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HYPOGLYCAEMIC EFFECT OF *Coccinia indica* (IVY GOURD) LEAVES AND ITS INTERACTION WITH GLIBENCLAMIDE IN DIABETIC RATS

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Thesis submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled **"HYPOGLYCAEMIC EFFECT OF *Coccinia indica* (IVY GOURD) LEAVES AND ITS INTERACTION WITH GLIBENCLAMIDE IN DIABETIC RATS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy **ELIZA JOSE**

CERTIFICATE

Certified that this thesis, entitled **"HYPOGLYCAEMIC EFFECT OF *Coccinia indica* (IVY GOURD) LEAVES AND ITS INTERACTION WITH GLIBENCLAMIDE IN DIABETIC RATS"** is a record of research work done independently by **Dr. Eliza Jose,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, associateship or fellowship to her.

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Dedicated to

Mother Mary

and my respectful teachers

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Introduction

1. INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by common feature of chronic hyperglycaemia with disturbance in carbohydrate, fat and protein metabolism. The consequences of diabetes include nephropathy, neuropathy and cardiovascular disorders. It was known to ancient Indian physicians as 'Madumeha'. It is caused by inherited or acquired deficiency of insulin production or resistance to the action of produced insulin (Fakeye *et al.*, 2007).

Diabetes is a major health problem in the developed as well as developing countries. It is ranked seventh among the leading causes of death, and third when all its fatal complications are taken into account (Trivedi *et al.*, 2004). The prevalence of diabetes for all age groups world wide was estimated to be 2.8 per cent in 2006 and will be 4.4 per cent by 2030. India ranks first in the estimated cases of diabetes with 31.7 million people with diabetes in 2000 and an estimated value of 79.4 million by 2030 (Wild *et al.*, 2004). In animals, diabetes mellitus occurs more frequently in dogs and cats, with an incidence of approximately 0.2-0.5 percent.

Treatment of diabetes mellitus consists fundamentally of managing the diet and/or therapies, which attempt to normalize metabolic activities, namely blood glucose levels. Although many drugs are available to manage diabetes, in most instances these are expensive and may also have adverse effects like hypoglycemia and obesity (Sharma *et al.*, 2008). In the present scenario, it is necessary to look for new drugs and strategies that can be used to manage this metabolic disorder.

Insulin therapy affords effective glycemic control but have drawbacks like ineffectiveness of oral administration, short shelf life, requirement of constant refrigeration and fatal hypoglycemia in case of excess dosage (Grover *et al.* 2000). Another major factor which could have implications with respect to insulin therapy is the presence of residual endogenous insulin secretion in type 2 diabetic patients

(Ryssy, 2001). Chronic treatment with sulphonyl ureas and biguanides are also associated with toxicity (Mallick *et al.*, 2006). Glibenclamide (sulphonyl urea) is one of the most commonly used oral hypoglycemic agent. It has some drawbacks in the treatment of diabetes. It has been reported that during chronic administration of sulphonylureas (glibenclamide) the insulin-releasing effect is prone to diminish or become lost because of decrease in proinsulin biosynthesis (Bailey, 1991). Further Babu *et al.* (2003) has cited that hypoglycemia is the most adverse effect of sulphonyl urea drugs. It was found by Tessier *et al.* (1994) in a survey on elderly diabetic patients that hypoglycaemic reactions were significantly more frequent with glibenclamide than with gliclazide. So this study focuses on finding an alternative which can minimize the side effects of hypoglycemic agents.

Plants have been the major source of drug for the treatment of diabetes mellitus in Indian system of medicine and other ancient systems of the world. India is a country with a vast reserve of natural resources and a rich history of traditional medicine. So research on medicinal plants is an important facet of biomedical research in India. More than 400 plants with glucose lowering effects are known. Out of many, only a few have been scientifically validated as per modern system of medicine. Phytomedicines are the natural answer to diabetes mellitus which are often locally available at affordable cost. The herbal market in the developed countries also is experiencing unprecedented growth. Hence the evaluation of hypoglycemic effect of plants that are easily and cheaply available is of much practical significance and utility. Phytomedicines are less expensive, easily available and have less side effects due to prolonged administration. All these highlights the importance of searching for an alternate therapy with plant derived drugs having not only insulinotropic effect but also increase insulin sensitivity. An ideal oral treatment for diabetes would be a drug that controls the glycemic level and prevents the development of atherosclerosis and other complications of diabetes.

Coccinia indica, which is grown abundantly in India, has been widely used in the traditional treatment of diabetes mellitus. There were reports on hypoglycemic action of pectin isolated from the fruit of *Coccinia indica.* Contradictory findings on the antidiabetic activity of different parts of *Coccinia indica* have been reported (Dhanabal *et al.,* 2004). The present study is directed towards investigation on the hypoglycemic, hypolipidemic and antioxidant effect of ethanolic extract of *Coccinia indica* leaves.

It is mentioned in ancient texts such as Charak Samhita as cited by Sharma *et al.* (2008) that a single herb exerts different actions on many diseases and that each herb may have a dominating effect and other comparatively subsidiary effect. Nowadays more and more people are using herbs and phytomedicines along with allopathic medicine. There is possibility of interaction between phytomedicine and allopathic medicine. These interactions may be partially additive, additive or synergistic, thereby the herbal drugs potentiates the action of the conventional drug. Conversely the herb may be directly antagonistic to the actions of the drug.

Recent trend observed in anti-diabetic treatment is the concomitant use of modern medicines and herbal remedies in the therapeutic regimen. These interactions may result in beneficial or adverse reactions (Blumenthal, 2000). So this study was taken with an objective of combining *Coccinia indica* with standard oral hypoglycemic drug, glibenclamide in the treatment of diabetes and to evaluate the and assess whether the combination can safely and effectively be used to obtain a sustained anti-diabetic effect. The study also aimed to evolve an effective and safe dose of the combination. A study was also conducted to assess the toxicity of *Coccinia indica* leaves to authenticate the safety of the drug.

Review of Literature

2. REVIEW OF LITERATURE

2.1. PHARMACOLOGICAL EFFECTS OF *Coccinia indica*

Khan *et al.* (1980) observed the glucose tolerance improving ability of *Coccinia indica.* According to the study human diabetic subjects who received homogenized and freeze-dried leaves of *Coccinia indica* for six weeks showed significant improvement in glucose tolerance when compared to the group which received placebo.

Hossain *et al.* (1992) studied the hypoglycemic effect of *Coccinia indica* in relation to the gluconeogenic enzyme, glucose-6-posphatase in normal fed and 48 hours starved rats. It was found that starvation induced three fold increase in the activity of glucose-6-phosphatase which was depressed by 19 per cent on feeding extract of *Coccinia indica* leaves. They suggested that the hypoglycemic effect of *Coccinia indica* is due to the depression of the key gluconeogenic enzyme glucose-6-phosphatase.

Shibib *et al.* (1993) conducted studies on the depression of the hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1-6-biphosphatase and elevation of both liver and red-cell shunt enzyme glucose-6-phosphatase dehydrogenase (G6PDH) by the oral administration of *Coccinia indica* leaf extract at a dose of 200 mg/kg body weight in streptozotocin induced diabetic rats. The results indicated that *Coccinia indica* extract lowered blood glucose by depressing its synthesis due to depression of the key gluconeogenic enzymes glucose-6-phosphatase and fructose-1-6-biphosphatase and also by enhancing glucose oxidation by the shunt pathway through activation of its principal enzyme G6PDH.

In diabetes, there is reduced activity of enzyme Lipoprotein lipase (LPL) and raised level of Glucose-6-phosphatase (G-6-Pase) and Lactate dehydrogenase (LDH). In human diabetic subjects, dried extract of *Coccinia indica* in doses of 500 mg/kg body weight orally corrected the elevated enzymes G-6-P ase, LDH in glycolytic pathway and restored the LPL activity in lipolytic pathway with the control of hyperglycaemia. This indicated the insulin like activity of the ingredients present in the same (Kamble *et al.*, 1998).

The effect of *Coccinia indica* on plasma antioxidants was reported by Venkateswaran and Pari (2003). They found that oral administration of ethanolic extract of *Coccinia indica* leaves for 45 days in streptozotocin induced diabetic rats resulted in significant reduction in plasma thiobarbituric acid reactive substances, hydro peroxides, vitamin E and ceruloplasmin and a significant increase in plasma vitamin C and reduced glutathione which clearly showed the antioxidant property of *Coccinia indica* leaf extract.

An investigation was made on the effect of *Coccinia indica* and *Abroma augusta* on glycemia, lipid profile and on indicators of end-organ damage in streptozotocin induced diabetic rats by Eshrat (2003). The diabetic rats were treated for eight weeks with 300 mg of water extract of combination of *A. augusta* and *C. indica*. The combination treatment showed good hypoglycemic and hypolipidemic effect and also corrected complications associated with diabetes such as retinopathy, neuropathy, nephropathy and musculopathy.

Daily administration of extract of *Coccinia indica* leaves at the dose rate of 200 mg/kg body weight for 45 days to streptozotocin induced diabetic rats decreased the concentrations of lipids and fatty acids *viz.*, palmitic, stearic and oleic acid (Pari and Venkateswaran, 2003).

Modulatory effect of *Coccinia indica* on aortic collagen was proven by Venkateswaran *et al.* (2003) by their studies on streptozotocin induced diabetic rats. The administration of *Coccinia indica* leaf extract for 45 days significantly reduced the accumulation and cross linking of collagen. The α/β and type I/type III ratio of pepsin soluble collagen were also restored by the treatment.

Dhanabal *et al.* (2004) conducted a study on the blood glucose level in alloxan-induced diabetic rats to test a few fractions and delineate the most active fraction of *Coccinia indica.* It was found that alcoholic extract of *Coccinia indica* was more active in reducing blood glucose level and among the fractions tested, only the toluene sub-fraction was found to be effective in reducing blood glucose level.

Rahuman and Venkatesan (2008) compared the larvicidal efficacy of different extracts of five cucurbitaceous plant leaf extract *viz.*, *Citrullus colocynthis*, *Coccinia indica*, *Cucumis sativus*, *Momordica charantia* and *Trichosanthes anguina* against the early fourth instar larvae of *Aedes aegypti* L and *Culex quinquefasciatus.* After 24 hour of exposure, the highest larval mortality was found in petroleum ether extract of *C. colocynthis*, methanolic extracts of *C. indica*, *C. sativus*, *Mommordica charantia* and acetone extract of *T. anguina.*

2.2. OTHER PLANTS HAVING HYPOGLYCEMIC AND ANTIOXIDANT EFFECT

Clinical evaluation of Saptarangi (*C.esculenta*) as a hypoglycemic agent in the treatment of diabetes mellitus in human diabetics was done by Kashyap

and Ahuja (1968). Crude extract of Saptarangi when administered orally at the dose of 25 g lead to fall in blood sugar by 15 per cent during four hour fasting.

The effect of *Trichosanthes dioica* seeds at dose rate of 7g/kg for two weeks on the total serum cholesterol, HDL cholesterol, triglycerides and phospholipids in normal and mild human diabetic subjects was studied by Sharma *et al.* (1990). The results showed a progressive fall in the fasting total serum cholesterol and triglycerides and increase in the levels of HDL cholesterol and phospholipids.

An experiment was conducted to study the effect of neem seed kernel powder on the activity of serum enzymes like alkaline phosphatase, acid phosphatase, lactate dehydrogenase, glucose 6-phosphatase and HMGCoA reductase activity in liver and intestine of alloxan diabetic rabbits by Bopanna *et al.* (1997). They observed that oral feeding of neem kernel powder (500 mg/kg) resulted in decrease in HMGCoA reductase activity and of lipogenic enzymes like glucose 6-phosphatase and lactate dehydrogenase and concluded that neem seed kernel powder has significant hypoglycemic and hypolipidemic effect.

Comparison of the effect of defatted seeds, whole crushed seeds and oil of *Prunus amygdalus* on lipid profile was made by Sunita and Singh (1997) in normal healthy rabbits. They observed that during 60 days of the treatment, the animals showed a progressive and significant reduction in serum total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, with increase in phospholipids and HDL cholesterol. The whole crushed seeds produced more marked effect and defatted seeds the least.

Effect of aqueous leaf extract of *Zizyphus jujube* on blood glucose level and lipid profile in diabetic rats was studied by Ignacimuthu and Amalraj (1998)

and found that the extract produced significant lowering of fasting blood glucose, serum cholesterol, serum triglycerides and phospholipids when administered for 30 days.

According to Krishnakumar *et al.* (1999) the administration of *Salacia oblonga* root extract in diabetic rats at 250 mg/kg dose for one month caused significant reduction in thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD) and hydro peroxides indicating the antioxidant activity of the extract.

Grover *et al.* (2000) studied the antihyperglycaemic effect of aqueous and alcoholic extracts as well as lyophilized powder of *Tinospora cordifolia* and *Eugenia jambolana* in experimental diabetes and found that maximum reduction of 73.5 per cent and 70.37 per cent in glucose levels was seen in animals receiving 200 mg/kg per day of lyophilized powder of *Eugenia jambolana* and 400 mg/kg per day of aqueous extract of *Tinospora cordifolia* respectively. On comparison, the reduction in hepatic and skeletal muscle glycogen content in diabetic animals was prevented to an extend by treatment with *Eugenia jambolana* indicating its ability to stimulate insulin release from β cells, but *Tinospora cordifolia* failed to exert any statistically significant changes in glycogen content.

Khosla *et al.* (2000) studied the effect of aqueous extract of *Azadirachta indica* leaves and seed oil on blood glucose levels in normal and alloxan induced diabetic rabbits and reported that the leaf extract at a rate of 500 mg/kg and seed oil 5 mg/kg led to significant fall in the concentration of blood glucose level in normal and alloxan induced diabetic rabbits.

Prince and Menon (2000) observed the effect of oral administration of aqueous extract of *Tinospora cordifolia* root extract (0.5 g/kg body weight) for 42 days in alloxan diabetic rats. There was an increase in body weight, total haemoglobin and hepatic hexokinase and a significant reduction in blood glucose and brain lipids.

The hypoglycemic effect of aqueous extract of *Boerhavia diffusa* leaves in alloxan induced diabetic rats was tested by Chude *et al.* (2001). When tested at 0, 2, 4, 6, 8 and 24 hours, the animals showed a non dose related decrease in blood glucose level at doses of 100,200 and 400 mg/kg bodyweight.

On the basis of the studies conducted by Kameswara and Appa (2001) on the effect of aqueous, ethanolic and hexane fractions of *Syzygium alternifolium* seeds in lowering blood glucose level, it was found that the aqueous extract produced maximum blood glucose lowering effect in both normal and alloxan diabetic rats and the maximum effect was obtained at the dose rate of 0.75 gram per kg body weight.

Rao *et al.* (2001) noticed that the aqueous extract of *Momordica cymbalaria* at a dosage of 0.5 g/kg body weight was showing maximal glucose lowering effect in alloxan induced diabetic rats.

Satyanarayana *et al.* (2001) studied the effect of various fractions of methanol extract of *Cocculus hirsutus* on blood glucose levels of diabetic rats and found that chloroform and ethyl acetate fractions showed significant hypoglycemic activity by reducing blood sugar levels from 338.6±21.9 to 280.0±18.9 mg/100 ml and 315.8±28.9 to 290.0±18.9 mg/100 ml respectively.

The influence of chronic treatment with *Enicostemma littorale* in non-insulin-dependent diabetic (NIDDM) rats was studied by Murali *et al.* (2002). They found that the extract at the dose rate of 2 g/kg orally for 6 weeks produced an increase in insulin sensitivity, normalized dyslipidaemia and provided nephroprotection in diabetic rats.

According to Puri *et al.* (2002), the administration of active fractions of Fenugreek seeds at the dose of 50 mg/kg for 15 days significantly attenuated the glucose tolerance curve and improvement in the glucose induced insulin response in sub diabetic and mild diabetic rabbits. Further the prolonged administration for 30 days in severely diabetic rabbits, the hypoglycemic effect was slow but sustained without any risk of developing severe hypoglycemia.

Sabu and Kuttan (2002) opined that methanolic extract of *Terminalia chebula, Terminalia belerica, Emblica officinalis* and their combination named 'Triphala' at a dose rate of 100 mg/kg body weight showed significant reduction in blood glucose. The extracts were found to scavenge the super oxides and inhibited the generation of lipid peroxides indicating that this preparation decreases the oxidative stress in diabetes.

Antidiabetic effect of *Gymnema monatum* leaves and its ability to combat oxidative stress in experimental diabetes was studied by Ananthan *et al.* (2003). Oral administration of 200 mg/kg body weight of the ethanolic extract of the leaves for 3 weeks in alloxan induced diabetic rats resulted in a significant reduction in blood glucose and increase in plasma insulin, where as the effect of 50 and 100 mg was not significant. The alcoholic extract also resulted in decreased free radical formation shown by increase in reduced glutathione (GSH), ascorbic acid (vitamin C) and α-tocopherol and decrease in lipid peroxides.

Daily administration of *Cassia kleinii* leaf extract at a dose of 200 mg/kg in streptozotocin induced diabetic rats improved liver glycogen content when compared to the diabetic control and also restored the serum glucose levels almost to nearer to the normal values by the 15th day of the treatment (Babu *et al.*, 2003).

Santhakumari *et al.* (2003) observed the modulation of oxidative stress parameters in streptozotocin induced diabetic rats by the treatment with *Piper betle* leaf powder suspension. Animals were made diabetic by single intraperitoneal injection of streptozotocin at the dose rate of 50 mg/kg body weight and the treatment was done with *P.betle* leaf powder at the rate of 75 mg and 150 mg/kg body weight orally for 30 days. There was a significant reduction in TBARS, hydro peroxides and α-tocopherol with a significant elevation in ascorbic acid in drug treated diabetic rats.

Babu *et al.* (2004) reported the antihyperglycaemic and antioxidant effect of 'hyponidd'- a herbomineral formulation composed of extracts of 10 medicinal plants- *Momordica charantia, Melia azadirachta, Pterocarpus marsupium, Tinospora cordifolia, Gymnema sylvestre, Enicostemma littorale, Emblica officinalis, Eugenia jambolana, Cassia auriculata and Curcuma longa.* Oral administration of hyponidd at 100 mg/kg and 200 mg/kg for 45 days resulted in significant reduction in blood glucose and increased levels of hepatic glycogen and total haemoglobin. Antioxidant property was shown by significant elevation of plasma reduced glutathione and vitamin C.

The level of blood glucose and malondialdehyde (lipid peroxide) in type 2 human diabetic patients was studied by Chandra *et al.* (2004) after the administration of *Emblica officinalis* commercial formulation (250 mg fruit powder) for 60 days. There was a decrease in blood glucose level from 203±82 mg/dl to189±55 mg/dl and a significant decrease in malondialdehyde levels from 7.55±2.3

nM to 5.36±0.8 nM. The study revealed that there is no direct relationship between blood glucose levels and malondialdehyde levels in type 2 diabetic patients.

Ravi *et al.* (2004) investigated the effect of *Eugenia jambolana* seed kernel on antioxidant defense system in streptozotocin induced diabetic rats by evaluating the activities of various antioxidant enzymes and histopathological examination of the pancreas. *Eugenia jambolana* rich in flavonoids accounted for the scavenging of free radicals and protective effect on antioxidant enzymes. The vascular degenerative changes in the islets of diabetic rats showed stages of regeneration in *Eugenia jambolana* treated group.

Sathyan (2004) found that oral feeding of alcoholic extract of *Azadirachta indica, Ocimum sanctum* and *Tinospora cordifoliae to* diabetic rats (200 mg/kg body weight) reduced the plasma blood glucose level from the pretreatment value of 250.87± 4.56 to 103.56± 6.78 mg/dl after 42 days of treatment.

The hypoglycemic and antioxidant effect of aqueous extract of *Aegle marmelos* in alloxan induced diabetic rats was studied by Upadhya *et al.* (2004). It was found that administration of *Aegle marmelos* at the dose rate of 500 mg/kg for four weeks decreased plasma glucose and urea level and increased the GSH level.

Trivedi *et al.* (2004) conducted studies on the hypoglycemic and hypolipidemic effect of Shilajit, a herbo-mineral drug in normal and alloxan induced adult albino rats. A significant reduction ($P<0.001$) in blood glucose level, total cholesterol and triglyceride was seen in normal rats at the dose of 100 mg/kg body weight after 4 weeks. In diabetic rats the peak reduction in blood glucose level was observed at the end of 2nd week of treatment which remained stable up to 4th week at

three dose rates of 50,100 and 200 mg/kg. Similar effects were also observed in cholesterol and triglycerides.

Vinuthan *et al.* (2004) were of the opinion that the hypoglycaemic effect of *Murraya koenigii* leaves in alloxan induced diabetic rats may be mediated through stimulating insulin synthesis and/or secretion from the β cells of pancreatic islets of Langerhans.

Akhani *et al.* (2005) investigated the antidiabetic activity of *Zingiber officinalis* Roscoe in streptozotocin-induced non-insulin dependent diabetic rats by administering the fresh juice (4ml/kg) and methanolic extract (0.5 g/kg) and found that *Zingiber officinalis* produced a significant ($p<0.05$) increase in area under curve of insulin ($AUC_{insulin}$) values. This may be due to the presence of galanolactone and gingerol in *Z. officinalis* which stimulate the secretion of insulin by competitively antagonizing 5-HT receptors.

Antia *et al.* (2005) opined that administration of aqueous extract of *Persea Americana* (100-200 mg/kg) in alloxan-diabetic rats produced a significant reduction in blood glucose level in a dose dependent manner after a single dose of the extract, as well as following prolonged treatment for 7 days.

Kaleem *et al.* (2005) studied the protective effects of lyophilized aqueous extract of *Piper nirgum* and *Vinca rosea* in alloxan induced diabetic rats by administering the extract at the dose of 0.5 ml per rat orally for 4 weeks. *Piper nirgum* and *Vinca rosea* were able to reverse the lipid peroxidation damage and normalized the altered antioxidant enzymes levels of the liver in addition to reversal of blood glucose level near to control values.

A comparative study on antidiabetic activity of methanolic extract and ethyl acetate extract of *Zingiber officinale* was done by Kadnur and Goyal (2005). They observed that antidiabetic activity was more on methanolic extract than ethyl acetate extract and it may be dependent on the concentration of 6-gingerol present in the extract which was 3.08 per cent and 1.64 per cent respectively.

The antioxidant properties of *Emblica officinalis* and its effect on oxidative stress in streptozotocin-induced diabetes was examined by Rao *et al.* (2005) by oral administration of polyphenol rich fraction of ethyl acetate extract at the dose of 40 mg/kg body weight for 20 days. The results showed strong free radical scavenging activity and inhibition of the production of advanced glycosylated end products revealing its antioxidant property.

Singh *et al.* (2005) examined the attenuating influence of dietary potato peel powder on hyperglycaemia and various oxidative stress associated biochemical parameters in diabetic rats. They found that incorporation of potato peel powder to diet (5 percent and 10 per cent) for four weeks showed a significant decrease in blood glucose level as well as normalized the activities of various antioxidant enzymes in liver and kidney of diabetic rats.

In alloxan induced diabetic mice, a significant lowering of blood glucose level was noticed after 24 days duration treatment with aqueous extract of *Cocculus hirsutus* leaves at doses of 250, 500 and 1000 mg/kg orally (Badole *et al.*, 2006). In oral glucose tolerance test (OGTT) the peak of serum glucose was significantly depressed at 30 minutes after glucose loading by *Cocculus hirsutus* extract at the dose of 1000 mg/kg.

Hypoglycemic effect of methylene chloride /methanol root extract of *Ceiba pentandra* in normal and diabetic rats was studied by Dzeufiet *et al.* (2006). In multiple dose studies by the administration of *C. pentandra* twice a day for 3 days at the dose rate of 40 and 75 mg/kg, the 14 h fasting blood glucose concentration was reduced by 59.8 per cent and 42.8 per cent with corresponding reduction of urine glucose levels by 95.7 per cent and 63.6 per cent respectively.

Administration of aqueous extract of *Aegle marmelos* seeds at the dose rate of 250 mg/kg in normal and healthy rats reduced the blood glucose level by 35.1 per cent after six hour and 41.2 per cent and 33.2 per cent in subdiabetic and mild diabetic rats after two hour in glucose tolerance test (GTT). The hypolipidemic effect in diabetic rats was evident by fall in level of total cholesterol by 25.49 per cent with increase of 33.43 per cent in high density lipoprotein (Kesari *et al.*, 2006)

The use of methanolic extract of the seeds of *Eugenia jambolana* and root of *Musa paradisiaca* for the management of streptozotocin induced diabetes mellitus was studied by Mallick *et al.* (2006). The remedial effect on blood glucose level as well as in the quantity of liver and skeletal muscle glycogen were more with composite extract than when used separately.

According to Rajasekaran *et al.* (2006) continued administration of *Aloe vera* leaf gel extract (300 mg/kg) in streptozotocin diabetic rats for 21 days resulted in significant reduction in fasting blood glucose, hepatic transaminases, plasma and tissue cholesterol, triglycerides, free fatty acids and phospholipids and significant improvement in plasma insulin.

On the basis of studies conducted by Resmi *et al.* (2006) on the antioxidant effect of *Albizzia lebbeck* in alloxan induced diabetic rats, it was

concluded that oral administration of *Albizzia lebbeck* twice a week for a period of four weeks could normalize the blood glucose and hepatic glycogen content and improve the antioxidant status.

Chandra *et al.* (2007) conducted studies to evaluate the protective effect of selected Indian herbal hypoglycemic agents like *Momordica charantia, Allium sativum, Azadirachta indica* and *Ocimum sanctum* on oxidative stress in streptozotocin induced diabetic rats. In addition to marked lowering of blood sugar level in diabetic rats, they inhibited lipid peroxidation and significantly reactivated the antioxidant enzymes and restored glutathione levels, reducing the oxidative load in diabetes mellitus.

Oral administration of aqueous extract of *Boswellia glabra* leaves and roots at the dose of 0.3 g/kg body weight in alloxan induced diabetic rats led to significant lowering of blood glucose in short term experiment of 3 hours and long term experiment of 28 days. The histopathological studies indicated a repair of the β cells of the islets of Langerhans after treatment which increased the insulin level and restored the homeostasis in the biochemical parameters like cholesterol, triglycerides, urea, creatinine and enzyme activities (Kavitha *et al.*, 2007)

Kesari *et al.* (2007) observed that after the administration of water extract of *Murraya koenigii* leaves at variable doses the hypoglycemic effect began after two hours and was maximum after four hours in alloxan induced diabetic rats. The effect was dose dependent up to 300 mg/kg equivalent of extract but decreased at 400 mg/kg.

