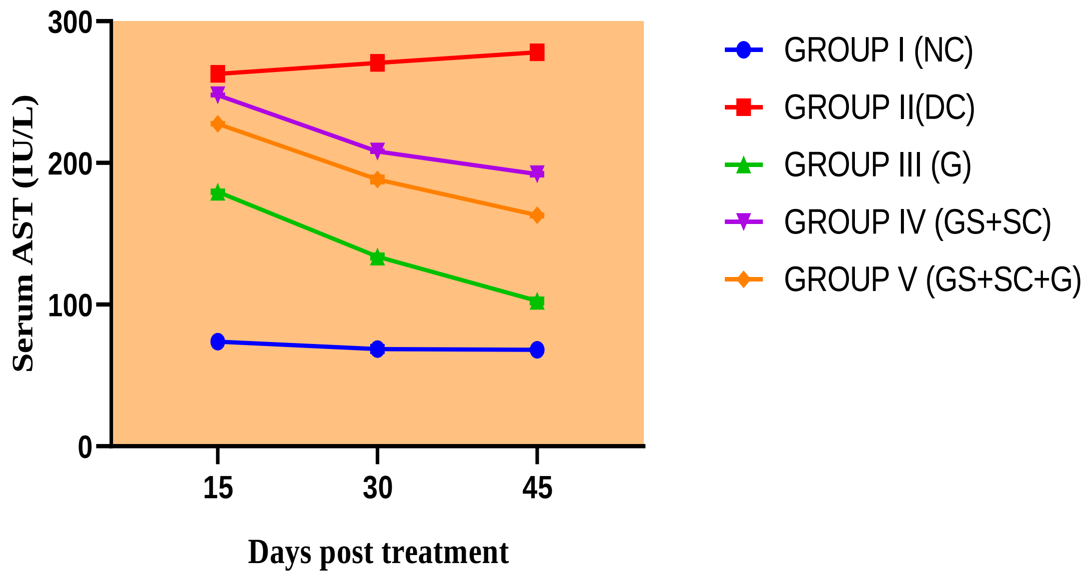


Table 5. The mean (±SE) serum triglyceride (mg/dL) values of different groups at different intervals of time

Groups	Days Post Treatment		
	15	30	45
Group I (NC)	107.42 ± 1.56^{ax}	106.54 ± 1.02^{ax}	106.62 ± 0.67^{ax}
Group II (DC)	234.97 ± 1.82^{bx}	269.44 ± 2.08^{by}	303.34 ± 0.68^{bz}
Group III (G)	200.51 ± 0.52^{cx}	162.57 ± 0.55^{cy}	107.71 ± 3.03^{az}
Group IV (GS+SC)	212.17 ± 0.50^{dx}	177.07 ± 1.75^{dy}	131.19 ± 1.21^{cz}
Group V (GS+SC+G)	185.75 ± 0.15^{ex}	151.49 ± 0.75^{ey}	111.24 ± 0.28^{az}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

Table 6. The mean (±SE) serum cholesterol (mg/dL) values of different groups at different intervals of time

Groups	Days Post Treatment		
	15	30	45
Group I (NC)	92.16 ± 0.65^{ax}	89.66 ± 0.88^{ay}	81.00 ± 0.77^{az}
Group II (DC)	161.33 ± 0.53^{bx}	179.00 ± 0.68^{by}	212.50 ± 1.61^{bz}
Group III (G)	126.50 ± 1.42^{cx}	116.50 ± 0.64^{cy}	108.00 ± 0.68^{cz}
Group IV (GS+SC)	147.50 ± 1.67^{dx}	130.00 ± 2.06^{dey}	117.50 ± 3.74^{cy}
Group V (GS+SC+G)	138.00 ± 2.84^{dx}	122.50 ± 1.67^{cey}	106.50 ± 0.38^{cz}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

Fig. 5: The mean (± SE) serum triglyceride (mg/dL) values of different groups at different intervals of time

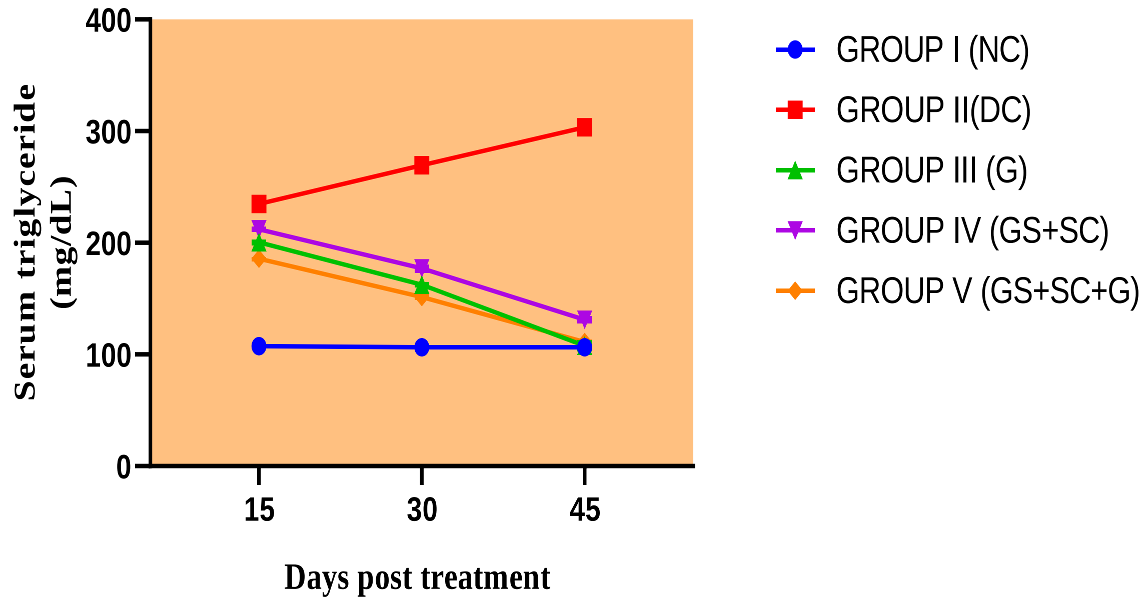


Fig. 6: The mean (± SE) serum cholesterol (mg/dL) values of different groups at different intervals of time

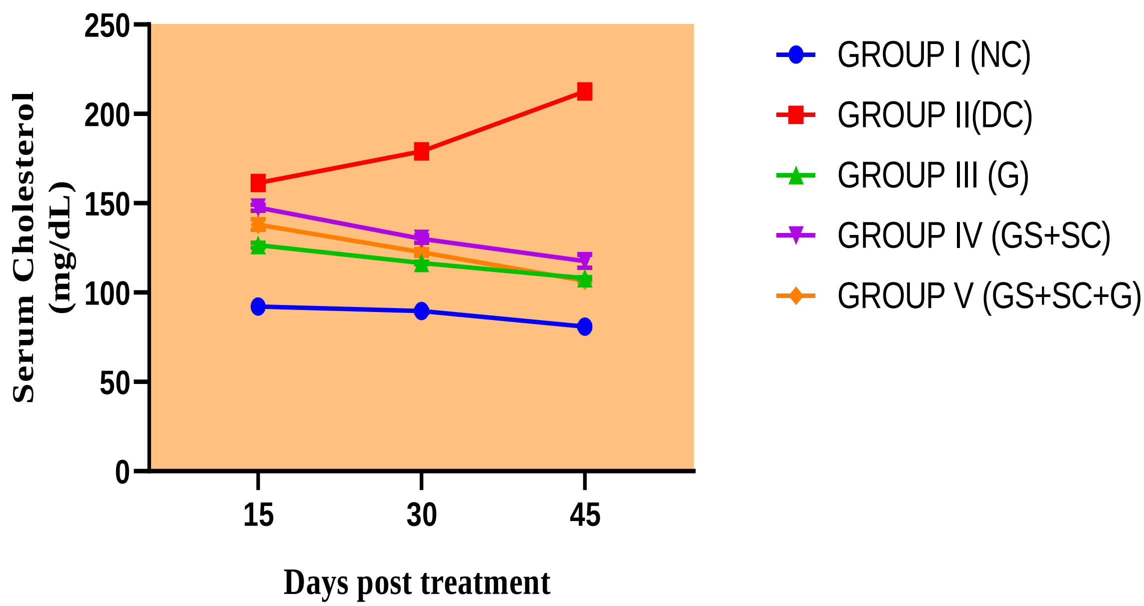


Table 7. The mean (±SE) haemoglobin (g%) values of different groups at different intervals of time

Groups	Days Post Treatment		
	15	30	45
Group I (NC)	14.24±0.22^{ax}	15.80±0.28^{ay}	15.44±0.04^{ay}
Group II (DC)	9.41±0.29^{bx}	7.96±0.19^{bxy}	7.21±0.25^{by}
Group III (G)	10.93±0.22^{cx}	12.25±0.18^{cx}	14.56±0.20^{cy}
Group IV (GS+SC)	11.01±0.05^{cx}	12.47±0.01^{cy}	13.65±0.27^{cz}
Group V (GS+SC+G)	11.03±0.19^{cx}	12.99±0.003^{dy}	13.85±0.09^{cz}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

Table 8. The mean (±SE) RBC count ($\times 10^{6}$ /µL) values of different groups at different intervals of time

Groups	Days Post Treatment		
	15	30	45
Group I (NC)	8.95±0.07^{ax}	9.76±0.05^{ay}	9.59±0.04^{ay}
Group II (DC)	5.72±0.08^{bx}	5.38±0.09^{bx}	5.12±0.06^{by}
Group III (G)	7.21±0.08^{cx}	9.43±0.07^{cy}	9.77±0.03^{az}
Group IV (GS+SC)	6.95±0.01^{cx}	7.67±0.03^{dy}	7.92±0.02^{cz}
Group V (GS+SC+G)	7.06±0.04^{cx}	7.37±0.11^{dy}	8.11±0.03^{dz}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

Fig. 7: The mean (± SE) haemoglobin (g%) values of different groups at different intervals of time

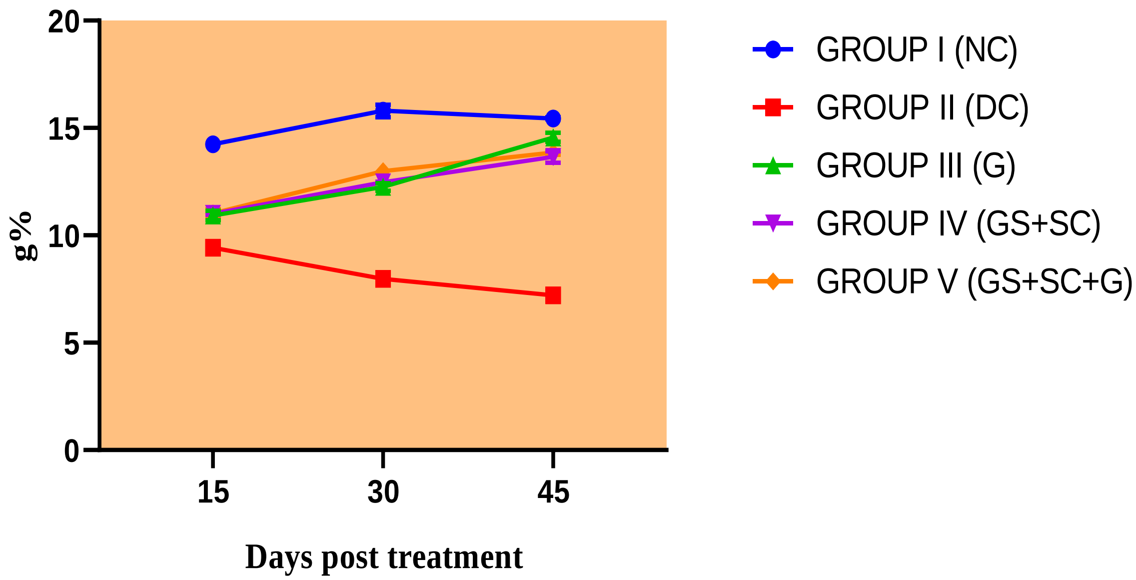


Fig. 8: The mean (± SE) RBC count ($x10^6/\mu L$) values of different groups at different intervals of time

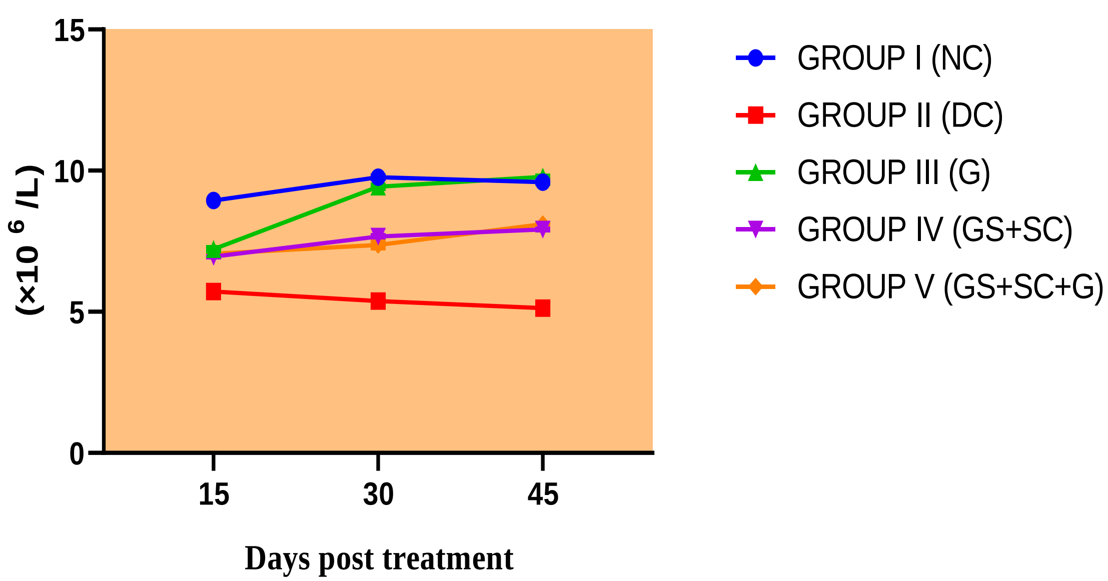


Table 9. The mean (±SE) WBC count (×10 ^{3} /µL) values of different groups at different intervals of time

Groups	Days Post Treatment		
	15	30	45
Group I (NC)	8.16 ± 0.04^{ax}	7.94 ± 0.10^{ax}	8.04 ± 0.18^{ax}
Group II (DC)	12.27 ± 0.20^{bx}	14.00 ± 0.44^{bxy}	14.09 ± 0.05^{by}
Group III (G)	10.45 ± 0.36^{cx}	10.02 ± 0.20^{cx}	8.69 ± 0.05^{ay}
Group IV (GS+SC)	10.86 ± 0.08^{cx}	10.47 ± 0.24^{cxy}	9.77 ± 0.20^{cy}
Group V (GS+SC+G)	10.26 ± 0.16^{cx}	10.01 ± 0.31^{cx}	7.87 ± 0.19^{ady}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

Table 10. The mean (±SE) platelet count (×10 ^{3} /µL) values of different groups at different interval of time

Groups	Days Post Treatment		
	15	30	45
Group I (NC)	445.17 ± 6.02^{ax}	443.55 ± 5.70^{ax}	452.03 ± 4.82^{ax}
Group II (DC)	$295.16 \pm 5.70^{\mathbf{bx}}$	282.68 ± 5.15^{bx}	266.83 ± 4.48^{by}
Group III (G)	327.47 ± 7.26^{cx}	366.27 ± 2.19^{cy}	404.45 ± 3.76^{cz}
Group IV (GS+SC)	321.34 ± 1.26^{cx}	348.16 ± 1.53^{dey}	412.24 ± 1.02^{cz}
Group V (GS+SC+G)	325.92 ± 5.12^{cx}	356.89 ± 8.39^{cey}	428.13 ± 2.10^{dz}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

Fig. 9: The mean (± SE) WBC count (x10^3/µL) values of different groups at different intervals of time

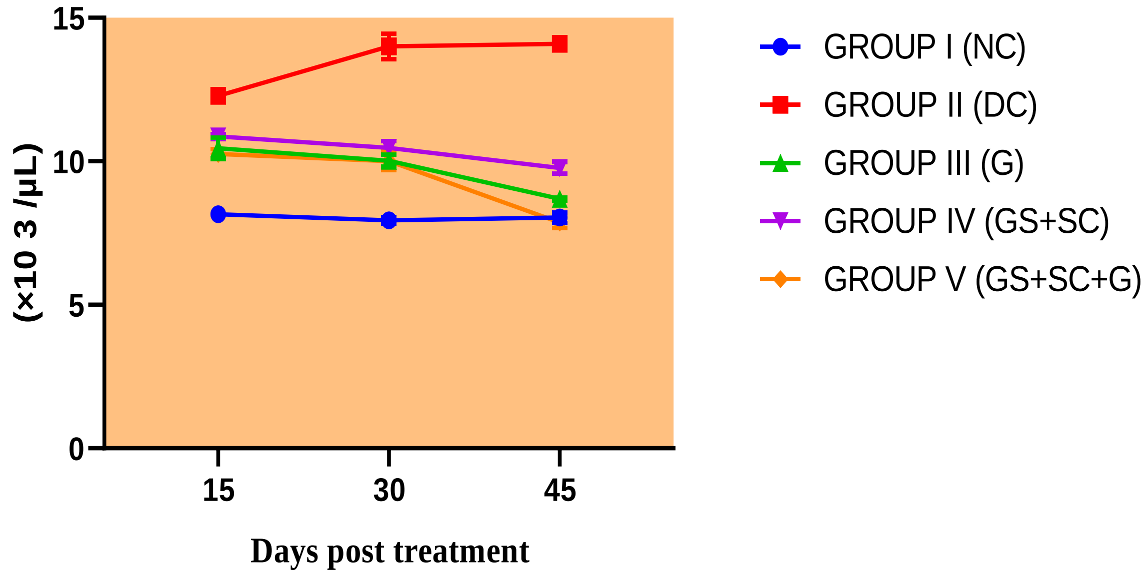


Fig. 10: The mean (± SE) platelet count (x10^3/µL) values of different groups at different intervals of time

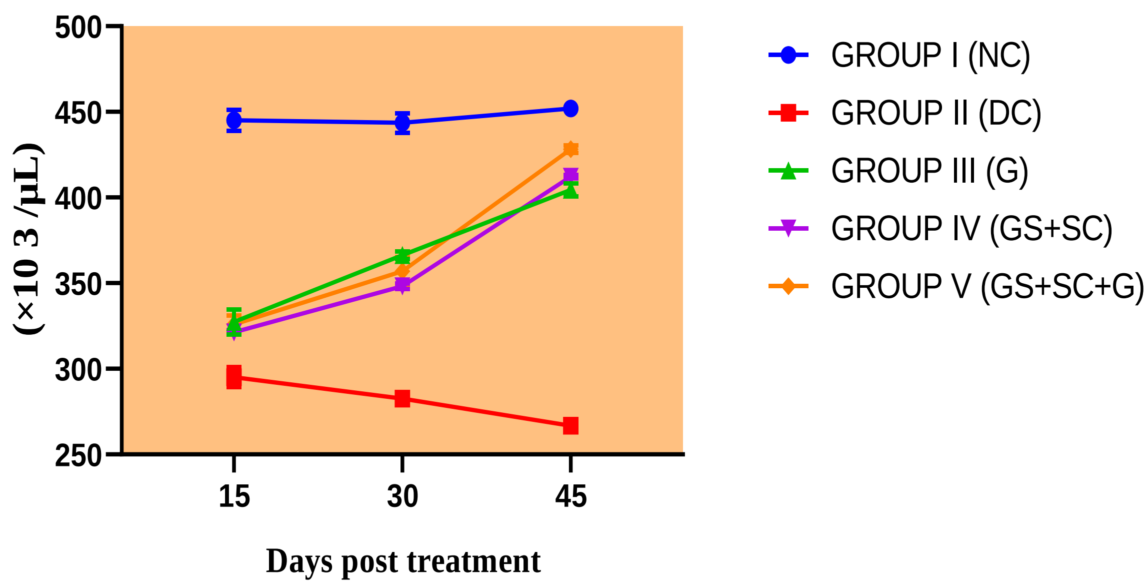


Table 11. The mean (± SE) values of activities of SOD (U/min/mg protein) in the liver of different groups at different intervals of time

Groups	Days Post Treatment		
	15	30	45
Group I (NC)	24.87 ± 0.45^{ax}	23.78 ± 0.77^{axy}	27.26 ± 0.82^{ay}
Group II (DC)	4.70 ± 0.07^{bx}	3.76 ± 0.05^{by}	3.65 ± 0.12^{by}
Group III (G)	6.71 ± 0.07^{cx}	8.305 ± 0.04^{cy}	10.41 ± 0.10^{cz}
Group IV (GS+SC)	5.04 ± 0.01^{dx}	6.69 ± 0.06^{dy}	7.32 ± 0.02^{dz}
Group V (GS+SC+G)	5.22 ± 0.03^{ex}	6.74 ± 0.01^{ey}	7.72 ± 0.02^{ez}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

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

Groups	Days Post Treatment		
	15	30	45
Group I (NC)	70.72 ± 0.48^{ax}	72.82 ± 0.78^{ay}	76.86 ± 0.34^{az}
Group II (DC)	23.66 ± 0.58^{bx}	21.27 ± 0.83^{by}	18.16 ± 0.20^{by}
Group III (G)	28.44 ± 0.12^{cx}	36.44 ± 0.64^{cy}	40.03 ± 0.17^{cz}
Group IV (GS+SC)	21.87 ± 0.42^{bx}	24.84 ± 0.10^{dy}	29.35 ± 0.31^{dz}
Group V (GS+SC+G)	23.31 ± 0.20^{bx}	27.94 ± 0.17^{ey}	31.78 ± 0.08^{ez}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

Fig. 11: The mean (± SE) values of activities of superoxide dismutase (U/min/mg protein) in the liver of different groups at different intervals of time

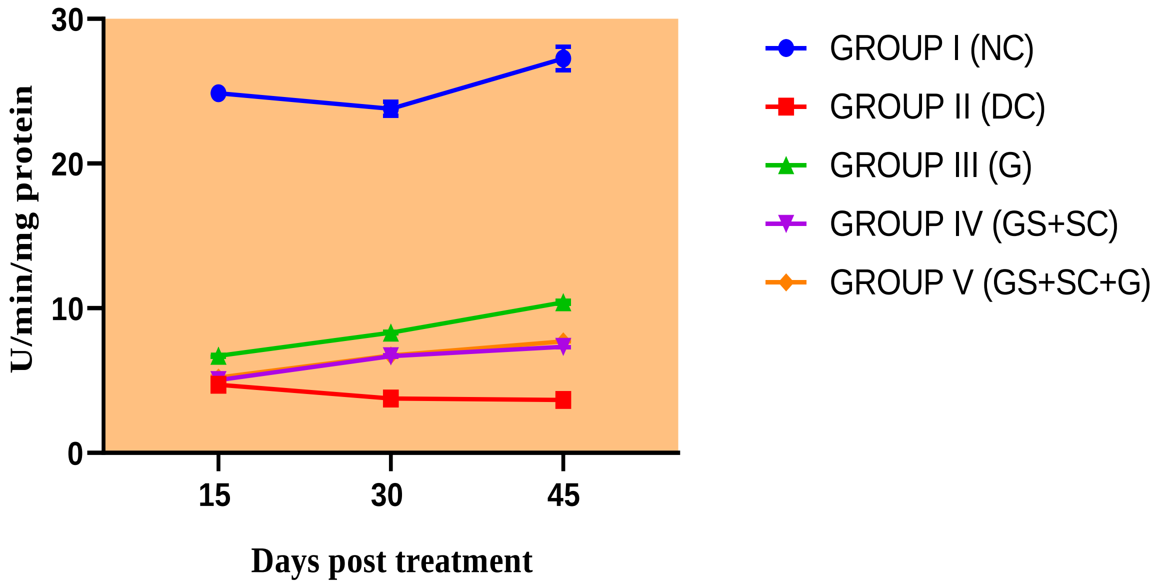


Fig. 12: The mean (± SE) values of activities of catalase (μ moles of H_2O_2/min/mg protein) in the liver of different groups at different intervals of time

