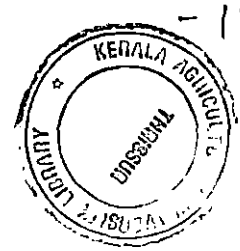
HYPOGLYCEMIC, HYPOLIPIDAEMIC AND CYTOPROTECTIVE EFFECTS OF LOTUS SEEDS (*Nelumbo nucifera*) IN SPRAGUE-DAWLEY RATS

JEYAMURUGAN. M.

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

Doctor of Philosophy

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur



2007

Department of Pharmacology and Toxicology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

DECLARATION

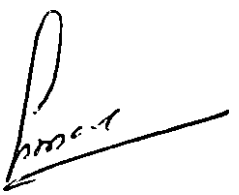
I hereby declare that this thesis entitled **"Hypoglycemic, hypolipidaemic and cytoprotective effects of lotus seeds (*Nelumbo nucifera*) in Sprague-Dawley rats"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.

Mannuthy

JEYAMURUGAN. M

CERTIFICATE

Certified that this thesis, entitled **"Hypoglycemic, hypolipidaemic and cytoprotective effects of lotus seeds (*Nelumbo nucifera*) in Sprague-Dawley rats"** is a record of research work done independently by **Jeyamurugan. M.,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associate ship to him.



Dr. A. M. Chandrasekharan Nair,
(Chairman, Advisory Committee)
Associate Professor and Head
Department of Pharmacology and Toxicology,
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur-680651.

CERTIFICATE

We, the undersigned members of the Advisory committee of Jeyamurugan. M., a candidate for the degree of Doctor of Philosophy in Pharmacology and Toxicology, agree that the thesis entitled **"Hypoglycemic, hypolipidaemic and cytoprotective effects of lotus seeds (*Nelumbo nucifera*) in Sprague-Dawley rats"** may be submitted by **Jeyamurugan. M.,** in partial fulfilment of the requirement for the degree.

Dr. A. M. Chandrasekharan Nair,
(Chairman, Advisory Committee)
Associate Professor and Head,
Department of Pharmacology and Toxicology,
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur-680651.

Dr .N. Gopakumar,
Professor and Head,
Department of Pharmacology and
Toxicology, Pookot.
(Member)

Dr. A. D. Joy,
Associate Professor,
Department of Pharmacology and
Toxicology.
(Member)

Dr. T. V. Aravindakshan,
Assistant Professor,
Department of Animal Breeding and
Genetics.
(Member)

Dr. N. Divakaran Nair,
Assistant Professor,
Centre of Excellence in Pathology.
(Member)

13/8

External Examiner

ACKNOWLEDGEMENT

To the greatest degree, I am indebted to my guide and Chairman of the Advisory Committee, ***Dr. A.M. Chandrasekharan Nair*** *, Associate Professor and head, Department of Pharmacology and Toxicology for his affectionate guidance, valuable suggestions, generous support and kindly help in all possible ways during the entire period of my study. Without his strong support and co-operation throughout the study, the successful completion of this work would not have been possible.*

I am greatly indebted to ***Dr. N. Gopakumar****, Professor and Head, Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Pookot and member of the advisory committee for the valuable help and whole hearted co-operation in carrying out the study.*

I am very much obliged to ***Dr. A.D. Joy****, Associate Professor, Department of Pharmacology and Toxicology and member of the advisory committee for his suggestions, earnest help, guidance and prompt review of thesis.*

I humbly place my respect and gratitude to ***Dr. T. V. Aravindakshan****, Assistant Professor, Department of Animal Breeding and Genetics and member of the advisory committee for his valuable suggestions, forthright views, patient and constructive review of the manuscript.*

I am grateful to ***Dr. N. Divakaran Nair,*** *Assistant Professor, Centre of Excellence in Pathology and member of the advisory committee for his continuous interest, valuable suggestions and support throughout the course of the thesis.*

I express my sincere thanks to ***Dr. K. Venugopalan****, Professor,* ***Dr. P.T.A. Usha****, Assistant Professor,* ***Dr. C.M. Aravindakshan*** *and* ***Shri. V.R. Reghunandanan****, Associate Professor, Department of Pharmacology and Toxicology for their mental support and encouragement during this study*

I would like to thank ***Dr. K.T. Prasanna Kumari,*** *Associate Professor, Department of Plant Breeding and Genetics, for the valuable offer to carrying out the project work given by Kerala State Council for Science and Technology and Environment.*

I greatly acknowledge the whole hearted help rendered by ***Mrs. K.S. Sujatha,*** *Assistant Professor and Head of the Department and* ***Mrs. Mercy,*** *Associate Professor, Department of Statistics for statistical analysis.*

I remember with gratitude the help and co-operation offered by ***Shri. P.R. Chandrasekhar,*** *Instrumentation Engineer and Head, Central Instrumentation lab.*

I am grateful to ***Dr. E. Nanu,*** *Dean i/c, College of Veterinary and Animal Sciences, Mannuthy for providing the facilities to conduct the research.*

I remain thankful to ***Zydus and Glenmark research centre*** *for kind supply of standard compound for my research work.*

I sincerely thank to ***Kerala State Council for Science, Technology and Environment*** *for providing financial assistance to the project entitled* ***"Comparative evaluation of red and white flowered ecotypes of sacred lotus (Nelumbo nucifera Gaertn)".***

I am obliged, thankful and grateful to my respected collegeue, ***Dr. Murugan, Dr. Rajaganapathi, Dr. Senthil kumar, Dr. Ajith and Dr. Shyam*** *for their help, valuable advice and guidance rendered to me during the period of my study.*

Persons can succeed in their life without brother but can't without friends. It is true with me. Nothing will be sufficient to show my deep sense of gratitude to my friends, ***Dr. Jerad Josep, Dr. Srinivasan, Dr. Shekar, Dr. Kowsikraj, Mr. Binoy, Mr. Jogesh Mohabatra, Mr. Jagatish Pati, Dr. Prabu, Dr. Madeshwaran, Dr. Shanmugasundaram, Dr. Sakthidevan, Sourave Banerjee,***

Debabrata Chanda, Arindum Gundu and Ravishanker *for the Valuable suggestions, priceless help, mental support and sustained encouragement rendered by them throughout the course of my research work.*

I gratefully acknowledge the whole hearted help and co-operation rendered by ***Dr. Vikram charavarthy, Dr. Vegateshwaralu, Dr. Bibu John, Dr. Senthil kumar, Dr. Blaji, Dr. Muthuramaliggam, Dr. Rishikesavan, Dr. Jagaveerapandian, Dr. Sivanesan, Dr. Ariprasath, Dr. Shekar, Dr. Arun, Dr. Sasikumar and Dr. Babu*** *during the course of the research work.*

I thankfully remember all those who directly or indirectly helped me and contributed to finalise the work.

I am indebted to ***Kerala Agricultural University*** *for awarding me the fellowship for the post graduate study.*

No words can implicitly express the deep gratitude to my beloved ***Mother, father, brother, son and my wife*** *for their love, affection, encouragement, prayers and blessings. I owe very much to them.*

JEYAMURUGAN. M

CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

Introduction

1. INTRODUCTION

Diabetes mellitus is characterized by chronic hyperglycemia and the development of specific microangiopathy in retina, renal glomerulus and peripheral nerve that evolves to blindness, end stage renal disease and debilitating neuropathies. There are two main forms of diabetes, type I (Insulin Dependent Diabetes Mellitus, IDDM) and type II (Non-Insulin Dependent Diabetes Mellitus, NIDDM). Type I diabetes is characterized by an absolute insulin insufficiency caused by the immunological destruction of pancreatic beta-cells. Type II diabetes is more complex in etiology and is characterized by a relative insulin deficiency, reduced insulin action and insulin resistance of glucose transport in skeletal muscle and adipose tissue (Guerra *et al.*, 2005).

Hyperglycemia and insulin resistance were reported to have an important role in the pathogenesis of macro vascular complications. One remarkable alteration in vascular function during diabetes is a decreased activity of vasodilators from endothelium such as nitric oxide and increased activity of vasoconstrictors such as angiotensin II and endothelin - I. The impairment of endothelial-dependent vasorelaxant responses has been reported in isolated aortic rings and the renal vasculature indicating that both conductance and resistance vessels are affected by endothelial dysfunction.

Oxidative damage has been suggested to be a contributory factor in the development and complications of diabetes. Some studies have shown decreased activity of antioxidant enzymes, reduced level of intracellular and serum glutathione (GSH), increase in cellular markers of lipid peroxidation in the renal cortex of diabetic animals and reversion of these alterations by dietary supplementation with vitamin E. A causal relationship between oxidative stress and endothelial dysfunction during diabetes is further supported by the findings that gene transfer and increased

expression of superoxide dismutase isoforms reverse endothelial dysfunction in diabetic aorta and that endothelial dysfunction is accompanied by decreased expression of superoxide dismutase in aortas of diabetic rats. Oxygen radicals contribute to the enhanced basal vascular tone, tubuloglomerular feedback, monocyte/macrophage infiltration and to the impaired endothelium-dependent relaxation in the diabetic kidney (Nascimento *et al.*, 2003).

Dyslipidaemia is common in patients with type II diabetes, associated with increased risk of coronary heart disease (CHD). In patients with diabetes, dyslipidaemia is characterised by elevated triglyceride levels, excessive postprandial lipaemia and increased level of low-density lipoprotein (LDL) particles and decreased level of high-density lipoprotein (HDL) cholesterol. Evidence indicates that abnormalities in proportion of triglycerides, LDL and HDL contribute to increased atherosclerotic risk in patients with diabetes (Taskinen, 2003).

Insufficient production of biologically active insulin is a common denominator in almost all forms of diabetes and the degree of insulin deficiency determines both the severity of the disease and the choice of therapy. Generally speaking, the total number of *beta*-cells reflects the balance between the loss and the proliferation of these cells. The number of functioning *beta* - cells in the islet organ is of decisive importance for the development, course and outcome of diabetes. Therefore, it would be of interest to examine the regulation of *beta* - cell growth and the factors that prevent or promote replacement of the lost islets. Although there has been much concentration of research on the understanding mechanisms of destruction of pancreatic islets, not much emphasis has been placed on the development, regeneration and maintenance of islet cells (Risbud and Bhonde, 2002).

The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world. The World Health Organization has also

recommended and encouraged this practice especially in countries, where access to the conventional treatment of diabetes is not adequate. There is an increased demand to use natural products with antidiabetic activity to avoid the side effects associated with modern medicines. The available literature shows that there are more than 400 plant species showing hypoglycemic activity. Though some of these plants have great reputation in the indigenous system of medicine for their antidiabetic activities, many remain to be scientifically established (Murthy *et al.*, 2004).

Hence, the present study was undertaken to investigate the hypoglycemic, hypolipidaemic and cytoprotective effects of red and white lotus seeds extract in Sprague-Dawley rats along with identification of the active principles present in the lotus seed extracts.

Review of literature

2. REVIEW OF LITERATURE

2.1. *Nelumbo nucifera*

Nelumbo nucifera (lotus) commonly known as kamala, thamari or padma is an aquatic herb found everywhere in the country. This plant is cultivated in India, for its elegant sweet scented flowers which are the national flower of India.

A formulation of medicines comprising of lotus seed is used in ayurvedic practice to treat the kidney and reproductive tract disorders. The rhizomes, flowers, stalk and leaves of lotus are used in the form of infusion in fever as refrigerant and diuretic (Mitra *et al.*, 1973). They detected alkaloids, steroids and reducing sugars in various extracts of rhizomes of *N.nucifera.*

Almost all parts of this plant are used in traditional medicinal practice to treat various diseases. The rhizomes are used as nutritive, mucilaginous, demulcent, diuretic, cholagogue and are effective in piles, dyspepsia and diarrhoea (Kritikar and Basu, 1975).

2.2. OTHER PHARMACOLOGICAL PROPERTIES OF LOTUS

Mazumder *et al.* (1992) reported that the petroleum ether extracts of seeds of *N.nucifera* administered to mice at a dose rate of 3 mg kg^{-1} body weight i.p. showed significant contraceptive, antiestrogenic and antiprogestonal activities. These findings indicated that the extract of *N.nucifera* seed could affect the estrus cycle by blocking the biogenesis of ovarian steroids at any intermediate stage and thereby the antifertility activity.

Wang *et al.* (1992) evaluated anti-arrhythmic effect of Liensinine (Lien), an alkaloid isolated from the green seed embryo of *Nelumbo nucifera* Gaertn. Its mechanism may be related to blockade of Ca^{2+} and Na^{+} influx.

In Chinese traditional medicine *N. nucifera* was one of the medicinal plants used for the treatment of hyperlipidaemia. The decoction of *N.nucifera* plant parts significantly reduced the serum triglyceride and cholesterol levels (Cour *et al.*, 1995).

In vitro antifungal and anti yeast activity of methanolic extract of rhizome of *N.nucifera* in doses of 250-350μg ml^{-1} was investigated against five different types of fungi and yeast by sensitivity test, disc diffusion test, spore germination technique and turbidity technique. The effect produced by the extract was comparable with the standard antifungal agent griseofulvin and found to be active against all organisms tested (Mukherjee *et al.*, 1995).

Mukherjee *et al.* (1995a) observed that the methanolic extract of *N.nucifera* at the dose rate of 100 and 600 mg kg^{-1} (p. o.) inhibited the castor oil induced diarrhoea. It reduced the gastrointestinal motility after administration of charcoal meal and had significant inhibition of PGE_2 induced enteropooling in rats. The results established the efficacy of *N.nucifera* rhizome as an antidiarrhoeal agent.

Gupta *et al.* (1996) proved the antisteroidogenic effect of seeds of *N.nucifera* (2.5-7.5 mg kg^{-1}, p.o.) in sexually immature female and male rats. It showed remarkable delay in sexual maturation in prepubertal female rats and significant reduction in sperm count and motility in male rats. An accumulation of cholesterol and ascorbic acid was also noted. These results indicated the suppression of steroidogenesis in both testes.

Mukherjee *et al.* (1996) reported that the methanolic extract of rhizomes of *N. nucifera* in doses of 200, 300 and 400 mg kg^{-1} i.p. showed reduction in spontaneous activity, decrease in exploratory behavior pattern by the head dip and Y – maze test. It also potentiated the pentobarbitone induced sleeping time in mice.

Mukherjee *et al.* (1996a) investigated the antipyretic activity of methanolic extract of rhizome of *N.nucifera* on normal body temperature and yeast induced pyrexia in rats. The extract in doses of 200, 300 or 400 mg kg^{-1} (p. o.) produced significant dose dependant lowering of normal temperature and yeast induced elevation of body temperature. The effect was comparable with the standard antipyretic drug, paracetamol (150 mg kg^{-1}, i.p.).

Mukherjee *et al.* (1997) reported that the ethanolic extract of rhizome of *N.nucifera* when administered orally reduced blood sugar level of normal glucose fed hyperglycemic and streptozotocin induced diabetic rats. The extract improved glucose tolerance and potentiated the action of exogenously administered insulin in normal rats.

Mukherjee *et al.* (1997a) studied the anti-inflammatory activity of the methanolic extract of *N. nucifera* rhizome in carrageenin and serotonin induced rat paw oedema. Methanol extract at doses of 200 and 400 mg kg^{-1} showed significant anti-inflammatory activity in both the models of inflammation in rats.

Yu and Hu (1997) evaluated neferine (Nef), a dibenzyl isoquinoline alkaloid isolated from *N. nucifera* Gaertn. on platelet aggregation and TXA_2/PGI_2 and cAMP/cGMP balance. Nef showed significant inhibition of rabbit platelet aggregation induced by ADP, collagen, arachidonic acid (AA) and platelet-activating factor (PAF). Nef was found to increase vascular 6-keto-PGF_1 alpha and platelet cAMP levels in a dose-dependent manner.

Extract of *N. nucifera* rhizome (RNN) was used as anti-diarrhoeal agent to combat the diarrhoea in experimental rats. The RNN extract in graded doses (100, 200, 400 and 600 mg kg^{-1}) reduced not only the frequency of defecation, wetness of

fecal dropping and PGE2 induced enteropooling but also the propulsive movements of charcoal meal (Talukder and Nessa, 1998).

Sinha *et al.* (2000) described the antipyretic potential of *N. nucifera* stalk extract on normal body temperature and yeast induced pyrexia in rats at dose level of 200 and 400 mg kg^{-1}. It caused a significant lowering of body temperature and yeast induced elevation of temperature.

Qian (2002) observed the antiarrhythmic effect of daurisoline and neferine which is an alkaloid isolated from *N. nucifera* Gaertn. Antiarrhythmic effect of daurisoline is more potent than that of dauricine.

Jung *et al.* (2003) assessed the antioxidant activity of *N. nucifera* stamens for their potential to scavenge stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, inhibition of total reactive oxygen species (ROS) generation, in kidney homogenates using 2',7'-dichlorodihydrofluorescein diacetate (DCHF-DA) and scavenge authentic peroxynitrites (ONOO-). Methanol (MeOH) extract of the stamens of *N. nucifera* showed strong antioxidant activity in the authentic peroxynitrites - system, and marginal activity in the DPPH and total ROS systems.