Krishna (2007) studied the hypoglycemic effect of *Pleurotus ostreatus* in alloxan-diabetic rats at different doses *viz.* 250, 500 and 1000 mg/kg

orally and found that most effective dose was 1000 mg/kg. The effect of combination of *Pleurotus ostreatus* with *Murraya koenigii* and *Aegle marmelos* were also evaluated and concluded that *P. ostreatus* exhibited a synergistic action with *M. koenigii* and *A. marmelos.*

Influence on hemorheological parameters in experimental diabetic rats by oral administration of aqueous extract of *Trigonella foenum-graecum* seeds was studied by Xue *et al.* (2007). There was a significant reduction in high and low shear rates of whole blood viscosity, ESR, plasma viscosity and platelet conglutination which showed its property to compact dyslipidemia and reduce the risk factor for the micro vascular complications of diabetes.

The phytochemical screening and evaluation of hypoglycemic effect of ethanolic extract of *Capparis sepiaria* leaves in streptozotocin induced diabetic rats by oral administration was done by Selvamani *et al.* (2008). At 12 hour post administration, the percentage of blood glucose lowering potential observed was 9.40 per cent, 13.57 per cent and 15.25 per cent at the dose of 100, 200 and 300 mg/kg respectively.

2.3. INTERACTION OF DRUGS

Miller (1998) reported that numerous herbal medicinals have been shown to affect blood glucose levels including fenugreek, garlic, ginseng and *Gymnema sylvestre* for patients with hypoglycemia and devil's claw, ginseng and licorice for patients with hyperglycaemia. He opined that more scientifically based studies evaluating efficacy and safety issues on the use of herbal medicines are needed which will prove to be a double-edged sword in which some herbal medicines

will fall into disfavour, while others will provide the basis for new and effective drugs.

The intake of five grams of Psyllium (husk of seeds of *Plantago ovate*) orally caused retardation in carbohydrate absorption which led to a reduction in insulin requirement in patients with type 1 diabetes. However this effect was weak and needed to be considered only in cases of long term use of bulk-forming laxatives. (ESCOP monographs 1999).

Blumenthal (2000) opined that the increasing use of herbs and phytomedicines and the growing interest in these by licensed healthcare professionals raised questions about their appropriate therapeutic uses, concerns about contraindications, potential adverse reactions and possible herb-drug interactions. The consumers using herbal supplements were 31 per cent and 48 per cent with prescription drugs and over-the-counter drugs respectively in North America.

Combination of Shilajit (a herbomineral preparation) with glibenclamide significantly enhanced the glucose-lowering effect of Shilajit (100 mg/kg) or glibenclamide *per se* in alloxan induced diabetic rats. Moreover, the effect of the combination treatment on the lipid profile was significantly more than that of glibenclamide alone, but was comparable to that produced by Shilajit(100 mg/kg) alone.(Trivedi *et al.*, 2004)

Palaian *et al.* (2006) pointed out that lack of accurate information regarding the safety profile and lack of information on exact constituents are the main problems in monitoring herbal drug toxicity and drug interaction between herbal medicine and allopathic medicine. They suggested that as herbal drugs are available at low cost and accessible even in remote locations, the use of indigenous drug need

more attention and it should be ensured that herbal drugs are prescribed after having correct drug information and a thorough drug history.

Fakeye *et al.* (2007) investigated on the individual and interaction influence of three variables, nature (N), dose administered (C) and duration of administration (D) using experimental factorial design at two levels – "high" and "low" – on blood glucose of diabetic rats on administration of ethanolic leaf extract of *Carica papaya* and two hypoglycemic agents, metformin and glimepiride . Leaf extract of *Carica papaya* significantly delayed the onset of hypoglycemic action of glimepiride and increased the hypoglycemic effect of metformin with the variables interacting differently for each drug-extract combinations.

Influence of oral administration of aqueous extract of fenugreek-seed powder in the pharmacodynamics and pharmacokinetics of gliclazide in rats/rabbits was studied by Satyanarayana *et al.* (2007). Fenugreek-seed powder extract produced hypoglycemia when given alone and prolonged the effect of gliclazide in combination during 1-2 h in normal and diabetic rats and during 2-8 hour in normal rabbits without hypoglycemic convulsions indicating that the combination can be used safely to obtain prolonged and sustained antidiabetic effect.

2.4. ALLOXAN INDUCED DIABETES

Lenzen and Mirzaie-Petri (1991) calculated the half maximal inhibitory concentration of alloxan for acotinase and glucokinase in sonically disrupted and intact isolated liver mitochondria and concluded that glucokinase rather than acotinase is the primary site for mediation of alloxan toxicity. They also

recognized the protective effect of glucose towards glucokinase through hindering the access of alloxan to the –SH groups of sugar binding site of glucokinase.

The activities of two enzymes *viz.* Na^{+} - K^{+} - ATP ase and succinic dehydrogenase in brain and liver of alloxan diabetic swiss albino mice were investigated by Mishra *et al.* (1995). It was seen that in alloxan induced diabetes there was a significant decrease in the activity of Na^{+} K^{+} -ATP ase reflecting reduced glucose transport across cell membrane and a significant enhancement in the activity of succinic dehydrogenase.

Ramakrishnan *et al.* (1996) examined the level of dopamine in seven discrete areas of rat brain 30 days after the induction of diabetes by single injection of alloxan and found that hippocampus, pons and medulla showed a greater increase in dopamine level in diabetic rats when compared with the control.

Szkudelski (2001) reported that alloxan and its reduced form, dialuric acid produced superoxide radicals which ultimately formed hydroxyl radicals which cause rapid destruction of β cells through different mechanisms like oxidation of essential -SH groups, inhibition of glucokinase, generation of free radicals and disturbances in intracellular calcium homeostasis.

Aleeva *et al.* (2002) counted pancreatic alpha and beta cells in alloxan induced diabetic rats and found that alloxan decreased the count of insulin producing beta cells but increased the number of glucagon secreting alpha cells in the pancreas by first week of induction of diabetes.

From their studies on mRNA expression of GLUT 2 (glucose trasporter-2), glucokinase, ß actin and proinsulin in pancreatic islets isolated from

alloxan-treated C57BL/6 mice, Walde *et al.* (2002) stated that GLUT 2 and glucokinase are the target molecules for alloxan.

Experiments conducted by Beppu *et al.* (2003) revealed the scavenging effect of *Aloe arborescens* Miller on free radicals generated by streptozotocin or alloxan. Boiled leaf skin and commercially available boiled aloin showed more potent free radical scavenging effects than non-boiled samples suggesting vegetable phenols involved in antioxidant effect.

Marika *et al.* (2004) investigated the effect of the preparation plaferon LB (preparation obtained from human placenta) on the morphological changes in pancreatic islets caused by experimental diabetes in rats produced by single intraperitoneal injection of alloxan at dose rate of 150 mg/kg. Study of H&E sections revealed that in diabetic control group part of langerhans islets have undergone sclerosis and hyalinosis.

Experiments conducted by Elsner *et al.* (2006) on RINmSF insulin-producing tissue culture cells showed that alloxan even at high millimolar concentrations, was not toxic to those cells which do not express the GLUT 2 glucose transporter in their plasma membrane. Further glutathione increased the toxicity of dialuric acid, the reduction product of alloxan and addition of a combination of SOD plus catalase completely abolished the toxicity of alloxan and dialuric acid.

2.5. TOXICOLOGICAL STUDIES ON PLANTS

Ezzat (1994) studied the toxic effect of *Nigella sativa* seeds on carbohydrate and key hepatic enzymes in normal albino rats after administration of the aqueous extract for 7 and 14 days at the dose of 10 ml/kg. The blood glucose

exhibited a significant decrease and serum insulin level and serum glutamate pyruvic acid transaminase increased significantly after 14 days. The results showed that the repeated use of *Nigella sativa* as therapeutic agent should not be encouraged.

Ginseng and Ashwagandha in the ratio of 10:1 were administered at the doses of 8.5, 12.75 and 17 mg/kg which were 4, 6 and 8 times the therapeutic dose respectively to albino rats for 90 days. The hematological, biochemical and histopathological studies done by sacrificing half of the animals after 45 days and rest after 90 days did not show any significant toxic effects. Hence the combination can be safely used in animals. (Aphale *et al.*, 1998).

Grover *et al.* (2000) assessed the white and red blood cell counts, hemoglobin, mean corpuscular volume, hematocrit, mean corpuscular hemoglobin and mean plasma glucose levels after administration of 200 mg/kg of lyophilized powder of aqueous extract of *Eugenia jambolana* and 400 mg/kg of aqueous extract of *Tinospora cordifolia* in mice for 60 days. There was no statistical difference in the values when compared with the normal which indicated that the extract was safe for therapeutic administration.

Babu *et al.* (2003) tested the alcoholic extract of *Cassia kleinii* leaves for its acute and short term toxicity in mice. At doses of 0.5, 1.0, 1.5 and 2 g/kg single oral administration of drug, the mortality and general behavior of the animal like grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion were observed periodically for 48 hours. They observed no gross behavioral changes and mortality and revealed the absence of acute toxicity even at high dose of *C. kleinii* (2g/kg body weight). In short term toxicity studies also, after 14 days, administration of extract did not show any toxic changes.

Lethal doses of fixed or volatile oils extracted from leaves or fruits of *Pimpinella anisum, Foeniculum vulgare, Sesamum indicum, Eugenia caryophyllata, Nigella sativa, Urtica pilulifera, Apium graveolens, Cuminum cyminum, Coriandrum sativum* and *Thymus fallax* were determined by Ozbek *et al.* (2004) by intraperitoneal injection with different concentrations in mice. The LD_{50} values were calculated by the method of probit analysis. The results indicated that the oils of *Sesamum indicum* and *Urtica pilulifera* were completely non lethal at doses of 12.8 ml/kg and was proved to be nontoxic.

The aqueous extract of leaves of *Cocculus hirsutus* was tested for acute oral toxicity in mice by Badole *et al.* (2006) according to Organization for Economic Co-operation and Development (OECD) guideline 425 and found that single dose up to 175 mg/kg body weight orally did not produce any mortality. Using a software for LD_{50} determination, doses up to 2000 mg/kg were found to be nonlethal and they stated that *C. hirsutus* did not have any acute oral toxicity.

Toxicological studies of *Momordica charantia* in normal and alloxan diabetic rats was conducted by Batran *et al.* (2006). In mice LD_{50} values were 91.90 and 362.34 mg/100 g for *Momordica charantia* juice and alcoholic extract respectively. The juice at the doses of 2.1, 5.7 and 9.2 mg/100 g and alcoholic extract at doses of 13.4, 24.8 and 36.2 mg/100 g did not show any significant effect in urea, creatinine, ALT, AST and AP in normal rats while in diabetic rats showed a significant decrease. This suggested that *Momordica charantia* was safe for therapeutic use in diabetes and possessed hypoglycemic, hepato-renal protective and hypolipidemic effect in alloxan induced diabetic rats.

The aqueous extract of stem bark of *Boswellia dalzielii* was evaluated for any toxicity by Etuk *et al.* (2006). Toxicity studies of the extract by oral

administration in rats showed that median lethal dose (LD_{50}) of the extract was greater than 3000 mg/kg. In sub acute toxicity studies for 28 days the extract did not produce any lethality in tested animals at doses of 900, 1800 and 2700 mg/kg. The hematological parameters also did not show any significant change except at highest dose of 2700 mg/kg which revealed that only prolonged oral administration of very high doses will be associated with toxicity.

Ozbek *et al.* (2006) conducted studies in mice to evaluate the median lethal dose of *Foeniculum vulgare* Miller Essential oil (FEO). The mortality was assessed 24 h, 48 h and 72 h after administration of FEO at increasing doses of 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 ml/kg body weight and LD_1, LD_{10}, LD_{50}, LD_{90} and LD_{99} of FEO were calculated as 0.449, 0.654, 1.038, 1.648 and 2.402 ml/kg body weight.

The toxicological evaluation of *Calycopteris floribunda* Lam. was done in calf, rabbit and rat by Sreekanth *et al.* (2006). Rabbits (25g/kg/day) and calves (35 g/kg/day) fed with fresh leaves showed morbidity and mortality with clinical signs like depression, downer status, polyuria and characteristic forelimb paresis (only in rabbits). It was found that the methanolic extract (0.6 g/kg) induced mortality in rabbits within 24 h and in rats the oral LD_{50} of the methanol extract was 0.384 g/kg body weight. The serum urea, serum glutamate, pyruvate transaminase, creatinine concentrations were elevated indicating liver and kidney damage. Thus the study revealed the hepatotoxic, nephrotoxic and cardiotoxic nature of *Calycopteris floribunda.*

The acute and sub acute toxicity of *Aegle marmelos* was evaluated by Veerapan *et al.* (2007). The LD_{50} values were calculated after intraperitoneal administration of drug at different doses of 50, 70, 90 and 100 mg/kg body weight. The sub acute toxicity studies were conducted by administering the extract for 14

consecutive days. The results showed the absence of any adverse effects on any organ and it was concluded that *A. marmelos* did not induce any short term toxicological effects.

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

The study was conducted in 56 adult Sprague-Dawley strain albino rats of both sexes weighing 180-200 g. The experiment was approved by the Institutional Animal Ethics Committee. The rats were procured from Small Animals Breeding Station, College of Veterinary and Animal Sciences, Mannuthy. All the animals were maintained in well ventilated cages in the laboratory under standard managemental conditions for one week, to get acclimatized with the new environment, before the commencement of the experiment. The experiment was carried out for a period of 60 days.

3.2 EXPERIMENTAL DESIGN

Fifty six rats were randomly divided into seven groups of eight animals each. The experimental design was as follows:

Group	Treatment
T_1	Normal control, administered with 3% Tween 80 p.o, daily from 16th day to 60th day
T_2	Diabetic control, administered with single dose of 10% alloxan at the dose of 130 mg/kg subcutaneously on zero day. From 16th day to 60th day 3% Tween 80 was administered orally.
T_3	Diabetic rats administered with ethanolic extract of leaf of *Coccinia indica* at the dose rate of 200 mg/kg orally daily from 16th day to 60th day.

T_4	Diabetic rats administered with glibenclamide at the dose rate of 0.25 mg/kg orally daily from 16th day to 60th day.
T_5	Diabetic rats administered with a combination of glibenclamide at the dose of rate of 0.125 mg/kg and ethanolic extract of *Coccinia indica* at the dose rate of 100 mg/kg orally from 16th day to 60th day
T_6	Diabetic rats administered with a combination of glibenclamide at the dose of rate of 0.125 mg/kg and ethanolic extract of *Coccinia indica* at the dose rate of 150 mg/kg orally from 16th day to 60th day
T_7	Diabetic rats administered with a combination of glibenclamide at the dose of rate of 0.125 mg/kg and ethanolic extract of *Coccinia indica* at the dose rate of 200 mg/kg orally from 16th day to 60th day

Blood glucose, serum cholesterol and serum triglycerides were estimated on 0th, 15th, 30th, 45th and 60th day. Body weight was also recorded on these days. On 60th day all the animals were sacrificed and liver and pancreas were collected. The pancreas was preserved in 10 per cent neutral buffered formalin for histopathological examination. For the estimation of biochemical parameters like lipid peroxides and reduced glutathione in liver and pancreas, the tissues were collected immediately after sacrificing and washed in ice cold normal saline (0.85 per cent).

3.3 PROCEDURE FOR INDUCTION OF DIABETES

All the animals were fasted overnight and their bodyweight and blood glucose was estimated on the next day (day zero) morning. A preliminary study was

conducted to fix the dose of alloxan * and it was found to be 130 mg/kg. Ten percent (w/v) alloxan solution was prepared in distilled water. All the treatment groups except the normal control were made diabetic by the subcutaneous injection of freshly prepared alloxan monohydrate at the dose rate of 130 mg/kg body weight. On 15th day, the blood glucose was estimated using O-toluidine method. Rats showing moderate hyperglycaemia (200-350 mg/100 ml) were selected for the study.

3.4 PREPARATION AND ADMINISTRATION OF DRUGS

The fresh leaves of *Coccinia indica* was collected and dried under shade. The dried leaves were coarsely powdered and extracted using ethanol in a soxhlet apparatus for 16 hours. The liquid extract so obtained was collected in a wide mouthed vessel and the solvent was allowed to evaporate by keeping them in a water bath at low temperature so as to obtain a semisolid extract. The crude extract thus prepared was kept in the refrigerator at 4° C for further use. A weighed quantity of the crude extract was homogenized with 3 per cent Tween 80 and was administered orally using an oro-gastric catheter to individual rats for 45 days based on their body weight.

Glibenclamide

Tab Daonil** (5 mg) was powdered and dissolved in distilled water so that 0.5 ml contains 0.025 mg of glibenclamide and given orally according to dose rate from 15th day to 60th day.

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**Aventis Ltd.

Fig. 1. *Coccinia indica* (Ivy gourd) with its fruit and flower

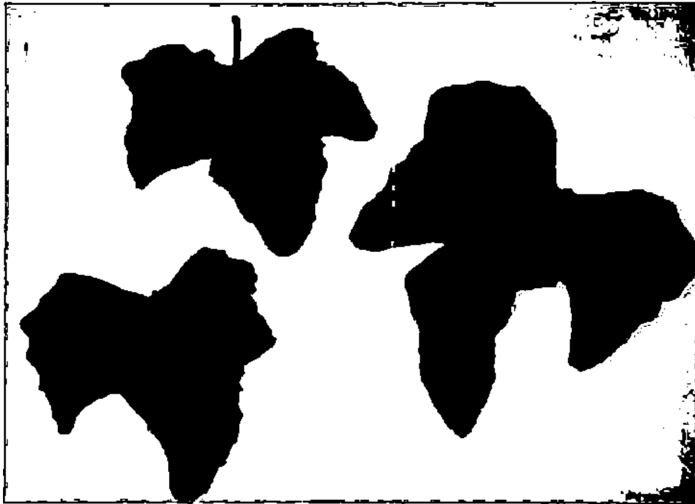


Fig. 2. *Coccinia indica* leaves

3.5 COLLECTION OF BIOLOGICAL SAMPLES

3.5.1 Blood

Blood was collected retro orbitally from the inner canthus of eye under light ether anaesthesia using heparinised capillary tubes. The dipotassium salt of Ethylene Diamine Tetra Acetic Acid (EDTA, 1 mg/ml) was used as anticoagulant and immediately used for estimation of blood glucose. The blood was collected on 0th, 15th, 30th, 45th and 60th day of the experiment.

3.5.2 Serum

Blood was collected in fresh vials without any anticoagulant and kept at room temperature for 10 minutes and in the refrigerator (4^{0} C) for 10 minutes. Then it was centrifuged at 5000 rpm for 10 minutes and the serum was separated for the estimation of cholesterol and triglycerides.

3.5.3 Liver and pancreas

On 60th day all the animals were sacrificed and liver and pancreas were collected, washed in ice cold normal saline and of biochemical parameters like lipid peroxides and reduced glutathione were estimated immediately. Pancreas was preserved in neutral buffered formalin for histopathological examination.

3. 6 ESTIMATION OF BIOCHEMICAL PARAMETERS

3. 6. 1 Blood Glucose

The blood glucose level was estimated by O-toluidine method as cited by Hyvarien and Nikila (1962).

3. 6. 1. 1 *Principle of O - Toluidine method*

Glucose reacts with O-toluidine in glacial acetic acid in the presence of heat to yield a blue green N-glucosylamine, the absorbance of which is measured at 625 nm.

3. 6. 1. 2 *Reagents*

1. Tungstic acid reagent
2. O-toluidine reagent
3. Glucose standard

Preparation of Reagents

1. Tungstic acid reagent

Dissolved one gram of polyvinyl alcohol in about 100 ml of distilled water with gentle warming. The solution was cooled and transferred into a one litre volumetric flask containing 11.1 gram of sodium tungstate previously dissolved in about 100 ml distilled water and mixed. In a separate vessel, 2.1 ml of concentrated sulphuric acid was added to about 300 ml of

distilled water and mixed. It was then added to the tungstate solution in one litre volumetric flask, mixed well and made up the volume with distilled water. The solution is stable for one year at room temperature.

2. O-toluidine Reagent

O-toluidine was redistilled to get a colourless solution. Five gram thiourea was added to 90 ml of O-toluidine and diluted to one litre with glacial acetic acid. It was stored in an amber coloured bottle in the refrigerator. The solution is stable for two years at refrigeration temperature.

3. Glucose standard (100mg/100ml)

Dissolved one gram reagent grade anhydrous glucose in one litre of distilled water containing 1.5 gram benzoic acid.

3. 6. 1. 3 ***Procedure***

Protein free filtrate was prepared by transferring 0.2 ml of the blood sample into a test tube containing 1.8 ml of the tungstic acid reagent. Mixed well and allowed to stand for 5 minutes and centrifuged at 3000 rpm for 10 minutes. Supernatant was collected and 0.5 ml was mixed with 2.5 ml of O-toluidine reagent in a glass stoppered test tube and mixed well. The blank was prepared by adding 0.5 ml of distilled water instead of the deproteinised blood to 2.5 ml of O-toluidine reagent. The standard was set by adding 50 μl of the glucose standard (100mg/100ml) to 450 μl of distilled water and 2.5 ml of O-toluidine reagent. Mixed well and placed all the loosely stoppered test tubes in a boiling water bath and boiled for 10 minutes. Then cooled by placing them in cold water bath. The optical density was measured at 625 nm in a

spectrophotometer. The concentration of glucose was calculated by the following formula.

$$\text{Glucose concentration (mg/dl)} = \frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times 100$$

3. 6. 2 Liver Glycogen

Liver glycogen was estimated as per the method explained by Carroll *et al.* (1956).

3. 6. 2.1 *Principle*

The liver tissue is digested by boiling it with 30 per cent Potassium hydroxide solution (to destroy alkali-liable carbohydrate) and the resulting red vine coloured solution is treated with anthrone reagent. The sulphuric acid medium of the anthrone reagent causes dehydration of the sugar to a furfural derivative which presumably condenses with anthrone to form a blue coloured compound. The colour produced is compared with a standard in a spectrophotometer at 620 nm.

3.6.2.2 *Reagents*

1. Anthrone reagent.
2. 95 percent ethanol.
3. 5 percent trichloroacetic acid
4. Glucose standard.

Preparation of Reagents

1. Anthrone reagent

A solution containing 0.05 per cent anthrone , one per cent thiourea and 72 per cent by volume sulphuric acid was used. For each litre of reagent, placed 280 ml of distilled water and added cautiously 720 ml of concentrated sulphuric acid of specific gravity 1.84 of highest purity. A mixture containing 500 mg of purified anthrone, 10 gm of highest purity thiourea and one litre of 72 percent sulphuric acid in a flask, warmed to 80-90^0 by occasionally shaking the flask to mix the contents, cooled and stored in a refrigerator.

2. Glucose standard

(a) Stock standard - Dissolved 100 mg of dry reagent grade anhydrous glucose in 100 ml of saturated benzoic acid solution.

(b) Working standard - Pipetted out 5 millilitre of the stock solution to a 100 ml volumetric flask and made up the volume with saturated benzoic acid solution. Two millilitre of this solution, containing 0.1 milligram of glucose, was used as the standard.

3. 6. 2. 3 *Procedure*

1. One gm of tissue sample was digested in 10 ml of 30 per cent boiling Potassium hydroxide solution
2. Then kept in boiling water bath for 15 minutes
3. One millilitre of the solution was taken and volume was made to 10 ml with distilled water

4. Again one millilitre of the solution was taken from it and volume was made to 10 ml with distilled water
5. From that 0.5 ml was taken and added 0.6 millilitre of 95 per cent ethanol and kept overnight for precipitation
6. After precipitation is completed, the tubes were centrifuged at 3000 rpm for 15 minutes and the clear supernatant was gently decanted.
7. 0.5 ml of distilled water was added to this and mixed thoroughly
8. A reagent blank was prepared by pipetting 0.5 millilitre of distilled water into a clean test tube. A Standard was prepared by pipetting 0.5 millilitre of standard glucose solution.
9. 2.5 millilitre of anthrone reagent was added to all test tubes and kept in cold water for cooling
10. Kept in boiling water bath for 15 minutes and then cooled under tap water
11. The colour developed was read immediately at 620 nm in a spectrophotometer. The concentration of glycogen was calculated using the following formula.

$$\text{Liver glycogen (mg/100g)} = \frac{\text{OD of unknown} \times 0.1 \times 100 \times 100 \times 0.9}{\text{OD of Standard} \times 0.1}$$

Where, 0.1 of numerator stands for concentration of standard

0.1 of denominator stands for weight of tissue

100 x 100 of numerator stands for dilution factor and conversion factor to grams

0.9 of numerator stands for conversion factor of glucose to glycogen

3. 6. 3 Total cholesterol

Cholesterol in serum was estimated by enzymatic CHOP-PAP method (Allain *et al* ., 1973) in semi automatic blood analyzer('Microlab 200') using Ecoline kit from E.Merck India Limited.

3.6.3.1 *Principle*

Cholesterol is oxidized in the presence of cholesterol oxidase (CHO) to cholesterol-3-one and hydrogen peroxide. Phenol and 4-amino antipyrene then combines with hydrogen peroxide by oxidative condensation in the presence of oxidase to produce red coloured quinone. The intensity thus produced is directly proportional to cholesterol concentration.

3.6.3.2 *Procedure*

Blank, standard and sample were prepared as follows :-

	Blank	Standard (200mg/dl)	Sample
Sample			10 µl
Standard		10 µl	
Distilled water	10 µl		
Reagent	1000 µl	1000 µl	1000 µl

Mixed, kept at 35^{0}C for 5 minutes and read the optical density in spectrophotometer at a wavelength of 500 nm.

Calculation

$$\text{Cholesterol (mg/dl)} = \frac{\text{Optical density of sample} \times \text{Conc.Std (mg/dl)}}{\text{Optical density of standard}}$$

3.6.4. Triglyceride

Triglyceride in serum was estimated by GPO-PAP method (Nussel and Arav, 1975) using kit from Merck.