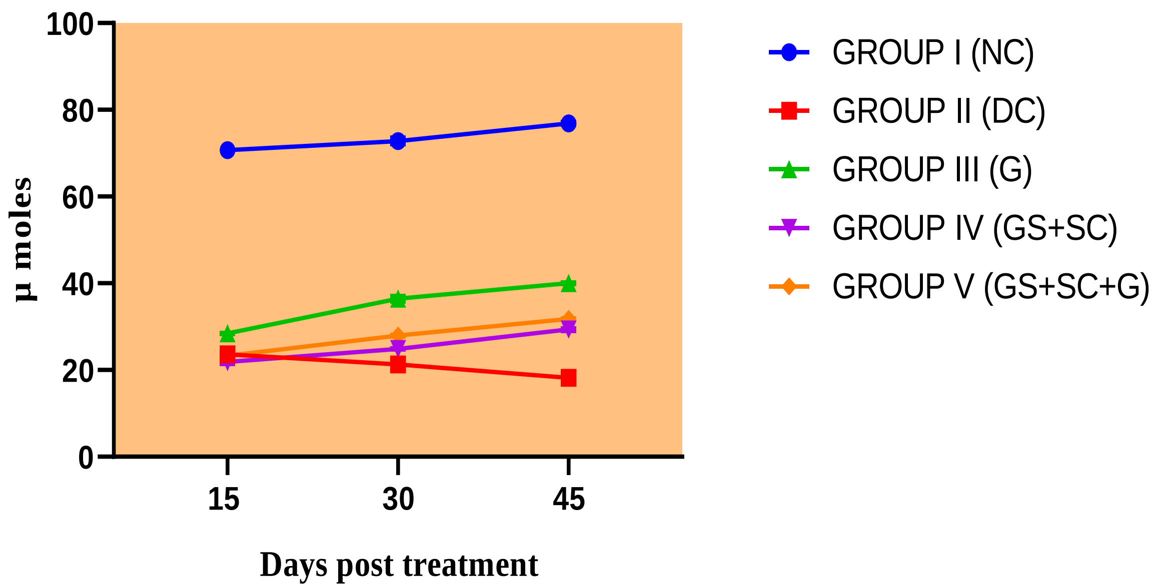


Table 13. The mean (± SE) values of activities of GPx (μM of glutathione utilized/min/mg protein) in the liver of different groups at different intervals of time

Groups	Days Post Treatment		
	15	30	45
Group I (NC)	38.16 ± 0.46^{ax}	38.24 ± 0.99^{ax}	43.06 ± 0.56^{ay}
Group II (DC)	16.61 ± 0.11^{bx}	15.27 ± 0.27^{by}	13.03 ± 0.17^{bz}
Group III (G)	22.56 ± 0.37^{cx}	24.24 ± 0.18^{cy}	28.24 ± 0.14^{cz}
Group IV (GS+SC)	16.45 ± 0.11^{bx}	17.95 ± 0.17^{dy}	19.63 ± 0.13^{dz}
Group V (GS+SC+G)	16.63 ± 0.04^{bx}	18.17 ± 0.02^{dy}	21.53 ± 0.23^{ez}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

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

Groups	Days Post Treatment		
	15	30	45
Group I (NC)	80.50 ± 0.34^{ax}	78.16 ± 0.54^{ay}	80.66 ± 0.71^{axy}
Group II (DC)	2.83 ± 0.16^{bx}	3.00 ± 0.25^{by}	3.66 ± 0.49^{bz}
Group III (G)	10.66 ± 0.42^{cx}	28.83 ± 0.83^{cy}	54.16 ± 2.34^{cz}
Group IV (GS+SC)	7.00 ± 0.36^{dx}	12.16 ± 0.47^{dy}	21.00 ± 0.36^{dz}
Group V (GS+SC+G)	8.50 ± 0.22^{ex}	15.66 ± 0.55^{ey}	26.00 ± 1.15^{ez}

Mean values with different superscripts differ significantly

Values are statistically significant at $P < 0.05$

Fig. 13: The mean (± SE) values of activities of glutathione peroxidase (µM of glutathione utilized/min/mg protein) in the liver of different groups at different intervals of time

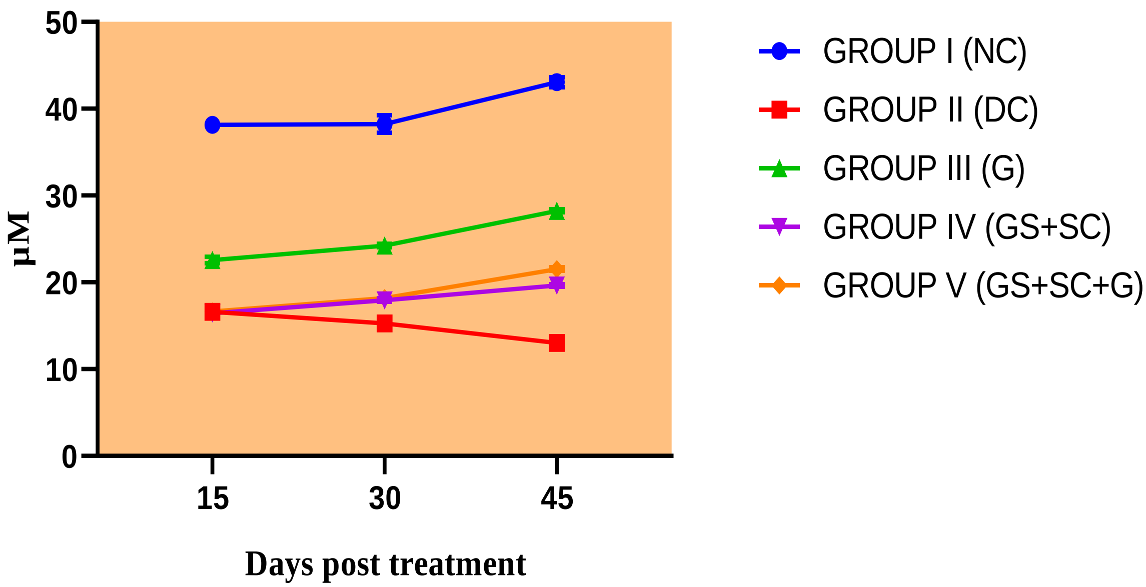


Fig. 14: The mean (± SE) percentage positivity of insulin secreting cells of different groups at different intervals of time

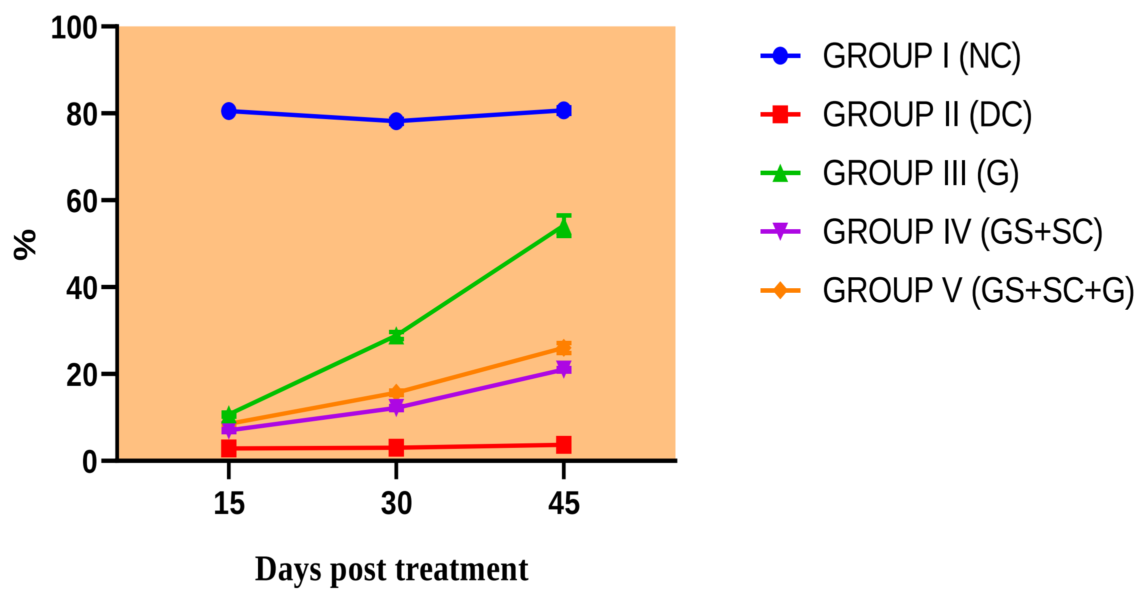


Plate 1: *Gymnema sylvestre* plant

Plate 2: *Swertia chirayita* plant

Plate 3: Comparison of normal control (Group I) and diabetic control (Group II) rats to other treatment groups (Group III, IV and V) on Day 45 of the experiment.

Plate 4: Diabetic control rats compared to Group IV and Group V rats on Day 45 of the experiment showing poor body condition with soiled, rough and dull hair coat.

Plate 5: Glibenclamide treated rats compared to Group IV and Group V rats on Day 45 of the experiment showing comparable improvement in body condition.

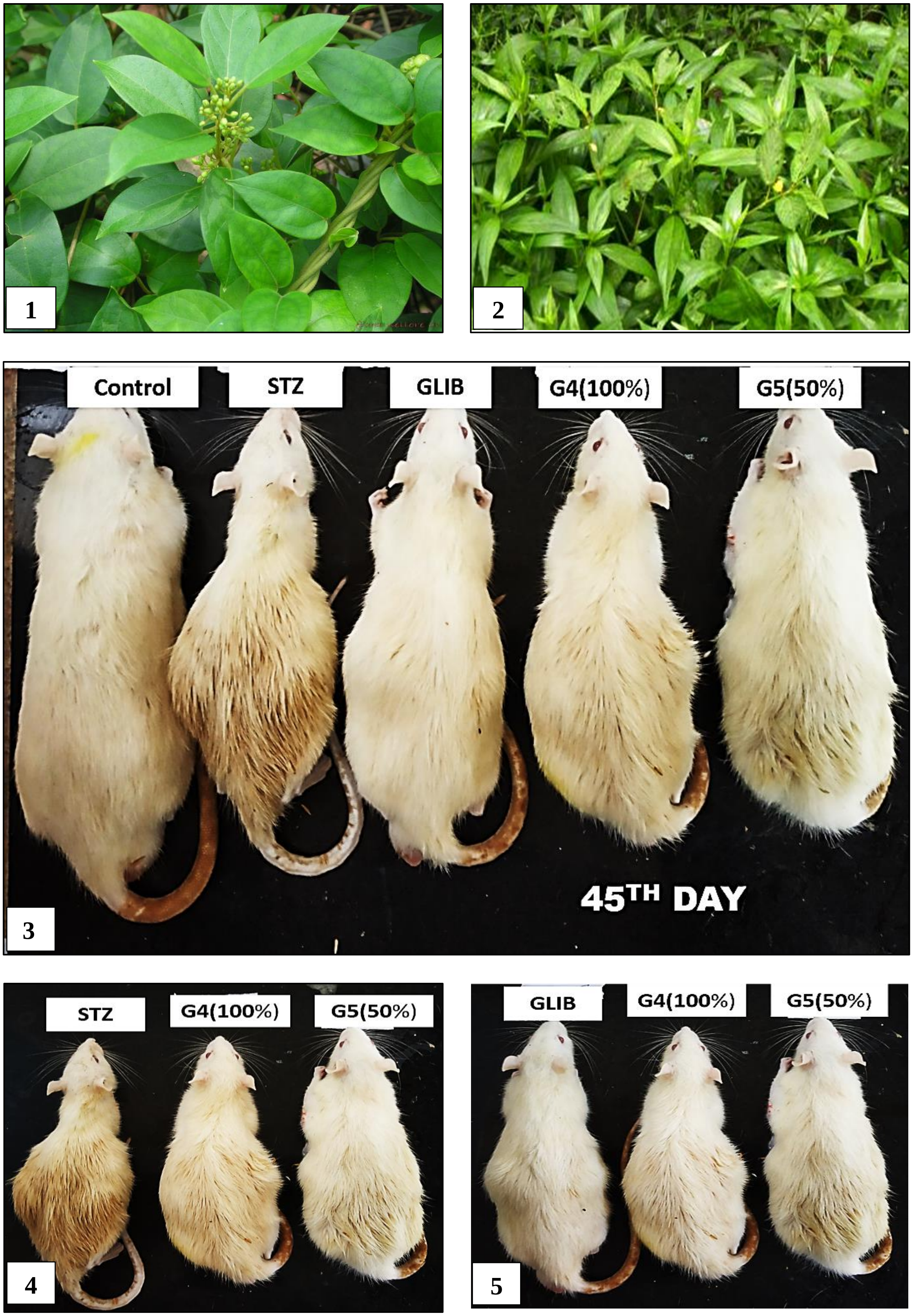

1
2
Control
STZ
GLIB
G4(100%)
G5(50%)
45TH DAY
3
STZ
G4(100%)
G5(50%)
4
GLIB
G4(100%)
G5(50%)
5

Plate 6: Pancreas from a control (Group I) rat showing normal architecture on 45^{th} day of the experiment.

Plate 7: Pancreas from a diabetic control (Group II) rat appearing as pale, thin gelatinous mass on 45^{th} day.

Plate 8: Pancreas from a glibenclamide treatment group (Group III) showing improvement in architecture on 45^{th} day.

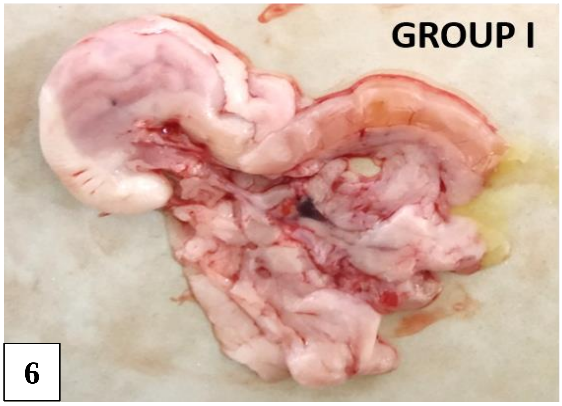
Plate 9: Pancreas from a Group IV rat showing improvement in architecture on 45^{th} day.

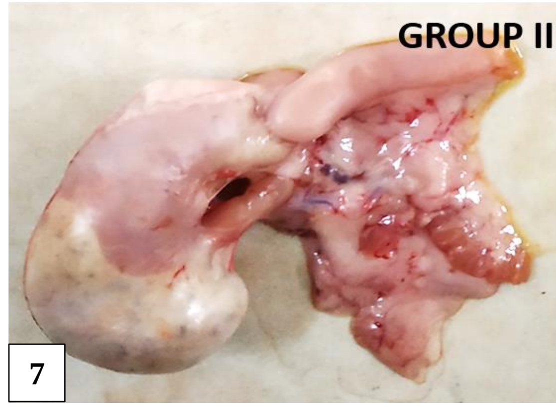
Plate 10: Pancreas from a Group V rat showing improvement on 45^{th} day.

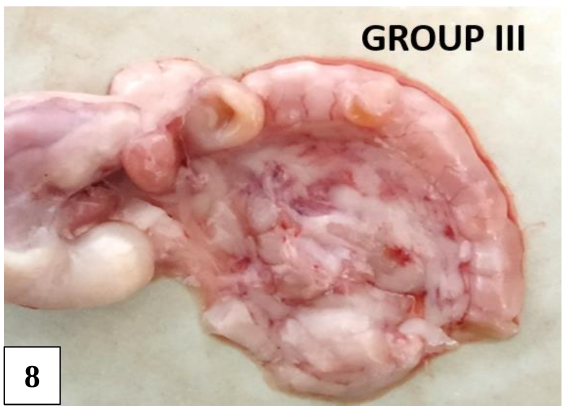
Plate 11: Liver from a control rat (Group I) showing normal appearance on 45^{th} day of the experiment.

Plate 12: Liver from a diabetic control (Group II) rat appearing enlarged and pale on 45^{th} day.

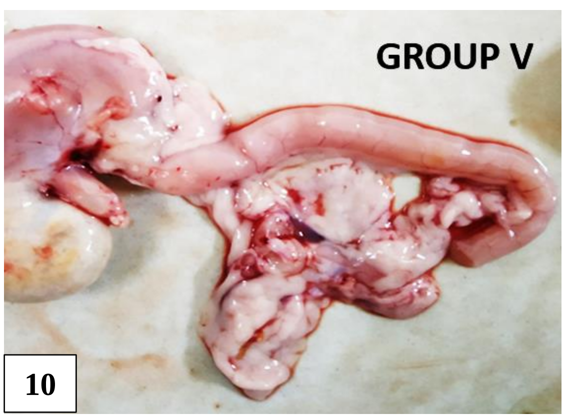
Plate 13: Liver from a treatment group (Group V) rat showing normal appearance of liver on 45^{th} day.


GROUP I
6

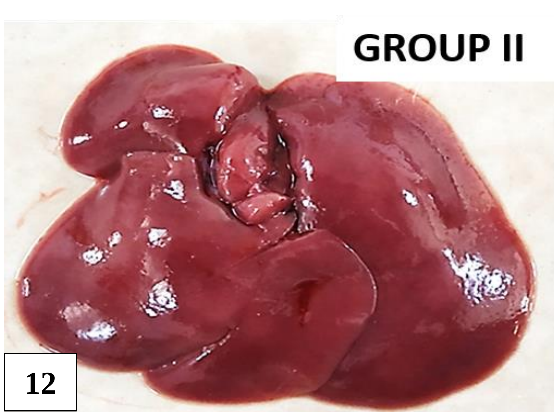

GROUP II
7


GROUP III
8


GROUP IV
9


GROUP V
10


GROUP I
11


GROUP II
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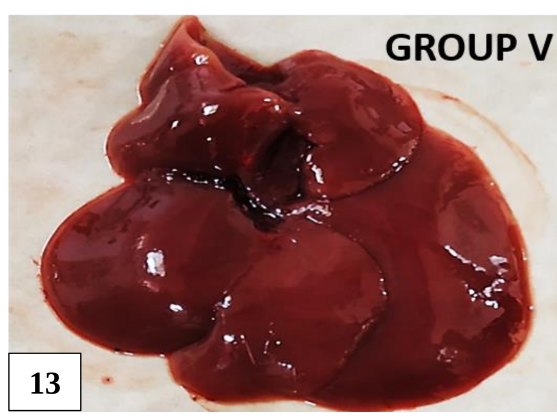

GROUP V
13

Plate 14: Section of pancreas from a normal control rat (Group I) showing well-formed islet of Langerhans.

H&E X 200

Plate 15: Section of pancreas from a normal control rat (Group I) showing normal islet of Langerhans with round to oval shaped, compactly arranged beta cells at the centre and alpha cells at the periphery.

H&E X 400

Plate 16: Section of liver from a normal control rat (Group I) on 45th day showing normal architecture.

H&E X 100

Plate 17: Section of kidney from a normal control rat (Group I) showing normal histological architecture of glomeruli and convoluted tubules.

H&E X 200

Plate 18: Section of heart from a normal control rat (Group I) showing compact arrangement of cardiac myocytes.

H&E X 200

Plate 19: Section of spleen from a normal control rat (Group I) showing normal architecture with well formed splenic corpuscle.

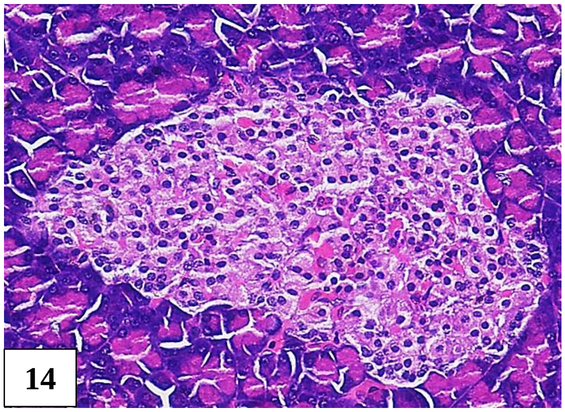
H&E X 100

Plate 20: Section of pancreas from a diabetic control rat (Group II) showing exocrine pancreatic necrosis and loss of architecture of islets with sparse cellularity on Day 15.

H&E X 200

Plate 21: Section of pancreas from a diabetic control rat (Group II) showing loss of normal architecture and degeneration and necrosis of β cell in islets of Langerhans on Day 15.

H&E X 200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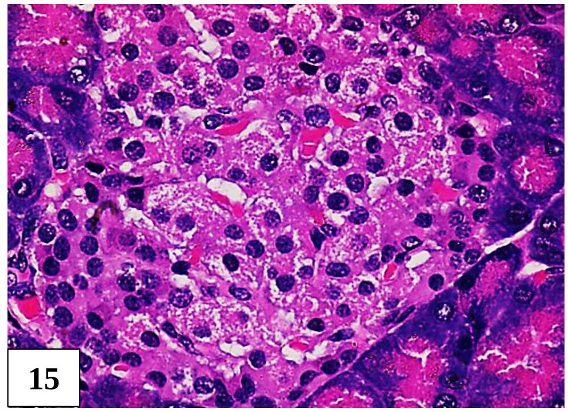
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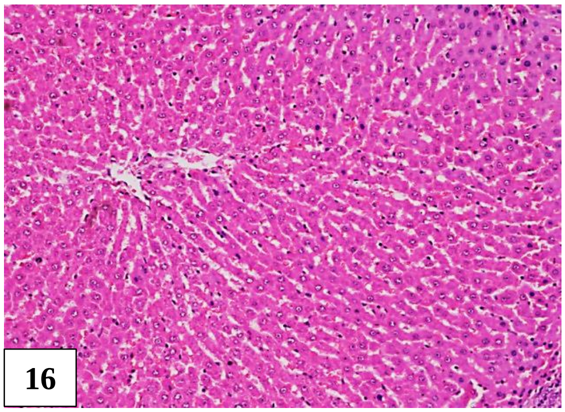
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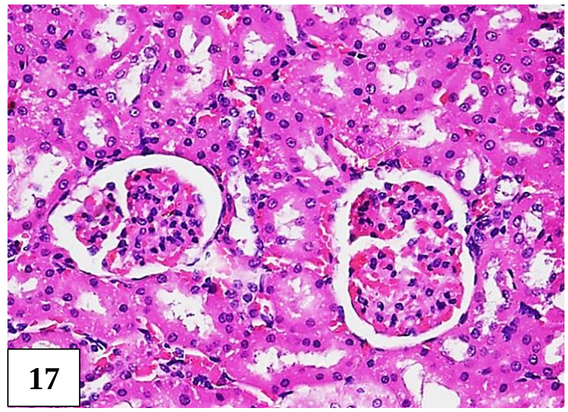
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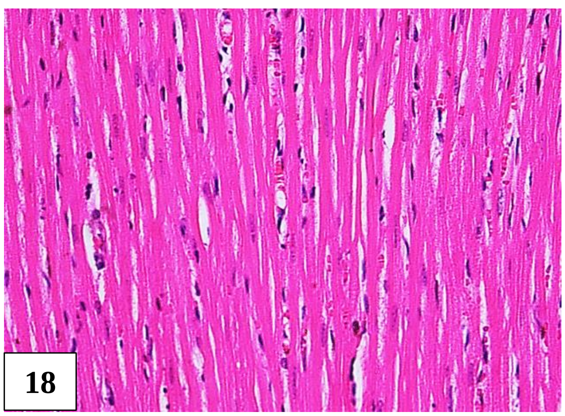
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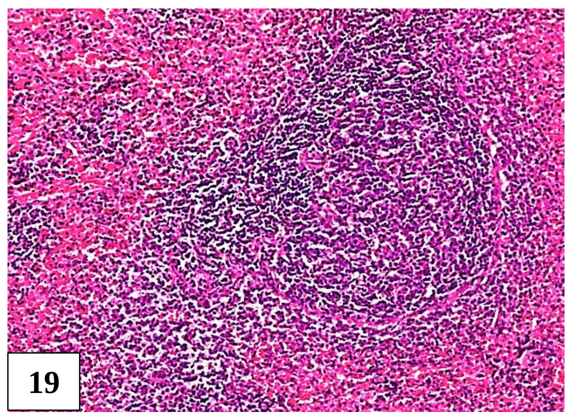
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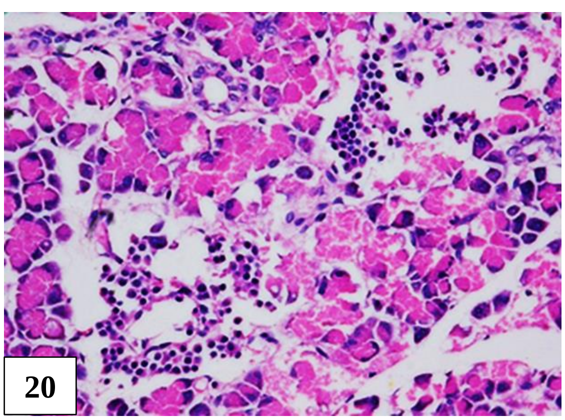
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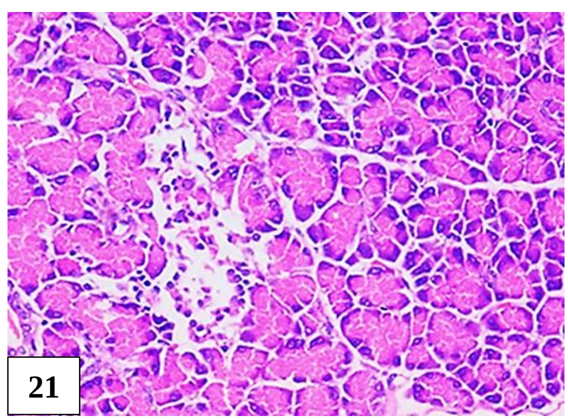

21

Plate 22: Section of pancreas from a diabetic control rat (Group II) showing loss of normal architecture with sparse cellularity, degeneration and necrosis of β cell on Day 15.