Sohn *et al.* (2003) studied the antioxidative and hepatoprotective effects of ethanol extracts from *N. nucifera* (ENN) seeds. Treatment of hepatocytes with *N. nucifera* (ENN) seeds inhibited both the production of serum enzymes and cytotoxicity by CCl_4. The genotoxic and cytotoxic effects of aflatoxin B_1 were also inhibited by *N.nucifera* (ENN) seeds in dose-dependent manners.

Wang *et al.* (2003) found that the methanolic extract of lotus plumule and blossom possessed strong reducing powers and free radicals scavenging abilities. However, only the methanolic extract of lotus plumule exhibited ion chelating properties.

The extracts from *N. nucifera* Gaertn, seeds were used in treatment of tissue inflammation in traditional Chinese medicine and inhibited peripheral blood mononuclear cells proliferation (PBMC) activated with phytohemagglutinin. By a bioassay-guided fractionation procedure, NN-B-4 identified from *N. nucifera* ethanolic extracts significantly suppressed activated PBMC proliferation. The inhibitory action of NN-B-4 did not involve direct cytotoxicity. In an attempt to further localize the point of arrest in the peripheral blood mononuclear cells proliferation, a set of key regulatory events leading to the cell proliferation, including cell cycle progression, production and gene expression of interleukin-2 (IL-2), IL-4, IL-10 and interferon-gamma (IFN-gamma) was examined. Cell cycle analysis indicated that NN-B-4 arrested the cell cycle progression of activated PBMC from the G1 transition to the S phase. The suppressant effects of NN-B-4 on proliferation of PBMC activated by PHA mediated through inhibition of early transcripts of PBMC, especially those of important like IL-2, IFN-gamma, and cdk4 and arrest of cell cycle progression (Liu *et al.*, 2004).

Kashiwada *et al.* (2005) evaluated the anti-HIV activity of benzylisoquinoline, aporphine, and bisbenzylisoquinoline alkaloids, including liensinine, negferine and isoliensinine isolated from the leaves and embryo of *N. nucifera*. It showed potent anti-HIV activities.

Rai *et al.* (2006) conducted studies on antioxidant activity of hydro alcoholic extract of *N. nucifera* seeds in carbon tetrachloride treated rats. Administration of hydro alcoholic extract of *N. nucifera* seeds to Wistar rats at 100 and 200 mg kg^{-1} body weight for 4 days prior to carbon tetrachloride treatment caused a significant dose dependent increase in the level of superoxide dismutase and catalase and a significant decrease in the level of thiobarbituric acid reactive substances, when compared to liver and kidney of carbon tetrachloride treated controls. These changes

observed at 100 mg kg^{-1} body weight were comparable to those observed for standard vitamin E at 50 mg kg^{-1} treatment.

2.3. OTHER INDIGENOUS PLANTS WITH ANTI-DIABETIC EFFECT

The studies conducted on methanolic extract of aerial parts of *Artemisia pallens* by Subramoniam *et al.* (1996) showed significant blood glucose lowering effect in glucose-fed hyperglycemic and alloxan-induced diabetic rats at the dose rate of 100 mg kg^{-1} p.o. In fasted normal rats the methanolic extract caused a moderate hypoglycemic effect at a higher dose (1000 mg kg^{-1} p.o.) and the aqueous extract (1000 mg kg^{-1} p.o.) was not showing the effect.

Soto *et al.* (1998) conducted studies on free-radical scavenging effect of Silymarin in alloxan induced diabetic rat. Silymarin increased pancreatic and blood reduced glutathione without changes in either hepatic reduced glutathione or blood glucose and prevents the increase in lipid peroxidation produced by alloxan. It also blunted the sustained increment in plasma glucose induced by alloxan.

The extract of the *Musa sapientum* flowers administered orally in rats at the dose levels of 0.15, 0.20 and 0.25 g kg^{-1} resulted in a significant reduction in blood glucose, glycosylated haemoglobin and an increase in total haemoglobin. The effect produced at the dose of 0.25 g kg^{-1} was significant. The result showed that extract of the *Musa sapientum* flowers has hypoglycaemic action (Pari and Maheswari, 1999).

Aqueous-ethanolic (50%, v: v) extract of *Punica granatum* L., flowers in alloxan-induced diabetic rats at the dose of 400 mg kg^{-1}, p.o. showed significant reduction in blood glucose (Jafri *et al.*, 2000).

Stanely *et al.* (2000) observed that oral administration of an aqueous extract of *Tinospora cordifolia* root to alloxan diabetic rats at the rate of 2.5, 5.0 and 7.5 g kg^{-1} p.o. for 42 days caused a significant reduction in blood glucose and brain lipids. The extract also caused an increase in body weight, total haemoglobin and hepatic hexokinase. The root extract also lowered hepatic glucose-6-phosphatase, serum acid phosphatase, alkaline phosphatase and lactate dehydrogenase in diabetic rats.

Aqueous extract of *Momordica cymbalaria* at a dosage of 0.5 g kg^{-1} p.o. has shown the maximal blood glucose lowering effect in diabetic rats. The same dosage did not produce any hypoglycemic activity in normal rats. The hypoglycemic activity of *Momordica cymbalaria* fruit was comparable with glibenclamide (Rao *et al.*, 2001).

Babu *et al.* (2002) evaluated anti-hyperglycemic activity of *Cassia kleinii* leaf extract in glucose fed normal and alloxan-induced diabetic rats. The alcohol extract of *Cassia kleinii* leaf (200 mg/kg p.o.) exhibited concentration dependent anti hyperglycemic effect in glucose loaded rats. However, the extract did not show hypoglycemic effect in fasted normal rats. In alloxan-induced diabetic rats the extract showed remarkable efficacy.

Chakrabarti *et al.* (2002) studied hypoglycemic and hypolipidemic effect of ethanolic extract of *Helicteres isora* roots and it was found to caused significant reduction in plasma glucose, triglyceride and insulin levels at the dose level of 300 mg kg^{-1} p.o. after 9 days of administration to insulin resistant and diabetic C57BL/KsJ and *db/db* mice. The extract also showed significant reduction in plasma triglyceride and insulin levels in normoglycemic and mildly hypertriglyceridemic Swiss albino mice without affecting plasma glucose level. In high fat fed hamster model, the extract showed significant reduction in plasma lipid levels.

Aqueous extract of *Enicostemma littorale* (15 g dry plant equivalent extract per kg) was assessed for its insulinotropic effect in alloxan induced diabetic rats. The extract caused significant increase in the serum insulin levels. The insulinotropic action of aqueous extract of *E. littorale* was further investigated using rat pancreatic islets. Extract has the potential to enhance glucose-induced insulin release at 11.1 mM glucose from isolated rat pancreatic islets. Incubation with Ca^{2+} chelator (EGTA) and Ca^{2+} channel blocker (nimodipine) did not affect the glucose-induced insulin release augmented by the extract. Maroo *et al.* (2002) concluded from this study that glucose lowering effect of aqueous extract of *E. littorale* associated with potentiation of glucose-induced insulin release through K^{+}ATP channel dependent pathway but did not require Ca^{2+} influx.

Murali *et al.* (2002) evaluated hypoglycemic effect of aqueous extract of *Enicostemma littorale* in non-insulin dependent diabetes mellitus rats and was shown significant decrease in both AUC glucose and AUC insulin values in treated group (2 g kg^{-1} p.o. daily for 6 weeks). Additionally treatment with *E. littorale* decreased the elevated cholesterol, triglyceride and creatinine levels in non-insulin dependent diabetes mellitus rats.

Vats *et al.* (2002) undertook investigation on hypoglycemic effect of the aqueous extract of *Pterocarpus marsupium* bark, alcoholic extract of seeds of *Trigonella foenum-graecum* and leaves of *Ocimum sanctum* in normal and alloxan-induced diabetic rats. The aqueous extract of *Pterocarpus marsupium* (1 g kg^{-1} p.o.) significantly lowered the blood glucose in alloxan diabetic rats 21 days after daily oral administration of the extract. Similarly, a hypoglycemic action was seen with alcoholic extract of *Trigonella foenum-graecum* in normal and diabetic rats.

Ananthan *et al.* (2003) observed that the treatment with alcoholic extract of *Gymnema montanum* leaf at the dose of 200 mg kg^{-1} p.o. for 3 weeks resulted in

significant reduction in blood glucose and an increase in plasma insulin levels. The effect of 50 and 100 mg kg^{-1} was not significant. The alcoholic extract also resulted in decrease in lipid peroxides and increased in reduced glutathione (GSH), ascorbic acid (Vitamin C) and alpha - tocopherol (Vitamin E). This result suggested anti diabetic and anti oxidant potency of *Gymnema montanum* leaf extract.

Hypoglycemic effect of ethanolic extract of *Cassia kleinii* leaf at the dose rate of 200 mg kg^{-1} p.o. produced significant reduction of blood glucose levels in streptozotocin diabetic rats. In addition extracts significantly reduced serum lipids, serum cholesterol and increased liver glycogen levels. However, the extract did not significantly influence the levels of serum insulin in both diabetic and normoglycemic rats (Babu *et al.*, 2003).

Hu *et al.* (2003) observed that a single administration of Gosha-jinki-gan (GJG 800 mg kg^{-1} p.o.) improved the glucose utilization and insulin resistance in STZ-induced diabetic rats.

The effect of cinnamon extract on the insulin action in awaked rats by the euglycemic clamp was investigated by Qin *et al.* (2003). They further analyzed possible changes in insulin signaling occurred in skeletal muscle. The rats were given with cinnamon extract orally for 3 weeks. After 3 weeks, cinnamon extract treated (30 and 300 mg kg^{-1}) rats showed a significantly higher glucose infusion rate (GIR) at 3 mU kg^{-1} per min insulin infusions compared with untreated control rat. There were no significant differences in insulin receptor (IR)-B, IR substrate (IRS)-1, and phosphatidyl inositol (PI) 3-kinase protein content between treated (300mg kg^{-1}) rats and controls. However, the skeletal muscle insulin-stimulated IR- B and the IRS-1 tyrosine phosphorylation levels were higher in rats treated with 300 mg kg^{-1}. These results suggest that the cinnamon extract would improve insulin action via increasing

glucose uptake *in vivo*, at least in part through enhancing the insulin-signaling pathway in skeletal muscle.

Study was conducted by Xie *et al.* (2003) to demonstrate anti-hyperglycemic activity of *Gymnema yunnanense* leaves extract in obese *ob/ob* and diabetic *db/db* mice. Daily intraperitoneal injections of the extract 100 mg kg^{-1} for 12 days in *db/db* and *ob/ob* showed significant reduction of fasting blood glucose levels compared to vehicle-treated mice. The glucose tolerance was also improved.

Colivicchi *et al.* (2004) conducted studies on the effects of dietary taurine on insulin- and adrenergic stimulated 2-deoxyglucose uptake by isolated adipocytes. The animals treated with taurine (free access to drinking water containing 3% taurine) 4 weeks before and 4 weeks after streptozotocin administration, prevented the loss of both insulin and adrenergic agonist stimulations of 2-deoxyglucose uptake, without affecting hyperglycemia.

Hypoglycemic and antihyperglycemic effects of flavonoid and saponin fractions of *Cogniauxia podoleana* Baillon were studied in normal and alloxan induced diabetic rats. The diethyl ether fraction of flavonoids (100 mg kg^{-1} p.o.) reduced the blood glucose levels to 40.0% in 3 h after oral administration to normal rats. In contrast, the oral administration of benzene and ethyl acetate fractions of saponins at a dose of 100 mg kg^{-1} to normal rats did not decrease the blood glucose levels. The diethyl ether fraction of flavonoids at a dose of 50 mg kg^{-1} p.o. was found to reduce hyperglycemic levels to 29.4 and 44.5% respectively, at 3 and 4 h after oral administration to alloxan-induced diabetic rats, whereas at a dose of 100 mg kg^{-1}, it decreased the levels of hyperglycemia to 41.4 and 70.4% respectively, after 3 and 4 h (Diatewa *et al.*, 2004).

Hypoglycemic effect of aqueous extracts of *Carum carvi* and *Capparis spinosa* L. fruit in normal and streptozotocin diabetic rats was studied by Eddouks *et al.* (2004). Oral administration of the aqueous *Carum carvi* and *Capparis spinosa* L. extracts (20 mg kg^{-1}) produced a significant decrease in blood glucose levels in streptozotocin diabetic rats both in a single dose and 14 day daily repeated dose treatment group. The blood glucose levels were nearly normalized 2 weeks after daily repeated oral administration of both aqueous *Carum carvi* and *Capparis spinosa* L. extracts (20 mg kg^{-1}). No significant changes on blood glucose levels were noticed in normal rats after both acute and chronic treatments with *Capparis spinosa* L. and *Carum carvi*. In addition, no changes were observed in basal plasma insulin concentrations after treatment with these plants in either normal or streptozotocin diabetic rats.

Maghrani *et al.* (2004) observed that aqueous extracts of *Fraxinus excelsior* seed and *Silybum marianum* aerial part produced significant reduction in blood glucose levels in normal and streptozotocin induced diabetic rats, after a single dose or 15 day daily doses, of the aqueous extracts at the rate of 20 mg kg^{-1} orally. In addition, no changes were observed in basal plasma insulin concentrations after both *Fraxinus excelsior* and *Silybum marianum* treatments in either normal and STZ diabetic rats indicating that these plants exert their pharmacological activity without affecting insulin secretion.

Maiti *et al.* (2004) showed that aqueous extract of seeds of *Tamarindus indica* Linn. (80 mg $100g^{-1}$ body weight p.o.) lowered fasting glucose level after 7 days treatment in streptozotocin induced diabetic male rat. Moreover, it produced a significant elevation in liver and skeletal muscle glycogen content and activity of liver glucose-6-phosphatedehydrogenase. Activities of liver glucose-6-phosphatase, liver and kidney glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate

transaminase (GPT) were decreased significantly in the aqueous extract supplemented group.

Studies were conducted by Murthy *et al.* (2004) and found that seed powder of *Datura metel* significantly reduced blood glucose levels in normal and alloxan-induced diabetic rats at the graded doses (25, 50 and 75 mg kg^{-1}, p.o.). The effect was found to be dose dependent.

Pari and Satheesh (2004) conducted study of hypoglycemic effect of aqueous solution of *Boerhaavia diffusa* L. in alloxan induced diabetic rats. *Boerhaavia diffusa* L. leaf extract (200 mg kg^{-1} p.o.) showed significant reduction in blood glucose and significant increase in plasma insulin levels. Additionally *Boerhaavia diffusa* L. leaf extract caused significant reduction of glycosylated haemoglobin and an increase in total haemoglobin level. The activities of the hepatic enzymes such as hexokinase was significantly increased and glucose-6-phosphatase, fructose-1, 6-bisphosphatase were significantly decreased. An oral glucose tolerance test (OGTT) was also performed in the same groups, in which a significant improvement was noted in glucose tolerance in rats treated with *Boerhaavia diffusa* L. leaf extract.

Sathyan (2004) assessed hypoglycemic effect of leaves of *Azadirachta indica*, *Ocimum sativum*, *Tinospora cordifolia* and their combination at 200 mg kg^{-1} in alloxan induced diabetic rats. In combination these three plants showed higher glucose reduction than that of used alone. It indicates synergistic effect of the combination

Hypoglycemic effect of Myrtle oil was assessed both in normal and diabetic rabbits. Myrtle oil significantly lowered blood glucose in alloxan-diabetic rabbits from fourth hour onwards at a dose of 50 mg kg^{-1} sublingually. However, Myrtle oil

did not affect serum insulin concentrations in normal and alloxan-diabetic rabbits. It reduced the serum triglyceride concentrations also (Sepici *et al.*, 2004).

Yadav *et al.* (2004) found that *Brassica juncea* seeds and *Murraya koenigii* leaves significantly reduced fasting serum glucose, insulin and cholesterol levels in a fructose-mediated non-genetic model of insulin resistance, after feeding of a fructose diet containing 10% *Brassica juncea* seeds powder for 30 days. On the other hand, a diet containing 15% *Murraya koenigii* leaves powder failed to exert any effect on these parameters.

Youn *et al.* (2004) conducted studies on hypoglycemic effect of the aqueous extract of *Commelina communis* L. leaf and stem on the activity of *alfa* - glucosidase *in vitro* and *in vivo*. It showed inhibitory activity of the *alfa* - glucosidase in a dose-dependent manner in *in vitro*. In *in vivo* it alleviated hyperglycemia caused by maltose or starch loading in normal and streptozotocin induced diabetic mice with better efficacy than that of acarbose. In addition, prolonged administration of *C. communis* L. extract tends to normalize hyperglycemia in streptozotocin induced diabetic mice.