3.6.4.1 *Principle*

Triglycerides are hydrolysed by lipase and liberated glycerol is phosphorylated with the help of glycerol kinase in presence of ATP to glycerol 3-phosphate. Glycerol-3-phosphate is oxidized in the presence of glycerol phosphate oxidase (GPO) to dihydroxy acetone phosphate and hydrogen peroxide. 4-chlorophenol and 4-amino antipyrene then combines with hydrogen peroxide by oxidative condensation in the presence of peroxidase to produce red coloured quinoneimine. The intensity of colour thus produced is directly proportional to triglyceride concentration.

3.6.4.2 ***Procedure***

Blank, standard and sample were prepared as follows

	Blank	Standard (200mg/dl)	Sample
Sample			10 µl
Standard		10 µl	
Distilled water	10 µl		
Reagent	1000 µl	1000 µl	1000 µl

Mixed, kept at 35^0C for 5 minutes and read the optical density in spectrophotometer at a wavelength of 500 nm.

Calculation

$$\text{Triglyceride (mg/dl)} = \frac{\text{Optical density of sample} \times \text{Conc.Std (mg/dl)}}{\text{Optical density of standard}}$$

3.6.5 Lipid peroxides in tissues

Level of lipid peroxides in tissue homogenate was determined by the method of Ohkawa *et al.* (1979).

3.6.5.1 *Principle*

Thiobarbituric acid reacts with lipid peroxides and malondialdehyde to form a red coloured pigment that can be determined by colorimetry. 1,1,3,3 tetra methoxy propane was used as a standard since it can be converted to malondialdehyde quantitatively by reacting with TBA.

3.6.5.2 *Reagents*

8.1 % Sodium dodecyl Sulphate

20 % Acetic acid solution, pH adjusted to 3.5 with sodium hydroxide

0.8 % aqueous solution of Thiobarbituric acid

1.15 % Potassium chloride

3.6.5.3 *Procedure*

Preparation of tissue homogenate:

Homogenates of liver and pancreas were prepared in a ratio of 1gram of wet tissue to 9 millilitre of 1.15 per cent Potassium chloride solution (10 % w/v) using a glass homogenizer. The tissue homogenate was centrifuged at 5000 rpm for 5 minutes and the supernatant was used for the estimation of lipid peroxides.

1. To 100 µl of the supernatant, added 200 µl of 8.1 per cent Sodium dodecyl Sulphate, 1.5 ml 20 acetic acid solution (pH 3.5) and 1.5 ml of 0.8 % aqueous solution of Thiobarbituric acid .
2. The mixture was made up to 4 ml with distilled water, and heated in a water bath at 95°C for 60 minutes.
3. After cooling under tap water, 1 ml of distilled water and 5 ml of n-butanol were added and shaken vigorously.

4. Centrifugation at 4000 rpm for 10 minutes, and absorbance of the organic layer was taken at 532 nm.

Preparation of standard curve:

Standard curve was prepared using concentrations varying from 0.5 nM to 5 nM of Tetra methoxy propane in deionised double distilled water by following the above procedure. A graph was plotted between optical density and concentration of the standards. The level of lipid peroxides were read directly from the standard curve, and expressed as nM of malondialdehyde per gram of wet tissue.

3.6.6 Reduced glutathione in tissue

Level of reduced glutathione in tissue homogenate was estimated by the method of Moron *et al.* (1979).

3.6.6.1 *Principle*

Reduced glutathione is measured by its reaction with DTNB to give a yellow coloured complex with an absorption maximum at 412 nm.

3.6.6.2 *Reagents*

0.2 M phosphate buffer, pH 8

25 % Trichloro acetic acid(TCA)

5 % Trichloro acetic acid

0.6 mM 5-5' dithiobis 2-nitrobenzoic acid (DTNB)

3.6.6.3 *Procedure*

Preparation of tissue homogenate:

Homogenates of liver and pancreas were prepared in a ratio of one gram of wet tissue to nine milliliters of 0.2 M phosphate buffer, pH 8 (10 % w/v) using a glass homogenizer. The tissue homogenate was centrifuged at 5000 rpm for 5 minutes and the supernatant was used for the estimation of reduced glutathione.

1. Added 125 µl of 25 per cent TCA to 500 µl of supernatant/serum for the precipitation of proteins.
2. The tubes were cooled on ice for 5 minutes.
3. The mixture was further diluted with 575 µl of 5 per cent TCA.
4. Centrifuged the tubes for 5 minutes and 300 µl of resulting supernatant was taken for the estimation of reduced glutathione.
5. The volume of aliquot was made up to 1 ml with 0.2 M phosphate buffer (pH, 8).
6. Added 2 ml of freshly prepared 0.6 mM DTNB to the tubes and the intensity of yellow colour formed was read at 412 nm.
7. The level of reduced glutathione was measured from the standard curve.

Preparation of standard curve:

Standard curve was prepared using concentrations varying from 1 µg to 10 µg of reduced glutathione which was dissolved in 5 per cent TCA. The volume of standard solution was made up to 1 ml with 0.2 M phosphate buffer (pH, 8). Added 2

ml of freshly prepared 0.6 mM DTNB to the tubes and the intensity of yellow colour formed was read at 412 nm. A graph was plotted between optical density and concentration of the standards. Knowing the optical density of the unknown samples, the corresponding concentration of GSH was read directly from the calibration curve and expressed as μg/g of wet tissue.

3.7 HISTOPATHOLOGICAL EXAMINATION OF PANCREAS

Representative samples of pancreas collected from the dissected animals were fixed in 10 per cent formalin. They were processed and paraffin embedded as described by Sheehan and Hrapchak (1980). The sections were stained with haematoxyline and eosin as per the technique followed by Bancroft and Cook (1984). The sections were examined in detail under light microscope.

3.8 PHYTOCHEMICAL SCREENING

The ethanol extract of *Coccinia indica* leaves were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure quoted by Harbone (1991).

3.8.1 Tests for detection of steroids

3.8.1.1 Salkowski Test

About five milligram of the extract was mixed with three millilitre of chloroform and then shaken with three millilitre of concentrated sulphuric acid.

3.8.1.2 Leiberman Burchard Test

About five milligram of the extract was mixed with three millitre of chloroform. Then five drops of acetic anhydride and one millilitre of concentrated sulphuric acid was added to it through the sides.

3.8.2 Tests for detection of alkaloids

About 0.5 gram of the extract was mixed with five millilitre ammonia solution and then extracted with equal volume of chloroform. To this, equal quantity of 0.1N hydrochloric acid was added. Acid layer was used for the following tests.

3.8.2.1 Mayer's Test

To one millilitre of acid layer obtained, a few drops of Mayer's reagent ((1.358 gram of Mercuric chloride dissolved in 60 ml of water and poured into a solution of five gram of potassium iodide in 10 ml of water; then made up the volume to 100 ml with distilled water) were added.

3.8.2.2 Hager's Test

To one millilitre of acid layer, a few drops of Hager's reagent (one gram of picric acid dissolved in 100 ml of water) were added and mixed.

3.8.2.3 Dragendroff's Test

Two drops of Dragendroff's reagent (solution of potassium bismuth iodide) was mixed with one millilitre of acid layer.

Dragendroff's reagent:

Stock solution (1): 0.6 gram of bismuth subnitrate was dissolved in two millilitre concentrated Hydrochloric acid and 10 millilitre of water was added.

Stock solution (2): six gram of potassium iodide was dissolved in 10 millilitre of water.

Both stock solutions were mixed together and then added seven millilitre of concentrated Hydrochloric acid and 15 millilitre of water. Sufficient amount of distilled water was added to make up the volume to 400 ml.

3.8.3 Tests for detection of Tannins

3.8.3.1 Ferric chloride test

To two milligram of the extract, three millilitre of one per cent ferric chloride solution was added and mixed.

3.8.4 Tests for detection of Flavonoids

3.8.4.1 Ferric chloride Test

To two millilitre of alcoholic solution of the extract, (0.5 gram extract in 10 ml methanol), a few drops of neutral ferric chloride solution was added and mixed.

3.8.4.2 Lead acetate Test

To two millilitre of alcoholic solution of the extract, (0.5 gram extract in 10 millilitre methanol), a few drops of 10 per cent lead acetate was added and mixed.

3.8.5 Tests for detection of Glycosides

3.8.5.1 Benedict's Test

To about one millilitre of the extract (0.5 gram of extract in one millilitre water), five millilitre of Benedict's reagent was added. The mixture was boiled for 10 minutes.

3.8.5.2 Sodium hydroxide Test

Mixed a small amount of the extract (about five milligram) in one millilitre water and added five to six drops of 10 per cent sodium hydroxide solution.

3.8.6 Tests for the presence of Phenolic Compounds

About five milligram of the extract was mixed with one millilitre of water and five drops of 10 per cent ferric chloride solution was added to it.

3.8.7 Tests for detection of Diterpenes

About five milligram of the extract was mixed with three millilitre of five per cent copper acetate solution.

3.8.8 Tests for detection of Triterpenes

3.8.8.1 Salkowski's Test

About three milligram of the extract was mixed with three millilitre of chloroform and then shaken with concentrated sulphuric acid.

3.8.8.2 Leiberman Burchardt Test

A few drops of acetic acid and one millilitre of concentrated sulphuric acid were added to three millilitre of chloroform solution of the extract (about three milligram of extract in three millilitre chloroform).

3.8.9 Tests for detection of Saponins

3.8.9.1 Foam Test

A small amount of the extract (about five milligram) was shaken with three millilitre of water.

3.9 EVALUATION OF TOXICITY

3.9.1 Evaluation of acute oral toxicity

The acute toxicity testing of *Coccinia indica* was carried out according to OECD Test Guideline 423.

3.9.1.1 Preparation of animals

Male albino mice were used for the study. The animals were randomly selected, marked to permit individual identification, and kept in cages for one week prior to dosing to allow for acclimatization to the laboratory conditions. Feed was withheld for 4 hours prior to administration of drug and 2 hours after the administration of drugs.

3.9.1.2 Preparation and administration of doses

The ethanolic extract of *Coccinia indica* leaves were made into an emulsion with 3 per cent Tween 80 prior to administration. The extract is given as a single dose using an oro-gastric tube. The starting dose selected was 300 mg/kg body weight. Three animals were used for each step. The dose levels upto 2000 mg/kg body weight with reference to OECD guideline was administered.

3.9.1.3 Observations for evaluation of acute oral toxicity

Animals were observed individually after dosing during the first 30 minutes, then at intervals during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days. Observations were made on toxicity signs like increased motor activity, anaesthesia, tremors, arching and rolling, clonic convulsions, ptosis, tonic extension, lacrimation, Straub reaction, exophthalmos, pilo-erection, salivation, muscle spasm, opisthotonus, writhing, hyperesthesia, depression, ataxia, stimulation, sedation, hypnosis, cyanosis and analgesia.

3.9.2 Determination of LD_{50}

The LD_{50} value depend on the route of administration (Akhila *et al.*, 2007). The values are found to increase with the following sequences of routes: intravenous, intraperitonal, subcutaneous and oral. In the present study intraperitoneal route was taken for evaluation of LD_{50}.

3.9.2.1 Preparation of animals and administration of drug

Wistar albino rats of both sex were used for the study. The animals marked to permit individual identification, and kept in cages for one week prior to dosing to allow for acclimatization to the laboratory conditions. The rats were randomly

divided into six groups, group I to VI of four each. Feed was withheld overnight prior to administration of drug and also 4 hours after the administration of the drug.

3.9.2.2 Preparation and administration of drug

The ethanolic extract of *Coccinia indica* leaves was dissolved in distilled water and was emulsified with Tween 80. The dilution was so made that each ml of the drug contained 200 mg of the extract. The group I to VI were administered with doses of 50, 100,200, 300, 500 and 2000 mg/kg intraperitonealy. The LD_{50} was calculated by Arithmetical method of Karber (Akhila *et. al.*, 2007, Turner 1965).

3.9.2.3 Observations for determination of LD_{50}

After 24 hours, the number of dead animals in a the groups were recorded. The LD50 was calculated by Arithmetic method of Karber.

LD_{50} = Least lethal dose - sum of { (a x b) / N}

Where, a = dose difference from previous group

b = mean mortality(mortality in the group + mortality in previous group / 2)

a x b = probit

3.9.3 Evaluation of sub acute toxicity

The sub acute toxicity of ethanolic extract of *Coccinia indica* leaf, if any was evaluated for a period of 14 days (Veerappan *et al.*, 2007, Turner 1965).

3.9.3.1 Preparation of animals

Thirty wistar albino rats were used for the study. The animals were weighing in a range of 110-140 grams and were divided randomly into group of six each. The

animals were kept in cages for one week prior to dosing to allow for acclimatization to the laboratory conditions.

3.9.3.2 Preparation and administration of drug

The ethanolic extract of *Coccinia indica* leaves was dissolved in distilled water and was finely emulsified with Tween 80. The dilution was so made that one millilitre of the drug contained 40 mg of the extract. The group I was kept as normal control. The group II, III IV and V were administered with doses of 50, 70, 90 and 100 mg/kg of the drug intraperitonealy for 14 consecutive days, one injection per day.

3.9.3.3 Observations for evaluation of subacute toxicity

The observations were made on zero day (before administration of the drug) and on 14th day(after administering the drug for 14 days). On zero day, body weight, biochemical parameters like serum Alanine amino transferase (ALT), Aspartate amino trasferase(AST), creatinine, total protein, glucose, hematological parameters like hemoglobin and packed cell volume were estimated for all groups. On 14th day in addition to the above parameters hematological parameters like RBC count, WBC count and differential leukocyte count were also estimated for all groups.

3.9.3.4 Alanine amino transferase (ALT) activity

Serum ALT activity was determined using commercially available kit, Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Procedure:

Reagent 1 and Reagent 2 were mixed in the ratio of 4:1 to prepare the working reagent and proceeded as follows.

Serum	100 µl
Working reagent	1000 µl

Mixed, after 1 minute, read the decrease in absorbance (ΔA) every minute for three minutes at 340 nm.

Calculation:

ALT activity in U/L = ΔA × 1746

3.9.3.5 Aspartate amino transferase (AST) activity

Serum AST activity was determined using commercially available kit, Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Procedure:

Reagent 1 and Reagent 2 were mixed in the ratio of 4:1 to prepare the working reagent and proceeded as follows.

Serum	100 µl
Working reagent	1000 µl

Mixed, after 1 minute, read the decrease in absorbance (ΔA) every minute for three minutes at 340 nm.

Calculation:

ALT activity in U/L = ΔA × 1746

3.9.3.6 Creatinine

Creatinine in serum was determined based on Jaffe kinetic method without deproteinisation in semi automatic blood analyzer ('Microlab 200') using Ecoline kit from E.Merck India Limited.

Principle:

Creatinine forms a yellow-orange compound in alkaline solution with picric acid. At a low concentration of picric acid as used in this method, precipitation of protein does not take place.

Reagents:

Reagent 1: Buffer solution

Reagent 2: Picric acid

Reagent 3: Creatinine standard

Procedure:

Reagent 1 and Reagent 2 were mixed in the ratio of 1:1 to prepare the working reagent, kept for at least 10 min. at room temperature and proceeded as follows.

Sample/standard	100 µl
Working reagent	1000 µl
Mix and read absorbance A1 after 60 sec, read absorbance A2 after further 120 sec.	

Calculation

Creatinine(mg/dl) = ΔA Sample / ΔA Std x Conc. Std.(mg/dl)

3.9.3.7 Total protein

Total protein in serum was estimated by biuret method using Lyphozyme kit from Beacon Diagnostics, India.

Principle:

In alkaline medium, total protein reacted with copper of biuret reagent causing an increase in absorbance. The increase in absorbance was due to the formation of violet coloured complex and it is directly proportional to the concentration of protein present in the sample.

Reagents:

Reagent 1: Biuret reagent

Reagent 2: Protein standard, 6 g/dl

Procedure:

Reagent	Blank	Standard	Test
Working reagent	1 ml	1 ml	1 ml
Standard	-	10 µl	-
Sample	-	-	10 µl
Distilled water	10µl	-	-
Mixed, incubated for 5 minutes at room temperature and the optical density was read at 546 nm against reagent blank.			

Calculation:

Total protein (g/dl) = Optical density of Sample/Optical density of Standard x 6

3.9.3.8 Glucose

Glucose was estimated photometrically by Glucose-oxidase-peroxidase (GOD-POD) method using commercial kit (Euro Diagnostics Pvt. Ltd).

Principle:

Glucose is oxidized by glucose oxidase to gluconic acid and H_2O_2 is liberated. The colorimetric indicator, quinoneimine is generated from 4-aminoantipyrine and phenol by H_2O_2 under the catalytic action of peroxidase. Intensity of colour generated is directly proportional to glucose concentration.

Procedure:

	Blank	Standard	Sample
Sample	---	---	10 µl
Standard	---	10 µl	---
Distilled water	10 µl	----	---
Reagent 1	1000 µl	1000 µl	1000 µl
Mixed and incubated for approximately 15 minutes at 37°C. The absorbance was read against the reagent blank at 546 nm with in 30 minutes.			

Calculation:

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard (mg/dl)}$$

3.9.3.9 Haemoglobin concentration

Haemoglobin was estimated by Acid Haematin method (Benjamin. 1985).

3.9.3.10 Volume of Packed Red Blood Cells (VPRC)

Volume of Packed Red Blood Cells was estimated by Wintrobe method (Benjamin, 1985).

3.9.3.11 Total Erythrocyte Count

Erythrocyte were counted by standard dilution technique using Hayems fluid. Counting of erythrocytes was done in the zone for erythrocytes in the haemocytometer placed under low power of microscope (Benjamin, 1985).

3.9.3.12 Total Leukocyte Count

Leucocytes were counted by standard dilution technique using Thomas fluid. Counting of leucocytes was done in the zone for leucocytes in the haemocytometer placed under low power of microscope (Benjamin, 1985).

3.9.3.13 Differential leukocyte count

Blood smears were prepared on clean glass slides and stained with Wrights stain. After staining, counting was done under oil immersion (Benjamin, 1985).

3.10 STATISTICAL ANALYSIS OF THE DATA

Data obtained were analyzed by the following statistical tools to determine the level of significance (Snedecor and Cochran, 1994). The value of $P<0.05$ was considered statistically significant.

1. Compared by analysis of variance (ANOVA) followed by Duncan's multiple range test
2. Weight gain for different groups was analyzed by analysis of co-variance (ANOCOVA)
3. Period wise comparison of means was done by paired t-test

Results

4. RESULTS

The present study was undertaken to evaluate the hypoglycemic, hypolipidemic and oxidative stress relieving effect of ethanolic extract of *Coccinia indica* leaves and its interaction with glibenclamide in alloxan induced diabetic rats. The toxicity of *Coccinia indica* leaves if any was also evaluated.

4.1 HYPOGLYCEMIC, HYPOLIPIDEMIC AND ANTIOXIDANT EFFECT OF *Coccinia indica* LEAVES ON ALLOXAN INDUCED DIABETIC RATS.

The study was conducted to evaluate the hypoglycemic, hypolipidemic and oxidative stress relieving effect of *Coccinia indica* at the dose rate of 200 mg/kg in a treatment course of 45 days.

4.1.1 Evaluation of general health improving and hypoglycemic effect of *Coccinia indica*

The body weight was recorded before giving alloxan (zero day), after induction of diabetes before starting the treatment (15th day), during the course of treatment (30th and 45th day) and at the end of the treatment (60th day). The body weight gain was taken as a criteria to assess the health status. The liver glycogen level which indicated the body reserve of energy was also estimated on 60th day to assess the health status of treatment groups. The blood glucose level was estimated before(zero day) and after (15th day) the induction of diabetes, during the course of treatment (30th and 45th day) and at the end of the treatment (60th day) to assess the hypoglycemic effect of *Coccinia indica* leaf.

4.1.1.1 Body Weight

The mean body weight recorded on day zero of groups I, II, III and IV were 173.75±3.10, 191.25±4.09, 195.63±2.74 and 195.00±2.83 g respectively. In this the group I differed from the other groups on zero day itself and so to nullify the effect of this on further results, univariate analysis of variance was used as the statistical tool for analysis of body weight. The mean values and percentage reduction in body weight are represented in Table 1 and 2 and Figure 3.

On 15th day, all the groups showed gradual decrease in body weight except the group I (normal control). The mean body weight obtained for the groups I to IV on 15th day were 172.50±4.63, 180.00±3.27, 181.86±3.77 and 180.00±3.13 g respectively.

On 30th day, the *Coccinia* treated group (group III) and the glibenclamide treated group (group IV) showed a slight increase in body weight and it was not significant ($p>0.05$) with the normal control (group I). The mean body weight recorded for groups I,II,III and IV on 30th day were 173.13±3.13, 168.75±2.27, 165.00±2.67 and 168.75±2.46 g respectively.

On 45th day the mean body weight obtained were 174.38±2.90, 162.50±1.89, 175.00±1.64 and 173.75±1.57 g respectively for group I to IV. There was a significant ($p<0.05$) increase in body weight in the treatment groups III and IV and the mean body weight of these groups were comparable with that of group II. The percentage increase in body weight of group III and IV were 7.69 and 6.92 respectively when compared with the diabetic control (group II).

On 60th day, the Coccinia treated group (group III) and glibenclamide treated group (group IV) showed a significant increase in body weight with respect to their body weight on 45th day. On 60th day, the mean body weight was significantly higher ($p<0.05$) for group III with respect to normal control (group II). In the group IV,

the mean body weight was comparable with respect to normal control. The percentage increase in body weight were 25.0 and 19.76 for group III and IV respectively when compared with the diabetic control. The mean body weight on 60th day were 176.25±4.43, 155.00±1.89, 193.75±2.46 and 185.63±2.58 g for group I to IV respectively.

4.1.1.2. Blood Glucose Level

Blood glucose level was estimated before giving alloxan (zero day) and on 15th,30th,45th and 60th day after alloxan administration. The mean values and percentage reduction in blood glucose are represented in Table 3 and 4 and Figure 4.

The blood glucose level of rats before giving alloxan(zero day) were 97.38±2.31, 94.75±3.21, 102.13±3.73, 91.75±1.39 mg/dl for groups I to IV respectively.

On 15th day, all the groups except normal control showed an increase in blood glucose level with a mean value of 296.25±14.18, 293.13±10.62 and 289.75±11.10 mg/dl respectively for groups II,III and IV. These values represents the alloxan induced diabetic blood glucose levels before giving various treatments. The mean blood glucose concentration for normal control was 93.38±2.88 mg/dl which varied significantly ($p<0.05$) from the diabetic induced groups.

On 30th day, that is 15 days after the beginning of treatment, the treatment groups III and IV showed significant ($p<0.05$) reduction in blood glucose level when compared with the diabetic control. The reduction in blood glucose level on 30th day by Coccinia (200 mg/kg) and glibenclamide (0.25 mg/kg) were comparable, for which the values obtained were 197.63±6.62, 195.38±3.95 mg/dl respectively. The percentage reduction in blood glucose was 28.72 and 29.53 for group III and IV respectively.

On 45th day there was a significant ($p<0.05$) reduction in blood glucose level for group III and IV on comparison with the diabetic control, though the treatments did not lower the blood glucose level to normal level. The blood glucose reduced

significantly ($p<0.05$) by 45.23 and 31.83 per cent for group III and IV respectively when compared with the diabetic control.

At the end of the experiment (on 60th day), the treatment groups showed a significant ($p<0.05$) fall in blood glucose level with percentage reduction being 50.64 and 46.06 for group III and IV respectively. Between the groups III and IV , group III treated with *Coccinia indica* at the dose rate of 200 mg/kg showed a greater decrease ($p<0.05$) in blood glucose value of 116.00±2.65 mg/dl. This was comparable with blood glucose value of 126.75±2.88 mg/dl produced by glibenclamide at the dose rate of 0.25 mg/kg.

4.1.1.3 Liver Glycogen

Liver glycogen was estimated after 45 days of the commencement of treatment and is presented in Table 9 and Figure 7. Group I had a mean liver glycogen value of 4.71±0.29 g%. There was a significant ($p<0.05$) reduction in liver glycogen levels in group II when compared with the group I and the value obtained was 2.40±0.31g%. The Coccinia treated group (group III) and glibenclamide treated group (group IV) showed a significant ($p<0.05$) increase in liver glycogen when compared to the group II (diabetic control). Increase in liver glycogen produced by groups III and IV were almost similar, the values obtained were 3.99±0.21 and 3.78±0.16 g% respectively. The percentage increase in liver glycogen were 66.25 and 57.50 for group III and IV respectively.