H&E X 400

Plate 23: Section of pancreas from a diabetic control rat (Group II) showing infiltration of inflammatory cells along with vascular congestion indicating pancreatitis on Day 15.

H&E X 200

Plate 24: Section of pancreas from a diabetic control rat (Group II) on 30th day showing increased cellularity with α cell hyperplasia.

H&E X 200

Plate 25: Section of pancreas from a diabetic control rat (Group II) on 45th day showing an improvement in exocrine and endocrine portion with increased α cell cellularity.

H&E X 200

Plate 26: Section of pancreas from a diabetic control rat (Group II) showing mild ductal epithelial hyperplasia on Day 45.

H&E X 400

Plate 27: Section of liver from a diabetic control rat (Group II) showing highly swollen hepatocytes with cytoplasmic vacuolations on Day 15.

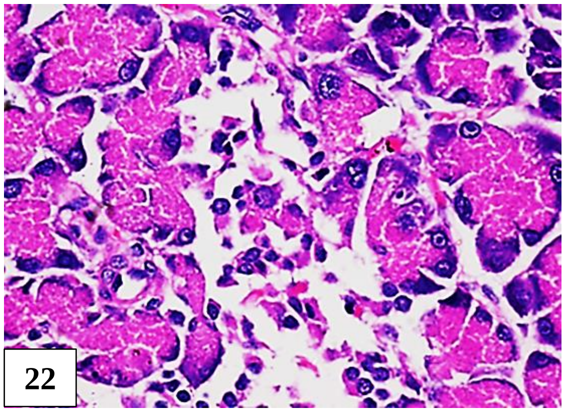
H&E X 400

Plate 28: Section of liver from a diabetic control rat (Group II) showing loss of hepatic cord architecture with multifocal areas showing apoptotic cells and bodies on Day 15.

H&E X 400

Plate 29: Section of liver from a diabetic control rat (Group II) showing highly swollen vacuolated cells and a focal necrotic area with infiltration of inflammatory cells on Day 15.

H&E X 200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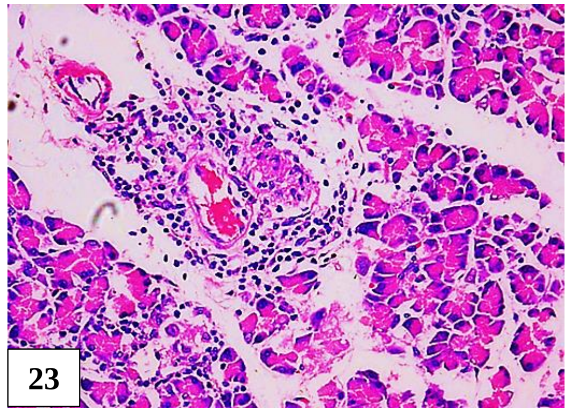
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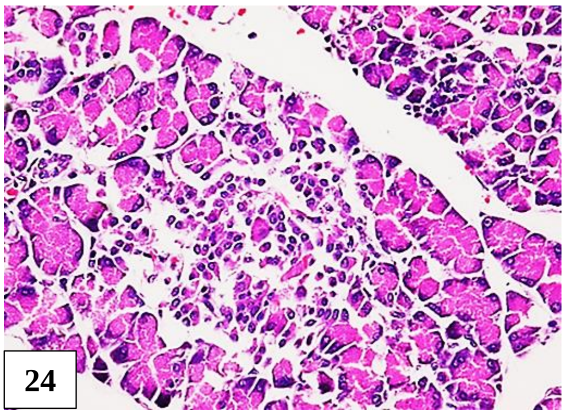
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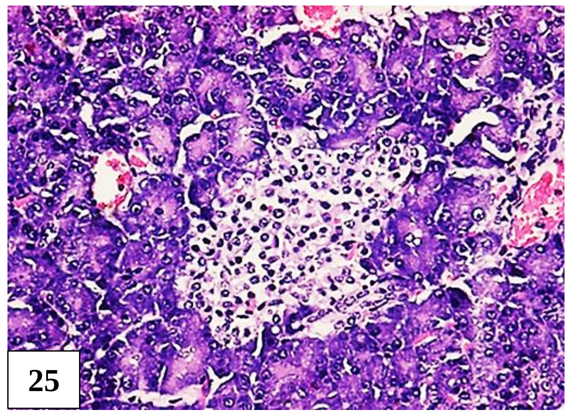
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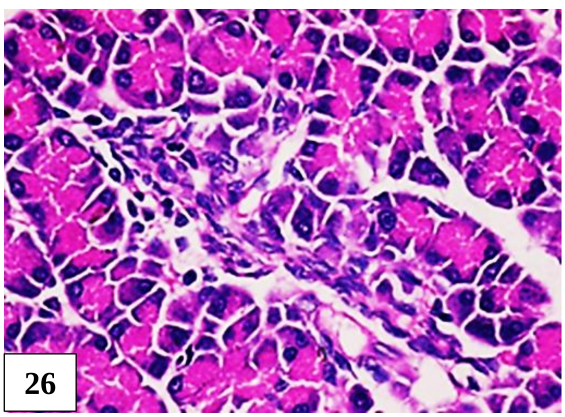
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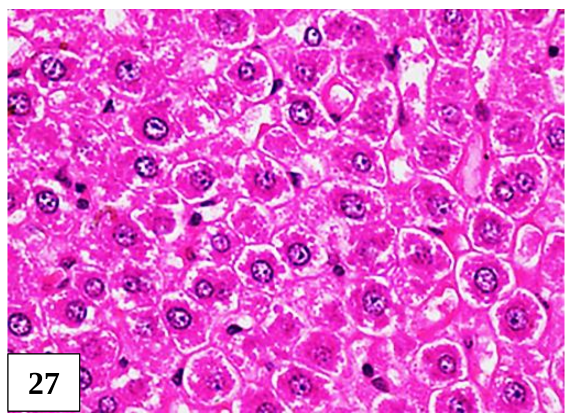
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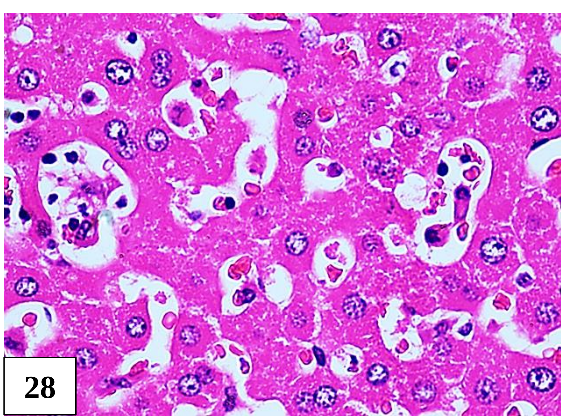
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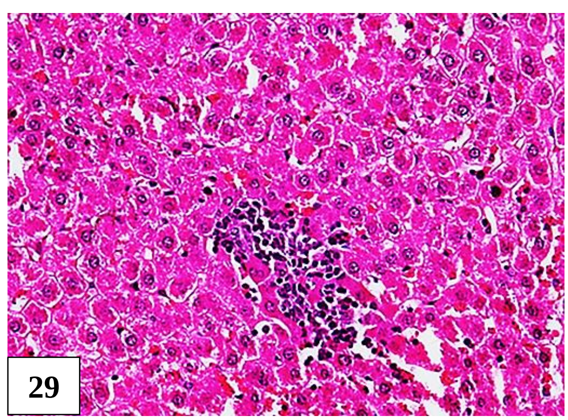
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Plate 30: Section of kidney from a diabetic control rat (Group II) showing vacuolar degeneration of tubular epithelial cells on Day 15.

H&E X 200

Plate 31: Section of heart from a diabetic control rat (Group II) showing multifocal myocardial degeneration and infiltration of inflammatory cells on Day 15.

H&E X 200

Plate 32: Section of spleen from a diabetic control rat (Group II) showing sparse cellularity with depletion of lymphoid cells in splenic corpuscles on Day 15.

H&E X 200

Plate 33: Section of liver from a diabetic control rat (Group II) showing multifocal areas of spaces with total loss of cells and occasional apoptotic bodies on Day 45.

H&E X 200

Plate 34: Section of kidney from a diabetic control rat (Group II) showing improvement in the architecture on Day 45.

H&E X 200

Plate 35: Section of heart from a diabetic control rat (Group II) showing vascular congestion on Day 45.

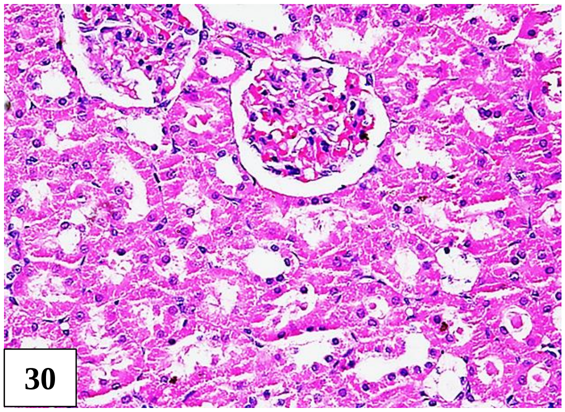
H&E X 200

Plate 36: Section of pancreas from a glibenclamide treatment group (Group III) on 15th day showing a small islet with mild α cell hyperplasia along with occasional cells with β cells morphology.

H&E X 400

Plate 37: Section of pancreas from a Group III rat on 30th day showing improvement in the size of the islet with more number of α cell and occasional cells with β cells morphology.

H&E X 400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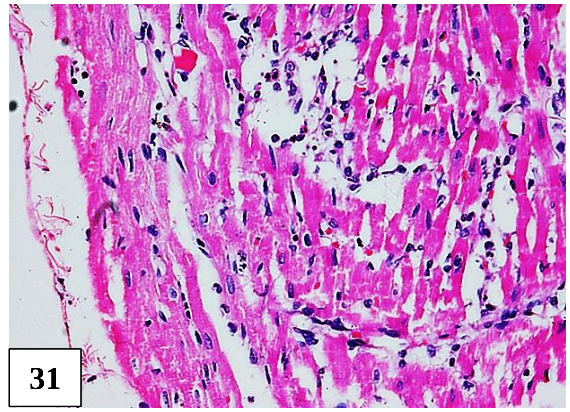
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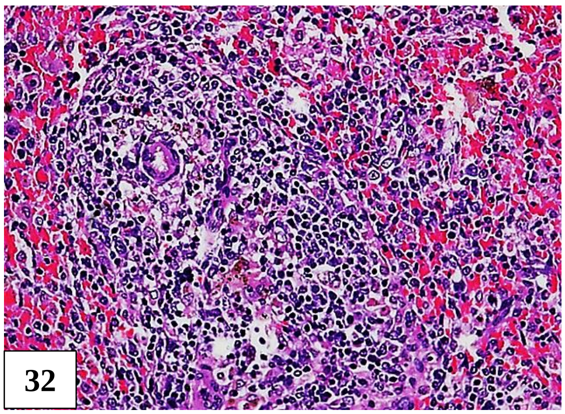
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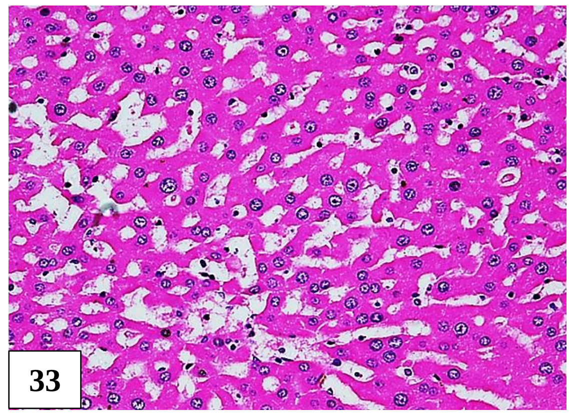
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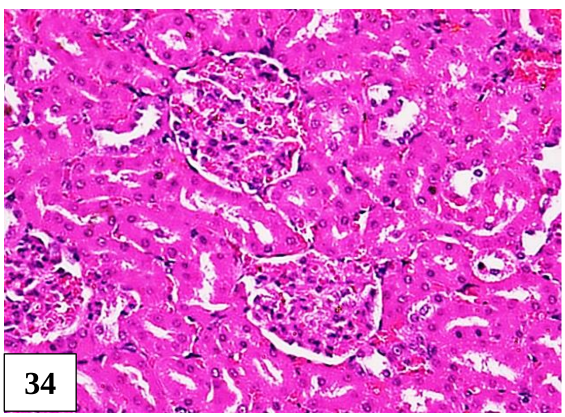
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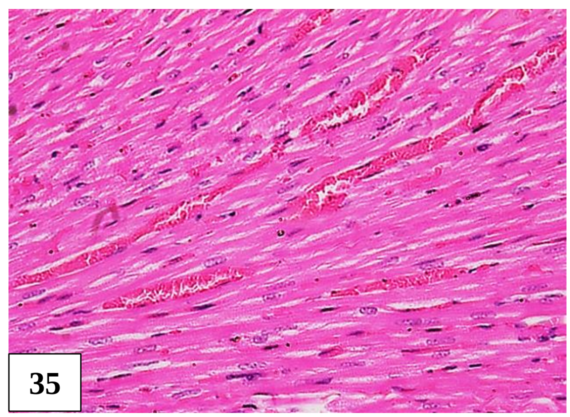
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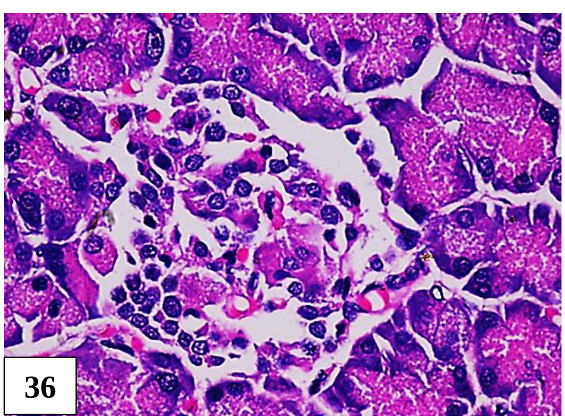
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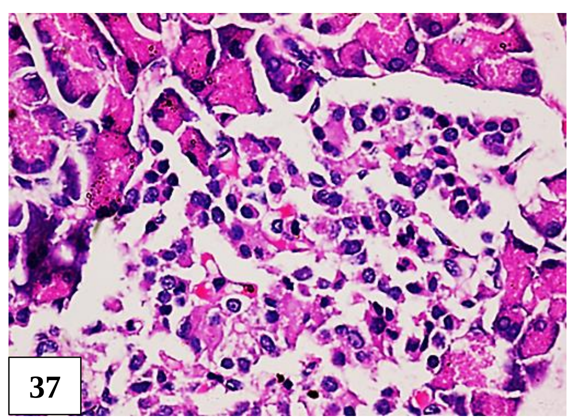
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Plate 38: Section of pancreas from a Group III rat on 45th day showing well formed islet with large number of β cells.

H&E X 400

Plate 39: Section of pancreas from a Group III rat on 45th day showing formation of multiple new islets.

H&E X 200

Plate 40: Section of liver from a Group III rat on 15th day showing highly swollen vacuolated hepatocytes and vascular congestion.

H&E X 400

Plate 41: Section of liver from a Group III rat on 45th day showing improvement in the architecture and reduced degenerative changes.

H&E X 400

Plate 42: Section of pancreas from treatment group (Group IV) on 15th day showing loss of normal architecture, degeneration and necrosis of β cell with sparse cellularity.

H&E X 400

Plate 43: Section of pancreas from a Group IV rat on 15th day showing loss of normal architecture of islet consisting of α cell and cystic empty spaces.

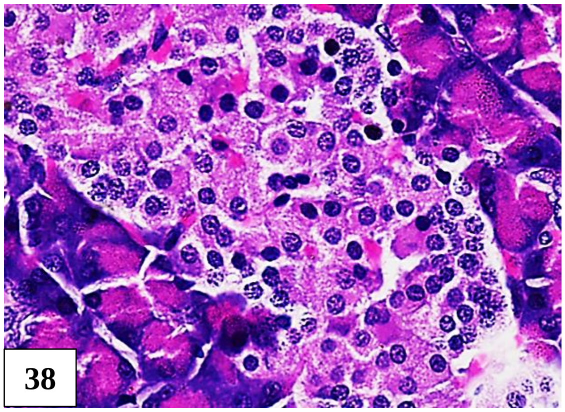
H&E X 400

Plate 44: Section of pancreas from a Group IV rat on 15th day showing exocrine pancreatic necrosis with infiltration of inflammatory cells.

H&E X 200

Plate 45: Section of pancreas from a Group IV rat on 30th day showing improvement in the cellularity in the islet with more number of α cell. Note persistence of cystic spaces.

H&E X 400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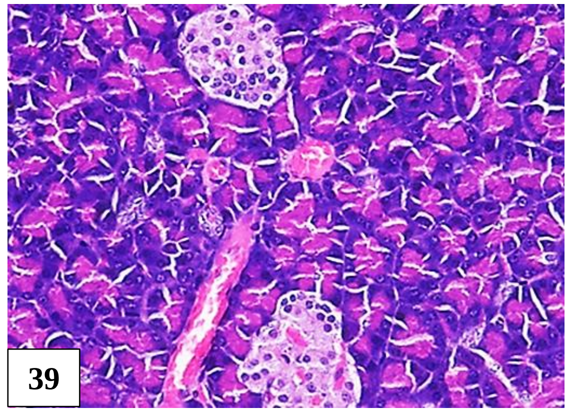
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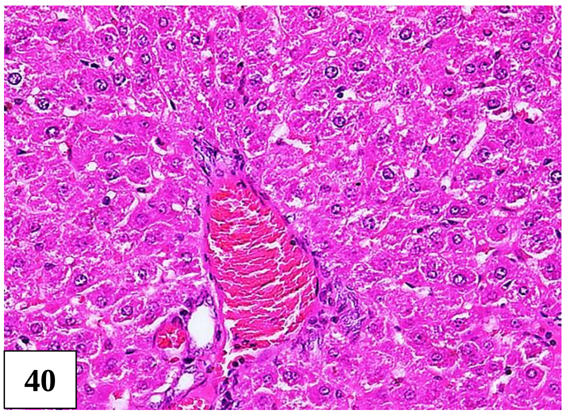
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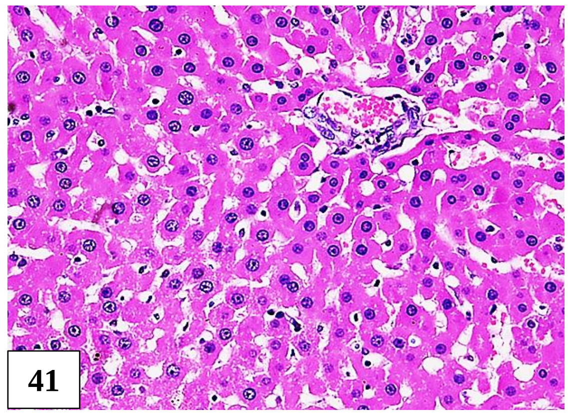
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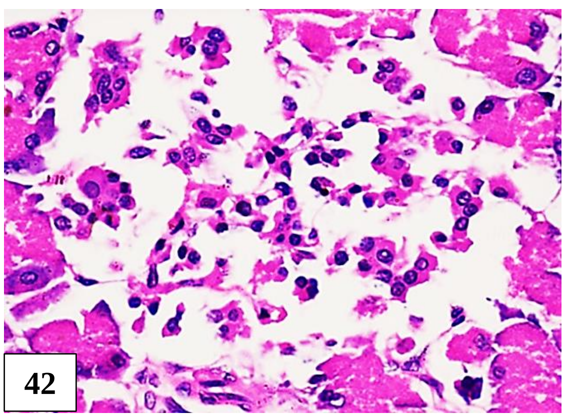
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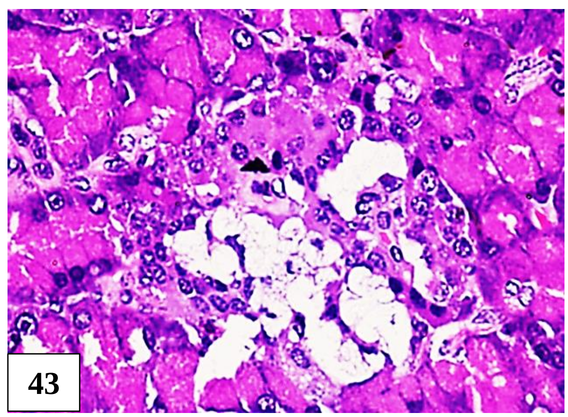
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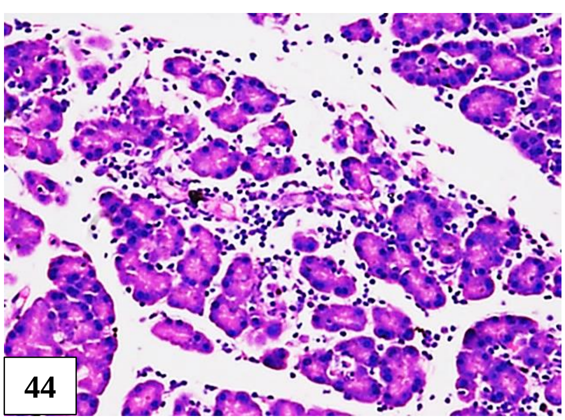
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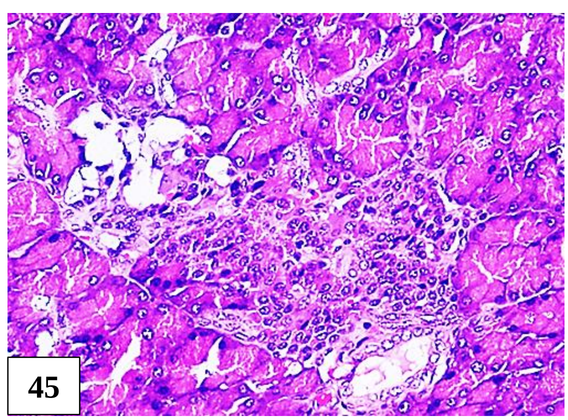
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Plate 46: Section of pancreas from a Group IV rat on 45th day showing moderately well formed islet with increase in cellularity and cells with β cell morphology.

H&E X 400

Plate 47: Section of liver from a Group IV rat on 15th day showing hepatocytes with vacuolar changes and occasional preapoptotic and apoptotic cells.

H&E X 400

Plate 48: Section of liver from a Group IV rat on 45th day showing moderate improvement in the architecture of the liver.

H&E X 200

Plate 49: Section of pancreas from treatment group rat (Group V) on 15th day showing loss of normal architecture with highly swollen, vacuolated and necrotic β cell and mild increase in α cell cellularity.

H&E X 400

Plate 50: Section of pancreas from a Group V rat on 15th day showing increased α cell cellularity with cystic spaces.

H&E X 400

Plate 51: Section of pancreas from a Group V rat on 30th day showing improvement in the cellularity with α cell and cells with β cell morphology in the islet.

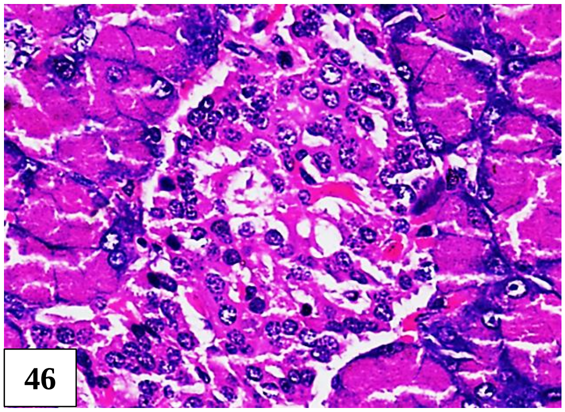
H&E X 400

Plate 52: Section of pancreas from a Group V rat on 45th day showing well formed islet with compact arrangement of cells. Note the cells with β cell morphology.

H&E X 400

Plate 53: Section of liver from a Group IV rat on 15th day showing swelling of hepatocyte with multifocal areas of necrosis and apoptotic cells and bodies.

