

**ANTIDIABETIC ACTIVITY OF HYDROETHANOLIC LEAF EXTRACT OF
Catharanthus roseus ON STREPTOZOTOCIN INDUCED DIABETIC
WISTAR RATS**

T H E S I S

Submitted

in partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

VETERINARY PHARMACOLOGY AND TOXICOLOGY

BY

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DECLARATION OF STUDENT

I hereby declare that the experimental research work and Interpretation of the thesis entitled "**ANTIDIABETIC ACTIVITY OF HYDROETHANOLIC LEAF EXTRACT OF *Catharanthus roseus* ON STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS.**" or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/ publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged

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CERTIFICATE

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LIST OF ABBREVIATIONS

%	Percent
<i>C. roseus</i>	<i>Catharanthus roseus</i>
@	At the rate of
<	Less than
>	Greater than
^o C	Degree centigrade
<i>ad lib</i>	Ad libitum
ADP-ribose	Adenosine diphosphate ribose
AEAL	Aqueous extracts of <i>Aerva lanata</i> Linn Juss
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AMP	Activated protein
AMPK	Activated protein kinase
AST	Aspartate aminotransferase
ATP	Adenosine tri phosphate
b wt	Body weight
BUN	Blood Urea Nitrogen
<i>C. tamala</i>	<i>Cinnamomum tamala</i>
Ca ²⁺	Calcium
CD	Critical Difference
Cm	Centimeter(s)
CPCSEA	Committee for the Purpose of Control and Supervision of Experiment on Animals
CRD	Completely Randomized Design
CTLEt	Aqueous extracts of <i>Cinnamomum tamala</i> leaves
DIME	<i>Dillenia indica</i> methanolic leaves extract
DI	decilitre (s)
DLA	Dog leukocyte antigen
DM	Diabetes mellitus

DMSO	Dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDTA	Ethylenediamine tetra acetic acid
EESD	Ethanolic extract of <i>Scoparia dulcis</i>
<i>et al.</i>	And associates
Etc.	And the others
fp2	Falcipain-2
GDM	Gestational diabetes mellitus
GH	Growth hormone
GLUT-2	Glucose transporter-2
gm	Gram (s)
H&E	Hematoxylin and eosin
H ₂ O ₂	Hydrogen peroxide
HA ⁺	Alloxan radical
Hb	Haemoglobin
HbA1c	Hemoglobin A1c
HCl	Hydrochloric acid
HDL-C.	High density lipid concentration
Hrs.	Hours
i/p	Intraperitoneal
IAEC	Institutional Animal Ethical Committee
IC50	Half maximal inhibitory concentration
IDDM	Insulin Dependent Diabetes Mellitus
IU	International Unit (s)
Kg	Kilogram (s)
L	Liter
LDL-C	Low density lipid concentration
M	Meter
MEAL	Methanol extracts of <i>Aerva lanata</i> Linn Juss
mg	Milligram (s)
min	Minutes
ml	Milliliter
mm	Millimeter
mRNA	Messenger Ribonucleic acid
MSA	Methane sulfinic acid

MT	Masson's trichome
NAD	Nicotinamide Adenosine Di-phosphate
NAD	Nicotinamide adenine dehydrogenase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
OH	Hydroxyl radical
p.o.	Per orally
PCV	Packed cell volume
RBC	Red blood cell
RNA	Ribonucleic acid
ROS	Reactive oxygen species
S.E.M.	Standard error of mean
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic Transaminase
SH	Sulfhydryl group
SITA	Sitagliptin
STZ	Streptozotocin
TRIG	Trigonelline
UK	United Kingdom
Viz.	Namely
VLDL-C	Very low density lipid concentration
β-cell	Beta cell
μ	Micro(n)
μg	Microgram (s)
μm	Micrometer
i.p.	Intra peritonially

INTRODUCTION

Diabetes mellitus is a metabolic syndrome caused due to absolute or relative deficiency of insulin secretion or action or both (Rao *et al.*, 2001). Diabetes was first documented by the Egyptians and is characterized by weight loss and polyuria. It is characterized by hyperglycemia, glycosuria, hyperlipaemia, negative nitrogen balance and sometimes ketonaemia (Tripathi, 2008). However, it was the Greek physician Aertaeus who coined the term Diabetes mellitus (DM). In Greek diabetes means “to pass through” and mellitus is the Latin word for honey (referring to sweetness). The presence of sugar in the urine of diabetics was demonstrated by Dobson in 1755 (Satoskar, 1999). Diabetes mellitus is now recognized as a serious global health problem. Westernized cultures and populations experiencing rapid acculturation are showing a sharp rise in non-insulin-dependent diabetes mellitus (Bennett and Knowler, 1980; Zimmer, 1998). In the year 2011, the International Diabetes Federation estimated that about 61.3 million people suffered from diabetes and projected that this figure is likely to go high up to 101.2 million by 2030.

Various epidemiological studies in India have shown that the prevalence and manifestations of diabetes are very high (Verma *et al.*, 1986; Ramchandran *et al.*, 1992; Ramaiya *et al.*, 1990) followed by China (Mohiuddin, 2012). The occurrence rates of diabetes for urban, semi urban and rural population vary from 5-15%, 4-6% and 3-5%, respectively, showing wide regional disparities with respect to different local settings. The rural population has exhibited a 3 times (2.4% to 6.4%) shift in the prevalence during last 14 years. Such rapidly increasing prevalence has remarkably narrowed the gap between rural and urban areas (Baruah *et al.* 2014).

Diabetes is categorized as type-1, type-2 and gestational diabetes. Type-1 diabetes, previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes, may account for 5 percent to 10 percent of all diagnosed cases of diabetes. Risk factors are less well defined for Type-1 diabetes than for Type-2 diabetes, but autoimmune, genetic, and environmental factors are involved in the development of this type of diabetes. Type-2 diabetes may account for about 90 percent to 95 percent of all diagnosed cases of diabetes. Type-2 diabetes was previously called non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes. Type-2 DM is a metabolic disorder

characterized by chronic hyperglycemia (elevated levels of plasma glucose), caused by inherited and/or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of insulin produced. The condition causes deregulation of carbohydrate, protein and fat metabolism. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro- and macro-vascular complications of diabetes, which are major causes of morbidity and death (Sangal, 2011). Gestational diabetes develops in 2 percent to 5 percent of all pregnancies but usually disappears when a pregnancy is over. Gestational diabetes occurs more frequently in African Americans, Hispanic/Latino Americans, American Indians and people with a family history of diabetes than in other groups. Obesity is also associated with higher risk. Women who have had gestational diabetes are at increased risk for later developing Type-2 diabetes. Such types of diabetes may account for 1 percent to 2 percent of all diagnosed cases of diabetes (Kelly, 2014).

Never the less the diabetes mellitus is also observed in animals. There is a role of genetic and environmental factors in feline and canine diabetes and the Type-2 diabetes is the most common form of diabetes in cats. High carbohydrate diets increase blood glucose and insulin levels and may predispose cats to obesity and diabetes (Rand *et al.*, 2004). The risk for development of diabetes increases about 2-fold in overweight cats and about 4-fold in obese cats (Laflamme, 2012). The clinical cases of diabetes mellitus are also reported in cattle and buffalo.

Diabetes has been a clinical model for general medicine. The primary defect in fuel metabolism results in widespread, multi-organ complications that ultimately encompass virtually every system of the body and every specialty of medicine. The feline diabetes mellitus closely resembles human type-2 diabetes mellitus in many respects including clinical, physiological, and pathological features of the disease. These features include age of onset of feline diabetes mellitus in middle age, association with obesity, residual but declining insulin secretion, development of islet amyloid deposits, loss of approximately 50% of cell mass, and development of complications in several organ systems including peripheral polyneuropathy and retinopathy (Henson and O'Brien., 2014).

Some evidence suggests that the incidence of diabetes in cats is increasing for the same reasons it is increasing in human (an increase in obesity

and a decrease in physical activity) (King *et al.* 1998; Prah *et al.* 2003). Diabetes typically occurs in dogs between 5 and 12 years of age, and is uncommon under 3 years of age. Breeds predisposed to diabetes include the Samoyed, Tibetan Terrier and Cairn Terrier, while others such as the Boxer and German Shepherd Dog seem less susceptible. These breed differences suggest a genetic component (Catchpole *et al.*, 2005).

Regardless of the underlying etiology, diabetic dogs and cats are hyperglycemic and glycosuric, which leads to the classic clinical signs of polyuria, polydipsia, polyphagia, and weight loss. Increased fat mobilization leads to hepatic lipidosis, hepatomegaly, hypercholesterolemia, hypertriglyceridemia, and increased catabolism (Rucinsky, 2010). Generally, canine diabetes mellitus is clinically diagnosed in dogs with 4-18 years of age, the median age at diagnosis being 7-9 years (Ciobotaru, 2013).

The modern allopathic, antidiabetic drugs like Tolbutamide, Chlorpropamide, Phenformin, Metformin, Repaglinide, Nateglinide, Rosiglitazone, Pioglitazone etc. are used for the treatment of diabetes mellitus, however these drugs have the disadvantage in causing less or more side effects like retinopathy, neuropathy, nausea, vomiting, flatulence, diarrhea, or constipation, headache, paresthesias, hyponatremia, flatulence, dizziness and joint pain etc. (Tripathi, 2003). So instead of allopathic drugs, herbal drugs are a great choice which is having more or less no side / adverse effects (Kokar and Mantha, 1998). The medicines of plant origin are free from the toxic properties. Though complementary and alternative medicine treatments are popular, scientific evidence support their application to diabetes care is scarce (Tripathi, 2007). Various types of antidiabetic drugs are available in the market for the treatment of diabetes mellitus, but none can be termed as an ideal one, due to their toxic side effects and, sometimes, diminution in response after prolonged use.

Therefore, there is always a need to search a drug which is better than existing and having fewer side effects. Traditional herbal drugs play an important part in the treatment of diabetes. If we were able to even identify some 5-6 herbal drugs that can reduce dose of insulin by increasing sensitivity, reducing insulin resistance, then we would have positively contributed in the treatment of diabetes. The Pharmaceutical Research and Development Committee report of Ministry of Chemicals, Government of India also underscores the importance of

traditional knowledge (Masheilkar, 1999). Herbal medicines are often used as therapeutic remedies in combination with allopathic drugs (Ramesh, 2003).

Herbal medicines can be relevant today only if they are applied and tested within the framework of modern sciences and subjected to the rigorous criteria for quality, safety and efficacy. Only then, herbal products can be comparable with modern medicines and can bring necessary confidence in prescribing.

Catharanthus roseus previously known as *Vinca rosea* (L.) is an ornamental shrub, belongs to the family *Apocynaceae*, is an erected procumbent herb or under shrub containing latex. That grows up to 30–100 cm in height. It is widely distributed around the World due to its high survivability in a variety of habitats and use as an ornamental plant (Bergen, *et al.*, 1996). *C. roseus* is used traditionally medicine for the treatment of diabetes in several countries of the world (Don, 1999) including like Nigeria (Ahmed, *et al.*, 2007), India (Ahmed, *et al.*, 2007, Nammi, *et al.*, 2003) South Africa, China, Mexico (Patel, *et al.*, 2012) and Malaysia (Ong, *et al.*, 2011). Roots and leaves of this plant contain more than 100 alkaloids. It has economic importance from its alkaloids. The two leaf alkaloids which are most important in medicine are vinblastine and vincristine (Jaleel *et al.*, 2008).

Fresh leaf juice and other part of *C. roseus* have been reported to reduce blood glucose in diabetic rabbits and others (Nammi *et al.*, 2003; Don, 1999) and it has gained acceptance from the pharmaceutical industries (Alxeandrova *et al.*, 2000, Heijden *et al.*, 2004). Significant antihyperglycemic activities of the alcoholic extract, (Chattopadhyay, 1999, Mostofa, *et al.*, 2007) aqueous extract, (Islam *et al.*, 2009) and the dichloromethane-methanol extract of *C. roseus* (Somananth *et al.*, 2001) have been reported in laboratory animals.

It has been reported that, ethanolic extract of the leaves and flower of *C. roseus* showed a dose-dependent lowering of blood sugar compare to the standard drug, Glibenclamide and aqueous extract lowered the blood glucose to about 20% in diabetic rats when compared to that of the dichloromethane and methanol extracts which lowered the blood glucose level to 49-58%. Therefore, to find antidiabetic activity of hydroethanolic leaf extract of *C. roseus* the present study was undertaken with the following objectives;

1. Qualitative analysis of phytochemical constituents in hydroethanolic leaf extract of *C. roseus*.
2. To study anti-diabetic property of hydroethanolic leaf extract of *C. roseus*.
3. To study hematological, biochemical and histopathological parameters in Streptozotocin (STZ) induced diabetic rats.

REVIEW OF LITERATURE

In recent years, many drugs have been isolated from natural source as the modern medicine system treats the symptoms and suppresses the disease but does little to ascertain the real cause. Many drugs which suppress or relieve some ailments usually have harmful side-effects. Drugs usually hinder the self-healing efforts of the body and make recovery more difficult. Therefore, the current scenario is to isolate active constituents present in the plant material to develop drugs having rare chances of adverse effects.

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia (high blood glucose concentration) caused by insulin deficiency, often combined with insulin resistance. Insulin is a hormone released from β cells of pancreas and convert glucose (source of energy in body) to glycogen thus maintaining the glucose levels in the body. The deficiency of insulin leads to increase of glucose levels in blood and urine. Diabetes can also give rise to other diseases like cataracts, cardiac problems, etc., there also occurs changes in biochemical parameters like cholesterol, urea, creatinine etc. The *Catharanthus roseus* contains 150 alkaloids including vincristine, vinblastine, ajmalicine, etc. The plant has been considered due to its wide range of pharmacological activity like anti-inflammatory, antimicrobial, antimitotic, antihypertensive, antifertility, antihypercholesterolemic, antimutagenic, antidiuretic, antifungal, antispasmodic, antiviral, cardio tonic, CNS depressant, antitumor, cytotoxic, antispermatogenic and anticancer activities. The study involves leaf of *Catharanthus roseus* for evaluating antidiabetic activity.

Pharmacological findings associated with “antidiabetic activity of hydroethanolic leaf extract of *catharanthus roseus* on streptozotocin induced diabetic wistar rats” have been well documented. The available literature is reviewed as to make out plan for research work.

2.1 Diabetes mellitus

Hawk and Bernard (1954) reported that two basic types of diabetes namely type 1 or insulin-dependent and type 2 or non-insulin-dependent diabetes. Type 1 commonly seen in juveniles is characterized by failure to produce insulin due to autoimmune destruction of beta-cells of the pancreas

while type 2 is usually adult-onset and is associated with insufficient production of insulin and loss of responsiveness by cells to insulin.

Papaspyros (1664) stated that diabetes mellitus is often called 'The silent killer', because it causes serious complications without serious symptoms and can affect many of major organs in the body.

Lernmark et al. (1981) said that diabetes can occur as a result of some secondary causes such as pancreatectomy and iron overload of beta-cells resulting from hemochromatosis. Other causes include excess cortisol production in Cushing's syndrome and excess growth hormone secretion in acromegaly and insulin-resistant syndrome.

Sharma (1983) stated that diabetes mellitus is referred as 'Madhumeha' in Ayurveda, Sushruta Sanhita and Charak Sanhita due to sweetishness of urine of diabetic patients. After going through the varied literature it is observed that many dietary supplements and herbs are used to effectively manage and control the elevated blood sugar levels in diabetic patients.

Spencer and Cudworth (1989) stated that diabetes is not a single disease but a syndrome that is characterized by a total or relative lack of insulin leading to persistent elevation of blood glucose as well as alteration in lipid and protein metabolism.

Kitchen and Roussel (1990) reported that hyperglycemia, acetonemia, ketonuria and glycosuria were documented as highly suggestive signs of diabetes mellitus in a bull. 2-fold in overweight cats and about 4-fold in obese cats.

Debra (1991) stated that the diabetes has been a clinical model for general medicine. The primary defect in fuel metabolism results in widespread, multi-organ complications that ultimately encompass virtually every system of the body and every specialty of medicine. It has been said that to know diabetes is to know medicine and health care. Although from a clinical standpoint this may be true, our increasing knowledge of the pathophysiology of the syndrome, together with the mechanisms of long term complications, has placed diabetes research at the frontier of immunology and molecular biology.

Kirithikar et al. (1995) stated that the diabetes mellitus is a complex and multifarious group of disorders that disturbs the metabolism of carbohydrates, fats and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. It is a chronic metabolic disorder characterized by hyperglycemia caused by insulin deficiency, often combined with insulin resistance. Insulin is a hormone released from the cells of pancreas and convert glucose to glycogen thus maintaining the glucose level in the body. The deficiency of insulin leads to increase of glucose level in blood and urine. Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complications that results in significant morbidity and mortality. It is a major disease characterized by derangement in carbohydrate, fat and protein metabolism, affecting nearly 10% of the population.

Williamson et al. (1996) said that normal healthy animals can be used for testing potential oral hypoglycemic agents. This is still a valid screening method which is often used in addition to diabetic animal models.

Satoskar et al. (1999) reported in the 17th century, Willis observation of the 17th century that the urine of diabetes as wonderfully sweet as if imbued with honey or sugar. The presence of sugar in the urine of diabetics was demonstrated by Dobson in 1755.

Georg and Ludvik (2000) said that diabetes mellitus (DM) is characterized by hyperglycemia due to disturbance in the group of metabolism of carbohydrates, fat and protein, resulting from defects in insulin secretion, action or both.

Beretta (2001) reported that, according to the World Health Organization (WHO), there are approximately 160,000 diabetics worldwide, the number of diabetics has double in the last few years and is expected to double once again in the year 2025.

Vats et al. (2004) reported that the diabetes mellitus is one of the common metabolic disorders with micro-and macrovascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world.

Rand et al. (2004) found that diabetes mellitus also occurs in animals, but more particularly in cats and dogs and to some extent in cattle and buffaloes. The type 2 diabetes is more common in cats and that the genetic as well as the environmental factors play a very important role in the development of it.

Iweala and Okeke (2005) diabetes characterized by the symptoms such as weakness, polyuria, excessive thirst as well as ketonemia, ketouria and ketosis due to altered metabolism of lipids and proteins. It is associated with abnormalities such as kidney failure, nervous defect, impotence, blindness, stroke and heart diseases. Abnormalities in lipid metabolism may contribute to excessive hepatic glucose through gluconeogenesis as well as abnormal drive from the autonomic nervous system.

Aylward (2005) mentioned that diabetes mellitus is a chronic metabolic disorder due to the relative deficiency of insulin secretion and varying degree of insulin resistance which is characterized by high circulating glucose.

Singh et al. (2010) stated that the diabetes is a disorder of carbohydrate, fat and protein metabolism caused due to attenuate production of insulin or due to its inhibitory action.

Braslasu et al. (2007) reported that the clinical symptoms of diabetes mellitus in wistar rats were: deeply deviance, hematuria, hyperglycemia, emaciation. Blood glucose values were 406.22 ± 24.85 mg/dl. They observed increase of albumin, triglyceride, creatine kinase and alkaline phosphatase levels.

Etuk (2010) reviewed that diabetes mellitus is a potentially morbid condition with high prevalence worldwide thus the disease constitutes a major health concern. Presently, it is an incurable metabolic disorder which affects about 2.8% of the global population.

Mohiuddin (2012) reported that the prevalence of diabetes is more in India followed by China.

Laflamme (2012) reported that the risk for development of diabetes increases about two fold in overweight cats and about four fold in obese cats.

Prasanna et al. (2012) reported that the diabetes is a condition primarily defined by the level of hyperglycemic giving rise to risk of micro vascular damage (retinopathy, nephropathy and neuropathy), significant morbidity due to specific diabetes related macrovascular complication (Ischemia heart disease, stroke and peripheral vascular disease), and diminished eminence of life.

IDF (2015) stated that the diabetes mellitus (DM) is a chronic disease, characterized and diagnosed by hyperglycemia. The disease frequently gives rise to various complications, some of them are serious. Globally, the prevalence of DM is high, about 415 million individuals, and it is projected by the International Diabetes Federation (IDF) that 642 million people will have the disease in 2040.

2.2 Antidiabetic agents

The synthetic oral hypoglycemic agents like Tolbutamide, Chlorpropamide, Phenformin, Metformin, Repaglinide, Nateglinide, Rosiglitazone, Pioglitazone etc. are used to treat the diabetes mellitus. However, these agents are having the adverse and side effects. During the past decade and especially in last few years some of the bioactive drugs isolated from hypoglycemic plants showed antidiabetic activity with more efficacy than synthetic oral hypoglycemic agents. Therefore, plants as folk remedies are widely used to treat diabetes mellitus. The plants with the most potent hypoglycemic effects include: *Murraya koenigii*, *Allium sativum*, *Bombax ceiba*, *Coccinia indica*, *Glycyrrhiza glabra*, *Memecylon umbellatum*, *Pterocarpus santalinus*, *Zingiber officinale*, *Zizyphus sativa*, *Terminalia catappa*, *Sida cordifolia* and *Cinnamomum tamala*.

Actually more than 12000 plants have been described to be experimentally or ethnopharmacologically used in the treatment of diabetes.

Anand et al. (1989) studied that an alcoholic extract of the leaves of *Zizyphus sativa Gaertn* plant showed dose-dependent reduction in blood glucose levels but failed to produce significant hypoglycemic activity in alloxan-diabetic rats.

Sheela and Augusti (1992) observed that Garlic contains S-allyl cysteine sulphoxide, a Sulphur containing amino acid, which produced significant blood glucose lowering activity in experimental diabetic animals.

Kumar et al. (1993) studied the Hypoglycemic activity of Pectin, isolated from the fruit of *Coccinia indica* in normal rats at a dose of 200 mg/100 g/day upon oral administration and it showed significant reduction in blood glucose and an increase in the liver glycogen level.

Amalraj and Ignacimuthu (1998) studied that an alcoholic extract of the leaves of *Memecylonum bellatum* (250 mg/kg) exerted significant serum glucose lowering effects in normal and alloxan-induced diabetic mice upon oral administration.

Kanth and Diwan (1999) studied that the methanol extract of root of *Sidacordifolia L.* plant has been reported to possess significant hypoglycemic activity.

Saleem et al. (1999) studied that *Bombax ceiba L.*, commonly known as silk cotton tree is distributed throughout India, particularly in Andhra Pradesh, usually occurring scattered in mixed deciduous forests. Shamiminaflavonolglucoside isolated from the leaves of the plant has been reported to possess significant hypoglycemic activity at 500 mg/kg in rats.

Chattopadhyay (1999) studied that the comparison of blood sugar lowering activity of four important medicinal plants (*Azadirachta indica*, *Gymnema sylvestre*, *Catharanthus roseus* and *Ocimum sanctum*) were carried out against normal and streptozotocin-induced diabetic rat models. The plant extracts decreased the blood sugar level in varying degrees. Blood sugar lowering unit (BLU) of activity of each leaf extract and tolbutamide was calculated by ED50 values. Statistical analysis revealed significant ($P < 0.05$) variation among the treatments as well as doses with regard to their blood sugar lowering capacity. *A. indica* leaf extract was found to have the most potent blood sugar-lowering activity followed by *C. roseus*, *G. sylvestre* and *O. sanctum*.

Kameswara Rao et al. (2001) studied that, the bark extracts of *Pterocarpus santalinus L.* at a dose of 0.25 g/kg body weight showed maximum

anti hyperglycemic activity but failed to produce any hypoglycemic activity in normal rats.

Nagappa et al. (2003) studied that the effect of petroleum ether, methanol, and aqueous extracts of the fruit of *Terminalia catappa* L. was examined on fasting blood sugar levels and serum biochemical parameters in alloxan-induced diabetic rats and produced significant hypoglycemic activity and alleviated the pancreatic necrosis produced by alloxan administration.

Venkateswaran et al. (2003) revealed that ethanolic leaf extract of *Coccinia indica* (200 mg/kg for 45 days) also produced modulatory effect on the aortic collagen content of STZ diabetic rats by reducing the accumulation and cross-linking of collagen.

Achyut et al. (2005) studied the aqueous extract of *Murraya koenigii* leaves for its hypoglycemic activity in normal and alloxan induced diabetic rabbits. Single administration of variable dose levels (200,300,400 mg/kg) of aqueous extract led to lowering blood glucose in normal as well as in diabetic rabbits. This plant is promising as it is widely and regularly used as a spice for food flavoring and as such it appears to be without any side effects and toxicity.

Nakagawa et al. (2004) reported that *Glycyrrhiza glabra* L. commonly known as licorice is a flavorful perennial herb that grows 3–7 ft high and has been used in food and medicinal remedies for thousands of years. The effect of hydrophobic flavonoids from this plant was studied on abdominal fat accumulation and blood glucose level in obese diabetic KK-A (y) mice. The findings indicated that the flavonoids have abdominal fat-lowering and hypoglycemic effects, possibly mediated through activation of peroxisome proliferator activated receptor-gamma (PPAR-gamma).

Akhani et al. (2004) studied that the juice of *Zingiber officinale* administered at a dose of 4 ml/kg p.o. daily for 6 weeks significantly prevented hyperglycemia and hyperinsulinemia in streptozotocin (STZ)-induced type I diabetic rats. It also produced a significant increase in insulin levels and a decrease in fasting glucose levels in diabetic rats.

Mostofa et al. (2007) studied that an aqueous extracts of *C. roseus* (Nyantara), *Azadirachta indica* (Neem), *Allium sativum* (Garlic) aqueous extracts of this plants were prepared and blood glucose lowering effect and improvement of body weight gain in Streptozotocin (50 mg/kg bwt i.p.) induced diabetic rats were measured and compared with that of a patent drug glimepiride in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh. Rats were administered *Catharanthus roseus*, *Azadirachta indica*, *Allium sativum* extracts at the dose rate of 1g/kg, 500 mg/kg and 1g/kg bwt orally for 14 days, respectively. Blood glucose level and body weight was measured by Glucotrend kit and Electronic balance and that compared with a patent drug Glimepride at a dose rate of 100 mg/kg bwt. The data were compared statistically by using student's unpaired *t*-test. The herbal preparations of these plants significantly increased body weight gain and decreased blood glucose as compared with the patent drug. The above study clearly indicated the significant antidiabetic activity of *C. roseus*, *Azadirachta indica* and *Allium sativum* and supports the traditional usage of the herbal preparations by Ayurvedic physicians for the therapy of diabetics.

Chakraborty and Das (2010) studied the hypoglycemic activity of aqueous leaf extract of *Cinnamomum tamala* against streptozotocin induced diabetes in wistar rats revealed that the extract possess both antihyperglycemic as well as antidiabetic properties.

Singh et al. (2010) reported hypoglycemic or anti-diabetic activity of different extracts of various plants evaluated in Alloxan induced diabetic rats. In their experiments 30 medicinal plants have been selected for thorough studies from various plants like- *Pterocarpus marsupium*, *Azadirachta indica*, *Allium sativum*, *Brassica juncea*, *catharanthus roseus* etc. They reported that many of them seem to act directly on pancreas and stimulate insulin level in blood and have been shown to display potent hypoglycemic activity.

Prasanna et al. (2012) studied aqueous and ethanol leaf extracts of *Vitex negundo* for its antidiabetic activity using alloxan induced diabetic model in rats. The aqueous extract showed ($P < 0.01$) significant activity than the ethanol extract at the tested dose level, which were comparable to glibenclamide, a standard antidiabetic drug.

2.3 Medicinal activity of *Catharanthus roseus*

Johnson et al. (1963) studied two of the common anti-cancer drugs of *C. roseus* which were derived from this plant namely vincristine and vinblastine (they are named after *Vinca*). Vincristine is used in the chemotherapeutic regime for Hodgkin's lymphoma while vinblastine is used for childhood leukemia. These vinca alkaloids bind to tubulin dimers and inhibiting microtubule structures of the cells, thus inhibiting the metaphase of cellular mitosis. Main side effects of these drugs are peripheral neuropathy, hair loss, hyponatremia and constipation.

Iweala and Okeke (2005) compared effects of the aqueous extracts of *C. roseus* and chlorpropamide (Diabenese) on the levels of serum cholesterol, total protein, lipid peroxidation, blood glucose and liver enzymes in alloxan-induced diabetic rats. Four groups namely A, B, C and D comprising of nine rats each were used. A and B were administered with chlorpropamide and *C. roseus* extracts respectively, while C and D served as diabetic and non-diabetic controls respectively. The results showed comparatively significant reductions ($P > 0.05$) in the levels of glucose, protein, cholesterol, lipid peroxidation and liver enzymes in the groups administered *C. roseus* extracts and chlorpropamide relative to the controls. The reductions were higher in the groups treated with *C. roseus* extract than in the groups treated with diabenese.

Nayak (2006) studied wound healing activity of *Vinca rosea* using the excision wound model in a streptozotocin induced diabetic rats. The animals were weight matched and placed into five groups ($n=6$ per group). Animals in groups 1 and 2 were normal control (Vaseline) and normal experimental (extract treated) respectively; those in groups 3 and 4 were the diabetic control and diabetic experimental batches. Diabetic animals in a reference group 5 were treated with topical mupirocin ointment. All animals were experimentally wounded on the posterior surface. The ethanol extract of *Vinca rosea* (100 mg kg⁻¹ body weight) was applied to animals of group 2 and 4 for ten days. Wounds were measured on days 1, 5 and 11. The granulation tissue formed on the wound was excised on the 11th day and used for the histology and biochemical work up. The wound size in animals of the *Vinca rosea* treated group were significantly reduced ($P < 0.001$) when compared with the diabetic control and mupirocin treated animals. Significant increase in the weight of the granulation tissue

($P < 0.001$) and the hydroxyproline content ($P < 0.001$) were also observed in extract treated animals. The result obtained is that the ethanol extract of *Vinca rosea* promotes significant wound healing and closure in diabetic rats compared with mupirocin and further evaluation of this activity in humans is suggested.

Nayak and Lexley (2006) reported that the extract of *C. roseus* significantly increased the wound breaking strength in the incision wound model compared with controls ($P < 0.001$). The extract-treated wounds were found to epithelialize faster, and the rate of wound contraction was significantly increased in comparison to control wounds ($P < 0.001$), Wet and dry granulation tissue weights, and hydroxyproline content in a dead space wound model increased significantly ($p < 0.05$). along with antidiabetic activity *c. roseus* has antimicrobial activity.

Jaleel (2008) stated that the roots and leaves of *C. roseus* plant contain more than 100 alkaloids. It has economic importance from its alkaloids. The two leaf alkaloids which are most important in medicine are vinblastine and vincristine, which are used in the treatment of cancer. Ajmalicine (antihypertension activity) and its oxidized form, serpentine (tranquilizer), are indole alkaloids obtained from the roots, with medicinal importance.