Table 1. Effect of ethanolic extract of *Coccinia indica* leaf on body weight in alloxan induced diabetic rats, grams

		0 day	15th day	30th day	45th day	60th day
Normal control	Mean	173.75	172.50[a]	173.13[b]	174.38[b]	176.25[b]
	S.E	3.10	4.63	3.13	2.90	4.43
Diabetic control	Mean	191.25	180.00[ab]	168.75[ab]	162.50[a]	155.00[a]
	S.E	4.09	3.27	2.27	1.89	1.89
Coccinia indica 200 mg/kg	Mean	195.63	181.86[ab]	165.00[ab]	*175.00[b]	*193.75[d]
	S.E	2.74	3.77	2.67	1.64	2.46
Glibenclamide 0.125 mg/kg	Mean	195.00	180.00[ab]	168.75[ab]	*173.75[b]	*185.63[c]
	S.E	2.83	3.13	2.46	1.57	2.58

*P<0.05,significant at 5 per cent level. Compared with diabetic control.
Means bearing the same superscript do not differ significantly

Table 2. Effect of ethanolic extract of *Coccinia indica* on percentage increase in body weight in alloxan-diabetic rats

Day	***Coccinia indica* 200 mg/kg**	**Glibenclamide 0.25 mg/kg**
30th	2.22	0.00
45th	7.69	6.92
60th	25.0	19.76

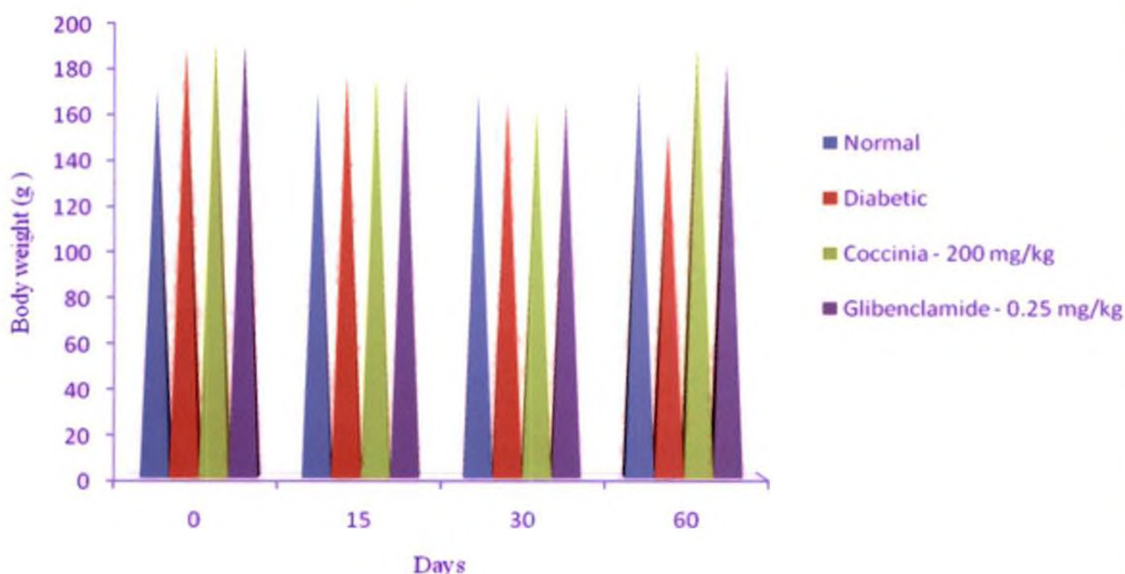


Fig.3-Effect of ethanolic extract of Coccinia indica leaves on body weight in diabetic rats
Body weight (g)
200
180
160
140
120
100
80
60
40
20
0
0
15
30
60
Days
Normal
Diabetic
Coccinia - 200 mg/kg
Glibenclamide - 0.25 mg/kg

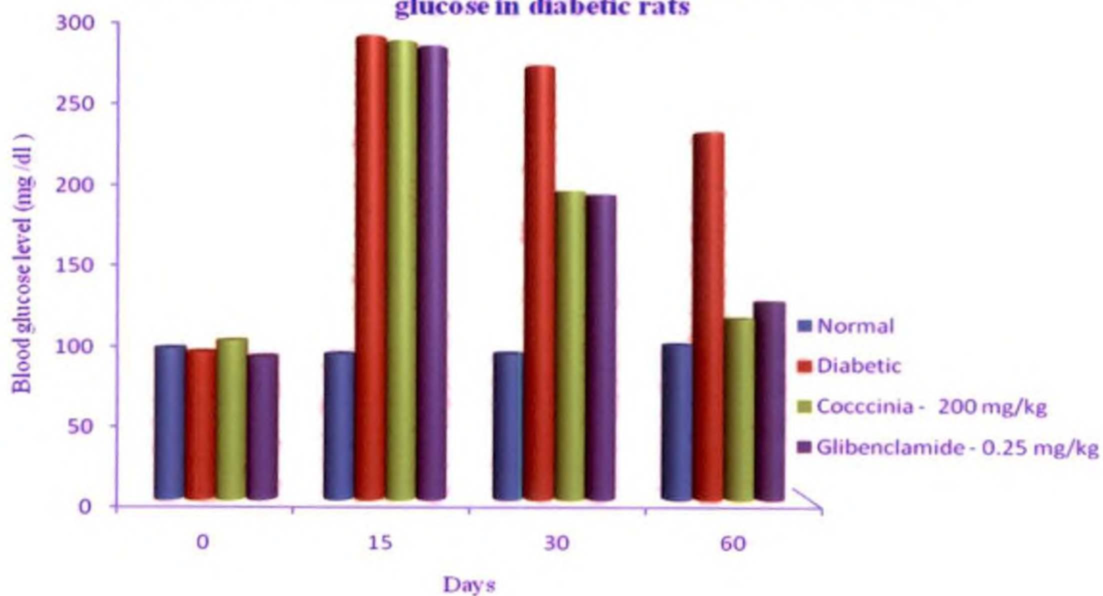


Fig.4-Effect of ethanolic extract of Coccinia indica leaves on blood glucose in diabetic rats
Blood glucose level (mg/dl)
300
250
200
150
100
50
0
0
15
30
60
Days
Normal
Diabetic
Cocccinia - 200 mg/kg
Glibenclamide - 0.25 mg/kg

Table 3. Effect of ethanolic extract of *Coccinia indica* leaf on blood glucose in alloxan induced diabetic rats, mg/dl

		0 day	15th day	30th day	45th day	60th day
Normal control	Mean	97.38	93.38[a]	93.50[a]	100.87[a]	99.25[a]
	S.E	2.31	2.88	1.85	2.87	2.02
Diabetic control	Mean	94.75	296.25[b]	277.25[d]	249.00[d]	235.00[d]
	S.E	3.21	14.18	11.84	9.67	7.17
Coccinia indica 200 mg/kg	Mean	102.13	293.13[b]	*197.63[bc]	*136.38[b]	*116.00[b]
	S.E	3.73	10.62	6.62	3.44	2.65
Glibenclamide 0.125 mg/kg	Mean	91.75	289.75[b]	*195.38[bc]	*169.75[c]	*126.75[c]
	S.E	1.39	11.10	3.95	2.91	2.88

*P<0.05,significant at 5 per cent level. Compared with diabetic control.
Means bearing the same superscript do not differ significantly

Table 4. Effect of ethanolic extract of *Coccinia indica* on percentage reduction in blood glucose in alloxan-diabetic rats

Day	***Coccinia indica* 200 mg/kg**	**Glibenclamide 0.25 mg/kg**
30th	28.72	29.53
45th	45.23	31.83
60th	50.64	46.06

4.1.2 Evaluation of Hypolipidemic Effect of *Coccinia indica*

4.1.2.1 Serum Cholesterol Level

Serum cholesterol levels estimated for groups I to IV on zero day,15th, 30th, 45th and 60th day are presented in Table 5 and 6 and Figure 5. The serum cholesterol level of rats on zero day was 48.25±1.77, 52.50±2.24, 47.00±2.56, 53.63±2.47 mg/dl respectively for groups I to IV respectively which did not vary significantly from one another.

On 15th day, all the groups except normal control showed an increase in serum cholesterol level with mean values of 88.25±2.80, 100.75±3.80 and 90.00±2.78 mg/dl for groups II,III and IV respectively which showed a significant ($p<0.05$) difference when compared with normal control (49.12±1.72 mg/dl).

On 30th day, there was a significant ($p<0.05$) decrease in serum total cholesterol value in groups III and IV compared to group II. The reduction in serum cholesterol level seen in groups III and IV were almost similar, for which the mean cholesterol values obtained were 90.75±4.22 and 88.13±2.47 mg/dl respectively. The percentage reduction in serum cholesterol was 4.72 and 7.47 for group III and IV respectively.

The treatment groups III and IV showed significant ($p<0.05$) decrease in serum cholesterol level compared with group II (diabetic control) on 45th day. Group II had a higher cholesterol value of 87.88±1.96 mg/dl and the serum cholesterol level reduced by 34.00 and 31.86 % for groups III and IV respectively when compared with the diabetic control group.

On 60th day, group III treated with Coccinia (200 mg/kg) showed a serum cholesterol level of 50.25±1.35 mg/dl which was comparable with that of group IV treated with glibenclamide (0.25 mg/dl) which showed a serum cholesterol value of 50.88±1.04 mg/dl. These values showed a significant ($p<0.05$) reduction in serum cholesterol level when compared to the diabetic control (86.75±2.30 mg/dl) and the

percentage reduction was 42.07 and 41.35 for group III and IV respectively. The serum cholesterol values on 60th day also showed significant ($p<0.05$) reduction in group III and IV when compared with serum cholesterol values of same respective groups on 15th day when analyzed by paired t-test.

4.1.2.2 Serum Triglyceride Level

The mean serum triglyceride values obtained on zero day were 101.00±2.87, 101.00±3.46, 99.50±2.59 and 97.50±2.82 mg/dl respectively for group I to IV which represented the normal serum triglyceride level before giving alloxan.

On 15th day, all the groups with the exception of normal control showed an increase in serum triglyceride level with a mean value of 162.25±7.69, 154.63±2.59 and 157.75±5.71 mg/dl respectively for groups II,III and IV.

On 30th day, the Coccinia (200 mg/kg) treated group (group III) and glibenclamide (0.25 mg/kg) treated group (group IV) showed a significant reduction of serum triglyceride than diabetic control group (group II). There was no significant difference in serum triglyceride between group III and IV, the value obtained were 152.13±4.97 and 147.13±4.19 mg/dl respectively.

On 45th day of the experiment, there was significant ($p<0.05$) lowering of serum triglyceride levels when compared with the 30th day values of both group III and group IV. The mean serum triglyceride values obtained were 115.88±2.95 and 128.50±2.85 mg/dl respectively for group III and IV and 163.00±6.69 mg/dl for group II. The percentage reduction in serum triglyceride values were 28.91 and 21.17 for group III and IV respectively.

Table 5. Effect of ethanolic extract of *Coccinia indica* leaf on serum cholesterol in alloxan induced diabetic rats, mg/dl

		0 day	15th day	30th day	45th day	60th day
Normal control	Mean	48.25	49.12[a]	53.25[a]	53.38[a]	53.50[a]
	S.E	1.77	1.72	2.19	1.53	1.54
Diabetic control	Mean	52.50	88.25[b]	95.25[c]	87.88[c]	86.75[c]
	S.E	2.24	2.80	3.29	1.96	2.30
Coccinia indica 200 mg/kg	Mean	47.00	*100.75[c]	90.75[bc]	*58.00[ab]	*50.25[ab]
	S.E	2.56	3.80	4.22	2.28	1.35
Glibenclamide 0.125 mg/kg	Mean	53.63	90.00[bc]	88.13[bc]	*59.88[ab]	*50.88[ab]
	S.E	2.47	2.78	2.47	3.04	1.04

*P<0.05,significant at 5 per cent level. Compared with diabetic control.
Means bearing the same superscript do not differ significantly

Table 6. Effect of ethanolic extract of *Coccinia indica* on percentage reduction of serum cholesterol in alloxan-diabetic rats

Day	**Coccinia indica leaf extract 200 mg/kg**	**Glibenclamide 0.25 mg/kg**
30th	4.72	7.47
45th	34.00	31.86
60th	42.07	41.35

Table 7. Effect of ethanolic extract of *Coccinia indica* leaf on serum triglyceride in alloxan induced diabetic rats, mg/dl

		0 day	15th day	30th day	45th day	60th day
Normal control	Mean	101.00	100.00[a]	98.63[a]	96.50[a]	98.75[a]
	S.E	2.87	3.40	2.51	3.13	2.10
Diabetic control	Mean	101.00	162.25[b]	168.75[c]	163.00[d]	162.25[b]
	S.E	3.46	7.69	6.98	6.69	9.42
Coccinia indica 200 mg/kg	Mean	99.50	154.63[b]	*152.13[b]	*115.88[b]	*105.88[a]
	S.E	2.59	2.59	4.97	2.95	2.82
Glibenclamide 0.125 mg/kg	Mean	97.50	157.75[b]	*147.13[b]	*128.50[c]	*104.63[a]
	S.E	2.82	5.71	4.19	2.85	2.60

*P<0.05,significant at 5 per cent level. Compared with diabetic control.
Means bearing the same superscript do not differ significantly

Table 8. Effect of ethanolic extract of *Coccinia indica* on s percentage reduction of serum triglyceride in alloxan-diabetic rats

Day	***Coccinia indica* leaf extract 200 mg/kg**	**Glibenclamide 0.25 mg/kg**
30th	9.84	12.8
45th	28.91	21.17
60th	34.74	35.51

Fig.5 - Effect of ethanolic extract of *Coccinia indica* leaves on serum cholesterol levels in diabetic rats

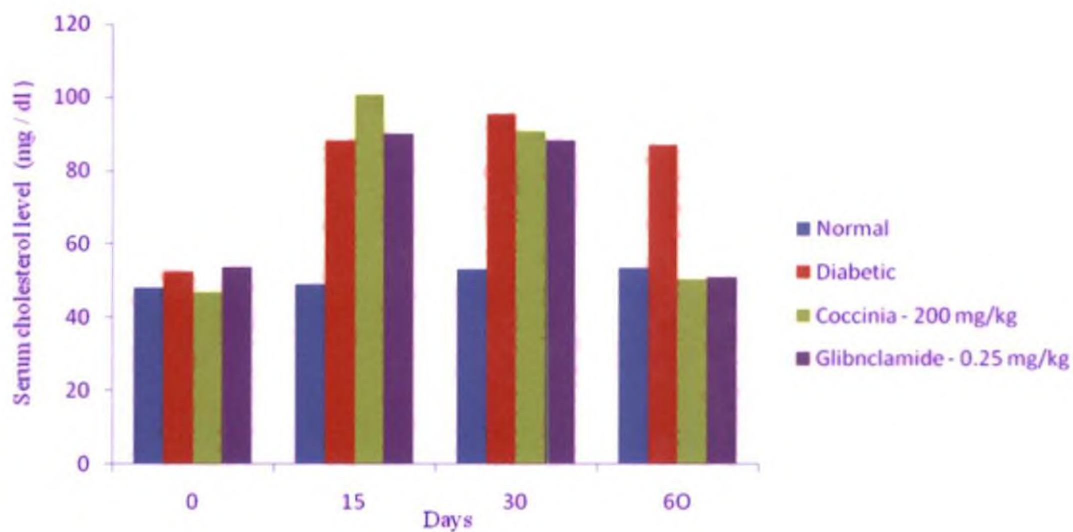
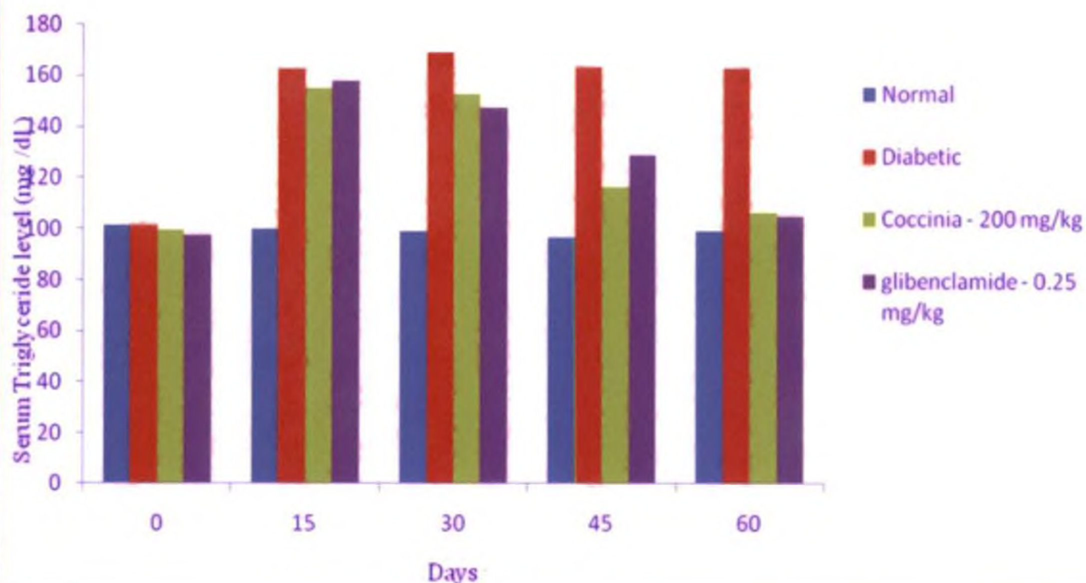


Fig.6 - Effect of ethanolic extract of *Coccinia indica* leaves on serum triglyceride levels in diabetic rats



On 60th day, after the end of the treatment, the serum triglyceride values of the treatment groups almost regained to that of normal control group and the mean triglyceride value of group III and IV were 105.88±2.82 and 104.63±2.60 mg/dl and that of group II was 162.25±9.42 mg/dl. The percentage reduction in serum triglyceride levels were 34.74 and 35.51 per cent for Coccinia treated and glibenclamide treated group respectively. There were no significant differences between the reduction of serum triglyceride by Coccinia (200mg/kg) and glibenclamide(0.25 mg/kg). Effect of Coccinia and glibenclamide treatments on serum triglyceride levels is presented in Table 7 and 8 and Figure 6.

4.1.3 Evaluation of Antioxidant Effect of *Coccinia indica*

4.1.3.1 Reduced Glutathione in Pancreas

After 45 days of the treatment, that is 60 days after the commencement of the experiment, reduced glutathione in pancreas was estimated. The mean values obtained were 577.63±5.29, 217.50±7.13, 463.13±17.37 and 493.13±12.68 μg / g for group I to IV respectively. The Coccinia treated group showed a significantly low ($p<0.05$) reduced glutathione values when compared with glibenclamide treated group. But both treatment groups showed significant increase in reduced glutathione values when compared to the diabetic control (group II).

4.1.3.2 Reduced Glutathione in Liver

The mean values of reduced glutathione in liver after 45 days of treatment were 492.50±7.01, 66.25±7.72, 290.00±11.80 and 313.75±20.71 μg / g respectively for group I to IV. There was significant ($p<0.05$) increase in reduced glutathione values with respect to diabetic control (group II) in Coccinia treated (group III) and glibenclamide

treated group (group IV) and both did not significantly vary each other. Effect of Coccinia and glibenclamide treatments on reduced glutathione levels is presented in Table 9 and Figure 8 and 9.

4.1.3.3 Lipid Peroxides in Pancreas

Lipid peroxides estimated in pancreas after the end of the treatment (60th day of experiment) showed mean values of 82.13±3.99, 336.63±9.91, 166.50±9.71 and 123.50±9.51 nM / g for groups I to IV respectively. There were significant ($p<0.05$) reduction in the lipid peroxides of pancreas in *Coccinia* (group III) treated group and glibenclamide (group IV) treated group. The percentage reduction in lipid peroxides in group III and IV were 50.54 and 63.31 respectively with respect to the diabetic control.

4.1.3.4 Lipid Peroxides in Liver

The mean values of lipid peroxides in liver obtained after 60 days of experiment was 140.88±3.49, 392.75±12.31, 212.50±3.74 and 197.38±7.99 nM / g for group I to IV respectively. There was significant ($p<0.05$) reduction in lipid peroxides of liver in group III and IV when compared to the diabetic control and the percentage reduction with respect to diabetic control was 45.89 and 49.74 for group III and IV respectively. The reduction in lipid peroxide levels produced at the end of the treatment with Coccinia (200 mg/kg) and glibenclamide (0.25 mg/kg) are presented in Figure 10 and 11 and Table 9.

Table 9. Effect of ethanolic extract of *Coccinia indica* leaf on liver glycogen(g%), reduced glutathione in pancreas and liver (µg / g) and lipid peroxides (nM / g) in pancreas and liver in alloxan induced diabetic rats on 60th day

		Liver glycogen	Reduced glutathione-pancreas	Reduced glutathione-liver	Lipid peroxides-pancreas	Lipid peroxides-liver
Normal control	Mean	4.71^{c}	577.63^{d}	492.50^{c}	82.13^{a}	140.88^{a}
	S.E	0.29	5.29	7.01	3.99	3.49
Diabetic control	Mean	2.40^{a}	217.50^{a}	66.25^{a}	336.63^{d}	392.75^{c}
	S.E	0.31	7.13	7.72	9.91	12.31
Coccinia indica 200 mg/kg	Mean	$*3.99^{b}$	$*463.13^{b}$	$*290.00^{b}$	$*166.50^{c}$	$*212.50^{b}$
	S.E	0.21	17.37	11.80	9.71	3.74
Glibenclamide 0.125 mg/kg	Mean	$*3.78^{b}$	$*493.13^{c}$	$*313.75^{b}$	$*123.50^{b}$	$*197.38^{b}$
	S.E	0.16	12.68	20.71	9.51	7.99

*$P<0.05$,significant at 5 per cent level. Compared with diabetic control.
Means bearing the same superscript do not differ significantly

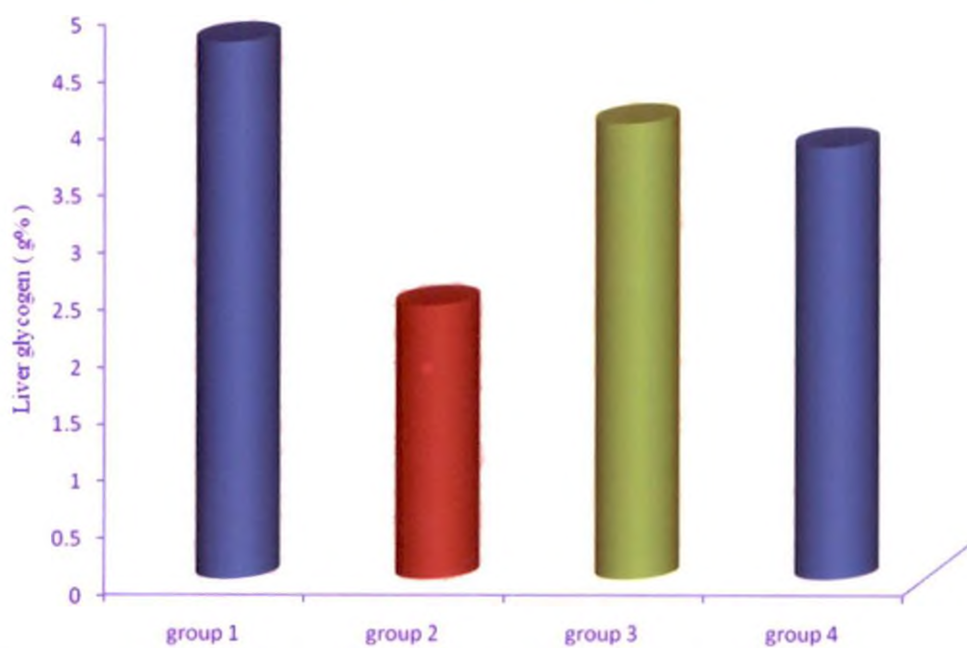


Fig.7 - Effect of ethanolic extract of Coccinia indica leaves on liver glycogen on 60th day
Liver glycogen (g%)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
group 1
group 2
group 3
group 4

Fig.8 - Effect of ethanolic extract of *Coccinia indica* leaves on reduced glutathione in pancreas on 60th day

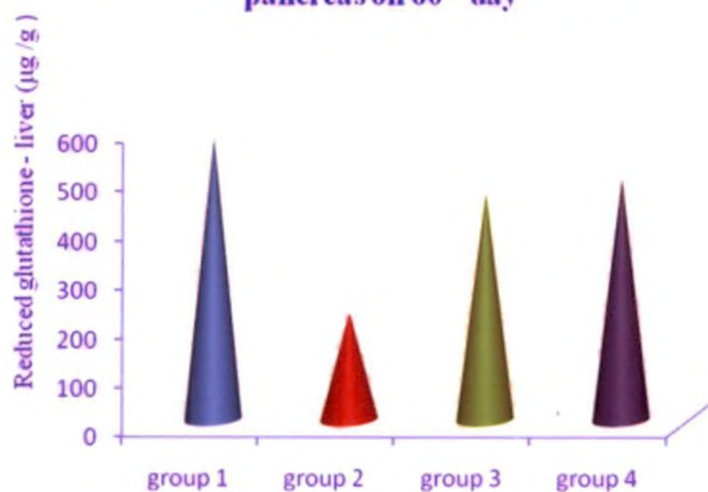


Fig.9 - Effect of ethanolic extract of *Coccinia indica* leaves on reduced glutathione in liver on 60th day

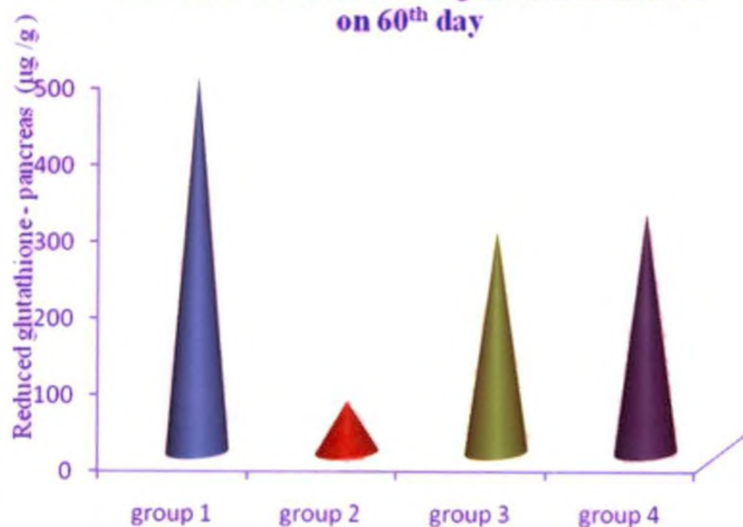


Fig.10 - Effect of ethanolic extract of *Coccinia indica* leaves on lipid peroxides in pancreas on 60th day

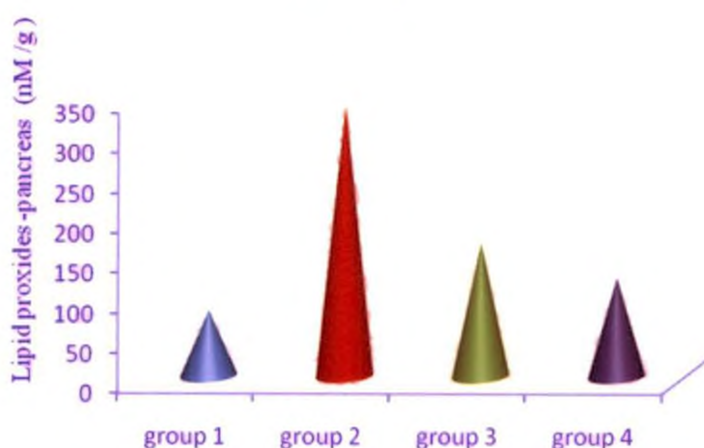
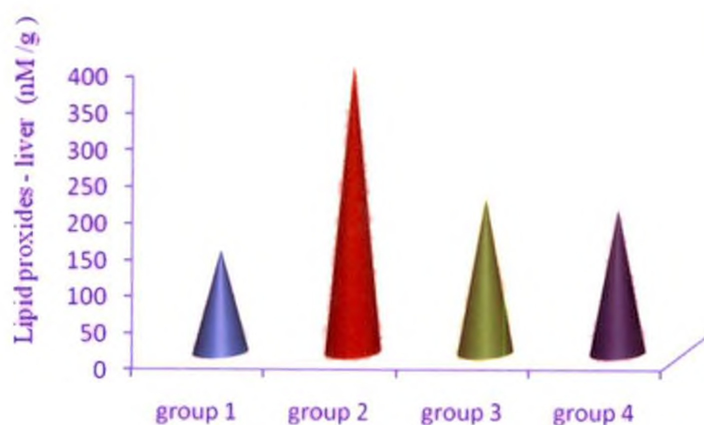


Fig.11 - Effect of ethanolic extract of *Coccinia indica* leaves on lipid peroxides in liver on 60th day



4.2 COMPARATIVE STUDY ON INTERACTION OF *Coccinia indica* WITH GLIBENCLAMIDE

Coccinia indica at three different doses of 100, 150 and 200 mg/kg was administered along with glibenclamide at the dose rate of 0.125 mg/kg to evaluate the interaction effect of *Coccinia* with glibenclamide.