H&E X 200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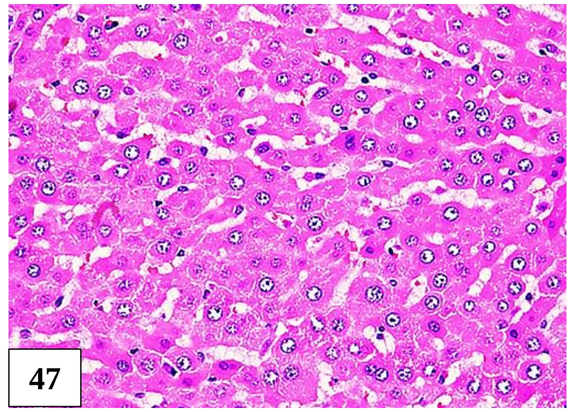
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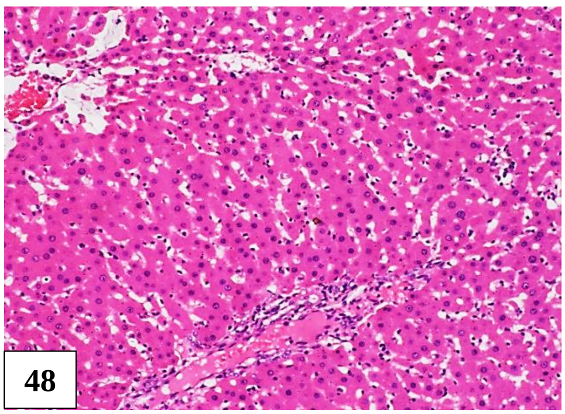
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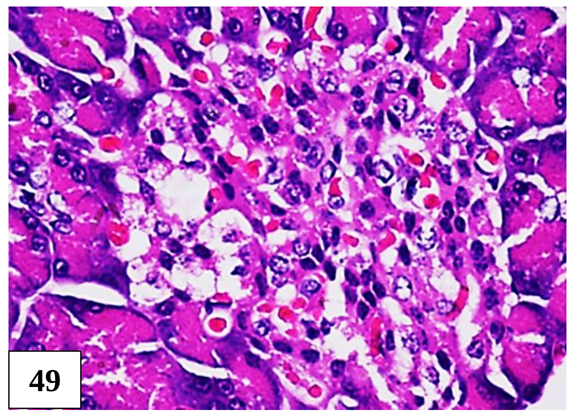
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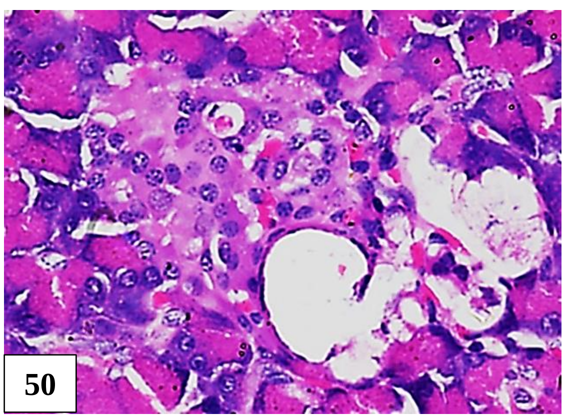
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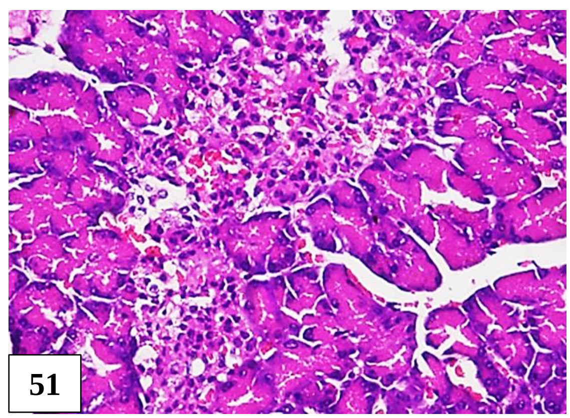
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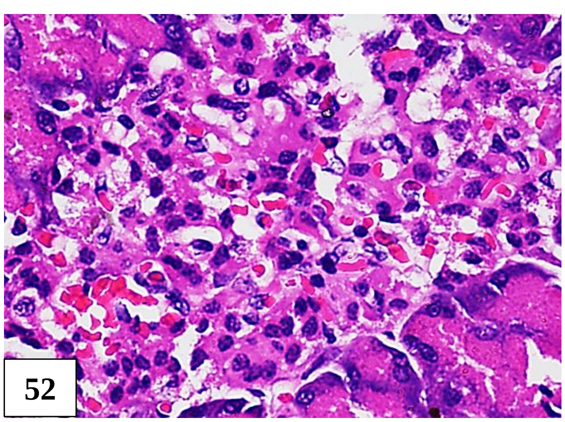
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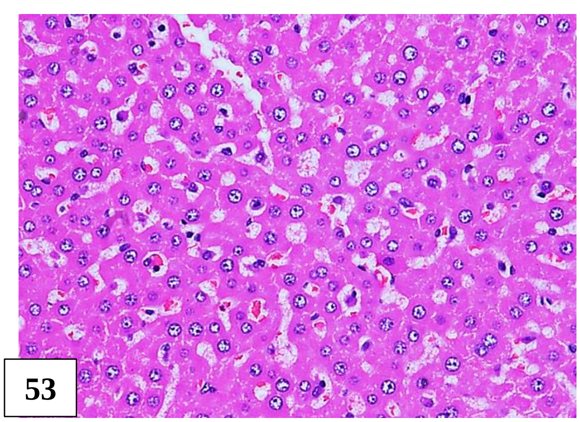
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Plate 54: Section of liver from a Group IV rat on 45th day showing improvement in architecture.

H&E X 200

Plate 55: Islets of Langerhans from the normal control rat (Group I) showing intensely stained insulin positive β cells.

IHC X 200

Plate 56: Pancreatic islet showing immune positive brownish granular reaction in the cytoplasm with an unstained nuclei.

IHC X 400

Plate 57: Section of pancreas from a diabetic control rat (Group II) on 15th day showing irregular and degenerated islet of Langerhans with mildly insulin positive degenerating β cells.

IHC X 400

Plate 58: Section of pancreas from a diabetic control rat (Group II) on 45th day showing occasional insulin positive β cells in a small islet.

IHC X 400

Plate 59: Section of pancreas from a Group III rat on 15th day showing a few insulin positive β cells in in the midst of unstained α cell.

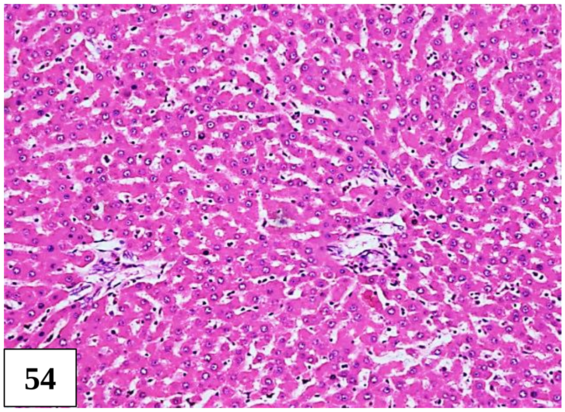
IHC X 400

Plate 60: Section of pancreas from a Group III rat on 45th day showing improvement in the number of insulin positive β cells in an islet.

IHC X 400

Plate 61: Section of pancreas from a Group III rat on 45th day showing insulin positive β cells in the newly formed islet along the sides of ductular epithelium.

IHC X 200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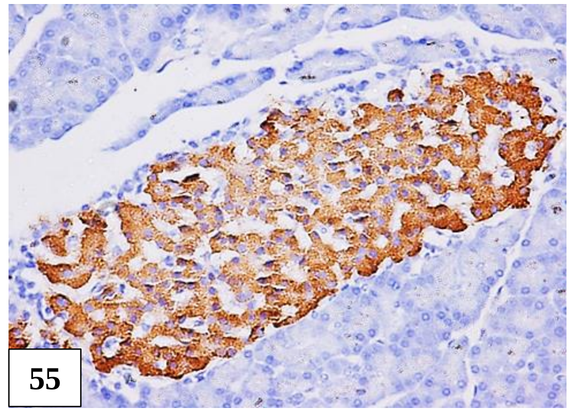
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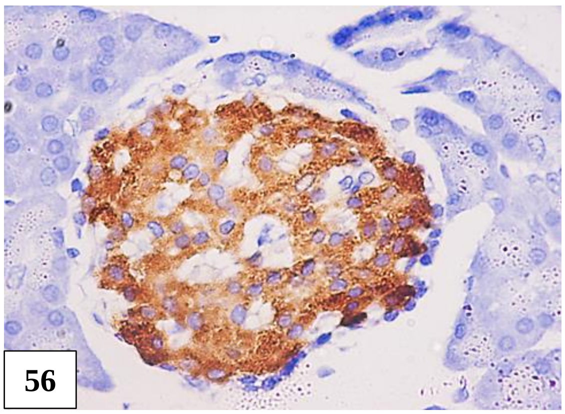
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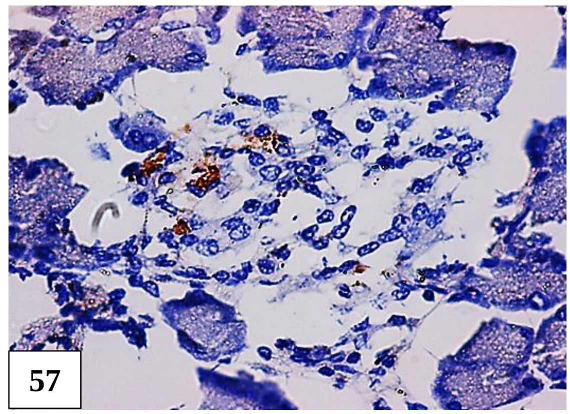
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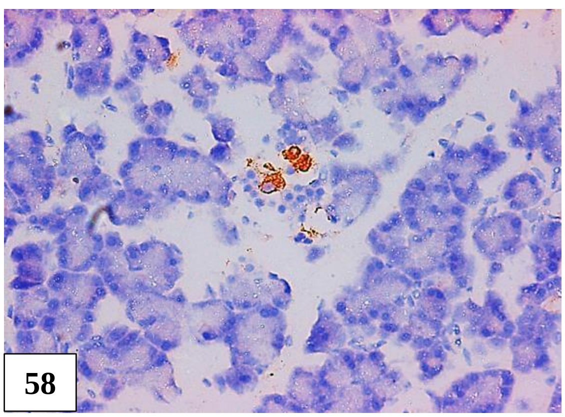
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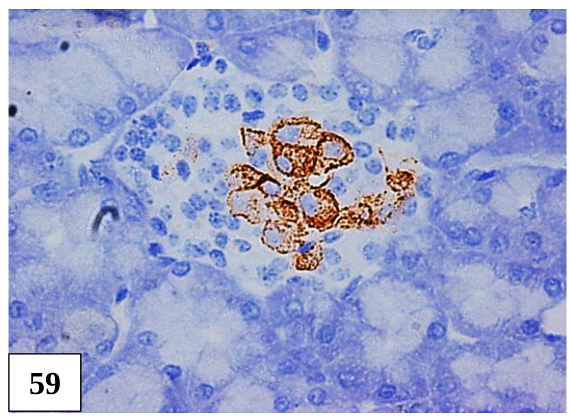
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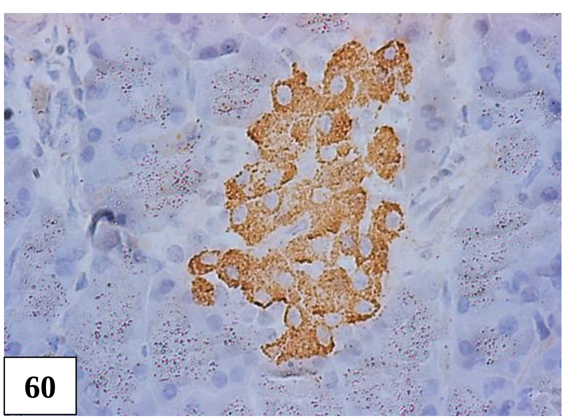
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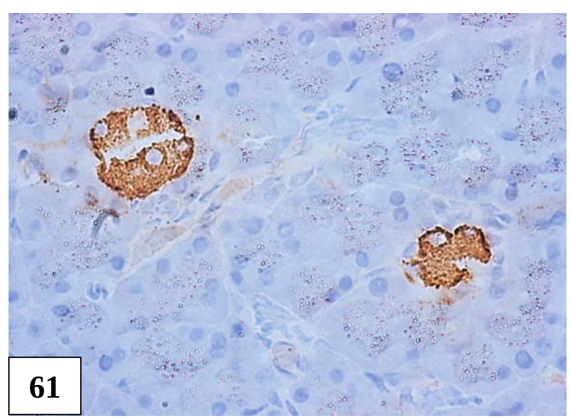
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Plate 62: Section of pancreas from a Group IV rat on 15th day showing occasional insulin positive β cells in the islet. Note increased number of unstained α cell.

IHC X 400

Plate 63: Section of pancreas from a Group IV rat on 45th day showing increased α cell cellularity and a few insulin positive β cells.

IHC X 400

Plate 64: Section of pancreas from a Group IV rat on 45th day showing well formed insulin positive β cells in the pancreas.

IHC X 200

Plate 65: Section of pancreas from a Group V rat on 15th day showing well formed few insulin positive β cells in the islet with sparse cellularity.

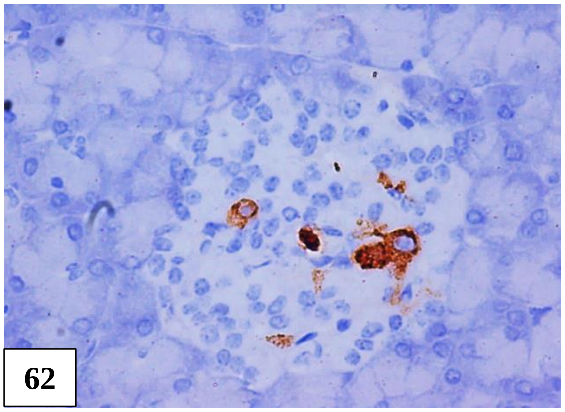
IHC X 400

Plate 66: Section of pancreas from a Group V rat on 45th day showing increase in number of insulin positive β cells in the core of the islet.

IHC X 400

Plate 67: Section of pancreas from a Group V rat on 45th day showing insulin positive β cells adjacent to pancreatic duct.

IHC X 200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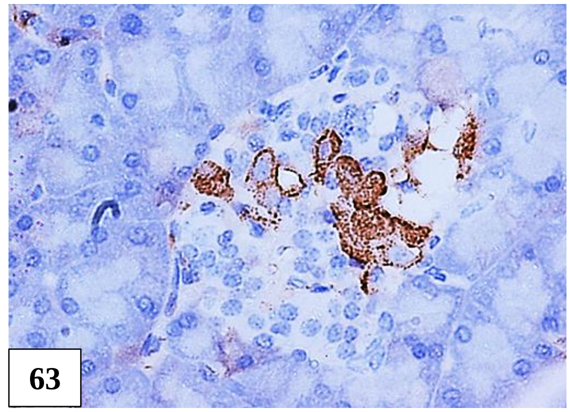
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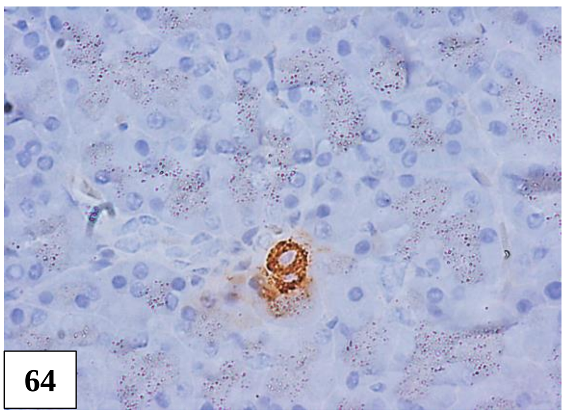
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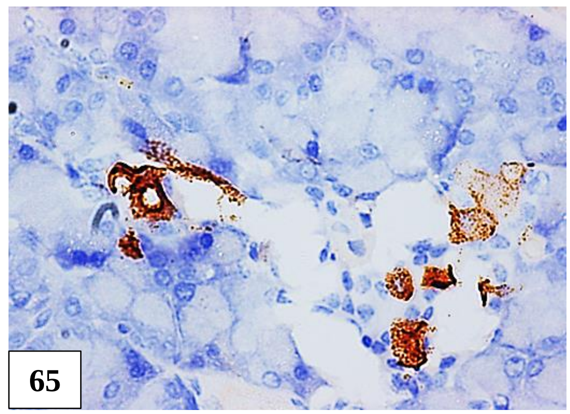
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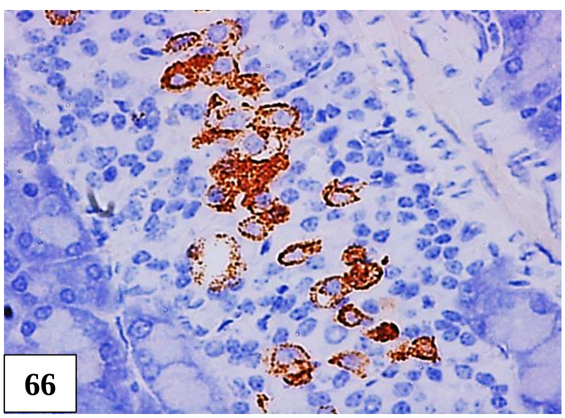
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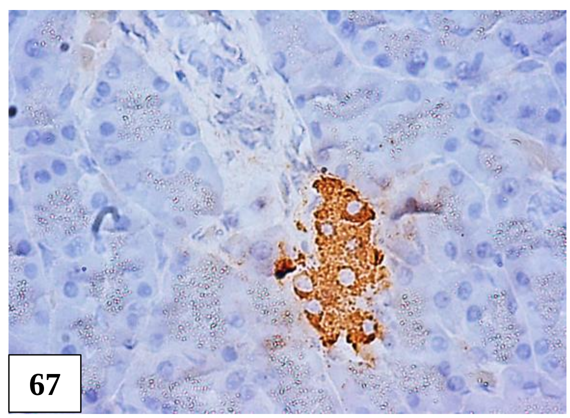
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Discussion

V. DISCUSSION

The results of studies on induced diabetes in rats with special emphasis on combined efficacy of *Gymnema sylvestre* and *Swertia chirayita* extracts are discussed as hereunder.

Diabetes mellitus is a chronic metabolic disorder of multiple etiologies characterized by a relative or absolute lack of insulin, resulting in hyperglycemia. Chronic hyperglycemia can lead to a variety of complications such as neuropathy, nephropathy and retinopathy and increased risk of cardiovascular disease (King. 2012). It affects essential biochemical activities in almost every cell in the body, increasing risk of diabetes in human being.

Diabetes is characterized by various clinical symptoms induced by hyperglycemia such as polyuria, polydipsia, weight loss, sometimes polyphagia and blurred vision. Hyperglycemia in diabetes is acute life threatening condition along with ketoacidosis or nonketotic hyperosmolar syndrome (American diabetes association, 2014).

According to International Diabetes Federation 2017, diabetes prevalence is presumed to rise from 425 million in 2017 to 629 million by 2045. Among the high income countries, 79% of global healthcare expenditure is being spent on diabetes alone, indicating that management of the diabetes is of huge economical importance (International Diabetes Federation, 2017).

At present, insulin therapy is the only satisfactory approach in diabetic mellitus, even though it is associated with various side effects like insulin resistance (Piedrola *et al*., 2001), anorexia, brain atrophy and fatty liver in chronic treatment (Weidmann *et al*., 1993). Treatment with sulphonylureas and biguanides are also associated with side effects (Rang and Dale, 1991). Long-term treatment with sulphonylurea may desensitize the β-cells of the pancreas and high concentrations of sulphonylurea may inhibit insulin biosynthesis *in vitro* and perhaps also in *in vivo* (Anderson and Borg, 1980 and Melander *et al*., 1987).

Currently, the focus is on using traditional methods for treatment and prevention of diabetes by use of herbal preparations. Herbs are used for the treatment of diabetes since ancient times and many useful herbs introduced in pharmacological and clinical trials have confirmed their blood sugar lowering effect and repair of ß-cells of islets of Langerhans (Gupta *et al*., 2008).

Gymnema sylvestre and *Swertia chirayita* are two such proven herbs shown to possess potent antidiabetic activity individually. Hence the present investigation was designed to carry out in Wistar albino male rats to explore the combined efficacy of *Gymnema sylvestre* and *Swertia chirayita* along with or without glibenclamide, a proven antidiabetic drug in streptozotocin induced diabetic rat model for a period of 45 days.

The antihyperglycaemic effect of combined herbal extract of *Gymnema sylvestre* and *Swertia chirayita* at dose rate of 200 mg/kg bw each (100% dosage) and in combination with glibenclamide at dose rate of 100 mg/kg bw each (50% dosage) were evaluated by the analysis of several parameters like body weights, serum liver enzymes like AST and ALT, serum lipids like triglyceride, cholesterol, liver antioxidant enzymes like catalase, superoxide dismutase and glutathione peroxidase, gross pathology and histopathology of pancreas, liver and other organs and immunohistochemistry for insulin in pancreas.

5.1 Induction of diabetes

In the present study, diabetes was induced in rats of Group II to V by the administration of streptozotocin (STZ) at the dose of 45 mg/kg by intra peritoneal route. All the rats of Group II to V showed hyperglycaemia with an increase in mean serum glucose levels ranging from 471.14 ± 1.75 mg/dL to 480.20 ± 2.53 mg/dL by 72 hours after STZ administration.

Streptozotocin (STZ) is a naturally occurring, antimicrobial agent produced by the bacterium *Streptomyces achromogenes* which is used as a chemotherapeutic alkylating agent (Vavra *et al.*, 1959). It is a known cytotoxic drug and induces diabetes by selective destruction of insulin producing beta cells upon uptake and therefore, is the first choice for induction of diabetes in animals experimentally (Karunanayake *et al.*, 1975: Li *et al*., 2001; Akbarzadeh *et al.*, 2007; Dhanush, 2009; Pragathi, 2011; Mallikarjuna *et al.*, 2013; Mudasir *et al*., 2013; Shesha Rao, 2013; Manjunatha, 2013; Gurikar, 2014; Kavitha Rani, 2015; Yaman *et al*., 2017 and Parasuraman *et al*., 2019).

Streptozotocin is taken up by pancreatic β-cells via glucose transporter GLUT2 and the cytotoxic effect has been attributed to alkylation of DNA leading to DNA damage (Delaney *et al.*, 1995 and Elsner *et al.*, 2000). The alkylating activity is related to its nitrosourea moiety.

Streptozotocin is also a nitric oxide (NO) donor and NO is reported to cause destruction of pancreatic islet cells contributing to STZ-induced DNA damage (Turk *et al.*, 1993; Morgan *et al.*, 1994 and Kroncke *et al.*, 1995). However, results of the several experiments provide the evidence that NO is not the only molecule responsible for the cytotoxic effect of STZ. Generation of reactive oxygen species induced by STZ also contributes to DNA fragmentation and evoke deleterious changes in the cells (Takasu *et al.*, 1991 and Bedoya *et al.*, 1996). Nitric oxide and reactive oxygen species can act seperately or may form highly toxic peroxynitrate to induce DNA damage (Szkudelski, 2001).

The DNA damage induces activation of poly ADP-ribosylation, a process more important for the diabetogenicity of STZ than just DNA damage itself. This process leads to depletion of cellular NAD^+ and ATP content. Enhanced ATP dephosphorylation supplies a substrate for xanthine oxidase resulting in formation of super oxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are generated. Further, STZ liberates toxic amounts of nitric oxide that inhibits acotinase activity and participates in DNA damage. As a result of STZ action, β-cells undergo destruction by necrosis or apoptosis (Szkudelski, 2001).

It can be stated that potent alkylating properties of STZ are the main reasons for its toxicity. However, the synergistic action of both NO and reactive oxygen species may also contribute to DNA fragmentation and other deleterious changes caused by STZ (Szkudelski, 2001).

5.2 Group I (NC)

In the present investigation, all animals in the normal control group remained healthy throughout the study period, as indicated by the results of all the evaluatory parameters.

5.3 Group II (DC)

The diabetic control rats remained hyperglycaemic during the entire period of the study and showed various biochemical and pathomorphological changes indicative of diabetes.

In the diabetic control animals a significant decrease in the mean body weight was observed throughout the experiment. The decrease was statistically significant from 15th day post STZ injection. The decrease in the body weight could be attributed to hypoinsulinism that occurs in diabetes. Similar findings were also noticed by several earlier workers in their studies (Pillion *et al.*, 1988; Dhanush, 2009; Pragathi, 2011; Mallikarjuna *et al*., 2013; Mudasir *et al.*, 2013; Shesha Rao, 2013; Manjunatha, 2013: Gurikar, 2014; Kavitha Rani, 2015; Al-attar and Alsalmi. 2019 and Parasuraman *et al*., 2019).

Insulin is an anabolic hormone and its deficiency causes catabolism of carbohydrates, proteins and fats leading to loss of body weight. The other factors contributory for the reduced weight include decreased protein synthesis in the absence of insulin, partly because of diminished transport of amino acids to the muscle (Warkins, 2003), loss of fluids leading to dehydration through glycosuric polyuria and altered uptake of glucose and glycogenesis by the target cell (Hakim *et al*., 1997 and Rubin and Strayer, 2008).