Raza et al. (2009) reported that the *C.roseus* possesses known antibacterial, antimicrobial, antifungal, antidiabetic, anticancer and antiviral activities. The different parts of *C. roseus* (leaf, stem, flower and root) were used and extracts were subjected to antibacterial assay. The extracts of *C. roseus* did not exhibit antibacterial activity against *Staphylococcus aureus*. Moreover, leaf, stem and flower extracts were also ineffective against *Pseudomonas aeruginosa*. The leave extract did not exhibit activity against *Corynebacterium diphtheria*, similarly, the crude extract of stem did not show activity against *Sigel boydii*. The most effective was the root extract, which exhibited broad-spectrum antibacterial activity against *Salmonella typhi* and *S. boydii* with the zone of inhibitions measuring 24 mm and 22 mm, respectively. The flower extract also showed activity against *C. diphtheria*.

Mohammed et al. (2011) concluded that *C. roseus* whole plant dichloromethane: methanol (1:1) extract is found to exhibit a significant antibacterial activity against, almost all the bacterial strains and antidiabetic

activity in alloxan induced diabetic rats. Isolation and molecular characterization of active constituents responsible for antibacterial and antidiabetic activity can be assessed for the bioactivity of the *C. roseus* plant extract and also to be explored for its activity against wide spectrum of microbes to develop it into a powerful antibiotic.

Hassan et al. (2011) investigated the *in vivo* antidiarrheal activity of *C. roseus* ethanolic leaf extract in Wistar rats. Castor oil was used to induce experimental diarrhea in rats pretreated with extracts administered at 200 and 500 mg/kg to determine antidiarrheal effect. Loperamide and atropine sulphate were used as standard drugs in the two experiments. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, tannins, triterpenes, flavonoids and saponins. The antidiarrheal effect of ethanolic extract of *C. roseus* revealed a dose dependant inhibition of castor oil induced diarrhea at doses of 200 and 500 mg/kg. On comparison of the extract with the negative control, there was a significant difference at 200 mg/kg ($P < 0.05$) and a more significant difference at 500 mg/kg ($P < 0.001$). Test extract's effect was further evaluated on intestinal transit and it exhibited dose dependant inhibition of transit motility of charcoal meal. The results indicated that the ethanol extract of *C. roseus* possesses significant antidiarrheal effect and corroborate the use of this herbal remedy as non-specific treatment for diarrhea in folk medicine

Elisa et al. (2012) studied the aqueous extract (250 mg/Kg) of flowers, leaves, roots, and stems from *C. roseus* and observed that it produced hypoglycemic effect in healthy and alloxan diabetic mice ($P < 0.05$). The aqueous leaf extract had the best hypoglycemic effect on healthy mice; meanwhile the best hypoglycemic effects on diabetic mice were presented by the aqueous stem extract.

Navitha et al. (2012) evaluated ethanolic extract of herb of *Catharanthus pusillus* was evaluated for hypoglycemic effect in Streptozotocin induced diabetic rats using both 18 hour fasted rat model and oral glucose tolerance test. A comparison was made between the actions of ethanolic extract *Catharanthus pusillus* and known anti diabetic drug Glibenclamide (5 mg/kg, p.o). The ethanolic extract of *Catharanthus pusillus* was administered at different doses to normal

and diabetic rats. The ethanolic extract at 500 mg/kg body weight dose level exhibited significant hypoglycemic activity ($P < 0.01$ and $P < 0.001$).

Balaabirami and Patharajan (2012) studied that *C. roseus* (periwinkle) an important medicinal plant for novel pharmaceuticals since most of the bacterial pathogens are developing resistance against many of the currently available anti-microbial drugs. Plants have proved to be significant natural resources for effective chemotherapeutic agents and offering a broad spectrum of activity with greater emphasis on preventive action. This study aims to investigate some of the anti-microbial, antifungal property of there leaves extract. The antimicrobial activity has been checked against microorganisms like *Escherichia coli* (MTCC - 443), *Klebsiella oxytoca* (MTCC - 4676), *Klebsiella pneumonia* (MTCC- 7162), *Proteus mirabilis* (ATCC -49565), *Pseudomonas aeruginosa* (MTCC -5210), *Salmonella typhimurium* (ATCC -13311), *Salmonella paratyphi* (MTCC-735), *Staphylococcus aureus* (MTCC -740) and 2425), *Aspergillus fumigatus* (MTCC -9657), *Candida albicans* (MTCC- 183), *Penicillium chrysogenum* (MTCC- 160), *Aspergillus flavus* (AFG3) and antifungal activity against *Aspergillus niger* (MTCC-2425) by disc diffusion method. The finding showed that this leaf extracts have the potential to inhibit the growth of bacteria and fungi. From the result, the leaves of this plant can be used as prophylactic agent in many of the diseases.

Soon et al. (2013) reported that the *in vitro* antioxidant and antidiabetic activities of the major alkaloids isolated from *C. roseus* leaves extract. Four alkaloids—vindoline I, vindolidine II, vindoline III and vindolinine IV—were isolated and identified from the dichloromethane extract (DE) of this plant's leaves. DE and compounds I–III were not cytotoxic towards pancreatic β -TC6 cells at the highest dosage tested (25.0 $\mu\text{g/mL}$). All four alkaloids induced relatively high glucose uptake in pancreatic β -TC6 or myoblast C2C12 cells, with III showing the highest activity. In addition, compounds II–IV demonstrated good protein tyrosine phosphatase-1B (PTP-1B) inhibition activity, implying their therapeutic potential against type 2 diabetes. Compound III showed the highest antioxidant potential in ORAC and DPPH assays and it also alleviated H_2O_2 -induced oxidative damage in β -TC6 cells at 12.5 $\mu\text{g/mL}$ and 25.0 $\mu\text{g/mL}$.

Gajalakshmi et al. (2013) studied that *C. roseus*, which is a potent medicinal plant possesses many of the pharmacological actions such as antimicrobial, antioxidant, anthelmintic, antifeedant, antisterility, antidiarrheal, antidiabetic effect etc. is used to treat many of the fatal diseases. Alkaloids were the major phytochemical constituent of the *C. roseus* plant and have different types possessing various medicinal uses. The pharmacology of the plant was found to be associated especially with the alkaloids that occupies almost most of the parts of the plant.

Balaji (2014) opined that the Plant Kingdom still remains to be the primary source of Medicine. It has been proved to be efficient enough to treat many diseases including the world's most dreadful disease tumor with nil or minimum side effects. Considerable studies have been carried out on ethnomedicinal plants of India; however, only few medicinal plants have attracted the interest of scientists. Concomitant with the preparation of clinically useful compounds from ancient plant remedies has been an increased interest in studying the pharmacological action of the plant alkaloids. *Vinca rosea* has been the thought of interest for most of the Pharmaceutical scientists for more than two decades. *Vinca rosea* is of importance due to its anti-tumor and anti-diabetic actions

Kabesh et al. (2015) revealed that phytochemical, antioxidant, antimicrobial and anticancer cell line study has been done using *Catharanthus roseus* plant water and methanol extracts. The antibacterial studies showed the antimicrobial activity against *Pseudomonas aeruginosa* and *Bacillus subtilis* among the pathogen used. The He La cell line cytotoxins study was also conducted and the cell death was 23%. GC-MS study revealed the presence of compounds in the extracts towards the antimicrobial, anticancer and anti-inflammatory effect

Aruna et al. (2015) reviewed *C. roseus* was native to the Indian Ocean island of Madagascar. This herb is now common in many tropical and subtropical regions worldwide, including the southern United States. It is a popular ornamental plant found in gardens and homes across the warmer parts of the world. The need for the novel pharmaceutical products out from the plant has attained a great interest in the present research world due to the cost and the

higher side effects that are associated with the chemically manufactured drugs. *C. roseus*, which is a potent medicinal plant has many of the pharmacological actions, which is used to treat many of the fatal diseases. Alkaloids were the major phytochemical constituent of the above medicinal plant and have different types possessing various medicinal uses.

Tolambiya and Mathur (2016) reported that the *C. roseus* is used in treatment of several diseases like diabetes, cancer, high blood pressure, asthma, inflammation, dysentery, brain imbalance, angiogenesis, malaria and other diseases that occur due to potent microorganisms. Though it's a native of Madagascar but it is found in most parts of the world. It is also appreciated for its long flowering period throughout the year. Its roots, stems and flowers are having active constituents to inhibit the growth of disease and other microorganism. Extracts from the dried or wet flowers and leaves of plants are applied as a paste on wounds in some rural communities. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia and Hodgkin's lymphoma. The fresh juice from the flowers of *C. roseus* is used to treat various skin problems e.g. dermatitis, eczema and acne. As *C. roseus* has mammoth potential to heal many syndromes due to its phytopharmaceutical assets and its intact fractions i.e. root, leaf, stem and flower are drug complaint. Still further research is required to find main utility in healing of diseases arising due to deadly viruses.

Nisar et al. (2016) revealed that *C. roseus* commonly known as evergreen herb is one of famous medicinal herb in the field of cancer treatment. It has many pharmacological properties such as anti-oxidant, anti-microbial, anti-diabetic, wound healing, anti-ulcer, hypotensive, antidiarrheal, hypolipidemic and memory enhancement. Alkaloids are one of major phytochemicals responsible for its anti-cancer properties followed by phenolic compounds such as flavonoids.

Mishra and Verma (2017) opined that the ayurveda is the Indian traditional system of medicine which focuses on the medical potential of plants, and *C. roseus* is a plant recognized well in Ayurveda. It is known for its antitumor, anti-diabetic, anti-microbial, anti-oxidant and anti-mutagenic effects. It is an evergreen plant first originated from islands of Madagascar. The flowers may vary in colour from pink to purple and leaves are arranged in opposite pairs. Also

reported that the *C. roseus* produces nearly 130 alkaloids mainly ajmalcine, vinceine, reserpine, vincristine, vinblastine and raubasine. Vincristine and Vinblastine are used for the treatment of various types of cancer such as Hodgkin's disease, breast cancer, skin cancer and lymphoblastic leukemia. It is an endangered species and need to be conserved using techniques like micropropagation. It has high medicinal value which need to be explored extensively.

Das and Sharangi (2017) stated that the genus *Catharanthus* consists of eight species of which seven are native to Madagascar and one, *C. pusillus*, to India. *C. roseus*, Madagascar periwinkle is one of the few pharmacological plants that have a long history of therapeutic voyage from Mesopotamian folklore of 2600 BCE till today playing a considerable role as herbal and traditional medicine of various diseases. As previously published reviews on Madagascar periwinkle mostly concentrate on the pharmaceutical and chemical compounds of the herb, lack of information about the other features of the species is extremely tangible. This organized review provided insights into agro technological, biological, ecological, and medicinal aspects (particularly anticancer compounds) of Madagascar periwinkle along with possible pathways and mode of action. The diverse medicinal and therapeutic potentials of *C. roseus* have been revealed that this magical herb may be utilized to its full potential and future research orientation may be directed towards it.

2.4 The antidiabetic activity of *Catharanthus roseus*

Chattopadhyay et al. (1991) stated that ethanolic extracts of the leaves and flower of *C. roseus* showed a dose-dependent lowering of blood sugar compare to the standard drug, glibenclamide. The hypoglycemic effect has appeared due to the result of the increase glucose utilization in the liver. The aqueous extract was found to lower the blood glucose to about 20% in diabetic rats when compared to that of the dichloromethane and methanol extracts which lowered the blood glucose level to 49-58%.

Chattopadhyay (1994) stated that the hypoglycemic activity of alkaloids isolated from *C. roseus* have been studied pharmacologically and a remedy derived from the plant has been marketed under the proprietary name Vinculin as a treatment for diabetes.

Don (1999) reported that the *C. roseus* belonging to dogbanes family Apocynaceae is known with various names in India and all over the world. Hot water decoction of the leaves and/or the whole plant is used for treatment of diabetes in several countries i.e. Brazil, Cook Islands, Dominica, England, Jamaica, Mozambique, Pakistan, Taiwan, Thailand and West Indies.

Singh et al. (2001) detected the hypoglycemic activity in dichloromethane: methanol extract (1: 1) of leaves and twigs of *C. roseus*, using streptozotocin-induced diabetic rat model. Extract at dose 500 mg/kg given orally for 7 and 15 days showed 48.6 and 57.6% hypoglycemic activity, respectively. Prior treatment at the same dose for 30 days provided complete protection against STZ challenge (75 mg/kg/i.p.x1). Enzymic activities of glycogen synthase, glucose 6-phosphate-dehydrogenase, succinate dehydrogenase and malate dehydrogenase were decreased in liver of diabetic animals in comparison to normal and were significantly improved after treatment with extract at dose 500 mg/kg p.o. for 7 days. Results indicate increased metabolism of glucose in treated rats. Increased levels of lipid peroxidation measured as 2-thiobarbituric acid reactive substances (TBARS) indicative of oxidative stress in diabetic rats were also normalized by treatment with the extract.

Andrade and Heinrich (2005) observed that in Mexico, diabetes mellitus is commonly treated with herbal extract and a total of 306 species have records of a popular use in the treatment of this endocrine syndrome. One of them is *C. roseus* that is employed as root infusion and it is commonly named as *vicaria*. Its plant is also known as “teresita” or “todo tiempo”. In the present study the organic and aqueous extracts from root, leaf, flower, stem, and an alkaloid-free aqueous stem fraction were evaluated for hypoglycemic activity in healthy or alloxan-induced diabetic mice.

Akhtar et al. (2007) stated that *C. roseus* (L.) is used traditionally in various regions of the world including Nigeria, India, Trinidad and Tobago to control diabetes.

John (2017) evaluated the antidiabetic activity of aqueous leaf extract of *Vinca rosea*. The aqueous extract at high dose (300mg/100g) body weight showed a significant hypoglycemic activity. Improvement in the body weight,

water and food consumption is also observed after the treatment with herbal extract.

Raja et al. (2008) showed, in vivo of anti-diabetic effect of aqueous extract of the leaves of *Vinca rosea* on alloxan induced diabetic rats. 7 days of the orally feeding *V.rosea* extract (400mg/kg) to alloxan (120 mg/kg, i.p., single dose) induced diabetic rats produced significant decrease in blood glucose level as compared to pathogenic diabetic rats. Further the aqueous extract treatment can significantly alter the pattern of glucose tolerance in normal and diabetic rats. The inhibitory effect on biochemical parameter (blood glucose level) induced by the Aqueous extract of *V.rosea* at a dose of 400 mg/kg was almost comparable to that of standard drug, glibenclamide 10 mg/kg.

Mohammed et al. (2010) studied antidiabetic activity of *Vinca rosea* methanolic whole plant extracts in alloxan induced diabetic rats for 14 days. The methanolic whole plant extract at high dose (500 mg/kg) exhibited significant antihyperglycemic activity than whole plant extract at low dose (300 mg/kg) in diabetic rats. The methanolic extracts also showed improvement in parameters like body weight and lipid profile as well as regeneration of β -cells of pancreas in diabetic rats. Histopathological studies reinforce the healing of pancreas, by methanolic *Vinca rosea* extracts, as a possible mechanism of their antidiabetic activity.

Manoharan et al. (2018) revealed that the ethanolic extract of *C. fenestratum* stem and *C. roseus* leaves have potent antihyperglycemic effect in alloxan-induced diabetic rats. *C. fenestratum* and *C. roseus* could therefore be used as an alternative remedy for diabetes mellitus and its complications.

Ohadoma and Michael (2011) investigated the interacting effects of co-administration of methanol leaf extract of *C. roseus* on the hypoglycemic activity of metformin as well as glibenclamide using experimental rats. The LD₅₀ was 121.32 mg/kg. The phytochemical studies indicated the presence of saponins, tannins, alkaloids, phlobatannins, flavonoids, triterpenoids, reducing sugars, anthraquinones and glycosides. All medicaments significantly reduced blood glucose levels when compared with control alone ($p < 0.05$) with highest percentage in blood glucose (64.86%) exhibited by metformin extract

combination. The leaf extract of *C.roseus* significantly increases the hypoglycemic effect of metformin.

Elisa et al. (2012) studied that aqueous extract (250 mg/Kg) of flowers, leaves, roots, and stems of *C. roseus* produced hypoglycemic effect in healthy and alloxan induced diabetic mice ($P < 0.05$).

Soon et al. (2013) reported that *C. roseus* is a herbal plant traditionally used by local populations in India, South Africa, China and Malaysia to treat diabetes.

Muralidharan (2014) revealed that the leaf extracts of *C. roseus* helps in regulating and maintaining the homeostatic metabolism in the body. Extracts were found to be effective as an anti- diabetic agent.

Khan (2015) studied that the antidiabetic activity of *C. roseus* and *C. alba* methanolic flower extracts in alloxan induced diabetic rats for 14 days and to compare the results of two varieties in reducing blood glucose levels. At the beginning of this study preliminary phytochemical investigation was carried out on methanolic extracts of flowers of both varieties of *Catharanthus*.

Waleed et al. (2015) concluded that the *C. roseus* plant ethanol extract was found to exhibit a significant anti hyperglycemic activity in streptozotocin (STZ)-induced diabetic rats.

Waleed et al. (2015) observed that the antidiabetic efficacy of ethanolic extract of *C. roseus* and an expression of GLUT-2 and GLUT-4 gene in streptozotocin induced diabetic wistar rats. The doses were administered orally at a rate of 100 and 200 mg/kg and detain the glucose transport system in liver for 4 weeks. The observed results showed a good positive correlation between intracellular calcium and insulin release levels in isolated islets of Langerhans. The supplementation of ethanolic extract of *C. roseus* significantly amplified the expression of GLUT gene mRNA by Real Time PCR in liver of diabetic rats.

Mishra and Verma (2017) studied that the ethanolic extracts of the leaves and flower of *C. roseus* showed a dose dependent lowering of blood sugar in comparable to the standard drug. Lowering of blood sugar in comparable to the standard drug glibenclamide. The Hypo glycemic effect has

appeared due to the result of the increase glucose utilization in the liver. The aqueous extract was found to lower the blood glucose of about 20% in diabetic rats when compared to that of the dichloromethane and methanol extracts which lowered the blood glucose level to 49-58%. The hypoglycemic effect has appeared due to the result of the increased glucose utilization in the liver. The hypoglycemic activities of alkaloids isolated from *C. roseus* have been studied pharmacologically and a remedy derived from the plant has been marketed under the proprietary name Vinculin as a treatment for diabetes.

Joyamma (2017) carried out study to evaluate the antidiabetic activity of aqueous leaf extract of *Vinca rosea*. The aqueous extract at high dose (300mg/100g) showed a significant hypoglycemic activity. Improvement in the body weight and water and food consumption is also observed after the treatment with herbal extract.

Shilpi and Baidyanath (2017) investigated hypoglycemic efficacy of methanol extract of *C. roseus* (Nayantara) of Apocyanaceae and *Opuntia ficusindica* of Cactaceae was tested on STZ induced mice diabetic models. The results clearly indicated that the diabetic control (DC) mice presented a significant lowering of body weight ($p < 0.001$) when compared with the normal control (NC) mice. The DC mice showed a significantly ($p < 0.001$) higher level of glucose (+279%), when compared with their normal control counterparts. Diabetic mice of both of the groups (DT150 and DT250) showed a reduction in glucose levels, when compared to the DC ones. The results clearly indicated that the methanol extract of *C. roseus* and *O. ficus indica* is antidiabetic in nature due to the presence of different types of active phytochemicals.

2.5 Induction of diabetes

For inducing diabetes mellitus in animals the chemical compounds or diabetic strains are used.

Diabetogenic drugs: These are the chemical agents which exert immediate toxic effect on the β -cells of the islets of Langerhans causing chronic though not necessarily lifelong diabetic state. Therefore, these substances have also been referred to as β cytotoxic agents or β cytotoxins as their action is restricted to the β -cells of the islets of Langerhans only.

White (1963) stated that the streptozotocin is an antimicrobial agent and has also been used as a chemotherapeutic alkylating agent

Rakieta et al. (1963) reported that in rats, streptozotocin (STZ) induces diabetes when used at doses ranging from 45 to 70 mg/kg. Also stated that the streptozotocin (STZ) was initially isolated from *Streptomyces achromogenes* in 1960, with its diabetogenic properties not described until 1963.

Rerup (1970) stated that a chemical compound that damages the pancreatic islets cells constitute a class of diabetogenic agents. He mentioned the compounds like alloxan and streptozotocin exhibit the most potent diabetic action and have been used widely for induction of experimental diabetes mellitus.

Akhtar and Ali (1984) reported that streptozotocin (STZ) is well known for its selective pancreatic islet cell toxicity and has been extensively used for the induction of diabetes mellitus in animals.

Kolb (1987) stated that the streptozotocin (STZ) is a highly selective pancreatic islet β -cell-cytotoxic agent that in the past was administered at a single high dose to cause, within 48 hr. complete β -cell necrosis and diabetes.

Piyachaturawat et al. (1988) studied that in rats, STZ injected in a dose of 65 mg/kg body weight effectively produced hyperglycemia and gastric mucosal ulcerations. The incidence and severity of lesions produced by STZ in pancreas, liver, kidney and GIT, progressively increased with time from one to six weeks post treatment.

Williamson et al. (1996) studied that the Single dose of STZ in sterile citrate buffer (e.g. pH 4.5 0.1M) may be used: mice 150 mg/kg; rats 80 mg/kg, administered intraperitoneally. Develops diabetes gradually and may be assessed after a few days, usually four days for mice and seven days for rats. Usually, a serum glucose level of about 180 – 500 mg/dl indicates the induction of diabetes mellitus. Sometimes diabetic animals are maintained on insulin if the experiments are not to commence immediately to prevent the animals' death.

Dolan (1997) stated that the streptozotocin is a permanent diabetes inducing drug. It is synthesized by a strain of the soil microbe *Streptomyces*

achromogenes (gram positive bacterium) with broad spectrum of antibacterial properties.

Rodrigues et al. (1997) observed that, at lower doses, STZ-induced diabetes is not stable, since spontaneous recovery occurs. Long-term studies on pathological changes related to hyperglycemia require a stable model of experimentally induced diabetes. Since different strains of the same animal species may differ in sensitivity to the diabetogenic effect of STZ.

Nurdiana et al. (1998) concluded that the multi low dose streptozotocin (MLD-STZ) methods at 40 mg/kg dose of STZ for five days showed significant diabetic condition. The BGL at week 3 was 334.16 ± 17.5 mg/dl and 325.7 ± 30.8 mg/dl at week 4, and no deaths occurred in experimental animals.

Pellegrino et al. (1998) revealed that non-insulin dependent diabetes mellitus (NIDDM) was induced by a single intraperitoneal injection of streptozotocin (60mg/kg) and nicotinamide (120mg/kg) to rats

Gajdosik et al. (1999) presented the sensitivity of young male Wistar rats, to the diabetogenic effect of streptozotocin (STZ) with regard to their health condition and mortality rates. Eight-week-old rats, weighing from 200 to 230 g, were randomized into five groups of eight animals. Streptozotocin was administered by i.v. injection in doses of 40, 50, 60 and 70 mg/kg body weight. The animals were kept on a standard diet with free access to water for 4 months. The highest STZ dose (70 mg/kg) was lethal to the animals, the doses of 50 and 60 mg/kg induced persistent hyperglycemia with glucose levels above 20 mm. Body weights of STZ treated rats from all experimental groups were significantly lower than those of control animals. Considerable polyuria was observed in all STZ treated rats. About 40 % of the STZ treated animals were found to develop overt cataract between days 90 and 100. At the end of the experiment, significant albuminuria was observed in the experimental groups administered 50 and 60 mg/kg STZ doses.

Singh et al. (2001) studied that the hypoglycemic activity was detected in dichloromethane: methanol extract (1:1) of leaves and twigs of *C. roseus* (family Apocynaceae), a traditionally used medicinal plant, using streptozotocin (STZ) induced diabetic rat model. Extract at dose 500 mg/kg given orally for 7 and 15

days showed 48.6 and 57.6% hypoglycemic activity, respectively. Prior treatment at the same dose for 30 days provided complete protection against STZ challenge (75 mg/kg/i.p.x1).

Szkudelski (2001) stated that the STZ was an antibiotic and antitumor agent, induces diabetes mellitus via reduction of nicotinamide adenine dinucleotide in pancreatic β -cells *in vivo*.

Balamurugan et al. (2003) reported that streptozotocin (STZ) is the most commonly used drug for induction of diabetes in rats.

Federiuk et al. (2004) stated that streptozotocin (STZ), 69% and alloxan (31%) most frequently used drugs and this model has been useful for the study of multiple aspects of the disease. Both drugs exert their diabetogenic action when they were administered parenterally (intravenously, intraperitoneally or subcutaneously). The dose of these agents required for inducing diabetes depends on the animal species, route of administration and nutritional status.

Braslasu et al. (2007) studied that the diabetes mellitus was induced to white wistar rats by intraperitoneal administration of streptozotocin – 60 mg/kg. From the 59 total rats to which Streptozotocin was only 33 (55.93%) have made diabetes mellitus. From those 33 rats, 5 have developed supra acute form, the death appeared on the first days from streptozotocin administration.

Akbarzadeh et al. (2007) studied to induce experimental diabetes mellitus by Streptozotocin in normal adult Wistar rats via comparison of changes in body weight, consumption of food and water, volume of urine and levels of glucose, insulin and C- peptide in serum, between normal and diabetic rats. Intravenous injection of 60mg/kg dose of Streptozotocin in adult wistar rats, makes pancreas swell and at last causes degeneration in Langerhans islet beta cells and induces experimental diabetes mellitus in the 2-4 days. Induction of experimental diabetes mellitus is indeed the first step in the plan of purification of pancreatic Langerhans islet cells of normal rats for transplanting under the testis subcutaneous of experimentally induced diabetic rats and for the induction of experimental diabetes in male adult rats weighted 250-300 grams (75-90 days), 60mg/kg of Streptozotocin was injected intravenously. Three days after degeneration of beta cells, diabetes was induced in all animals.

Guneli et al. (2008) suggested that intraperitoneal administration of melatonin for 5 days is a potentially beneficial agent to reduce testicular damage in adult male rats with streptozotocin (STZ)-induced diabetes, probably by decreasing oxidative stress.

Paik et al. (2008) reported that, injection of a single high dose of streptozotocin (200 mg/kg body weight) induced rapid and permanent hyperglycemia in mice. In contrast, the injection of the same total dose divided into multiple “subdiabetogenic” doses (40 mg/kg per day for five consecutive days) caused the development of delayed but progressive hyperglycemia only in the thymus competent mice. Female mice were less susceptible to streptozotocin at both doses.

Zafar et al. (2009) observed that the STZ given intravenously or intraperitoneally to laboratory mice in multiple sub-diabetogenic doses induces pronounced pancreatic insulinitis with eventual destruction of insulin secreting beta cells and diabetes mellitus. In an experimental study in rats, streptozotocin given intraperitoneally in a dose of 45 mg/kg body weight of animals, effectively produced hyperglycemia. Histopathology of kidney showed lesions similar to human glomerulosclerosis, glomerular membrane thickening, arteriolar hyalinization and tubular necrosis. Increased levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and pseudocholesterase (PChE) were observed in the kidney. It seems that the diabetic complications in the kidney are likely to be associated with alterations in enzyme levels.

Vivek (2010) reported that the streptozotocin is an unusual aminoglycoside containing nitrosamine group discovered in 1959 as an antibiotic, now marketed as a generic drug. The nitrosamines group enables the metabolite to act as a nitric oxide (NO) donor. NO is an important messenger molecule involved in many physiological and pathological processes in the body. Streptozotocin is widely used to induce diabetes in rodent models by inhibition of β -cell O-GlcNacase

Mohammed et al. (2011) studied antidiabetic activities of whole plant extract of *C. roseus* Dichloromethane: methanol (1:1) extract was used for carrying out *in-vivo* antidiabetic activity. The study on antidiabetic activity

involves induction of diabetes to all male Wister albino rats using alloxan monohydrate (80mg/kg body/weight) except control group followed by treatment of diabetic rats with extract (500mg/kg body weight) daily for 14 days. The results were compared with standard drug Glibenclamide (5 mg/kg body weight) by measuring glucose levels and body weights of all animals. Glucose level was found to decrease and body weight was increased in extract treated and standard treated groups when compared to diabetic group.

Deeds et al. (2011) found that the streptozotocin (STZ)-induced diabetes mellitus (DM) offers a very cost-effective and expeditious technique that can be used in most strains of rodents, opening the field of DM research to an array of genotypic and phenotypic options that would otherwise be inaccessible. Despite widespread use of STZ in small animal models, the data available concerning drug preparation, dosing and administration, time to onset and severity of DM, and any resulting morbidity and mortality are often limited and inconsistent. Because of this, investigators inexperienced with STZ-induced diabetes may find it difficult to precisely design new studies with this potentially toxic chemical and account for the severity of DM it is capable of inducing. Until a better option becomes available, attempts need to be made to address shortcomings with current STZ-induced DM models.

Palanisamy et al. (2011) studied that the diabetes was induced by intraperitoneal injection of streptozotocin (45mg/kg) to albino wistar rats. ellagic acid (50 mg/kg and 100 mg/kg) dissolved in 0.2% dimethyl sulfoxide was administered orally to diabetic rats using an intragastric tube daily for a period of 35 days. A significant increase in plasma glucose, blood glycosylated hemoglobin and hexokinase activity and decrease in plasma insulin and C-peptide, blood hemoglobin, glycogen (liver and muscle) and the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in liver and kidney were observed in STZ-induced diabetic rats. Oral administration of ellagic acid these biochemical parameters to near normal.

Waleed et al. (2015) concluded that the *C. roseus* plant ethanol extract was found to exhibit a significant anti hyperglycemic activity in streptozotocin (STZ)-induced diabetic rats. The results from blood glucose, serum biochemical estimation and glucose transport gene (GLUT-2 and GLUT-4) mRNA indicated

that the *C. roseus* has renewing and healing ingredients as it could reverse most blood and tissue changes caused by STZ-induced diabetes in rats.

Khan (2015) studied antidiabetic activity of *C. roseus* and *Catharanthus Alba* methanolic flower extracts in alloxan induced diabetic rats for 14 days and compared the results. Fasting blood glucose estimations and body weight measurements were carried out on 0th, 1st, 7th and 14th day of the experiment. On 14th day the serum was separated and analyzed for biochemical parameter variations. The methanolic flower extracts at high dose (400 mg/kg) exhibited significant ($p < 0.01$) anti-hyperglycemic activity than methanolic flower extracts at low dose (200 mg/kg) in diabetic rats. The methanolic flower extracts also showed improvement in parameters like body weight and lipid profile as well as regeneration of β -cells of pancreas in diabetic rats.

Khaleel et al. (2015) induced experimental diabetes mellitus by Streptozotocin in normal adult wistar rats via comparison of changes in body weight, consumption of food and water and levels of glucose between normal and diabetic rats. For this intraperitoneal injection of 40mg/kg dose of streptozotocin in adult wistar rats, makes pancreas swell and at last causes degeneration in Langerhans islet beta cells and induces experimental diabetes mellitus in the 48-72 hours. The diabetic and normal animals were kept in the metabolic cages separately and their body weight, consumption of food and water and the levels of serum glucose in all animals were measured and then these quantities were compared. After one week of inducing diabetes by single rapid intraperitoneal injection 40mg/ kg BW, the blood glucose levels are measured with glucometer, in normal rats the glucose levels was 125+10 mg/dl, in diabetic rats it was 386+45mg/dl.