4.2.1 Interaction of *Coccinia* with glibenclamide on general health improving and hypoglycemic effect

4.2.1.1 Body weight

The mean body weight recorded on zero day of groups I, II, V, VI and VII were 173.75±3.10, 191.25±4.09, 195.63±3.59, 194.38±3.59 and 196.25±2.63 g respectively. In this the group I differed from the other groups on zero day itself and so to nullify the effect of this on further results, univariate analysis of variance was used as the statistical tool for analysis of body weight. The mean values and percentage reduction are recorded are represented in the Table 10 and Figure 12.

On 15th day, after the induction of diabetes, all the groups showed gradual decrease in body weight except group I (normal control). The mean body weight obtained for groups I, II, V, VI and to VII on 15th day were 172.50±4.63, 180.00±3.27, 175.63±2.41, 178.75±3.63 and 187.50±4.53 g respectively.

The mean body weight recorded for groups I,II,V,VI and VII on 30th day were 173.13±3.13, 168.75±2.27, 160.63±3.05, 161.87±3.53 and 162.50±3.66 g respectively. On 30th day, group V, VI and VII did not show any significant ($p>0.05$) increase in body weight when compared with group II.

On 45th day the mean body weight obtained were 174.38±2.90, 162.50±1.89, 185.00±3.27, 181.88±4.43 and 176.25±2.63 g respectively for group I, II, V, VI and VII. There was a significant increase in body weight in the treatment groups

V, VI and VII and the mean body weight of these groups were comparable with that of normal control (group I). The percentage increase in body weight of group V,VI and VII were 13.84, 11.93 and 8.46 respectively when compared with the diabetic control (group II).

On 60th day, the mean body weight of the treatment groups almost gained back to normal body weight (before the induction of diabetes) and they did not differ significantly from mean body weight of zero day. The mean body weight obtained for group V,VI and VII were 198.13±2.30, 196.88±4.53 and 197.50±2.50 respectively. The body weight increased by 27.83, 27.01 and 27.41 per cent for group V,VI and VII respectively when compared with the diabetic control (group II).

*4.2.1.2. **Blood glucose level***

Blood glucose level was estimated before giving alloxan (zero day) and on 15th, 30th, 45th and 60th day after alloxan administration.

The mean blood glucose levels of rats before giving alloxan (zero day) were 97.38±2.31, 94.75±3.21, 101.63±3.23, 96.50±3.62 and 92.00±2.11 mg/dl for groups I,II, V,VI and VII respectively.

On 15th day, the mean values of blood glucose were 93.38±2.88, 296.25±14.18, 296.88±9.82, 284.13±11.38 and 273.88±11.04 mg/dl for group I, II, V, VI and VII respectively. All the groups differed significantly($p<0.05$) from group I (normal control) and there were no significant differences among the groups II, V, VI and VII.

On 30th day, that is 15 days after the beginning of treatment, the treatment groups V, VI and VII showed significant ($p<0.05$) lowering in blood glucose level when compared with the diabetic control. The reduction in blood glucose level was more marked in group VII which was evident from the percentage reduction of 42.33 when compared to 26.64 and 35.17 of group V and VI respectively. The mean blood glucose

values of group I, II, V, VI and VII were 93.50±1.85, 277.25±11.84, 203.38±5.15, 179.75±2.67 and 159.88±5.12 mg/dl respectively.

The mean blood glucose values on 45th day were 100.87±2.87, 249.00±9.67, 184.00±6.40, 152.00±8.50 and 126.63±4.14 mg/dl respectively for group I,II,V,VI and VII respectively. The treatment groups V,VI and VII showed a significant ($p<0.05$) reduction in mean blood glucose level when compared with the diabetic control(group II). The percentage reduction in mean blood glucose level were 26.10, 38.96 and 49.14 respectively for group V, VI and VII respectively.

At the end of the experiment (on 60th day), the treatment groups showed a significant ($p<0.05$) fall in blood glucose level with percentage reduction being 33.46, 42.55 and 53.83 for group V, VI and VII respectively. Among the groups V, VI and VII, group VII treated with a combination of *Coccinia indica* at the dose rate of 200 mg/kg and glibenclamide at the dose rate of 0.125 mg/kg showed the lowest blood glucose value of 108.50±2.54 mg/dl. The mean blood glucose values were 99.25±2.02, 235.00±7.17, 156.38±3.33 and 135.00±2.50 mg/dl for group I, II, V and VI respectively. The reduction in blood glucose levels produced at the end of the treatment with combination treatments of *C. indica* and glibenclamide are presented in Table 11, 12 and Figure 13.

4.2.1.3 Liver glycogen

Liver glycogen was estimated after 45 days of the commencement of treatment and are presented in Table 17 and Figure 16. Group I had a mean liver glycogen value of 4.71±0.29 g%. There was a significant($p<0.05$) reduction in liver glycogen level of group II compared to group I, the value obtained was 2.40±0.31g%. Group V, VI and VII produced a significant($p<0.05$) increase in liver glycogen when compared to group II. Increase in liver glycogen of group VII was most marked with a

Table 10. Interactive effect of ethanolic extract of *Coccinia indica* leaf and glibenclamide on body weight in alloxan induced diabetic rats, grams

Groups		0 day	15th day	30th day	45th day	60th day
I	Mean	173.75	172.50[a]	173.13[b]	174.38[b]	176.25[b]
	S.E	3.10	4.63	3.13	2.90	4.43
II	Mean	191.25	180.00[ab]	168.75[ab]	162.50[a]	155.00[a]
	S.E	4.09	3.27	2.27	1.89	1.89
V	Mean	195.63	175.63[ab]	160.63[a]	*185.00[c]	*198.13[c]
	S.E	3.59	2.41	3.05	3.27	2.30
VI	Mean	194.38	178.75[ab]	161.87[a]	*181.88[bc]	*196.88[c]
	S.E	3.59	3.63	3.53	4.43	4.53
VII	Mean	196.25	187.50[b]	162.50[a]	*176.25[b]	*197.50[c]
	S.E	2.63	4.53	3.66	2.63	2.50

*$P<0.05$,significant at 5 per cent level. Compared with diabetic control.

Means bearing the same superscript do not differ significantly

Table 11. Interactive effect of ethanolic extract of *Coccinia indica* leaf and glibenclamide on blood glucose in alloxan induced diabetic rats, mg/dl

Groups		0 day	15th day	30th day	45th day	60th day
I	Mean	97.38	93.38^{a}	93.50^{a}	100.87^{a}	99.25^{a}
	S.E	2.31	2.88	1.85	2.87	2.02
II	Mean	94.75	296.25^{b}	277.25^{e}	249.00^{e}	235.00^{e}
	S.E	3.21	14.18	11.84	9.67	7.17
V	Mean	101.63	296.88^{b}	$*203.38^{d}$	$*184.00^{d}$	$*156.38^{d}$
	S.E	3.23	9.82	5.15	6.40	3.33
VI	Mean	96.50	284.13^{b}	$*179.75^{c}$	$*152.00^{c}$	$*135.00^{c}$
	S.E	3.62	11.38	2.67	8.50	2.50
VII	Mean	92.00	273.88^{b}	$*159.88^{b}$	$*126.63^{b}$	$*108.50^{ab}$
	S.E	2.11	11.04	5.12	4.14	2.54

*P<0.05,significant at 5 per cent level. Compared with diabetic control.
Means bearing the same superscript do not differ significantly

Table 12. Interactive effect of ethanolic extract of *Coccinia indica* leaf and glibenclamide on blood glucose in alloxan-diabetic rats, percentage reduction

Day	**Group V**	**Group VI**	**Group VII**
30th	26.64	35.17	42.33
45th	26.10	38.96	49.14
60th	33.46	42.55	53.83

Fig.12-Interactive effect of ethanolic extract of *Coccinia indica* leaves and glibenclamide on body weight in diabetic rats

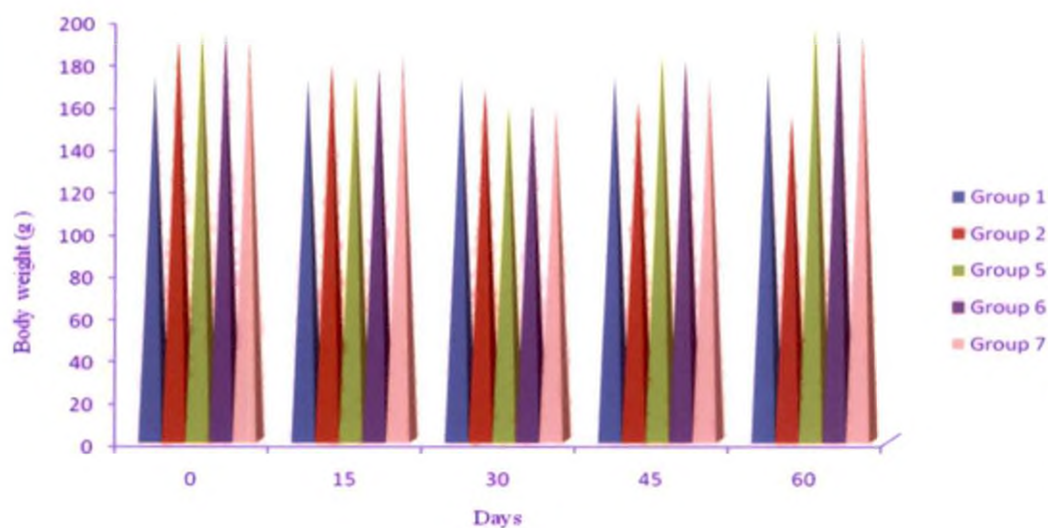
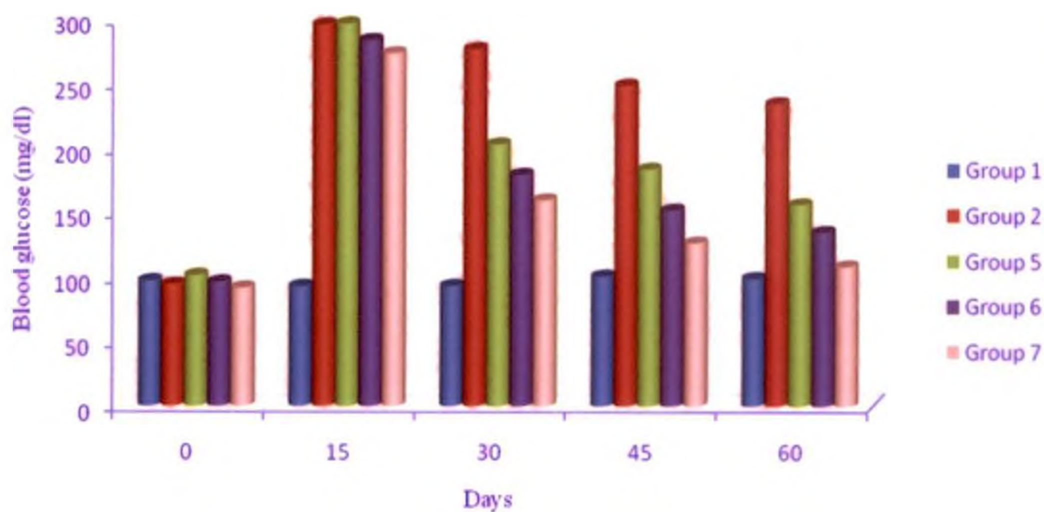


Fig.13-Interactive effect of ethanolic extract of *Coccinia indica* leaves and glibenclamide on blood glucose in diabetic rats



percentage increase of 70.83 with respect to diabetic control (group II) and that of group VI and VII were 51.64 and 67.08 respectively. The mean liver glycogen value of group V, VI and VII were 3.66±0.23, 4.01±0.38 and 4.10±0.19 g% respectively.

4.2.2 Interaction of *Coccinia* with glibenclamide on hypolipidemic effect

4.2.2.1 Serum cholesterol level

Serum cholesterol levels estimated for groups I, II, V, VI and VII on zero day, 15th, 30th, 45th and 60th day are presented in Table 13, 14 and Figure 14. The serum cholesterol level of rats on zero day were 48.25±1.77, 52.50±2.24, 49.25±3.02, 43.63±1.94 and 46.13±1.90 mg/dl respectively for groups I, II, V, VI and VII which do not vary significantly from one another.

On 15th day, all the groups except normal control showed an increase in cholesterol level. The mean serum cholesterol value were 88.25±2.80, 100.13±4.86, 99.13±3.21 and 96.88±2.91 mg/dl for groups II, V, VI and VII respectively which varied significantly with normal control value of 49.12±1.72 mg/dl.

On 30th day, there was a significant ($p<0.05$) decrease in serum total cholesterol value in groups V, VI and VII compared to group II. The reduction in serum cholesterol level seen in groups V, VI and VII were almost similar, for which the values obtained were 85.38±3.50, 89.63±1.86 and 90.88±2.34 mg/dl for group V, VI and VII respectively. The mean cholesterol value of diabetic control was 95.25±3.29 mg/dl.

The treatment groups V, VI and VII showed significant ($p<0.05$) decrease in serum cholesterol level compared to group II (diabetic control) on 45th day. Group II had a higher cholesterol value of 87.88±1.96 mg/dl and the percentage reduction of

Table 13. Interactive effect of ethanolic extract of *Coccinia indica* leaf and glibenclamide on serum cholesterol in alloxan induced diabetic rats, mg/dl

Groups		0 day	15th day	30th day	45th day	60th day
I	Mean	48.25	49.12[a]	53.25[a]	53.38[a]	53.50[b]
	S.E	1.77	1.72	1.53	1.53	1.54
II	Mean	52.50	88.25[b]	95.25[c]	87.88[d]	86.75[c]
	S.E	2.24	2.80	3.29	1.96	2.30
V	Mean	49.25	100.13[d]	*85.38[b]	*64.38[c]	*53.00[b]
	S.E	3.02	4.86	3.50	2.90	2.41
VI	Mean	43.63	99.13[cd]	89.63[bc]	*61.38[bc]	*50.13[ab]
	S.E	1.94	3.21	1.86	2.97	2.46
VII	Mean	46.13	96.88[bcd]	90.88[bc]	*55.88[ab]	*46.00[a]
	S.E	1.90	2.91	2.34	1.66	1.85

*P<0.05,significant at 5 per cent level. Compared with diabetic control.
Means bearing the same superscript do not differ significantly

Table 14. Interactive effect of ethanolic extract of *Coccinia indica* leaf and glibenclamide on serum cholesterol in alloxan-diabetic rats, percentage reduction

Day	**Group V**	**Group VI**	**Group VII**
30th	10.36	5.9	4.59
45th	26.74	30.15	36.41
60th	38.9	42.21	46.97

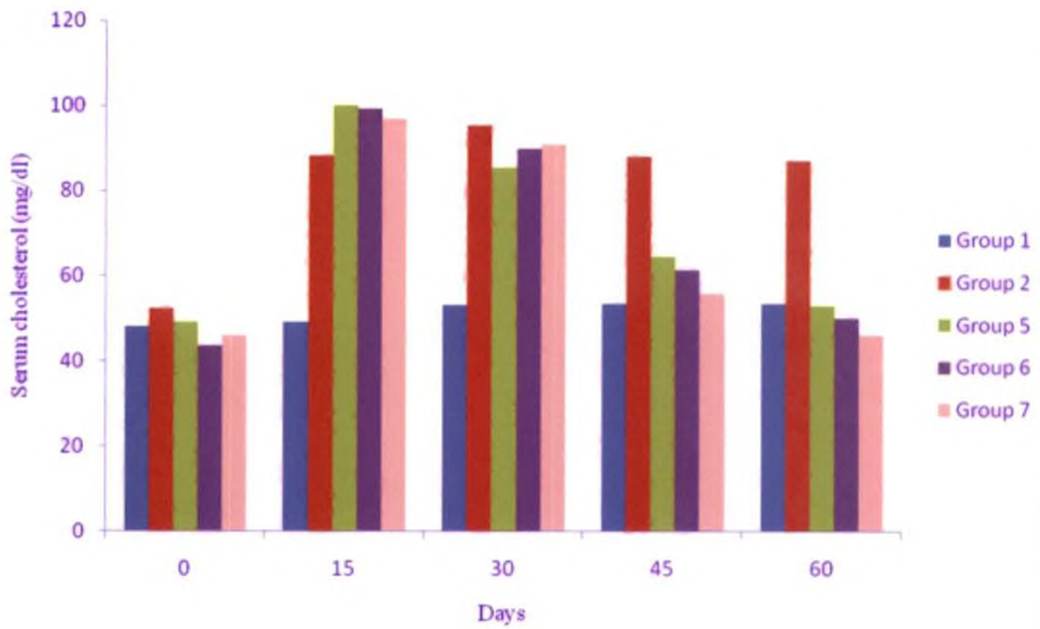


Fig.14-Interactive effect of ethanolic extract of *Coccinia indica* leaves and glibenclamide on serum cholesterol in diabetic rats
120
100
80
60
40
20
0
Serum cholesterol (mg/dl)
0
15
30
45
60
Days
Group 1
Group 2
Group 5
Group 6
Group 7

serum cholesterol level was 26.74, 30.15 and 36.41 for group V, VI and VII respectively when compared with the diabetic control group.

On 60th day, there was a significant ($p<0.05$) decrease in mean serum cholesterol values of group V, VI and VII and the mean value of these groups were comparable with that of normal control (group I). The mean serum cholesterol values of group I, II, V, VI and VII were 53.50±1.54, 86.75±2.30, 53.00±2.41, 50.13±2.46 and 46.00±1.85 mg/dl respectively. The percentage reduction of serum cholesterol when compared with the diabetic control (group II) were 38.9, 42.21 and 46.97 for group V, VI and VII respectively.

4.2.2.2 Serum triglyceride level

The mean values obtained on zero day were 101.00±2.87, 101.00±3.46, 98.63±2.88, 90.36±2.35 and 97.71±1.14 mg/dl for group I, II, V, VI and VII respectively which represented the normal serum triglyceride level before giving alloxan.

On 15th day, all the groups except normal control showed an increase in serum triglyceride level with a mean value of 162.25±7.69, 159.13±5.56, 162.25±4.74 and 160.25±4.80 mg/dl for groups II, V, VI and VII respectively.

On 30th day, group V, VI and VII showed a significant ($p<0.05$) reduction of serum triglyceride when compared with diabetic control group(group II). There was no significant difference in serum triglyceride among group V, VI and VII and the value obtained were 148.63±5.03, 147.38±4.40 and 134.50±2.54 mg/dl respectively.

On 45th day of the experiment, there was a significant lowering of serum triglyceride level when compared with the 30th day values of group V, VI and VII. The mean serum triglyceride values obtained were 128.63±5.09, 125.38±2.34 and 104.25±2.82 for group V, VI and VII respectively and group VII showed the most significant reduction compared to group II.

Table 15. Interactive effect of ethanolic extract of *Coccinia indica* leaf and glibenclamide on serum triglyceride in alloxan induced diabetic rats, mg/dl

Groups		0 day	15th day	30th day	45th day	60th day
I	Mean	101.00	100.00[a]	98.63[a]	96.50[a]	98.75[a]
	S.E	2.87	3.40	2.51	3.13	2.10
II	Mean	101.00	162.25[b]	168.75[d]	163.00[d]	162.25[b]
	S.E	3.46	7.69	6.98	6.69	9.42
V	Mean	98.63	159.13[b]	*148.63[bc]	*128.63[c]	*110.75[a]
	S.E	2.88	5.56	5.03	5.09	1.92
VI	Mean	90.36	162.25[b]	*147.38[bc]	*125.38[bc]	*104.25[a]
	S.E	2.35	4.74	4.40	2.34	1.59
VII	Mean	97.71	160.25[b]	*134.50[bc]	*104.25[a]	*101.50[a]
	S.E	1.14	4.80	2.54	2.82	2.52

*P<0.05,significant at 5 per cent level. Compared with diabetic control.
Means bearing the same superscript do not differ significantly

Table 16. Interactive effect of ethanolic extract of *Coccinia indica* leaf and glibenclamide on serum triglyceride in alloxan-diabetic rats, percentage reduction

Day	Group V	Group VI	Group VII
30th	11.92	12.66	20.29
45th	21.09	23.08	36.04
60th	31.74	35.75	37.44

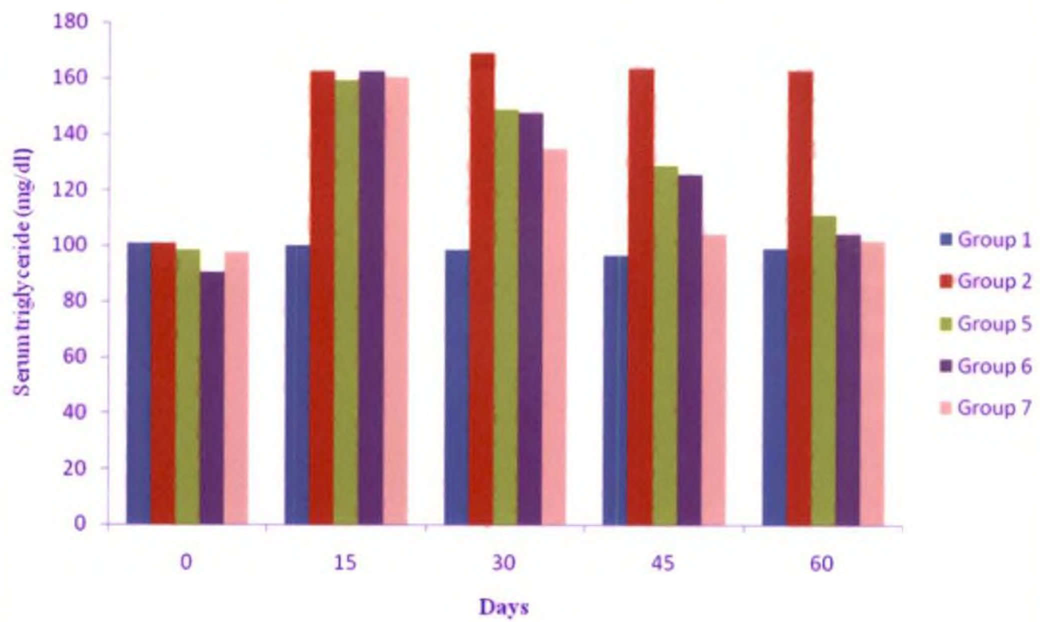


Fig.15-Interactive effect of ethanolic extract of Coccinia indica leaves and glibenclamide on serum triglyceride in diabetic rats
180
160
140
120
100
80
60
40
20
0
Serum triglyceride (mg/dl)
0
15
30
45
60
Days
Group 1
Group 2
Group 5
Group 6
Group 7

The percentage reduction in serum triglyceride values were 21.09, 23.08 and 36.04 for group V, VI and VII respectively with respect to the diabetic control.

After the end of the treatment, the serum triglyceride values of the treatment groups almost reached back to normal and the mean triglyceride value of group V, VI and VII were 110.75±1.92, 104.25±1.59 and 101.50±2.52 mg/dl respectively and there was no significant difference in serum triglyceride level among the treatment groups. The percentage reduction in serum triglyceride level were 31.74, 35.75 and 37.44 for group V, VI and VII respectively. The reduction in serum triglyceride level obtained for different groups on 60th day is presented in Table 15, 16 and Figure 15.

4.2.3 Interaction of *Coccinia* with glibenclamide on antioxidant effect

4.2.3.1 Reduced glutathione in pancreas

After 45 days of the treatment, that is 60 days after the commencement of experiment, reduced glutathione in pancreas were estimated. The mean values obtained were 577.63±5.29, 217.50±7.13, 455.63±7.53, 470.63±3.46 and 491.25±5.15 µg / g for group I , II, V, VI and VII respectively.

All the groups showed a significant ($p<0.05$) increase in reduced glutathione values when compared with the diabetic control (group II). The group VI and VII showed higher increase in reduced glutathione values than group V.