Zhang *et al.* (2004) studied the hypoglycemic and anti-diabetic effect of *Rehmannia glutinosa* (roots) oligosaccharide in glucose-induced hyperglycemic and alloxan induced diabetic rats. It was found that pretreatment of *Rehmannia glutinosa* oligosaccharide (100 mg kg^{-1} for 3 days, i.p.) in normal rats, a partial prevention of hyperglycemia caused by glucose (2 g kg^{-1} i.p.), while adrenalectomy induced hyperglycemia was not prevented. In alloxan-induced diabetic rats, *Rehmannia glutinosa* oligosaccharide (100 mg kg^{-1} for 15 days, i.p.) showed a significant decrease in blood glucose level and hepatic glucose-6-phosphatase activity with an increase in hepatic glycogen content. The results indicated that oligosaccharide of

Rehmannia glutinosa Libosch. exerted a significant hypoglycemic effect in normal and alloxan-induced diabetic rats.

Studies conducted by Aguilar *et al.* (2005) showed that aqueous extracts and raw juice of *Ibervillea sonorae* root at a dose of 600 mg kg^{-1} exert significant reduction in blood glucose level after intraperitoneal administration in healthy mice. On the other hand dichloromethane extract showed a marked hypoglycemic activity, in healthy and alloxan induced diabetic rats after oral administration at a dose of 300 mg $kg^{-1}day^{-1}$.

Anandharajan *et al.* (2005) analysed the influence of *Pterocarpus marsupium* methanolic extract and isoflavone isolated from *Pterocarpus marsupium* on a battery of cellular targets GLUT-4, PPARγ and PI_3 kinase. The significant glucose uptake showed by *Pterocarpus marsupium* crude and pure was comparable with insulin and Rosiglitazone. Elevation of GLUT-4 and PPARγ gene expression in parallel with glucose uptake supported the *in vitro* glucose uptake activity of *Pterocarpus marsupium* methanolic extract and *Pterocarpus marsupium* isoflavone.

Hypoglycemic activity of aqueous and ethanolic extract of *Caesalpinia bonducella* seed shell and kernel was studied by Chakrabarti *et al.* (2005) using physiological hyperglycemic model, type I and type II sub acute diabetic animal. They noticed that extracts from *Caesalpinia bonducella* at the dose of 250 mg kg^{-1} p.o. showed potent hypoglycemic activity in chronic type II diabetic model.

Eddouks *et al.* (2005) undertook investigation on the hypoglycemic activity of aqueous extract of *Chamaemelum nobile* aerial part in normal and streptozotocin-induced diabetic rats. Oral administration of *Chamaemelum nobile* aqueous extract (20 mg kg^{-1}) reduced blood glucose levels in single and 15 days daily repeated dose

in both normal and streptozotocin-induced diabetic rats. Basal plasma insulin concentrations remain unchanged after treatment.

Ju *et al.* (2005) conducted studies to investigate the potential role of creatine in GLUT4 gene expression in rat skeletal muscle. Female Wistar rats were fed with chow containing 2% creatine monohydrate *ad libitum* for 3 weeks showed significant increase in GLUT4 protein levels in extensor digitorum longus triceps, and epitrochlearis muscles compared with muscles from normal rat fed with chow alone.

Treatment with aqueous extract of *Murraya koenigii* leaves at the dose levels 200, 300 and 400 mg kg^{-1} p.o. led to a lowering of blood glucose level in normal as well as in diabetic rabbits. Kesari *et al.* (2005) found the dose dependent reduction in glucose level after a single oral administration of aqueous extract by improvement in glucose tolerance.

The leaves powder of *Eucommia ulmoides* (Du-zhong) was added to a standard diet at the rate of 1% level and was evaluated for its anti diabetic activity in streptozotocin induced diabetic rats. The powdered leaves were given for 3 weeks. The blood glucose levels were significantly reduced in the Du-zhong leaves powder supplemented and water extract of the powdered Du-zhong leaves treated groups than in the untreated group (Lee *et al.*, 2005).

Study conducted by Muruganandan *et al.* (2005) showed that mangiferin (10 and 20 mg kg^{-1} i.p. for 28 days) lowered the blood glucose level in streptozotocin (STZ) induced diabetes rat. Further, mangiferin (10 and 20 mg kg^{-1}, i.p.) showed significant antihyperlipidemic and antiatherogenic activities as evidenced by significant decrease in plasma total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C) levels coupled together with elevation of highdensity lipoprotein cholesterol (HDL-C) level and diminution of atherogenic index in diabetic rats.

Musabayane *et al.* (2005) undertook investigation on the hypoglycemic effect of ethyl acetate extract of *Syzygium cordatum* leaf in non-diabetic and streptozotocin (STZ)-induced diabetic rats. The *Syzygium cordatum* leaf extract was administered at the dose of 6 mg 100^{-1} gm body weight orally for 4 weeks. Oral administration of the extract did not affect plasma glucose concentration in STZ-diabetic rats after 4 weeks although it significantly increased hepatic glycogen content in comparison with untreated STZ-diabetic rats.

Ruzaidi *et al.* (2005) conducted studies on ethanolic extract of cocoa at the dose levels of 1%, 2% and 3% and found that it significantly reduced the serum glucose levels compared to the control when it supplemented in feed at 1% and 3% levels for 4 weeks in streptozotocin-diabetic rats. Furthermore, supplementation at 1 and 3% cocoa extract reduced the level of total cholesterol and triglycerides. Interestingly, this study showed that serum HDL- cholesterol increased significantly in diabetic rats fed with 2% cocoa extract, while the LDL- cholesterol decreased significantly in the 1% treated group.

Schaan *et al.* (2005) examined the effects of nervous activity on expression of GLUT2, the major glucose transporter in proximal renal tubules, which participates in renal glucose handling. Study was carried out in diabetic and non diabetic rats with normal, low, or high renal sympathetic nervous activity (RSNA). Compared with controls, diabetes rats increased the GLUT2 protein content in kidney. The renal denervation-caused decrease in RSNA, reduced GLUT2 content in both normal and diabetic rats

Shirwaikar *et al.* (2005) conducted studies on the antidiabetic potential of the alcoholic extract of *Coscinium fenestratum* Colebr stem in the STZ- nicotinamide induced type II diabetic model. Administration of alcoholic extract to normal and

experimental diabetic rats at the dose 250 mg kg^{-1} p.o. and 500 mg kg^{-1} p.o. for 12 days caused significant reduction in fasting blood glucose levels in the normal as well as in the treated diabetic animals. Serum insulin levels were not altered. A significant reduction in serum lipid profiles, thiobarbituric acid reactive substance levels, glycosylated hemoglobin and increased in liver glycogen were noted.

The studies conducted by Singh *et al.* (2005) on effect of potato peel –powder in diabetic rats revealed its antidiabetic and antioxidant property. Diabetic rats fed with potato peel –powder supplemented diet at 5% and 10% for 4 weeks showed a significant decrease in blood glucose levels. In addition it also reduced significantly the hypertrophy of liver and kidney of STZ-diabetic rats and also normalized the activities of serum ALT, AST, hepatic and renal MDA and GSH, as well as activities of various antioxidant enzymes in liver and kidney of diabetic rats.

Studies conducted by Sy *et al* (2005) observed acetone extracts of the leaves of *Vernonia colorata* at the dose level of 100 mg kg^{-1} p.o. caused significant reduction in blood glucose in normoglycemic and alloxan-induced diabetic rats. In contrast, the hexane extract of the leaves of *Vernonia colorata* increased significantly the glycemia in normoglycemic rats.

2.4. OTHER INDIGENOUS PLANTS WITH PANCREATIC BETA CELLS PROLIFERATION EFFECT

Effect of flavonoid fraction (XE) extracted from the bark of *Pterocarpus marsupium* Roxb. (Leguminoceae) was studied in normal and alloxanised albino rats. The flavonoid fraction did not show a consistent effect on normal blood sugar levels but it effectively reversed the alloxan induced changes in the blood sugar level and the beta-cell population in the pancreas (Chakravarthy *et al.*, 1980).

Ahmed *et al.* (1998) undertook investigation on effect of *Momordica charantia* fruit juice on the distribution and number of alfa, beta and delta cells in the pancreas of streptozotocin induced diabetic rats. The results indicated that there was a significant increase in the number of beta cells in *M. charantia* treated animals when compared with untreated diabetic control. However, their number was still significantly less than that of normal rats. There was also a significant increase in the number of delta cells in streptozotocin diabetic rats compared to non-diabetic rats. This increase in the number of delta cells was not affected by *M. charantia* treatment. The number of alfa cell did not change significantly in *M. charantia*-treated rats.

Hardikar and Bhonde (1999) studied islet neogenic effect of cytosolic extracts from the regenerating pancreas in streptozotocin diabetes in BALB:c mice. Islet neogenesis was observed in the cytosoloic extract treated animals and confirmed by increasing circulating insulin concentrations, islet area and subsequent decrement in fasting glucose. Histological analysis of the pancreas in the cytosolic extract treated group revealed numerous tiny neo-islets as compared to the larger mature islets in the non-diabetic controls.

Study was conducted by Ogneva and Martinova (2002) on the potential morphogenic activities of the growth factors FGF1 (Fibroblast growth factor 1), FGF2 (Fibroblast growth factor 2) and FGF7 (Fibroblast growth factors 7), in pancreatic cells from streptozotocin diabetic newborn rats. The most prominent stimulatory effect was found after application of FGF2 and FGF7 at the dose of 100 ng l^{-1} in endocrine and exocrine pancreatic cells of diabetic rats. It is concluded that FGF2 and FGF7 might act as putative key-signaling molecules in the differentiation of pancreatic cells.

Degirmenci *et al.* (2005) observed acarbose and *Rumex patientia* caused significant morphological changes of pancreatic beta cells in streptozotocin induced

diabetic rats. The rats treated with STZ alone showed mitochondrial vaculation and swelling as well as dilatation of endoplasmic reticulum in the beta cells. No pathological changes were observed in the STZ + acarbose group. In the STZ + *Rumex patientia* group, reduced swelling in mitochondria of beta cells was noticed.

2.5. OTHER INDIGENOUS PLANTS WITH HYPOLIPIDAEMIC EFFECT

Mini (1992) evaluated hypolipidaemic effect of aqueous extract of *Allium sativum* pulp and aqueous extract of *Embilica officinalis* fruits in rabbits. It showed significant reduction in plasma total cholesterol and triglyceride levels.

Hypolipidemic effect of *Trigonella foenum qraecum* (Fenugreek) was studied by Khosla *et al.* (1995) in normal and alloxan-induced diabetic rats. Rats were treated with unroasted and roasted powdered seeds at the dose of 2 g kg^{-1} and 6 g kg^{-1}. It produced significant fall in various serum lipids like total cholesterol, triglycerides, LDL and VLDL cholesterols in normal rats as well as diabetic rats increased HDL cholesterol was also noticed in diabetic rats.

Bopanna *et al.* (1997) observed that treatment with neem kernel powder along with glibenclamide significantly decreased the concentration of serum lipids, blood glucose and activities of serum enzymes like alkaline phosphatase, acid phosphatase, lactate dehydrogenase, liver glucose-6-phosphatase and HMG CoA reductase activity in liver and intestine of alloxan diabetic rabbits. The results suggest a significant antidiabetic and antihyperlipaemic effect of the neem kernel powder.

Prince *et al.* (1999) evaluated the hypolipidemic effect of an aqueous extract of *Tinospora cordifolia* roots. Administration of the extract of *T. cordifolia* roots (2.5 and 5.0 g kg^{-1}) for 6 weeks resulted in a significant reduction in serum and tissue

cholesterol, phospholipids and free fatty acids in alloxan diabetic rats. The root extract at a dose of 5.0 g kg^{-1} body weight showed highest hypolipidemic effect.

Dhandapani *et al.* (2002) studied the hypolipidaemic and hypoglycemic effect of *Cuminum cyminum* supplementation in alloxan diabetic rats. It was found that oral administration of 0.25 g kg^{-1} body weight of *C. cyminum* for 6 weeks to diabetic rats resulted in significant reduction in blood glucose and an increase in total haemoglobin and glycosylated haemoglobin. It also showed significant reduction in plasma and tissue cholesterol, phospholipids, free fatty acids and triglycerides.

Le *et al.* (2004) studied the hypolipidemic and hypoglycemic effect of petroleum ether extract of *Nigella sativa* seeds in the normal rats. It was found that *Nigella sativa* treated rats had lower fasting plasma levels of insulin and triglycerides and higher HDL-cholesterol as compared to controls at the end of the 4-weeks treatment.

Prince *et al.* (2004) observed the antidiabetic and antihyperlipidemic effect of an alcoholic extract of *Syzigium cumini* seeds (JSEt) in alloxan diabetic rats. Oral administration of alcoholic extracts of *Syzigium cumini* seeds to diabetic rats at the dose of 100 mg kg^{-1} resulted in a significant reduction in blood glucose and urine sugar and lipids in serum and tissues in alloxan diabetic rats. The extract also increased total haemoglobin. The effect of alcoholic extracts of *Syzigium cumini* seeds was similar to that of insulin.

Studies conducted on aqueous extract of *Cissus sicyoides* by Viana *et al.* (2004) revealed the hypoglycemic and anti-lipemic effects in alloxan-induced diabetes in rats. The treatment of diabetic rats with *Cissus sicyoides* for 7 days (100 and 200 mg kg^{-1}, p.o.) significantly reduced the blood glucose levels by 25 and 22%

respectively. While no changes were seen in total cholesterol levels. A significant decrease in plasma triglyceride levels was noticed.

Ethanolic extract of *Zingiber officinale* Roscoe was assessed for its lipid lowering and antioxidant potential in streptozotocin induced diabetes in rats. Ethanolic extract of *Zingiber officinale* (200 mg kg^{-1}) fed orally for 20 days produced significant antihyperglycemic effect in diabetic rats. It also lowered serum total cholesterol, triglycerides and increased the HDL-cholesterol levels when compared with diabetic rats. *Zingiber officinale* extract treatment lowered the liver and pancreas thiobarbituric acid reactive substances (TBARS) (Bhandari *et al.*, 2005).

Fuliang *et al.* (2005) found that ethanol and water extracts of propolis (*Apis mellifera* L.) decreased cholesterol, triglyceride, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol in serum of rats with diabetic. An increased serum level of high-density lipoprotein cholesterol was noticed.

2.6. OTHER INDIGENOUS PLANTS WITH ANTI-OXIDANT EFFECT

Cytoprotective effect of *Aloe arborescens* Miller var. *natalensis* Berger (Kidachi aloe in Japanese) in streptozotocin and alloxan induced diabetic rats were studied by Beppu *et al.* (2003). It was found that kidachi aloe components inhibited the destruction of rat pancreatic islet beta -cells by streptozotocin and alloxan.

Mohamad *et al.* (2004) conducted studies on antioxidant effect of low doses of vanadate in combination with *Trigonella foenum graecum* seed powder in diabetic rats. It was found that vanadate (0.6 mg ml^{-1}) and combined dose of vanadate (0.2 mg ml^{-1}) increased activities of superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase in liver and kidney of diabetic rats after 21days of treatment.

Protective role of motilla – moriles appellation red wine in experimental diabetes induced by the injection of streptozotocin in male Wistar rats were studied by Montilla *et al.* (2004). After 4 weeks of treatment lipid peroxidation levels, reduced glutathione content and antioxidative enzyme activity were determined in kidney, liver and pancreas. A significant reduction in lipid peroxidation level elevated reduced glutathione content and antioxidative enzyme activity was noted.

Studies conducted by Pari and Latha (2004a) showed that aqueous extract of *Scoparia dulcis* had significant cytoprotective effect on the occurrence of oxidative stress in the brain of rats during diabetes. When they administered aqueous extract of *Scoparia dulcis* orally (200 mg kg^{-1}) for 6 weeks it caused significant increase in the activities of plasma insulin, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase and reduced glutathione. A significant decrease in lipid peroxidation and hydroperoxide formation in brain was noticed.

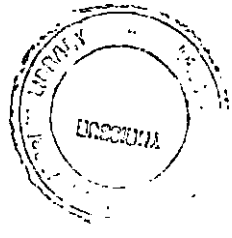
Protective role of quercetin on beta cell damage in experimental streptozotocin induced diabetes in rats was studied by Coskun *et al.* (2005). The decreased activities of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase activities in diabetic animals were regained by the treatment of quercetin in pancreas. Additionally quercetin treatment significantly decreased the elevated malondialdehyde and nitric oxide. It increased the insulin levels and preserved islet cells in diabetic rats.

Manonmani *et al.* (2005) observed that treatment with aqueous extract of *Cassia fistula* (Linn.) flowers (10 ml kg^{-1} p.o.) in alloxan induced diabetic rats, significantly increased antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione in heart tissue. Additionally appreciable decrease in peroxidation products *viz.,* thiobarbituric acid reactive substances, conjugated dienes, hydroperoxides was also observed in heart

tissues. The results suggested the significant anti oxidant effect of aqueous extract of *Cassia fistula* (Linn.) flowers.