Hikmah et al. (2015) revealed the profile of diabetic blood glucose level in rat using a stratified dose streptozotocin (STZ-SD) and multi-low dose streptozotocin (MLD-STZ) induction methods. 27 Wistar rats were divided into three groups, the control group, SD-STZ group, and MLD-STZ group. The induction of experimental animal was using the method of SD-STZ and MLD-STZ for five consecutive days intraperitoneally. The SD-STZ method using a stratified dose of 40, 35, 30, 25, and 20 mg/Kg of body weight for five days. MLD-STZ methods using a dose of 20 mg/Kg of body weight for five times. The

measurement of blood glucose level (BGL) and body weight (BW) in the animal was made before and on days 2, 4, 7, and 14 after STZ induction. The results of this study indicate that the diabetic conditions (BGL \geq 300 mg/dl) on the SD-STZ induction method was more stable than MLD-STZ method, and no deaths occurred in experimental animals.

2.6 Mechanism of action of streptozotocin

Arison *et al.* (1967) reported that streptozotocin is diabetogenic. Again, this insulinopenia syndrome, called 'streptozotocin diabetes' is caused by the specific necrosis of the pancreatic beta cells and streptozotocin has been the agent of choice for the induction of diabetes mellitus in animals ever since.

Magee and Swann (1969) reported that the effects of STZ on different organs has various biological actions, including the production of acute and chronic cellular injury, carcinogenesis, teratogenesis and mutagenesis.

Rerup (1970) observed that the streptozotocin features four important biological properties as evidenced by its antibiotic, β -cell (beta)-cytotoxic, oncolytic, as well as oncogenic effects. This product is an antineoplastic antibiotic and is used mainly in the treatment of pancreatic (islet cell) tumors. It is used for the treatment of malignant insulinoma.

Dorr and Fritz (1980) revealed that the streptozotocin (STZ) is a naturally occurring nitrosourea with molecular weight of 265 and empirical formula of C₁₄H₂₇N₅O₁₂. It is widely used to induce insulin dependent diabetes mellitus in experimental animals because of its toxic effects on islet beta cells.

Kolb (1987) showed that the diabetogenic effect of streptozotocin was due to selective destruction of pancreatic islet β cells. As a result of this action, the animals experience insulin deficiency, hyperglycemia, polydipsia, and polyuria, all of which are characteristic of human diabetes mellitus type1.

Piyachaturawat *et al.* (1991) stated that the STZ is a nitrosourea compound which generally shares similar fate of disposition with other nitrosoureas and is a drug of choice in islet cell carcinoma and malignant carcinoid tumors. It is diabetogenic, hepatotoxic, nephrotoxic and also causes gastric ulceration.

Gu et al. (1997) reported that the diabetogenic action of STZ was the direct result of irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin.

Miodrag et al. (1998) studied cellular and molecular requirements for beta 3-cell damages in an immune-mediated toxin induced insulin-dependent diabetes mellitus in the model of multiple low-dose streptozotocin-induced diabetes in rats and mice. It was found that strain-related susceptibility to diabetes induction correlated with a higher level of IL-2, IFN- γ , and TNF- α production, whereas such differences were not observed when IL-1 and NO production by macrophages were analyzed; elimination of immune regulatory RT6+T cells that increases IFN- γ production, enhances susceptibility to MLD-STZ-induced diabetes; mercury-induced Th2 cells downregulated the disease; IFN- γ -mediated macrophage activation to produce proinflammatory cytokines rather than NO is an important event in early diabetogenic effects of invading macrophages; inhibition of IL-1 activity downregulates diabetes induction; and generation of NO in beta 3 cells appears to be important for diabetogenic effects. Taken together, data indicate that MLD-STZ diabetes is induced by Th-1 lymphocytes that secrete soluble effector molecules that activate macrophages and promote destruction of β 3 cells possibly by both nitric oxide and nonnitric oxide-mediated mechanisms.

Holemans and Assche (2003) reported that streptozotocin prevents DNA synthesis in mammalian and bacterial cells. In the bacterial cells, it renders special reaction with cytosine groups, resulting in degeneration and destruction of DNA. The biochemical mechanism results in mammalian cell death. Streptozotocin prevents cellular reproduction with a much smaller dose than the dose needed for inhibiting the substrate connection to the DNA or inhibiting many of the enzymes involved in DNA synthesis

Katakam et al. (2005) reported that rats treated with a single dose of streptozotocin (STZ; 50 mg/kg) showed acute upregulation of serum Osteopontin (OPN) levels and pancreatic OPN mRNA and protein. Serum OPN dropped by the end of day 7 but was still higher than prediabetic levels. Pancreatic mRNA and protein showed a similar pattern. Twenty-four hours after STZ injection, the

intensified OPN expression was localized towards the periphery of the islets and surrounded the remaining insulin-positive cells.

Lenzen (2008) studied that the streptozotocin are toxic glucose analogues that preferentially accumulate in pancreatic beta cells via the GLUT2 glucose transporter. In the presence of intracellular thiols, especially glutathione, alloxan generates reactive oxygen species (ROS) in a cyclic redox reaction with its reduction product, dialuric acid. Autoxidation of dial uric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalysed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells, which have a particularly low antioxidative defence capacity, and the ensuing state of insulin-dependent 'alloxan diabetes'. As a thiol reagent, alloxan also selectively inhibits glucose-induced insulin secretion through its ability to inhibit the beta cell glucose sensor glucokinase. 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. The targeting of mitochondrial DNA, thereby impairing the signalling function of beta cell mitochondrial metabolism, also explains how streptozotocin is able to inhibit glucose-induced insulin secretion.

Zafar et al. (2009) concluded that the streptozotocin through its direct alkylating action can cause cellular necrosis and selective destruction of the beta cells producing hyperglycemia at a dose of 45 mg/kg body weight. It may also be stated that streptozotocin by producing diabetes (hyperglycemia) and hypoinsulinemia alters various metabolic and enzymatic functions of kidney resulting in various pathologic lesions. It may also be concluded that the diabetic complications in kidney are associated with alterations in enzyme levels.

Teeraporn (2011) reported that streptozotocin probably the most widely used agents producing insulin dependent diabetes mellitus and non-insulin dependent diabetes mellitus in experimental animals. Streptozotocin is a glucosamine nitrosourea compound.

Singh et al. (2001) stated that the streptozotocin enters the B 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, B cells undergo the destruction by necrosis.

Saini and Sharma (2013) reported that the STZ-induced diabetes was characterized by severe loss in body weight. The decrease in body weight in diabetic rats showed that the loss or degradation of structural proteins was due to diabetes. Structural proteins are known to contribute to body weight. STZ-induced diabetes may be due to vitiate glucose oxidation and reduction of insulin biosynthesis and secretion. The toxicity of STZ is due to DNA alkylation of its methyl nitrosourea moiety, mainly O at 6 position of guanine. The transfer of methyl group from STZ to the DNA molecule causes damage which results in fragmentation of DNA and functional defects of the beta cells. Moreover, STZ has potential to act as an intracellular nitric oxide (NO) donor and generates reactive oxygen species (ROS). The synergistic action of both NO and ROS may also contribute to DNA fragmentation and other deleterious changes caused by STZ.

Goud et al. (2015) studied that the streptozotocin is a permanent diabetogenic compound, produced by the gram positive soil bacterium *Streptomyces achromogenes* that exhibits broad spectrum of antibacterial properties. STZ induces diabetes mellitus in laboratory animals by killing insulin-producing pancreatic β - cells. Streptozotocin is a toxic glucose analogue that preferentially accumulate in pancreatic beta cells via the low affinity glucose transporter GLUT2. The toxic effector mechanism of STZ starts with its decomposed products and the free radicals generated, which destroy the pancreatic β - cells by alkylating DNA, impairing mitochondrial system and inhibiting O-GlcNAcase.

Furman (2015) reported the streptozotocin (STZ) as an antibiotic that produces pancreatic islet β -cell destruction and is widely used experimentally to

produce a model of type 1 diabetes mellitus (T1DM). Detailed in this unit were protocols for producing STZ-induced insulin deficiency and hyperglycemia in mice and rats. Also described protocols for creating animal models for type 2 diabetes using STZ.

2.7 Serum biochemistry in diabetes

Nabi et al. (2014) studied dysfunctions in streptozotocin (STZ) induced diabetic rats. The average levels of glucose, triglycerides, cholesterol, creatinine, urea in the plasma of diabetic and control rats were 302.6 ± 87.5 vs 100.7 ± 11.5 mg/dL, 174.9 ± 18.6 vs 82.2 ± 10.2 mg/dL; 250.8 ± 22.3 vs 165.2 ± 24.1 mg/dL; 0.94 ± 0.19 vs 0.81 ± 0.05 mg/dL; 77.1 ± 9.7 vs 26.8 ± 5.8 mg/dL, respectively. The mean values of aspartate transaminase (AST) and alanine transaminase (ALT) in diabetic and control rats were 141.4 ± 28.0 vs 61.6 ± 18.6 IU/L and 61.4 ± 13.6 vs 48.5 ± 6.0 IU/L, respectively. Biochemical parameters measured in diabetic rats varied significantly ($p < 0.001$) compared to those of control rats. Plasma indices such as triglycerides, cholesterol, creatinine, urea, AST and ALT had no relation with the functions of neutrophils. However, multidimensional scaling found a close relation between plasma cholesterol and phagocytic activity of neutrophils from diabetic rats.

Sharma et al. (2010) studied the influence of the ethanolic extract of *Ficus glomerata* in albino rats and observed that the serum urea, creatinine and cholesterol levels were decreased significantly in a dose related fashion by ethanolic extract of *Ficus glomerata* (at 250 and 500 mg/kg) due to 10 days of treatment, whereas serum protein level was increased significantly when compared to the diabetic control group.

Zulfiker et al. (2010) studied the effect of ethanolic extract of *Scoparia dulcis* (EESD) on the blood glucose levels in the normal and diabetic mice. The result indicated that the antidiabetic effect of EESD was promising. They observed that after a single dose load of 200 mg/kg glucose in mice, there was a significant drop of fasting blood glucose level ($p < 0.05$) during the 3 hr. study period, where as in case of chronic administration, highly significant variation ($p < 0.001$) was observed between investigational and diabetic control mice in lowering fasting blood glucose level. The extract significantly lowered blood glucose level at both doses (100 mg/kg & 200 mg/kg body weight) and showed

maximum reduction; at 100 mg/kg it was found 31.87% and 200 mg/kg it was 46.97% on 14th day, whereas reduction of 50.74% was found for metformin on day 14 as a peak. The short-term and long-term experiment was done to find out the effect of EESD on blood glucose level in alloxan induced diabetic mice. This showed that the plant extract was found to be highly effective in blood sugar lowering activity in alloxan-induced hyperglycemic mice.

Jayanthi et al. (2010) reported that the effect of daily oral administration of *C. roseus* leaf dichloromethane: methanol (1:1) extracts (500 mg/ body weight) for 20 days on blood glucose and hepatic enzymes in normal and alloxan induced diabetic rats. A significant ($P < 0.05$) increased body weight and decreased blood glucose, urea, cholesterol levels of the test animals showed that the extract exhibited anti hyperglycemic activity and increased in protein and glycogen ($P < 0.01$) were observed in diabetic rats treatment with *C. roseus* leaf dichloromethane methanol extract when compared to diabetic rats. The activity of the hepatic enzymes such as hexokinase was significantly ($P < 0.01$) increased and glucose 6-phosphatase and fructose 1, 6- bisphosphatase were significantly ($P < 0.05$) decreased by the administration of *C. roseus* leaf in diabetic rats when compared to normal rats.

Kemasari et al. (2011) conducted the experiment to understand the effect of *Mangifera indica* leaf extract in the alloxan induced diabetic rats. They found that there is reduction in plasma total protein, albumin and globulin levels of the alloxan induced rats. They are of the opinion that the decrease in protein may be due to microproteinuria and albuminuria, which is an important clinical marker of diabetic nephropathy and / or may be due to increased protein catabolism. The lack of insulin also reduces RNA and mRNA, which is another factor for the reduction of total protein. Significant increase in renal parameters like urea, uric acid and creatinine in alloxan induced group indicates impaired renal function i.e. due to decreased excretion of urea, uric acid and creatinine in the urine, which in turn may be due to the basement membrane injury. They observed that the treatment with *Mangifera indica* leaf extract produced significant improvement in the levels of urea, uric acid and creatinine. ALT and AST are the specific markers to assess hepatocellular damage leading to liver cell necrosis. During these studies the alanine aminotransferase and aspartate aminotransferase activities were assessed as it is the more specific index of liver

cell damage in humans and in experimental animals. Thus lowering of these enzymes content in serum was a definite indication of hepatoprotective action of *Mangifera indica* leaf extract.

Mohammed et al. (2011) observed that biochemical parameters like Urea, Creatinine, Total Cholesterol and Alkaline Phosphatase levels increased considerably in diabetes induced rats and values for dichloromethane: methanol (1:1) whole plant extract were found to be 43.33, 0.36, 120 and 85 mg/dl respectively which is nearer to standard and normal group values.

Johnkennedy et al. (2012) carried out the investigations to know the effect of the leaf extract of *Gongronema latifolium* on the serum cholesterol. Hypercholesterolemia has been reported to occur in alloxan induced diabetic rats. However, they observed that the oral administration of leaf extract of *Gongronema latifolium* restored the level of cholesterol in diabetic rats. They reported that the hypolipidemic effect of *Gongronema latifolium* extract may be attributed to inhibition of endogenous synthesis of lipids probably by potentiating the secretion of insulin and due to the presence of several bioactive hypolipidemic principles and their synergistic properties. Furthermore, it was observed that serum urea and creatinine levels in diabetic rats treated with the extract of *Gongronema latifolium* was significantly reduced, while the serum protein and albumin got increased. This indicated that *Gongronema latifolium* practically offers protection against alloxan induced renal toxicity.

Borgohain et al. (2012) studied the effect of ethanolic extract of fruit pulp of *Terminalia chebula*. They observed that the ethanolic extract of fruit pulp of *Terminalia chebula* lowered the blood glucose levels in alloxan induced diabetic rats. The extract when administered orally at the dose of 100mg/kg/day for four weeks produced statistically significant ($P < 0.01$) reduction in blood glucose levels in diabetic albino rats as compared to diabetic control. However, there was no difference between normal control and normal test group after four weeks of the treatment. This indicated that the *Terminalia chebula* is antihyperglycemic, however does not lower the normal blood glucose levels.

Luka and Mohammed (2012) reported that the aqueous extract of *Mangifera indica* at a dose of 400 mg/kg body weight reduced significantly ($P < 0.05$) the blood glucose level in alloxan induced diabetic rats, which suggests

that the plant may have hypoglycemic effect. The mechanism of action of the extract however is not yet understood. Since alloxan is known to induce diabetes by destroying completely the pancreatic islet of β -cells, which produces insulin. Therefore, certainly the aqueous extract of *Mangifera indica* does not act by stimulating insulin release from the pancreatic β -cells. Hence, the mechanism of action of aqueous extract of *Mangifera indica* is peripheral utilization of glucose just like insulin. This suggests that some of the active principles in the extract are responsible for the hypoglycemic action and are analogs of the insulin. They also studied that the effect of aqueous extract of *Mangifera indica* leaf on serum total protein level. The result shows that the diabetic control rats had significant ($P < 0.05$) low serum total protein level compared with the normal control rats, while the diabetic treated showed a significant ($P < 0.05$) increase in serum protein level compared to the diabetic control group. The effect of the aqueous extract of *Mangifera indica* leaf on serum cholesterol levels of normal and alloxan-induced diabetes rats. The result showed that aqueous extract of *Mangifera indica* had a significant ($p < 0.05$) reduction of serum total cholesterol levels in alloxan-induced diabetic rats treated with the extract compared to the diabetic group.

Rajan et al. (2013) conducted the hypoglycemic studies using ethanol extract of *Blepharis repens (vahl)* in male wistar rats. They reported that alloxan causes a significant elevation in the level of blood glucose in rats and that on administration of ethanol extract of *Blepharis repens (vahl)* at the dose of 100 and 200 mg/kg body weight significantly decreased the blood glucose level of diabetic rats after twenty one days of treatment, indicating that it has hypoglycemic properties. Further, they also observed that it possess the hypolipidimic and hepatoprotective properties just like the standard drug glibenclamide. They also observed the effect of ethanol extract of *Blepharis repens (vahl) Roth* on protein, SGOT and SGPT levels in diabetic rats. They stated that a significant reduction in serum protein, albumin and globulin were observed in alloxan induced diabetic rats, when compared to control and glibenclamide treated rats. On administration of ethanol extract of *Blepharis repens (vahl) Roth* to the diabetic rats, they observed the protein, albumin and globulin levels were restored to normal. During these studies it was also revealed that the levels of SGPT and SGOT in alloxan induced diabetic rats were elevated. They were of the opinion that the increase in the SGPT and SGOT levels is due to leaking out of enzymes from the tissues and migrating into the

circulation by the adverse effect of alloxan. However, the whole plant ethanol extracts of *Blepharis repens (vahl) Roth* regulated the activity of SGPT, SGOT and ALP in Liver of rats intoxicated with alloxan.

Rathor et al. (2013) studied the antidiabetic effect of the hydro alcoholic extract and chloroform extract of *Ecbolium ligustrinum*. They observed that, the hydro alcoholic extract and chloroform extract of *Ecbolium ligustrinum* has the antidiabetic properties. Further, when hydro alcoholic extract and chloroform extract of *Ecbolium ligustrinum* were compared for their antidiabetic activity in comparison to active control, particularly glibenclamide the result showed that their potential was lesser but significant (** $p < 0.01$) than the standard drug at chronic level.

Yakubu and Ogunro (2014) revealed that the administration of alloxan significantly ($p < 0.05$) elevated the blood glucose level of the rats when compared with the non-diabetic rats treated with distilled water. The blood glucose level of the diabetic rats treated with distilled water went on increasing and by the 15th day of the experimental period, it was increased by 72.9%. The extract caused dose dependent significant ($p < 0.05$) reduction of blood glucose levels at the doses of 18, 36 and 72 mg/kg body weight. The reduction by the end of the exposure period did not compare well with the non-diabetic rats treated with distilled water. However, glibenclamide with the dose of 2.5 mg/kg body weight significantly ($p < 0.05$) reduced the blood glucose levels of the diabetic animals to values that compared well with non-diabetic distilled water treated control.

Muralidharan (2014) studied that the feeding with aqueous extract of leaves of *C. roseus* commonly called as periwinkle in *alloxan*- induced diabetic rats significantly ($P < 0.001$) decreased blood glucose levels and has brought down TC, LDL, VLDL and TG close to normal level. In control rats, fed with the experimental leaves did not show any hypoglycemia effect and no significant body weight changes were found indicating that *C. roseus* has anti- diabetic activity.

Aruljiothi and Samipillai (2016) presented the blood glucose and serum protein in the alloxan induced diabetic rats. The alloxan was induced to rats to analyse the blood glucose. The plant extract, *C. roseus* was treated to alloxan induced rats to reduce the blood glucose and the serum protein recovered the

normal level. The present study was concluded that the *C. roseus* reduce the blood glucose level in the rats.

2.8 Hematology in diabetes

Emmanuel et al. (2010) carried out the hematological studies to know the influence of the methanol fraction of *C. occidentalis* leaves on hemoglobin and hepatic glycogen in diabetic rats. They reported that the Glycosylated hemoglobin levels were significantly elevated and the total hemoglobin, hepatic glycogen were decreased in diabetic rats as compared with normal rats. The oral administration of methanol fraction of *C. occidentalis* leaves (COLMF) (200 mg/kg b wt.) maintained all these parameters to near normal status in diabetic rats. They also reported that the hyperglycemia has an important role in the pathogenesis of long-term complications and the excess glucose present in blood reacts with hemoglobin causing decrease in the total hemoglobin level.

Kemasari et al. (2011) stated that in the diabetic rats there is increased formation of glycosylated hemoglobin. Therefore, in the diabetic subjects the total hemoglobin levels are much lower than the normal level.

Akter et al. (2014) carried out the hematological studies in the alloxan induced diabetic mice and found that the total erythrocyte count and hemoglobin content were significantly ($P < 0.001$) reduced as compared to control group. However, by treatment with neem and spirulina both total erythrocyte count and hemoglobin were significantly ($P < 0.001$) increased than diabetic control mice.

2.9 Histopathological alterations in diabetes

Puranik et al. (2010) conducted the histological examination and found that the endocrine pancreas in *Tinospora cordifolia* (Willd.) treated diabetic rats did not reveal any evidence of regeneration of beta cells of islets of Langerhans. The histology section of the pancreas of the drug treated control rats showed the normal architecture of the islets of Langerhans with the granulated beta cells appearing dark. The histology of the pancreas in diabetic rats showed small and shrunken islets of Langerhans. However, the destruction of beta cells was observed in this section. The histology of the pancreas in *Tinospora cordifolia* (Willd.) treated diabetic rats showed a similar architecture as that of diabetic rats.

There was no considerable change in the architecture of the islets of Langerhans after the *Tinospora cordifolia* (Willd.) treatment. They also observed that there was no regeneration of beta cells after the *Tinospora cordifolia* (Willd.) treatment, indicating that the antihyperglycemic activity of *Tinospora cordifolia* (Willd.) is independent of insulin secretion by pancreatic beta cells.

Emmanuel et al. (2010) carried out the histological studies and found that the control rat showed normal architecture with islet of Langerhans which are formed of numerous compactly arranged cells, whereas the diabetic rat showed pyknotic nuclei and dark nuclei, and few cells at the periphery had round or ovoid nuclei. However, the section of rat treated with methanol fraction of *C. occidentalis* leaves extract 100mg/kg b wt. showed shrunken nuclei and mild inflammatory cells, the rats treated with methanol fraction of *C. occidentalis* leaves 200 mg/kg b wt showed normal architecture with shrunken nuclei. Section of rat treated with Glibenclamide showed islet cells with vascular nuclei and pyknotic nuclei.

Mohammed et al. (2011) studied histopathology of pancreas and showed damage of islets of Langerhans in diabetic rats when compared to normal but shows healing of pancreas in diabetic rats treated with whole plant extract of *C. roseus* and standard glibenclamide drug (5mg/kg body wt.).

Kumar et al. (1993) on photo micrographic studies found that the liver showed normal hepatic cells with well-preserved cytoplasm, nucleus, nucleolus and central vein in normal rats. In case of diabetic rats, the normal lobular structure was preserved and the central vein was prominently congested, focal areas of hemorrhage a fatty changes and normal portal tract were evident. As were in diabetic rats treated with *Dillenia indica* methanolic leaves extract (DIME) @500 mg/kg, the hepatocytes, portal tracts and central veins appeared normal. The histology of pancreas of normal rats showed normal acini and normal cellular population in the islets of Langerhans of the pancreas. However, in the diabetic animals treated with the DIME it was observed that there was extensive damage to islets of Langerhans and reduction in the dimensions of islets were observed in diabetic rats which were restored toward normal cellular population size of islets by DIME 500 mg/kg treatment. Histology of kidney in normal animals showed normal structure. In diabetic rats, mild thickening of the basement

membrane of the arterioles of glomeruli along with mild change of density of mesangial mesangium were observed. No other significant changes were seen. After DIME 500 mg/kg treatment, these changes were improved towards normal condition.

Samad et al. (2013) performed the histopathology of islets of Langerhans and revealed that size of Langerhans islets in diabetic control group had a significant difference in comparison with all treatment groups ($P < 0.05$). There was no significant difference in size of Langerhans islets between the Streptozotocin and normal saline treated rats and alone Streptozotocin treated rats. They also studied the effect of hydroalcoholic extract of *N. sativa* on the number of islets and the number of islet cells and islets diameter (μm) in pancreatic tissues of the normal and diabetic rats. The light microscopy evaluation of the pancreas of control rats showed normal pancreatic islet structure. However, the pancreas from untreated diabetic rats disclosed that the islets were comparatively small, shrunken, degenerated (hydropic degeneration) and necrotized

Kamble and Bodhankar (2013) reported that after eight weeks of study examination of renal sections stained with hematoxylin and eosin (H&E) and Masson's trichome (MT), did not show changes in the architecture of the renal tissue of non-diabetic rats. While, the renal tissue sections of diabetic animals showed severe glomerular injury, tubular swelling. The MT stained sections of kidney of the diabetic animals showed glomerular fibrosis and peritubular fibrosis. In contrast kidney sections of trigonelline (TRIG) treated animals showed moderate injury, tubular swelling with H&E stained. The MT stained renal tissue of TRIG treated animals showed mild glomerular fibrosis and moderate peritubular fibrosis. The H&E and MT stained renal tissue of sitagliptin (SITA) treated animals showed mild glomerular necrosis, moderate tubular swelling and moderate glomerular fibrosis and mild peritubular fibrosis. The treatment with (TRIG + SITA) showed mild glomerular fibrosis, absence of tubular swelling with peritubular fibrosis.

Madan et al. (2014) observed the islet of Langerhans in the control group, where in a large number of β cells distributed throughout the islet of Langerhans. As Where in the diabetic group, a decrease in the number of β cells

of the islets of Langerhans was observed in comparison to the control group. It was observed that the few functional β cells and α cells were more prominent in the islets of Langerhans from the diabetic groups treated with Diabecon, Madhurakshak (Mdr), (a polyherbal formulation containing extracts from twelve different herbs viz., *Pterocarpus marsupium*, *Cinnamomum tamala*, *Eugenia jambolana*, *Gymnema sylvestre*, *Piper nigrum*, *Azadiracheta indica*, *Trigonella foenumgraecum*, *Momordica charantia*, *Phyllanthus emblica*, *Terminalia chebula*, *Terminalia bellerica* and shudh shilajit) Mdr Concentrate and Mdr Powder at concentrations of 250 mg/kg and 600 mg/kg body weight, respectively. The damaged β cells seen after the initial induction of diabetes were no longer observed after treatment with Diabecon and Mdr. The recovery of necrotic β cells was especially more pronounced after treatment with 600 mg/kg of Mdr Concentrate when compared to Mdr powder.

Latha et al. (2014) conducted the histopathological studies of liver and kidney in normal, diabetic and diabetes treated with the *C. fimbriata* groups. They observed that the normal histological liver section shows the well-arranged cells and clear central vein. In the diabetic group, it was found that there was the complete destruction of hepatocytes, degeneration of central vein, fatty degeneration and various size vacuoles. However, it was observed that the histopathological changes are restored near to normal in the *C. fimbriata* treated group. The normal kidney section shows the well-arranged cells and compact glomerular basement membrane. While, the diabetic groups showed the endocytic vacuoles in the proximal tubules and the thickening of the glomerular basement with glomerulosclerosis. However, these histopathological changes were restored to normal by the treatment with *Caralluma fimbriata*.

Rajesh et al. (2014) conducted the histopathology of kidney, liver and pancreas in normal, diabetic and diabetes treated with methanol extracts of *Aerva lanata* (Linn.) Juss. (MEAL) and aqueous extracts of *Aerva lanata* (Linn.) Juss. (AEAL) groups. They observed that the normal kidney section shows the well-arranged cells and the compact glomerular basement membrane. While, the diabetic groups showed that the endocytic vacuoles are characteristically seen in the proximal tubules and there was the thickening of the glomerular basement with glomerulosclerosis. The damage was recovered with the treatment of MEAL and AEAL at the dose of 400 mg/kg. The normal histological liver section

showed the well-arranged cells and clear central vein. However, the diabetic group showed the complete destruction of hepatocytes, degeneration of central vein, fatty degeneration and various size vacuoles. These histopathological changes are restored near to normal after treatment with higher dose of MEAL and AEAL. The histopathological examination of hematoxylin and eosin stained sections of kidney, liver and pancreas of all the rats from vehicle control group revealed normal histological pattern. The diabetic control rats administered with Streptozotocin 60 mg/kg bd wt, revealed multifocal hepatocellular hypertrophy with intracytoplasmic micro vesicular steatosis, bridging coagulative necrosis of centrilobular hepatocytes characterized by cytoplasmic eosinophilia, karyolysis and mononuclear cell infiltration in the liver, atrophy of 50 – 60 % of islets of Langerhans accompanied by marked vacuolar degeneration of islet cells and mild to moderate multifocal mononuclear cell infiltration in the pancreas

Khan (2015) revealed histological examination of *Catharanthus roseus* methanolic flower extracts in alloxan induced diabetic rats and showed extensive damage to the islets of Langerhans and reduced dimensions of islets in animals treated with alloxan only whereas partial restoration of normal cellular population and enlarged size of β -cells with hyperplasia was observed by methanolic extracts. Restoration of normal cellular population size of islets with hyperplasia by glibenclamide was also observed.

2.10 Antidiabetic activity of the metformin

Tucker et al. (1981) reported the optimal oral metformin dose for many diabetic patients as 2 g/day. After a single oral dose, metformin is rapidly distributed to many tissues following partial absorption by the small intestine, but the luminal concentration in the gastrointestinal tract remains high. The peak plasma concentration occurs in 3 hr. (increasing from 1.0 to 1.6 mg/ml [about 6 to 10 mM] after a 0.5 g dose and to 3 mg/ml [about 18 mM] after a 1.5 g dose) with a mean plasma half-life of about 20 hr.).

Amitra (1990) stated that the Metformin was a hypoglycemic drug effective in the treatment of non-insulin-dependent diabetes mellitus and increasingly used in Canada and Europe. Effects on intestinal glucose absorption, insulin secretion, and hepatic glucose production are insufficient to explain its hypoglycemic action, with most evidence suggesting that the major

effect of the drug is on glucose utilization. In vivo and in vitro studies have demonstrated that metformin stimulates the insulin induced component of glucose uptake into skeletal muscle and adipocytes in both diabetic individuals and animal models. This increase is more significant in diabetic than in nondiabetic animals, suggesting an enhanced action of the drug in the hyperglycemic state. The increase in glucose uptake is also reflected in an increase in the insulin-dependent portion of glucose oxidation. Potential sites of action of metformin are the insulin receptor and the glucose transporters. Although metformin increases insulin binding in various cell types, this effect is not universal and does not correlate with stimulation of glucose utilization. In contrast, direct effects of the drug on the glucose transport system have been demonstrated. Metformin elevates the uptake of nonmetabolizable analogues of glucose in both nondiabetic rat adipocytes and diabetic mouse muscle. In the latter, the stimulatory effect of the drug is additive to that of insulin. In human and rat muscle cells in culture, metformin increases glucose-analogue transport independently of and additive to insulin, suggesting an insulin-independent action. Most of these results suggest that the basis for the hypoglycemic effect of this biguanide is probably at the level of skeletal muscle by increasing glucose transport across the cell membrane

Wilcock and Bailey (1994) studied bio distribution in mice using ¹⁴C-labeled metformin and showed accumulation mainly in the gastrointestinal tract, kidney, and liver.