The mean (± SE) serum glucose values were observed to be drastically increased in the present investigation, compared to normal control animals at different intervals of the study indicating hyperglycaemia. The increase in serum glucose values was in accordance with those of previous reports (Karunanayake *et al*., 1975; Li *et al*., 2001;

Akbarzadeh *et al*., 2007, Mir *et al*., 2008; Dhanush, 2009; Mallikarjuna *et al*., 2013; Pragathi, 2011; Mudasir *et al.*, 2013; Shesha Rao, 2013: Manjunatha, 2013; Gurikar, 2014: Rani *et al*., 2016; Parasuraman *et al*., 2019 and Hsiao *et al.,* 2019). This elevation in the serum glucose level could be attributed to insulin deficiency caused by selective destruction of β cells of islets of Langerhans by STZ which in turn causes loss of glucose homeostasis (Magee and Swann, 1969; Tjalve *et al*., 1976; Takasu *et al*., 1991; Gu *et al*., 1997 and Lenzen, 2007).

Insulin, generally enables the cells to take up glucose from the blood and also helps in the utilization of the glucose in the cells by glycolysis, tricarboxylic acid cycle, hexose monophosphate shunt, and glycogenesis. Hypoinsulinism in STZ diabetes leads to decreased glucose transport across the cells and its utilization contributing for hyperglycaemia (Karunanayake *et al*., 1974; Shabib *et al*., 1993; Sarkar *et al*., 1996; Ahmed *et al*., 2001; Li *et al*., 2001; Rao *et al*., 1999; Akbarzadeh *et al*., 2007; Dhanush, 2009; Pragathi, 2011; Mallikarjuna *et al.*, 2013; Mudasir *et al.*, 2013; Shesha Rao, 2013; Manjunatha, 2013; Gurikar, 2014; Kavitha Rani, 2015; Zhang *et al*., 2016 and Parasuraman *et al*., 2019).

In the present study, the mean (± SE) values of serum cholesterol and triglycerides were found to be progressively increasing throughout the experimental period. Hyperlipidemia, characterized by hypercholesterolemia and hypertriglyceridemia has been reported to be a consistent feature in experimental STZ and alloxan induced diabetes in rats (Hardman and Limberd, 2001). Hyperlipidemia in diabetes can occur through various metabolic derangements mainly in response to hypoinsulinemia or resistance to insulin.

Insulin deficiency induced lipolysis of the adipose tissue and mobilization of fatty acids, its excess esterification to triglycerides and conversion to cholesterol in liver can account for hyperlipidemia (Hem, 1977; Choi *et al*., 1991; Platel *et al*., 1993; Sharma *et al*., 1997; Prince *et al*., 1999; Rao *et al*., 1999; Ahmed *et al*., 2001; Fernandes *et al*., 2007; Dhanush, 2009; Pragathi, 2011; Mudasir *et al.*, 2013; Mallikarjuna *et al.*, 2013; Shesha Rao, 2013; Manjunatha, 2013; Gurikar, 2014; Kavitha Rani, 2015 and Al-attar and Alsalmi. 2019).

A progressive increase in the mean serum values of ALT and AST was observed in diabetic control group (Group II) in the present study. Serum alanine aminotransferase and serum aspartate aminotransferase are the leakage enzymes present in the cytosol and organelles of hepatocytes and an elevation in serum levels indicate active hepatic damage. Streptozotocin has been reported to induce both plasma membrane and organellar membrane damage especially that of rough endoplasmic reticulum and mitochondria following its uptake by hepatocytes through GLUT-2 (Mudasir *et al.*, 2013). Experimental studies have shown that subtle membrane changes are sufficient to allow passage of intracellular enzymes to the extracellular space. Usually a very high concentration gradient exists between the hepatocytes and the sinusoidal space for enzymes. Cell damage increases permeability causing cytosolic isoenzymes to spill into the sinusoids and from there into the peripheral blood (Hearse, 1979; Virdi *et al*., 2003; Zafar *et al*., 2009; Mudasir *et al.*, 2013; Mallikarjuna, 2013; Shesha Rao, 2013; Manjunatha, 2013; Gurikar, 2014; Kavitha Rani, 2015 and Parasuraman *et al*., 2019).

The mean haemoglobin values of diabetic control rats in the present study were found to decline progressively from 15th day of treatment onwards. Decreased haemoglobin content observed in diabetic rats might be due to increased formation of glycosylated haemoglobin. In 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 thereby reduce the levels of haemoglobin in diabetic animals (Pari *et al*., 2001; Dhanush, 2009 and Mallikarjuna *et al.*, 2013, Mudasir *et al*., 2013, Shesha Rao, 2013, Manjunatha, 2013; Gurikar, 2014 and Kavitha Rani, 2015).

In the current study there was a moderate decrease in TEC count in the diabetic rats from 15th to 45th day post treatment. The results indicated that these rats suffered from anemia which might be due to the toxic effect of STZ used for induction of diabetes. Anemia could be attributed to the destruction of RBCs and reduced rate of its release from the bone marrow to blood (Helal *et al.*, 2005 and Rabbani *et al.*, 2010). Also, elevated levels of glucose could result in the peroxidation of membrane lipids of RBC leading to destruction of erythrocytes in diabetes mellitus (Jain *et al.*, 1990). Abnormal glycation, that adversely affects hemoglobin and membrane proteins in erythrocytes could also lead to the destruction of erythrocytes in diabetes mellitus (Kennedy and Baynes, 1984).

An elevation in total TLC count was observed in diabetic rats in the current study. A meagre elevation observed may be a stress leukogram in response to induced diabetes by STZ. Similar observation was also made by several earlier worker (Ramadan *et al*., 2009; Papazafiropoulou, 2010 and El-Baky, 2013). In addition a low grade inflammatory reaction associated with complications of diabetes also may be responsible for increase in leukocyte count (Itembong *et al*., 2010 and Akter *et al*., 2014).

There was also a decrease in total platelet count in diabetic rats in the present study. Diabetes mellitus is associated with accelerated atherosclerosis that exists in a prothrombotic state with elevated platelet function. Diabetes influences the platelet functions by activation of platelet adhesion which causes loss of platelets from circulation (Ogbodo *et al.*, 2007; Akthar *et al.*, 2012 and El-Baky, 2013).

In the current study, the SOD, CAT and GPx activities were significantly reduced in the liver of diabetic rats from 15th to 45th day. Similar observations were also made in alloxan and STZ induced diabetic rat models by several early workers (Singh *et al.*, 2010; Pragathi, 2011; Manjunath, 2013; Kavitha Rani, 2015 and Al-attar and Alsalmi. 2019). Compromised antioxidant system, denoted by increased lipid peroxidation and decreased levels of both non-enzymatic and enzymatic antioxidants, is a feature of diabetes and such changes have been noticed in alloxan or STZ induced diabetes (Bonnefont-Rousselot *et al.*, 2000 and Maritim *et al.*, 2003). Persistent hyperglycemia during diabetes leads to production of free radicals or impaired antioxidant defenses via several mechanisms (Saxena *et al.*, 1993; Maritim *et al.*, 2003 and Valko *et al.*, 2007). Decline in the activities of antioxidant enzymes in the liver of diabetic animals indicates the extent of free radical induced damage during STZ induced hyperglycemia (Resmi *et al.*, 2006 and Kaleem *et al.*, 2006). In 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 consonance with those of earlier workers (Akbarzadeh *et al.,* 2007; Mallikarjuna *et al.,* 2013; Dhanush, 2009; Pragathi, 2011; Mudassir *et al.,* 2013; Manjunath, 2013; Kavitha Rani, 2015 and Muzumbukilwa *et al*., 2019).

The clinical signs of diabetes result from metabolic disorder associated with hyperglycaemia due to decreased insulin level. When hyperglycaemia exceeds the renal threshold for reabsorption, glycosuria occurs which further induces an osmotic diuresis and then results in polyuria with a profound loss of water and electrolytes. Increased level of glucose in blood depletes intracellular water and triggers osmoreceptor of the thirst centre of the brain leading to polydipsia. In addition, the catabolism of protein and fats due to insulin deficiency induces a negative energy balance which leads to polyphagia. Despite the polyphagia, catabolic effects persist resulting in weight loss and muscle weakness (Kumar *et al.*, 2010; Mallikarjuna, 2009; Dhanush, 2009; Pragathi, 2011; Mudassir, 2011; Manjunath, 2013; Kavitha Rani, 2015 and Al-attar and Alsalmi. 2019).

Grossly, pancreas appeared slightly atrophied and showed progressive decrease in size from 15^{th} day onwards which appeared as a thin gelatinous strip on 45^{th} day in the present study. The progressive decrease in the size of pancreas may be attributable to the cytotoxic effect of streptozotocin on β cells of islets as well as damage to exocrine portion (Jelodar *et al*., 2005; Mir *et al.*, 2008; Atangwho *et al*., 2010; Pragathi, 2011; Mallikarjuna *et al.,* 2013; Mudasir *et al.,* 2013; Shesha Rao, 2013; Manjunatha, 2013; Gurikar, 2014; Kavitha rani, 2015; Yaman *et al*., 2017 and Al-attar and Alsalmi. 2019).

Grossly, liver appeared pale, soft and enlarged from 15th day of the treatment which could be attributed to diabetes induced liver damage (Ohno *et al*., 2000; Dhanush, 2009; Zafar *et al*., 2009; Mallikarjuna *et al.*, 2013 and Kavitha Rani, 2015).

The spleen was comparatively smaller than the normal control rats. The other organs such as kidneys, intestine, lungs and heart did not show any appreciable gross lesions throughout the study period.

The diabetic control rats in the present study revealed microscopicly lesions in both exocrine and endocrine components. In the endocrine portion there was a reduction in the number of islets which were irregular with loss of demarcation with the adjacent exocrine portion. The islets revealed depletion of β-cells with either necrosis or apoptosis and altered distribution of α and β-cells. In some islets hypercellularity was observed with increase in the number of α-cells during later period of experimentation. The exocrine portion revealed loss of architecture and acini were lined by vacuolated, degenerating and necrotic cells. Some of the lobules which were intact revealed large number of apoptotic cells. The histopathological findings observed in the pancreas were in accordance with those of many earlier workers (Li *et al*., 2001; Sharma *et al*., 2003; Zhang *et al*., 2003; Jelodar *et al*., 2005; Akbarzadeh *et al*, 2007; Mir *et al.,* 2008; Atangwho *et al*., 2010; Dhanush, 2009; Pragathi, 2011; Mallikarjuna *et al.,* 2013; Mudasir *et al.,* 2013; Shesha Rao, 2013; Manjunatha, 2013; Kavitha Rani, 2015 and Yaman *et al*., 2017 and Al-attar and Alsalmi. 2019).

In the present study, the damage to the exocrine acinar cells could be probably due to secondary response to excessive free radical liberation and antioxidant depletion in STZ cytotoxicity (Pragati, 2011). 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 (West *et al.,* 1996; Szkudelski, 2001; Akbarzadeh *et al.,* 2007 and Zafar *et al.,* 2009).

The vacuolar degeneration, obliteration of sinusoidal spaces along with individual hepatocyte necrosis, cell lysis, fragmentation of nucleus and infiltration of inflammatory cells observed in liver and in kidneys vacuolar changes in the tubular cells could be attributed to the STZ induced cytotoxicity. These changes were in accordance with those of earlier workers like Muzumbukilwa *et al*. (2019) and Parasuraman *et al*. 2019. Streptozotocin, in addition to pancreatic β-cells also damages the hepatocytes and renal cells through GLUT-2 transporter expressed on these cells and with generation of free radicals (Mir *et al*., 2008). Increased formation of hydroxyl radicals in the liver of STZ-induced diabetic rats has been reported by Ohkuwa *et al*. (1995) which cause lipid peroxidation and cell damage.

Microscopically, spleen revealed 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 and Pragati, 2011). Histopathological examination of brain, heart, lungs, intestine and lymph nodes did not show any significant lesions throughout the study period.

5.4 Group III (G)

Glibenclamide is a second generation sulfonylurea used in the treatment of non insulin dependent diabetes. It’s hypoglycemic effect is mainly due to stimulation of insulin release from pancreatic beta cells and sensitization of the peripheral tissues to insulin (Sweetman, 2002).

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 Ca^{2+} to enter the cell. Increased intracellular Ca^{2+} concentration enhances the binding of Ca^{2+} 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 and Ling *et al*., 2006).

In the present study, Group III diabetic rats were treated daily with glibenclamide at a dose rate of 600 µg/kg bw from 3rd day onwards. The animals showed improvement in their body weight which was significantly ($P<0.05$) higher compared to diabetic control rats from 15th day post-treatment which was also the observation of several earlier workers (Virdi *et al*., 2003; Dhanush, 2009; Pragathi, 2011; Mallikarjuna *et al.*, 2013; Mudasir *et al.*, 2013; Shesha Rao, 2013; Manjunatha, 2013; Kavitha rani, 2015; Parasuraman *et al*., 2019 and Lawal *et al.,* 2019). The improvement could be attributed to the effect of glibenclamide in enhancing insulin secretion by β-cells of pancreas and also increasing sensitization of the peripheral tissues to insulin (Sweetman, 2002).

In the present study, the serum glucose levels in glibenclamide treated rats were significantly improved from 15th day onwards compared to diabetic control group. These findings were in concurrence with those of Virdi *et al.* (2003), Fernandes *et al*. (2007), Abdollahi *et al*. (2010), Dhanush, (2009), Pragathi, (2011), Mallikarjuna *et al.* (2013), Mudasir *et al.* (2013), Shesha Rao (2013); Manjunatha (2013), Kavitha rani, (2015) Parasuraman *et al*. (2019) and Lawal *et al.*, (2019).

The improvement in glucose concentration in circulation could be attributed, as indicated earlier to glibenclamide induced insulin secretion which improves sensitivity of β-cells to glucose and in turn potentiates insulin secretion (Virdi *et al*., 2003; Fernandes *et al*., 2007 and Abdollahi *et al*., 2010). In addition, inhibition of gluconeogenesis, ketogenesis, and stimulation of glucose transport, glycogen synthase activity and glycerol-3-p-acyl transferase activity also could be contributory for improvement in serum glucose level (Rabbani *et al.*, 2010).

Glibenclamide has been reported to increase synthesis of GLUT-2 protein which improves glycogen uptake in the liver, thereby reduce glucose concentration in circulation and also reduce endogenous glucose production (Luzi and Pozza, 1997). Glibenclamide increases sensitization of the peripheral tissue to insulin and also the number of insulin receptors (Hribal *et al.*, 2001). However, in the present study, the glucose levels failed to reach the normal levels inspite of glibenclamide treatment, which could probably be due to the failure of β cells to acquire optimum functional status and also could be failure of treatment with glibenclamide to rectify severe β cells damage by STZ.

Serum cholesterol and serum triglyceride levels were observed to be significantly reduced in the glibenclamide treated rats compared to diabetic control rats in the present study. This could be on an account of decreased glucose level following glibenclamide induced insulin release. It was also reported that glibenclamide induced alleviation of hypertriglyceridemia was due to acute reduction in triglyceride of intestinal origin. The above finding was in accordance with the findings of many earlier workers (Virdi *et al*., 2003; Fernandes *et al*., 2007; Dhanush, 2009; Mallikarjuna, 2009; Manjunath, 2013; Lakshami, 2015; Kavitha Rani, 2015; Chika and Yahaya. 2019 and Parasuraman *et al*., 2019).

The mean serum ALT and AST values were reduced in glibenclamide treatment group in comparison with diabetic rats. However, it was observed that the values were higher compared to normal control animals which indicated that glibenclamide treatment did not completely reverse the liver damage caused by STZ in the present study. This was substantially supported by the persistence of mild degree of hepatic damage till the end of the study.

Similarly in glibenclamide treated group, there was a significant improvement in Hb concentration in comparison with the diabetic control group from 30th day of the study. Improvement in the Hb concentration could be attributed to antihyperglycaemic effect of glibenclamide on improvement in insulin level, thus reducing non-enzymatic glycosylation of haemoglobin (Rubin and Strayer, 2008).

Group III rats also showed a significant improvement in the blood parameters such as TEC, TLC and total platelet counts when compared to diabetic control animals which could be due to marginal amelioration of diabetic symptoms by glibenclamide.

The mean values of SOD, CAT and GPx activity in the liver of glibenclamide treated rats were significantly improved compared to diabetic rats. These findings are in concurrence with those of Manjunath (2013) and Kavitha Rani (2015). The antioxidant activity of glibenclamide is well documented in the literature. Earlier studies indicated that glibenclamide possesses direct mechanism to enhance the levels of SOD, CAT, glutathione-s-transferase (GST) besides reducing the LPO in the STZ diabetic animals (Altan *et al*., 1997; Elmali *et al.,* 2004 and Bukan *et al.*, 2004). Also, the improvement could be attributed to the hypoglycemic effect of glibenclamide by increasing the release of insulin thereby reversing the diabetes induced changes, suggesting that glibenclamide may directly increase liver antioxidant enzymes.

The intensity of various clinical symptoms decreased gradually with glibenclamide treatment. The reduction in the severity of clinical symptoms could be attributed to the effect of glibenclamide on improving basal glucose levels by increasing insulin secretion, insulin action and β-cell proliferation (Koltermann *et al.,* 1984; Ling *et al.,* 2006; Rubin and Strayer, 2008; Manjunath, 2013, Kavitha Rani, 2015; Lakshmi, 2015 and Parasuraman *et al*., 2019)

Gross pathological changes observed in the glibenclamide treated rats reduced progressively from 15^{th} to 45^{th} post treatment and the observations were in agreement with the findings of Ananthan *et al.* (2003). This could be attributed to alleviation of diabetes complications by the improved levels of insulin by glibenclamide treatment.

There was a progressive improvement in the microscopic pathology of pancreas of glibenclamide treated rats from 15^{th} to 45^{th} day of treatment. Guiot *et al*. (1994) and Wang *et al*. (2008) attributed the improvement to increased proliferation as well as recruitment of subpopulation of β-cells and thereby increase in the β cell mass upon treatment with glibenclamide. This could be possibly due to insulin induced regeneration of endocrine pancreas, resulting in improved histological appearance, size and number of islets (Adewole and Ojewole, 2007). In addition, as indicated by Paris *et al.* (2004), the improved β-cell mass could be on an account of β-cell neogenesis from ductal epithelial cells. This is well substantiated by ductal hyperplasia and presence of newly formed small clusters of β-cells or individual cells adjacent to ductal hyperplastic tissue with insulin synthesis observed microscopically.

Liver section revealed progressive improvement in the architecture from 15th day onwards in the glibenclamide treated rats and the observation are in accordance with those of Luzi and Pozza (1997). Studies using [3H]-glibenclamide boluses have suggested that hepatocytes possess specific binding sites that may be relevant in mediating the action of the drug on the liver. Additional studies have shown that the drug has a positive action on glycogen deposition with direct action on the synthesis of GLUT-2 rather than GLUT-4 proteins and at the glycogen phosphorylase level. The effect of glibenclamide on the insulin level and altered metabolism of various macromolecules could be the reason for improvement in the microscopic architecture of liver (Luzi and Pozza, 1997; Manjunath, 2013; Kavitha Rani, 2015; Lakshmi, 2015 and Parasuraman *et al*.,2019).

Spleen revealed persistence of lymphocytic depletion on 15th day with improvement of cellular mass on 30th and 45th day compared to diabetic animals. Improvement in hyperglycaemic state, antioxidant level and insulin level could be contributory for improvement in lymphocytic population by reducing the free radical injury (Elmali *et al.,* 2004).

5.5 Group IV (GS+SC) and Group V(GS+SC+G)

In the present study a combination of *Gymnema sylvestre* and *Swertia chirayita* along with or without glibenclamide was used to alleviate the streptozotocin induced diabetes in rats. Both *Gymnema sylvestre* and *Swertia chirayita* have been reported to possess antidiabetic activity, but antidiabetic effect in combination of these two herbs has not been explored. Hence, the present study was taken up to study the combined antidiabetic efficacy of these two herbs and to explore whether there is any synergistic or antagonistic effect in alleviating diabetic changes in streptozotocin induced diabetes in Wistar albino rats. An attempt was also made to see the possibility of partial / complete replacement of oral hypoglycemic drug like glibenclamide with these extracts for the control of diabetes mellitus.

In the current study, Group IV (GS+SC) diabetic rats were treated with *Gymnema sylvestre* and *Swertia chirayita* extracts in combination at 200 mg/kg bw each and Group V (GS+SC+G) diabetic rats were treated with *Gymnema sylvestre* and *Swertia chirayita* extracts in combination at 100 mg/kg bw each along with glibenclamide at 300 μg/kg bw.

Gymnema sylvestre, is a woody climber plant of Asclepiadaeceae family, native to southern India, known to lower blood glucose level and alleviate diabetic symptoms (Bailey and Day, 1989; Shigematsu *et al.*, 2001; Leach, 2007 and Matheka and Alkizim, 2012). Its leafy extracts are widely used in ayurvedic formulations and recommended as a treatment for diabetes mellitus.

The plant has been reported to suppress glucose absorption in the small intestine of rats, reduce plasma glucose increment in the oral sucrose tolerance test, lower blood glucose level and alleviate diabetic symptoms in type-2 diabetes (Shigematsu *et al.,* 2001). The herb accounts for its sweet inactivation property to the presence of triterpene saponins known as gymnemic acids, gymnema saponins, and gurmarin (Tiwari *et al*., 2014). The possible mechanisms by which *Gymnema sylvestre* exerts its hypoglycaemic effects have been reported to be through increasing secretion of insulin, promoting regeneration of islet cells, increasing utilization of glucose through increased activities of enzymes responsible for utilization of glucose by insulin dependent pathways and by inhibition of glucose absorption from intestine (Kanetkar *et al.,* 2007 and Tiwari *et al*., 2014).

Swertia chirayita is a critically endangered medicinal herb that grows at high altitudes in the sub temperate regions of the Himalayan regions of india and belongs to the family Gentianaceae. It is commonly called an Chiretta, Bhunimba, Kairata and Bitter stick (Joshi and Dhawan, 2005) and used for various aliments by tribes.

Swertia chirayita is a potent hypoglycemic agent and antioxidant in nature and contain many phytochemicals such as alkaloids, flavonoids, steroids, glycosides, triterpenoids, saponins, xanthones and ascorbic acid. The main phyto chemical ingredients are swertiamarin, amarogentin, swechirin, mangiferin, sweroside, gentianine, amaroswerin, oleanolic acid, swertanoone and ursolic acid (Joshi and Dhawan, 2005; Kumar *et al.,* 2014 and Aleem and Kabir 2019).

A xanthone isolated from the hexane fraction of *Swertia chirayita* identified as swerchirin (1:8 dihydroxy 3:5 dimethoxy xanthone) was reported to have potent hypoglycemic activity (Saxena *et al.* 1991 and Bajpai *et al.* 1991). The possible mechanism by which the *Swertia chirayita* exerts its hypoglycemic effect was reported to be through stimulation of insulin release from the islets of pancreas and increase in the insulin action (Saxena *et al*., 1991; Kumar *et al.*, 2014 and Thomson *et al.*, 2014). Mangiferin is an another phytochemical with hypoglycemic activity which enhances peripheral usage of glucose, stimulates the insulin release, and promotes β cell repair and regeneration (Bhowmik *et al*., 2009; Phoboo *et al*., 2010).

The rats of Group IV and V in the current study showed progressive improvement in body weight which was significantly ($P < 0.05$) higher when compared to diabetic control animals. However, the increase in body weights of rats remained lesser compared to the normal control and glibenclamide treatment rats throughout the study period. The improvement in body weight could be attributed to the alleviation of diabetic symptoms and hyperglycemia, by improvement in insulin production, insulin stimulated glucose transport across the cells and anabolic effect of insulin by *Gymnema sylvestre* and *Swertia chirayita* as reported by Pragathi (2011) and Mallikarjuna *et al*. (2013) for *Gymnema sylvestre* and Manjunath (2013) and Verma *et al*. (2013) for *Swertia chirayita*.

Among the treatment groups (Group IV and V), the Group V rats treated with half dose of extract along with glibenclamide at half dose revealed significant ($P < 0.05$) improvement in body weight compared to that of Group IV rats. The result of the study indicated that there was no dose dependent improvement in the mean body weight and improvement observed in Group V could be attributed to the addition of glibenclamide, at half dose.

It was observed that there was a significant ($P < 0.05$) reduction in the serum mean glucose values in Group IV and V rats compared to diabetic control (Group II). This could be attributed to the anti hyperglycaemic effect of phytochemical constituents of *Gymnema sylvestre* and *Swertia chirayita* as reported by Pragathi (2011) and Mallikarjuna *et al*., (2013) for *Gymnema sylvestre* and Manjunath (2013) and Verma *et al*. (2013) for *Swertia chirayita*. However, the mean glucose values were significantly (P < 0.05) higher compared to glibenclamide treatment group and control group throughout the study period.

Among the treatment groups (Group IV and V), the mean glucose values were significantly (P < 0.05) reduced in Group V rats treated with extract along with glibenclamide compared to that of Group IV rats treated with combination of extracts alone at higher dose. The result of the study indicated that there was no dose dependent improvement in the glucose value and improvement observed in Group V could be attributed to the addition of glibenclamide.