The effect of caffeic acid phenethyl ester, on cardiac tissue in streptozotocin induced diabetic rats was studied by Okutan *et al.* (2005). Diabetic animals treated with 10 μmol kg^{-1} i.p. dose of caffeic acid phenethyl ester per day for 8 weeks exhibited significant increase in superoxide dismutase, catalase and glutathione peroxidase levels and reduction of malondialdehyde levels in the cardiac tissues. The result revealed the cytoprotective effect of caffeic acid phenethyl ester by increasing the antioxidant enzyme levels.

The effect of ethanolic extracts of three *Viscum album* subspecies, ssp. *album*, ssp. *austriacum*, ssp. *abietis*, on antioxidant activity was studied in streptozotocin-induced diabetic rats. The extract at the dose of 500 mg kg^{-1} p.o. significantly increased the antioxidant enzymes levels in liver, kidney and heart tissues (Orhan *et al.*, 2005).



Materials and methods

3. MATERIALS AND METHODS

3. 1. EXPERIMENTAL ANIMALS

The study was conducted in male Sprague-Dawley rats weighing 150 - 200 g purchased from the Small Animal Breeding Station, Kerala Agricultural University, Mannuthy. The animals were reared under standard environmental conditions (22 to 28^0 C, 60-70 per cent relative humidity, 12 hr dark/light cycle) and fed with standard rat feed and water *ad libitum*.

3. 2. PREPARATION AND ADMINISTRATION OF LOTUS SEEDS EXTRACT

3. 2. 1. Preparation of Ethanolic Extract

The seeds of red and white types of lotus (*Nelumbo nucifera*) were collected fresh and were dried in the shade at room temperature. The dried seeds were then powdered well in a pulveriser. The powdered seeds were subjected to extraction 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 it in a water bath at low temperature so as to obtain a semi-solid mass. The yield of the extract was 10 per cent for both red and white lotus seed. The crude extract thus prepared was kept in a refrigerator at 4^0C for further use.

3. 2. 2. Preparation of Formulation and Dosing

Ethanolic extract of red and white lotus seeds and respective reference drug were weighed separately and homogenized with the help of a mortar and pestle in appropriate volume of vehicle (Gum acacia 2.5% for the first 5 experiments and normal saline for 6^{th} experiment) so as to get a concentration of 600 mg kg^{-1}. The formulation was administered orally at

Fig.1. lotus (*Nelumbo nucifera*) Red type flower and seed

Fig.2. lotus (*Nelumbo nucifera*) White type flower and seed

the rate of 600 mg kg^{-1}, once daily for 28 days. To ensure accurate dosing, suspension was prepared freshly and administered by slow syringing through the orogastric tube.

3. 3. EXPERIMENTAL DESIGN

Six experiments were carried out in the present investigation. In each experiment the animals were divided into five groups.

3. 3. 1. Hypoglycemic Effect of Red and White Lotus Seeds Extract in Insulin Dependent Diabetes Mellitus (Type I diabetes mellitus) Model

Thirty Sprague - Dawley male rats were used for this study. They were divided into five groups of six each. Insulin dependent diabetes mellitus (Type I) was induced (in group 2, 3, 4 and 5) by administration of a single dose of alloxan at the rate of 130 mg kg^{-1} i.p. (dissolved in 0.01 M citrate buffer, pH 4.5) after 12 hours fasting. Since alloxan is capable of producing fatal hypoglycemia due to massive pancreatic insulin release rats were given 20% glucose solution (15-20 ml) intraperitonially after 6 hours of alloxan administration and they were provided with 5% glucose for drinking for the succeeding 24 hours to prevent hypoglycemia. After 2 weeks rats having fasting (12 hours) blood glucose levels ranging from 200-270 mg dl^{-1} were used for the study (Prince *et al.,* 2004).

3. 3. 1. 1. Treatment schedule

Group 1: Normal control – Non diabetic rats were administered with vehicle alone at the rate of 1ml kg^{-1} p.o. for 28 days.

Group 2: Diabetic control – Diabetic rats were administered with vehicle alone at the rate of 1ml kg^{-1} p.o. for 28 days.

Group 3: Diabetic rats were administered with red lotus seeds extract at the rate of 600 mg kg^{-1} p.o. for 28 days.

Group 4: Diabetic rats were administered with white lotus seeds extract at the rate of 600 mg kg^{-1} p.o. for 28 days.

Group5: Diabetic rats were administered with reference drug metformin at the rate of 200 mg kg^{-1} p.o. for 28 days.

Blood glucose levels were estimated on 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment. On 28^{th} day oral glucose tolerance test was carried out. Liver glycogen and GLUT 2 gene expression in kidney was carried out on 29^{th} day.

3. 3. 2. Hypoglycemic Effect of Red and White Lotus Seeds Extract in Non - Insulin Dependent Diabetes Mellitus (Type II diabetes mellitus) Model

Thirty Sprague - Dawley male rats were used for this study. They were divided into five groups of six each. Non insulin dependent diabetes mellitus (Type II) was induced (in group 2, 3, 4 and 5) by administration of single dose of alloxan in 6 days old Sprague - Dawley male rat pups. The pups received alloxan monohydrate at the rate of 200 mg kg^{-1} i.p. (dissolved in 0.01 M citrate buffer, pH 4.5) after 16 hours fasting. At the age of 8 weeks there was significant increase in fasting (12 hours) blood glucose levels ranging from 200-290 mg dl^{-1} were used for the study (Kodama *et al.*, 1993).

3. 3. 2. 1. Treatment schedule

Group 1: Normal control – Non diabetic animals were administered with vehicle alone at the rate of 1ml kg^{-1} p.o for 28 days.

Group 2: Diabetic control - Diabetic animals were administered with vehicle alone at the rate of 1ml kg^{-1} p.o. for 28 days.

Group 3: Diabetic rats were administered with red lotus seeds extract at the rate of 600 mg kg^{-1} p.o. for 28 days.

Group 4: Diabetic rats were administered with white lotus seeds extract at the rate of 600 mg kg^{-1} body weight p.o. for 28 days.

Group5: Diabetic rats were administered with reference drug metformin at the rate of 200 mg kg^{-1} p.o. for 28 days.

Blood glucose levels were estimated on 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment. On 28^{th} day oral glucose tolerance test was carried out. Liver glycogen and GLUT 4 gene expression in skeletal muscle was carried out on 29^{th} day.

3. 3. 3. Pancreatic Beta Cells Proliferating Effect of Red and White Lotus Seeds Extract in Partial (70%) Pancreatectomy model

Thirty six Sprague - Dawley male rats were used for this study. They were divided into six groups of six each. Rats in group 2, 3, 4, 5 and 6 were anesthetized with thiopental sodium at the rate of 35 mg kg^{-1} i.p. The abdomen was opened through an upper midline incision. The spleen and the entire splenic portion of the pancreas were surgically removed keeping the mesenteric portion of pancreas intact. This resulted in ~70% pancreatectomy conformed by weighing the removed portion of pancreas during a pilot study. Sham operation was performed in rats of group one by removing the spleen while leaving the pancreas intact. The incision was closed using 5-0 silk sutures (Leon *et al.*, 2003). After one week rats having fasting (12 hours) blood glucose levels ranging from 130-150 mg dl^{-1} were used for the study.

3. 3. 3. 1. Treatment schedule

Group 1: Sham operated control- Rats were administered with vehicle alone at the rate of 1ml kg^{-1} p.o. for 28 days.

Group 2: Pancreatectomized rats were administered with vehicle alone at the rate of 1ml kg^{-1} p.o. for 28 days.

Group 3: Pancreatectomized rats were administered with red lotus seeds extract at the rate of 600 mg kg^{-1} p.o. for 28 days.

Group 4: Pancreatectomized rats were administered with white lotus seeds extract at the rate of 600 mg kg^{-1} p.o. for 28 days.

Group 5: Pancreatectomized rats were administered with reference drug metformin at the rate of 200 mg kg^{-1} body weight p.o. for 28 days.

Group 6: Pancreatectomized rats were administered with reference drug nicotinamide at the rate of 500 mg kg^{-1} i.p. for 28 days.

Blood glucose levels were estimated on 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day. Pancreas was collected for histopathological analysis on 28^{th} day after sacrificing the animals.

3. 3. 4. Hypocholesterolemic Effect of Red and White Lotus Seeds Extract in Hypercholesterolemic Model

Thirty Sprague - Dawley male rats were used for this study. They were divided into five groups of six each. Rats in group 2, 3, 4 and 5 were fed high cholesterol diet (standard diet supplemented with 1% cholesterol and 0.5% sodium cholate) for 7 days before starting the experiment and through out the experimental period. On 0 day (after 7 days high cholesterol diet feeding) total cholesterol was estimated. There was a significant increase in fasting total cholesterol levels ranging from 200-230 mg dl^{-1} (hypercholesterolemia) and were used for this study (Visavadiya and Narasimahacharya, 2005).

3. 3. 4. 1. Treatment schedule

Group 1: Normal control – Non hypercholesterolemic rats were administered with vehicle alone at the rate of 1ml kg^{-1} p.o. for 28 days.

Group 2: Hypercholesterolemic control – Hypercholesterolemic rats were administered with vehicle alone at the rate of 1ml kg^{-1} p.o. for 28 days.

Group 3: Hypercholesterolemic rats were administered with red lotus seeds extract at the rate of 600 mg kg^{-1} p.o. for 28 days.

Group 4: Hypercholesterolemic rats were administered with white lotus seeds extract at the rate of 600 mg kg^{-1} p.o. for 28 days.

Group5: Hypercholesterolemic rats were administered with reference drug simvastatin at the rate of 20 mg kg^{-1} p.o. for 28 days.

Total cholesterol and HDL levels were estimated on 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment. LDL levels were calculated from standard formula (Noda *et al.*, 2000). On 29^{th} day liver total lipid level was estimated.

3. 3. 5. Hypotriglyceridemic Effect of Red and White Lotus Seeds Extract in Hypertriglyceridemic Model

Thirty Sprague - Dawley male rats were used for this study. They were divided into five groups of six each. Rats in group 2, 3, 4 and 5 were given with high fat diet (Standard rat feed 44.85%, Casein 21.15%, Soya oil 31.5% and Mineral mixture 2.5%) for 21 days before starting the experiment and through out the experimental period. On 0 day (after 21 days of high fat diet feeding) triglyceride level was estimated and there was significant increase in fasting triglyceride levels ranging from 200-240 mg dl^{-1}. These hypertriglyceridemic rats were used for this study (Akiyama *et al.*, 1996).

3. 3. 5. 1. Treatment schedule

Group 1: Normal control – Non hypertriglyceridemic rats were administered with vehicle alone at the rate of 1ml kg^{-1} p.o. for 28 days.

Group 2: Hypertriglyceridemic control – Hypertriglyceridemic rats were administered with vehicle alone at the rate of 1ml kg^{-1} p.o. for 28 days.

Group 3: Hypertriglyceridemic rats were administered with red lotus seeds extract at the rate of 600 mg kg^{-1} p.o. for 28 days.

Group 4: Hypertriglyceridemic rats were administered with white lotus seeds extract at the rate of 600 mg kg^{-1} p.o. for 28 days.

Group 5: Hypertriglyceridemic rats were administered with reference drug fenofibrate at the rate of 30 mg kg^{-1} for p.o. 28 days.

Serum triglycerides and plasma free fatty acids were estimated on 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment. On 29^{th} day liver total lipid level was estimated.

3. 3. 6. Cytoprotective Effect of Red and White Lotus Seeds Extract on Pancreatic Beta Cells in Repeated Alloxan Induced Pancreatitis Model

One hundred and twenty Sprague - Dawley male rats were used for this study. They were divided into four large groups (A, B, C and D) of thirty animals each and each group was further divided into five small groups of six animals each. All the animals in each large group were sacrificed on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively. Rats were administered with alloxan monohydrate at the rate of 100 mg kg^{-1} i.p. (dissolved in 0.01 M citrate

buffer, pH 4.5) for first 5 days of 28 days experiment except normal control. For first 5 days animals were fasted for 12 hrs daily.

3. 3. 6. 1. Treatment schedule

Group 1: Normal control – Six animals from each large group served as normal control and were administered with normal saline 1ml kg^{-1} p.o. for 7, 14, 21 and 28 days respectively. They were sacrificed on respective days after dosing.

Group 2: Alloxan monohydrate treated control - Six animals from each large group were administered with alloxan monohydrate and subsequently normal saline 1ml kg^{-1} p.o. for 7, 14, 21 and 28 days respectively and they were sacrificed on respective days after dosing.

Group 3: Six animals from each large group were administered with alloxan monohydrate and subsequently red lotus seeds extract at the rate of 600 mg kg^{-1} p.o for 7, 14, 21 and 28 days respectively and they were sacrificed on respective days after dosing.

Group 4: Six animals from each large group were administered with alloxan monohydrate and subsequently white lotus seeds extract at the rate of 600 mg kg^{-1} p.o. for 7, 14, 21 and 28 days respectively and they were sacrificed on respective days after dosing.

Group 5: Six animals from each large group were administered with alloxan monohydrate and subsequently metformin at the rate of 200 mg kg^{-1} p.o. for 7, 14, 21 and 28 days respectively and they were sacrificed on respective days after dosing.

Every week 30 animals were sacrificed and pancreas was collected for estimation of superoxide dismutase, catalase, gutathione peroxidase and lipid peroxidation. Pancreas was collected for histopathological analysis after sacrificing the animals.

3. 4. COLLECTION OF BIOLOGICAL SAMPLES

3. 4. 1. Blood

Blood was collected from retro orbital plexus in the inner canthus of the eye under light ether anesthesia using sodium heparinised capillary tubes (microhaematocrit capillaries). Blood was collected in fresh test tubes containing disodium salt of ethylene diamine tetra acetic acid as anticoagulant (1mg ml^{-1}).

3. 4. 2. Serum

Blood was collected in fresh tubes without any anticoagulant and kept at 4^0C for half an hour. Then it was centrifuged at 3000 rpm for 20 minutes. The serum was pipetted into a tube and used freshly for estimation of total cholesterol, HDL and triglycerides.

3. 4. 3. Plasma

Blood was collected in fresh tubes with anticoagulant (disodium salts of EDTA, 1mg ml^{-1}) and kept at 4^0C for half an hour. Then it was centrifuged at 3000 rpm for 20 minutes. The plasma was pipetted out into a tube and used for the estimation free fatty acids.

3. 4. 4. Pancreas and Liver

The animals were euthanized by chloroform and dissected out. The pancreas was collected and used for the estimation of super oxide dismutase, catalase, glutathione peroxidase and lipid peroxidation. Glycogen and total lipid was estimated in liver tissue.

3. 4. 5. Kidney and skeletal muscle

The animals were euthanized by decapitation and dissected out. The kidney and skeletal muscle were collected and used freshly for GLUT 2 and GLUT 4 gene expression respectively.

3. 5. ESTIMATION OF BIOCHEMICAL PARAMETERS

3. 5. 1. Blood Glucose

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

3. 5. 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.

3. 5. 1. 2. Preparation of Reagents

Tungstic acid reagent

Dissolved one gram of polyvinyl alcohol in about 100 ml of distilled water with gentle warming. Cooled and transferred into a one litre volumetric flask containing 11.1 gm of sodium tungstate previously dissolved in about 100 ml distilled water and mixed by swirling. In a separate vessel, 2 ml of conc. H_2SO_4 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.

O-toluidine Reagent

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

Glucose standard

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

3. 5. 1. 3. Procedure

Protein free blood 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 stopper test tube and mixed well. The blank was prepared by adding 0.5 ml of distilled water instead of the deprotenised blood to 2.5 ml of O-toluidine reagent. The standard was set by adding 0.05 ml of the glucose standard to 0.45 ml of distilled water and 2.5 ml of O-toluidine reagent. Mixed well and placed all the loosely stoppered test tube 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 625nm in a spectrophotometer. The concentration of glucose was calculated by the following formula.

$$\text{Glucose concentration (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

3. 5. 2. Liver Glycogen

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

Reagents

1. Anthrone reagent.
2. 95 % ethanol.
3. 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 ml of the stock solution to a 100 ml volumetric flask and made up the volume with saturated benzoic acid solution. Two ml of this solution, containing 0.1 mg of glucose, were used as a standard.

3. 5. 2. 1. Procedure

1. Two gm of tissue sample was homogenized in a Teflon homogenizer with 5 ml of 5% trichloroacetic acid for 3 minutes.
2. The homogenate was filtered by using Whatman filter paper (No 40) and the filtrate was collected in a conical flask.
3. Transferred the residue quantitatively to the homogenizer and homogenized again with 5 ml of 5% trichloroacetic acid for 1 minute. Two more extractions were done in the same manner and all filtrate was pooled and mixed thoroughly.
4. One ml of the trichloroacetic acid filtrate was pipetted into a fresh test tube and added 5 ml of 95% ethanol.
5. The tubes were capped with clean rubber stoppers and placed in a water bath at 37^0C for 3 hours.