Costa et al. (2008) reported that the biguanide metformin is an antihyperglycemic agent used in the treatment of type-2 diabetics. However, the mechanism of action of metformin is still unclear. Studies have shown that metformin activates AMP activated protein kinase (AMPK) in liver cells and skeletal muscle of rats. Recent studies indicate that AMPK plays a role in the regulation of glucose and lipid metabolism. The AMPK is an enzyme that promotes energy availability and is activated by skeletal muscle contraction and during myocardial ischemia; it is involved in the stimulation of glucose transport and fatty acid oxidation.

Choi et al. (2008) mentioned that the metformin, a biguanide antihyperglycemic agent, is widely used in the management of type 2 diabetes

mellitus. It lowers the blood glucose concentration without causing hypoglycemia. They observed that after intravenous (at doses of 0.25–1.0 g) and oral (at doses of 0.5–1.5 g) administration of metformin to four healthy volunteers, the terminal half-lives of the drug were 1.52–4.50 h, 78.9–99.9% of the dose were excreted in the urine via active renal tubular secretion. Further they found that the absorption of the drug was not complete (20–30% of the oral dose were recovered from the feces) possibly due to an active, saturable absorption process, and the extent of absolute oral bioavailability of the drug was 33–55%. The Metformin does not bind to human plasma proteins.

Arunachalam et al. (2012) observed the changes in blood glucose level of diabetic rats treated with metformin and the diabetic untreated rats. The treatment of diabetic rats with the Metformin HCL floating tablet formulation (FM3), Metformin Hcl (100 mg/kg) and Metformin Hcl SR (100 mg/kg) significantly decreased the blood glucose level compared to untreated diabetic rats. The treatment of diabetic rats with the FM3 significantly reduced the blood glucose levels compare to the Metformin Hcl and Metformin Hcl SR treated rats. The islets of alloxan induced diabetic rats showed extensive necrotic changes followed by fibrosis and atrophy. The Alloxan diabetic rats treated with Metformin Hcl minimum degree of necrotic and fibrotic changes of islets of Langerhans. The necrotic and fibrotic changes were not detected in the rats were treated with FM3 and Metformin Hcl SR.

Saleh et al. (2013) observed that a single i.p. injection of Streptozotocin (55 mg/kg) produced an elevation of plasma glucose level which was evidenced 48 h after administration. The elevation was found to be persistent during the period of investigation and reached the average value of 376% of the normal one, 16 days after Streptozotocin administration. The oral treatment of hyperglycemic rats with antidiabetic drugs either Rosiglitazone (0.5 mg/kg/day) or Metformin (150 mg/kg/day) for 2 weeks succeeded to cause a decrease in the elevated plasma glucose level reaching nearly the normal values.

Rena et al. (2013) reported that according to national and international guidelines, metformin is the recommended first-line oral therapy for the treatment of type 2 diabetes. This is down to several factors, including the impressive safety record of the drug, having been in clinical use for over 50 years and the

fact that metformin treatment is weight neutral. In addition, there are likely to be other beneficial effects, including a reduction in cardiovascular disease and mortality compared with non-intensive treatment and a possible reduction in cancer incidence, which has been seen in some, but not all, studies. As metformin was discovered in the era before modern target-based drug discovery, the molecular details of its mechanism of action were not established before it was used clinically and these continue to be an area of vigorous research. Chemically, biguanides such as metformin are composed of two guanidine groups joined together with the loss of ammonia. Anti-hyperglycemic effects have been observed in response to many, but not all, guanidine-containing compounds. Whilst this in itself is of considerable biological value, it also starts to pave the way towards a more targeted approach to therapy and novel drug development. The most widely accepted model of the antihyperglycemic action of metformin is that suppression of hepatic gluconeogenesis occurs principally as a consequence of mitochondrial inhibition. The pharmacokinetics of metformin, which is predominantly targeted to the liver, might help to account for this to some extent.

Mohanraghupathy *et al.* (2013) conducted the studies to understand the effect of glipizide and metformin combination on blood glucose levels of diabetic rats. They found that the combination of glipizide and metformin can effectively lower the blood glucose levels as compared to the alone glipizide.

Velmani *et al.* (2013) studied the influence of the metformin in the diabetic rats. They found that the rats treated with Streptozotocin (STZ) -induced diabetes and had fasted exhibited a significant elevation in serum glucose levels after 48 hours. The administrations of metformin (250 mg/kg body weight) caused significant reductions ($P < 0.001$) in the serum glucose levels during the 21-day treatment. The rats treated with standard metformin showed improved anti-hyperglycemic activity. The serum total protein concentrations of STZ-induced diabetic rats were lowered as a result of the diabetic state of the experimental rats. However, gradual increases in serum protein were observed during the 21-day test for the rats administered metformin. The analyses of the biochemical parameter revealed an elevation of the serum marker enzyme in the STZ-induced diabetic groups, indicating significant increases in SGPT levels. As did the metformin-treated group significant decreases ($P < 0.001$) in the SGPT levels

to near pre-diabetic levels were observed. The rats treated with metformin showed no hepatic abnormalities, and the arrangements of the hepatocytes in the liver were almost normal. Administrations of metformin to diabetic rats protected the majority of the organs.

American Diabetes Association (2014) reported that metformin had been an important drug for treatment of type 2 diabetes (T2D) for decades. It is the most widely used oral antihyperglycemic agent and is currently recommended as first line therapy for all newly diagnosed T2D patients.

Rena *et al.* (2017) reported metformin a widely-used drug that results in clear benefits in relation to glucose metabolism and diabetes-related complications. The mechanisms underlying these benefits are complex and still not fully understood. Physiologically, metformin has been shown to reduce hepatic glucose production, yet not all of its effects can be explained by this mechanism and there is increasing evidence of a key role for the gut. At the molecular level the findings vary depending on the doses of metformin used and duration of treatment, with clear differences between acute and chronic administration. Metformin has been shown to act via both AMP activated protein kinase (AMPK)-dependent and AMPK independent mechanisms; by inhibition of mitochondrial respiration but also perhaps by inhibition of mitochondrial glycerophosphate dehydrogenase, and a mechanism involving the lysosome. In the last 10 years, we have moved from a simple picture, that metformin improves glycaemia by acting on the liver via AMPK activation, to a much more complex picture reflecting its multiple modes of action. More work is required to truly understand how this drug works in its target population: individuals with type 2 diabetes.

Bailey (2017) reviewed that the metformin (dimethylbiguanide) had become the preferred first-line oral blood glucose-lowering agent to manage type 2 diabetes. Its history linked to plant *Galega officinalis* (also known as goat's rue), a traditional herbal medicine in Europe, found to be rich in guanidine, which, in 1918, was shown to lower blood glucose. Guanidine derivatives, including metformin, were synthesized and some (not metformin) were used to treat diabetes in the 1920s and 1930s but were discontinued due to toxicity and the increased availability of insulin. Metformin was rediscovered in the search for

antimalarial agents in the 1940s and, during clinical tests, proved useful to treat influenza when it sometimes lowered blood glucose. This property was pursued by the French physician Jean Sterne, who first reported the use of metformin to treat diabetes in 1957. However, metformin received limited attention as it was less potent than other glucose-lowering biguanides (phenformin and buformin), which were generally discontinued in the late 1970s due to high risk of lactic acidosis. Metformin's future was precarious, its reputation tarnished by association with other biguanides despite evident differences. The ability of metformin to counter insulin resistance and address adult-onset hyperglycemia without weight gain or increased risk of hypoglycemia gradually gathered credence in Europe, and after intensive scrutiny metformin was introduced into the USA in 1995. Long-term cardiovascular benefits of metformin were identified by the UK Prospective Diabetes Study (UKPDS) in 1998, providing a new rationale to adopt metformin as initial therapy to manage hyperglycemia in type 2 diabetes. Sixty years after its introduction in diabetes treatment, metformin has become the most prescribed glucose-lowering medicine worldwide with the potential for further therapeutic applications.

2.11 Phytochemical studies of antidiabetic plants

Hatapakki et al. (2005) revealed from the phytochemical investigation that the major chemical constituents of the ethanol extract of *Cassia auriculata* were sterols, flavonoids, triterpenoid and tannins. The plants containing sterols are reported to possess antidiabetic activity. The presence of sterol; β Sitosterol in ethanol extract could be attributed for the observed antidiabetic activity.

Zulfiker et al. (2010) conducted the phytochemical analysis of the ethanolic extract of *Scoparia dulcis*. These studies revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids and tannins in the plant extracts. On the basis of these findings opined that the ethanolic extract of *Scoparia dulcis* may also have brought about antihyperglycemic action through stimulation of β -cells of islets of Langerhans to release more insulin and this effect may be due to its constituents like saponins, flavonoids and glycosides. Since, the flavonoids and terpenes isolated from the other antidiabetic medicinal plants have been found to stimulate secretion or possess an insulin like-effect they postulated the extract bears insulin like action.

Aswar and Kuchekar (2011) conducted the phytochemical screening of *Caesalpinia bonducella* (Roxb.) seeds hydro-alcoholic extract. They revealed the presence of carbohydrates, proteins, amino acids, glycosides, flavonoids, saponins, steroids and triterpenoids etc. in the hydro-alcoholic extract of *Caesalpinia bonducella* (Roxb.) seeds.

Siddaiah et al. (2011) performed the phytochemical analysis of the methanolic extract of the leaves of *Ximenia americana*. They reported that the major chemical constituents of the extract and some of this active principle including flavonoids are known to be used for the treatments of diabetes. Further they mentioned that it is possible that the presence of flavonoids and tannins are responsible for the antidiabetic activity.

Sharma and Kumar (2011) conducted the phytochemical studies of the fruit extract of *Rubus ellipticus* plant and found that the extract possess the flavonoids and tannins as the active principles. They claimed that the presence of the flavonoids and tannins in the extracts are responsible for the antidiabetic activity.

Mohammed et al. (2011) studied that the phytochemical screening of whole plant extract of *C. roseus*, revealed chemical analysis showed the presence of alkaloids and carbohydrate antidiabetic activities

Kumavat et al. (2012) performed the preliminary phytochemical analysis of *Cassia javanica* leaves and revealed the presence of reducing sugars, proteins, alkaloids, tannins, glycosides, sterols, flavonoids, and saponins as the active principles. They stated that the antidiabetic activity of the plant is due to these active principles.

Luka and Mohammed (2012) screened leaf extract of the plant *Mangifera indica* and revealed that the alkaloids, tannins, saponins, cardiac glycosides, resins, sterols are present in the extract. They claimed that the antidiabetic properties of the plant are due the presence of the said active principles.

Elisa et al. (2012) revealed that the qualitative tests used to identify phytochemical constituents on organic and aqueous extracts from the different

parts of the *C. roseus* plant. Alkaloids were detected in the dichloromethane extracts as well as the methanol extracts from flowers, leaves, stems, and roots. The aqueous root extract was also positive to alkaloid test. Terpenoids/sterols were detected in the hexane extracts from flower, leaf, and stem of *C. roseus*. They were also detected in dichloromethane extracts from leaf, stem, and root. The methanol extracts from leaf and root were positive to terpenoids/sterols test. The polyphenolic tests were positive in the methanol extract from flower, leaf, stem, and root. In the aqueous extracts from flower, leaf, and stem polyphenolic compounds were detected. Flavonoids compounds were detected in the methanol extracts from flower, leaf, stem, and root. Glycosides were detected in the methanol extracts from leaf and stem as well as in the aqueous extracts from flower and leaf. Saponins were not detected in any extracts

Rajan et al. (2013) obtained the different whole plant extracts of the plant *Blepharis repens* using Soxhlet apparatus. The percentage yields were 1.24% in petroleum ether, 1.11% in chloroform, 2.1% in ethyl acetate and 5.3% in ethanol and 4.5%w/w. They conducted the phytochemical screening of all the extracts of *Blepharis repens (vahl) Roth* and found that all the extracts contains carbohydrates, alkaloids, phytosteroids, flavonoids, tannins, saponins, fixed oils as the active principles.

Yadav et al. (2013) reported that the phytochemical evaluation of *C. roseus*. The Phytochemical Screening revealed alkaloids, saponins, flavanoids, carbohydrates and anthraquinone glycosides were present in *Catharanthus roseus*.

Rathor et al. (2013) investigated the hydroalcoholic and chloroform extract of *Ecbolium Ligustrinum* for the presence of the active principles. They found that various chemical constituents mainly tannins, flavanoids, glycosides, proteins are present in hydroalcoholic extracts, whereas alkaloids, sterols, proteins, glycosides and flavanoids are present in the chloroform extract. They stated that the proteins, glycosides and flavanoids isolated from the hydroalcoholic extract are responsible for antidiabetic and antioxidant properties.

Hridi et al. (2013) performed the qualitative phytochemical analysis of *H. benghalensis* stem extract and found that the extract contains tannins, saponins, flavonoids, carbohydrates and gums, reducing sugars, alkaloids, and

terpenoids. They reported that the flavonoids and tannins in the extract are having the anti-diabetic properties.

Wagay et al. (2013) observed that the antimicrobial activity and phytochemical analysis of Acetone extract of *C. roseus* whole plant against the wound isolates. Two different solvents such as ethanol and methanol were used to extract the bioactive compounds from the whole plant of *C. roseus* and screened for their antimicrobial activity against the isolated wound pathogens under well diffusion method. The maximum antibacterial activity was observed in crude Ethanolic extract of *C. roseus* against *Pseudomonas aeruginosa*. Qualitative analysis of phytochemical screening reveals the presence of Flavonoids, Tannin, Alkaloids and Terpenoids.

Natava et al. (2014) performed the phytochemical screening of the various extracts of *H. zeylanicum* and revealed the presence of various components in the extract such as steroids, triterpenes, saponins, alkaloids, carbohydrates, flavonoids, tannins and glycosides. Among them flavonoids and tannins were the most prominent ones.

Rajesh et al. (2014) carried out the phytochemical analysis of *Aerva lanata* (Linn.) Juss and observed that chemical constituents like alkaloid, terpenoids, steroids, tannins and phenolic are present in the plant. They also mentioned that these active principles bear the antidiabetic properties.

Shahin et al. (2014) studied that Qualitative analysis of *C. roseus* plant confirms the presence of various phytochemicals like alkaloids, flavonoids, terpenoids, saponins, steroids, carbohydrates, anthaquinone glycosides etc. in different extracts of its leaves and flowers. Some minerals have also been identified in the leaves and flowers of the plant by ICP-MS techniques.

Kabesh et al. (2015) investigated the phytochemical analysis of aqueous and methanol extracts of *Catharanthus roseus*. The enzymatic and non-enzymatic (DPPH) method was employed to analyze the antioxidant property. Qualitative analysis of phytochemical screening reveals the presence of Alkaloids, Phenol, Saponins and Protein. Further presence of phytochemicals were detected by Thin Layer Chromatography (TLC), which is the standard technique for separating organic compounds. The extracts were purified using

Column chromatography (silica gel). The fraction 3 was subjected to GC-MS analysis to the compounds presents in the extract.

Nayaka and Babu (2015) studied that the importance of feather compost as bio-fertilizer to increase the fertility of soil and growth of *C. roseus*. The screening of primary and secondary metabolites was carried out in the effect of feather compost on the growth of a *C. roseus* plant. The presence of feather compost has significantly increased primary and secondary metabolites as compared to control one. The crude ethanol extract of *C. roseus* plant leaves analyzed over TLC, shows prominent orange brown colour band indicating the presence of vincristine to that of standard vincristine sample. The compound was identified with alkaloid extraction of Et-OH in mass spectrum. Comparing the mass spectrum obtained with database the molecular formula C₄₆H₅₇N₄O₁₀ and molecular ion peak at 825 was found.

Amanda et al. (2016) reported the phytochemical analysis of *C. roseus* were positive for organic acids, reducing sugars, phenols and tannins, depsides and depsidones, steroids and triterpenoids, alkaloids and saponins.

Nisar et al. (2016) studied that the *C.roseus* plant used for different disease treatment such as skin cancer, Hodgkin's disease, breast cancer, and lymphoblastic leukemia. Conferring the comparative abundances of different phytochemicals, physiochemical and fluorescence analysis from *C. roseus*. Some of the chemical constituent's tests have been performed for phytochemical analysis. Fluorescence analysis was done by using dried powder mixed with different chemicals and have been observed under UV light (365 nm) and ordinary light. Total ash, acid insoluble ash, and moisture have been selected for physiochemical analysis. The precipitate formation or colour change was used as analytical answers for phytochemical analysis. The result of fluorescence analysis observed under UV light (365nm) and ordinary light by observing colour changes in sample. The percentage of loss of weight on drying, total ash and acid insoluble ash were obtained by employing standard methods of analysis for the physiochemical test. Extraction of *C. roseus* plant using deep eutectic solvent gave positive results.

MATERIALS AND METHODS

The research work was carried out at the Department of Pharmacology and Toxicology, Nagpur Veterinary College, Nagpur. The work was aimed to study the anti-diabetic activity of hydroethanolic leaf extract of *Catharanthus roseus* on streptozotocin induced-diabetes wistar rats. The parameters like hematological, biochemical and histopathology were studied. Further, qualitative analysis of phytochemical constituents in hydroethanolic leaf extract of *Catharanthus roseus* was studied. The study was carried out for the period of 28 days.

3.1 Experimental Animals:

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of social justice and empowerment, government of India. The proposed research work was conducted on 50 rats of wistar strain, which were procured from the CPCSEA recognized Laboratory Animal Breeding Center (National institute of Bioscience, Pune). Wistar rats with equal sex ratio weighing around 150-200 gm were used for the present study.

3.2 Housing:

All the rats were kept under standard managerial conditions as per the norms of CPCSEA. They were provided with cycle of 12 hr. light and 12 hr. dark period. All the animals were maintained in polypropylene cages, with rice husk as bedding material. In each cage, 3-4 rats were kept.

3.3 Feeding:

The animals were provided with *ad lib* balanced pelleted feed which was procured from National Institute of Bioscience, Pune and wholesome drinking water throughout the experiment.

3.4 Bedding Material:

Clean and autoclaved dried rice husk was used as a bedding material for the experimental animals. Bedding material was changed on every alternate day.

3.5 Herbal Medicine:

Hydroethanolic leaf extract of *C. roseus* was prepared and was used as herbal medicine.

3.5.1 Selection and Collection of Plant Material

The branches containing fresh green leaves were selected for experiment and procured from matured and naturally grown plants in the month of February. The selected plants was located in the premises of teaching veterinary clinical complex, Nagpur veterinary college, Nagpur.

3.5.2 Identification and authentication of plant

The collected plant material was identified by the Botany Department of Rashtrasant Tukdoji Maharaj University, Nagpur with the assistance of expert botanist. The plant was identified as *C. roseus* and herbarium sheet with appropriate information regarding identified plant species were filled up by botanist and given authentication number **10193**. One herbarium sheet was deposited in the respective department for future reference and records. The picture of herbarium sheet of twinge of *C. roseus* with identification details is shown in Plate1.

3.5.3 Processing and extraction of the plant material:-

The collected plant leaves were air dried under shed at normal room temperature. Dried leaves were subjected to grinding and converted into somewhat coarse powder form. The dried powder of matured leaves of *C. roseus* was processed for extraction by using soxhlet extraction method.

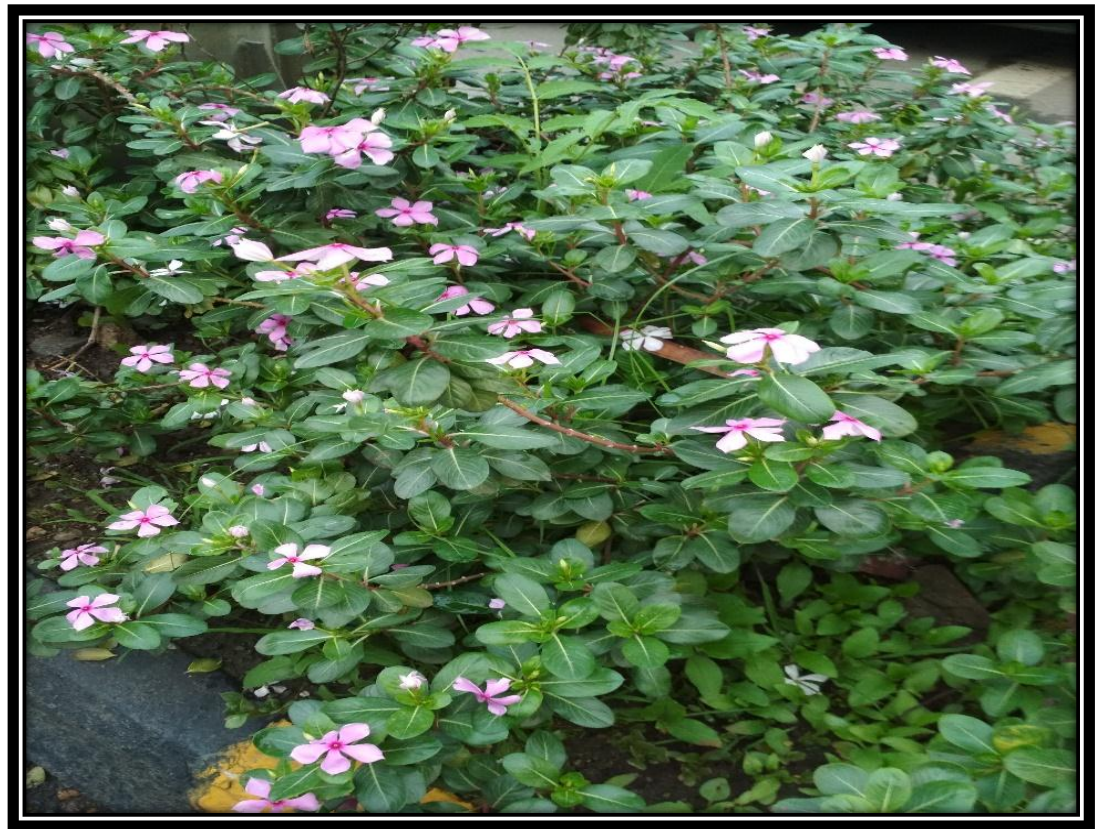


Plate I: Showing plant of *Catharanthus roseus*

Soxhlet Apparatus

The coarsely powdered leaves of *C. roseus* were used for extraction with 70% ethanol in soxhlet apparatus. The extract was allowed to evaporate on the water bath till it become semisolid and then dried in vacuum desiccator.

3.5.4 Phytochemical study for the qualitative analysis of certain active principles

The phytochemical study was undertaken for qualitative determination of various active constituents of hydroethanolic leaf extract of *C. roseus* as per the method described by **Rosenthaler (1930) and Raman (2006)**

A: Test for alkaloids

About 50 milligrams of extract was stirred with dilute hydrochloric acid and filtered. The filtrate was then subjected to following tests.

a) Mayer's test

Few 1-2 milliliters of filtrate were taken in a test tube and few drops of Mayer's reagent were added alongside wall of test tube. Formation of white or creamy precipitate indicates positive test.

Mayer's reagent: Mercuric chloride (1.358 grams) was dissolved in 60 milliliters of water and potassium iodide (5 grams) was dissolved in 10 milliliters of water. These two solutions were mixed and volume was made up to 100 milliliters with water.

b) Wagner's reagent: (Rosenthaler, 1930)

To the 1-2 milliliters of filtrate, Wagner's reagent was added. Appearance of brown flocculent precipitate indicates the presence of alkaloids.

Wagner's reagent: Iodine 1.27 grams and 2 grams of potassium iodide were dissolved in 5 milliliters distilled water and solution was further dissolved in water to make final volume 100 milliliters.

c) Hager's test

1-2 milliliters of filtrate were taken in a test tube and one or two milliliters of Hager's reagent were added. A prominent yellow color precipitate indicates positive test.

Hager's reagent: A saturated aqueous solution of picric acid was made

(d) Dragendroff's test

To 1-2 milliliters of filtrate, 1 or 2 millilitres of Dragendroff's reagent were added. A prominent yellow precipitate indicates positive test.

Dragendroff's reagent: It was prepared by mixing solution A (17 grams of Bismuth subnitrate + 200 grams of tartaric acid + 800 milliliters of distilled water) and solution B (160 grams of potassium iodide + 400 milliliters of distilled water) in 1:1 proportion (V/V).

B: Test for carbohydrates

Extracts (100 milligrams each) were dissolved individually in 5 milliliters of water and filtered. The filtrates were then subjected to following test.

(a) Fehling's test:

About 1 millilitre of filtrate was taken in a test tube and added with 1 millilitre of Fehling A and 1 millilitre of Fehling B solution and mixed well by shaking. The test tube was heated on water bath for 2 minutes. Appearance of red precipitate indicates positive test.

Fehling - A solution: Copper sulphate (34.66 grams) was dissolved in distilled water and volume made up to 500 milliliters.

Fehling - B solution: Potassium sodium tartarate (173 grams) and sodium hydroxide (50 grams) were dissolved in water and volume made up to 500 milliliters.

(b) Benedict's test

About 0.5 milliliter of filtrate was taken in a test tube and 0.5 milliliter of Benedict's reagent was added. The mixture was heated over boiling water bath for 2 minutes. A characteristic colored precipitate indicates test as positive.

Benedict's reagent: Sodium citrate (173 grams) and sodium carbonate (100 grams) were dissolved in 800 milliliters of distilled water and boiled to make it clear. Copper sulphate (17.3 grams) dissolved in 100 milliliters distilled water was added to it.

C: Test for glycosides

The extract (50 milligrams) was dissolved in concentrated hydrochloric acid for 2 hrs. on water bath, this hydrolysate was filtrated and filtrate was used for following test.

a) Legal's test

About 50 milligrams of extract was taken in a test tube and small amount of pyridine was added to it and mixed well. After that sodium nitroprusside followed by 10% sodium hydroxide was added. Development of pink color indicates positive test.

D: Test for saponins

The extract (50 milligrams) was taken in stoppered test tube and finally diluted up to 20 milliliters by adding distilled water. The tube was shaken for 15 minutes and observed for formation of foam. A two centimeter foam layer indicates positive test.

E: Test for amino acids

The extract (100milligrams) was dissolved in 10 milliliters of distilled water and filtered through Whatman filter paper no. 1 and filtrate was again used for following test

a) Ninhydrin Test

Two milliliter of filtrate was taken in test tube and few drops of ninhydrin solution were added. A characteristic purple color indicates positive test for presence of amino acids.

F: Test for proteins

a) Xanthoprotein Test (Rosenthaler, 1930)

50 milligram residue was taken in 2 milliliter of water and to it 0.5 milliliter concentrated nitric acid was added. The appearance of (white or yellow) precipitate indicate presence of proteins.

b) Biuret Test (Rosenthaler, 1930)

50 milligram of residue was taken in water and 1 ml of 1 % solution of sodium hydroxide was added followed by a drop of 1 % solution of copper sulphate. Violet – pink colour development indicates the presence of proteins.

G: Test for phytosterols

a) Salkowski's test (Rosenthaler, 1930)

50 milligrams of extract was taken in 2 milliliters of chloroform and sulphuric acid was added alongside of test tube and test tube was shaken. Red color development in the chloroform layer and greenish yellow fluorescence in the lower layer indicates presence of sterols.

H: Test for phenolic compounds

(a) Ferric chloride test

About 50 milligrams of extract were dissolved in 5 milliliters of distilled water and transferred to test tube and to this 5% neutral ferric chloride solution was added. Development of dark green color indicates presence of phenolic compounds.

(b) Lead acetate test

About 50 milligrams extract was dissolved in distilled water and 3

milliliters of 10% lead acetate solution were added. A bulky white precipitate indicates presence of phenolic compounds.

I: Test for flavonoids (Rosenthaler, 1930)

A 50 milligrams of residue was dissolved in 5 millilitres of ethanol (95%) and treated with a 3-5 drops of concentrated hydrochloric acid and 0.5 gram of magnesium metal turnings. Development of either pink or red color indicates presence of flavonoids

G: Test for resins (Rosenthaler, 1930)

The alcoholic extract was dissolved in alcohol. To this, a 3-5 drops of water were added. The appearance of turbidity was considered as a positive test.

K: Test for anthraquinones

Bontrager's Test

A 50 mg of the extract was boiled for a few minutes with 5 ml of 10% sulphuric acid and filtered immediately. The filtrate was cooled and shaken with benzene. The benzene layer was separated and shaken with half of its volume to 10% ammonia. The ammonical layer acquiring pink colour indicates the presence of anthraquinones.

3.5 Reference Drug: Metformin:

Metformin was used as the reference drug for evaluating the antidiabetic activity. Glyciphage tablets (250 mg), formulated by Franco Indian pharmaceutical Pvt. Ltd., 20, Dr. E. Moses Road, Mumbai- 400 011, were powdered and dissolved in distilled water.

The rats were allowed for acclimatization for 15 days to the environment, prior to the start of the experiment a separate identification mark was given to each rat by using picric acid. A total of fifty Wistar rats, used in this study were divided into five groups containing ten rats in each group having equal sex ratio.

3.7 Induction of Diabetes:

Streptozotocin (STZ) was dissolved in citrate buffer (pH - 4.5) and injected intraperitoneally @ 40 mg/kg body wt. to induce diabetes in rats. The animals were fasted for 16 hours prior to STZ injection, and after the injection 5% sucrose supplemented for 24 hours in order to prevent the animal from fatal hypoglycemia. One week after administration of STZ injection, blood glucose levels were checked using commercial digital glucometer. The animals with a blood glucose level of more than 300 mg/dl were considered diabetic and such animals were included in final study.

3.8 Design of experiment

Diabetics Rats were divided into 4 groups T₂, T₃, T₄ and T₅ each group comprising of 10 rats with equal sex ratio. Additional group T₁ comprising of 10 non-diabetic rats having equal sex ratio involved in the design as a normal control group. Group T₂ received streptozotocin @ 40 mg/kg body wt. i.p. Group T₃ received metformin @ 100 mg /kg body wt. referral standard drug and T₄& T₅ were treated with C.roseus @ 200, 400 mg/ kg body wt. respectively.

Table 1 Design of Experiment

Sr. No.	Group	No. of Rats	Treatment	Dose (mg/kg wt.) Route of administration	Duration of treatment
1.	T ₁	10	Non-diabetic control (Normal saline)	At equivalent dose volume of 1.0 ml p.o.	Daily for 28 days
2.	T ₂	10	Diabetic control (streptozotocin)		
3.	T ₃	10	Diabetes + Metformin (Std. drug control)	Metformin @ 100 mg/kg p.o.	Daily for 28 days

4.	T ₄	10	Diabetes + C. <i>roseus</i> leaves extract	<i>C. roseus</i> @ 200 mg/kg p. o.	Daily for 28 days
5.	T ₅	10	Diabetes + C. <i>roseus</i> leaves extract	<i>C. roseus</i> @ 400 mg/kg p.o.	Daily for 28 days

Glucose Estimation: The blood glucose levels was measured on 0th, 14th and 28th day by commercial available glucose estimation strip.

Body Weight:

Body weight of animal will be measured on 0th, 7th, 14th, 21th and 28th day of experiment.