In the present study, there was a progressive improvement in serum AST and ALT levels of Groups IV and V diabetic rats which were significantly (P < 0.05) lesser compared to Group II diabetic control rats. However, the mean ALT and AST values remained significantly (P < 0.05) higher compared to normal control and glibenclamide treatment groups. This observation indicates that *Gymnema sylvestre* and *Swertia chirayita* lack sufficient hepatoprotective effect and could only partially reverse the STZ induced hepatic damage. Mild improvement observed could be due to restored tissue antioxidant level and decreased lipid peroxidation in liver preventing free radical injury (Nagalekshmi *et al.* 2011; Manjunath, 2013 and Kavitha Rani, 2015). Among treatment groups (Group IV and V), the mean serum ALT and AST values were significantly (P < 0.05) reduced in the

Group V rats treated with combination of herbal extracts along with glibenclamide compared to that of Group IV rats.

In the present study Group IV and V rats showed a significant ($P < 0.05$) improvement in serum cholesterol, triglyceride levels when compared with diabetic control rats, and the values were comparable to the glibenclamide group on 45th day of the study. The decrease in the serum cholesterol and triglyceride level could be attributed to the gymnemic acid which influences the faecal excretion of lipids (Shigematsu *et al*., 2001 and Kanetkar *et al*., 2007), inhibition of intestinal absorption of oleic acid (Wang *et al*., 1998), reduced fatty acid assimilation in small intestine (Tiwari *et al*., 2014) and the presence of bellidifolin and swerchirin, the xanthenoids in *Swertia chirayita* which decrease the serum cholesterol and triglyceride levels (Basnet *et al.,*1994; Tian *et al.,* 2008 and Manjunath, 2013). Among Group IV and V rats, Group V rats treated with the combination of herbal extracts along with glibenclamide revealed better improvement in mean serum cholesterol, triglyceride values when compared to Group IV rats.

In the present study there was a significant ($P < 0.05$) progressive improvement in the mean haemoglobin values of Group IV and V rats when compared to diabetic control rats. On 45th day the values were comparable to that of glibenclamide treatment group, however, the values were significantly lesser compared to the normal control group.

Similarly, gradual significant ($P < 0.05$) improvement was seen in blood cell parameters like total erythrocytes, leukocytes and platelet values in Group IV and V rats compared to diabetic control animals. This improvement in blood cell parameter could be attributed to blood glucose lowering and haematopoietic property of the phytoconstituents present in the *Swertia chirayita* extract. A xanthone swerchirin isolated from the herb has shown to induce proliferative response in bone marrow cells in mice and the beneficial effects of swerchirin on haemopoiesis may be related to stimulation of colony stimulating factor and other haematopoietic growth factors (Ya *et al.*, 1999).

The hepatic antioxidant enzymes levels of SOD, CAT and GPx in Groups IV and V rats progressively improved from 15^{th} day to 45^{th} day indicating that there was reduction in oxidative stress caused by STZ induced diabetes in the treatment groups and the values were significantly ($P < 0.05$) higher compared to the diabetic control rats. However, the values remained significantly lesser compared to glibenclamide treatment and normal control rats. This improved antioxidant activity could be attributed to the presence of flavanoids and triterpenoids of the *Gymnema sylvestre* herb which inhibits DPPH, scavenge superoxide, hydrogen peroxide and lipid peroxidation and enhance the cellular antioxidant defense (Pragathi, 2011; Kang *et al.* 2012; Mallikarjun *et al.* 2013 and Rani *et al*. 2016) and *Swertia chirayita* extracts contain many naturally occurring polyhydroxy xanthones and flavonoids that have been associated with wide range of biological and pharmacological activities including antioxidant and antidiabetic activities (Surayawanshi *et al.,* 2006). Six xanthone derivaties obtained from *Swertia* species identified as bellidifolin, methylbellidifolin, swertianin, methylswertianin, norswertianin and desmethylbellidifolin were shown to possess different antioxidant activities (Yamahara *et al.*, 1991), like radical scavenging activity.

In Group V diabetic rats treated with combined herbal extract along with glibenclamide showed significantly ($P < 0.05$) better improvement in the hepatic antioxidant enzyme level over Group IV rats.

There was a decrease in the severity of the clinical signs in both the groups and also grossly, a significant appreciable improvement was observed in the appearance of pancreas. Microscopically, also there was a gradual improvement in the architecture of pancreas in both the Groups (IV and V), with an improvement in the number, size and shape of the islets and architecture of exocrine portion.

On 15^{th} day, the pancreatic islets appeared sparse in number, irregularly shaped and showed effects of STZ in the form of loss of β cell, with some cells showing cell swelling, cytoplasmic vacuolation, necrosis and apoptosis.

On Day 45, appreciable improvement in the architecture of islets and exocrine portion was observed with increase in size and number of islets. Hypercellularity with increased number of α cells and cells with the β cell morphology was observed however with persistence mild cytotoxic effects of STZ.

The improvement in the pancreatic architecture could be attributed to the effect of *Gymnema sylvestre* and *Swertia chirayita* on pancreatic islets resulting in promotion of beta cell subset regeneration or repair of damaged cells and in regeneration of pancreas (Baskaran *et al*., 1990 and Kanetkar *et al.*, 2007, Manjunath, 2013). The reduction in the pancreatic damge also could be attributed to the improved antioxidant levels observed in these groups by 45^{th} day of the study.

Microscopically, liver revealed progressive improvement in the architecture from STZ induced liver damage from 15^{th} day in both Group IV and V. However, mild vacuolar degeneration, necrosis and mononuclear cell infiltration were still persistent on the 45th day.

Among the treatment groups (Group IV and V), Group V rats treated with combination of herbal extracts along with glibenclamide showed better results in alleviating cytotoxic effects of STZ on liver parenchyma when compared to Group IV rats. This could be inferred that, addition of glibenclamide to the combined extract treatment could be responsible for the better performance of Group V rats compared to Group IV rats. The study showed that there was no dose dependent effect and both Group IV and V showed comparable results.

5.6 Immunohistochemical demonstration and quantification of insulin positive beta cells in pancreas

In the present study, immunohistochemical staining of pancreas for insulin revealed polyhedral shaped beta cells with dark brown coloured granules in the cytoplasm and unstained round to oval shaped nucleus. The functional beta cells revealed a large number of granules densely occupying the cytoplasm which varied in their size. Occasional beta cells with sparsely scattered granules were also observed. In normal control rats the mean percentage value of beta cells with insulin was 80.66 ± 0.71 on 45th day of the study.

In a normal individual, the beta cells are the most abundant cells found in the islets constituting approximately 65-80 per cent of the endocrine cells with 15-20 per cent of alpha cells, 4 per cent of delta cells and 1 per cent of pancreatic polypeptide cells.

In the present study, there was a drastic decline in the number of insulin positive cells in the islets of Langerhans in diabetic rats owing to the specific destruction of beta cells by STZ. On 15th day of treatment the mean percentage of insulin positive cells reduced to 2.83 ± 0.16 which slightly increased to 3.66 ± 0.49 on 45th day. The results of the present study, indicated that there was a slight increase in β cell mass due to regeneration of β cells by replication of pre existing β cells by 45th day in response to chronic hyperglycemia as also reported by Dor *et al.* (2004), Georgia and Bhushan (2004), Brennand *et al.* (2007) and Meier *et al.* (2008).

In the glibenclamide treatment group (III), there was a progressive increase in the number of immune positive cells with a mean percentage value of 54.16 ± 2.34 at 45th day of the study. The finding indicated that glibenclamide improved the number of beta cells in STZ diabetic rats on treatment for a period of 45 days. The possible reason for the increase in the number could be sulphonylurea induced insulin release from surviving cells which in turn stimulate and enhance beta cell proliferation, maturation and functional activity of cells, as experimentally proved by Ling *et al.* (2006) and Adewole and Ojewole, (2007). Similar observation was also made by Pragathi (2011), Mudasir *et al.* (2013), Manjunatha, (2013) and Shesha Rao, (2013) in their respective studies on efficacy of various plant extracts in STZ induced diabetes model.

In Group IV and V diabetic rats treated with combination of *Gymnema sylvestre* and *Swertia chirayita* and along with glibenclamide respectively also showed a significant improvement in the number of insulin positive cell when compared to diabetic control rats, with mean percentage values of 7.00 ± 0.36 and 8.50 ± 0.22 on 15th day to 21.00 ± 0.36

and 26.00 ± 1.15 on 45th day. However, the mean percentage values were significantly lesser compared to that of glibenclamide treatment and normal control on all the days of observation. Among the treatment groups, Group V rats showed better improvement compared to Group IV diabetic rats.

The improvement in architecture of pancreatic islets in combined herbal extracts treatment rats could be attributed to the effect of phytochemicals of *Gymnema sylvestre* and *Swertia chirayita* which have been reported to have direct action on pancreatic β-cells, resulting in promotion of regeneration of β-cell subsets or repair of damaged cells, thereby increase in insulin positive cells in the islets (Manjunath, 2013 and Kavitha Rani, 2015).

The perusal of literature did not reveal any reports on the effect of combination of *Gymnema sylvestre* and *Swertia chirayita* herb and there combined effect along with glibenclamide in alleviating STZ induced diabetic changes. The results of serum biochemistry, haematological parameters and antioxidant activity of the present study indicated that there was no dose dependent improvement and among the treatment groups Group V rats treated with combination of herbal extracts along with glibenclamide at 50% dosage revealed significantly better improvement than Group IV rats treated with combination of herbal extracts at 100% dosage. The improvement observed could be attributed to the addition of glibenclamide to the combined extracts in Group V rats at half dose.

Ayurvedic remedies for diabetes are usually mixed formulations containing blood sugar lowering herbs in combinations with immunomodulators and detoxicants. Polyherbal formulations have plant-based pharmacological agents which may exert synergistic, potentiative, agonistic antagonistic actions by virtue of their diverse active principles within themselves (Sujatha *et al*., 2012). Many medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another. Interaction between herbs and drugs may decrease or increase the pharmacological or toxicological effects of either components (Manjunath, 2013).

In the present study the combination of the *Gymnema sylvestre* and *Swertia chirayita* did not show synergistic effect in alleviating STZ induced changes in diabetes, in comparison with the results of individual herbs conducted by earlier workers like Manjunath, (2013) and Kavitha Rani, (2015). The diabetic rats treated with combination of herbal extract along with glibenclamide showed better improvement than diabetic rats treated with combination of herbal extracts alone. These improvements could be attributed to the addition of glibenclamide rather than combined herbal extract alone.

Summary

VI. SUMMARY

The present study was conducted to study the combined antidiabetic efficacy of *Gymnema sylvestre* and *Swertia chirayita* extracts alone and along with glibenclamide in streptozotocin induced diabetic rats and compared with oral hypoglycemic agent glibenclamide. The study was conducted for a period of 45 days under optimal managemental conditions.

Fifty male Wistar albino male rats were divided into five groups with ten rats in each group. Experimental diabetes was successfully induced by a single intra-peritoneal injection of streptozotocin at 45 mg/kg bw except in normal control rats. The rats were hyperglycemic by 72 hours with average serum glucose value of more than 200 mg/dL.

The various groups in the present study included normal control (Group I), diabetic control (Group II), diabetic rats treated with glibenclamide at 600 µg/kg bw (Group III), diabetic rats treated with combination of *Gymnema sylvestre* and *Swertia chirayita* at 200 mg/kg bw each (100% dosage) (Group IV) and diabetic rats treated with combination of *Gymnema sylvestre* and *Swertia chirayita* at 100 mg/kg bw (50% dosage) along with glibenclamide at 300 µg/kg bw (50% dosage) (Group V).

In order to study anti hyperglycemic effect, treatments were administered by gavaging on a daily basis for a period of 45 days. Blood and serum samples were collected on 3^{rd}, 15^{th}, 30^{th} and 45^{th} day and tissue samples for histopathology and antioxidant evaluation were collected by sacrificing two animals on 15^{th} and 30^{th} day and the remaining six animals on 45^{th} day of the experiment.

The various groups of rats were subjected to physiological and biochemical parameters such as body weight, serum glucose, cholesterol, triglyceride, ALT, AST, and haematological parameters like haemoglobin, TEC, TLC, platelet count, hepatic antioxidant levels of SOD, CAT, GPx and the findings were correlated and confirmed with histopathology.

A progressive decrease in mean body weight of diabetic control rats was observed throughout the study period which was significantly ($P<0.05$) lesser compared to normal control and other treatment groups (Group IV and V), where in all other treatment groups showed significant ($P<0.05$) improvement in body weight. Among treatment groups the mean body weight of Group V rats were comparable to glibenclamide control. However, not on par with the normal control rats.

High levels of serum glucose were recorded in all the experimental treatment groups on 3rd day, which gradually decreased by 15th, 30th and 45th day of the study. The diabetic group showed a gradual increase in the mean serum glucose levels and it remained significantly higher ($P<0.05$) when compared to the normal control. In other treatment groups (Group IV and V), there was a progressive decrease in serum glucose level which was significantly ($P<0.05$) higher compared to the normal control and glibenclamide treatment groups. Among the treatment groups, Group V rats treated with combination of herbal extracts along with glibenclamide showed better anti hyperglycemic effect.

The serum AST and ALT values in diabetic control rats progressively increased throughout the study period which were significantly ($P<0.05$) higher compared to normal control. The treatment groups (Group III to V) showed a progressive decrease in mean

ALT and AST values, however, the values were significantly (P<0.05) higher than glibenclamide and normal control rats, but Group V rats showed significantly (P<0.05) better improvement in ALT and AST values.

A significant (P<0.05) increase in mean serum triglyceride and cholesterol levels was observed in the diabetic control group on all the interval of the study when compared to normal control rats. In treatment groups (Group IV and V) a progressive decrease in serum triglyceride and cholesterol levels were observed. However, Group V rats treated with combination of herbal extracts along with glibenclamide showed better improvement which was comparable to that of glibenclamide treatment group on 45th day of the study.

The diabetic control rats showed a significant (P<0.05) decrease in the mean haemoglobin levels when compared to normal control rats. In all the other treatment groups (Group III, IV and V) a significant improvement in haemoglobin values was observed compared to diabetic control rats. However, the mean haemoglobin values were not on par with that of normal control. Group V rats treated with combination of herbal extracts along with glibenclamide showed numerically better improvement over Group IV rats.

The diabetic control rats showed a significant (P<0.05) decrease in TEC, TLC, platelet counts compared to the normal control rats. A progressive improvement in blood cell count was observed in treatment groups (Group IV and V) which was significantly (P<0.05) higher compared to diabetic control. Combination of herbal extracts along with glibenclamide treatment group (Group V) showed better improvement in blood cell counts compared to groups treated with combination of herbal extracts alone.

The hepatic antioxidant levels of CAT, SOD and GPx in diabetic control rats were significantly ($P<0.05$) decreased compared to normal control on all the days of examination. The treatment groups (Group IV and V) showed significant ($P<0.05$) progressive improvement in hepatic antioxidant levels when compared to diabetic control. However, the values were significantly lesser ($P<0.05$) compared to the glibenclamide treatment and normal control rats.

The clinical signs in STZ induced diabetic control rats were restlessness, polyuria, polydipsia, polyphagia, weight loss, rough hair coat and mild diarrhoea. The severity of the signs was progressive as the experiment advanced. All the other treatment group (Group III, IV and V) rats showed gradual reduction in the severity of clinical signs.

In the present study, pancreas was slightly congested, atrophied and appeared as thin gelatinous strip grossly in the diabetic control rats. Liver was pale, soft and friable. Other organs such as kidney, spleen, heart, lungs, intestine and brain did not show any gross lesions throughout the study. Similar lesions were observed grossly in treatment group also but the extent of the lesions was progressively decreased.

In diabetic control, microscopic lesions were noticed in both exocrine and endocrine portions. There was reduction in the size and number of islets of Langerhans. Compact arrangement of β and α cells was completely lost and islets were irregular in shape and size and there was total loss of β cells with α cells unaffected. Several islets of Langerhans also showed presence of apoptotic cells.

The exocrine pancreas showed loss of normal lobular architecture with reduction in the lobular size. Acinar cells showed vacuolar degeneration, necrosis and apoptosis, with infiltration of inflammatory cells.

Similar lesions were present in all the treatment groups on 15^{th} day, and there was a progressive improvement in the architecture of both endocrine and exocrine portion of pancreas in all the treatment groups at the end of the study. Streptozotocin induced effects on islets gradually subsided in the glibenclamide treatment group and the number of islets with compact arrangement of β and α cells increased.

Herbal extract treatment groups (Group IV and V) also showed progressive improvement in the architecture of both endocrine and exocrine portions. There was an increase in number and size of the islets, consisting of cells with β cell morphology. The improvement observed in Group V rats treated with combination of herbal extracts along with glibenclamide was better than Group IV rats treated with combination of herbal extracts alone.

Histopathology of liver revealed swelling of hepatocytes with increase in the cytoplasmic granularity, vacuolations, presence of necrotic and apoptotic hepatocytes and obliteration of sinusoidal spaces on 15^{th} day. These changes persisted till the end of the study in diabetic control rats.

All other treatment groups (Group III, IV and V) showed partially reduced streptozotocin induced changes in the liver. However, improvement was not on par with that of normal control rats.

Histopathologically, in diabetic control rats kidney showed increased granularity in tubular epithelial cells with congestion, heart showed congestion along with degeneration of myocardial cells and infiltration of inflammatory cells. In spleen, lymphatic depletion was observed. Other organs did not show any appreciable changes throughout the study.

Immunohistochemical demonstration of insulin showed drastic reduction in the number of insulin positive cells in the diabetic group. There was significant improvement in the number of insulin positive cells in various treatment groups. Glibenclamide treated rats (Group III) revealed an appreciable improvement in the endocrine portion compared to all other treatment groups (Group IV and V). Groups V rats treated with combination of herbal extracts along with glibenclamide showed better regeneration of β-cell population than Group IV rats treated with combination of herbal extracts alone. However, the improvement was not on par with that of glibenclamide treatment and normal control rats.

Conclusions

- Diabetes mellitus can be effectively induced in Wistar albino rats by single intraperitoneal injection of streptozotocin at the dose rates of 45 mg/kg body weight.
- Glibenclamide substantially alleviates the effects of STZ in diabetic rats. However, the improvement is not on par with that of normal control animals.
- Treatment with combination of *Gymnema sylvestre and Swertia chirayita* can only partially alleviate the STZ induced diabetic effects.
- No synergistic and no dose dependent improvement is present in combination of *Gymnema sylvestre* and *Swertia chirayita* extarcts.
- Supplementation of glibenclamide at half dose along with combined extracts has better antidiabetic effect.
- It could be concluded from the study that, combination of herbal extracts has only mild antidiabetic effect and cannot be used as a sole diabetes therapy and it cannot replace conventional anti diabetic drug glibenclamide in treatment of diabetes.

Bibliography

VII. BIBLIOGRAPHY

ABDOLLAHI, M., ZUKI, A.B.Z., GOH, Y.M., REZAEIZADEH, A. and NOORDIN, M.M., 2010. The effects of *Momordica charantia* on the liver in streptozotocin induced diabetes in neonatal rats. *Afr. J. Biotech.,* **9**(31): 5004-5012

ADEGHATE, E., HAMEED, R.S., PONERY, A.S., TARIQ, S., SHEEN, R.S., SHAFFI ULLAH, M. and DONÁTH, T., 2010. Streptozotocin Causes Pancreatic Beta Cell Failure via Early and Sustained Biochemical and Cellular Alterations. *Exp. Clin. Endocrinol. Diabetes*., **118**: 699 – 707

ADEWOLE, S.O. and OJEWOLE, J.A., 2007. Insulin-induced immunohistochemical and morphological changes in pancreatic beta-cells of streptozotocin-treated diabetic rats. *Methods Find. Exp. Clin. Pharmacol.,* **29**(7): 447-455

AHMED, A.B.A., RAO, A.S. and RAO, M. V., 2010. In vitro callus and in vivo leaf extract of *Gymnema sylvestre* stimulate β cells regeneration and anti-diabetic activity in Wistar rats. *Phytomed*., **17:** 1033–1039

AHMED, I., LAKHANI, M.S., GILLETT, M., JOHN, A. and RAZA, H., 2001. Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic *Momordica charantia* (karela) fruit extract in streptozotocin induced diabetic rats. *Diab. Res. Clin. Pract.,* **51:**155-161

AKBARZADEH, A., NOROUZIAN, D., MEHRABI, M.R., JAMSHIDI, S.H., FARHANGI, A., ALLAH, A., VERDI, S.M.A., MOFIDIAN, I. and LAME, B.R., 2007. Induction of diabetes by streptozotocin in rats. *Ind. J. Clin. Biochem.*, **22** (2): 60-64

AKHTAR, N. and HAQQ, T.M., 2012. Current nutraceuticals in the management of osteoarthritis: a review. *Ther. Adv. Musculoskelet Dis.*, **4** (3): 181–207

AKTER, F., RAHMAN, M.M., MOSTOFA, M. and CHOWDHURY, E.H., 2014. Antidiabetic effect of neem and spirulina in alloxan induced diabetic mice. *Int.J.Curr.Res.Aca.Rev.*, **2**(4): 124-134

AL-ATTAR, A.M. and ALSALMI, F.A., 2019. Effect of Olea europaea leaves extract on streptozotocin induced diabetes in male albino rats. *Saudi J. Bio. Sci.*, **26**:118-128

ALEEM, A and KABIR, H., 2019. Review on *Swertia chirata* as traditional uses to its pyhtochemistry and phrmacological activity. *J. Drug. Delivery & Therapeutics*., **8**(5-s):73-78

AL-NAHDI, A.M.T., JOHN, A. and RAZA, H., 2019. Streptozotocin-induced Molecular and Metabolic Targets in Pancreatic Beta-cell Toxicity. *Hamdan. Med. J.*, **12**(2):65-71

ALTAN, N., YIGIT, S., ELMALI, E., MALHATUN, E., ROTA, S. and KILIC, N., 1997. Effect of the sulphonylurea glyburide on superoxide dismutase in streptozotocin-induced diabetic rat muscle. *Gen. Pharmacol.*, **28:** 795–796

AMERICAN DIABETES ASSOCIATION., 2014. Diagnosis and classification of diabetes mellitus. *Diabetes Care*., **37**(1): 581-590

ANANTHAN, R., MUNIAPPAN, L., LEELAVINOTHAN, P., KUNGA, M.R., CHOKKANNA, G.B. and VENGATESH, N.B., 2003. Effect of *Gymnema montanum* on blood glucose, plasma insulin, and carbohydrate metabolic enzymes in alloxan-induced diabetic rats. *J. Med. Food.*, **6**(1): 43-49

ANDERSON, A. and BORG, L.A.H., (1980). Effects of glizipide on the insulin production by isolated mouse pancreatic islets. *Acta. Endocrinol*., **239**: 37-41

ANDERSON, N.V. and LOW, D.G., 1990. Incidence of diabetes mellitus in animals. *Anim. Hosp.*, **1**: 89-90

ANONYMOUS., 2019. Evaluation of some plant constituents for immunomodulatory effect. Chapter 2, Plant profile: *Gymnema sylvestre* R.Br and *Swertia chirata Buch.-Ham. 26-33*

ATANGWHO, I.J., EBONG, P.E., EGBUNG, G.E., AKPASO, M.I. and ASUQUO, E.E., 2010. Histological effect of combined extracts of *Vernonia amygdalina* and *Azadirachta indica* on normal and diabetic rats: The pancreas and liver. *Res. J. Agri. and Biol. Sci.,* **6**: 514-521

BABU, P.S. and PRINCE, P.S.M., 2004. Antihyperglycaemic and antioxidant effect of Hyponid, an ayurvedic herbomineral formulation in streptozotocin-induced diabetic rats. *J. Pharm. Pharmacol.*, **56**: 1435-1442

BAILEY, C.J. and DAY, C., 1989. Traditional plant medicines as treatments for diabetes. *Diab.Care*., **12**: 553-564

BAJPAI, M.B., ASTHANA, R.K., SHARMA, N.K., CHATTERJEE, S.K. and MUKHERJEE, S.K., 1991. Hypoglycemic effect of swerchirin from the hexane fraction of *Swertia chirayata*. *Planta Med.,* **57:** 102-104

BARAL, R., RAND, J.S., CATT, M. and FARROW, H.A., 2003. Prevalence of feline diabetes mellitus in a feline private practice. *J. Vet. Intern. Med.*, **17**: 433

BASKARAN, K., KIZAR, A.B., RADHA, S.K. and SHANMUGASUNDARAM, E.R., 1990. Antidiabetic effect of a leaf extract from *Gymnema sylvestre* in non-insulin dependent diabetes mellitus patients. *J. Ethnopharmacol.,* **30**: 295-390

BASNET, P., KADOTA, S., SHIMIZU, M. and NAMBA, T., 1994. Bellidifolin a potent hypogycemic agent in streptozotocin (STZ)-induced diabetic rats from *Swertia japonica*. *Planta Med.,* **60:** 507-511

BAYNES, J.W., 1991. Role of oxidative stress in comlications of diabetes mellitus. *Diabetes.,* **40:** 405.