6. After precipitation is completed, the tubes were centrifuged at 3000 rpm for 15 minutes and the clear supernatant was gently decanted.
7. The tubes were placed in an inverted position for 10 minutes to allow complete drainage of ethanol from packed glycogen.
8. The glycogen was dissolved by the addition of 2 ml of distilled water, the water was added in a manner that will wash down the sides of the tube.
9. A reagent blank was prepared by pipetting 2 ml of water into a clean test tube. A standard was prepared by pipetting 2 ml of standard glucose solution.
10. Ten ml of anthrone reagent was added into each tube which were kept in a boiling water bath to a depth a little above the level of the liquid in the tubes for 15 minutes and then removed and cooled in a cold water bath to room temperature.
11. The resulting colour was read immediately at 620nm in a spectrophotometer. The concentration of glycogen was calculated using the following formula.

$$\text{Glycogen (mg/100 gm of tissue)} = \frac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} \times 0.1 \frac{\text{Volume of extract}}{\text{gm of tissue}} \times 90$$

3. 5. 3. GLUT 2 and GLUT 4 Gene Expressions

3. 5. 3. 1. Total RNA extraction

Total RNA extraction was done as per the method explained by Chomczynski and Sacchi (1987).

1. 100 mg of kidney tissue and skeletal muscle were homogenized for GLUT2 and GLUT4 RNA extractions respectively.
2. One ml of water saturated phenol followed by 200 µl of choloroform-isoamyl alcohol mix (freshly prepared in the ratio of 49:1) was added to the homogenizer containing 100 mg of tissue.

3. Mixed thoroughly and incubated in ice for 15 minutes.
4. It was centrifuged at 10000 rpm for 20 minutes at 4^0C.
5. The upper aqueous phase was transferred to another tube and precipitated the RNA by adding 1 ml of 100% isopropanol. It was incubated at -20^0C for 30 minutes.
6. Centrifuged at 10000 rpm for 20 minutes at 4^0C and the supernatant was discarded.
7. The pellet obtained was resuspended in 0.3 ml of denaturation solution and RNA was precipitated by adding 0.3 ml of 100% isopropanol and incubated at -20^0C for 30 minutes
8. It was centrifuged at 10000 rpm for 20 minutes at 4^0C and supernatant was discarded.
9. The RNA pellet was resuspended in 75% ethanol and incubated at room temperature for 15 minutes.
10. Again centrifuged at 10000 rpm for 20 minutes at 4^0C and supernatant was discarded.
11. RNA pellet in the test tube was dissolved in 200 µl of DEPC water and incubated for 15 minutes at 55^0C and later used for cDNA synthesis.

3. 5. 3. 2. cDNA synthesis

cDNA synthesis and RT-PCR was done as per the method described by Saiki *et al.* (1985).

1. Five µl of total RNA was added to sterile RNase free ependrof tube [Ependrof tubes were soaked with chloroform for 30 minutes and autoclaved (121^0C/15 lbs) for 15 minutes]. The volume was made upto 9 µl by adding 4 µl of sterile water.
2. One µl of Oligo $(dT)_{18}$ primer was added to diluted total RNA.
3. The vials were incubated at 65^0C for 10 minutes and at room temperature for 2 minutes.

4. Then the reagents were added in the following order

   1 µl RNase inhibitor

   1µl 0.1M DTT

   4 µl RT buffer (5x)

   2.0 µl 30 mM dNTP mix

   0.5 µl M-MuLV Reverse Transcriptase

   1.5 µl sterile water

6. The solution was mixed thoroughly and incubated at 37^0C for 1hour and 95^0C for 2 minutes.
7. Then it was placed in ice immediately.
8. One µl of 0.5M EDTA was added and extraction was done by using phenol- Choloroform mixture.

   1. 10 µl of Phenol – Chloroform - Isoamyl alcohol (25:24: 1) was mixed gently and centrifuged at 10000 rpm for 10 minutes.
   2. The upper aqueous phase was transferred to another tube and mixed with chloroform - isoamyl alcohol (24: 1). Mixed gently and centrifuged at 10000 rpm for 30 minutes. This step was repeated once again.
   3. The upper aqueous phase was collected and mixed with 0.6 volume of ice cold isopropanol. Mixed gently and centrifuged at 10000 rpm for 15 minutes.
   4. Supernatant was discarded and pellet was resuspended in TE buffer [100mM tris HCl (pH 8.0) and 0.01mM EDTA (pH 8.0)]
   5. Samples were stored at -20^0C.

3. 5. 4. Selection of Primer

Primers were designed with the help of Primer – 3 software.

Gene	Primer Sequence		Amplicon Size (bp)
GLUT 2	Forward primer	GACATCGGTGTGATCAATGC	292
	Reverse primer	TGATCCTTCCGAGTTTGTCC	
GLUT 4	Forward primer	AGCAGCTCTCAGGCATCAAT	296
	Reverse primer	CTCAAAGAAGGCCACAAAGC	
Beta actin	Forward primer	AGCCATGTACGTAGCCATCC	294
	Reverse primer	GCCATCTCTTGCTCGAAGTC	

3. 5. 4.1. Amplification of GLUT2, GLUT4 and Beta actin gene

Amplification of GLUT2, GLUT4 and beta actin genes (as an internal control) were carried out as per the method cited by Sambrook *et al.* (1989).

The reagents were added in the following order

1. 2 µl cDNA product
2. 5 µl 10x PCR buffer
3. 1 µl 30 mM dNTP mix
4. 1 µl Forward primer (100 ng/ µl)
5. 1 µl Reverse primer (100 ng/ µl)
6. 0.5 µl - (1 U Taq DNA polymerase)
7. 39.5 µl of RNAse free water

3. 5. 4. 2. Programme for PCR

Annealing temperature was standardized with a gradient PCR (PTC – 200 Thermal cycler MJ Research, Inc MA USA)

1. Initial denaturation at 94^0C for 2 minutes
2. Denaturation at 94^0C for 45 seconds
3. Annealing temperature at 52^0C for 30 seconds
4. Primer Extension at 72^0C for 1minutes
5. Step 2 to 4 repeated for 34 times
6. Final Extension 72^0C for 2 minutes
7. Hold at 4^0C

3. 5. 4.3. Electrophoresis of PCR Product (Agarose Gel Electrophoresis)

The agarose gels were prepared in the horizontal gel tray of the electrophoresis apparatus. A 1.5 % of agarose gel was prepared for good resolution of DNA fragments.

1.5 gram of agarose (Molecular Biology grade) was mixed with 100 ml of 1X TAE buffer and boiled in a microwave oven until the solution was clear. The solution was cooled to about 60°C, mixed with ethidium bromide (10 mg ml^{-1} in water) to a final concentration of 0.5 μg ml^{-1} and poured into the mould to a thickness of 3-5mm. The combs were placed in the slot avoiding any air bubbles and withdrawn after the gel was set and positioned for electrophoresis.

The gel was placed in a horizontal tank with one litre of 1X TAE buffer sufficient just to cover the gel. An aliquot of 10 μl of the PCR product was mixed with 5 μl of loading buffer (50 mM Tris, 5 mM EDTA, 50% glycerol, 0.1% Bromophenol Blue) and was loaded into the wells of the gel. Electrophoresis was done for 1 hour at 60 volts. The gel was placed on a transilluminator to view the products.

TAE buffer composition (50X)

Tris base - 48.4 gm

Glacial acetic acid - 11.42 ml

0.5 M EDTA (pH 8.0) – 20 ml

Make upto 1000 ml with distilled water.

3. 5. 4. Total cholesterol

Estimation of total cholesterol in serum was carried out according to the methods of Allian *et al.* (1974).

3. 5. 4. 1. Procedure

1. Ten µl serum was dispensed into serum tubes.
2. 1000 µl of reagent solution was dispensed into all the tubes containing serum and standard.
3. Mixed well and kept for 5 minutes at 37^0C.
4. After 5 minutes reading was taken at 505nm.
5. The concentration of total cholesterol was calculated using the following formula.

$$\text{Total cholesterol Conc (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

3. 5. 5. LDL cholesterol

LDL cholesterol level was calculated by the following formula suggested by Noda *et al.* (2000).

$$\text{LDL cholesterol} = \text{Total cholesterol} - \left[\text{HDL cholesterol} + \left(\text{Triglyceride}/5\right)\right]$$

3. 5. 6. HDL cholesterol

Estimation of HDL cholesterol in serum was carried out according to the methods of Bachorik *et al.* (1976).

3. 5. 6. 1. Procedure

1. 300 µl of serum was added to 300 µl of HDL precipitating reagent.
2. Mixed well and kept for 10 minutes at room temperature. Mixed again and centrifuged for 10 minutes at 4000 rpm.
3. 50 µl HDL supernatant was dispensed into a test tubes and in another tube 50 µl standard was dispensed.
4. 1000 µl of reagent solution was dispensed into all the tubes containing HDL supernatant and standard.
5. Mixed well and kept for 5 minutes at 37^0C.
6. After 5 minutes reading was taken at 505nm.
7. The concentration of HDL was calculated using the following formula.

$$\text{HDL cholesterol Conc (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 50 \times 2$$

2 = Dilution factor of the sample

3. 5. 7. Triglyceride

Estimation of triglycerides in serum was carried out according to the methods of Bucolo and David (1973).

3. 5. 7. 1. Procedure

1. Ten µl serum was dispensed into serum tubes.
2. 1000 µl of reagent solution was dispensed into all the tubes containing serum and standard.
3. Mixed well and kept for 5 minutes at 37^0C.
4. After 5 minutes reading was taken at 505nm.

5. The concentration of triglycerides was calculated using the following Formula:

$$\text{Triglyceride Conc (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

3. 5. 8. Liver Total Lipid

3. 5. 8. 1. Lipid extraction

Lipids were extracted from the tissues by the method of Folch *et al.* (1957) using chloroform - methanol mixture (2:1 v/v).

1. Two gram of liver tissue was homogenised in 7:0 ml of methanol using Teflon homogeniser. The contents were filtered with the help of Whatman filter paper (No 40). The content on the paper was scrapped off and homogenised with 14 ml of chloroform- methanol mixture.

2. This was again filtered into a flask and the residue was successively homogenised in chloroform – methanol (2:1 v/v) and each time this extract was filtered and it was repeated for two times. The pooled filtrate was evaporated to dryness.

3. The dried residue of lipid was dissolved in 5 ml of chloroform–methanol mixture (2:1 v/v) and transferred into a centrifuge tube, 2 ml of 0.1 M potassium chloride was added, shaken well and centrifuged at 3000 rpm for 10 minutes.

4. The upper aqueous layer containing gangliosides was discarded. The chloroform layer was mixed with 1.0 ml of chloroform–methanol–potassium chloride mixture (1:10:10 v/v) and then centrifuged again. This washing was repeated thrice and each time, the upper layer was discarded.

5. The lower layer was made up to 5.0 ml with chloroform–methanol mixture (2:1 v/v) and used for the analysis of liver total lipid.

Estimation of total lipid in liver extract was carried out according to the methods of Zoeliner (1962)

3. 5. 8. 2. Lipid estimation procedure

1. 2.5 ml of conc H_2SO_4 was dispensed into test tubes and 100 µl of extract was added.

2. It was shaken vigorously and kept in boiling water bath for 10min.

3. 50 µl conc H_2SO_4 digested extract was added to 1000 µl of working reagent and shaken vigorously.

4. Mixed well and kept for 15 minutes at 37^0C.

5. After 15 minutes reading was taken at 520nm.

6. The concentration of total lipid was calculated using the following formula.

$$\text{Total lipid (mg/100gm of wet tissue)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 750$$

3. 5. 9. Free Fatty Acid

Non-esterified free fatty acids in plasma were estimated by the method of Hron and Menahan (1981).

Reagents

1. Extraction solvent – Chloroform: Heptane: Methanol (200: 150: 7)
2. Activated silicic acid

3. Copper nitrate solution- 0.05 M
4. Triethanolamine – 0.1 M
5. Sodium hydroxide – 1 M
6. Copper – Triethanolamine reagent (Cu-TEA): Ten ml of copper nitrate solution was mixed with 10 ml of triethanolamine and 6 ml of sodium hydroxide and diluted to 100 ml, then 33 g of sodium chloride was added and the pH was adjusted to 8.1 with Conc Hcl.
7. Diphenyl carbazide solution - 0.5 M in methanol
8. Palmitic acid - 2 mM

3. 5. 9. 1. Procedure

1. Six ml of chloroform- heptane-methanol mixture (200:150: 7) was added into a screw cap test tube containing 50 µl of plasma.
2. 330 mg of activated silicic acid was added to the tubes and shaken 30 times by hand, allowed to stand at room temperature for 15 minutes and shaken again 6 times.
3. It was centrifuged at 3000 rpm for 15 minutes.
4. After centrifugation, the solvent containing the free fatty acid was decanted into another tube containing 2 ml of Cu-TEA solution.
5. The tubes were shaken vigorously on a mechanical shaker for 20 minutes and centrifuged at 3000 rpm for 15 minutes.
6. One ml of the upper organic phase was then pipetted into a clean test tube and 0.5 ml of diphenylcarbazide solution was added.
7. The resulting colour was read immediately at 550nm in a spectrophotometer.

$$\text{Free fatty acid (mM)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 2$$

3. 5. 10. Superoxide Dismutase

The levels of Superoxide dismutase in pancreatic tissues were estimated by the method of Mimami and Yoshikawa (1979).

Reagents

1. Tris cacodylic acid buffer (50 mM, pH8.2)

   Tris cacodylic acid 50 Mm

   Diethylene triamine penta acetic acid

   Nitroblue tetrazolium 0.1 M

   Triton X 100 - 0.001 percent.

   All reagents were mixed in equal quantities and the pH was adjusted to 8.2 using 0.1N sodium hydroxide.

2. Pyrogallol 0.2 mM

3. 5. 10. 1. Procedure

1. Freshly excised pancreas 250 mg was homogenized with 10 volumes of 0.9 percent sodium chloride followed by centrifugation at 400 rpm for 10 minutes at 4^0C to harvest the supernatant.
2. The assay mixture consists of 1.4 ml of 50 mM tris cacodylic acid buffer, 1.4 ml of 0.2 mM pyrogallol and 0.2 ml of enzyme preparation.
3. Blank contained distilled water instead of enzyme preparation.
4. The absorbance due to auto oxidation of pyrogallol was read at 420nm using spectrophotometer.
5. The values were expressed in units mg^{-1} of protein after quantifying the protein content of supernatant by method of Lowry *et al.* (1951).

3. 5. 11. Catalase

The activity of catalase in pancreatic tissue was determined by the method of Sinha (1972).

Reagents

1. Phosphate buffer: 0.01 M, pH 7.0
2. Hydrogen peroxide: 0.2 M
3. Dichromate-acetic acid: Potassium dichromate (5%) was mixed with glacial acetic acid in the ratio of 1:3. From this 1 ml was diluted again with 4 ml acetic acid.
5. Standard H_2O_2: 0.1 ml of 0.2 M H_2O_2 was diluted to 100 ml using distilled water.

3. 5. 11. 1. Procedure

1. To 0.9 ml of phosphate buffer 0.1 ml of tissue homogenate and 0.4 ml of hydrogen peroxide were added into a test tube.
2. After 60 seconds 2.0 ml of dichromate acetic acid mixture was also added. The tubes were kept in boiling water bath for 10 minutes and the colour developed was read at 620nm against blank.
3. The values were expressed in units mg^{-1} of protein after quantifying the protein content of supernatant by method of Lowry *et al.* (1951).

3. 5. 12. Glutathione Peroxidase

Glutathione peroxidase was estimated in pancreatic tissue by the method of Rotruck *et al.* (1973).

Reagents

1. Tris Buffer 0.4 M, pH 7.0
2. Sodium azide solution 10 mM
3. Trichloroacetic acid 10%
4. EDTA 0.4 mM
5. Hydrogen peroxide solution 20 mM

6. Glutathione solution 2 mM
7. Ellman's reagent: 19.8 mg of dithionitro bis benzoic acid in 100 ml of 1% sodium citrate solution.

3. 5. 12. 1. Procedure

1. To 0.2 ml of tris buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of tissue homogenate were added.
2. To this mixture, 0.2 ml of glutathione followed by 0.1 ml of hydrogen peroxide was added.
3. The contents were mixed well and incubated at 37°C for 10 minutes along with a tube containing all the reagents except sample.
4. After 10 minutes the reaction was arrested by the addition of 0.5 ml of 10% TCA and then centrifuged.
5. One ml of the supernatant was taken and to this 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer was added.
6. The yellow colour developed was read at 412nm. The values were expressed in units mg^{-1} of protein after quantifying the protein content of supernatent by method of Lowry *et al.* (1951).

3. 5. 13. Thiobarbituric acid reactive substances (TBARS)

The levels of lipid peroxidation in pancreatic tissues were estimated by the method of Fraga *et al.* (1988).