3.9 Drug treatments

Group T₁ was fed with normal saline only. Group T₂ was given single dose streptozotocin @ 40mg/kg body weight, intraperitoneally, and no treatment was given .Group T₃ was treated with metformin @ 100 mg/kg body weight, during the period of experiment. The groups T₄ & T₅ were administered with hydroethanolic leaf extract of *Catharanthus roseus* @ 200 and 400 mg/kg body weight, which was dissolved in distilled water. In group T₃, T₄ & T₅ treatment was given orally in the volume of 1 ml by using gavage needle. The treatment was given for the period of 28 days consecutive days.

3.10: Collection of material for Hematological and Biochemical parameters

The blood was collected on 0th, 14th and 28th days of the experiment. Blood was collected by retro bulbar method in glass vials containing 1% ethylene diamine tetra acetic acid (EDTA) for hematological estimation and in clot activator tube for biochemical estimation. One week after administration of the streptozotocin to the rats from the treatment groups T₂, T₃, T₄, and T₅ blood collections was considered as the 0th day collection.

3.11 Biochemical Estimations

Blood samples were collected in non-heparinized tubes and were kept undisturbed. The serum was separated and stored at -20°C for subsequent analysis. Biochemical estimations were done on the auto analyzer by using commercial reagent kits for,

- I) serum blood glucose
- II) serum total cholesterol
- III) serum total proteins
- IV) serum AST and ALT
- V) Blood Urea Nitrogen (BUN)

3.12 Hematology

The collected blood samples in 1% EDTA were subjected to hematological studies such as hemoglobin concentration (Hb), using Sahli's Method (acid hematin) and Packed Cell Volume (PCV) by micro tube method as mentioned by Benjamin (1965).

3.13 Histopathological Examination

On the last day of experiment i.e., on 28th day, after collection of blood the animals were sacrificed for histopathological examinations. The pancreas, liver and kidney of each rat were examined grossly. Thereafter, pancreas, liver and kidney tissues were removed for histological studies. The tissues were washed with normal saline and were fixed in 10% buffered formalin immediately upon removal. They were gradually dehydrated, embedded in paraffin, cut into 5 μm sections and stained with Hematoxylin and Eosin for histopathological examination according to standard procedure as described by Ross *et al.* (1989).

3.14 Statistical Analysis

All the values in the test are presented as Means \pm SEM. Statistical differences between the means of the various groups were evaluated using Anova two – factor with replication. A 'P' value of less than 5% was considered to be statistically significant ($P < 0.05$). The data generated were analyzed statistically by standard statistical procedure.

RESULTS AND DISCUSSION

The present study was undertaken to study the antidiabetic activity of hydroethanolic leaf extract of *Catharanthus roseus* on streptozotocin induced diabetes in Wistar rats at Department of Veterinary Pharmacology and Toxicology, Nagpur veterinary college, Nagpur during a period of 28 days.

The present study includes observations on hydroethanolic leaf extract of *C. roseus* on streptozotocin induced diabetes in Wistar rats. Group 'T₁' served as negative control and was treated with distilled water only @ 1.0 ml p.o. Group 'T₂' served, as positive control, which was injected with streptozotocin @40mg/kg bdwt, i/p. Group 'T₃' received Metformin @ 100 mg/kg bdwt as a referral standard drug and group T₄ and T₅ were treated with hydroethanolic leaf extract of *Catharanthus roseus* @ 200 and 400 mg/kg bdwt respectively. The efficacy of the extracts was determined by analyzing various serobiochemical, hematological and histopathological parameters. The results of treatment groups are compared with streptozotocin induced diabetic control group (T₂) and are presented discussed in this chapter.

4.1. Extractability percentage

The leaf powder of *C. roseus* was subjected to hydroethanolic extraction. The colour, consistency and extractability percentage of hydroethanolic extract are presented in Table 2. The extractability, consistency and colour were found to be 19 %, semisolid and dark olive green, respectively (Plate IV).

4.2. Phytochemistry

The results of qualitative phytochemical analysis of hydroethanolic leaf extract of *Catharanthus roseus* were presented in Table 3. The phytochemical analysis of hydroethanolic leaf extract revealed the presence of alkaloids, proteins, amino acids, saponins, phytosterols, phenolic compound, resins, tannins, glycosides.



Plate II: Showing dried leaves of plant *Catharanthus roseus*

4.3 Biochemical estimations:

The initial day for blood collection in case of rats from the Group T₁ was considered as the 0th day, while the day one week after the administration of streptozotocin for the rats from the treatment groups T₂, T₃, T₄ and T₅ was considered as the 0th day. Further blood samples were collected from the rats of all groups after 14th and 28th day's for the hematological, biochemical and blood glucose estimation.

The biochemical estimations like blood glucose by digital glucometer, serum blood glucose, serum total cholesterol, serum total protein and AST, ALT and BUN were conducted. The result obtained are presented in in Table 4 to 10. The mean biochemical values of the group T₁ was considered as the normal biochemical values for the rats from the rest of the groups T₂, T₃, T₄ and T₅. The results obtained are presented in table 2.

Table 2: Solvent used, Quantity, Colour, Consistency and Extractability Percentage for a hydroethanolic leaf extract of *C. roseus*.

Sr. No	Content	Hydroethanolic extract
1	Solvent used	Ethanol 70%
2	Quantity	50 gm
3	Colour	Dark olive green
4	Consistency	Semi-Solid
5	Extractability	19 %

Table 3: Qualitative phytochemical analysis of hydroethanolic leaf extracts of *C. roseus*.

Sr. No.	Active principle	Test applied	Observation	Result
1	Phytosterols	Salkowskistest	Development of red colour in chloroform layer	Present

2	Alkaloids	A. Dragondroffs reagent	Development of prominent yellow precipitate	Present
		B. Wagners reaction	Appearance of brown flocculent precipitation	Present
		C. Hager's test	Development of prominent yellow precipitate	Present
		D. Mayer's test	Formation of white or creamy precipitate	Present
3	Proteins and Amino acids	A. Xanthoprotein test	White precipitate was formed	Present
		B. Biuret test		Absent
		C. Ninhydrin test	Development of purple colour.	Present
4	Carbohydrate	A. Benedict's reagent		Absent
		B. Fehling's reagent		Absent
5	Glycosides	Legal's test	Development of pink colour	Present
6	Saponins	Foam test	Formation of foam layer	Present
7	Phenolic compounds	A. Lead acetate test	Formation of white precipitate	Present
		B. Ferric chloride test	Occurrence of green colouration of filtrate	Present
8	Flavonoids	Test for flavonoids		Absent
9	Resins	Test for resins	Turbidity was appeared	Present
10	Tannins	A. Lead acetate test	Formation of precipitate	Present
		B. Ferric chloride test	Occurrence of green colouration of filtrate	Present
11	Anthraquinones	Bontragers test		Absent



Plate III: Showing dried leaves powder of plant *Catharanthus roseus*

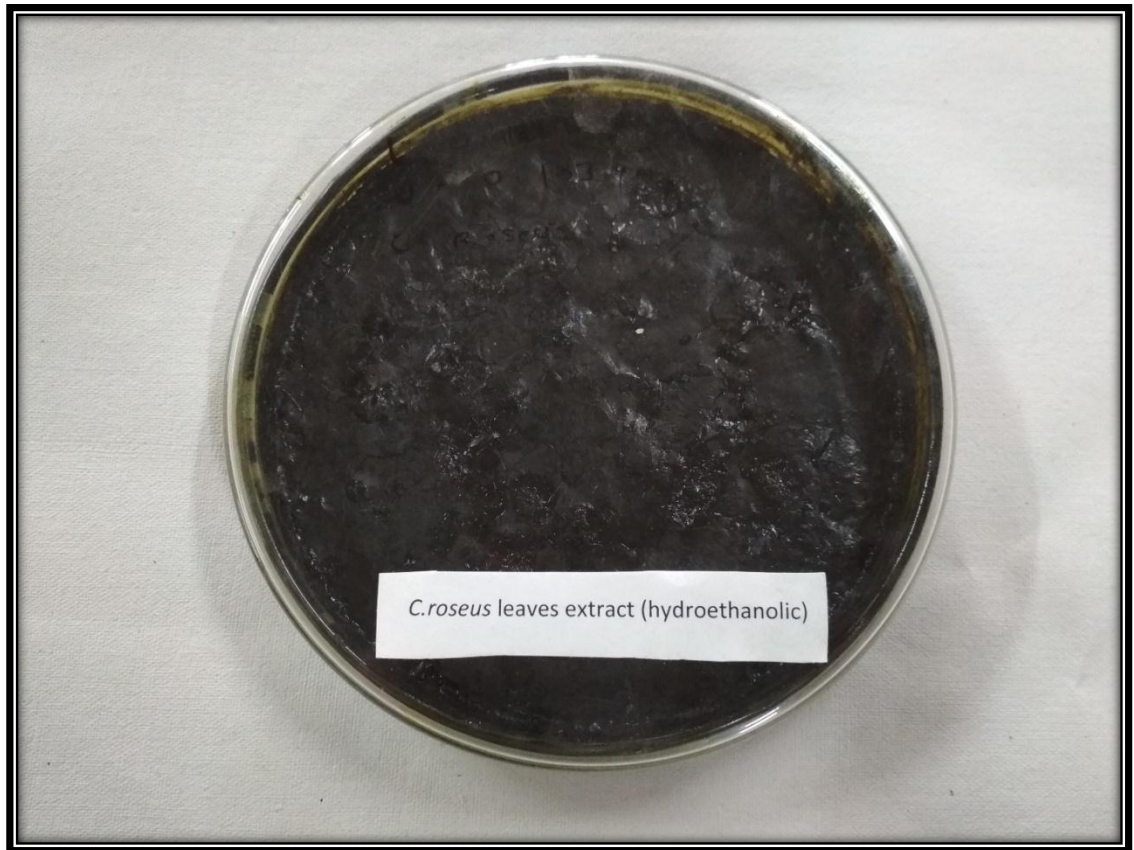


Plate IV: Showing hydroethanolic leaf extract of plant *Catharanthus roseus*

4.3.1 Blood glucose levels:

In the present study rat blood glucose levels were estimated by digital glucometer individually and also using biochemical kits supplied by M/S using auto analyzer model as early as possible after blood collections.

The blood glucose levels obtained by both the method were almost equal with minor variation and hence, the values obtained by using the digital glucometer were included the present study.

The table 4 and fig. 1 depicts the mean blood glucose levels on 0th, 14th and 28st day of the rats from the groups T₁, T₂, T₃, T₄ and T₅. The blood glucose levels of the group T₁ differ significantly with other group T₂, T₃, T₄ and T₅. The mean blood glucose values of groups T₁, T₂, T₃, T₄ and T₅ on 0th day were the blood glucose levels in 100.5 ± 3.65 , 320 ± 1.5 , 327 ± 1.66 , 325 ± 2.8 and 318.5 ± 1.82 mg/dl respectively. The values in group T₂ to T₅ did not differ significantly, it indicating development of diabetes in all the rats belonging to these groups.

The mean blood glucose values of groups T₁, T₂, T₃, T₄ and T₅ on 14th day were 99.45 ± 2.28 , 332 ± 2.1 , 225 ± 4.03 , 281 ± 2.22 and 220.64 ± 3.87 mg/dl respectively. This indicates that the standard reference antidiabetic drug metformin was found to be more effective when administered daily at the dose rate of 100mg/kg b. wt. than hydroethanolic leaf extract of *C. roseus* at the dose of 200 mg/kg b wt. However, at the dose rate of 400 mg / kg hydroethanolic leaf extract of *C. roseus* was found to be more effective than standard reference antidiabetic drug metformin. The blood glucose levels of all treatment groups when compared with each other reveals that the group T₅ shows good improvement in the blood glucose levels (220.64 ± 3.87 mg/dl) than the groups T₃ (225 ± 4.03 mg/dl) and T₄ (281 ± 2.22 mg/dl).

In comparison to the 14th day, the 28th day of treatment ,rats in different groups showed blood glucose level which were maintained in group T1, little increase in diabetic control (T2), significantly decreased in std. drug treated group (T3) and also significant decrease in *C.roseus* treated groups T4 & T5. The mean blood glucose values of groups T1, T2, T3, T4 and T5 on 28th day were 99.26 ± 1.05 , 348 ± 3.14 , 139 ± 1.33 , 166.58 ± 2.45 , and 137.49 ± 2.51 mg/dl respectively. From these findings it is observed that on 28st day there is no

significant variation in the blood glucose levels of T₃ (139 ± 1.33 mg/dl) and T₅ (137.49 ± 2.51mg/dl) and was significantly lower than the blood glucose of T₄ (166.58 ± 2.45 mg/dl). This is indicative that the treatment with treatment with leaf extract of *C. roseus* (400mg/kg) was as effective as metformin (100mg/kg) in controlling the blood glucose levels of the diabetic rats.

Streptozotocin is responsible for an autoimmune process that results in disruption of the langerhanes islet beta celled. Which results in the toxicity of beta celled with emergence of clinical diabetes within 2-7 day or even upto one week depending upon the dose of streptozotocin.

In the present study streptozotocin @ 40 mg/ kg body wt was used intraperitoneally in rats to produce diabetes. And rats showed increase in blood glucose levels clinically more than 300 mg/dl after a period of one week indicated development of clinical diabetes.

Balamruga *et al.* (2003) used STZ @ 40 mg/kg to produce diabetes in rats STZ @ 60 mg/ kg have been used by Akbarzadeh (2007) and Braslasu *et al.* (2007) whereas palanisamy *et al.* (2011) used STZ @ 45 mg / kg, Furman (2015) used at @ 65 mg /kg and saleh *et al.* (2013) used STZ @ 55 mg /kg to produce clinical diabetes in rats.

The treatment of diabetic rats with metformin @ 100 mg/ kg b wt. reduced the elevated blood glucose level indicating its hypoglycemia property. Choi *et al* (2008) used metformin in the rats @ dose rate of 100 mg / kg b.wt and Saleh *et.al* (2013) used @ dose rate of 150 mg/ kg for its hypoglycemic property. Metformin which is complex drugs having multiple sides of action and molecular mechanisms. Metformin acts directly or indirectly on liver to lower glucose production and acts on the guts to increase glucose utilization, increase GLP-1 and alter the microbiome. At molecular levels its inhibites the mitochondrial respiratory chain in liver leading to activation of AMPK, enhancing insulin activity and lowering CAMP. Thus, reducing the expression gluconeogenetic enzyme (Rena *et al.* 2017).

The rats treated with hydroethanolic leaf extract of *C. roseus* @ dose rate of 200 & 400 mg/kg b.wt cause significance decreased in blood glucose level. On 28th day in the present study *C. roseus* @ 400 mg/kg showed better results as

Table 4: Effect of hydroethanolic leaf extract of *C. roseus* on mean Blood glucose levels observed in different groups (mg/dl)

Sr. No.	Group	Treatment	0 th day	14 th day	28 th day
1	T ₁	Normal Control	^A 100.5 ± 3.65 ^a	^A 99.45 ± 2.28 ^a	^A 99.26 ± 1.05 ^a
2	T ₂	Diabetic Control	^B 320 ± 1.5 ^a	^E 332 ± 2.1 ^b	^D 348 ± 3.14 ^c
3	T ₃	Std. Drug control @100mg/kg bd. Wt.	^C 327 ± 1.66 ^c	^C 225 ± 4.03 ^b	^B 139 ± 1.33 ^a
4	T ₄	<i>C. roseus</i> @ 200 mg/kg bd. Wt.	^C 325 ± 2.8 ^c	^D 281 ± 2.22 ^b	^C 166.58 ± 2.45 ^a
5	T ₅	<i>C. roseus</i> @ 400 mg/kg bd. Wt.	^B 318.5 ± 1.82 ^c	^B 220.64 ± 3.87 ^b	^B 137.49 ± 2.51 ^a

Values are Mean ± SE for 6 rats in each group. Values not sharing common superscript differ significantly at p < 0.05.

CD Value for days= 7.319476

CD value for treatment=4.22

ANOVA: Mean Blood Glucose observed in different groups (mg/dl)

Source of variation	SS	DF	MS	F
Treatment	513846.9	4	128461.7	3171.872
Days	150654.2	2	75327.11	1859.916
Interaction	137131.6	8	17141.46	423.2429
Error	3037.521	75	40.50028	-
Total	804670.2	89	-	-

Fig 1: Mean Blood Glucose Levels by digital glucometer observed in different groups (mg/dl)

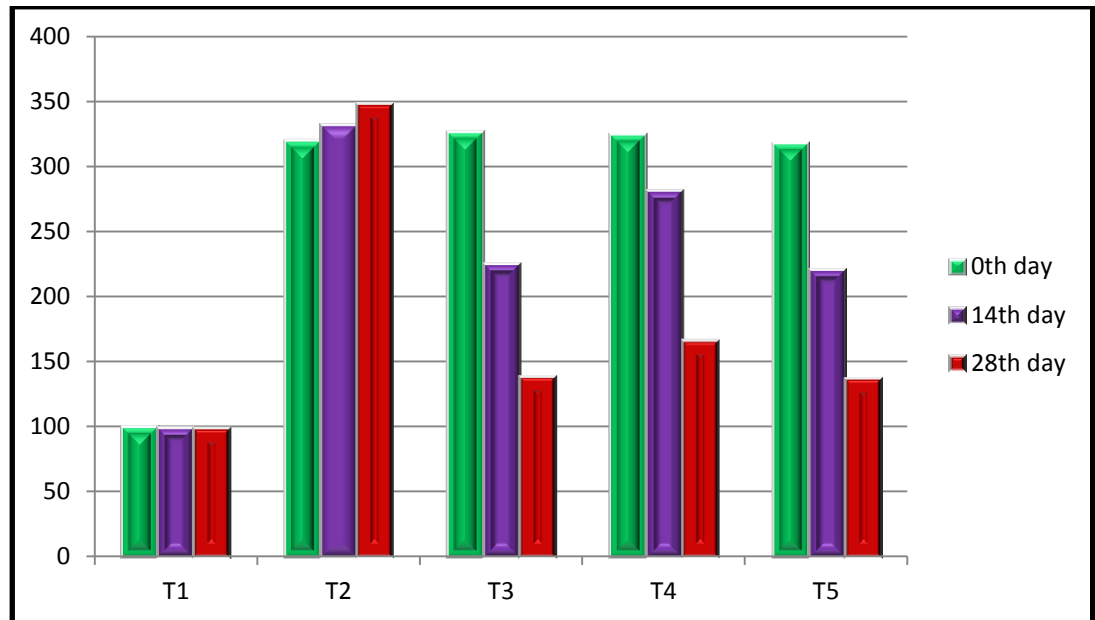
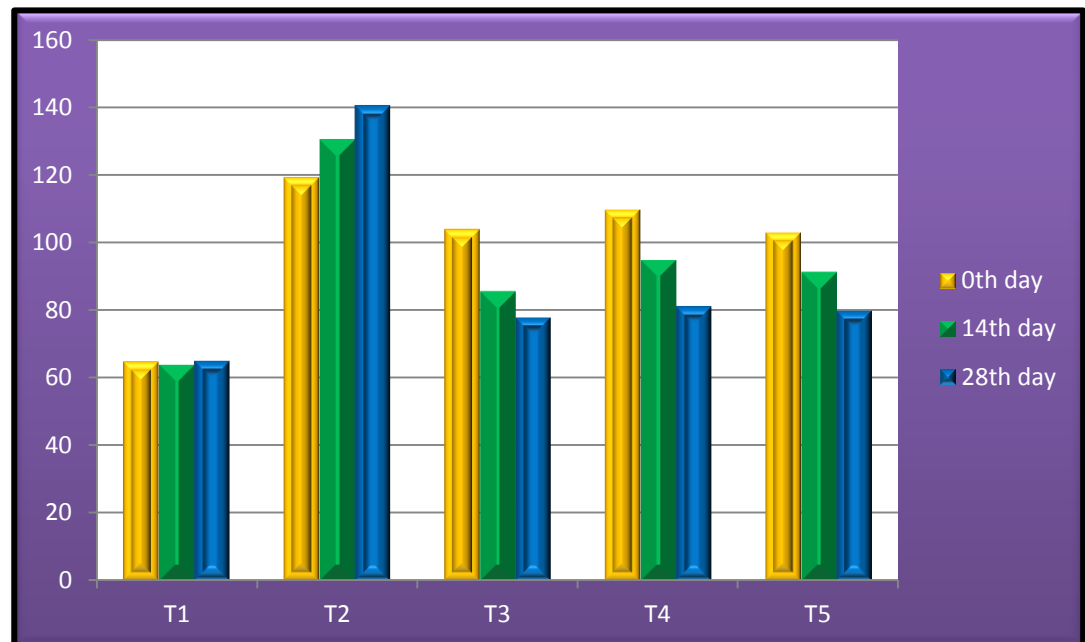


Fig 2: Mean Serum Total Cholesterol Levels observed in different groups (mg/dl)



treatment. Whereas, in all the treatment groups (T₁, T₂ & T₃) total cholesterol level decreased as depicted in the table 5.

The table 5 and fig. 3 shows the mean serum total cholesterol levels of different groups on 0th, 14th and 28st days of the experiment. The mean serum total cholesterol values of groups T₁, T₂, T₃, T₄ and T₅ on 0th day were 64.83 ± 0.73, 119.35 ± 1.45, 104 ± 1.56, 109.83 ± 1.44 and 103.03 ± 0.54 mg/dl respectively. There was significant difference in the mean serum total cholesterol levels of the group T₁ and other groups T₂, T₃, T₄ and T₅. The mean serum total cholesterol values of groups T₁, T₂, T₃, T₄ and T₅ on 14th day of the experiment were 63.87 ± 0.77, 130.55 ± 0.97, 85.65 ± 2.05, 94.94 ± 0.79 and 91.33 ± 0.66 mg/dl respectively. Total cholesterol levels reduced to the remarkable extent in metformin treated group followed by levels in *C. roseus* (400 mg/ kg) *C. roseus* (200 mg/kg) treated groups. The daily treatment of the metformin (100mg/kg) to the group T₃ declined the increased serum total cholesterol levels as compared to the daily treatment of the hydroethanolic leaf extract of *C. roseus* at the dose of 200 and 400 mg/kg to the groups T₄ and T₅ respectively.

The mean serum total cholesterol values of groups T₁, T₂, T₃, T₄ and T₅ on 28th day of the experiment were 65.05 ± 0.54, 140.51 ± 1.10, 77.86 ± 1.46, 81.17 ± 1.23 and 79.82 ± 2.31mg/dl respectively. The trend of declining the total cholesterol levels in all three treatments was maintained on 28 day of treatment, as on 14th day, indicating better effects by metformin treated groups followed by *C. roseus* 400 mg & 200 mg / kg. However, on 28th day of treatment rats in metformin and *C. roseus* (400 mg/ kg) treated groups did not show significance difference indicating competitions for reducing the total cholesterol levels. The treatment with metformin (100mg/kg) daily up to 28thday offered to group T₃ caused significant decrease in the serum total cholesterol levels when compared to the treatment of hydroethanolic leaf extract of *C. roseus* at the dose of 200 and non-significant decrease @ 400 mg/kg to the groups T₄ and T₅ respectively.

There was progressive rise in serum total cholesterol levels on 14th and 28th day of the diabetic rats from the group T₂ indicating the development of hypercholesterolemia. Whereas, in normal group there was no change in the total serum cholesterol levels. Similarly, Nabi *et al.* (2004) and John Kennedy *et al.*

(2012) reported that hypercholesterolemia occurs in streptozotocin induced diabetic rats. There is proportionate decrease in mean serum total cholesterol levels of the rats from group T₄ and T₅ which received the treatments with the hydroethanolic leaf extract of *C. roseus* at the dose of 200 and 400 mg/kg b wt respectively daily for 28 days. These findings are in accordance with the observations of Iweala and Okeke (2005), Jayanthi *et al.* (2010), Mohammed *et al.* (2011) recorded control of increasing cholesterol leads due to *C.roseus* in diabetic rats.

4.3.3 Serum total protein levels

The table 6 and fig 4 depicts the mean serum total protein levels of all the groups on 0th, 14th and 28th days of the experiment. The mean serum total protein values of groups T₁, T₂, T₃, T₄ and T₅ on 0th day were 7.53 ± 0.08, 6.12 ± 0.05, 5.86 ± 0.04, 6.30 ± 0.03 and 6.31 ± 0.03 g/dl respectively. There is significant difference in the serum total protein of the group T₁ and all treated groups at 5% level of significance. The groups T₂, T₃, T₄ and T₅ shows decline in the serum total protein levels than the normal level, after development of the diabetes in rats.

The mean serum total protein values of groups T₁ to T₅ on 14th day were 7.79 ± 0.05, 5.7 ± 0.03, 6.29 ± 0.04, 6.51 ± 0.01 and 6.51 ± 0.01 g/dl respectively. There is significant difference between all the groups except group T₄ and T₅ at 5% level of significance.

The serum total protein values of groups T₁ to T₅ on 28th day were 7.87 ± 0.10, 5.25 ± 0.09, 7.23 ± 0.04, 7.15 ± 0.01 and 7.52 ± 0.06 g/dl respectively. There is significant difference in the mean serum total protein levels of all the groups at 5% level of significance. The observations on 28th day revealed that the trend of the treatment effect was more in *C. roseus* treated group @ 400 mg followed by metformin & *C. roseus* treated group @ 200 mg /kg doses.

Reduction in plasma protein due to diabetes in rats may be due to microproteinuria & albuminuria which is an important clinical markers of diabetic nephropathy or it may also be due to increase protein catabolism lack of insulin also reduces RNA and mRNA which is another factor for the reduction of total proteins ,as quoted by kemasari *et al.* (2011).

treatment. Whereas, in all the treatment groups (T₁, T₂ & T₃) total cholesterol level decreased as depicted in the table 5.

The table 5 and fig. 3 shows the mean serum total cholesterol levels of different groups on 0th, 14th and 28st days of the experiment. The mean serum total cholesterol values of groups T₁, T₂, T₃, T₄ and T₅ on 0th day were 64.83 ± 0.73, 119.35 ± 1.45, 104 ± 1.56, 109.83 ± 1.44 and 103.03 ± 0.54 mg/dl respectively. There was significant difference in the mean serum total cholesterol levels of the group T₁ and other groups T₂, T₃, T₄ and T₅. The mean serum total cholesterol values of groups T₁, T₂, T₃, T₄ and T₅ on 14th day of the experiment were 63.87 ± 0.77, 130.55 ± 0.97, 85.65 ± 2.05, 94.94 ± 0.79 and 91.33 ± 0.66 mg/dl respectively. Total cholesterol levels reduced to the remarkable extent in metformin treated group followed by levels in *C. roseus* (400 mg/ kg) *C. roseus* (200 mg/kg) treated groups. The daily treatment of the metformin (100mg/kg) to the group T₃ declined the increased serum total cholesterol levels as compared to the daily treatment of the hydroethanolic leaf extract of *C. roseus* at the dose of 200 and 400 mg/kg to the groups T₄ and T₅ respectively.

The mean serum total cholesterol values of groups T₁, T₂, T₃, T₄ and T₅ on 28th day of the experiment were 65.05 ± 0.54, 140.51 ± 1.10, 77.86 ± 1.46, 81.17 ± 1.23 and 79.82 ± 2.31mg/dl respectively. The trend of declining the total cholesterol levels in all three treatments was maintained on 28 day of treatment, as on 14th day, indicating better effects by metformin treated groups followed by *C. roseus* 400 mg & 200 mg / kg. However, on 28th day of treatment rats in metformin and *C. roseus* (400 mg/ kg) treated groups did not show significance difference indicating competitions for reducing the total cholesterol levels. The treatment with metformin (100mg/kg) daily up to 28thday offered to group T₃ caused significant decrease in the serum total cholesterol levels when compared to the treatment of hydroethanolic leaf extract of *C. roseus* at the dose of 200 and non-significant decrease @ 400 mg/kg to the groups T₄ and T₅ respectively.

There was progressive rise in serum total cholesterol levels on 14th and 28th day of the diabetic rats from the group T₂ indicating the development of hypercholesterolemia. Whereas, in normal group there was no change in the total serum cholesterol levels. Similarly, Nabi *et al.* (2004) and John Kennedy *et al.*

Table 5: Effect of hydroethanolic leaf extract of *C. roseus* on mean Serum Total Cholesterol levels observed in different groups (mg/dl)

Sr. No.	Group	Treatment	0 th day	14 th day	28 th day
1	T ₁	Normal Control	^A 64.83 ± 0.73 ^a	^A 63.87 ± 0.77 ^a	^A 65.05 ± 0.54 ^a
2	T ₂	Diabetic Control	^D 119.35 ± 1.45 ^a	^E 130.55 ± 0.97 ^b	^D 140.51 ± 1.10 ^c
3	T ₃	Std. Drug control @100mg/kg bd. Wt.	^B 104 ± 1.56 ^c	^B 85.65 ± 2.05 ^b	^B 77.86 ± 1.46 ^a
4	T ₄	<i>C. roseus</i> @ 200 mg/kg bd. Wt.	^C 109.83 ± 1.44 ^c	^D 94.94 ± 0.79 ^b	^C 81.17 ± 1.23 ^a
5	T ₅	<i>C. roseus</i> @ 400 mg/kg bd. Wt.	^B 103.03 ± 0.54 ^c	^C 91.33 ± 0.66 ^b	^{BC} 79.82 ± 2.31 ^a

Values are Mean ± SE for 6 rats in each group. Values not sharing common superscript differ significantly at p < 0.05.

CD values for days = 3.62

CD values for treatment =2.09

ANOVA: Mean Serum Total Cholesterol levels observed in different groups (mg/dl)

Source of variation	SS	DF	MS	F
Treatment	39630.88	4	9907.72	997.1688
Days	1980.058	2	990.029	99.6421
Interaction	5672.196	8	709.0245	71.36023
Error	745.1887	75	9.93585	-
Total	48028.32	89	-	-

Table 6: Effect of hydroethanolic leaf extract of *C. roseus* on mean Serum Total Protein levels observed in different groups (g/dl)

Sr. No.	Group	Treatment	0 th day	14 th day	28 th day
1	T ₁	Normal Control	^D 7.53 ± 0.08 ^a	^D 7.79 ± 0.05 ^b	^D 7.87 ± 0.10 ^c
2	T ₂	Diabetic Control	^B 6.12 ± 0.05 ^c	^A 5.7 ± 0.03 ^b	^A 5.25 ± 0.09 ^a
3	T ₃	Std. Drug control @100mg/kg bd. Wt.	^A 5.86 ± 0.04 ^a	^B 6.29 ± 0.04 ^b	^B 7.23 ± 0.04 ^c
4	T ₄	<i>C. roseus</i> @ 200 mg/kg bd. Wt.	^C 6.30 ± 0.03 ^a	^C 6.51 ± 0.01 ^b	^B 7.15 ± 0.01 ^c
5	T ₅	<i>C. roseus</i> @ 400 mg/kg bd. Wt.	^C 6.31 ± 0.03 ^a	^C 6.51 ± 0.01 ^b	^C 7.52 ± 0.06 ^c

Values are Mean ± SE for 6 rats in each group. Values not sharing common superscript differ significantly at p < 0.05.