BEDOYA, F.J., SOLANO, F. and LUCAS, M., 1996. N-monomethyl-arginine and nicotinamide prevent streptozotocin-induced double strand DNA break formation in pancreatic rat islets. *Experientia.,* **52:** 344-347

BHOWMIK, A., KHAN, L.A., AKHTER, M. and ROKEYA B., 2009. Studies on the antidiabetic effects of *Mangifera indica* stem-barks and leaves on nondiabetic, type 1 and type 2 diabetic model rats. *Bangladesh. J. Pharmacol.,***4**:110-114

BHOWMIK, A., MOSIHUZZAMAN, M., KABIR, Y. and ROKEYA, B., 2018. Antihyperglycemic activity of *Swertia chirata* on nstz-t2dm rats: a chronic study. *J. Pharmaceutical. Res. Int.* **22**(5): 1-11

BONNEFONT-ROUSSELOT, D., BASTARD, J.P., JAUDON, M.C. and DELATTRE, J., 2000. Consequences of the diabetic status on the oxidant/antioxidant balance. *Diabetes Metab.,* **26**(3): 163-76

BRAHMACHARI, G., MONDAL, S., GANGOPADHYAY, A., GORAI, D., MUKHOPADHYAY, B., SAHA, S. and BRAHMACHARI, A.K., 2004. Swertia (Gentianaceae): Chemical and pharmacological aspects. *Chem. Biodivers.,* **1**(11): 1627-1651

BRENNAND, K., HUANGFU, D., and MELTON, D., 2007. All β cells contribute equally to islet growth and maintenance. *PLoS Biol.,* **5**

BUKAN, N., SANCAK, B., BILGIHAN, A., KOSOVA, F., BUGDAYCI, G. and ALTAN, N., 2004. The effects of the sulphonylurea glyburide on glutathione peroxidase, superoxide dismutase and catalase activities in the heart tissue of streptozotocin induced diabetic rat. *Methods Find. Exp. Clin. Pharmacol.,* **26:** 579–522

CALIBORNE, A., 1985. Catalase Activity. *In*: Handbook of Methods for Oxygen Radical Research. *Edt*. Greenwald, R. A., Boca Raton., CRC Press., *pp* 283-284

CENNET, R. and EBUBEKIR., 2010. Effect of streptozotocin on biochemical parameters in rats. *Asian J. Chem*., **22** (3): 2375-2378

CHANDRASEKAR, B., BAJPAI, M.B. and MUKHERJEE, S.K., 1990. Hypoglycemic activity of *Swertia chirayita* (Roxb ex Flem) Karst. *Indian J. Exp. Biol.,* **28**(7): 616-618.

CHEN, Y., HUANG, B., HE, J., HAN, L., ZHAN, Y. and WANG, Y., 2011. *In vitro* and *in vivo* antioxidant effects of the ethanolic extract of *Swertia chirayita*. *J. Ethnopharmacol.,* **136**(2): 309-315.

CHIKA, A and YAHAYA, A., 2019. Effect of co-administration of glibenclamide and methanolic stem extract of *Anisopus mannii* N.E.Br. (*Apocynaceae*) on glucose homeostasis and lipid profile in streptozotocin/nicotinamide-induced diabetic rats. *National J. Physio. Pharmacy and Pharmacology*., **9**(10):1045-1051.

CHIS, I.C., UNGUREANU, M.I., MARTON, A., SIMEDREA, R., MURESAN, A., POSTESCU, I.D. and DECEA, N., 2009. Antioxidant effects of a grape seed extract in a rat model of diabetes mellitus. *Diab. Vasc. Dis. Res.*, **6**(3): 200-204

CHOI, J.S., YOKOZAWA, T. and OURA, H., 1991. Improvement of hyperglycemia and hyperlipemia in streptozotocin diabetic rats by a methanolic extract of *Prunus daidiana* stems and its main component, prunin. *Planta Med*., **57:** 208-211

CHOPRA, R.N., NAYAR, S.L. and CHOPRA, I.C. 1956. Glossary of Indian Medicinal Plants, CSIR, New Delhi, p. 237.

DELANEY, C.A., DUNGER, A., MATTEO, M.D., CUNNINGHAM, J.M., GREEN, M.H. and GREEN, I.C. 1995. Comparison of inhibition of glucose-stimulated insulin secretion in rat islets of Langerhans by streptozotocin and methyl and ethyl nitrosoureas and methane sulphonates. Lack of correlation with nitric oxide-releasing or alkylating ability. *Biochem, Pharmacol .,* **50**: 2015-2020

DHANUSH, K.B., 2009. Pathomorphological and biochemical evaluation of hypoglycaemic effects of *Eugenia jambolona* and *Tinospora cordifolia* in experimentally induced diabetes in rats. M.V.Sc. thesis, KVAFSU, Bidar, Karnataka, India.

DOR, Y., BROWN, J., MARTINEZ, O.I. and MELTON, D.A. 2004. Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. *Nature.*, **429**: 41– 46

EL-BAKY, A.A.A., 2013. Clinicopathological effect of extract on Streptozotocininduced diabetes in rats. *World J. Med. Sci*. **8** (3): 205-211

ELMALI, E., ALTAN, N. and Bukan, N., 2004. Effect of the sulphonylurea glibenclamide on liver and kidney Antioxidant enzymes in Streptozocin-induced diabetic rats. *Drugs R D.*, **5** (4): 203-208

EL-SHERBINY, M., ELADL, M.A., RANADE, A.V., GUIMEI, M. and GABR, H., 2019. Functional beta-cells derived from umbilical cord blood mesenchymal stem cells for curing rats with streptozotocin-induced diabetes mellitus. *Sing. Med. J.,*1-21

ELSNER, M., GULDBAKKE, B., TIEDGE, M., MUNDAY, R. and LENZEN, S., 2000. Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. *Diabetologia.*, **43**: 1528-1533

EREJUWA, O.O., SULAIMAN, S.A., WAHAB, M.S.A., SIRAJUDEEN, K.N., SALLEH, S. and GURTU, S., 2011. Glibenclamide or metformin combined with honey improves glycemic control in streptozotocin-induced diabetic rats. *Int. J. Biol. Sci.*, **7:** 244-252

FELIG, G., BERGMAN, M. and FELIG, C., 1995. The Endocrine Pancreas: Diabetes mellitus, 3rd ed. MacGraw-Hill, New York. pp 1107-1250

FERNANDES, N.P., LAGISHETTY, C.V., PANDA, V.S. and NAIK, S.R., 2007. An experimental evaluation of the antidiabetic and antilipidemic properties of a standardized Momordica charantia fruit extract. *BMC Complement. Altern. Med.*, **7:** 29-32

GANDA, O.P., ROSSI, A.A. and LIKE, A.A., 1976. Studies on streptozotocin diabetes. *Diab.*, **25**: 595-603

GEORGIA, S., and BHUSHAN, A., 2004. B cell replication is the primary mechanism for maintaining postnatal β cell mass. *J. Clin. Invest*., **114**: 963–968

GU, D., ARNUSH, M. and SARVETNIC, N., 1997. Endocrine/exocrine intermediate cells in Streptozotocin treated Ins-IFN-gamma transgenic mice. *Pancreas.,* **15**(3): 246-250

GUIOT, Y., HENQUIN, J.C. and RAHIER, J., 1994. Effects of glibenclamide on pancreatic β cell proliferation *in vivo. Eur.J. Pharmacol.,* **261**(1):157-161

GUNASEKARAN, V., SRINIVASAN, S. and RANI, S.S., 2019. Potential antioxidant and antimicrobial activity of *Gymnema sylvestre* related to diabetes. *J. Med. Plants. Stud***.,** **7**(2): 5-11

GUPTA, R., KUMAR G.B., SAMTA, J. and SAXENA, A.M., 2008. An overview of Indian novel traditional medicinal plants with antidiabetic potentials. *African. J. Trad. CAM.,* **5** (1):1 – 17

GURIKAR, R.Y., 2014. Pathomorphological and biochemical evaluation of effect of chromium in streptozotocin induced diabetes in rats. Thesis submitted to KVAFSU for Master of Veterinary Sciences in Veterinary Pathology.

HAKIM, Z.S., PATEL, B.K. and GOYAL, R.K., *1997.* Effects of chronic ramipril treatment in streptozotocin-induced diabetic rats. *Ind. J. Physiol. Pharmacol.,* ***41:*** *353-360*

HARDMAN, J.G. and LIMBERD, L.E., 2001. Insulin, oral hypoglycemic agents and the pharmacology of the endocrine pancreas. *In: Goodman and Gilman's: The Pharmacological Basis of Therapeutics.,* 10th E*dn.* Mcgraw-Hill Company Limited, USA., *pp* 1383-1399

HEARSE, D.J., 1979. Cellular damage during myocardial ischemia: Metabolic changes leading to enzyme leakage. In: Hearse, D.S., Leiris, J. and Loisance, D., Editors. Enzymes in cardiology. Chichester, John Wiley and Sons., *pp* 1-2

HELAL, E.G.E., GAWISH, A.S.M. and KAHWASH, A., 2005. Some hematological studies on diabetic rats treated with certain hypoglycemic plants. *The Egypt. J. Hospital Medicine.,* **19:** 179-188

HEM, D.A., 1977. Exploration and exploitation. In: D.A. Hem, Editor, Biologically active substances, John Wiley and Sons, Chichester, New York., *pp* 209

HOENIG, M., 2002. Comparative aspects of diabetes mellitus in dogs and cats. *Mol. Cell Endocrinol.*, **197**(1-2): 221-229

HRIBAL, M.L., D'ALFONSO, R., GIOVANNONE, B., LAURO, D., LIU, Y.Y., BORBONI, P., FEDERICI, M., LAURO, R. and SESTI, G., 2001. The sulfonylurea glimepiride regulates intracellular routing of the insulin-receptor complexes through their interaction with specific protein kinase C isoforms. *Mol. Pharmacol*., **59**: 322-330

HSIAO, C.C., LIN, C.C., HOU, S.Y., YANG KO, J. and WANG, C.J., 2019. Low-energy extracorporeal shock wave ameliorates streptozotocin induced diabetes and promotes pancreatic beta cells regeneration in a rat model. *Int. J. Mol. Sci.,* **20**(4934):1-12

INTERNATIONAL DIABETES FEDARATION., 2017. *Diabetes atlas,* 8th edition. http://www.idf.org/, accessed on june 20, 2019.

ITEMOBONG, S.E., ITEM, J.A., HENRY, D.A., ITORO, F.U., OBOSO, E.E. and PATRICK, E.E., 2010. Effects of ethanol extract of *Azadirachta indica* leaves on some immunological and haematological parameters of diabetic Wistar rats. *African J. Pharma. pharmacol*., **4**(3): 104-108

JAIN, S.K, LEVINE, S.N., DUETT, J. and HOLLIER, B., 1990. Elevated lipid peroxidation levels in red blood cells of streptozotocin-treated diabetic rats. *Metabolism.,* **39**(9): 971-975

JELODAR, G., MALEKI, M., MOTADAYEN, M.H. and SIRUS, S., 2005. Effect of fenugreek, onion and garlic on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats. *Ind. J. Med. Sci*., **59**: 64- 69

JOSHI, P. and DHAWAN, V., 2005. *Swertia chirayita* — an overview. *Curr.Sci.,* **89**: 635-640

KAKKAR, R., MANTHA, S.V., RADHI, J., PRASAD, K. and KALRA, J., 1998. Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. *Clin. Sci.,* **94**(6): 623-632

KALEEM, M., ASIF, M., AHMED, Q.U. and BANO, B., 2006. Antidiabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin-induced diabetic rats. *Singapore Med. J.,* **47**(8): 670-675

KANETKAR, P., SINGHAL, R. and KAMAT, M., 2007. *Gymnema sylvestre*: A Memoir. *J. Clin. Biochem. Nutr*., **41**: 77–81

KANG, M.H., LEE, M.S., CHOI, M.K., MIN, K.S and SHIBAMOTO, T., 2012. Hypoglycemic activity of *Gymnema sylvestre* extracts on oxidative stress and antioxidant status in diabetic rats. *J. Agri. Food. Chem.*, **60** (10): 2517–2524

KANTE, K. and REDDY, C.S., 2013. Antidiabetic activity of *Dolichos lablab* (seeds) in streptozotocin nicotinamide induced diabetic rats. *Hygeia J. D. Med*., **5**(1): 32-40

KAR, A., CHOUDHARY, B.K. and BANDYOPADHYAY, N.G., 2003. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. *J. Ethnopharmacology.,* **84**:105-108

KARUNANAYAKE, E.H., BAKER, J.R., CHRISTIAN, R.A., HEARSE, D.J. and MELLOWS, G., 1976. Autoradiographic study of the distribution and cellular uptake of (14C)-streptozotocin in the rat. *Diabetologia.,* **12**:123–128

KARUNANAYAKE, E.H., HEARSE, D.J. and MELLOWS, G., 1974. The synthesis of Streptozotocin and its distribution and excretion in the rat. *Biochem. J.,* **142:** 673-683

KARUNANAYAKE, E.H., HEARSE, D.J. and MELLOWS, G., 1975. The metabolic rate and elimination of streptozocin. *Biochem.Soc. Trans.,* **3**: 410-414

KAVITHA RANI, B., 2015. Pathomorphological and biochemical studies on the effect of *gymnema sylvestre* and chromium in induced diabetes in rats. Ph.D. thesis, KVAFSU, Bidar, Karnataka, India.

KAVITHA, K.N. and DATTATRI, A.N., 2013. Experimental Evaluation of antidiabetic activity of *Swertia Chirata* – Aqueous Extract. *J. Pub. Health. Med. Res*., **1**(2): 71-75

KAVITHA, K.N. and DATTATRI, A.N., 2015. An evaluation of antidiabetic potential of methanolic extract of *Swertia chirata* in streptozotocin induced diabetic rats**.** *RJPBCS*.**, 6** (1): 620-625

KAWADA, J., 1992. New hypotheses for the mechanisms of streptozotocin and alloxan inducing diabetes mellitus. *Yakugaku Zasshi*., **112**(11): 773-791

KENNEDY, L. and BAYNES, J.W., 1984. Non-enzymatic glycosylation and the chronic complications of diabetes: an overview. *Diabetologia.,* **26**(2): 93-98

KING, JF, A., 2012. The use of animal models in diabetes research. *Brit. J. Pharm*., **166**: 877-894.

KIRTIKAR, K.R. and BASU, B.D. 1933. Indian Medicinal Plants, Ed. L. M. Basu, Allahabad, Vol. 3, p. 1663.

KOLTERMANN, O.G., GRAY, R.S., SHAPIRO, G., SCARLETT, J.A., GRIFFIN, J. and OLEFSKY, J.M., 1984. The acute and chronic effects of sulfonylurea therapy in type II diabetic subjects. *Diabetes,* **33**: 346–354

KRONCKE, K.D., FEHSEL, K., SOMMER, A., RODRIGUEZ, M.L. and KOLB-BACHOFEN, V., 1995. Nitric oxide generation during cellular metabolization of the diabetogenic N-methyl-N-nitroso-urea streptozotocin contributes to islet cell DNA damage. *Biol. Chem. Hoppe-Seyler.*, **376:** 179-185

KUMAR, M., ALOK, S., JAIN, S.K. and VERMA, A., 2014. Antidiabetic activity of plants with their phytoconstituents: a review. Int. J. Pharmacognsy., **1**(1): 9-22

KUMAR, P., RANI, S., ARUNJYOTHI, B., CHAKRAPANI, P. and ROJARANI, A., 2017. Evaluation of antidiabetic activity of *Gymnema sylvestre* and *Andrographis paniculata* in streptozotocin induced diabetic rats. *Int. J. of Pharmacognosy and Phytochemical. Res*., **9**(1); 22-25

KUMAR. S.N., MANU, U.V. and MANI, I., 2010. An open label study on the supplementation of *Gymnema sylvestre* in type 2 diabetics. *J.Diet.Suppl.*, **7**(3): 273-282

LACHMAN, L., LIEBERMAN, H.A. and KANIG, J.L., 1986. The theory and practice of industrial pharmacy, Varghese publishing house, Bombay, *3rd Edi.*, pp 790

LAHA, S. and PAUL, S., 2019. *Gymnema sylvestre* (Gurmar): A Potent Herb with Anti-Diabetic and Antioxidant Potential. *Pharmacogn. J*., **11**(2):201-206

LAKSHMI, H.A., 2015. Studies on induced diabetes in rats and evaluation of efficacy of *Azadirachta indica* in its control. M.V.Sc thesis, Karnataka veterinary animal and fisheries science university, Bidar, India.

LAWAL, S.K., ADENIJI, A.A., SULAIMAN, S., AKAJEWOLE, M.M., BUHARI, M.O. and OSINUBI, A.A., 2019. Comparative effects of glibenclamide, metformin and insulin on fetal pancreatic histology and maternal blood glucose in pregnant streptozotocin- induced diabetic rats. *Afri Health Sci.*, **19**(3): 2491-2504

LEACH, M.J., 2007. *Gymnema sylvestre* for diabetes Mellitus: a systematic review. *J. Altern. Complement. Med.*, **13**(9): 977-983

LEBOVITZ, H.E. and FEINGLOS, M.N., 1983. The oral hypoglycemic agents. *In*: Ellenberg, M. and Rifkin, H., *Edn*. Diabetes mellitus, theory and practice, 3rd *Edn*. Medical Examination Publishing, New Hyde Park, New York, 591–610

LENZEN, S., 2007. Alloxan and streptozotocin diabetes. *In*: Peschke E (*ed*) Endokrinologie III VorträgeimRahmen des Projektes "Zeitstrukturenendokriner Systeme". [Endocrinology III lectures within the time structures of endocrine systems project framework]. Abhandlung der Sächs. Akad. Wiss., Mathnaturwiss Klasse, Verlag der Sächsischen Akademie der Wissenschaften, Leipzig, commissioned by S. HirzelVerlag, Stuttgart/ Leipzig., *pp* 119–138

LENZEN, S., 2008. The mechanisms of alloxan- and streptozotocin-induced diabetes**.** *Diabetologia.,* **51**(2): 216-226

LEWIS, C and BARBIERS, A.R., 1959. Streptozotocin, a new antibiotic. *In vitro* and *in vivo* evaluation. *Antibiot. Ann.,* **7**: 247–254

LI, Z., KARLSSON, F.A. and SANDLER, S., 2001. Islet loss and alpha cell expansion in type I diabetes induced by multiple low-dose streptozotocin administration in mice. *J. Endocrinol.,* **165**: 93-99

LING, Z., WANG, Q., STANGE, G., VELD, P.I. and PIPELEERS. D., 2006. Glibenclamide Treatment Recruits β-Cell Subpopulation into Elevated and Sustained Basal Insulin Synthetic Activity. *Diabetes.,* **55:** 78-85

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RAMDALL, R.J., 1951. Total protein estimation. *J. Biol. Chem.,* **193:** 265

LUNA, L.G., 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, *Edn* 3rd, McGraw Hill Book Co., London.

LUZI, L. and POZZA, G., 1997. Glibenclamide: an old drug with a novel mechanism of action. *Acta. Diabetol.,* **34:** 239-244

MAGEE, P.N. and SWANN, P.F., 1969. Nitroso compounds. *Br. Med. Bull*., **25**: 240 - 244

MALL, G,K., MISHRA, P,K. and PRAKASH, V., 2009. Antidiabetic and Hypolipidemic Activity of *Gymnema sylvestre* in Alloxan induced Diabetic Rats. *Global J. Biotech. Biochem*., **4** (1): 37-42

MALLIKARJUNA, G., 2009. Pathomorphological and biochemical evaluation of hypoglycaemic effects of *Momardica charantia* and *Gymnema sylvestre* in experimentally induced diabetes in rats. M.V.Sc. thesis, KVAFSU, Bidar, Karnataka, India

MALLIKARJUNA, G., SUGUNA RAO. and SATYANARAYANA, M.L., 2013. Pathomorphological and biochemical evaluation of hypoglycaemic effects of *Momardica charantia* and *Gymnema sylvestre* in experimentally induced diabetes in rats. *Int. J. Agri. Biol. Res*., **3**(1): 51-57

MANJUNATHA, K.P., 2013. Pathomorphological studies on induced Diabetes in rats and evaluation of Antidiabetic efficacy of *Pterocarpus marsupium* and *Swertia chirayita* extracts. PhD thesis, KVAFSU, Bidar, Karnataka, India.

MARITIM, A.C., SANDERS, R.A. and WATKINS, J.B., 2003. Diabetes, oxidative stress, and antioxidants: a review. *J. Biochem. Mol. Toxicol.*, **17**(1): 24-38

MARKLUND, S.L. and MARKLUND, G., 1974. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem*., **47:** 469-474

MATHEKA, D.M. and ALKIZIM, F.O., 2012. Complementary and alternative medicine for type 2 diabetes mellitus: Role of medicinal herbs. J. *Diab. Endocrinol.,* **3**(4): 44-56

MEHTA, J.L., RASOULI, N., SINHA, A.K. and MOLVI, B., 2006. Oxidative stress in diabetes: A mechanistic overview of its effects on atherogenesis and myocardial dysfunction. *Int. J. Biochem. Cell. Biol.*, **38**: 794

MEIER, J. J., BUTLER, A.E., SAISHO, Y., MONCHAMP, T., GALASSO, R. BHUSHAN, A., RIZZA, R.A. and BUTLER, P.C., 2008. B-cell replication is the primary mechanism subserving the postnatal expansion of β-cell mass in humans. *Diab.,* **57**: 1584–1594

MELANDER, A., BITZEN, P.O. and FABER, O., 1987. Sulphonylurea antidiabetic drugs: An update of their clinical pharmacology and rational therapeutic use. *Drugs,* **37**: 58-72

MIR, S.H., BAQUI, A., BHAGAT, R.C., DARZI, M.M. and SHAH, A.W., 2008. Biochemical and histomorphological study of streptozotocin-induced diabetes mellitus in rabbits. *Pak. J. Nutr.,* **7:** 359- 364

MOMIN, A., 1987. Role of indigenous medicine in primary healthcare. In: Proceedings of first international seminar on Unani medicine. New Delhi, India, 54.

MORGAN, N.G., CABLE, H.C., NEWCOMBE, N.R. and WILLIAMS, G.T., 1994. Treatment of cultured pancreatic-cells with streptozotocin induces cell death by apoptosis. *Biosci. Rep.,* **14:** 243-250

MUDASIR, 2011. Pathomorphological and biochemical evaluation of hypoglycaemic effects of *Momardica charantia* and cow urine in experimentally induced diabetes in rats. M.V.Sc. thesis, KVAFSU, Bidar, Karnataka, India.

MUDASIR, S.A., SUGUNA RAO., BYREGOWDA, S.M., SATYANARAYANA, M.L., SHRIDHAR, N.B. and PRAGATHI, B.S., 2013. Evaluation of hypoglycemic effect of *Momordica charantia* extract in distilled water in streptozotocin-diabetic rats. *Braz. J. Vet. Pathol.,* **6**(2): 56-64

MUKHERJEE, B. and MUKHERJEE, S.K., 1987. Blood Sugar Lowering Activity of *Swertia chirata* (Buch-Ham) Extract. *Pham. Biol.,* **25**(2): 97-102

MUKHERJEE, S.K. and MUKHERJEE, S.S., 1966. Therapeutic advance in diabetes mellitus through ages. *J. Res. Ind. Med.,* **1**: 91-112.

MUZUMBUKILWA, W.T., NLOOTO, M. and OWIRA, P.M.O., 2019. Hepatoprotective effects of Moringa oleifera Lam (Moringaceae) leaf extracts in streptozotocin-induced diabetes in rats. *J. Functiona.l Foods.,* **57**:75–82

NAGALEKSHMI, R., MENON, A., DHANYA, K., CHANDRASEKHARAN., KRISHNAN, C. and NAIR, K. 2011. Hepatoprotective activity of Andrographis Paniculata and Swertia Chirayita. *Food. Chem. Toxi*., **49** (2011): 3367–3373

OGBODO, S. O., CHUKWURAH, E. F., OKORO, I.L. and OKEKE, A. C., 2007. Platelet number and functions in diabetic patients in Enugu, Nigeria. *Pharmacologyonline*, **2**: 300-306.

OHKUWA, T., SATO, Y. and NAOI, M., 1995. Hydroxyl radical formation in diabetic rats induced by streptozotocin. *Life. Sci*., 56:1789-1798

OHNO, T., HORIO, F., TANAKA, S., TERADA, M., NAMIKAWA, T. and KIYCH, J., 2000. Fatty liver and hyperlipidemia in IDDM of streptozotocin treated shrews. *Life Sci.,* **123**: 125-131

OKABAYASHI, Y., TANI, S., FUJISAWA, T., KOIDE, M., HASEGAWA, H., NAKAMURA, T., FUJII, M., OTSUKI, M., 1990. Effect of *Gymnema sylvestre*, R.Br. on glucose homeostasis in rats. *Diab. Res. Clin. Pract*., **9**: 143-148

OUBRE, A.Y., CARLSON, T.J., KING, S.R. and REAVEN, G.M., 1997. From plant to patient: an ethnomedical approach to the identification of new drugs for the treatment of NIDDM. *Diabetologia.,* **40**(5): 614-617.