Reagents

1. Tricholoroacetic acid (TCA) - 15%
2. Hydrochloric acid (HCl) - 0.25 N
3. Thiobarbituric acid (TBA) - 0.38% in hot water
4. TCA: TBA: HCl reagent –Solution 1, 2 and 3 were mixed freshly in ratio of 1: 1: 1.

5. Stock standard - 4.8 mM: 0.079 ml of 1, 1, 3, 3 tetra methoxy propane was diluted to 100 ml distilled water.

3. 5. 13. 1. Procedure

1. Two hundred milligrams of tissue was homogenized in 9 ml of Tris–HCl buffer (pH 7.5), 1.0 ml of the tissue homogenate was treated with 2.0 ml of TBA-TCA-HCl reagent and mixed thoroughly.
2. The mixture was kept in a boiling water bath for 15 minutes.
3. After cooling, the tubes were centrifuged at 3000 rpm for 10 minutes and the supernatant was taken for measurement.
4. The absorbance of chromophore was read at 535nm against the reagent blank.

Values were expressed as **mM/100 g wet tissue**

3. 6. HISTOPATHOLOGICAL EXAMINATION OF PANCREAS

3. 6. 1. Histopathological Examination

Representative samples of pancreas obtained from the dissected animals were fixed in 10 percent neutral buffered formalin. They were then processed and paraffin embedded sections were taken as described by Sheehan and Hrapchak, 1980. The sections were stained with Haematoxylin and Eosin as per the technique followed by Bancroft and Cook, 1984. The slides were also stained with special stain (Aldehyde fuchsin and Phloxine B - Gomori method) to demonstrate alpha and beta pancreatic cells (Sheehan and Hrapchak, 1980). The sections were examined in detail under light microscope and lesions were classified.

3. 7. PHYTOCHEMICAL SCREENING

The ethanolic extracts of seeds from red and white variety of lotus were tested for the presence of various active principles namely steroids,

alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure quoted by Harborne (1991).

3. 7. 1. Tests for Detection of Steroids

3. 7. 1. 1. Salkowski test

About 5 mg of the extract was mixed with 3 ml of chloroform and then shaken with about 3 ml concentrated sulphuric acid. Development of red colour indicates the presence of steroids.

3. 7. 1. 2. Liberman Burchardt test

About 5 mg of the extract was mixed with 3 ml of chloroform. Then five drops of acetic anhydride and 1 ml of concentrated sulphuric acid were added to it through the sides. Development of a reddish ring at the junction of two layers indicates the presence of steroids.

3. 7. 2. Tests for Detection of Alkaloids

About 5 mg of the extract was mixed with 5 ml of ammonia and then extracted with equal volume of chloroform. To this, 5 ml of diluted hydrochloric acid was added. The acid layer obtained was used for chemical tests for the alkaloids.

3. 7. 2. 1. Mayer's test

To 1 ml of acid extract, few drops of Mayer's reagent (1.358 g of mercuric chloride dissolved in 60 ml of water and poured into a solution of 5 g of Potassium iodide in 10 ml of water and then made up the volume to 100 ml with distilled water) was added. Development of a creamy white precipitate indicates the presence of alkaloids.

3. 7. 2. 2. Wagner's test

Few drops of Wagner's reagent (2 g of iodine and 6 g of potassium iodide dissolved in 100 ml of water) were added to 1 ml of the acid extract.

Development of reddish brown precipitate indicates the presence of alkaloids.

3. 7. 2. 3. Hager's test

To 1 ml of the acid extract, few drops of Hager's reagent (1 g of picric acid dissolved in 100 ml of water) were mixed. Development of yellow precipitate indicates the presence of alkaloids.

3. 7. 2. 4. Dragendroff's test

Few drops of Dragendroff's reagent [Stock solution (1) 0.6 grams of bismuth sub nitrate was dissolved in 2 ml of concentrated hydrochloric acid and 10 ml of water was added. Stock solution (2) six grams of potassium iodide was dissolved in 10 ml of water. Then both the stock solutions (1) and (2) were mixed together and then it was mixed with 7 ml of concentrated hydrochloric acid and 15 ml of water. Sufficient amount of distilled water was added to the mixture to make up the volume to 100 ml] was mixed with 1 ml of acid extract. Development of a reddish brown precipitate indicates the presence of alkaloids.

3. 7. 3. Test for Detection of Phenolic compounds

About 5 mg of the extract was mixed with 1 ml of water, five drops of ten per cent ferric chloride was added to it. Development of dark blue colour indicates the presence of phenolic compounds.

3. 7. 4. Tests for Detection of Tannins

3. 7. 4. 1. Ferric chloride test

Two milligram of the extract was mixed with 3 ml of one per cent ferric chloride solution. Development of a blue, green or brownish colour indicates the presence of tannins.

3. 7. 4. 2. Gelatin test

About 0.5 g of the extract was mixed with few drops of one per cent solution of gelatin containing ten per cent sodium chloride. Development of a white precipitate indicates the presence of tannins.

3.7. 5. Tests for Detection of Flavonoids

3. 7. 5. 1. Ferric chloride test

To 2 ml of alcoholic solution of the extract (0.5 g extract in 10 ml methanol), few drops of neutral ferric chloride solution was mixed. Development of green colour indicates the presence of flavonoids.

3. 7. 5. 2. Lead acetate test

To 2 ml of alcoholic solution of the extract (0.5 g extract in 10 ml methanol), few drops of neutral ten per cent lead acetate was mixed. Development of a yellow precipitate indicates the presence of flavonoids.

3. 7. 6. Tests for Detection of Glycosides

3. 7. 6. 1. Sodium hydroxide test

A small amount of the extract (about 5 mg) was mixed with 1 ml of water and added 5-6 drops of sodium hydroxide solution (10 %). Development of a yellow colour indicates the presence of glycosides.

3. 7. 6. 2. Benedict's test

To about 1 ml of the extract (0.5 g extract in 1 ml of water), 5 ml of Benedict's reagent was added. The mixture was boiled for two minutes. Development of brown to red colour indicates the presence of glycosides.

3. 7. 7. Test for Detection of Diterpenes

About 5 mg of the extract was mixed with 3 ml of copper acetate solution (5 %). Development of green colour indicates the presence of diterpenes.

3. 7. 8. Tests for Detection of Triterpenes

3. 7. 8. 1. Salkowski test

About 5 mg of the extract was mixed 3 ml of chloroform and then it was shaken with 3 ml of concentrated sulphuric acid. Development of yellow colour in lower layer on standing indicates the presence of triterpenes.

3. 7. 8. 2. Lieberman Burchardt test

Few drops of acetic acid and 1 ml concentrated sulphuric acid were added to 3 ml of chloroform solution of the extract (about 3 mg extract in 3 ml chloroform). Development of deep red ring at the junction of two layers indicates the presence of triterpenes.

3. 7. 9. Test for Detection of Saponins

3.7. 9. 1. Foam test

A small amount of the extract (about 5 mg) was shaken with 3 ml of water. Development of the foam that persists for ten minutes indicates the presence of saponins.

3. 8. STATISTICAL ANALYSIS

The data obtained were subjected to statistical analysis using one way ANOVA followed by Dunnet comparison tests descried by Snedecor and Cochran (1985).

Results

4. RESULTS

The present study was undertaken to evaluate the hypoglycemic, hypolipidaemic and cytoprotective effects of red and white lotus seeds extract in male Sprague-Dawley rats. The results obtained were statistically analysed and presented in Tables 1 to 37 and Figures 1 to 24.

4.1. HYPOGLYCEMIC EFFECT OF RED AND WHITE LOTUS SEEDS EXTRACT IN INSULIN DEPENDENT (TYPE I) AND NON INSULIN DEPENDENT (TYPE II) DIABETIC ANIMAL MODELS

The effect of ethanolic extract of red and white lotus seeds on blood glucose levels in alloxan induced type I diabetes are expressed in Table 1 and Figure 3.

In type I diabetes the rats treated with ethanolic extract of red lotus seeds at the dose rate of 600 mg kg^{-1} reduced blood glucose levels by 7.83, 14.63, 17.44 and 22.90 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 2). On the other hand white lotus seeds at the same dose rate reduced the blood glucose levels by 9.90, 16.23, 22.23 and 26.60 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 2). It was significant ($P < 0.01$) from 14^{th} day onwards (Table 1).

The effect of lotus seeds on liver glycogen is shown in Table 3 and Figure 4. Red and white lotus seeds extract showed significant ($P < 0.05$ and $P < 0.01$) increase in liver glycogen levels by 25.25 and 32.07 % respectively (Table 6). The mean values of GLUT2 gene expression and oral glucose tolerance is showed in Table 4, Figure 5 and Table 5, Figure 6 respectively. The GLUT 2 gene expression in kidney was significantly ($P < 0.01$) reduced by both red and white lotus seeds extract by 25.90 and 34.16 % respectively (Table 6). Oral glucose tolerance was significantly

($P < 0.01$) improved by 24.64 and 25.78 % by red and white lotus seeds extract respectively (Table 6).

Metformin treated group at the dose rate of 200 mg kg^{-1} reduced blood glucose levels by 15.15, 23.75, 26.68 and 31.22 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 2). It was significant on 7 ($P<0.05$), 14, 21 and 28 days ($P < 0.01$) treatment (Table 1). The mean value of liver glycogen and GLUT2 gene expression is shown in Table 3, Figure 4 and Table 4, Figure 5 respectively. Significant ($P < 0.01$) increase in liver glycogen and decrease ($P<0.01$) in GLUT2 gene expression in kidney by 49.30 and 44.34 % (Table 6) respectively was noticed after 28 days treatment. The mean value of glucose tolerance is shown in Table 5, and Figure 6. Metformin significantly ($P < 0.01$) improved glucose tolerance also by 27.19 % (Table 6).

The effect of ethanolic extract of red and white lotus seeds on blood glucose levels in alloxan induced type II diabetes are expressed in Table 7 and Figure 7.

In type II diabetes the rats treated with ethanolic extract of red lotus seeds at the dose rate of 600 mg kg^{-1} reduced blood glucose levels by 5.66, 9.93, 16.06 and 22.69 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 8). It was significant from 21($P < 0.01$) days treatment (Table 7). On the other hand white lotus seeds at the same dose rate reduced blood glucose levels by 8.09, 11.07, 19.06 and 26.85 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 8). It was significant ($P < 0.01$) from 21^{st} day onwards (Table 7).

The effect of lotus seeds on liver glycogen is shown in Table 9 and Figure 8. Red and white lotus seeds extract also showed significant ($P < 0.05$ and $P < 0.01$) increase in liver glycogen levels by 22.11 and 33.27 % respectively (Table 12). The mean values of GLUT4 gene expression and oral glucose tolerance is showed in

Table 10, Figure 9 and Table 11, Figure 10 respectively. The GLUT4 gene expression in skeletal muscle was significantly ($P < 0.01$) increased by both red and white lotus seeds extract by 19.37 and 40.04 % respectively (Table 12). Oral glucose tolerance was significantly ($P < 0.01$) improved by 24.76 and 25.82 % by red and white lotus seeds extracts respectively (Table 12).

Metformin treated group at the dose rate of 200 mg kg^{-1} showed reduction in blood glucose levels by 14.08, 17.58, 24.75 and 34.33 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 8). It was significant on 7 ($P<0.05$), 14, 21 and 28 days ($P < 0.01$) treatment (Table 7). The mean value of liver glycogen and GLUT4 gene expression is showed in Table 9, Figure 8 and Table 10, Figure 9 respectively. Significant ($P < 0.01$) increase in liver glycogen and GLUT4 gene expression in skeletal muscle by 63.49 and 61.25 % (Table 12) respectively was noted. The mean value of glucose tolerance is shown in Table 11 and Figure 10. Metformin also significantly ($P < 0.01$) improved glucose tolerance by 27.81 % (Table 12).

4.2. PANCREATIC BETA CELLS PROLIFERATION EFFECT OF RED AND WHITE LOTUS SEEDS EXTRACT IN PANCREATECTOMIZED ANIMAL MODEL

The effect of ethanolic extract of red and white lotus seeds on blood glucose levels in pancreatectomized rats are presented in Table 13 and Figure 11.

Pancreatectomized rats treated with ethanolic extract of red lotus seeds at the dose rate of 600 mg kg^{-1} reduced blood glucose levels by 0.06, 0.94, 8.91 and 13.33 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 14). It was significant on 21($P < 0.05$) and 28 ($P < 0.01$) days treatment (Table 13).

Table 1. Effect of ethanolic extract of red and white lotus seeds on blood glucose levels in type I diabetic rats, mg/dl

		0 day	7th day	14th day	21st day	28th day
Normal control	Mean	98.48	97.22	96.42	99.16	99.60
	S.E	4.89	3.70	3.12	2.43	3.17
Diabetic control	Mean	240.81	244.66	248.60	246.40	246.82
	S.E	9.40	8.97	7.52	8.35	8.16
Red lotus seed extract 600 mg/kg	Mean	241.95	225.49	212.23**	203.43**	190.28**
	S.E	9.93	9.16	9.28	9.51	9.78
White lotus seed extract 600 mg/kg	Mean	246.14	220.42	208.26**	191.62**	181.16**
	S.E	9.24	9.87	8.63	6.48	7.76
Metformin 200 mg/kg	Mean	247.15	207.60*	189.56**	180.65**	169.77**
	S.E	10.05	9.51	7.29	7.29	7.51

** P <0.01 , *P <0.05 Compared to diabetic control

Table 2. Effect of ethanolic extract of red and white lotus seeds on blood glucose levels in type I diabetic rats, percentage reduction

Day	Red lotus seed extract 600 mg/kg	White lotus seed extract 600 mg/kg	Metformin 200 mg/kg
7th	7.83	9.90	15.15
14th	14.63	16.23	23.75
21st	17.44	22.23	26.68
28th	22.90	26.60	31.22

Table 3. Effect of ethanolic extract of red and white lotus seeds on liver glycogen levels in type I diabetic rats, mg/100gm tissue

	Normal control	Diabetic control	Red lotus seed extract 600 mg/kg	White lotus seed extract 600 mg/kg	Metformin 200 mg/kg
Mean	54.44	30.42	38.10*	40.18**	45.42**
S.E	2.90	1.52	1.32	1.87	2.11

** P <0.01 , *P <0.05 Compared to diabetic control

Table 4. Effect of ethanolic extract of red and white lotus seeds on GLUT2 in type I diabetic rats, band density (AU)

	Normal control	Diabetic control	Red lotus seed extract 600 mg/kg	White lotus seed extract 600 mg/kg	Metformin 200 mg/kg
Mean	55.07	84.38	62.53**	55.55**	46.97**
S.E	1.49	2.02	1.08	1.66	1.89

** P <0.01 Compared to diabetic control

GLUT - Glucose transporter

Table 5. Effect of ethanolic extract of red and white lotus seeds on oral glucose tolerence test in type I diabetic rats, AUC (0 - 120 min), mg/dl.min

	Normal control	Diabetic control	Red lotus seed extract 600 mg/kg	White lotus seed extract 600 mg/kg	Metformin 200 mg/kg
Mean	19402.67	36015.33	27142.16**	26732.16**	26223.00**
S.E	273.71	110.20	637.62	550.44	195.71

** P <0.01 , Compared to diabetic control

AUC - Area Under Curve, min-minutes

Table 6. Effect of ethanolic extract of red and white lotus seeds on liver glycogen, glucose tolerence and GLUT 2 levels in type I diabetic rats, in percentage

	Red lotus seed extract 600 mg/kg	White lotus seed extract 600 mg/kg	Metformin 200 mg/kg
Liver glycogen (increase)	25.25	32.07	49.30
Glucose tolerence (increase)	24.64	25.78	27.19
GLUT 2 (reduction)	25.90	34.16	44.34