CD values for days=0.18

CD values for treatments = 0.09

ANOVA: Mean Serum Total Protein levels observed in different groups (g/dl)

Source of variation	SS	DF	MS	F
Treatment	38.55361	4	9.638402	376.1605
Days	5.492162	2	2.746081	107.172
Interaction	10.38023	8	1.297528	50.63898
Error	1.921733	75	0.025623	-
Total	56.34773	89	-	-

Fig 3: Mean Serum Total Protein Levels observed in different groups (g/dl)

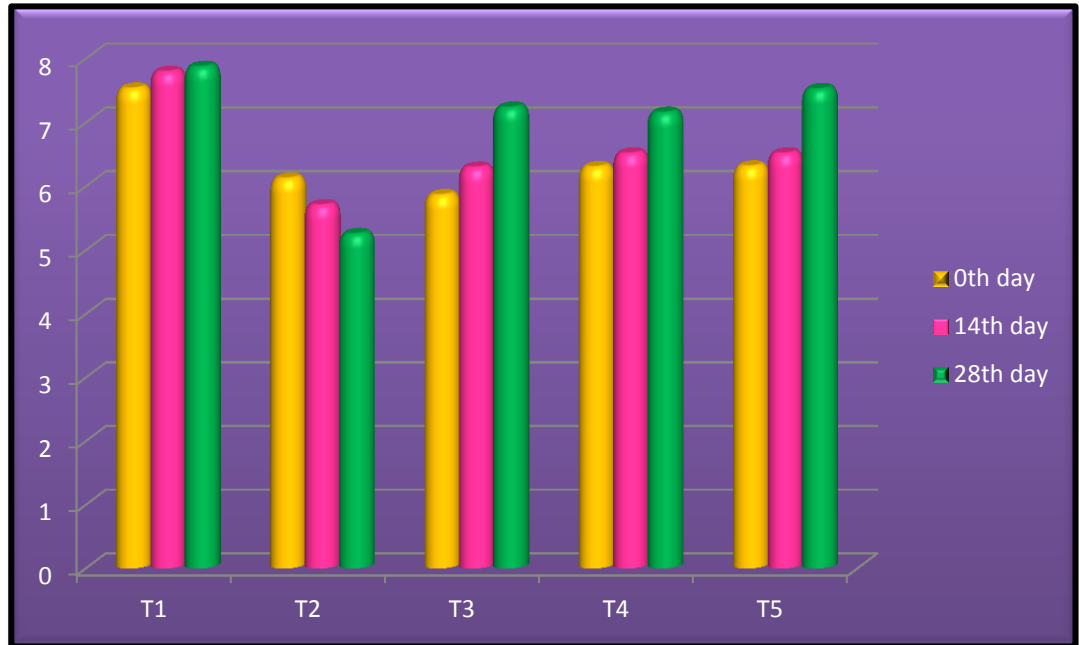
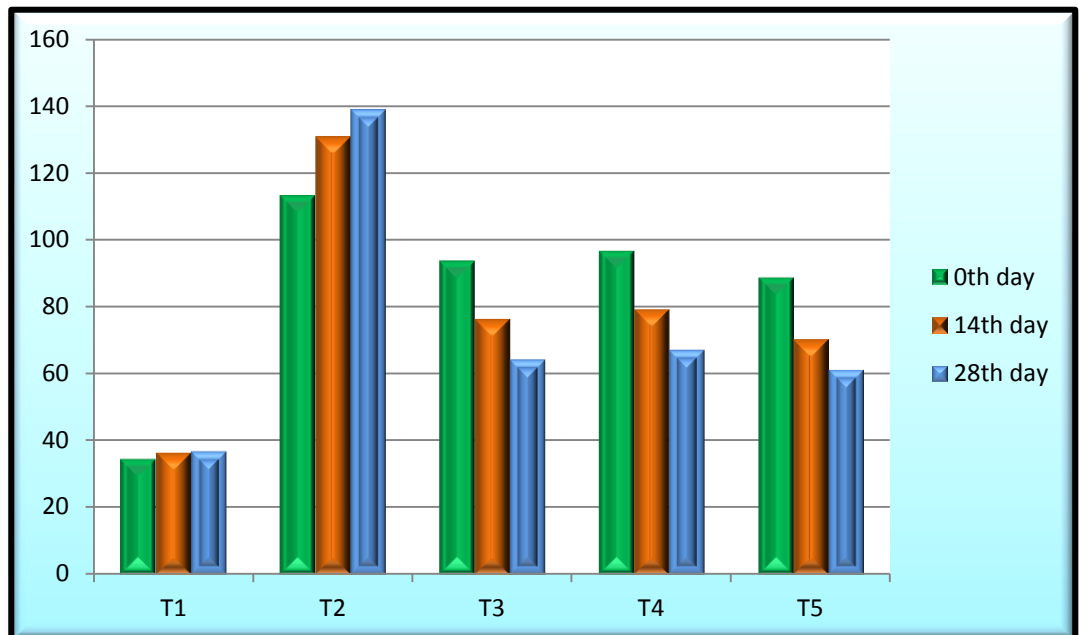


Fig 4: Mean AST Levels observed in different groups (IU/L)



Total proteins might be decrease due to decrease protein synthesis as stated by (Jayanthi *et al.* 2010). The treatment due to *C. roseus* @ 200 & 400 mg/ kg b.wt reverted back an increase level of total protein in diabetic rats. There is significant decrease in the serum total protein levels of the diabetic rats (Kemasari *et al.* 2011). The present studies indicates that the diabetes when treated with hydroethanolic leaf extract of *C. roseus* are effective in improving the lowered serum total protein level of the diabetic rats and that the effect of hydroethanolic leaf extract of *C. roseus* is dose dependent. This has relevance with the findings of Iweala and Okeke (2005), Jayanti *et al.* (2010) and Aruljiothi and Samipilai (2016).

4.3.4 Serum Glutamic Oxaloacetic Transaminase (SGOT) / AST Levels

The table 7 and fig 5 shows the mean AST levels of different groups on 0th, 14th and 28th day of the experiment. The mean AST values of groups T₁, T₂, T₃, T₄ and T₅ on 0th day were 34.55 ± 0.28, 113.37 ± 0.75, 93.78 ± 0.79, 96.77 ± 0.90 and 88.86 ± 0.77 IU/L respectively. There is significant difference in the mean AST levels of the group T₁ and all other groups at 5% level of significance. The mean AST levels of the treatment groups increased above the normal value and were observed between 113.37± 0.75 to 88.86 ±0.77.

The mean AST values of groups T₁, T₂, T₃, T₄ and T₅ on 14th day were 36.46 ± 0.24, 130.93 ± 1.62, 76.39 ± 1.36, 79.31 ± 2.18 and 70.34 ± 0.60 IU/L respectively. There is significant difference in the mean AST levels of all the groups at 5% level of significance. The treatment with hydroethanolic leaf extract of *C. roseus* (400mg/kg) to the group T₅ was found to be highly effective in decreasing the AST levels as compared to the treatments with metformin (100 mg/kg) and hydroethanolic leaf extract of *C. roseus* (200mg/kg) to the groups T₃and T₄ respectively.

The mean AST values of groups T₁, T₂, T₃, T₄ and T₅ on 28th day were 36.84 ± 0.28, 138.98 ± 1.78, 64.28 ± 0.95, 67.15 ± 0.97 and 61.18 ± 0.92 IU/L respectively. There is significant difference in the mean AST levels of all the groups at 5% level of significance. The treatment with hydroethanolic leaf extract of *C. roseus* (400mg/kg) to the group T₅ was found to be highly effective in decreasing the AST levels. Whereas, the treatments with metformin (100 mg/kg)

Table 7: Effect of hydroethanolic leaf extract of *C. roseus* on mean AST levels observed in different groups (IU/L)

Sr. No.	Group	Treatment	0 th day	14 th day	28 th day
1	T ₁	Normal Control	^A 34.55 ± 0.28 ^a	^A 36.46 ± 0.24 ^a	^A 36.84 ± 0.28 ^a
2	T ₂	Diabetic Control	^E 113.37 ± 0.75 ^a	^E 130.93 ± 1.62 ^b	^E 138.98 ± 1.78 ^c
3	T ₃	Std. Drug control @100mg/kg bd. Wt.	^C 93.78 ± 0.79 ^c	^C 76.39 ± 1.36 ^b	^C 64.28 ± 0.95 ^a
4	T ₄	<i>C. roseus</i> @ 200 mg/kg bd. Wt.	^D 96.77 ± 0.90 ^c	^D 79.31 ± 2.18 ^b	^D 67.15 ± 0.97 ^a
5	T ₅	<i>C. roseus</i> @ 400 mg/kg bd. Wt.	^B 88.86 ± 0.77 ^c	^B 70.34 ± 0.60 ^b	^B 61.18 ± 0.92 ^a

Values are Mean ± SE for 6 rats in each group. Values not sharing common superscript differ significantly at p < 0.05.

CD Values for days=3.11

CD values for treatment = 1.79

ANOVA: Mean Serum AST levels observed in different groups (IU/L)

Source of variation	SS	DF	MS	F
Treatment	76791.62	4	19197.91	2614.817
Days	2097.185	2	1048.593	142.8217
Interaction	7665.893	8	958.2366	130.5149
Error	550.6477	75	7.341969	-
Total	87105.35	89	-	-

and hydroethanolic leaf extract of *C. roseus* (200mg/kg) to the groups T₃ and T₄ respectively showed similar results.

The above observations reveals that the AST levels of the group T₂ (Diabetic Control) got increased significantly up to the 28th day of the experiment. The significant increases in the AST level shows liver damage under insulin deficiency and have been related with increased gluconeogenesis and ketogenesis during diabetes and it might be associate with the leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of streptozotocin (Zafar *et al.* 2009 and Nabi *et al.* 2013). However, the treatment with metformin caused consecutive decrease in the levels of AST till the end of the experiment. Similarly, the AST levels of the diabetic rats from the groups T₄ and T₅ got decreased significantly which were treated with the hydroethanolic leaf extract of *C. roseus* @ 200 and 400 mg/kg b wt respectively daily for 28 days. The rats treated with 70 % ethanolic extract of leaves of *C. roseus* at the dose of 200 and 400 mg/kg b wt caused significant decrease in the AST levels on 28th day of trial in the present study which is indication of regeneration of Linn cells.

4.3.5 Serum Glutamic Pyruvic Transaminase (ALT) Levels

The table 8 and fig 6 shows the mean ALT levels of all the groups on 0th, 14th and 28th day of the experiment. The mean ALT values of groups T₁, T₂, T₃, T₄ and T₅ on 0th day were 29.45 ± 0.31, 134.55 ± 0.60, 97.48 ± 1.16, 109.66 ± 1.05 and 96.15 ± 0.30 IU/L respectively. There was a significant ($P \leq 0.5$) increase in the mean ALT level of all treated groups i.e. T₂, T₃, T₄ and T₅. On 0th day treatment as compared two groups one animals indicating increase in levels because of the streptozotocin induced diabetes.

The mean ALT values of groups T₁, T₂, T₃, T₄ and T₅ on 14th day were 28.63 ± 0.63, 138.93 ± 0.44, 73.16 ± 1.24, 80.73 ± 1.97 and 72.30 ± 1.10 IU/L respectively. There was a significant decrease in ALT levels of the groups T₃, T₄ and T₅, when compared with the group T₂ (diabetic control). The treatment with hydroethanolic leaf extract of *C. roseus* (400mg/kg) to the group T₅ was found to be equally effective to decline the increased levels of the ALT as compared to the treatment with metformin (100 mg/kg).

Table 8: Effect of hydroethanolic leaf extract of *C. roseus* on mean ALT levels observed in different groups (IU/L)

Sr. No.	Group	Treatment	0 th day	14 th day	28 th day
1	T ₁	Normal Control	^A 29.45 ± 0.31 ^a	^A 28.63 ± 0.63 ^a	^A 29.01 ± 0.42 ^a
2	T ₂	Diabetic Control	^D 134.55 ± 0.60 ^a	^D 138.93 ± 0.44 ^b	^E 144.18 ± 1.13 ^c
3	T ₃	Std. Drug control @100mg/kg bd. Wt.	^B 97.48 ± 1.16 ^c	^B 73.16 ± 1.24 ^b	^C 64.5 ± 0.40 ^a
4	T ₄	<i>C. roseus</i> @ 200 mg/kg bd. Wt.	^C 109.66 ± 1.05 ^C	^C 80.73 ± 1.97 ^b	^D 68.15 ± 0.58 ^a
5	T ₅	<i>C. roseus</i> @ 400 mg/kg bd. Wt.	^B 96.15 ± 0.30 ^c	^B 72.30 ± 1.10 ^b	^B 61.28 ± 0.63 ^a

Values are Mean ± SE for 6 rats in each group. Values not sharing common superscript differ significantly at p < 0.05.

CD values for days = 2.59

CD values for treatment = 1.49

ANOVA: Mean Serum ALT levels observed in different groups (IU/L)

Source of variation	SS	DF	MS	F
Treatment	110512.5	4	27628.12	5432.357
Days	6459.751	2	3229.876	635.0718
Interaction	6579.996	8	822.4995	161.7233
Error	381.4383	75	5.085843	-
Total	123933.7	89	-	-

Fig 5: Mean ALT Levels observed in different Groups (IU/L)

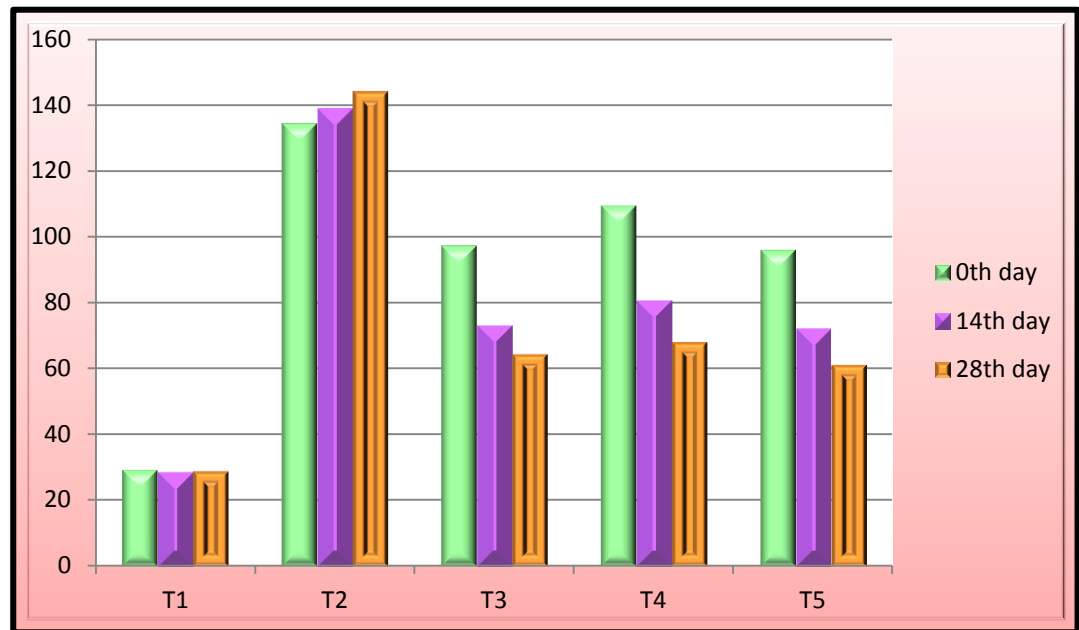
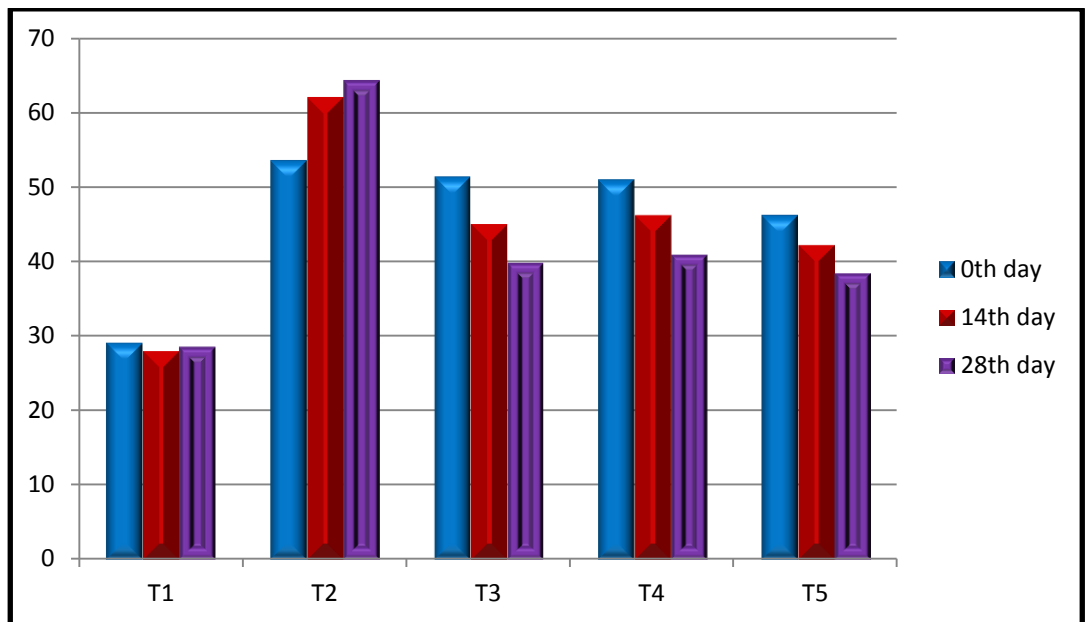


Fig 6: Mean Blood Urea Nitrogen Levels observed in different groups (mg/dl)



The mean ALT values of groups T₁, T₂, T₃, T₄ and T₅ on 28th day were 29.01 ± 0.42, 144.18 ± 1.13, 64.5 ± 0.40, 68.15 ± 0.58 and 61.28 ± 0.63 IU/L respectively. There is significant difference in the mean ALT levels of the groups viz. T₁, T₂, T₃, T₄ and T₅ at 5 % level of significance. There is a significant decrease in ALT levels of the groups T₃, T₄ and T₅, when compared with the group T₂ (diabetic control). The treatment with hydroethanolic leaf extract of *C. roseus* (400mg/kg) to the group T₅ was found to be equally effective for decreasing the increased levels of the ALT as compared to the treatments with metformin (100 mg/kg) and more effective as compared to hydroethanolic leaf extract of *C. roseus* (200mg/kg) treated group T₄.

From the above findings it is observed that there was significant increase in ALT levels of the group T₂ up to 28 days of the experiment. The levels of ALT in streptozotocin induced diabetic rats significantly increased than normal which was agreement with Zafar *et al.* (2009) and Nabiet *al.* (2004). In metformin-treated group significant decrease (P < 0.5) in the ALT levels to near pre-diabetic levels were observed (Velmani *et al.* 2013). In the present studies it was found that the metformin (100mg/kg) and the hydroethanolic leaf extract of *C. roseus* at both doses also decreased the elevated ALT levels of the diabetic rats. The treatment with the hydroethanolic leaf extract of *C. roseus* (400 and 200 mg/kg) was effective than that of metformin at the dose of 100mg/kg b wt.

4.3.6 Blood Urea Nitrogen (BUN) Levels (mg/dl)

As shown in table 9 and fig 7 the mean BUN values of groups T₁, T₂, T₃, T₄ and T₅ on 0th day were 29.13 ± 0.20, 53.57 ± 0.48, 51.37 ± 0.50, 50.98 ± 1.71 and 46.22 ± 0.29 mg/dl respectively. There is a significant difference in the mean BUN levels of the group T₁ and groups T₂, T₃, T₄ and T₅ at 5% level of significance. The mean BUN levels of the groups T₂, T₃, T₄ and T₅ is significantly higher than the group T₁ (Normal control), indicating an increased because of diabetes.

The mean BUN values of groups T₁, T₂, T₃, T₄ and T₅ on 14th day were 27.98 ± 0.74, 62.05 ± 1.53, 45.04 ± 0.46, 46.27 ± 0.48 and 42.24 ± 0.21 mg/dl respectively. There is significant variation in the mean BUN levels of all the groups T₁, T₂, T₃, T₄ and T₅ at 5% level of significance. The treatment with hydroethanolic leaf extract of *C. roseus* (400 and 200 mg/kg) to the group T₅ was

Table 9: Effect of hydroethanolic leaf extract of *C. roseus* on mean BUN levels observed in different groups (mg/dl)

Sr. No.	Group	Treatment	0 th day	14 th day	28 th day
1	T ₁	Normal Control	^A 29.13 ± 0.20 ^a	^A 27.98 ± 0.74 ^a	^A 28.53 ± 0.55 ^a
2	T ₂	Diabetic Control	^D 53.57 ± 0.48 ^a	^D 62.05 ± 1.53 ^b	^D 64.30 ± 1.70 ^b
3	T ₃	Std. Drug control @100mg/kg bd. Wt.	^C 51.37 ± 0.50 ^c	^C 45.04 ± 0.46 ^b	^C 39.84 ± 0.65 ^a
4	T ₄	<i>C. roseus</i> @ 200 mg/kg bd. Wt.	^C 50.98 ± 1.71 ^c	^C 46.27 ± 0.48 ^b	^C 40.9 ± 0.67 ^a
5	T ₅	<i>C. roseus</i> @ 400 mg/kg bd. Wt.	^B 46.22 ± 0.29 ^c	^B 42.24 ± 0.21 ^b	^B 38.39 ± 0.43 ^a

Values are Mean ± SE for 6 rats in each group. Values not sharing common superscript differ significantly at p < 0.05.

CD values for days =2.44

CD values for treatment = 1.41

ANOVA: Mean Serum BUN levels observed in different groups (mg/dl)

Source of variation	SS	DF	MS	F
Treatment	9038.086	4	2259.522	500.746
Days	226.6962	2	113.3481	25.11974
Interaction	1051.402	8	131.4252	29.12592
Error	338.4233	75	4.512311	-
Total	10654.61	89	-	-

found to be more effective to cause decrease in BUN as compared to the treatments with metformin (100 mg/kg).

The mean BUN values of groups T₁, T₂, T₃, T₄ and T₅ on 28th day were 28.53 ± 0.55, 64.30 ± 1.70, 39.84 ± 0.65, 40.9 ± 0.67 and 38.39 ± 0.43 mg/dl respectively. There is significant variation in the mean BUN levels of all the groups T₁, T₂, T₃, T₄ and T₅ at 5% level of significance. The treatment with hydroethanolic leaf extract of *C. roseus* (400 mg/kg) to the group T₅ significantly lowered the BUN levels and was found to be highly effective as compared to the treatments with metformin (100 mg/kg) and hydroethanolic leaf extract of *C. roseus* (200 mg/kg) to the groups T₃ and T₄ respectively.

From the above observations it is observed that the mean levels of BUN were significantly increased in the diabetic rats of the group T₂ up to the end of experiment of 28 days. Kemasari *et.al*, (2011) who observed that there is significant increase in renal parameters like urea, uric acid and creatinine in alloxan induced diabetic rats which indicate the impaired renal function. The treatment with metformin (100 mg/kg) and hydroethanolic leaf extract of *C. roseus* at 200 and 400 mg/kg caused significant decrease in the BUN levels on 14th and 28th day of the experiment. However, the treatment with hydroethanolic leaf extract of *C. roseus* (400 mg/kg) caused significant decrease in the BUN levels as comparable to that of the treatments of metformin (100 mg/kg). These observations are in relevance with Mohammed *et al.* (2011).

4.4 Hematology

4.4.1 Hemoglobin (Hb) Levels (g/dl)

The table 10 and fig 8 depicts the mean hemoglobin levels of the groups T₁, T₂, T₃, T₄ and T₅ on 0th, 14th and 28th day of the experiment. The mean hemoglobin values of groups T₁, T₂, T₃, T₄ and T₅ on 0th day were 13.18 ± 0.23, 10.11 ± 0.10, 10.12 ± 0.02, 10.3 ± 0.06 and 10.28 ± 0.07 g/dl respectively. There is significant decrease in the mean hemoglobin levels of groups T₂, T₃, T₄ and T₅ at 5% level of significance as compare to T₁ on 0th day of experiment.

The mean hemoglobin values of groups T₁, T₂, T₃, T₄ and T₅ on 14th day were 13.43 ± 0.20, 9.36 ± 0.07, 12.25 ± 0.07, 12.69 ± 0.19 and 14.23 ± 0.04 g/dl

respectively. There is significant difference in the mean hemoglobin levels of all the groups T₁, T₂, T₃, T₄ and T₅ at 5% level of significance. The treatments with the hydroethanolic leaf extract of *C. roseus* at the dose of 400 mg/kg improved the lowered hemoglobin levels of the diabetic rats and brought the levels comparable to that in control group (T₁) rats. The treatments with the metformin (100 mg/kg) and the hydroethanolic leaf extract of *C. roseus* (200mg/kg) also significantly improved the hemoglobin levels of the diabetic rats and the levels were close to normal.

The mean hemoglobin values of groups T₁, T₂, T₃, T₄ and T₅ on 28th day were 13.51 ± 0.15, 9.02 ± 0.04, 12.83 ± 0.08, 12.80 ± 0.12 and 14.52 ± 0.08 g/dl respectively. There is significant difference in the mean hemoglobin levels of all the groups T₁, T₂, T₃, T₄ and T₅ at 5% level of significance. The treatments with the hydroethanolic leaf extract of *C. roseus* at the dose of 400 mg/kg increased the lowered hemoglobin levels of the diabetic rats and the effect was such that the hemoglobin levels were found to be more than the normal levels. The treatments with the metformin (100 mg/kg) and the hydroethanolic leaf extract of *C. roseus* (200mg/kg) also significantly improved the hemoglobin levels of the diabetic rats, which were near to the normal levels.

The above results indicate that the hemoglobin levels decreased significantly in the diabetic rats and are corresponding to the findings of Emmanuel *et al.* (2010) and Kemasari *et al.* (2011). The decreased level of hemoglobin observed in diabetic rats might be due to increased formation of glycosylated hemoglobin. It has been reported that subjects the total hemoglobin levels are much lower than normal, as stated by Kemasari *et al.* (2011). The standard drug Metformin (100 mg/kg) treated rats of group T₃ showed the significant improvement in the hemoglobin levels. However, the treatments with hydroethanolic leaf extract of *C. roseus* at the dose of 400 mg/kg to the groups T₄ showed significant increase in the hemoglobin levels of the diabetic rats as compared to the treatments with metformin (100 mg/kg) and the hydroethanolic leaf extract of *C. roseus* (200 mg/kg) to the group T₃ and T₄ respectively. The hydroethanolic leaf extract of *C. roseus* has improved the hemoglobin levels of the diabetic rats.

Table 10: Effect of hydroethanolic leaf extract of *C. roseus* on mean Haemoglobin levels observed in different groups (g/dl)

Sr. No.	Group	Treatment	0 th day	14 th day	28 th day
1	T ₁	Normal Control	^B 13.18 ± 0.23 ^a	^D 13.43 ± 0.20 ^a	^C 13.51 ± 0.15 ^a
2	T ₂	Diabetic Control	^A 10.11 ± 0.10 ^b	^A 9.36 ± 0.07 ^a	^A 9.02 ± 0.04 ^a
3	T ₃	Std. Drug control @100mg/kg bd. Wt.	^A 10.12 ± 0.02 ^a	^B 12.25 ± 0.07 ^b	^B 12.83 ± 0.08 ^c
4	T ₄	<i>C. roseus</i> @ 200 mg/kg bd. Wt.	^A 10.3 ± 0.06 ^a	^C 12.69±0.19 ^b	^B 12.80 ± 0.12 ^b
5	T ₅	<i>C. roseus</i> @ 400 mg/kg bd. Wt.	^A 10.28 ± 0.07 ^a	^E 14.23 ± 0.04 ^b	^D 14.52 ± 0.08 ^b

CD values for days = 0.34

CD values for treatment = 0.21

ANOVA: Mean Hemoglobin levels observed in different groups (g/dl)

Source of variation	SS	DF	MS	F
Treatment	165.5427	4	41.38568	453.9429
Days	55.99428	2	27.99714	307.0894
Interaction	64.01384	8	8.00172	87.76777
Error	6.8377	75	0.091169	-
Total	301.318	89	-	-

4.4.2 Packed Cell Volume (PCV) Levels (%)

The table 11 and fig 9 depicts the mean PCV levels of the groups T₁, T₂, T₃, T₄ and T₅ on 0th, 14th and 28th day of the experiment. The mean PCV values of groups T₁, T₂, T₃, T₄ and T₅ on 0th day were 39.55 ± 0.69, 30.34 ± 0.32, 30.37 ± 0.07, 30.90 ± 0.20 and 30.85 ± 0.22 % respectively. There is significant difference in the mean PCV levels of the group T₁ and groups T₂, T₃, T₄ and T₅ at 5% level of significance. The levels of PCV of the groups T₂, T₃, T₄ and T₅ does not varied significantly. There is significant decline in the mean PCV levels of the groups T₂, T₃, T₄ and T₅ on 0th day of the experiment than the group T₁ (Normal Control).

The mean PCV values of groups T₁, T₂, T₃, T₄ and T₅ on 14th day were 40.29 ± 0.62, 28.10 ± 0.21, 36.77 ± 0.22, 38.09 ± 0.59 and 42.71 ± 0.13 % respectively. There is significant difference in the mean PCV levels of the groups T₁, T₂, T₃, T₄ and T₅ at 5% level of significance. There is significant improvement in the mean PCV levels of the diabetic rats treated with the hydroethanolic leaf extract of *C. roseus* (400mg/kg) followed by treatments with the metformin (100 mg/kg) and the hydroethanolic leaf extract of *C. roseus* (200mg/kg).

The mean PCV values of groups T₁, T₂, T₃, T₄ and T₅ on 28th day were 40.55 ± 0.47, 27.08 ± 0.14, 38.5 ± 0.25, 38.41 ± 0.37 and 43.58 ± 0.24 % respectively. There is significant difference in mean PCV levels of the groups T₁, T₂, T₃, T₄ and T₅ at 5% level of significance. The treatments with the hydroethanolic leaf extract of *C. roseus* 400 mg/kg increased the lowered PCV levels of the diabetic rats and were found to be higher than the normal levels. The treatments with the metformin (100 mg/kg) and the hydroethanolic leaf extract of *C. roseus* (200 mg/kg) to the groups T₃ and T₄ significantly improved the PCV levels of the diabetic rats; however the effect was lesser than that of the treatments with the hydroethanolic leaf extract of *C. roseus* 400 mg/kg.