PAPAZAFIROPOULOU, A., KARDARA, M., SOTIROPOULOS, A., BOUSBOULAS, S., STAMATAKI, P. and PAPPA, S., 2010. Plasma glucose levels and white blood cell count are related with ankle Brachial Index in Type 2 diabetic subjects. *Hellenic J Cardiol.,* **51**: 402-406

PARASURAMAN, S., CHING, T,H., LEONG, C.H. and BANIK, U, 2019. Antidiabetic and antihyperlipidemic effects of a methanolic extract of *Mimosa pudica* (Fabaceae) in diabetic rats. *Egyptian J. Basic Applied Sci*., 6(**1**):137–148.

PARI, L., RAMAKRISHNAN, R. and VENKATESWARAN, S. 2001. Antihyperglycaemic effect of Diamed, a herbal formulation in experimental diabetes in rats. *J. Pharm. Pharmacol.,* **53:** 1139–1143

PARIS, M., TOURREL-CUZIN, C., PLACHOT, C. and KTORZA, A., 2004. Review: Pancreatic β cell neogenesis revisited. *Exptl. Diab. Res.*, **5:** 111-121

PERSAUD, S.J., AL-MAJED, H., RAMAN, A. and JONES, P.M., 1999. *Gymnema sylvestre* stimulates insulin release in vitro by increased membrane permeability. *J. Ethnopharmacol.,* **163**: 207-212

PFEIFER, M.A., HALTER, J.B., GRAF, R. and PORTE, D.Jr., 1980. Potentiation of insulin secretion to non-glucose stimuli in normal man by tolbutamide. *Diabetes.,* **29:** 335-340

PHOBOO, S., PINTO, M.D.S., BHOWMIK, P.C., JHA, P.K. and SHETTY, K., 2010. Quantification of major phytochemicals of *Swertia chirayita*, a medicinal plant from nepal. *An Int. J. Ecol.,* **17:** 59-68

PIEDROLA, G., NOVO, E., ESCOBER, F. and ROBLES, G.R., 2001. White blood cell count and insulin resistance in patients with coronary artery disease. *Ann. Endocrinol*., **62**: 7-10

PILLION, D.J., JENKINS, R.L., ATCHISON, J.A., STOCKARD, C.R., CLEMENTS R.S. and GRIZZLE, W.E., 1988. Paradoxical organ-specific adaptations to streptozotocin diabetes mellitus in adult rats. *Am. J. Physiol.,* **254:** 749-755

PLATEL, K., SHRUPALEKER, K.S. and SRINIVASAN, K., 1993. Effect of dietery intake of freeze-dried bitter gourd (*Momordica charantia*) on growth and blood constituents in albino rats. *Die Nahrung*, **37**(2): 156-160

PRAGATHI, B.S., 2011. Evaluation of hypoglycaemic effect of *Gymnema sylvestre* and cow urine in diabetic rat model. M.V.Sc.thesis, KVAFSU, Bidar, Karnataka, India.

PRAGATHI, B.S., SUGUNA RAO., BYREGOWDA, S.M., SATYANARAYANA, M.L., NAGARAJ, B.N., ANSAR KAMRAN, C., AYOUB, S.M. and PURUSHOTHAM. K.M., 2015. Antidiabetic effect of *Gymnema sylvestre* in streptozotocin induced diabetes in rats. *Braz. J. Vet. Pathol.*, **8**(2): 36 – 45.

PRAHL, A., GLICKMAN, L., GUPTIL, L., GLICKMAN, N. and TETRICK, M., 2003. Time trends and risk factors for diabetes mellitus in cats. *J. Vet. Intern. Med.*, **17:**434.

PRINCE, P.S.M., MENON, V.P. and GUNASEKARAN, G., 1999. Hypolipidaemic action of *Tinospora cordifolia* roots in alloxan diabetic rats. *J. Ethnopharmacol*., **64**: 53-57

RABBANI, S.I., DEVI, K. and KHANAM, S., 2010. Protective role of glibenclamide against nicotinamide-streptozotocin induced nuclear damage in diabetic Wistar rats. *J. Pharmacol. Pharmacother.*, **1**(1): 18-23

RAHEJA, B.S., 1977. Oral hypoglycemic agent in the management of Maturity-Onset Diabetes-A: *Reprint. Reassessment. Journal J. J. Group Hospitals Grant Medical College.*, **22**: 1-9.

RAJESH, C.S., RAJENDRA, H., VENKATESH, P., ANAND, A.S. and KISHAN PRASAD, H.L., 2017. Anti-hyperglycemic effect of *Swertia chirata* root extract on indinavir treated rats. *National. J. Phys. Pharmacy. Pharmacology*., **7**(6): 569-573.

RAMADAN, G., EL-BEIH, N. M. and ABD EL-DHAFFAR, E. A., 2009. Modulatory effects of black v. green tea aqueous extract on hyperglycemia, hyperlipidaemia and liver dysfunction in diabetic and obese rat models. *Br. J. Nutr*., **102**: 1611-1619

RAND, J. S., FLEEMAN, L. M., FARROW, H.A., APPLETON, D.J. and LEDERER, R., 2004. Canine and feline diabetes mellitus: nature or nurture? *J. Nutr.,* **134**(8):2072-2080.

RANG, H. P. and DALE, M. M., 1991. The Endocrine System Pharmacology. Longman, Harlow., pp 504-508

RANI, K.B., SUGUNA RAO., SATYANARAYANA, M.L., BYREGOWDA, S.M., NARAYANASWAMY, H.D., ANSAR KAMRAN, C., SHRIDHAR, N.B and GURIKAR, R.Y., 2016. Assessment of hepatic antioxidant enzymatic activity with *Gymnema sylvestre* and chromium in Streptozotocin induced diabetic female rats. *Frontier J. Vet. Anim. Sci.,* **5**(1): 5-15

RAO, B.K., KESAVULU, M.M., RAO, C.A. and GIRI, R., 1999. Antidiabetic and hypolipidemic effects of *Momordica cymbalaria* in alloxan diabetic rats. *J. Ethnopharmacol.*, **67**:103-109

RAZA, H. and JOHN, A., 2012. Streptozotocin-Induced Cytotoxicity, Oxidative Stress and Mitochondrial Dysfunction in Human Hepatoma HepG2 Cells. *Int. J. Mol. Sci.,* **13**(5): 5751-5767

RENU, A., KUMAR SUNIL., KUMAR DINESH., MALIK AJAY and KUMAR TARUN., 2011. Antidiabetic activity of ethanolic extract of *Swertia chirayita* Buch ham.*IRJP*., **2**(1):230-232

RESMI, C.R., VENUKUMAR, M.R. and LATHA, M.S., 2006. Antioxidant activity of *Albizzia lebbeck* (Linn.) Benth. in alloxan diabetic rats. *Indian J. Physiol. Pharmacol.,* **50**(3): 297-302

ROSAC, C., 2002. The pathophysiological basis of efficacy and clinical experience with the new oral antidiabetic agents. *J. Diab. Compli*., **16:** 123-132

ROTRUCK, J.T., POPE, A.L., GANTHER, H.E., SWANSON, A.B., HAFEMAN, D.G. and HOCKSTRA, W.G., 1973. Selenium: Biochemical role as a component of glutathione peroxidase. *Sci*., **179:** 588- 598

RUBIN, R. and STRAYER, D.S., 2008. Rubin Pahology: Clinicopathological foundation of medicine, Lippincott Williams and Wilkins publication. *pp* 231

SAHA, P., MANDAL, S., DAS, A., DAS, P.C. and DAS, S., 2004. Evaluation of the anticarcinogenic activity of Swertia chirata Buch.Ham, an Indian medicinal plant, on DMBA-induced mouse skin carcinogenesis model. *Phytother. Res.,* **18**(5): 373-378

SAHU, N., MAHATO S.B., SARKAR, S.K., and PODDAR, G., 1996. Triterpenoid Saponins from Gymnema sylvestre. *Phytochem*., **41**:1181–1185

SARKAR, S., PRANAVA, M. and MARITA, A.R., 1996. Demonstration of the hypoglycemic action of *Momordica charantia* in a validated animal model of diabetes. *Pharmacol. Res.*, **33:**1-4

SATHYA, S., KOKILAVANI, R. and GURUSAMY, K., 2008. Hypoglycemic effect of *Gymnema sylvestre* (retz.,) R. Br leaf in normal and alloxan induced diabetic rats. *Ancien . Sci of Life*., **28**(2) :12-14

SAXENA, A.M., BAJPAI, M.B. and MUKHERJEE, S.K., 1991. Swerchirin induced blood sugar lowering of streptozotocin treated hyperglycemic rats. *Indian J. Exp. Biol.,* **29**(7): 674-675

SAXENA, A.M., BAJPAI, M.B., MURTHY, P.S. and MUKHERJEE, S.K., 1993. Mechanism of blood sugar lowering by a swerchirin-containing hexane fraction (SWI) of *Swertia chirayita*. *Indian J. Exp. Biol.,* **31**(2): 178-181

SAXENA, A.M., MURTHY, P.S. and MUKHERJEE, S.K., 1996. Mode of action of three structurally different hypoglycemic agents: A comparative study. *Indian J. Exp. Biol.*, **34**(4): 351-355

SAXENA, A.M., SAMTAJOHRI., PRIYASHARMA. and RAHUL, G., 2007. Blood sugar lowering activity of *Swertia chirayita* (roxb. ex. flem) karst in different experimental rat models. *Flora and Fauna.*, **13**(2): 415-418

SCARTEZZINI, P. and SPERONI, E., 2000. Review on some plants of Indian traditional medicine with antioxidant activity. *J.Ethnopharmacol.*, 71: 23–42

SCHNEDL, W.J, FERBER. S., JOHNSON, J.H. and NEWGARD, C.B., 1994. STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. *Diabetes*., **43**: 1326-1333

SEKAR, B.C., MUKHERJEE, B., CHAKRAVARTI, B. and MUKHERJEE S.K., 1987. Effect of different fractions of *Swertia chirayita* on the blood sugar level of albino rats. *J. Ethnopharmacol.,* **21**(2): 175-181

SERRANO-MARTIN, X., PAYARES, G. and MENDOZA-LEON, A., 2006. Glibenclamide, a blocker of K+ (ATP) channels shows antileishmanial activity in experimental murine cutaneous leishmaniasis. *Antimicrob. Agents Chemother.*, **50**(12): 4214-4216

SETH, S.D. and SHARMA, B., 2004., Medicinal plants of India. *Indian J. Med. Res*.**,** **120:**9-11

SHABIB, B.A., KHAN, L.A. and RAHMAN, R., 1993. Hypoglycemic activity of *Coccinia indica* and *Momordica charantia* in diabetic rats; depression of the hepatic glyconeogenic enzymes glucose 6 phosphatase and fructose 1,6 bisphosphatase and elevation of both liver and red cell count enzyme glucose-6- phosphate dehydrogenase. *Biochem. J.*, **292**: 267-270

SHANMUGASUNDARAM, E.R.B., GOPINATH, K.L., SHANMUGASUNDARAM, K.R. and RAJENDRAN, V.M., 1990. Possible regeneration of the islets of langerhans in streptozotocin diabetic rats given *Gymnema sylvestre* leaf extracts. *J. Ethnopharmacol.*, **30**: 265-279

SHANMUGASUNDARAM, E.R.B., VENKATASUBRAMANYAM, M., VIJENDRAN, N. and SHANMUGASUNDARAM, K.R., 1988. Effect of an isolate from *Gymnema sylvestre,* R.Br. in the control of diabetes mellitus and the associated pathological changes. *Ancient. Sci. Life.,* **7**:183- 194

SHARMA, A.K. and GUPTA, R., 2017. Anti-hyperglycemic activity of aqueous extracts of some medicinal plants on wistar rats. *J, Diabetes, Metab.,* **8**(7):1-7

SHARMA, S.B., NASIR, A., PRABHU, K.M., MURTHY, P.S. and DEV, G., 2003. Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of *Eugenia jambolana* in alloxan-induced diabetic rabbits. *J. Ethnopharmacol.,* **85**(2-3): 201-206

SHARMA, S.R., DWIVEDI, S.K. and SWARUP, D., 1997. Hypoglycaemic, antihyperglycaemic and hypolipidemic activities of *Cesalpinia bonducella* seed in rats. *J. Ethnopharmacol.,* **58**: 39-44

SHESHA RAO., 2013. Evaluation of antidiabetic effect of *Trigonella foenum graecum* and *Coccinia indica* in induced diabetes in rats with special emphasis on pathomorphological changes. PhD thesis, KVAFSU, Bidar, Karnataka, India.

SHIGEMATSU, N., ASANO, R., SHIMOSOKA, M. and OKAZAKI, M., 2001. Effect of long term administration with Gymnema sylvestre R. Br on plasma and liver lipid in rats. Biol. Pharm. Bull., 24: 643-664

SINGH, P.K., BAXI, D., BANERJEE, S. and RAMACHANDRAN, A.V., 2010. Therapy with methanolic extract of *Pterocarpus marsupium* Roxb and *Ocimum sanctum* Linn reverses dyslipidemia and oxidative stress in alloxan induced type I diabetic rat model. *Exp. Toxicol. Pathol.,* **64**(5): 441-448

SINGH, P.P., AMBIKA. and CHAUHAN, S.M., 2012. Activity-guided isolation of antioxidant xanthones from *Swertia chirayita* (Roxb.) H. Karsten (Gentianaceae). *Nat. Prod. Res.,* **26**(18): 1682-1686

SUGIHARA, Y., NOJIMA, H., MATSUDA, H., MURAKAMI, T., YOSHIKAWA, M. and KIMURA, I., 2000. Antihyperglycemic effects of gymnemic acid IV, a compound derived from *Gymnema sylvestre* leaves in streptozotocin diabetic mice. *J. Asian. Natur. Product. Res*., **2**(4): 321-327

SUJATHA, S., SHALIN, J.J. and JOSEPH, B., 2012. Complementary therapeutic potential: a focus on polyherbal products in hyperglycemia. *Int. J. Pharmaceutical. Sci. Res*., **3**(4): 957-966

SUMAN, R.K., MANJUSHA, K. BORDE., IPSEETA, R.M. and DESHMUKH, Y.A., 2015. Antidiabetic activity of *Gymnema sylvestre* leaves extract on streptozotocin indiced experimental diabetic rats. *Indo American J. Pharmaceutical Res.,* **5**(5): 2054-2060

SURYAWANSHI, S., MEHROTRA, N., ASTHANA, R.K. and GUPTA RC., 2006. Liquid chromatography/tandem mass spectrometric study and analysis of xanthone and secoiridoid glycoside composition of *Swertia chirata*, a potent antidiabetic. *Rapid Commun. Mass Spectrom.,* **20**(24): 3761-3768

SWEETMAN, S.C., 2002. Martindale: The complete drug reference. Pharmaceutical Press, London.

SZKUDELSKI, T., 2001. The mechanism of alloxan and streptozotocin action in β cells of the rat pancreas. *Physiol. Res.,* **50**: 536-546.

TAKASU, N., KOMIYA, I., ASAWA, T., NAGASAWA, Y. and YAMADA, T., 1991. Streptozotocin- and alloxan-induced H_2O_2 generation and DNA fragmentation in pancreatic islets. H_2O_2 as mediator for DNA fragmentation. *Diabetes.,* **40:** 1141-1145

TASA, S., SARANDOLB, E., ZIYANOKA, S., ASLANB, K. and DIRICANB, M., 2005. Effects of green tea on serum paraoxonase/arylesterase activities in streptozotocin-induced diabetic rats. *Nutr. Res*., **25**: 1061-1074

TELCI, A., CAKATAY, U., SALMAN, S., SATMAN, I. and SIVAS, A., 2000. Oxidative protein damage in early stage Type 1 diabetic patients. *Diabetes Res. Clin. Pract.,* **50**(3): 213-223.

THOMSON, J. HEATHER-ANNE., OPEOLU, O.OJO., PETER, R, FLATT. and YASSER, H. A. ABDEL WAHAB., 2014. Antidiabetic actions of aqueous bark extract of *Swertia chirayita* on insulin secretion, cellular glucose uptake and protein glycation. *J. Exp.Integr Med.*, **4**(4): 268-272.

TIAN, L.Y., CHEN, J.C. and HUANG, F.J., 2008. Simultaneous determination of four active components in Swertia by RP-HPLC. *Chin. J. Nat. Med.,* **6:** 444-449

TIWARI, P., MISHRA, B.N. and SANGWAN, N.S., 2014. Phytochemical and pharmacological properties of *Gymnema sylvestre*: an important medicinal plant. *Biomed. res. Int*., 1-18

TJÄLVE, H., WILANDER, E. and JOHANSSON, E.B., 1976. Distribution of labeled streptozotocin in mice: uptake and retention in pancreatic islets. *J. Endocrinol.,* **69**: 455-456

TURK, H.M., SEVINC, A., CAMCI, C., CIGLI, A., BUYUKBERBER, S., SAVLI, H. and BAYRAKTAR, N., 2002. Plasma lipid peroxidation products and antioxidant enzyme activities in patients with type 2 diabetes mellitus. *Acta Diabetol.,* **39**(3): 117-122.

TURK, J., CORBETT, J.A., RAMANADHAM, S., BOHRER, A. and MCDANIEL, M.L., 1993. Biochemical evidence for nitric oxide formation from streptozotocin in isolated pancreatic islets. *Biochem. Biophys. Res. Commun.,* **197**(3): 1458-1464

VALKO, M., LEIBFRITZ, D., MONCOL, J., CRONIN, M.T., MAZUR, M. and TELSER, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.*, **39**(1): 44-84

VARIYAR, M.C. and GUPTA, S.S. 1964. Experimental studies on pituitary diabetes IV. Effect of *Gymnema sylvestre* and *Coccinia indica* against the hyperglycemia response of somatotrophin and corticotrophin hormones. *Ind. J. Med. Res*., **52**: 200-207

VAVRA, J.J., DE BOER, C., DIETZ, A., HANKA, L.J. and SOKOLSKI, W.T., 1959. Streptozotocin, a new antibacterial antibiotic. *Antibiot. Annu.,* **7**: 230-235.

VERMA, V.K., SARWA, K.K. and KAMARUZ ZAMAN, M.D., 2013. Antihyperglycemic activity of *Swertia chirayita* and *Andrographis paniculata* plant extracts in streptozotocin-induced diabetic rats. *Int J Pharm Pharm Sci,* **5**(3), 305-311.

VIJAYANAND, R.A. and SATISH B.B., 2012. Anti-diabetic effects of Gymnema sylvestre extract on streptozotocin induced diabetic rats and possible β-cell protective and regenerative evaluations. *Digest. J. Nanomaterials. Biostructures*., **7**(1): 135 – 142

VIRDI, J., SIVAKAMI, S., SHAHANI, S., SUTHAR, A.C., BANAVALIKAR, M.M. and BIYANI, M.K., 2003. Antihyperglycemic effects of three extracts from *Momordica charantia*. *J. Ethnopharmacol*., **88:** 107-111

WANG, L., LUO, H., MIYOSHI, M., IMOTO, T., HIJI, Y. and SASAKI, T., 1998. Inhibitory effect of gymnemic acid on intestinal absorption of oleic acid in rats. *Can. J. Physiol. Pharmacol*., **76**:1017-1023

WANG, Q., HEIMBERG, H., PIPELEERS, D., LING, Z., 2008. Glibenclamide activates translation in rat pancreatic beta cells through calcium-dependent mTOR, PKA and MEK signalling pathways. *Diabetologia.,* **51**: 1202–1212

WARKINS, P.J., 2003. Diabetes mellitus, types and complications. *In*: P. J. Walkins *Edn* ABC of Diabetes, 5th *edn*., BMJ Press., *pp* 5-95.

WEIDMANN, P., BOEHLEN, L.M. and COURTEN, D.E.M., 1993. Pathogenesis and treatment of hypertension associated with diabetes mellitus. *Am. Heart J.*, **125**:1498-1513

WEST, E., SIMON, O.R. and MORRISON, E.Y., 1996. Streptozotocin alters pancreatic beta-cell responsiveness to glucose within six hours of injection into rats. *West. Indian. Med. J.*, **45**: 60-62

WIER, G.C., CLORE, E.T., ZMACHINSKI, C.J. and BONNER-WEIR, S., 1981. Islet secretion in a new experimental model for noninsulin dependent diabetes. *Diab.*, **30**:590-595

WILD, S., ROGLIC, G., GREEN, A., SICREE, R. and KING, H., 2004. Global Prevalence of Diabetes. *Diabetes Care.*, **27:** 1047–1053

YA, B.Q., NIAN, L.C., LI, C. and GEN, X.P., 1999. Protective effect of swerchirin on hematopoiesis in 60Co-irradiated mice. *Phytomedicine.*, **6**(2): 85-88

YAMAHARA, J., KOBAYASHI, M., MATSUDA, H. and AOKI, S., 1991. Anticholinergic action of *Swertia japonica* and an active constituent. *J. Ethnopharmacol.*, **33**(1-2): 31-35

YAMAMOTO, H., UCHIGATA, Y. and OKAMOTO, H., 1981. Streptozotocin and alloxan induce DNA strand breaks and poly(ADP-ribose) synthetase in pancreatic islets. *Nature.*, **294**: 284-286

YAMAN, T., UYAR, A., CELIK, I., ALKAN, E.E., KELES, O.F. and YENER, Z., 2017. Histopathological and immunohistochemical study of antidiabetic effects of heracleum persicum extract in experimentally diabetic rats. *Ind. J. Pha. Edu. Res.*, **51**(3): 450-457

YAVUZ, O., CAM, M., BUKAN, N., GUVEN, A. and SILAN, F., 2003. Protective effect of melatonin on -cell damage in streptozotocin-induced diabetes in rats. *Acta. histochem.*, **105**(3): 261–266

ZAFAR, M., NAEEM-UL-HASSAN, N.S., AHMED, M. and KAIMKHANI, Z.A., 2009. Altered liver morphology and enzymes in streptozotocin induced diabetic rats. *Int. J. Morphol.*, **27**(3): 719-725

ZHANG, F., YE, C., LI, G., DING, W., ZHOU, W., ZHU, H., CHEN, G., LUO, T., GUANG, M., LIU, Y., ZHANG, D., ZHENG, S., YANG, J,. GU, Y., XIE, X. and LUO, M., 2003. The rat model of type 2 diabetic mellitus and its glycometabolism characters. *Ex. Anim.*, **52:** 401–407

ZHANG, Y. and DAVID, S.R., 2001. Glibenclamide's action in the hypothalamus alters peripheral glucose homeostasis. *Eur. J. Pharmacol., pp.* 424

ZHANG, Y., FENG, F., CHEN, T., LI, Z. and SHEN, Q.W., 2016. Antidiabetic and antihyperlipidemic activities of Forsythia suspensa (Thunb.) Vahl (fruit) in streptozotocin-induced diabetes mice. *J. Ethnopharmacol*. 192:256–263.

ZHOU, J., KANG, X., LUO, Y., YUAN, Y., WU, Y., WANG, M. and LIU, D., 2019. Glibenclamide-Induced Autophagy Inhibits Its Insulin Secretion-Improving Function in β Cells. *Int. J. End.,* 1-9

ZHOU, Y.S., GAO, Y., GUO, X.H., LI, B., WANG, S. and CHI, J.M., 2004. Effects of timely insulin treatment on protection of beta cells in a rat model of type 2 diabetes mellitus. *Chin. Med. J.,* **117**(10): 1523-1529

Abstract

VIII. ABSTRACT

The present study was undertaken to evaluate the antidiabetic efficacy of *Gymnema sylvestre* and *Swertia chirayita* in combination with or without glibenclamide in streptozotocin induced diabetes in rats. The various groups in this study included normal control (Group I), diabetic control (Group II), diabetic rats treated with glibenclamide (Group III) at 600 µg/kg bw, diabetic rats treated with combination of *Gymnema sylvestre* and *Swertia chirayita* (Group IV) at 200 mg/kg bw each and diabetic rats treated with a combination of herbal extracts along with glibenclamide (Group V) at 100 mg/kg bw each and 300 µg/kg bw respectively. There was a significant improvement in the evaluatory parameters such as body weights, serum glucose, ALT, AST, cholesterol, triglyceride, blood parameters and hepatic antioxidant enzymes in treatment groups when compared to diabetic control. Histopathology and immunohistochemistry of pancreas also showed an improvement in the number of islet and β cells by 45th day in the treatment groups compared to diabetic control. Glibenclamide treatment substantially alleviated the effects of STZ in diabetic rats. However, the improvement was not on par with that of normal control rats. Combination of *Gymnema sylvestre* and *Swertia chirayita* with or without glibenclamide treatment only partially alleviated the STZ induced diabetic effects. Among the treatment groups, combination of herbal extract along with the glibenclamide at half dose showed better antidiabetic effect. No synergistic and no dose-dependent improvement was observed in the combination of *Gymnema sylvestre* and *Swertia chirayita* extract treatment. Combination of herbal extracts showed only mild antidiabetic effect hence cannot replace conventional anti diabetic treatment with glibenclamide.

Key words: Diabetes, *Gymnema sylvestre*, *Swertia chirayita*, glibenclamide, streptozotocin, rats, immunohistochemistry