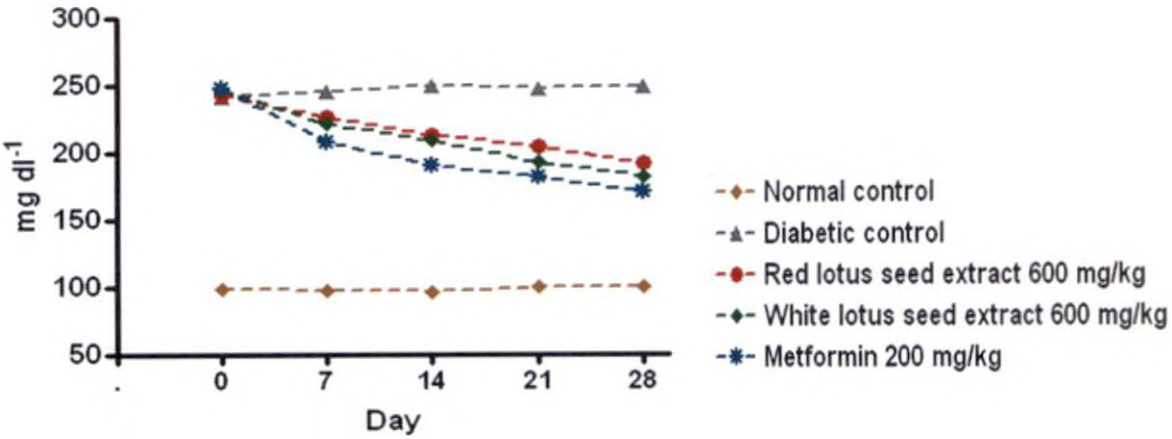
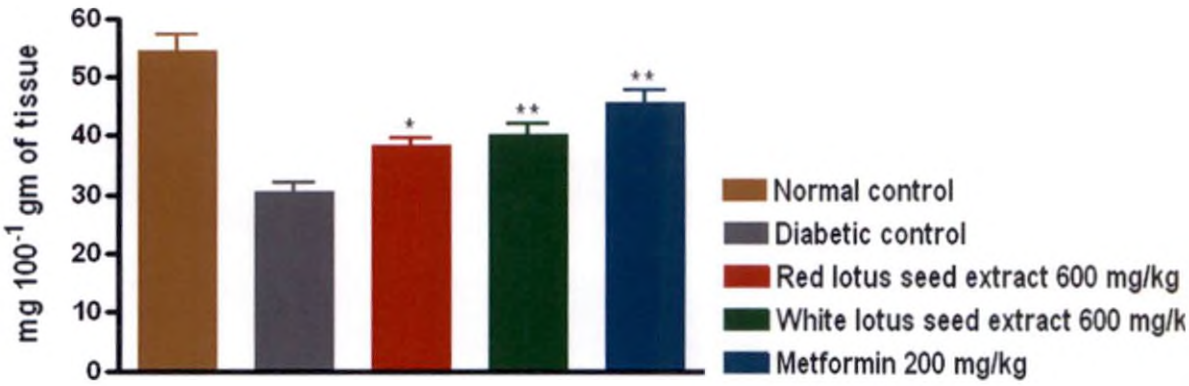


Fig. 3. Effect of ethanolic extract of red and white lotus seeds on blood glucose levels in type I diabetic rats

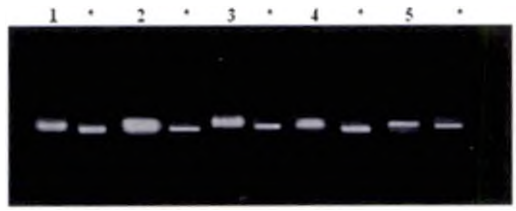


*P<0.05, **P<0.01 compared to diabetic control

Fig. 4. Effect of ethanolic extract of red and white lotus seeds on liver glycogen levels in type I diabetic rats

Fig. 5. Effect of ethanolic extract of red and white lotus seeds on GLUT 2 gene expression in type I diabetic rats

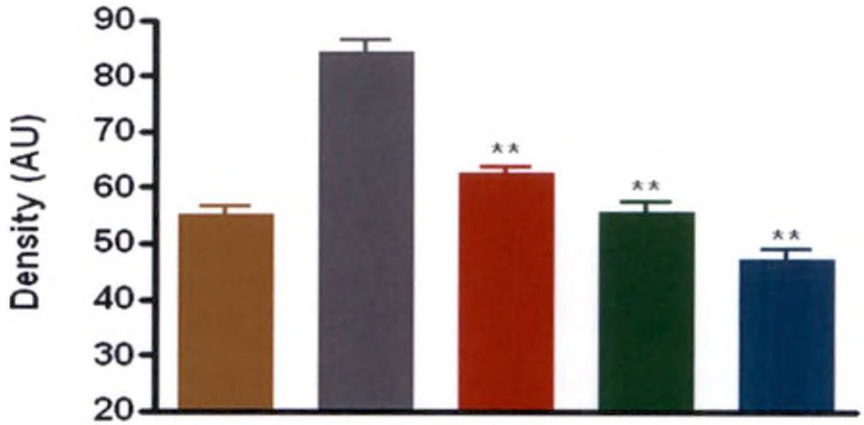
A



1. Normal control
2. Diabetic control
3. Red lotus seed extract 600 mg/kg
4. White lotus seed extract 600 mg/kg
5. Metformin 200 mg/kg

*. Beta actin

B



**P<0.01 compared to diabetic control

Normal control
Diabetic control
Red lotus seed extract 600 mg/kg
White lotus seed extract 600 mg/kg
Metformin 200 mg/kg

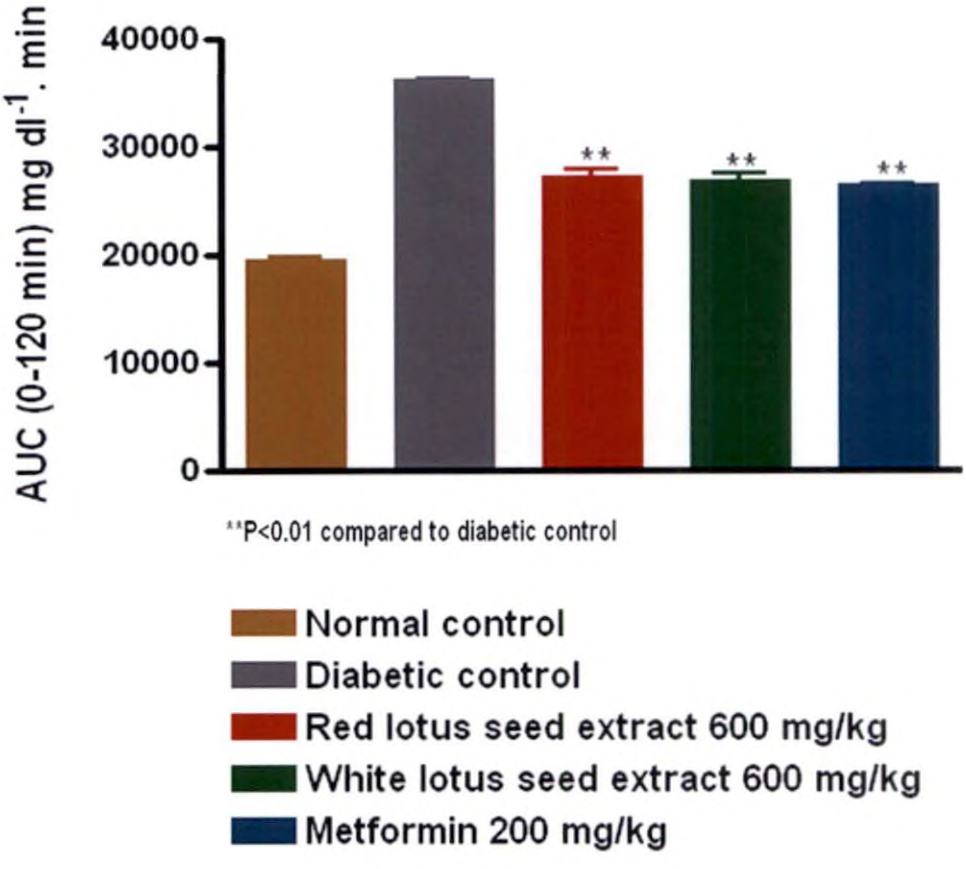


Fig. 6. Effect of ethanolic extract of red and white lotus seeds on OGTT in type I diabetic rats

Table 7. Effect of ethanolic extract of red and white lotus seeds on blood glucose levels in type II diabetic rats, mg/dl

		0 day	7th day	14th day	21st day	28th day
Normal control	Mean	99.47	101.14	100.09	100.00	98.23
	S.E	2.29	2.56	2.58	3.20	2.01
Diabetic control	Mean	262.37	263.51	260.29	262.71	259.79
	S.E	8.18	7.77	7.94	7.79	8.16
Red lotus seed extract 600 mg/kg	Mean	264.78	248.60	234.44	220.53**	200.84**
	S.E	10.82	11.98	12.35	14.50	13.44
White lotus seed extract 600 mg/kg	Mean	265.64	242.19	231.46	212.63**	190.03**
	S.E	8.07	6.32	7.07	7.14	7.73
Metformin 200 mg/kg	Mean	261.94	226.40*	214.54**	197.68**	170.60**
	S.E	6.75	6.87	7.50	7.42	5.01

** P <0.01 , *P <0.05 Compared to diabetic control

Table 8. Effect of ethanolic extract of red and white lotus seeds on blood glucose levels in type II diabetic rats, percentage reduction

Day	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Metformin 200 mg/kg
7th	5.66	8.09	14.08
14th	9.93	11.07	17.58
21st	16.06	19.06	24.75
28th	22.69	26.85	34.33

Table 9. Effect of ethanolic extract of red and white lotus seeds on liver glycogen levels in type II diabetic rats, mg/100g tissue

	Normal control	Diabetic control	Red lotus seed extract 600 mg/kg	White lotus seed extract 600 mg/kg	Metformin 200 mg/kg
Mean	58.21	28.44	34.73*	37.90**	46.50**
S.E	2.11	1.15	1.24	1.82	1.13

** P <0.01 , *P <0.05 Compared to diabetic control

Table 10. Effect of ethanolic extract of red and white lotus seeds on GLUT4 in type II diabetic rats, band density (AU)

	Normal control	Diabetic control	Red lotus seed extract 600 mg/kg	White lotus seed extract 600 mg/kg	Metformin 200 mg/kg
Mean	84.60	72.90	87.03**	102.09**	117.56**
S.E	1.74	1.33	0.92	0.95	1.25

** P <0.01 Compared to diabetic control

GLUT - Glucose transporter

Table 11. Effect of ethanolic extract of red and white lotus seeds on oral glucose tolerence test in type II diabetic rats, AUC (0 - 120 min), mg/kg.min

	Normal control	Diabetic control	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Metformin 200 mg/kg
Mean	19402.50	36256.17	27278.00**	26893.50**	26174.50**
S.E	251.08	165.19	603.93	534.41	228.64

** P <0.01 , Compared to diabetic control

AUC - Area Under Curve, min-minutes

Table 12. Effect of ethanolic extract of red and white lotus seeds on liver glycogen, glucose tolerence and GLUT 4 levels in type II diabetic rats, percentage increase

	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Metformin 200 mg/kg
Liver glycogen	22.11	33.27	63.49
Glucose tolerence	24.76	25.82	27.81
GLUT 4	19.37	40.04	61.25

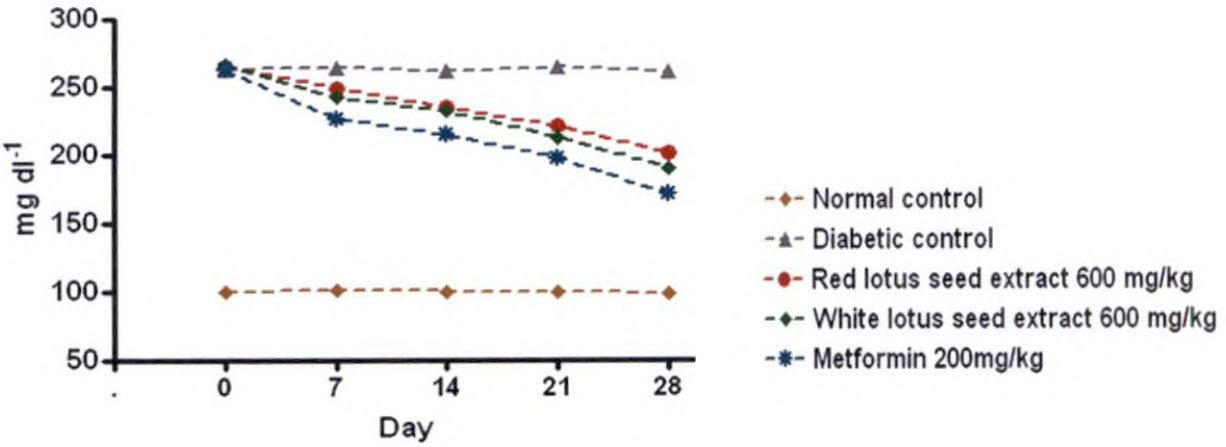
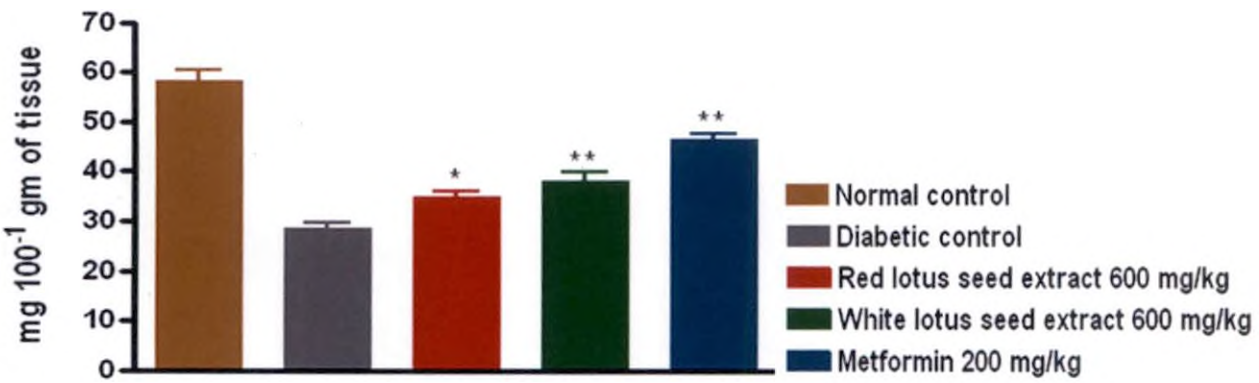


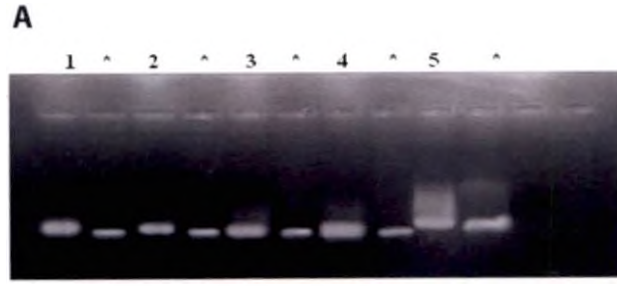
Fig. 7. Effect of ethanolic extract of red and white lotus seeds on blood glucose levels in type II diabetic rats



*P<0.05, **P<0.01 compared to diabetic control

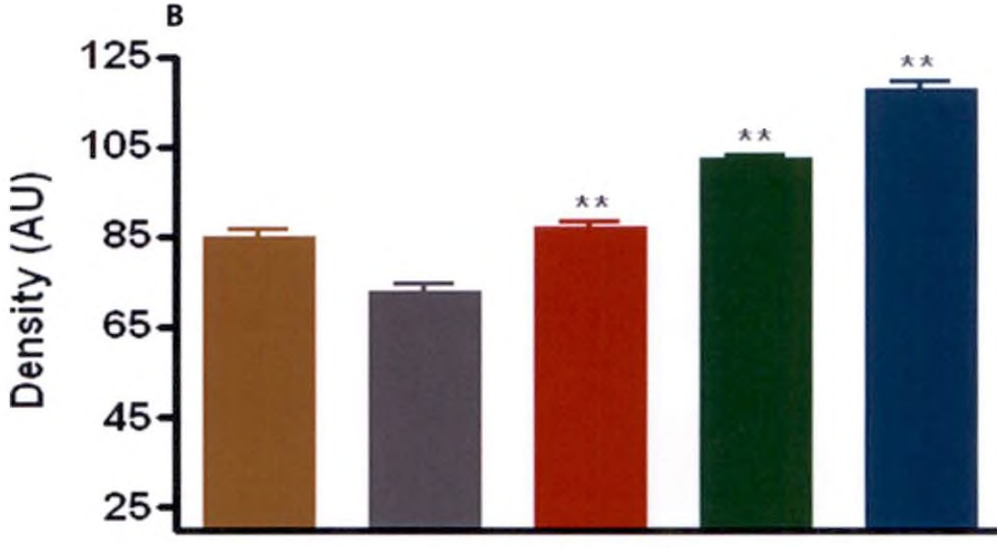
Fig. 8. Effect of ethanolic extract of red and white lotus seeds on liver glycogen levels in type II diabetic rats

Fig. 9. Effect of ethanolic extract of red and white lotus seeds on GLUT 4 gene expression in type II diabetic rats



1. Normal control
2. Diabetic control
3. Red lotus seed extract 600 mg/kg
4. White lotus seed extract 600 mg/kg
5. Metformin 200 mg/kg

*. Beta actin



**P<0.01 compared to diabetic control

Normal control
Diabetic control
Red lotus seed extract 600 mg/kg
White lotus seed extract 600 mg/kg
Metformin 200 mg/kg