4.2.3.2 Reduced glutathione in liver

The mean values of reduced glutathione in liver after 45 days of treatment were 492.50±7.01, 66.25±7.72, 435.00±10.18, 443.75±5.96 and 452.50±6.48 µg / g respectively for group I, II, V, VI and VII. There was a significant ($p<0.05$) increase in

Table 17. Interactive effect of ethanolic extract of *Coccinia indica* leaf and glibenclamide on liver glycogen, reduced glutathione in pancreas and liver and lipid peroxides in pancreas and liver in alloxan induced diabetic rats

Groups		Liver glycogen (g%)	Reduced glutathione pancreas (µg / g)	Reduced glutathione liver (µg / g)	Lipid peroxides pancreas (nM / g)	Lipid peroxides liver (nM / g)
I	Mean	4.71^{c}	577.63^{e}	492.50^{c}	82.13^{a}	140.88^{a}
	S.E	0.29	5.29	7.01	3.99	3.49
II	Mean	2.40^{a}	217.50^{a}	66.25^{a}	336.63^{c}	392.75^{b}
	S.E	0.31	7.13	7.72	9.91	12.31
V	Mean	3.66^{b}	455.63^{b}	435.00^{b}	113.38^{b}	157.25^{a}
	S.E	0.23	7.53	10.18	2.20	3.78
VI	Mean	4.01^{b}	470.63^{bcd}	443.75^{b}	115.63^{b}	150.50^{a}
	S.E	0.38	3.46	5.96	3.30	5.57
VII	Mean	4.10^{c}	491.25^{cd}	452.50^{b}	107.50^{b}	145.13^{a}
	S.E	0.19	5.15	6.48	2.78	3.94

*P<0.05,significant at 5 per cent level. Compared with diabetic control.
Means bearing the same superscript do not differ significantly

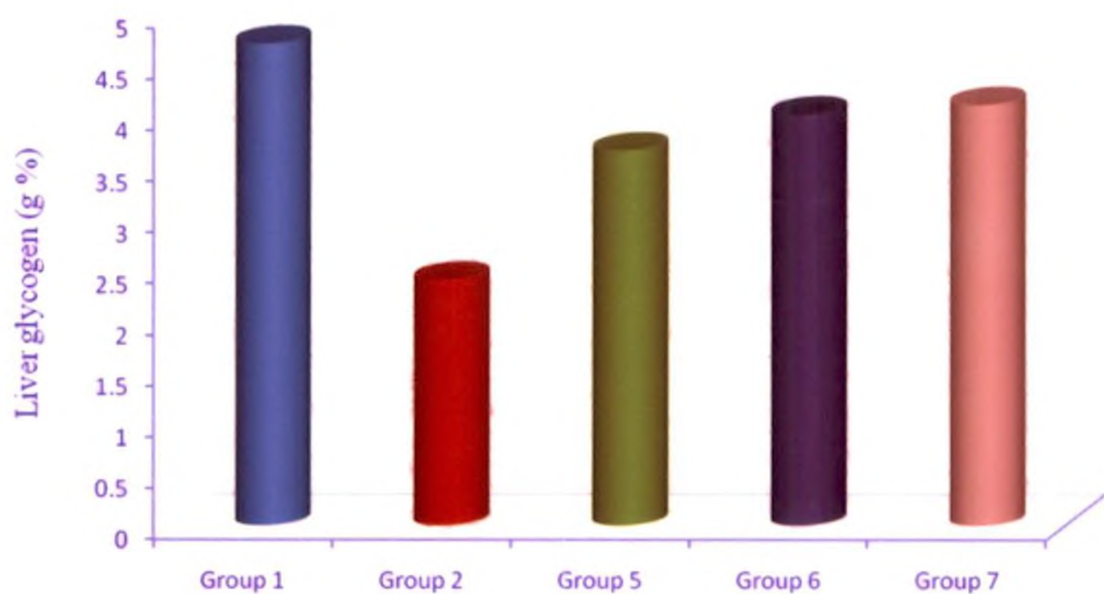


Fig.16-Interactive effect of ethanolic extract of Coccinia indica leaves and glibenclamide on liver glycogen on 60th day
Liver glycogen (g %)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Group 1
Group 2
Group 5
Group 6
Group 7

Fig.17 - Interactive effect of ethanolic extract of *Coccinia indica* leaves and glibenclamide on reduced glutathione in pancreas on 60th day

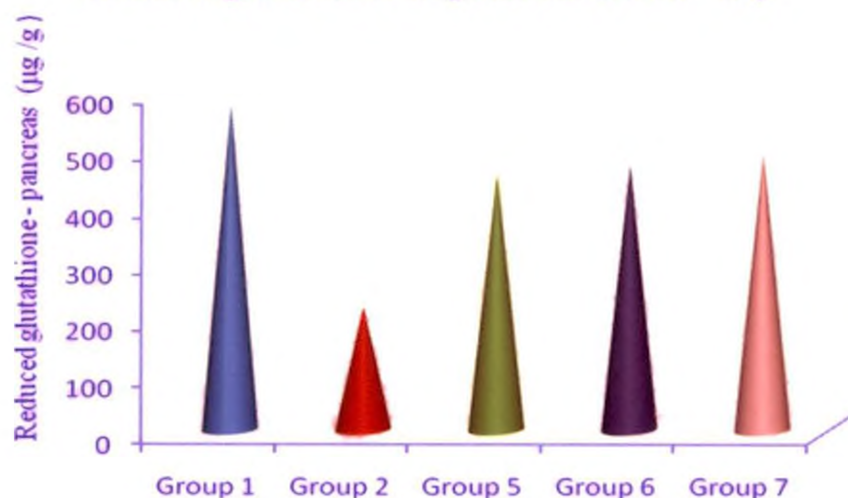
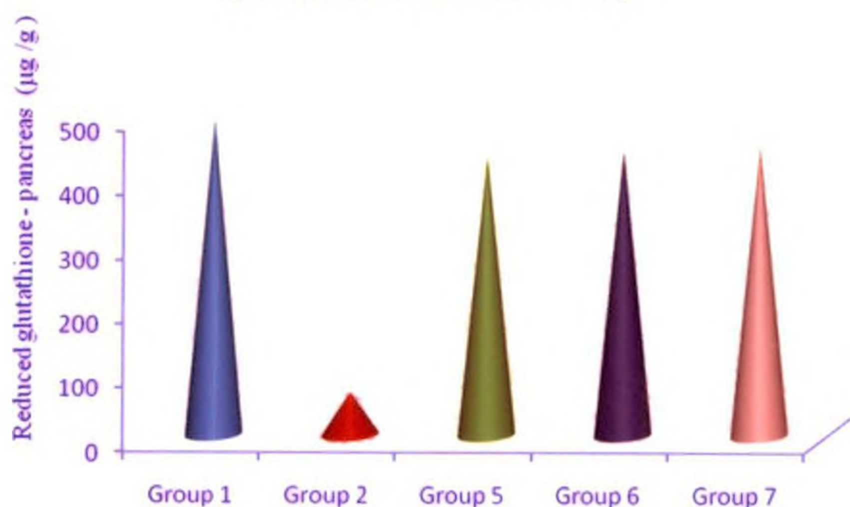


Fig.18 - Interactive effect of ethanolic extract of *Coccinia indica* leaves and glibenclamide on reduced glutathione in liver on 60th day



reduced glutathione values with respect to diabetic control (group II) in group V, VI and VII and they did not significantly vary with one another. Interactive effect of Coccinia and glibenclamide treatments on reduced glutathione levels is presented in Table 17 and Figure 17 and 18.

4.2.3.3 Lipid peroxides in pancreas

Lipid peroxides estimated in pancreas after the end of the treatment (60th day) showed mean values of 82.13±3.99, 336.63±9.91, 113.38±2.20, 115.63±3.30 and 107.50±2.78 nM / g for group I, II, V, VI and VII respectively. There was a significant($p<0.05$) reduction in the lipid peroxides of pancreas in group V, VI and VII and they did not significantly vary one another. The percentage reduction in lipid peroxides in group V, VI and VII were 66.32, 65.65 and 68.07 respectively with respect to the diabetic control.

4.2.3.4 Lipid peroxides in liver

The mean values of lipid peroxides in liver obtained after 45 days of treatment were 140.88±3.49, 392.75±12.31, 157.25±3.78, 150.50± 5.57 and 145.13±3.94 nM / g for group I, II, V, VI and VII respectively. There was significant reduction in lipid peroxides of liver in group V, VI and VII when compared to the diabetic control and the percentage reduction with respect to diabetic control were 59.96, 61.79 and 63.05 for group V, VI and VII respectively. The reduction in lipid peroxide levels produced by the interactive effect of Coccinia and glibenclamide are presented in Figure 19 and 20 and Table 17.

Fig.19 - Interactive effect of ethanolic extract of *Coccinia indica* leaves and glibenclamide on lipid peroxides in pancreas on 60th day

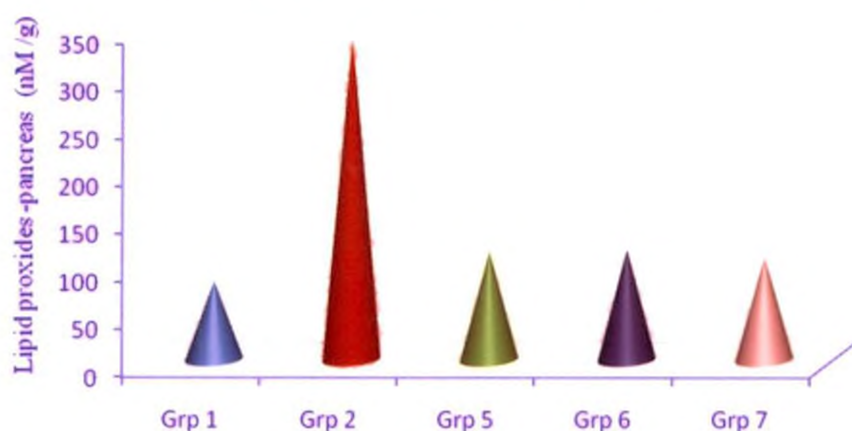
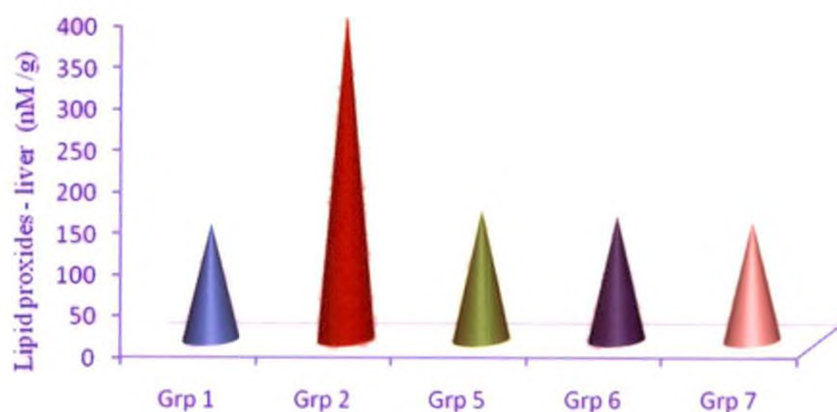


Fig.20 - Interactive effect of ethanolic extract of *Coccinia indica* leaves and glibenclamide on lipid peroxides in liver on 60th day



4.3 HISTOPATHOLOGICAL EXAMINATION OF PANCREAS

4.3.1 Normal control

The islets of control rats appeared to be in the form of well defined spherical islands of lightly stained cells separated from the surrounding parenchyma by connective tissue fibers.

4.3.2 Diabetic control

There was disruption of the spherical shape of the islet and the cells in the islets of alloxan induced diabetic rats were destroyed. These changes were further substantiated by the biochemical parameters like elevated blood glucose, serum triglycerides and cholesterol.

4.3.3 Treatment groups

The cells in the islet showed regenerative hypertrophy and hyperplasia. As regards the overall histological picture, the sections of the pancreas from both Coccinia and glibenclamide treatment groups revealed marked improvement in terms of the size of the islets as well as the number of cells in each islets. This finding was further corroborated by the biochemical results.

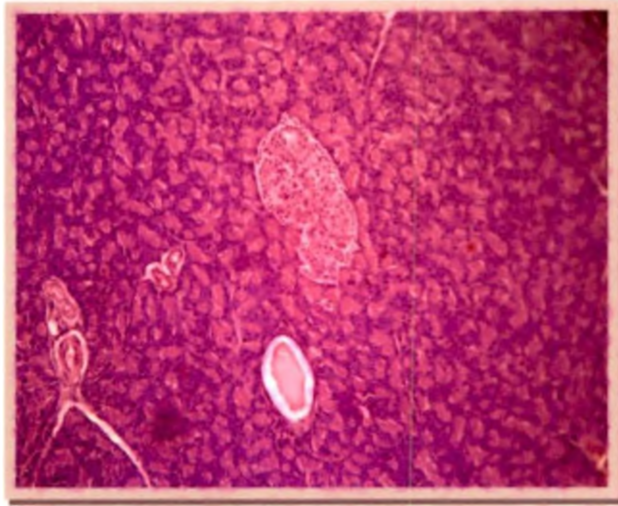


Fig. 21. Normal control group (H &E x 100)

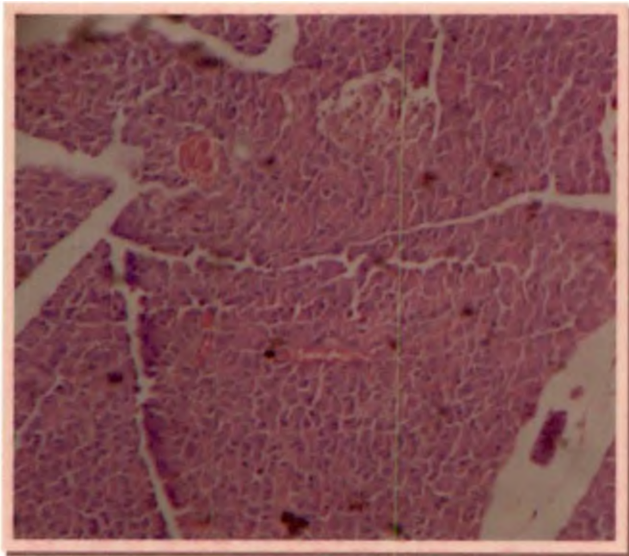


Fig. 22. Diabetic control group (H &E x 100)

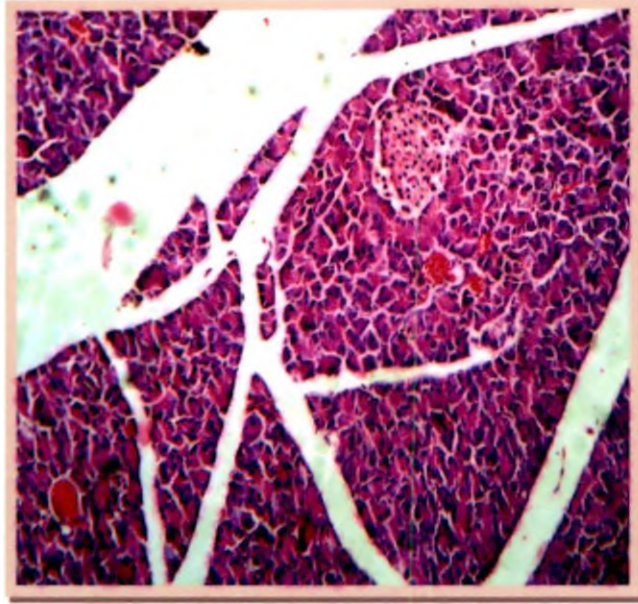


Fig.23. Coccinia (200 mg/kg) treated group (H&E x 100)

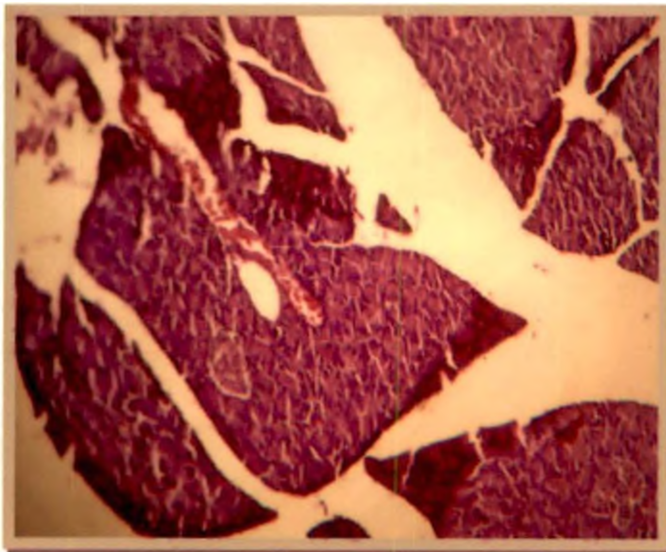


Fig. 24. Glibenclamide (0.25 mg/kg) treated group (H&E x 100)

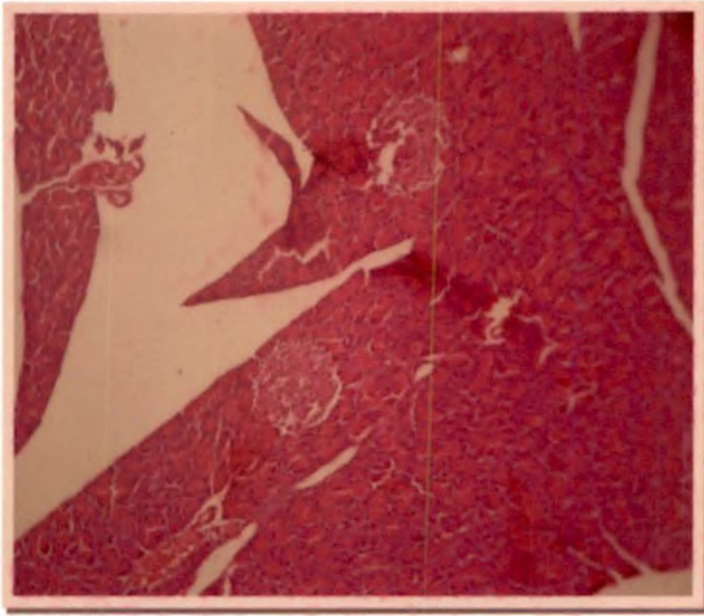


Fig. 25. Coccinia (200 mg/kg) + glibenclamide (0.125 mg/kg) treated group (H&E x 100)

4.4 PHYTOCHEMICAL SCREENING

4.4.1 Steroids

No red colour and red ring were obtained in the Salkowski test and Lieberman Burchadt test respectively. Thus steroids were not detected in the ethanolic extract of *Coccinia indica* leaves.

4.4.2 Alkaloids

No creamy white precipitate in Mayer's test and no characteristic yellow coloured precipitate with Hager's test were obtained with the extract. With Wagner's reagent, no characteristic reddish brown precipitate was obtained. Dragendroffs test also gave a negative result. So no detectable levels of alkaloids were detected in the ethanolic extract of *Coccinia indica.*

4.4.3 Tannins

No characteristic brownish green colour obtained in ferric chloride test and white precipitate in gelatin test. It indicated the absence of tannins in the ethanolic extract of *Coccinia indica.*

4.4.4 Flavonoids

Absence of green colour in the ferric chloride test and characteristic yellow coloured precipitate in lead acetate test indicated the absence of flavonoids in the ethanolic extract of *Coccinia indica* leaves.

4.4.5 Glycosides

A red colour was obtained in the Benedict's test indicated the presence of glycosides in the sample. A yellow colour was obtained by mixing the extracts with sodium hydroxide reagent, which also indicated the presence of glycosides in ethanolic extract of *Coccinia indica* leaves.

4.4.6 Phenolic compounds

The extract mixed with 10 percent ferric chloride produced no characteristic dark blue colour, which indicated the absence of phenolic compounds in *Coccinia indica* ethanolic extract.

4.4.7 Diterpenes

Diterpenes were not detected in *Coccinia indica* ethanolic extract as indicated by absence of green colour when mixed with copper sulphate solution.

4.4.8 Triterpenes

For *Coccinia indica* ethanolic extract, lower layer turned to yellow on standing as per Salkowski test, and by Leiberman Burchadt's test, a deep ring appeared at the junction of the two layers. These results indicated the presence of triterpenes in the extract.

4.4.9 Saponins

In the foam test, foam was not persisted for 10 minutes, which indicated that no saponins were present in the extract.

Table 18. Results of phytochemical screening of *Coccinia indica* leaf extract

Sl. No	Active principle	*Coccinia indica*
1	Steroids	Absent
2	Alkaloids	Absent
3	Tannins	Absent
4	Flavonoids	Absent
5	Glycosides	Present
6	Phenolic compounds	Absent
7	Diterpenes	Absent
8	Triterpenes	Present
9	Saponins	Absent

4.5 TOXICITY EVALUATION OF ETHANOLIC EXTRACT OF *Coccinia indica* LEAF

4.5.1 Acute oral toxicity

According to the OECD guideline 423, the doses starting from 300 mg/kg was administered. The doses upto 2000 mg/kg did not show any toxic symptoms for 14 days after the administration of drug. Hence it is referred that the drug did not produce any acute oral toxicity.

4.5.2 LD_{50}

There was no mortality in the group I, II, III, IV, V and VI for doses upto 2000 mg/kg body weight. So LD_{50} is higher than 2000 mg/kg by intraperitoneal route. As per the instructions in OECD Guidelines 423, testing for LD_{50} dose higher than 2000 mg/kg was not done due to reasons of animal welfare concern.

4.5.3 Sub acute toxicity

During and after the course (14th day) of study, no abnormal behavioural or toxic symptoms were observed in any of the animals. Mortality was also not seen in any of the group. There was no reduction in feed intake and changes in physical alertness of animals in any of the groups.

4.5.3.1 Body weight

The mean body weight of group I, II, III, IV and V on zero day were 120.83±2.71, 120.00±3.65, 120.00±2.89, 121.67±3.07 and 116.67±2.47 g respectively. There was no significant difference in the body weight before (zero day) and after the course of study (14 days) and the mean value obtained on 14th day were 124.17±2.71, 120.83±2.71, 124.17±2.01, 125.83±2.01 and 123.33±2.11 g for group I, II, III, IV and V respectively. The results obtained are presented in the Table 19.

4.5.3.2 Serum alanine amino transferase level

The results obtained are presented in Table 19. The serum ALT levels before the commencement of toxicity study were 50.67±2.69, 52.33±1.71, 48.50±2.05, 52.83±2.40 and 50.33±2.89 U/L respectively for groups I to V. After 14 days of the experiment, the levels of ALT in treatment groups were comparable to the normal control (group I). The mean ALT values on 14th day were 49.33±2.39, 52.33±2.09, 53.83±1.51, 49.00±3.34 and 49.50±2.16 U/L for group I, II, III, IV and V respectively.

4.5.3.3 Serum aspartate amino transferase

The mean serum AST levels on zero day were 157.83±9.12, 157.33±6.46, 159.83±5.71, 158.67±7.89 and 159.33±6.17 U/L for group I, II, III, IV and V respectively. There were no significant differences in serum AST levels for group II, III, IV and V when compared to group I (normal control) after the treatment. The mean AST levels recorded after the treatment were 159.33±3.16, 152.67±5.71, 157.17±8.06, 157.17±5.85 and 157.83±4.29 U/L for group I to V respectively. The results obtained are presented in the Table 19.

4.5.3.4 Serum creatinine

The results of the effect of ethanolic extract of *Coccinia indica* leaf by intraperitoneal administration on creatinine are presented in the Table 20. On zero day the mean serum creatinine values of group I, II, III, IV and V were 1.80±0.13, 1.58±0.12, 1.73±0.13, 1.47±0.13 and 1.62±0.13 mg/dl respectively. On 14th day after treatment, the mean serum creatinine levels were 1.57±0.12, 1.67±0.12, 1.62±0.14, 1.64±0.15 and 1.70±0.13 mg/dl respectively for group I, II, III, IV and V. The mean

Table 19. Evaluation of toxicological effect of intraperitoneal administration of *Coccinia indica* with respect to body weight and serum enzymes activity

		Body weight (grams)		Serum ALT level (U/L)		Serum AST level (U/L)	
		0th day	14th day	0th day	14th day	0th day	14th day
Group I	Mean	120.83	124.17	50.67	49.33	157.83	159.33
	S.E	2.71	2.71	2.69	2.39	9.12	3.16
Group II	Mean	120.00	120.83	52.33	52.33	157.33	152.67
	S.E	3.65	2.71	1.71	2.09	6.46	5.71
Group III	Mean	120.00	124.17	48.50	53.83	159.83	157.17
	S.E	2.89	2.01	2.05	1.51	5.71	8.06
Group IV	Mean	121.67	125.83	52.83	49.00	158.67	157.17
	S.E	3.07	2.01	2.40	3.34	7.89	5.85
Group V	Mean	116.67	123.33	50.33	49.50	159.33	157.83
	S.E	2.47	2.11	2.89	2.16	6.17	4.29

Table 20. Evaluation of toxicological effect of intraperitoneal administration of *Coccinia indica* with respect to serum creatinine, serum glucose and serum total protein

		Serum Creatinine (mg/dl)		Serum Glucose (mg/dl)		Serum Total protein (g/dl)	
		0th day	14th day	0th day	14th day	0th day	14th day
Group I	Mean	1.80	1.57	84.50	84.17	4.33	4.17
	S.E	0.13	0.12	3.05	3.79	0.21	0.17
Group II	Mean	1.58	1.67	81.50	78.83	4.17	4.17
	S.E	0.12	0.12	5.03	3.10	0.17	0.17
Group III	Mean	1.73	1.62	80.50	83.67	4.00	4.33
	S.E	0.13	0.14	3.89	2.69	0.00	0.21
Group IV	Mean	1.47	1.64	82.00	84.33	4.33	4.17
	S.E	0.13	0.15	3.54	4.26	0.21	0.17
Group V	Mean	1.62	1.70	81.83	81.50	4.17	4.17
	S.E	0.13	0.13	4.16	4.14	0.17	0.17

creatinine values of group II, III, IV and V did not vary significantly from group I (normal control).

4.5.3.5 Serum glucose

The results obtained are presented in the Table 20. The mean glucose level on zero day was 84.50±3.05, 81.50±5.03, 80.50±3.89, 82.00±3.54 and 81.83±4.16 mg/dl for group I, II, III, IV and V respectively. The mean blood glucose level after 14 days were 84.17±3.79, 78.83±3.10, 83.67±2.69, 84.33±4.26 and 81.50±4.14 mg/dl for group I, II, III, IV and V respectively. The serum glucose levels of the groups did not differ significantly.

4.5.3.6 Serum total protein

The mean serum total protein for group I, II, III, IV and V were 4.33±0.21, 4.17±0.17, 4.00±0.00, 4.33±0.21 and 4.17±0.17 g/dl respectively on zero day and they did not differ significantly. On 14th day the mean serum total protein value were 4.17±0.17, 4.33±0.21, 4.17±0.17 and 4.17±0.17 g/dl for group II, III, IV and V respectively which did not vary significantly from the mean total protein value 4.17±0.17 g/dl of group I(normal control). The results obtained are presented in the Table 20.

4.5.3.7 Haemoglobin concentration

The mean values are presented in the Table 21. The mean haemoglobin concentration of group I, II, III, IV and V before the administration of drug were 16.08±0.37, 15.92±0.35, 16.75±0.21, 16.58±0.37 and 16.58±0.24 g/dl respectively. After the experiment the mean haemoglobin concentration were 16.67±0.25, 16.58±0.35, 16.83±0.31, 16.25±0.38 and 16.42±0.35 g/dl for group I, II, III, IV and V respectively. The values were with in normal range on both zero day and 14th day in all the groups.

4.5.3.8 Volume of Packed red cell (VPRC)

The results obtained are represented in Table 21. The mean VPRC values of group I, II, III, IV and V were 48.83±0.95, 50.67±1.02, 47.67±0.88, 50.00±1.44 and 49.33±0.87 mm respectively on the zero day. On the 14th day, the mean VPRC value obtained were 50.67±0.92, 50.17±1.14, 50.00±1.18, 50.50±1.18 and 49.50±0.92 mm respectively for group I, II, III, IV and V. There were no significant differences in the mean VPRC value among the groups after the experiment.

4.5.3.9 Total erythrocyte count

The results obtained on 14th day are presented in the Table 21. The mean values were 6.27±0.23, 6.61±0.19, 6.54±0.23, 6.61±0.32 and 6.84±0.14 X 10^6 /µl for group I, II, III, IV and V respectively. There were no significant differences in group II, III, IV and V when compared to the normal control (group I).