The above results suggest that the PCV levels get significantly declined in the diabetes. The standard drug Metformin treated rats of group T₃ were made significant improvement in the PCV levels on consecutive period of days of the experiment. The diabetic rats from the groups T₄ and T₅ treated with hydroethanolic leaf extract of *C. roseus* at the dose of 400 mg/kg and 200 mg/kg respectively showed significant increase in the PCV levels of the diabetic rats

Table 11: Effect of hydroethanolic leaf extract of *C. roseus* on mean PCV levels observed in different groups (%)

Sr. No.	Group	Treatment	0 th day	14 th day	28 th day
1	T ₁	Normal Control	^A 39.55 ± 0.69 ^a	^D 40.29 ± 0.62 ^a	^C 40.55 ± 0.47 ^a
2	T ₂	Diabetic Control	^A 30.34 ± 0.32 ^b	^A 28.10 ± 0.21 ^a	^A 27.08 ± 0.14 ^a
3	T ₃	Std. Drug control @100mg/kg bd. Wt.	^A 30.37 ± 0.07 ^a	^B 36.77 ± 0.22 ^b	^B 38.5 ± 0.25 ^c
4	T ₄	<i>C. roseus</i> @ 200 mg/kg bd. Wt.	^A 30.9 ± 0.20 ^a	^C 38.09 ± 0.59 ^b	^B 38.41 ± 0.37 ^b
5	T ₅	<i>C. roseus</i> @ 400 mg/kg bd. Wt.	^A 30.85 ± 0.22 ^a	^E 42.71 ± 0.13 ^b	^D 43.58 ± 0.24 ^b

Values are Mean ± SE for 6 rats in each group. Values not sharing common superscript differ significantly at p < 0.05.

CD value for days= 1.041

Cd values for treatment = 0.60

ANOVA: Mean PCV levels observed in different groups (%)

Source of variation	SS	DF	MS	F
Treatment	1489.884	4	372.4711	453.9429
Days	503.9485	2	251.9742	307.0894
Interaction	576.1245	8	72.01557	87.76777
Error	41.5393	75	0.820524	-
Total	2631.497	89	-	-

Fig 7: Mean Haemoglobin Levels observed in different groups (g/dl)

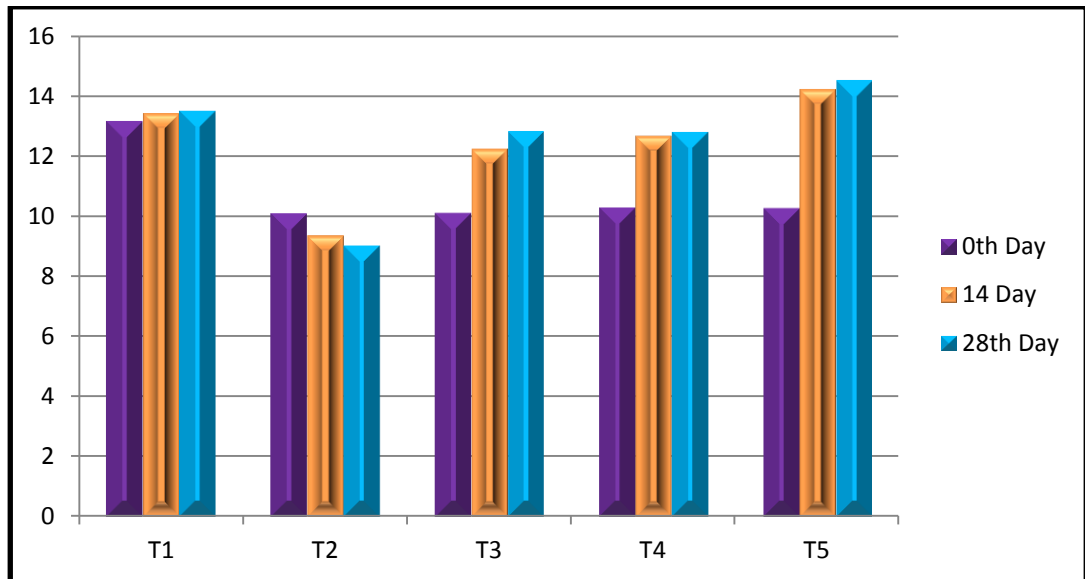


Fig 8: Mean Packed Cell Volume Levels observed in different groups (%)

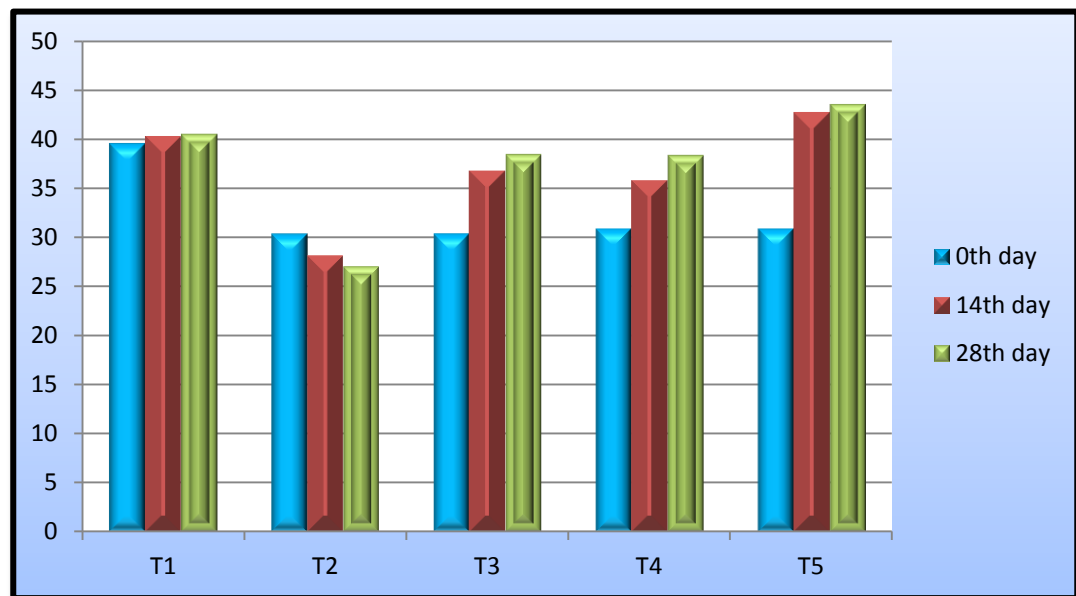


Table 12: Effect of hydroethanolic leaf extract of *C. roseus* on mean Body Weight (G) observed in different groups

Sr. No.	Group	Treatment	0 th day	7 th day	14 th day	21 th day	28 th day
1	T ₁	Normal Control	^{AB} 158.33 ± 2.78 ^a	^B 163.83 ± 2.63 ^{ab}	^C 169.33 ± 2.44 ^b	^C 172.83 ± 2.32 ^b	^{BC} 170.66 ± 2.13 ^b
2	T ₂	Diabetic Control	^A 156.33 ± 0.61 ^c	^A 152.66 ± 0.84 ^{bc}	^A 148.33 ± 2.96 ^b	^A 143.33 ± 2.96 ^{ab}	^A 138.83 ± 3.49 ^a
3	T ₃	Std. Drug control @100mg/kg bd. Wt.	^C 162 ± 1 ^a	^B 163.16 ± 1.32 ^a	^B 164.66 ± 0.88 ^a	^B 166.5 ± 0.84 ^{ab}	^{BC} 171.16 ± 0.74 ^a
4	T ₄	<i>C. roseus</i> @ 200 mg/kg bd. Wt.	^C 161.66 ± 2.78 ^a	^B 163 ± 2.67 ^a	^B 164.83 ± 2.58 ^{ab}	^B 166.66 ± 2.53 ^{ab}	^B 169 ± 2.52 ^b
5	T ₅	<i>C. roseus</i> @ 400 mg/kg bd. Wt.	^C 164.16 ± 2 ^a	^{BC} 165.66 ± 1.47 ^a	^{BC} 167.66 ± 1.81 ^{ab}	^{BC} 169.83 ± 1.62 ^{ab}	^C 172.66 ± 1.25 ^b

Values are Mean ± SE for 6 rats in each group. Values not sharing common superscript differ significantly at p < 0.05.

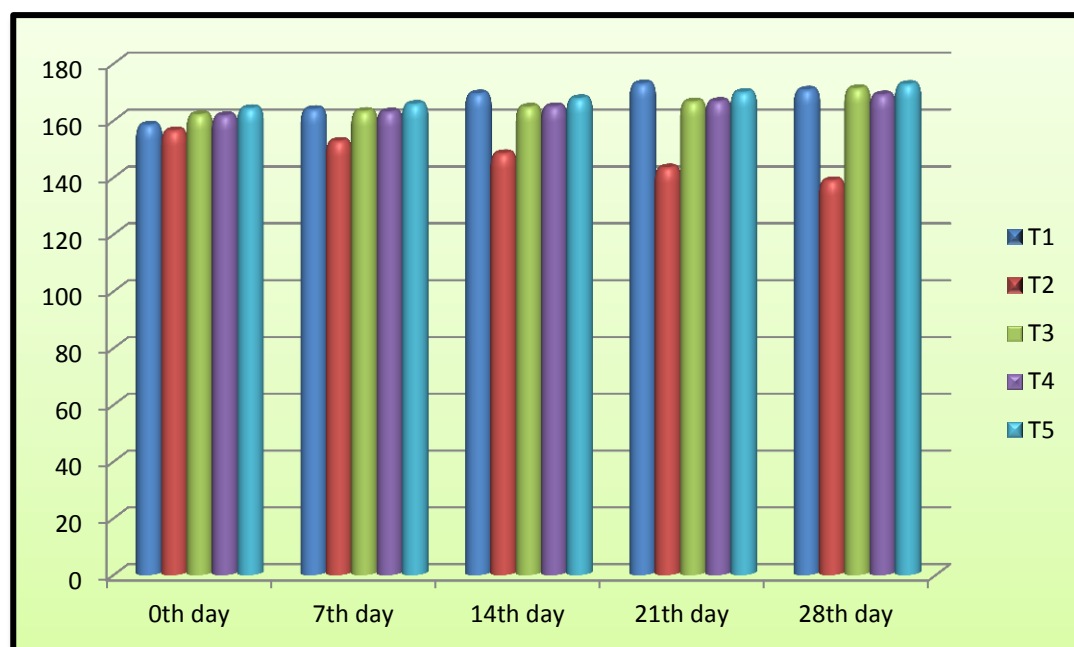
CD values for days =5.84

CD values for treatment =3.37

ANOVA: Mean Body Weight observed in different groups

Source of variation	SS	DF	MS	F
Treatment	8367.107	4	2091.777	79.68066
Days	311.5067	4	77.87667	2.966504
Interaction	2484.16	16	155.26	5.914216
Error	3281.5	125	26.252	-
Total	14444.27	149	-	-

Fig 9: Mean Body weight observed in different groups (gms/week)



than the rats from the normal group T₁ and diabetic group T₂ on 28th day of the experiment.

4.5 Physical Parameters

4.5.1 Body Weight

As shown in table 12, there was a constant increase in body weights of rats from normal group (T₁). Whereas in diabetic control group of rats (T₂) there was a consistent decrease in weight from its initial weight of 156.33±0.61 to 138.83±3.49 on 28th days of study. In treated groups it has been observed that the average weight were increased up to 28th day of treatment, however, this increased was less as compared to weights of normal group of rats at the same time in all the treated groups (T₃, T₄ and T₅) weights of rats were significantly increased as compared to diabetic group (T₂) from the results obtained it is evident that the weight loss in this diabetic rats was due to emaciation, in appetite as a diabetic complications on stated by Braslasu *et al.* (2007). As per the results obtained in the present study metformin @ 100 mg/kg body weight not only control the loss of weight but also caused gain in the weights from its 162±1 to 171.16±0.74. The groups of rats under study treated with *C. roseus* @ 400 mg/kg and 200 mg/kg showed comparable results with the standard drug metformin and could present the loss in body weight and also there was an increases in weights of rats from 164.16±2 to 172.66±1.25 and from 161.66±2.78 to 169±2.52 respectively.

The improvement in the body weight of the diabetic rats with the treatment of the hydroethanolic leaf extract of *C. roseus* is in agreement with the observations made by Nayak (2006), Jayanthi *et al.* (2010) and Hikmah *et al.* (2015).

4.6 Histopathological Study

Histopathological alterations in pancreas, liver and kidney were studied in experimental rats which were sacrificed on 28st day of the experiment. No histopathological lesions of any significance were noticed in any organ of rats from normal control group.

4.6.1 Pancreas

The pancreas from diabetic control group showed severe destruction of β -cells in islets of Langerhans, which indicated selective destruction of β -cells as attempted by streptozotocin. Severe necrosis and decreased population of β -cells in islets of Langerhans along with the severe fibrosis was noticed in rats from the diabetic control group (Plate X). The treatment with metformin (100 mg/kg) showed normal architecture of islets of Langerhans and improved β -cells population as normal (Plate XIII). The treatment with hydroethanolic leaf extract of *C. roseus* (200 mg/kg) showed mild to moderate necrosis and decreased β -cell population in islets of Langerhans (Plate XVI). The treatment group given hydroethanolic leaf extract of *C. roseus* @ 400 mg/kg showed improvement in β -cell population as normal as standard drug treatment and was showing normal architecture of islets of Langerhans and acini (Plate XIX).

The finding of the present study are in agreement with Puranik *et al.* (2010) who also observed small and shrunken islets of Langerhans and destruction of beta cells in the pancreas of diabetes induced rat. Piyachaturawat *et al.* (1988) studied that in rats, STZ injected in a dose of 65 mg/kg body weight effectively produced hyperglycemia and gastric mucosal ulcerations. The incidence and severity of lesions produced by STZ in pancreas, liver, kidney and GIT, progressively increased with time from one to six weeks post treatment. The rats treated with COLMF 200 mg/kg b wt showed normal architecture with shrunken nuclei (Emmanuel *et al.* 2010). Histopathology of pancreas showed damage of islets of langerhans in diabetic rats when compared to normal but shows healing of pancreas in diabetic rats treated with whole plant extract of *C. roseus* and standard glibenclamide drug Mohammed *et al.* (2011).

The methanolic extracts also showed improvement in parameters like body weight and lipid profile as well as regeneration of β -cells of pancreas in diabetic rats. Histopathological studies reinforce the healing of pancreas, by methanolic *Vinca rosea* extracts, as a possible mechanism of their antidiabetic activity (Mohammed *et al.* 2010).

4.6.2 Liver

The liver section from diabetic control group revealed severe congestion between lobules and fatty changes in hepatocytes (Plate IX). The sections from the treatment with metformin (100 mg/kg) revealed normal architecture of hepatocytes (Plate VIII). The sections from the treatments with hydroethanolic leaf extract of *catharanthus roseus* 200 mg/kg showed mild fatty changes and mild degenerative changes in hepatocytes (Plate XIV) respectively. However, liver from the treatments with hydroethanolic leaf extract of *catharanthus roseus* (400 mg/kg) showed the normal architecture of hepatocytes (Plate XVII). These observations corroborate with Piyachaturawat *et al.* (1988) who also noted congestion, haemorrhages and fatty changes in liver of streptozotocin induced diabetic rats. The rats treated with metformin showed no hepatic abnormalities and the arrangements of the hepatocytes in the liver were almost normal (Velmani *et al.* 2013). Histopathological changes are restored near to normal in *C. fimbriata* treated and diabetes induced group (Latha *et al.* 2014).

4.6.3 Kidney

The kidney sections from the streptozotocin induced diabetic group showed severe hemorrhages in interstitial spaces along with tubular necrotic areas and glomerulopathies (Plate IX). The sections from the treatment with metformin (100 mg/kg) revealed normal architecture of glomeruli and tubules (Plate XII). Mild hemorrhages, vacuolar degenerative changes and tubular necrosis were observed in group treated with hydroethanolic leaf extract of *catharanthus roseus* (200 mg/kg) (Plate XV). While the sections from the treatment with hydroethanolic leaf extract of *catharanthus roseus* (400 mg/kg) revealed normal architecture of glomeruli and tubules (Plate XVIII). These observations are in agreement with Piyachaturawat *et al.* (1988). The incidence and severity of lesions produced by STZ in pancreas, liver, kidney and GIT, progressively increased with time from one to six weeks post treatment Piyachaturawat *et al.* (1988).

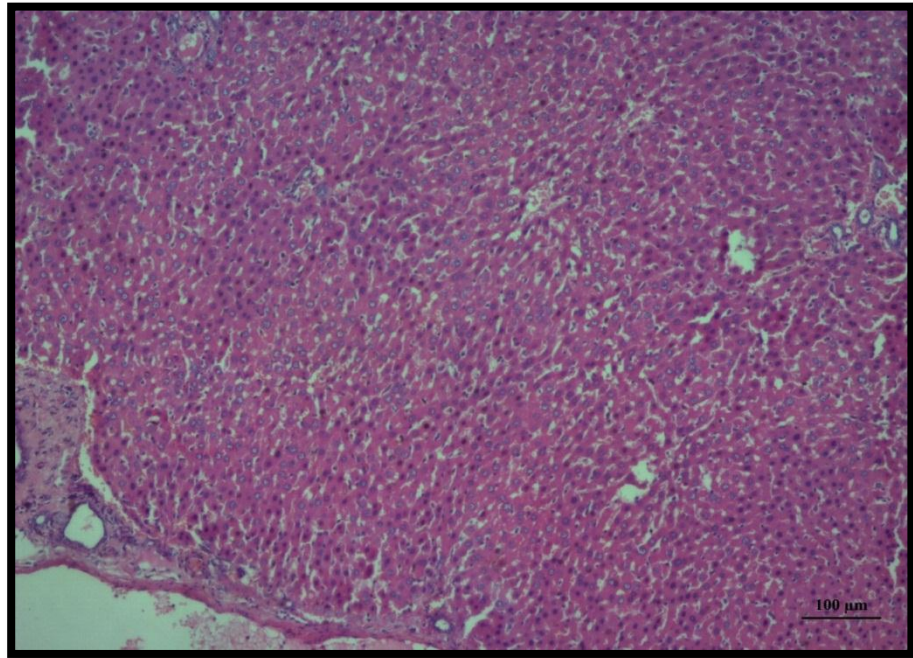


Plate V: A microphotograph showing normal structure of liver (Group T1) (H & E).

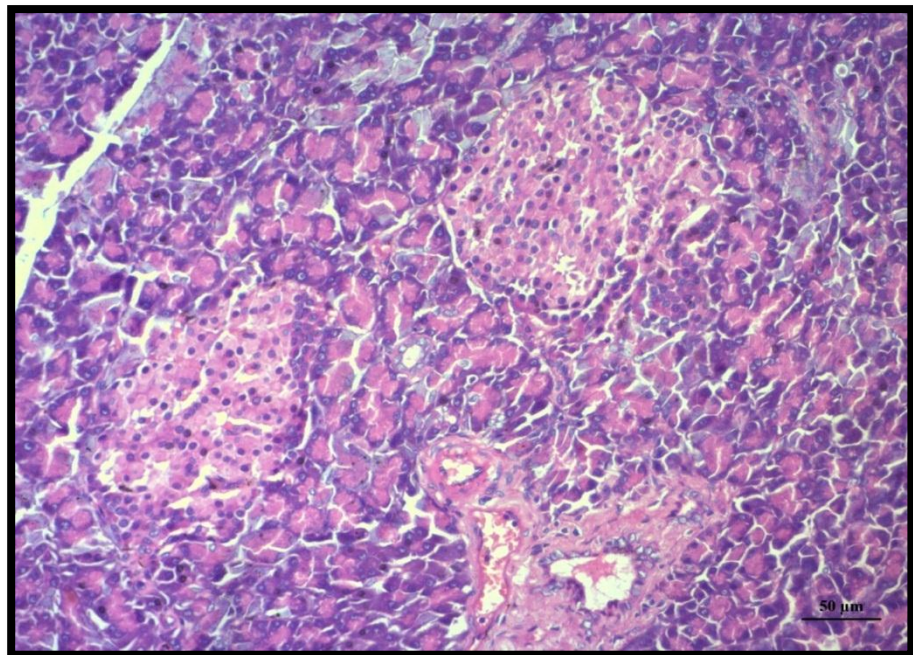


Plate VI: A microphotograph showing normal structure of pancreas (Group T1) (H & E).

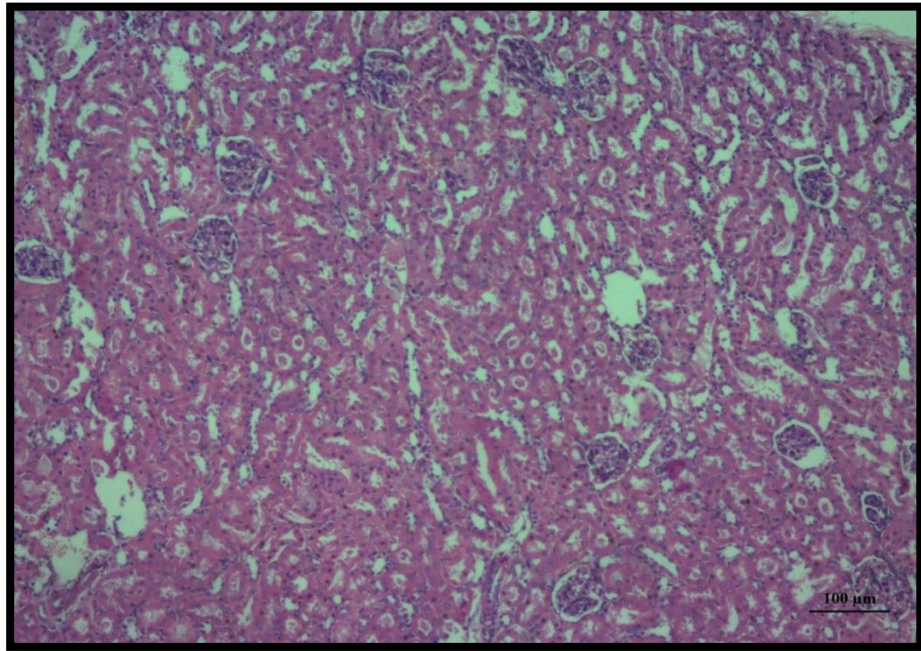


Plate VII: A microphotograph showing normal structure of kidney (Group T1) (H & E).

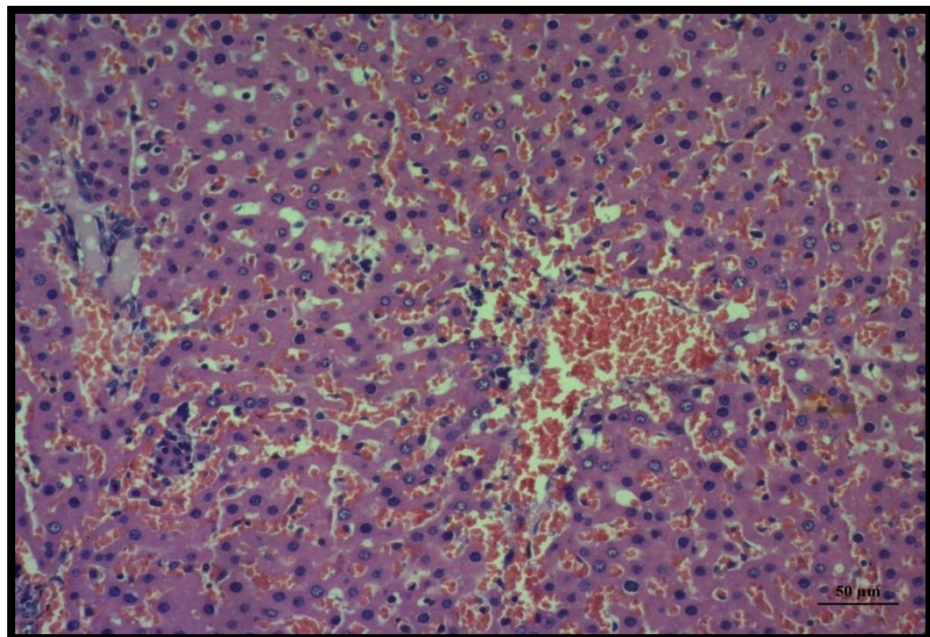


Plate VIII: A microphotograph of liver showing severe sinusoidal congestion, hemorrhages & hepatocytes appear swollen with more eosinophilic cytoplasm in streptozotocin induced diabetes (Group T2) (H & E).

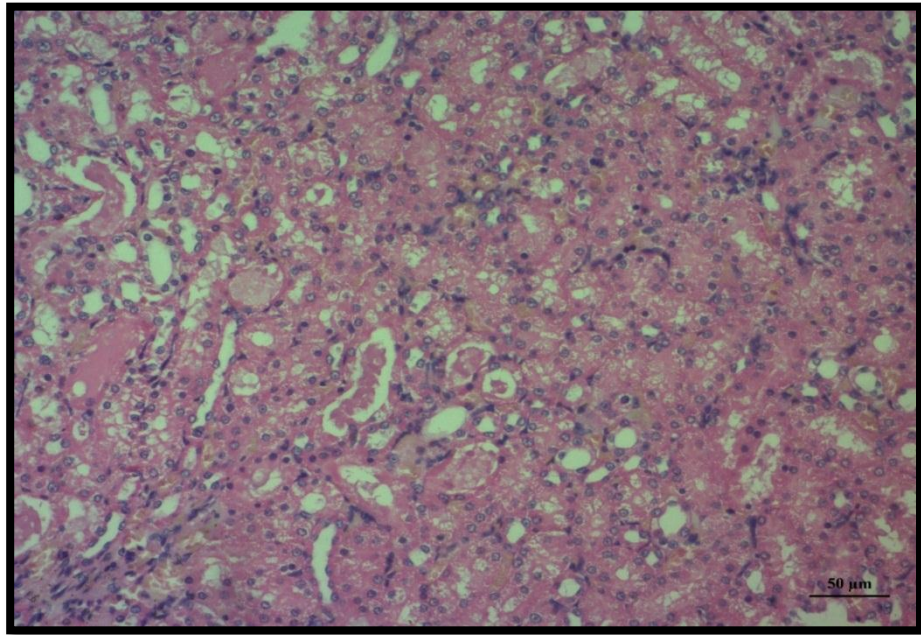


Plate IX: A microphotograph of kidney showing severe swelling of epithelial cells & severe hemorrhages interstitial space in streptozotocin induced diabetes (Group T2) (H & E).

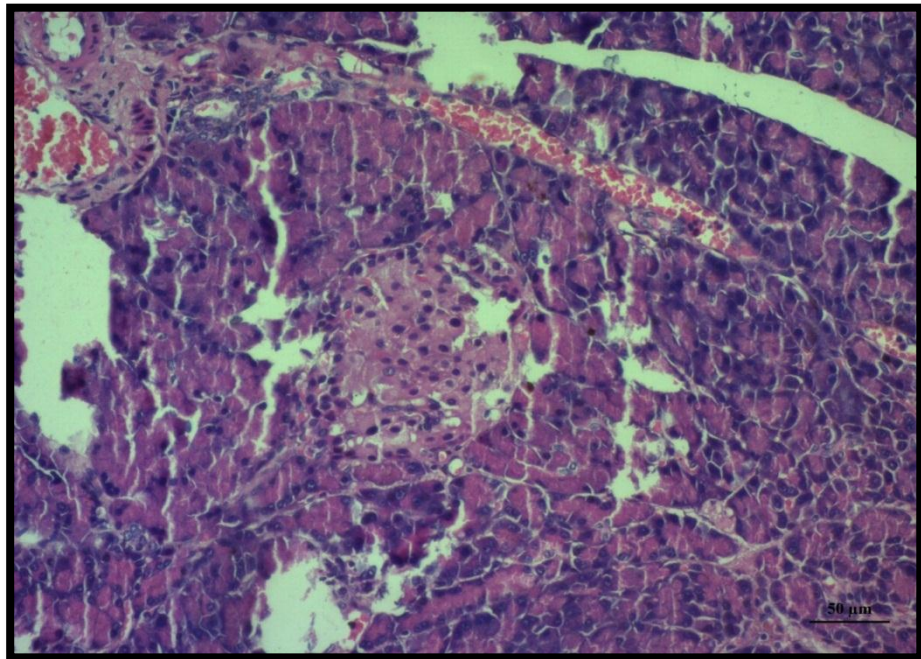


Plate X: A microphotograph of pancreas showing beta cells damaged, necrosis of beta cells and reduced population of beta cells in streptozotocin induced diabetes (Group T2) (H & E).

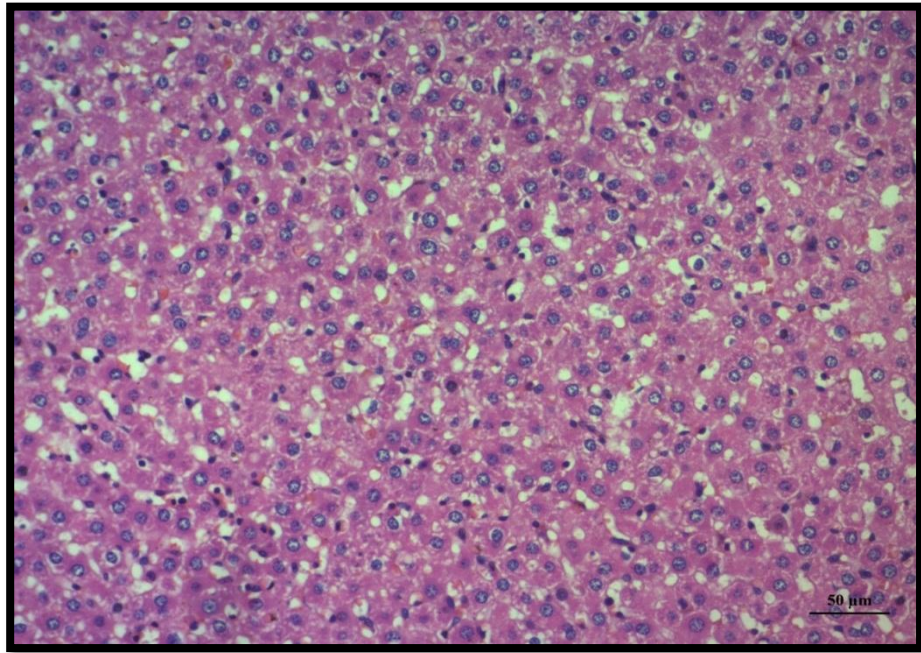


Plate XI: Section of liver showing normal orientation of hepatocytes in treatment with metformin (100 mg/kg) (Group T3) (H & E).

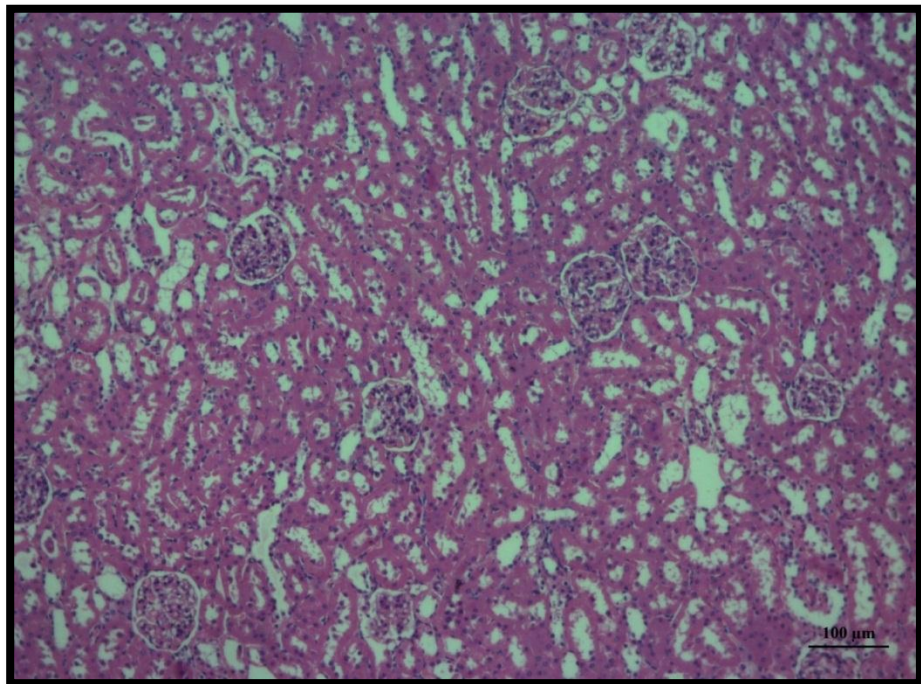


Plate XII: Section of kidney revealed normal orientation of glomeruli and tubules in treatment with metformin (100 mg/kg) (Group T3) (H & E).