4.5.3.10 Total leucocyte count

The mean total leucocyte count obtained for group I, II, III,IV and V on 14th day were 6.10±0.14, 6.25±0.16, 6.59±0.15, 6.04±0.22 and 6.43±0.15 X 10^3 /µl respectively. The results obtained are presented in Table 21. The results indicate that the groups did not differ significantly.

4.5.3.11 Differential leucocyte count

The lymphocytes showed a mean value of 75.83±1.28, 76.50±1.45, 72.67±1.69, 64.33±1.52 and 61.67±1.43 % for group I, II, II, IV and V respectively on 14th day. The values obtained are presented in the Table 22. The groups differed significantly in the lymphocyte value. The lymphocyte value of group II and III were comparable with the normal control (group I). Group IV and V showed a significant ($p<0.05$) reduction in lymphocyte value when compared with the normal control (group I).

Table 21. Evaluation of toxicological effect of intraperitoneal administration of *Coccinia indica* with respect to hematological parameters

		Haemoglobin (g/dl)		Packed cell volume (mm)		Total erythrocyte count($10^6/\mu l$)	Total leucocyte count($10^3/\mu l$)
		0th day	14th day	0th day	14th day	14th day	14th day
Group I	Mean	16.08	16.67	48.83	50.67	6.27	6.10
	S.E	0.37	0.25	0.95	0.92	0.23	0.14
Group II	Mean	15.92	16.58	50.67	50.17	6.61	6.25
	S.E	0.35	0.35	1.02	1.14	0.19	0.16
Group III	Mean	16.75	16.83	47.67	50.00	6.54	6.59
	S.E	0.21	0.31	0.88	1.18	0.23	0.15
Group IV	Mean	16.58	16.25	50.00	50.50	6.61	6.04
	S.E	0.37	0.38	1.44	1.18	0.32	0.22
Group V	Mean	16.58	16.42	49.33	49.50	6.84	6.43
	S.E	0.24	0.35	0.87	0.92	0.14	0.15

Table 22. Evaluation of toxicological effect of intraperitoneal administration of *Coccinia indica* with respect to Differential leucocyte count.

		Differential leucocyte count (%) 14th day				
		Lymphocyte	Neutrophil	Monocyte	Basophil	Eosinophil
Group I	Mean	75.83	20.83	1.83	0.50	1.00
	S.E	1.28	1.25	0.31	0.22	0.37
Group II	Mean	76.50	20.67	1.67	0.67	0.50
	S.E	1.45	1.41	0.33	0.21	0.22
Group III	Mean	72.67	23.67	2.17	0.50	1.00
	S.E	1.69	1.76	0.31	0.22	0.89
Group IV	Mean	64.33	32.17	2.00	0.67	0.83
	S.E	1.52	1.78	0.37	0.21	0.31
Group V	Mean	61.67	35.17	1.83	0.50	0.83
	S.E	1.43	1.49	0.31	0.22	0.31

The mean neutrophil value on 14th day were 20.83±1.25, 20.67±1.41, 23.67±1.76, 32.17±1.78 and 35.17±1.49 % for group I, II, III, IV and V respectively. Group II and III did not differ significantly from group I (normal control). Group IV and V showed a significant increase in neutrophil value when compared with group I. The mean neutrophil value obtained are presented in the Table 22.

There was no significant difference in the monocyte value on 14th day for group II, III, IV and V when compared with group I (normal control). The mean monocyte value were 1.67±0.33, 2.17±0.31, 2.00±0.37 and 1.83±0.31 % for group II, III, IV and V respectively and 1.83±0.31 % for group I (normal control). The results obtained are presented in the Table 22.

The mean basophil value were 0.50±0.22, 0.67±0.21, 0.50±0.22, 0.67±0.21 and 0.50±0.22 % for group I, II, III, IV and V respectively and they did not differ significantly. The mean eosinophil value were 1.00±0.37, 0.50±0.22, 1.00±0.89, 0.83±0.31 and 0.83±0.31 % for group I, II, III, IV and V respectively and there were no significant differences among the groups.

Discussion

5. DISCUSSION

Diabetes mellitus is an endocrine disease involving heterogeneous metabolic disorders of carbohydrates, fats and proteins. This disease is a pandemic in both developed and developing countries. The present regimen to treat diabetes is by exercise, modifying diet and by the use of oral hypoglycemic agents or by insulin therapy. Although many drugs are available to manage diabetes, in most instances these are expensive and may also have adverse effects like hypoglycemia and obesity (Sharma *et al.*, 2008). Screening of herbs for hypoglycemic effect will be of great significance in this context. Hence the present study was conducted to evaluate the hypoglycemic effect of *Coccinia indica* leaves.

In combination therapy, the dosage of allopathic medicine can be reduced so that the toxicity can also be minimized. Hence this study was conducted to find the interactive effect of *Coccinia indica* leaves with glibenclamide and to derive a safe and effective dose of the combination. A study was also conducted to assess the toxicity of *Coccinia indica* leaves to authenticate the safe use of the drug.

5.1 SCREENING OF *Coccinia indica* FOR ACTIVE PRINCIPLES

The screening of ethanolic extract of *Coccinia indica* revealed the presence of triterpenes and glycosides. A similar result was reported by Purintrapiban *et al.* (2006) in the phytochemical screening of aqueous extract of *Coccinia indica* stem. In the present study, screening revealed the presence of triterpenoid compounds and free carbohydrates and/or bound sugars in the form of glycosidic compounds.

The presence of flavonoids, saponins, phenols, tannins and terpenoids in the phytochemical screening of the crude hydromethanolic extract of the leaves of *Coccinia grandis* were reported by Umamaheswari *et al.* (2008).

Wongtodsaporn and Assawasiripong (1979) studied the active principles in *Coccinia indica* leaves by extracting fresh leaves with 80 per cent ethanol and further reextracting with

petroleum ether and chloroform respectively. Chemical tests indicated the presence of flavonoids and steroid in petroleum ether extract. Flavonoids, steroids and organic compounds with mild reducing property were found to be present in chloroform extract and reducing sugar and amino acids in aqueous ethanolic extract.

Two novel triterpenoid saponins with insulin-like activity, termed assamicin I and II, were isolated from the roots of *Aesculus assamica* were isolated by Sakurai *et al.* (2002) which inhibited release of free fatty acids from epinephrine-treated rat adipocytes and enhanced glucose uptake into 3T3-L1 adipocytes. So it can be suggested that the presence of triterpenes present in the *Coccinia indica* may have insulin like activity which is responsible for its hypoglycemic effect.

5.2 HYPOGLYCEMIC, HYPOLIPIDEMIC AND ANTIOXIDANT EFFECT OF *Coccinia indica* LEAF ON ALLOXAN INDUCED DIABETIC RATS.

All the animals became diabetic on 15th day of alloxan administration. The mean blood glucose were raised and the average value ranged between 273.88±11.04 to 296.25±14.18, mg/dl in the groups II to VII when compared to 93.38±2.88 mg/dl of normal control group. Elsner *et al.* (2006) explained that the cytotoxic glucose analogue alloxan, after selective accumulation in pancreatic beta cells which are weakly protected against oxidative stress, destroys the insulin producing cells and causes a state of insulin dependent diabetes mellitus through reactive oxygen species mediated toxicity in rodents. The hypoglycemic, hypolipidemic and oxidative stress relieving effect of *Coccinia indica* was evaluated in alloxan induced diabetic rats.

5.2.1 Evaluation of general health improving and hypoglycemic effect of *Coccinia indica*

There was weight loss in alloxan induced diabetic animals as cited by Chougale *et al.* (2007). The liver glycogen content was also reduced in alloxan-induced diabetic rats. This can be substantiated by the findings of Baker *et al.* (1952). He observed that there was complete suppression of the incorporation of labeled glucose into the liver glycogen of alloxan treated diabetic rats. Further, Gold (1970) suggested that alloxan induced diabetes resulted in loss of the

capability of liver to activate glycogen synthetase *in vitro.* Based on these findings, the body weight and liver glycogen level were taken as parameters for assessing general health improvement by *Coccinia indica*. Blood glucose was estimated to assess the glycemic state of the animal.

5.2.1.1 Body Weight

The diabetic rats showed a rapid reduction in body weight. The diabetic control group also showed a loss of body weight from 180.00±3.27 gram to 155.00±1.89 gram. Similar results were reported by Surana *et al.*(2008) where the mean body weight of diabetic control reduced from 188.8±0.92 to 175.6±1.92 gram. The metabolic disorders in diabetes is due to insulin insufficiency which can be either the deficiency or resistance. Due to this, glucose cannot be utilized for energy purpose and fat and proteins are catabolised which results in weight reduction in diabetic animals. This is supported by the findings of Rajkumar and Govindarajulu (1991) that in diabetic rats, non availability of glucose leads to utilization of fat and structural proteins, resulting in reduction in body weight. Further the lack of insulin which is an anabolic hormone decreases the anabolic processes like glycogenesis, lipogenesis and protein synthesis which results in loss of body weight. Protein synthesis is decreased in all tissues due to absolute or relative deficiency of insulin (an anabolic hormone) in alloxan-induced diabetic rats (Ananthi *et al.*, 2003).

The Coccinia treated group (group III) had a mean body weight of 165.00±2.67 gram on 30th day which had an initial body weight of 181.86±3.77 gram on 15th day. The reduction in body weight may be due to induction of diabetes and as reported by Khan *et al.* (1980) and due to the slow action of Coccinia it requires more time to regain the body weight. This was evident from the observations on 45th and 60th day where the body weight gradually improved to 175.00±1.64 and 193.75±2.46 gram respectively.

Increase in body weight was observed by Ananthan *et al.* (2003) in diabetic rats by the administration of ethanolic extract of *Gymnema montanum* leaf. They suggested that the presence of active constituents like gymnnmagnin and gymnemic acids in *Gymnema montanum* may be responsible for the blood glucose lowering and insulin stimulatory effect which in turn

improved the glycemic control resulting in better weight gain when compared to the diabetic control. In the phytochemical analysis of *Coccinia indica,* the presence of triterpenes were detected. It has been demonstrated that triterpenoids have an insulin-like activity *in-vitro* and *in-vivo* (Sakurai *et al.*, 2002). The increase in body weight in Coccinia treated group might be due to the insulin-like activity of triterpenoids present in Coccinia with the resultant stimulatory effect on anabolic processes like protein synthesis and lipogenesis which produced the weight gain.

The initial mean body weight in group IV which was treated with glibenclamide at the dose rate of 0.25 mg/kg body weight was 195.00±2.83 gram. The treatment with glibenclamide for 45 days after the induction of diabetes showed significant improvement in body weight but the response was less when compared with group III treated with *Coccinia indica* at the dose rate of 200 mg/kg body weight. Glibenclamide is an oral hypoglycemic agent which comes under the class sulphonyl urea and have the action of stimulating pancreatic cells to enhance the production of insulin. The enhanced insulin production with the resultant stimulation of anabolic processes may be responsible for the increase in weight of glibenclamide treated group. Further glibenclamide at the dose rate of 0.25 mg/kg did not show the obesity forming tendency, which is one of the side effects normally experienced while treating diabetes with sulphonyl ureas. The obesity forming tendency of sulphonyl urea was observed in the study of Ogbonnia *et al.*(2008) where the diabetic rats treated with glibenclamide at the dose rate of 600 µg/kg body weight increased the mean body weight from 152.5±2.5 gram to 169.69±0.2 gram.

Thus Coccinia (200 mg/kg) and glibenclamide (0.25 mg/kg) improved the weight of alloxan diabetic rats and Coccinia was found to be more effective in regaining the body weight compared to glibenclamide.

5.2.1.2 Blood Glucose

The mean blood glucose levels prior to alloxan administration were similar among all the four groups. After fifteen days of alloxan administration, about three fold elevation of blood glucose was observed. This may be due to the formation of reactive oxygen species by alloxan with a simultaneous increase in cytosolic calcium concentration which caused rapid destruction

of insulin producing ß cells (Szkudelski, 2001). Glutamine and alanine are the major aminoacid precursors for gluconeogenesis and they are controlled by insulin. In diabetes there is increased conversion of alanine to glucose, contributing to the enhanced rate of gluconeogenesis. It is hypothesized that the factor responsible for the development of most of the complications of diabetes is the prolonged exposure of tissues to elevated concentrations of glucose. Prolonged hyperglycaemia results in the formation of advanced glycation end products (Hardman and Limbird, 1996).

The mean blood glucose levels of diabetic control increased from 94.75±3.2 mg/dl to 296.25±14.18 mg/dl. This is similar to the results obtained by Vinuthan *et al.* (2004) where the mean blood glucose level of diabetic rats was raised upto 261.78±7.49 mg/dl. They opined that this was due to the destruction of beta cells of pancreas by alloxan.

The group III treated with Coccinia at the dose rate of 200 mg/kg showed a lower blood glucose value of 197.63±6.62 mg/dl by 30th day, 136.38±3.44 mg/dl by 45th day and 116.00±2.65 mg/dl by 60th day as against 293.13±10.62 mg/dl on 15th day. This showed the hypoglycemic effect of *Coccinia indica* which was effective at the dose rate of 200 mg/kg body weight.

Khan *et al.* (1980) cited that the active principles of *Coccinia indica* may have insulin like activity. The phytochemical analysis revealed the presence of triterpenes and glycosides. Dhanabal *et al.* (2004) isolated the fractions of alcoholic extract of *Coccinia indica* and found that only the toluene sub-fraction was effective in reducing the blood glucose level and the active principles in this fraction were found to be triterpenes. It has been demonstrated that triterpenes have an insulin-like activity (Sakurai *et al.*, 2002). The hypoglycemic effect of *Coccinia indica* may be due to the insulin like activity of triterpenes present in it. Kamble *et al.* (1998) estimated the activity of the enzymes glucose 6-phosphatase, lactate dehydrogenase (LDH) and lipoprotein lipase (LPL) after administration of *Coccinia indica* in diabetic patients and postulated that the ingredients present in the extract of *C. indica*, act like insulin, correcting the increased activity of glucose 6-phosphatase and LDH in glycolytic pathway and restoring the LPL activity in lipolytic pathway which control the hyperglycaemia. Glucose 6-phosphatase converts glucose 6-phosphate to free glucose. The correction of elevated level of glucose 6-phosphatase in diabetes by *C. indica* favours anabolism of glucose in body. Similarly insulin is

essentially an anabolic hormone which favours anabolism of carbohydrates, fat and proteins. This explains the insulin like activity of *C. indica* and this activity might be responsible for the hypoglycemic effect of *C. indica*. This can be further supported by the findings of Shibib *et al.* (1993) that *Coccinia indica* and *Momordica charantia* caused depression of key gluconeogenic enzymes glucose 6-phosphatase and fructose-1,6-bisphosphatase and enhancement of glucose oxidation by the shunt pathway through activation of its principal enzyme glucose 6-phosphatase dehydrogenase. Eshrat (2003) opined that the hypoglycemic effect of *Coccinia indica* and *Abroma augusta* either alone or in combination may be due to increase in blood insulin level by the extracts and stimulation of liver and extrahepatic tisssues to utilize glucose.

The reduction in blood glucose by *Coccinia indica* may be further supported by the histopathological studies of the pancreas where the islets of Langerhans showed marked improvement in terms of the size as well as the number of cells in each islets. Similar results were observed by Kavitha *et al.* (2007) where islet cells of pancreas in alloxan-induced diabetic rats treated with *Boswellia glabra* root recovered to that of the normal control with more number of cells in the islet than that of the diabetic control.

Khosla *et al.* (2000) reported the hypoglycemic action for aqueous extract of *Azadirachta indica* leaves where the mean blood glucose values reduced from 285.00±2.63 to 149.14±3.13 mg/dl. They opined that the hypoglycemic effect of *A. indica* may be due to increased release of insulin from beta cells of pancreas similar to that of sulphonyl ureas. It may also be partly due to its action on extrapancreatic sites, that is by increased peripheral glucose utilization or by direct metabolic effect on tissues particularly on liver.

Akhthar *et al.*(2007) observed that alloxan-treated animals receiving the extracts of *Coccinia cordifolia* and *Catharanthus roseus* showed rapid normalization of blood glucose levels in comparison to the control and it was explained by the possibility that surviving beta-cells were exerted to release more insulin by *C. cordifolia* and *C.roseus*.

The ethanolic extract of *Bacopa monnieri* produced significant lowering of blood glucose in alloxan induced diabetic rats(Ghosh *et al.*, 2008). They suggested that the insulin- like action of the extract might have caused an increase in peripheral glucose utilization and thereby decreased blood glucose concentration.

Glibenclamide treated group showed a significant decrease in blood glucose level at a lower dose rate of 0.25 mg/kg by significantly reducing the blood glucose level from 289.75±11.10 to 126.75±2.88 mg/dl. This can be explained by the action of sulphonyl ureas which cause stimulation of insulin secretion from pancreatic beta cells (Ravi *et al.*, 2004). Glibenclamide comes under sulphonyl urea class of oral hypoglycemic drugs, which inhibits ATP-sensitive K^+ channels causing depolarization of the pancreatic beta cells resulting in insulin secretion. Lowering of blood glucose is brought about by the enhanced production of insulin.

Thus the study revealed that Coccinia at the dose rat of 200 mg/kg body weight produced significant hypoglycemic effect which is better than that of glibenclamide at the dose rate of 0.25 mg/kg body weight.

5.2.1.3 Liver Glycogen

Liver is an important organ with both endocrine and exocrine function. It helps in controlling postprandrial hyperglycaemia and is involved in the synthesis of glycogen.

Insulin insufficiency in diabetes will enhance glucose production in liver by increasing gluconeognesis and glycogenolysis. It also inhibit hepatic glucose uptake and impair the normal capacity of liver to synthesize glycogen. This can also be explained with the reports of Hikino *et al.* (1989) that decreased enzymatic activity of glucokinase, hexokinase and phosphofructokinase in diabetic animals resulted in depletion of liver and muscle glycogen. Glucokinase is found in association with glucose transporter molecule-2 in liver and pancreatic beta cells and catalyses the phosphorylation of glucose to glucose 6-phosphate which is needed for the facilitated diffusion of glucose into cells. The liver glucokinase gene is regulated by insulin. So insulin insufficiency will cause a decreased activity of glucokinase resulting in decreased influx of glucose into liver and subsequent reduction in synthesis of glycogen. In the present study, group II (diabetic control) showed a significant decrease in liver glycogen levels. The mean liver glycogen value of diabetic control was 2.40±0.31 gram per cent which was significantly lower than that of normal control. Reduction in liver glycogen might be due to alloxan induced islet cell damage and the resultant insufficient production of insulin.

The treatment with Coccinia at the dose rate of 200 mg//kg body weight increased the liver glycogen to 3.99±0.21 gram per cent when compared to 2.40±0.31gram per cent in diabetic control. Similar results were got for Grover *et al.* (2000) in the treatment of diabetic rats with decoction of kernels of *Eugenia jambolana* where the glycogen content was raised to 22.60±4.71 mg/g when compared to 8.81±1.41 mg/g in diabetic rats. He evaluated the content of glucose 6-phosphate and also the activity of glucokinase, hexokinase and phosphofructokinase and found that these were elevated after the treatment with *Eugenia jambolana*. He stated that the reason for increase in liver glycogen was due to the increase of glucose 6-phosphate content and increased activity of the enzymes glucokinase, hexokinase and phosphofructokinase.

The increase in liver glycogen in Coccinia treated group may be due to the insulin-like activity of the active constituents like triterpenes. Sakurai *et al.* (2002) had demonstrated the insulin-like activity of triterpenes. Because of the insulin like activity Coccinia may stimulate hepatic glucose uptake and also may inhibit gluconeogenesis and glycogenolysis in liver. The improvement in liver glycogen content can be further supported by the findings of Shibib *et al.* (1993) that *Coccinia indica* and *Momordica charantia* caused depression of key gluconeogenic enzymes glucose 6-phosphatase and fructose-1,6-bisphosphatase and enhancement of glucose oxidation by the shunt pathway through activation of its principle enzyme glucose 6-phosphatase dehydrogenase. The increase in liver glycogen by Coccinia might be due to the suppression of gluconeogenesis in liver and enhancement of hepatic glucose uptake.

Treatment with glibenclamide also showed an improved liver glycogen content of 3.78±0.16 gram per cent when compared to 2.40±0.31 gram per cent of diabetic control. This is in concordance with the results of Fernandes *et al.* (2007) where in the treatment with glibenclamide at the dose rate of 4 mg/kg raised the liver glycogen content to 1.8±0.1gram per cent with respect to 1.0±0.2 gram per cent in diabetic control. Glibenclamide acts on pancreatic beta cells and stimulate pancreatic insulin secretion. Insulin has anabolic action and decreases gluconeogenesis and glycogenolysis. The stimulation of insulin secretion by pancreas will also enhance hepatic glucose uptake. The increase in liver glycogen by glibenclamide might be due to the stimulated action of insulin which helped in increased glucose uptake into the liver and

enhancement of anabolic pathway for glucose in liver and subsequent conversion of glucose into liver glycogen.

Thus we can see Coccinia at the dose rat of 200 mg/kg body weight produced significant increase in liver glycogen content when compared to the diabetic control. This was comparable to the increase in liver glycogen content produced by glibenclamide at the dose rate of 0.25 mg/kg body weight.

5.2.2 Evaluation of hypolipidemic effect of *Coccinia indica*

In alloxan induced diabetes there is reduction of insulin due to the destruction of insulin producing pancreatic beta cells. One of the important action of insulin is the inhibition of lipolysis by inhibiting hormone sensitive lipase. Insulin insufficiency stimulates hormone sensitive lipase and causes increased mobilization of free fatty acids from the fat deposits leading to abnormally high concentration of serum lipids. So in diabetes there is marked elevation of lipids leading to hyperlipidemia. Moreover other hormones such as glucagon and catacholamines, known to increase during diabetes, further increased the serum lipids by stimulating lipolysis (El-Soud *et al.*, 2007). So serum cholesterol and triglycerides were estimated to assess the effectiveness of the treatment in combating diabetes

5.2.2.1 Serum Cholesterol and Triglyceride

On day zero, the serum cholesterol levels estimated in all groups were almost similar. On 15th day, all the groups (II, III and IV) except normal control showed a substantial increase in serum cholesterol level. In accordance to this agrees with the observations of Surana *et al.* (2008), who noted elevated blood lipids especially cholesterol and triglycerides in diabetic condition.

The serum cholesterol and triglyceride in the treatment group (group III) were increased to 100.75±3.80 mg/dl and 154.63±2.59 mg/dl respectively on 15th day after induction of diabetes from a mean value of 47.00±2.56 mg/dl. This was similar to the observations of Sharma *et al.*(2003) where the serum cholesterol and triglyceride were increased to 105.50±11.40 mg/dl

and 165.00±14.50 mg/dl respectively after the induction of diabetes. The marked hyperlipidemia observed may be due to the consequence of uninhibited actions of lipolytic hormones on the fat depots.

By 60th day the mean serum cholesterol value of the treatment groups (III and IV) regained to the normal levels of 50.25±1.35 and 50.88±1.04 mg/dl respectively. There was also a significant reduction in serum triglyceride level in group III and IV which were 105.88±2.82 and 104.63±2.60 mg/dl respectively on 60th day when compared to 154.63±2.59 and 157.75±5.71 mg/dl on 15th day. The results showed that the treatment with Coccinia and glibenclamide regained the mean cholesterol and triglyceride to normal levels when compared to the diabetic control (162.25±9.42 mg/dl). Similar results were reported by Rajasekaran *et al.* (2006) who evaluated the beneficial effect of *Aloe vera* leaf gel extract on lipid profile status in streptozotocin induced diabetic rats. They found that there was a reversal in the increased levels of cholesterol, triglycerides and phospholipids after the treatment. They suggested that the reduction may be due to increased clearance and decreased production of the major transporters of endogenously synthesized cholesterol and triglycerides.

The present study showed a decrease in mean cholesterol value by the treatment with Coccinia. This may be due to the presence of triterpenes. Triterpenes are demonstrated to have insulin-like activity (Sakurai *et al.*, 2002). The insulin-like activity of the active constituents like triterpenes might have lead to the suppression of lipolysis and mobilization of free fatty acids from the fat deposits. This can be further supported by the findings of Lu *et al.* (2009) that the total triterpene acid fraction from *Folium eriobotryae* produced a good hypolipidemic profile.

In glibenclamide treated group there was a marked reduction in both serum cholesterol and triglycerides. This may be due its stimulatory effect of glibenclamide on pancreatic beta cells to secrete insulin. The resultant increase in the level of insulin might have prevented the lipolysis in fat tissues and thus corrected the elevated lipid level in diabetes.

Thus Coccinia (200 mg/kg) showed marked hypolipidemic effect and was comparable with that of glibenclamide (0.125 mg/kg).

5.2.3 Evaluation of antioxidant effect of *Coccinia indica*

Reduced glutathione and lipid peroxides in pancreas and liver were estimated for assessing the antioxidant effect of *Coccinia indica* leaves. Reduced glutathione protects the cells from the toxic effect of reactive oxygen species or peroxidative damage and contributes to the elimination of organic peroxides and foreign compounds (Chandra *et al.*, 2007). So reduced glutathione in liver and pancreas were taken as a parameter for measuring antioxidant status.

5.2.3.1 Reduced Glutathione in Tissues

Reduced glutathione (GSH) is essential to maintain structural and functional integrity of cells. It is a potent free radical scavenger within the islet of beta cells and is an important factor against the progressive destruction of beta cell. Apart from its direct free radical scavenging properties and abilities to conjugate with several electrophilic intermediates that are capable of initiating lipid peroxidation, GSH acts as the physiological co-substrate of the conjugating enzyme systems. In diabetic control the reduced glutathione levels were reduced to 217.50±7.13 µg/g when compared to 577.63±5.29 µg/g of normal control. This can be explained by the findings of Elsner *et al.* (2006) that in the presence of intracellular thiols, especially glutathione, alloxan generates "reactive oxygen species" (ROS) in a cyclic reaction between this and its reduced form, dialuric acid. The cytotoxic action of alloxan is initiated by free radicals formed in this reaction. Decreased glutathione levels and the generation of ROS have been considered to be an indicator of increased oxidative stress. The decreased levels of plasma GSH in diabetes may be due to increased utilization in trapping the oxy radicals (Venkateswaran and Pari, 2003).