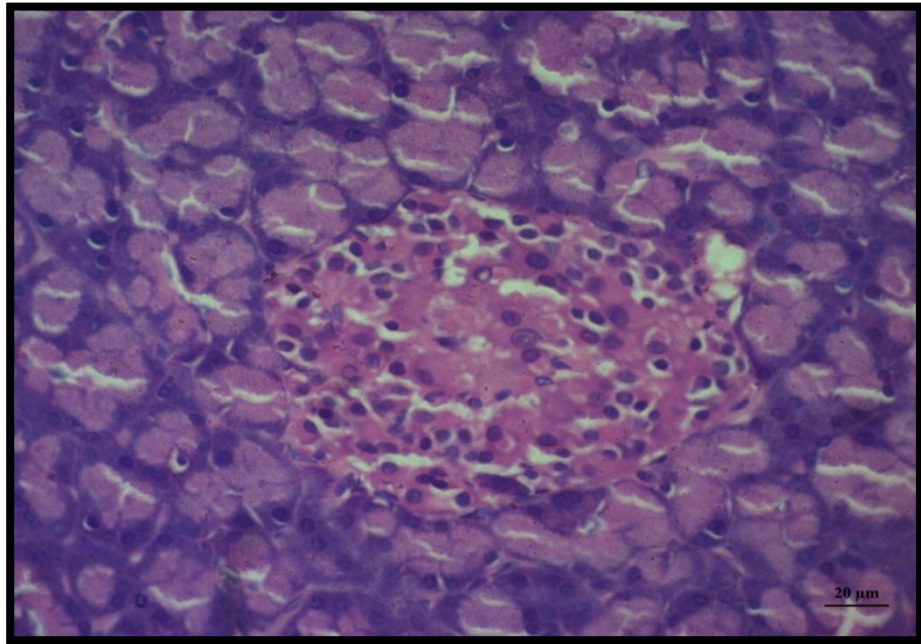


Plate XIII: Section of pancreas showing normal alfa & beta cells with increase the population of beta cells in treatment with metformin (100 mg/kg) (Group T3) (H & E).

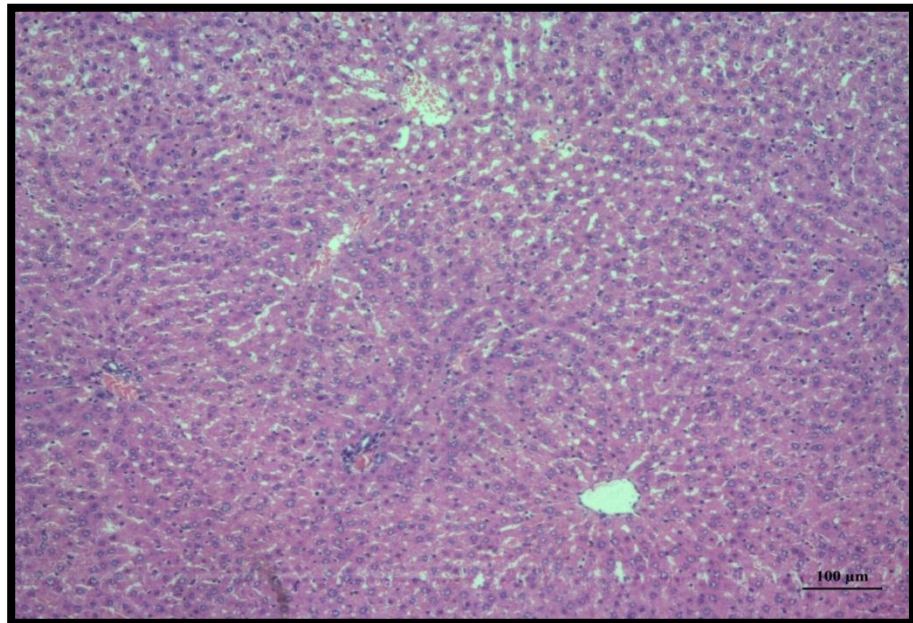


Plate XIV: Section of liver showing mild cynoidal congestion, the hepatocytes showed mild fatty changes & mild degree of degenerative changes in treatment with hydroethanolic leaf extract of *C. roseus* (200 mg/kg)(Group T4) (H & E).

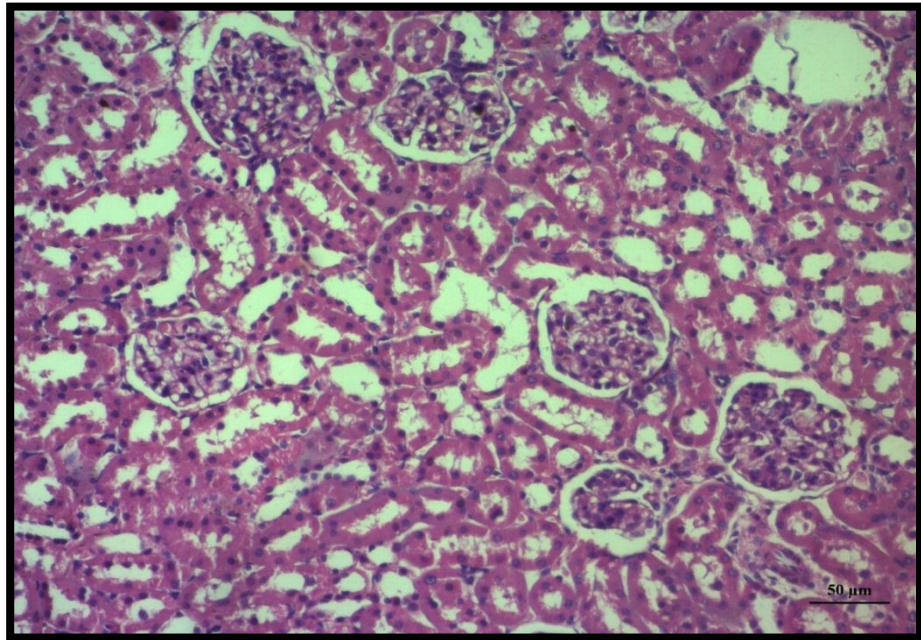


Plate XV: Section of kidney show mild to moderate necrosis & vacuolar degenerative changes in treatment with hydroethanolic leaf extract of *C. roseus* (200 mg/kg)(Group T4) (H & E).

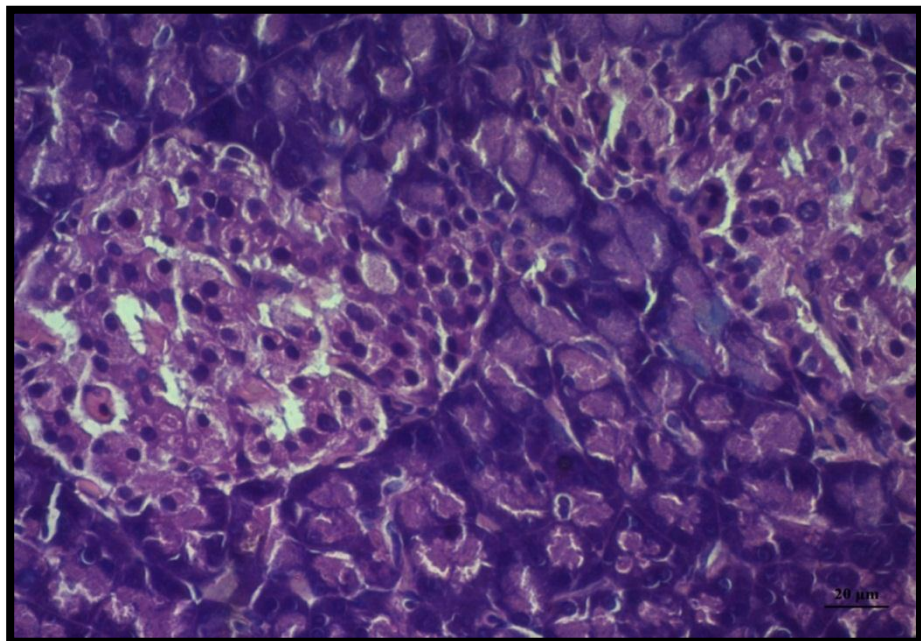


Plate XVI: Section of pancreas showing reduced area of islets of Langerhans, only few of the beta cells are necrosed other beta cells are quite healthy in treatment with hydroethanolic leaf extract of *C. roseus* (200 mg/kg) (Group T4) (H & E).

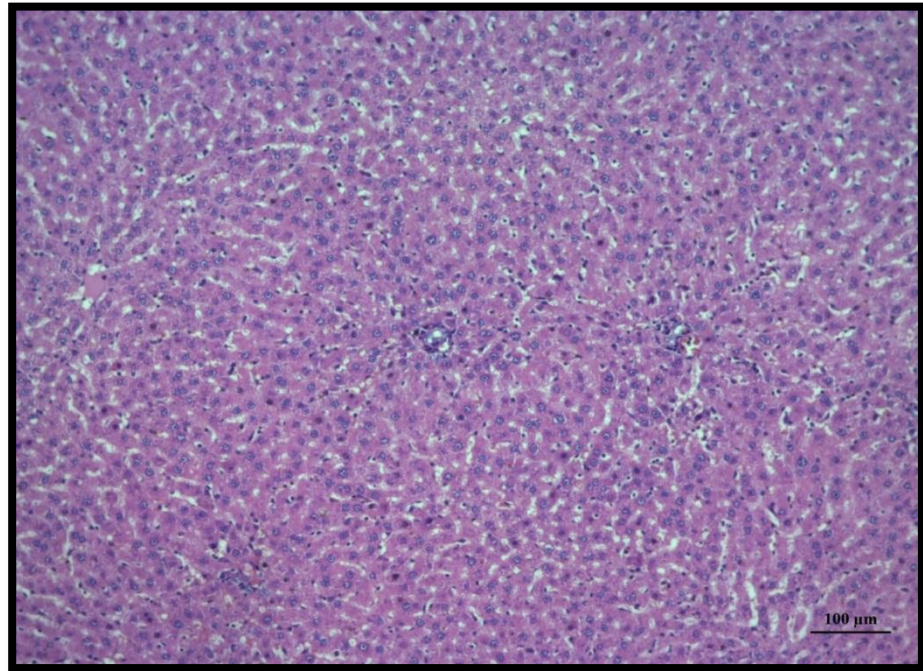


Plate XVII: Section of liver showing normal orientation of hepatocytes in treatment with hydroethanolic leaf extract of *C. roseus* (400 mg/kg) (Group T5) (H & E).

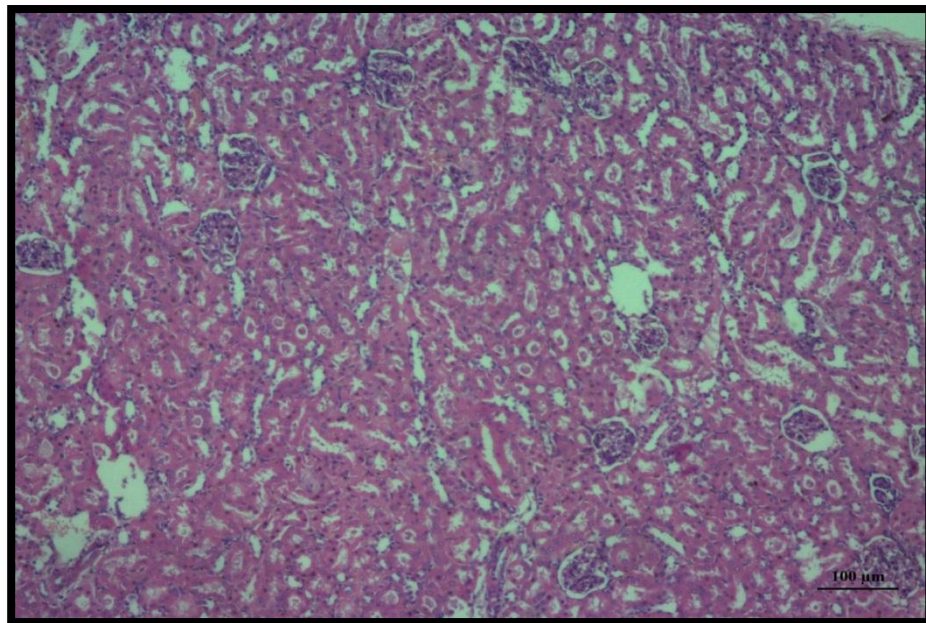


Plate XVIII: Section of kidney revealed normal orientation of glomeruli & tubules in treatment with hydroethanolic leaf extract of *C. roseus* (400 mg/kg) (Group T5) (H & E).

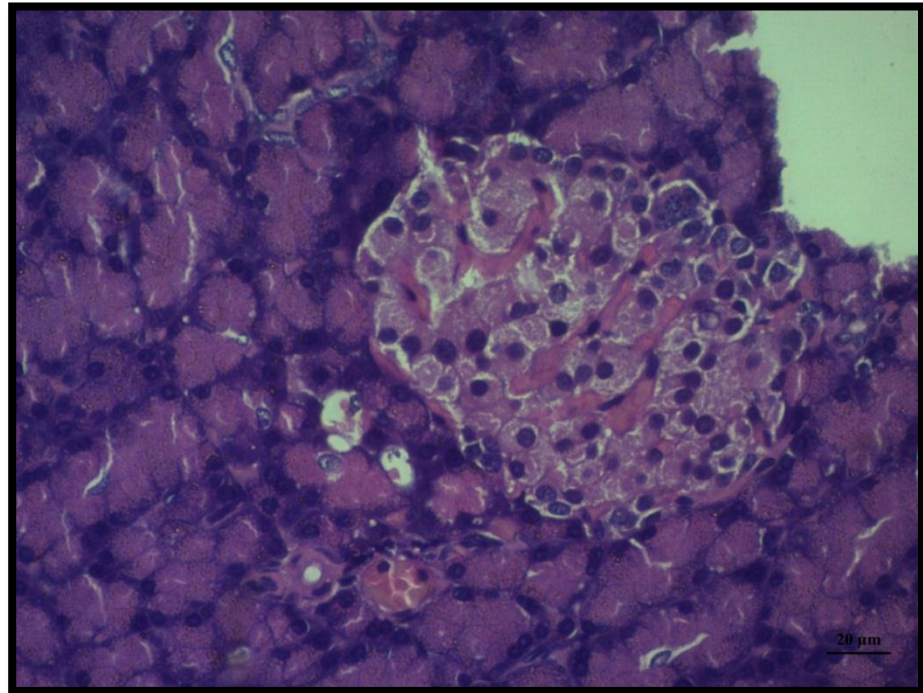


Plate XIX: Figure showing normal structure of pancreas and beta cells appear healthy in treatment with hydroethanolic leaf extract of *C. roseus* (400 mg/kg) (Group T5) (H & E).

SUMMARY AND CONCLUSION (S)

The present study was undertaken to study the diabetic effects caused by the Streptozotocin and to evaluate the efficacy of Hydroethanolic leaf extract of *C. roseus* on Streptozotocin induced diabetes in Wistar rats. While studying the antidiabetic properties of the hydroethanolic leaf extract of *C. roseus*, the percent extractability and qualitative phytochemical analysis of the extract was undertaken. The extractability percent was found to be 19 percent and the phytochemical analysis revealed the presence of the active principles like sterols, alkaloids, proteins, amino acids, saponins, tannins, glycosides, resin and Phenolic compounds. The parameters for studying the antidiabetic effects of the hydroethanolic leaf extract of *C.roseus* included the serobiochemical, hematological and histopathological changes in the pancreas, liver and kidney. The experiment was conducted for the period of 28days. A total of fifty Wistar rats having equal sex ratio were used in this study. The rats were divided into five groups containing ten rats in each group having equal sex ratio. The rats were allowed for acclimatization for a period 15 days prior to the start of the experiment. A separate identification mark was given to each rat by using picric acid. The five groups formed were labeled as T₁, T₂, T₃, T₄ and T₅.The rats from Group 'T₁' served as negative control and were treated daily with normal saline, while the rats from the group T₂, T₃, T₄ and T₅ were induced diabetes by intraperitoneal injection of Streptozotocin (40 mg/kg). Group T₂ served as positive control (Diabetes); Group 'T₃' received daily standard reference drug Metformin @ 100 mg/kg bd wt; Group T₄ and T₅ were treated with Hydroethanolic leaf extract of *C. roseus* @ 200 and 400 mg/Kg bd wt. respectively. During the experiment, blood was collected on 0th,14th and 28th day for biochemical and hematological studies. Weight of the animal were recorded individually at the end of every week till 28 th day of experiment. All the rats from each group were sacrificed on 28th day for the histopathological studies of pancreas, liver and kidney.

The results obtained in the present study indicated that the hydroethanolic leaf extract of *C. roseus* at dose of 200 and 400 mg/kg bd wt has the hypoglycemic effect. However, the hydroethanolic leaf extract of *C. roseus* at dose 400 mg/kg bd wt has more hypoglycemic effect as compared to the hypoglycemic effect at dose of 200 mg/kg bd wt. The magnitude of

hypoglycemic effect of Hydroethanolic leaf extract of *C. roseus* at the dose of 400 mg/kg bd wt was found to be completely the same when compared to the hypoglycemic effect by the standard reference drug metformin (100 mg/kg). The levels of total cholesterol, AST, ALT and BUN were increased in Streptozotocin induced diabetic rats on 0th day from the diabetic and all three treated groups. However, the Hydroethanolic leaf extract of *C.roseus* at dose of 200 and 400 mg/kg bd wt and metformin (100 mg/kg) decreased the elevated levels of total cholesterol, AST, ALT and BUN in Streptozotocin induced diabetic rats from 14th day onwards up to 28th day of the experiment. The levels of total protein were decreased in Streptozotocin induced diabetic rats on 0th day from the groups T₂, T₃, T₄ and T₅. While, the Hydroethanolic leaf extract of *C. roseus* at dose of 200 and 400 mg/kg bd wt and metformin (100 mg/kg) caused improvement in the total protein levels in diabetic rats from 14th day onwards up to the end of experiment.

The levels of hemoglobin and PCV were decreased in Streptozotocin induced diabetic rats on 0th day from the groups T₂, T₃, T₄ and T₅. However, the Hydroethanolic leaf extract of *C. roseus* at dose of 200 and 400 mg/kg bd wt and metformin (100 mg/kg) caused improvement in the hemoglobin and PCV levels in diabetic rats from 14th day onwards up to the end of experiment. Hydroethanolic leaf extract of *C.roseus* (400 mg/kg) caused more improvement in the hemoglobin and PCV levels as compared to that of metformin (100 mg/kg).

During the period of 28 days of the experiment it was observed that there was decrease in the body weight in diabetic rats from group T₂, T₃, T₄ and T₅ on 7th day as compared to the healthy rats from group T₁. However, the treatment with Hydroethanolic leaf extract of *C.roseus* at dose of 200 and 400 mg/kg bd wt and metformin (100 mg/kg) caused progressive improvement in the body weight in diabetic rats from 14th day up to 28th day of the experiment. , Hydroethanolic leaf extract of *C.roseus* (400 mg/kg) and metformin (100 mg/kg) showed marked improvement in the body weight of diabetic rats from group T₅ and T₃ respectively .

From the overall observation & result obtained in the present study, It is concluded that:

1. The blood glucose levels were increased in the rats due to Streptozotocin induced diabetes and were decreased by the treatment with the metformin (100 mg/kg) and the Hydroethanolic leaf extract of *C. roseus* at the dose rate of 400 mg / kg followed by 200 mg/kg as used in the present study. This indicates that the Hydroethanolic leaf extract of *C. roseus* possess antidiabetic property.
2. The biochemical parameters (viz. cholesterol, AST, ALT, BUN and total protein) heamatological parameters (Hb, & pcv) and body weights of rats were altered due to streptozotocin induced diabetes and corrected to normal with the treatment of *C.roseus* @ 400 mg /kg and the efficacy was comparable to the treatment with metformin @ 100 mg / kg. The *C.roseus* dose rate at 200 mg/kg was effective at lesser extent.
3. Hydroethanolic leaf extract of *C. roseus* possess phytochemicals such as alkaloids, proteins, amino acids, saponins, phytosterols, phenolic compound, resins, tannins, glycosides.

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VITA

The author Dr. Sneha Gurudas Dongare was born on 11th April 1993 in Etapalli, district, Gadchiroli, Maharashtra. She obtained first class in 10th and 12th from State board and completed graduation B.VSc and A.H. from College of Veterinary and Animal Sciences, Nagpur. During graduation she represented university at 10th Maharashtra state inter university youth festival in Folk dancing indradhanushya -2012 SRTM University (Nanded).

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2. National seminar on “Role of excipients in drug product design” at Gurunanak College of pharmacy, Nagpur.
3. Participated in National Post Graduate Students Conference On Peeping In Poultry Paradigm (NPSC) at Nagpur Veterinary College, Nagpur during 25th-26th April 2017.

THESIS ABSTRACT

- a) Title of the thesis : **“ANTIDIABETIC ACTIVITY OF HYDROETHANOLIC LEAF EXTRACT OF *Catharanthus roseus* ON STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS”.**
- b) Full name of student : **DONGARE SNEHA GURUDAS**
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- e) Year of award of degree : **2018**
- f) Major subject : **Veterinary Pharmacology and Toxicology**
- g) Total number of pages on the thesis : 92
- h) Number of words in the abstract : 466
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ABSTRACT

The present study was conducted in 50 Wistar rats, weighing around 150-200 g which were divided into five groups (T₁, T₂, T₃, T₄ and T₅) each comprising of ten rats having equal sex ratio. The prime objective of experiment was to study the antidiabetic activity of hydroethanolic leaf extract of *C. roseus* on streptozotocin induced-diabetes in Wistar rats. Diabetes was induced by

administering streptozotocin @ 40 mg/kg body weight in groups T₂, T₃, T₄ and T₅. Whereas, T₁ group was maintained as normal control. The group T₂ was maintained as diabetic control. The group T₃ was treated with metformin (standard antidiabetic drug) at 100 mg/kg bd wt. Groups T₄ and T₅ were treated with hydroethanolic leaf extract of *C. roseus* @ 200 and 400 mg/kg b. wt. respectively. In the experiment, blood was collected on 0th, 14th and 28th day for biochemical and hematological studies and at the end of experiment all the rats were sacrificed for histopathological studies.

All the streptozotocin induced diabetic rats from the treatment groups showed increased blood glucose levels. It indicates damage to β -cells of islets of Langerhans by the streptozotocin causing disturbance in insulin secretion and thereby leading to increase in blood glucose levels. Treatment to the diabetic rats from group T₄ and T₅ by hydroethanolic leaf extract of *C. roseus* caused significant decrease in the elevated blood glucose levels of the rats from these groups on 14th and 28th day of the experiment.

Hematological, biochemical and body weight parameters levels altered due to the streptozotocin induced diabetes were observed to be normal range by the treatment with *C. roseus* leaf extract @ 400 mg/ kg followed by the dose rate of 200 mg/ kg.

Streptozotocin induced diabetic rats showed no morphological changes in pancreas, liver and kidneys. The predominant histopathological alterations in pancreas of diabetic rats were complete destruction of β cells of islets of Langerhans with severe fibrosis, necrotic changes and reduction in the population of β cells of islets. The liver of the diabetic rats exhibited severe congestion in between lobules, severe fatty changes in hepatocytes, degenerative changes in hepatocytes and vacuolization of hepatocytes. There were severe hemorrhages in interstitial spaces along with tubular necrotic areas and glomerulopathies including degenerative changes in tubular epithelium and vacuolar changes in the kidneys of the diabetic rats. The histopathological alterations in respect of pancreas, liver and kidneys of diabetic rats from groups T₃, T₄ and T₅ were similar to the diabetic rats from group T₂, but where of milder degree. By using hydroethanolic extract complete reversal of the histological changes were obtained. The overall inference of the study is that the

hydroethanolic leaf extracts of *C. roseus* at all the doses viz. 200 and 400 mg/kg bd wt. used showed protective effect against the damage caused by streptozotocin induced diabetes.

प्रबंध सारांश

अ) प्रबंध शिर्षक	:	“विस्तार जातीच्या उंदरामध्ये सदाफुलीच्या पानाचा पाण्यामधील अर्काचा स्ट्रेप्टोजोटोसीन मधुमेहावर संरक्षणात्मक प्रभाव”
ब) विद्यार्थ्यांचे संपूर्ण नाव	:	डोंगरे स्नेहा गुरूदास
क) मुख्य मार्गदर्शकाचे संपूर्ण नाव आणि पत्ता	:	डॉ. ए. पि. सोमकुवर सहयोगी प्राध्यापक औषधनिर्माण व विषशास्त्र विभाग नागपूर पशुवैद्यकीय महाविद्यालय, नागपूर.
ड) पदवी	:	स्नातकोत्तर पदवी (एम. व्ही. एस. सी.)
इ) पदवी प्रदान करण्याचे वर्ष	:	२०१८
फ) मुख्य विषय	:	औषधनिर्माण शास्त्र व विषशास्त्र
ग) प्रबंधामधील एकूण पाने	:	९२
ह) प्रबंध सारांशमधील एकूण शब्द	:	३५०
ई) विद्यार्थ्यांची सही	:	
ज) शोध प्रबंध पुढे पाठविणाऱ्या अधिकाऱ्याची सही, नाव व पत्ता	:	सहयोगी अधिष्ठाता नागपूर पशुवैद्यकीय महाविद्यालय, नागपूर

सारांश

सदर अभ्यास हा सुमारे १५०—२०० ग्रॅम वजन असणाऱ्या विस्तार जातीच्या ५० उंदरावर करण्यात आला व त्यांना टी १ ते टी ५ या पाच गटात विभागले, प्रत्येक गटात १० उंदिर ठेवण्यात आले. स्ट्रेप्टोजोटोसीन मधुमेहाविरोधी सदाफुलीच्या पानांचा हायड्रोईथॅनालीक

अर्काचा संरक्षणात्मक परिणाम पाहणे हा सदर अभ्यासाचा मुख्य उद्देश आहे. मधुमेह हा स्ट्रेप्टोझोटोसीन ४० मि. ग्रॅ./किलो प्रमाणात टोचुन उत्पन्न करण्यात आला. गट टी.१ फक्त सामान्य पाणी देवून सामान्य नियंत्रण गट म्हणून ठेवण्यात आला. गट टी.२ हा मधुमेह नियंत्रण गट म्हणून केवळ तुलना करण्यासाठी ठेवला होता. गट टी.३ मध्ये मेटफॉर्मिन (मधुमेह विरोधी औषध) १०० मिग्रॅ./किलो देवून उपचार करण्यात आला. गट टी.४ व टी.५ यांना सदाफुलीच्या पानांचा हायड्रोईथॅनॉलीक अर्क प्रत्येकी २०० आणि ४०० मिग्रॅ./किलो देवून उपचार केला. प्रयोगादरम्यान, जैवरासायनिक घटक आणि रक्त तपासणी घटकांचे निरीक्षण करण्यासाठी शुन्य, चौदा आणि अठ्ठाविसाव्या दिवशी रक्ताचे नमुने जमा करण्यात आले आणि प्रत्येक गटातील सर्व उंदीर हे अवयवांमधील विकृती शास्त्रीय बदल अभ्यास करण्यासाठी अपर्ण करण्यात आले.

सर्व उपचार गटातील उंदरांचे मधुमेहामुळे रक्तातील शर्कराची पातळी वाढली. इन्सुलिनच्या स्त्राव करणाऱ्या आईसलेट्स च्या बी पेशीचे नाश/नुकसान झाले. चौदा व अठ्ठाविस दिवसाच्या उपचारानंतर रक्तातील शर्करेची पातळी गट टी ४ व टी ५ मध्ये लक्षणीय कमी झाली.

उंदरामध्ये प्रयोगिक रित्या निर्मित मधुमेहामुळे रक्तातील जैवरासायनिक घटक, रक्ततपासणी व वजना मधील बदल मोठ्या प्रमाणात आढळून आले. परंतु, सदाफुलीच्या पाणांच्या अर्क दिल्यामुळे मधुमेहामुळे झालेले सर्व बदल पुर्वरत होण्यास मदत झाली. यामध्ये सदाफुलीच्या पाणांच्या अर्काची मात्रा ४०० मि. ग्रॅ./कि. जास्त प्रभावी तर त्यापाठोपाठ २०० मि. ग्रॅ./कि. ही मात्रा प्रभावी ठरली.

स्ट्रेप्टोझोटोसीन मधुमेहामध्ये उंदरांचे स्वादुपिंड, यकृत आणि मुत्रपिंड यात रूपशास्त्रीय कोणताही बदल दिसला नाही. स्वादुपिंडामध्ये प्रबळ विकृती शास्त्रीय बदल दिसले त्यामध्ये आयलेट्स च्या बी पेशीच्या संख्येमध्ये घट दिसून आली, पेशी समुह नष्ट झाले आणि इन्सुलिनच्या स्त्रावात कपात झाली. यकृतामध्ये हिपॅटोसाईट्स मध्ये तीव्र फॅटी बदल, हिपॅटोसाईट्स ची झीज आणि हिपॅटोसाईट्स च्या व्हॅक्युओलर दरम्यान तीव्र रक्तसंचय. मुत्रपिंडांमध्ये पेशीचा थर व्हॅक्युओलर ची झीज, ट्युबुलर इपीथेलियल पेशींचा नाश आणि

तीव्र रक्त स्राव असे बदल दिसले. गट टी३, टी४ व टी५ मध्ये विकृती शास्त्रीय बदल गट टी२ च्या प्रमाणात लक्षणिय कमी होते सदाफुलीच्या पांनांचा हायड्रोईथॅनालीक अर्काचा वापर करून विकृती शास्त्रीय बदलामध्ये पुर्ण परावर्तन होऊन सामान्य झाले. अभ्यासातील सदाफुलीच्या पांनांचा हायड्रोईथॅनालीक अर्काचे सर्व मात्राचे (२०० आणि ४०० मिग्रॅ/किलो) स्ट्रेप्टोझोटोसीन मधुमेहाद्वारे झालेल्या नुकसानावर संरक्षणात्मक प्रभाव दिसुन आले आहे.