The reduced glutathione in pancreas were raised to 463.13±17.37 and 493.13±12.68 µg/g for Coccinia and glibenclamide treated groups respectively when compared to 217.50±7.13 µg/g for diabetic control. Similar results were also obtained for reduced glutathione in liver where the values obtained were 290.00±11.80 and 313.75±20.71 µg/g for Coccinia and glibenclamide treated groups respectively when compared to 66.25±7.72 µg/g for diabetic control. This is similar to the results obtained for Krishnakumar *et al.* (1999) where the reduced glutathione levels were raised to 379.31±12.9 and 383.41±13.0 mM/100 g for *Salacia oblonga* extract and

glibenclamide treated groups respectively when compared to 357.86±9.5 μg/g in diabetic control. They stated that increase in the glutathione content may be one of the factors responsible for the inhibition of lipid peroxidation.

Chandra *et al.* (2007) observed an increase in glutathione content in diabetic rats treated with *Allium sativum, Azadirachta indica, Momordica charantia and Ocimum sanctum.* They stated that this may be due to increased biosynthesis of GSH *via* accumulation of GSH synthase by these herbal preparations. They suggested that the active components of *A. Sativum* (allicin and diallyl sulfides), *A. Indica* (nimbin and azapirones), *M. Charantia* (charantin and other compounds) and *O.sanctum* (apigenin, linalool and luteolin) may be involved in the restoration of the antioxidant status by regulation of enzymes and GSH in diabetic rats.

The increase in reduced glutathione content by treatment with Coccinia may be due to the antioxidant property of the triterpenes present in it. This can be supported by the findings of Geetha *et al.* (1998) that the triterpenes present in the stem bark of *Crataeva nurvala* increased the blood glutathione after the treatment. The effect of triterpenes-betulin and lupeol on the membrane peroxidation and antioxidant systems in red blood cells was evaluated by Vidya *et al.* (2000) and found that they increased the level of antioxidants such as reduced glutathione, glutathione peroxidase and catalase.

The increase in the reduced glutathione in tissues by glibenclamide showed its antioxidant effect. This may be effected by the direct action of glibenclamide on antioxidant enzymes like catalase and superoxide dismutase as found out by Elmali *et al.* (2004).

The distinct diminution in reduced glutathione content of tissues in diabetic rats and its subsequent attainment of near normalcy on administration of Coccinia revealed the protection offered by *Coccinia indica* in combating oxidative insult due to diabetes

5.2.3.2 Lipid peroxides in tissues

Lipid peroxides play an important role in aging, atherosclerosis and in a number of diabetic complications. Cell damage is caused by initiation of lipid peroxidation due to free radicals. Thiobarbituric acid reactive substances (TBARS) are produced during lipid peroxidation. Therefore high levels of TBARS indicate cell membrane damage (Janero, 1990).

The lipid peroxides level in pancreas and liver were 336.63±9.91 and 392.75±12.31 nM//g respectively in diabetic control groups when compared to 82.13±3.99 and 140.88±3.49 nM/g of pancreas and liver respectively of normal rats. The higher lipid peroxide level in diabetic rats could probably be associated with decreased antioxidant defense potential (Kaleem *et al.*, 2005).The decreased defense potential in lipid peroxidation may be due to the cell membrane damage by the free radicals. In the treatment groups with Coccinia and glibenclamide, the lipid peroxides in pancreas were reduced to 166.50±9.71 and 123.50±9.51 nM/g respectively. Liver lipid peroxides were reduced to 212.50±3.74 and 197.38±7.99 nM/g respectively for Coccinia and glibenclamide treated groups respectively. Similar results were got for Ravi *et al.* (2004) where the level of TBARS (lipid peroxides) in pancreatic tissue were reduced to 56.40±5.6 and 52.68±6.8 mM/100 gram by treatment with *Eugenia jambolana* seed kernel and glibenclamide respectively when compared to 70.91±9.3 mM/gram of diabetic control. They suggested that the protective effect of *Eugenia jambolana* seed kernels could be due to the antioxidant effect of flavonoids present in the seeds, which act as strong superoxide radical and singlet oxygen quenchers.

This reduced value of lipid peroxides obtained after treatment with Coccinia and glibenclamide can be explained with the increase in values of reduced glutathione after the treatment. Reduced glutathione scavenges free radicals and renders protection against lipid peroxidation caused by free radicals (Chaturvedi and Segale, 2007). So the elevation in reduced glutathione by the treatment with Coccinia and glibenclamide might have helped in the scavenging of free radicals which caused cell membrane damage and death. This might have subsequently reduced the raised lipid peroxides. The tendency of the lipid peroxides to retrieve towards near normal values in Coccinia and glibenclamide treated rats unveiled their anti-lipid peroxidative potential.

5.2 COMPARATIVE STUDY ON INTERACTION OF *Coccinia indica* WITH GLIBENCLAMIDE

The effect of combination of *Coccinia indica* at doses of 100, 150 and 200 mg/kg body weight (group V, VI and VII respectively) with glibenclamide at a reduced dose rate of 0.125 mg/kg were evaluated to study the interaction of Coccinia with glibenclamide.

5.2.1 Interaction of *Coccinia indica* with glibenclamide on general health improving and hypoglycemic effect

5.2.1.1 Body weight

The mean body weight obtained for the combination treatment with *Coccinia indica* and glibenclamide were 198.13±2.30, 196.88±4.53 and 197.50±2.50 gram for group V, VI and VII respectively. The results showed that there was significant increase in the body weight in the treatment groups (V, VI and VII) when compared with the mean body weights after the induction of diabetes. The regaining of body weight is higher in the combination treatment when compared to gain in body weight in groups treated with Coccinia (193.75±24.6 g) or glibenclamide (185.63±2.58 g) alone. Similar results were obtained by Bopanna *et al.* (1997) where the gain in body weight observed were higher for combination treatment with glibenclamide and neem seed kernel powder than treatment with neem seed kernel powder or glibenclamide alone.

The better gain in body weight by the combination treatment with Coccinia and glibenclamide may be due to the better glycemic control and utilization of resources by their additive action. This may be explained by evaluating the mechanism of action of Coccinia and glibenclamide. As discussed earlier, Coccinia might have insulin-like activity. This in combination with the insulin-release stimulating effect of glibenclamide might have helped in better utilization of glucose by the body and helped in forming body carbohydrates, protein and fat which was as a result of anabolism favouring effect of insulin.

5.2.1.2 Blood Glucose

The blood glucose values obtained on 60th day with combination of glibenclamide (0.125 mg/kg) with *Coccinia indica* at increasing dose rates of 100, 150 and 200 mg/kg body weight were 156.38±3.33, 135.00±2.50 and 108.50±2.54 mg/dl respectively. From this it seen that the combination produced dose dependent hypoglycemic effect. The lowest dose of Coccinia (100 mg/kg) in combination with glibenclamide (0.125 mg/kg) produced lesser hypoglycemic effect

when compared to higher doses of Coccinia. The combination of glibenclamide (0.125 mg/kg) with the highest dose of Coccinia (200 mg/kg) produced the most effective hypoglycemic effect (108.50±2.54 mg/dl). So it is evident form the results that the combination of Coccinia (200 mg/kg body weight) with glibenclamide (0.125 mg/kg) helped in better reduction of blood glucose than Coccinia or glibenclamide alone. This can be explained by the probable additive action of Coccinia and glibenclamide when used together. The mechanism by which glibenclamide produced its hypoglycemic effect is through the stimulation of insulin secretion from the pancreatic beta cells. Coccinia probably might have produced its hypoglycemic effect by its insulin-like activity of active constituents like triterpenes present in it. Thus the insulin release stimulating effect of glibenclamide together with the insulin like activity of triterpenes present in Coccinia helped in better hypoglycemic effect than when used alone.

A similar result was obtained for Trivedi *et al.* (2004) where the combination of Shilajit (a herbomineral preparation) at the dose rate of 100 mg/kg body weight with glibenclamide at the dose rate of 5 mg/kg body weight produced a better hypoglycemic effect than shilajit (100 mg/kg) or glibenclamide(5 mg/kg) alone. They suggested that this may be due to the extrapancreatic action of shilajit in addition to its pancreatic action.

From this result it is evident that none of the combination of Coccinia with glibenclamide produced severe hypoglycemia. Blumenthal (2000) has cited that the administration of *Aloe vera* juice orally in the morning and at bedtime to 36 diabetic patients for 42 days increased the hypoglycemic effect of glibenclamide. The present study showed that the combination treatment with Coccinia (200 mg/kg) and glibenclamide (0.125 mg/kg) produced better reduction in blood glucose levels but it did not produce severe hypoglycemia and hence the combination can be safely used for treatment.

Glibenclamide has some drawbacks in the treatment of diabetes. It has been reported that during chronic administration of sulphonylureas the insulin-releasing effect is prone to diminish or become lost (Bailey, 1991). Further Babu *et al.* (2003) has cited that hypoglycemia is the most worrisome effect of sulphonyl urea drugs. It was found by Tessier *et al.* (1994) in a survey on elderly diabetic patients that hypoglycaemic reactions were significantly more frequent with glibenclamide than with gliclazide. In the present study reduced dose of glibenclamide(0.125 mg/kg) abolished the chances of hypoglycemia caused by sulphonyl urea (glibenclamide) when

taken in excessive doses. So from this study we can see that Coccinia (200 mg/kg) in combination with glibenclamide (0.125 mg/kg) not only produced most efficient hypoglycemic effect but also helped in preventing the adverse effects of glibenclamide.

5.2.1.3 Liver Glycogen

The mean glycogen levels were slightly higher for the group treated with a combination of Coccinia and glibenclamide. In group VII (combination of Coccinia at the dose rate of 200 mg/kg and glibenclamide at the dose rate of 0.125 mg/kg), the liver glycogen value was almost restored to that of normal control group (group I). This agrees with the findings of Mallick *et al.* (2006) where the restoration of liver glycogen to normal control level was observed after administration of composite extract of *Eugenia jambolana* and *Musa paradisiaca.* They suggested that the possible mechanisms of antidiabetognic action of these extracts is by modulating glycogen metabolism which may be effected by the insulinotropic effect of these extracts.

The better liver glycogen values by the combination of Coccinia (200 mg/kg) and glibenclamide (0.125 mg/kg) may be due to combined effect of insulin-like activity of Coccinia and insulin secretion stimulatory effect of glibenclamide. The insulin-like activity of Coccinia might have helped in increased uptake of glucose into liver and its conversion into glycogen and this effect was further potentiated by the insulin secretion stimulating effect of glibenclamide. Thus the overall effect in improving glucose utilization by the combination of Coccinia and glibenclamide might have helped in the better liver glycogen level.

5.1.2 Interaction of *Coccinia* with glibenclamide on hypolipidemic effect

5.1.2.1 Serum Cholesterol and triglyceride

The treatment with combination of Coccinia and glibenclamide restored the mean cholesterol values almost to normal levels. The restoration of mean cholesterol values almost to normal levels were also seen in treatment with Coccinia and glibenclamide alone. The results of

serum triglyceride values after combination treatment of Coccinia with glibenclamide were similar to that obtained for serum cholesterol. Both combination treatment and treatment with Coccinia or glibenclamide alone restored the serum triglyceride values almost to normal levels.

In the combination treatment of neem seed kernel with glibenclamide, there was a significant decrease in serum cholesterol level than produced by glibenclamide alone (Bopanna *et al.*, 1997). They suggested that the hypoglycemic and and antihyperlipemic effect of neem seed kernel with glibenclamide may b a synergistic combination.

Trivedi *et al.* (2004) observed that combination of glibenclamide with shilajit (a herbomineral preparation) failed produce significant improvement in lipid profile than that produced by shilajit alone. He opined that the improvement in lipid profile by glibenclamide in diabetic rats may be due to better glycemic control which is a secondary mechanism in controlling lipid status in body. Since glibenclamide acted by secondary mechanism, further improvement in the lipid profile was not observed when used with shilajit.

Coccinia might be producing its hypolipidemic effect by its insulin-like activity which caused the suppression of lipolysis. Combination of Coccinia with glibenclamide failed to produce significant improvement in the lipid profile level than that produced by Coccinia *per se.* This may be explained on the basis that improvement in the lipid profile by glibenclamide in diabetic rats may be due to better glycemic control (Trivedi *et al.*, 2004). Since glibenclamide may be acting by secondary mechanism, further improvement in lipid profile was not observed when used with Coccinia.

5.1.3 Interaction of *Coccinia* with glibenclamide on antioxidant effect

5.1.3.1 Reduced Glutathione in Tissues

The reduced glutathione in pancreas and liver were increased significantly by the combination treatment with Coccinia and glibenclamide when compared to the diabetic control. The increase in reduced glutathione content by the antioxidant property of the triterpenes present in Coccinia in combination with direct action of glibenclamide on antioxidant enzymes like

catalase and superoxide dismutase might have together caused improvement in reduced glutathione content.

5.1.3.2. Lipid Peroxides in Tissues

There was significant decrease in lipid peroxides level in pancreas and liver and this lipid peroxide reducing effect was comparable with that of Coccinia and glibenclamide treatment alone. The elevation in reduced glutathione by the treatment with Coccinia and glibenclamide might have helped in the scavenging of free radicals which caused cell membrane damage and death. This might have subsequently reduced the raised lipid peroxides.

Lack of further improvement in antioxidant status by the combination when compared to Coccinia and glibenclamide alone may be due to the reason that the decreased dose of glibenclamide (0.125 mg/kg) may not be effective enough to produce any significant improvement in antioxidant effect.

5.3 TOXICITY EVALUATION OF ETHANOLIC EXTRACT OF *Coccinia indica* LEAF

5.3.1 Acute oral toxicity

The ethanolic extract of *Coccinia indica* leaves did not show any oral toxicity upto 2000 mg/kg for 14 days. Hence according to OECD guideline 423, the drug has no acute oral toxicity. Similar results were reported by Badole *et al.* (2006) who evaluated the acute oral toxicity of aqueous extract of leaves of *Cocculus hirsutus* following the OECD guidelines. The results showed that the doses upto 2000 mg/kg were nonlethal and that all animals were alive, healthy and active during the observation period of 14 day post administration of highest dose of 2000 mg/kg body weight.

5.3.2 LD_{50}

The results showed that the LD_{50} is higher than 2000 mg/kg body weight by intraperitoneal route. Akhila *et al.* (2007) cited that the values of LD_{50} were found to increase with the following sequences of routes: intravenous, intraperitoneal, subcutaneous and oral. So a

high LD_{50} value greater than 2000 mg/kg at intraperitoneal route also indicated that the extract had a wide margin of safety.

5.3.3 Sub acute toxicity

Behavioral, biochemical and hematological parameters were estimated to evaluate the subacute toxicity of ethanolic extract of *Coccinia indica* leaves. Observation on behavioral changes and estimation of serum biochemical parameters were done by Sreekanth *et al.* (2006) for the toxicological evaluation of *Calycopteris floribunda.* Grover *et al.* (2000) evauated the hematological parameters to assesss the safety of aqueous extract of *Eugenia jambolana* and *Tinospora cordifolia* for therapeutic administration.

5.3.3.1 Behaviour

No abnormal behavioural were observed in any of the animals. Toxic symptoms like increased motor activity, anaesthesia, tremors, arching and rolling, clonic convulsions, ptosis, tonic extension, lacrimation, Straub reaction, exophthalmos, pilo-erection, salivation, muscle spasm, opisthotonus, writhing, hyperesthesia, depression, ataxia, stimulation, sedation, hypnosis, cyanosis and analgesia were not noticed in any of the animals. These observations proved that Coccinia was free of any toxic behavioural changes.

5.3.3.2 Body weight

There was no significant difference between the mean body weights on day zero and 14th day. The results of the toxicological studies of aqueous stem bark extract of *Boswellia dalzielii* showed a significant reduction in percentage weight gain when compared with the control group. Contrary to this there was no adverse effect on mean body weight gain in groups administered with various doses of ethanolic extract of *Coccinia indica* leaves. So the extract can be considered safe with respect to its effect on body weight.

***5.3.3.3 Serum Alanine Amino Transferase level* (ALT)**

Serum glutamate pyruvate transaminase, which is also known as alanine amino trasfrase(ALT) is a specific liver marker enzyme and when there is damage to liver cells, the cell integrity will be lost and increased levels of these enzymes were released in to blood. In the

present study, there was no increase in the serum ALT level before and after the treatement. The results showed that *Coccinia indica* did not produce any liver damage after subacute administration. This is similar to the findings of Batran *et al.* (2006), where the alcoholic extract of *Momordica charantia* at the dose rate upto a higher limit of 36.2 mg/100 g did not show any significant change in ALT level.

5.3.3.4 Serum Aspartate Amino Trasferase (AST)

AST is also a liver specific enzyme, which higher level indicate liver damage, cardiac injury, infarction and muscle injury (Mc. Gregor *et al.*, 2003). In the present study, after the subacute administration of *Coccinia indica* for 14 days, there was no significant increase in the serum AST level. So there was no possible liver damage caused by administration of *Coccinia indica* leaves. This is similar to the findings of Batran *et al.* (2006) where the administration of alcoholic extract of *Momordica charantia* did not produce any significant increase in serum AST level.

5.3.3.5 Serum Creatinine

Decreased serum creatinine and serum urea levels have been considered the most important manifestations of severe tubular necrosis of kidney. There was no significant difference in the serum creatinine level on 14th day when compared to the values on zeroth day. A significant rise in serum creatinine was obtained by Sreekanth *et al.* (2006) after the administration of leaves of *Calycopteris floribunda* in rats which indicated that the extract produced some toxic effect on kidney. As the administration of *Coccinia indica* did not cause any significant change in serum creatinine, Coccinia was proved to be free of any toxic effect on kidney.

5.3.3.6 Serum Glucose

The mean serum glucose values did not show any significant difference after 14 days when compared to the glucose values before the beginning of experiment. Batran *et al.* (2006) evaluated the toxic effects of *Momordica charantia* juice and found that there was a significant reduction in the serum glucose levels. In the present study the *Coccinia indica* leaves did not

reduce the serum glucose levels and thus it was found that the extract did not interfere with glucose absorption and utilization in normoglycemic animals.

5.3.3.7 Serum Total Protein

Protein synthesis is one of the functions of the liver and when liver is damaged, a reduction in protein synthesis will be observed. In the present study there was no significant change in the serum total proteins on 14th day when compared with the values on day zero. This is similar to the values obtained by Manna *et al.* (2004) where there was no significant difference in the total proteins after administration of alpha cypermethrin orally as a single dose. As there was no change in the total protein value after the administration of Coccinia extract , it was found not affecting the liver function.

5.3.3.8 Hematological Parameters

Hematological parameters like hemoglobin concentration, volume of packed red cells(VPRC), total leukocyte count(TLC), total erythrocyte count(TEC) and differential leukocyte count(DLC) were estimated on 14th day. There was no significant difference in the values of hemoglobin concentration, VPRC, TLC and TEC for all the treatment groups (II, III IV and V) when compared with normal control (group I). This showed that ethanolic extract of *Coccinia indica* did not possess any toxic effect at the dose levels administered. This agrees with the observations made by Etuk *et al.* (2006) after administration of *Boswellia dalzielii* where there was no significant change in the hematological parameters. In the differential leukocyte count an increase in neutrophil count was seen in group IV and V administered with higher doses of Coccinia (90 and 100 mg/kg body weight intraperitonealy respectively). This may be due to the acute inflammatory process taking place in the body as a result of irritation due to the intraperitoneal administration of the extract at higher doses and can not be considered as a toxic effect of *Coccinia indica.*

Summary

6. SUMMARY

The present study was undertaken to evaluate the hypoglycemic, hypolipidemic and antioxidant effect of ethanolic extract of *Coccinia indica* leaves. The study also envisaged to evaluate the interactive effect of *Coccinia indica* with glibenclamide and also its toxicity. A study was also carried out to assess the toxicity of *C. indica.*

Fifty six adult Sprague-Dawley strain of albino rats weighing 180-200 grams were randomly divided into seven groups of eight animals each. Group I served as normal control. All the groups except normal control were made diabetic by the subcutaneous injection of alloxan at the dose rate of 130 mg/kg body weight. The blood glucose level of all groups were checked on 15th day. Rats showing moderate hyperglycaemia (200-350 mg/dl) were selected for the study. Group II was kept as diabetic control.

Group III was administered with *C. indica* at the dose rate of 200 mg/kg body weight and group IV with glibenclamide at the dose rate of 0.25 mg/kg body weight orally for 45 days. Group V, VI and VII were administered with the extract of *C. indica* at the dose rate of 100, 150 and 200 mg/kg body weight respectively in combination with glibenclamide at the dose rate of 0.125 mg/kg body weight.

Body weight, blood glucose, serum cholesterol and triglyceride of all groups were noted on 15th, 30th, 45th and 60th day. Liver glycogen, reduced glutathione and lipid peroxides in pancreatic and hepatic tissues were estimated after sacrificing the animals on 60th day.

All the treatment groups except the diabetic control group showed a gradual increase in body weight and by the end of the experiment, regained to the original level as it was at the beginning of the experiment. For diabetic control there was a gradual decrease in the body weight throughout the experiment.

The blood glucose level was almost restored in group III and IV after 45 days of treatment with *Coccinia indica* and glibenclamide respectively. In group V, VI and VII where the combination of *C. indica* and glibenclamide were administered at increasing doses of *C. indica* (100, 150 and 200 mg/kg), the blood glucose level reduced significantly and group VII

showed better reduction in blood glucose when compared with group V and VI. Group VII showed the highest reduction in blood glucose among all the treatment groups.

Serum cholesterol level was found to be highest in group II (diabetic control). All the treatment groups showed a significant reduction in serum cholesterol compared to group II and there is no significant difference among the groups. However group VII (combination of *C. indica* (200 mg/kg) and glibenclamide (0.125 mg/kg) showed a more marked reduction in serum cholesterol.

The serum triglyceride showed a gradual reduction in all the treatment groups after the commencement of the treatment and returned to normal levels by the end of the treatment (60th day). The triglyceride level of the diabetic group remained high throughout the experiment.

Group II showed the lowest liver glycogen at the end of the experiment. The increased liver glycogen produced by group III, IV, V, VI and VII were almost similar. Group VII (combination of *C. indica* at the dose of 200 mg/kg and glibenclamide at the dose of 0.125 mg/kg) was found to be more effective in restoring liver glycogen values.

A decrease in reduced glutathione content was observed in liver and pancreas of diabetic control group (group II) at the end of the experiment, but it was significantly increased in all other treatment groups. The lipid peroxides in pancreas and liver showed a higher value on 60th day in diabetic control group, but a reduced value was noted in all other treatment groups.

The results of the present study confirmed the hypoglycemic, hypolipidemic and antioxidant property of *C. indica.* Combination of *C. indica* with glibenclamide (*C. indica* at the dose of 200 mg/kg body weight and glibenclamide at the dose of 0.125 mg/kg body weight) is more effective in producing the hypoglycemic effect than *C. indica* or glibenclamide alone which suggests that *C. indica* can be effectively used with glibenclamide. Further the results showed that the dose of glibenclamide can be reduced by the combination of *C. indica* with glibenclamide.

Acute oral toxicity was evaluated in albino mice with single oral dose of the extract upto 2000 mg/kg body weight (OECD guidelines 423) showed no toxicity. The sub acute toxicity was tested in four groups of animals by giving 50, 70, 90 and 100 mg/kg body weight

intraperitoneally. The biochemical and hematological parameters showed no significant difference. The results revealed that *C. indica* did not possess any sub acute toxicity.

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HYPOGLYCAEMIC EFFECT OF *Coccinia indica* (IVY GOURD) LEAVES AND ITS INTERACTION WITH GLIBENCLAMIDE IN DIABETIC RATS

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ABSTRACT

The present study was undertaken to assess the hypoglycemic, hypolipidemic, antioxidant effect, interactive effects with glibenclamide and toxicity of ethanolic extract of *Coccinia indica* leaves.

The experiment was conducted in seven groups of eight adult Sprague-Dawley rats each. Group I served as the normal control. All the groups except normal control were made diabetic by subcutaneous injection of alloxan at the dose rate of 130 mg/kg body weight and group II was kept as diabetic control. The groups III and IV were administered with the extract of *C. indica* at the dose rate of 200 mg/kg and glibenclamide at the dose rate of 0.25 mg/kg respectively for 45 days. The groups V, VI and VII were administered with a combination of *C. indica* at the dose rate of 100, 150 and 200 mg/kg respectively with glibenclamide at the dose rate of 0.125 mg/kg body weight for 45 days.

Blood glucose, serum cholesterol and serum triglyceride and body weight were noted on day zero, 16th, 30th, 45th and 60th day and liver glycogen on 60th day. The antioxidant effect was assessed by estimation of reduced glutathione and lipid peroxides in pancreas and liver. Representative samples of pancreas were also subjected to histopathological examination.

The diabetic control showed significant increase in the level of blood glucose, serum cholesterol and serum triglyceride level and also a significant reduction in body weight. All the other groups showed a gradual increase in body weight after 30 days of treatment. The groups III and IV restored the blood glucose levels after 45 days. The serum cholesterol, serum triglyceride, reduced glutathione

and lipid peroxide levels in pancreas and liver were also restored indicating the hypolipidemic and antioxidant effect of the treatments.

The groups V, VI and VII, in which the combinations of *C. indica* with glibenclamide were used, regained the blood glucose levels in 45 days of treatment. Group VII showed most marked effect and is found to be better than group III. The combination treatments produced a marked reduction in serum cholesterol and triglyceride levels and there was restoration of lipid peroxides and reduced glutathione in pancreas and liver.

The ethanolic extract of *C. indica* leaves were screened for acute oral toxicity and sub acute toxicity. The administration of the extract upto 2000 mg/kg body weight (OECD guidelines 423) did not show any acute oral toxicity in mice. The sub acute toxicity studies conducted in wistar rats, did not show any toxic symptoms like mortality, change in biochemical, hematological parameters. The results revealed that *C. indica* did not possess any sub acute toxicity. These toxicity studies indicated the safety of ethanolic extract of *C. indica* as a drug in the treatment of diabetes.

From the study it can be concluded that *C. indica* at the dose of 200 mg/kg body weight produced better hypoglycemic effect when compared with glibenclamide at the dose rate of 0.25 mg/kg body weight. Further the combination of *C. indica* (200 mg/kg) with glibenclamide (0.125 mg/kg) showed a more effective hypoglycemic, hypolipidemic and antioxidant effect than *C. indica* or glibenclamide alone and the combination can be safely used in the treatment of diabetes.