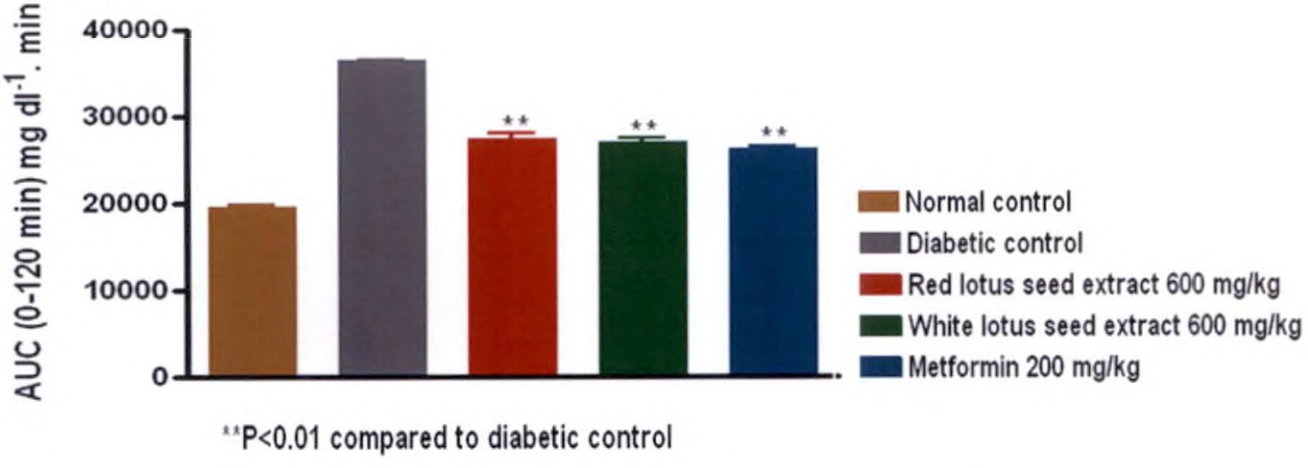


Fig. 10. Effect of ethanolic extract of red and white lotus seeds on OGTT in type II diabetic rats

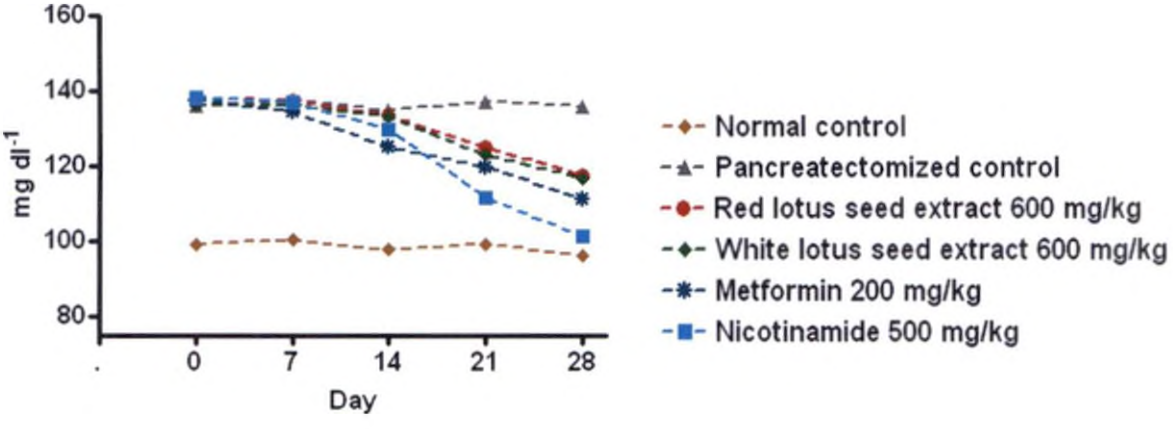


Fig. 11. Effect of ethanolic extract of red and white lotus seeds on blood glucose levels in pancreatectomized rats

Table 13. Effect of ethanolic extract of red and white lotus seeds on blood glucose levels in pancreatectomized rats, mg/dl

		0 day	7th day	14th day	21st day	28th day
SHAM control	Mean	99.17	100.26	97.73	99.21	96.05
	S.E	3.77	3.06	2.42	1.65	1.50
pancreatectomized control	Mean	136.08	137.52	134.68	137.09	135.65
	S.E	2.10	3.03	2.33	1.16	1.88
Red lotus seed extract 600 mg/kg	Mean	137.75	137.44	133.42	124.87*	117.57**
	S.E	3.06	4.01	3.92	2.91	3.08
White lotus seed extract 600 mg/kg	Mean	136.17	136.07	133.08	122.86**	116.67**
	S.E	1.58	1.28	1.37	2.52	1.18
Metformin 200 mg/kg	Mean	137.50	134.36	124.92	119.63**	111.00**
	S.E	2.49	3.34	2.99	3.09	2.83
Nicotinamide 500 mg/kg	Mean	138.08	136.92	129.80	111.43**	101.20**
	S.E	2.80	3.54	3.70	3.77	5.04

** P <0.01 , *P <0.05 Compared to diabetic control

Table 14. Effect of ethanolic extract of red and white lotus seeds on blood glucose levels in pancreatectomized rats, percentage reduction

Day	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Metformin 200 mg/kg	Nicotinamide 500 mg/kg
7th	0.06	1.06	2.30	0.44
14th	0.94	1.19	7.25	3.63
21st	8.91	10.38	12.73	18.71
28th	13.33	14.00	18.18	25.40

On the other hand white lotus seeds at the same dose rate reduced blood glucose levels by 1.06, 1.19, 10.38 and 14.00 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table. 14). It was significant from 21^{st} ($P < 0.01$) day onwards (Table. 13). Metformin treated group at the dose rate of 200 mg kg^{-1} showed reduction in blood glucose levels by 2.30, 7.25, 12.73 and 18.18 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 13). The Nicotinamide treated group at the dose rate of 500 mg kg^{-1} reduced blood glucose levels by 0.44, 3.63, 18.71 and 25.40 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table. 14). It was significant ($P < 0.01$) from 21^{st} day onwards (Table 13).

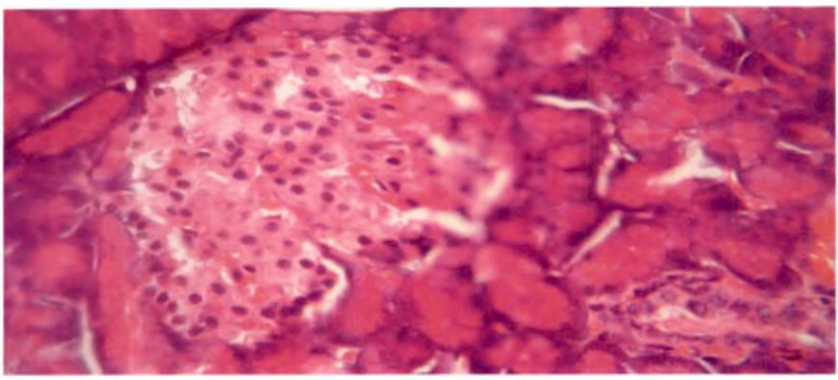
Histopathological examination of pancreas revealed that there was significant proliferation of beta cells and increasing number of active cells, in rats treated with extract, Metformin and Nicotinamide (Figure 12, A- L).

4.3 HYPOCHOLESTEROLEMIC EFFECT OF RED AND WHITE LOTUS SEEDS EXTRACT IN HYPERCHOLESTEROLEMIC ANIMAL MODEL

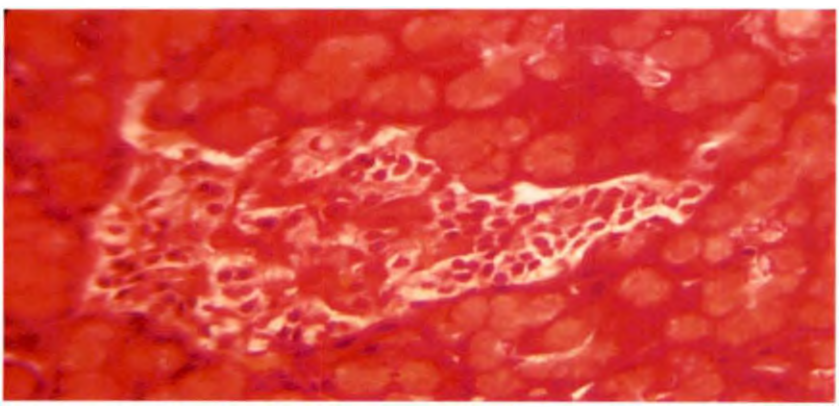
The effect of ethanolic extract of red and white lotus seeds on total cholesterol (Table. 15 and Figure. 13), LDL-cholesterol (Table 17 and Figure 14) and HDL-cholesterol (Table. 19 and Figure 15) in high cholesterol diet induced hypercholesterolemic rats are presented in respective tables and figures.

Hypercholesterolemic rats treated with ethanolic extract of red lotus seeds at the dose rate of 600 mg kg^{-1} reduced serum total cholesterol levels by 4.08, 19.01, 27.01 and 32.96 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table. 16). On the other hand white lotus seeds at the same dose rate reduced serum total cholesterol levels by 6.42, 22.03, 28.13 and 35.95 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table. 16). It was significant ($P < 0.01$) from 14^{th} day onwards (Table. 15).

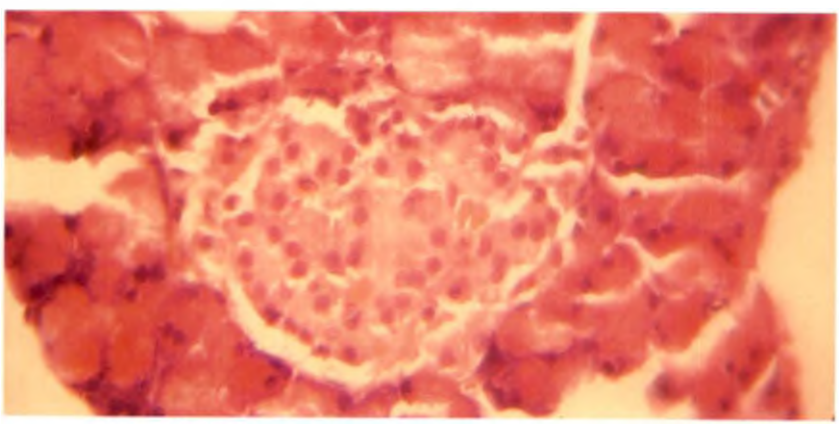
Fig.12. Effect of ethanolic extract of red and white lotus seeds on histopathological changes in pancreas of pancreatectomized rats (H&E and Aldehyde fuchsin and Phloxine B, A-J.L : 160 x , K-400x)



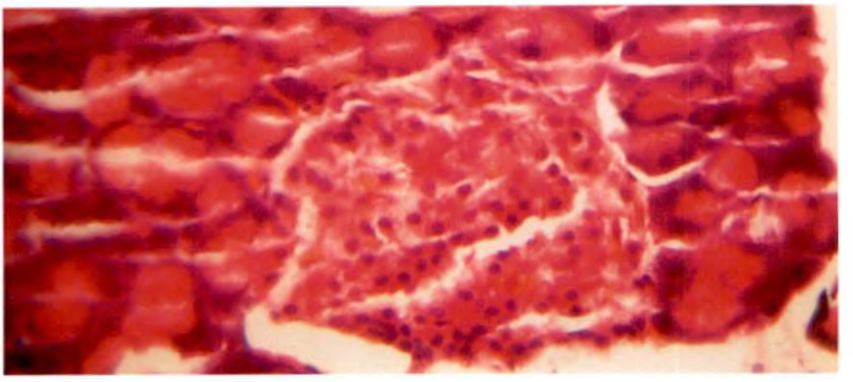
Normal control (Vehicle 1ml/kg)



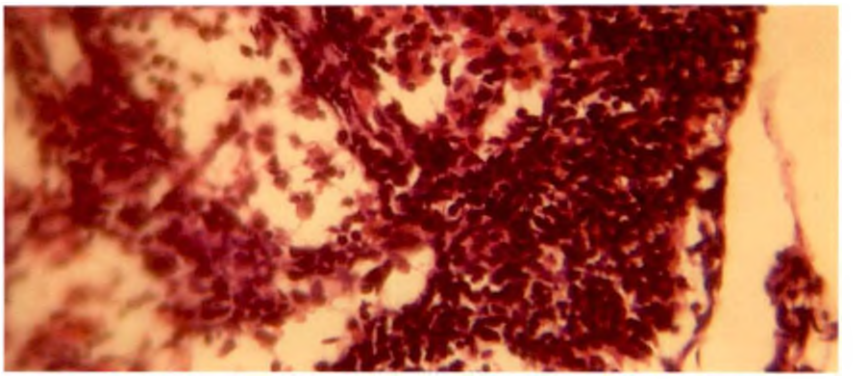
Pancreatectomized control (Vehicle 1ml/kg)



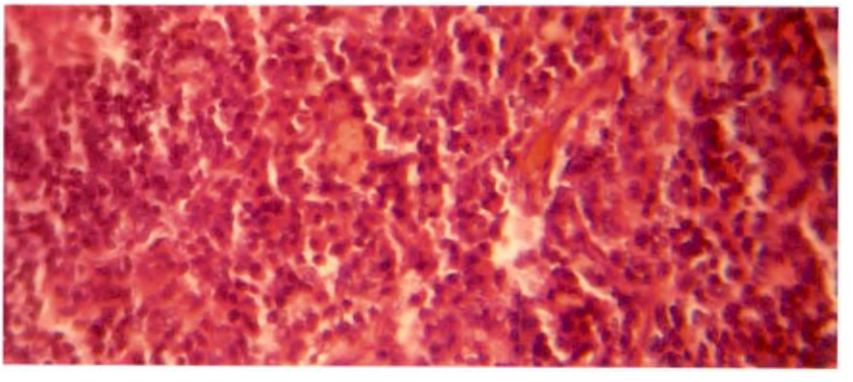
Red lotus seed extract 600 mg/kg for 28 days



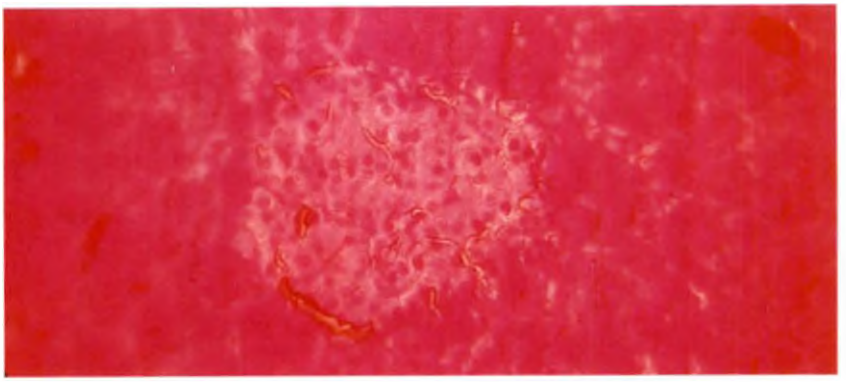
White lotus seed extract 600 mg/kg for 28 days



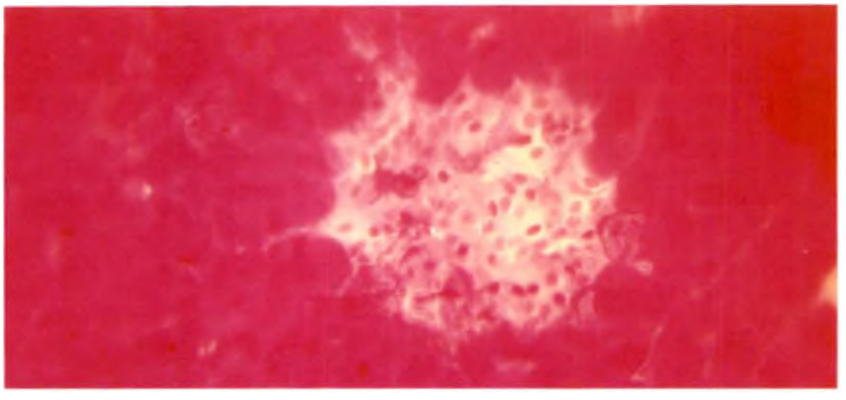
Metformin 200 mg/kg for 28 days



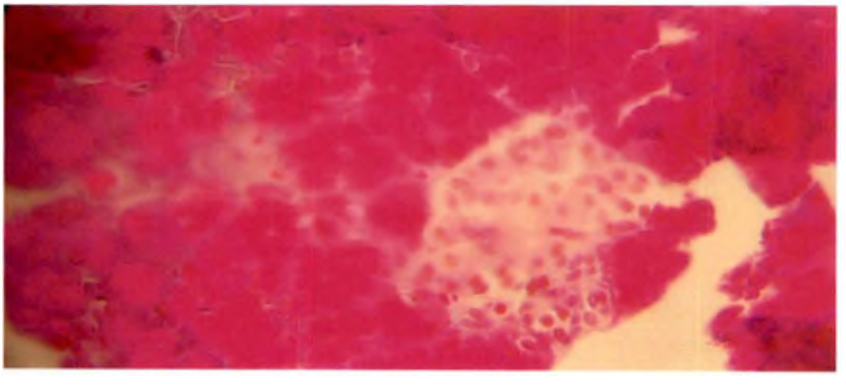
Nicotinamide 500 mg/kg for 28 days



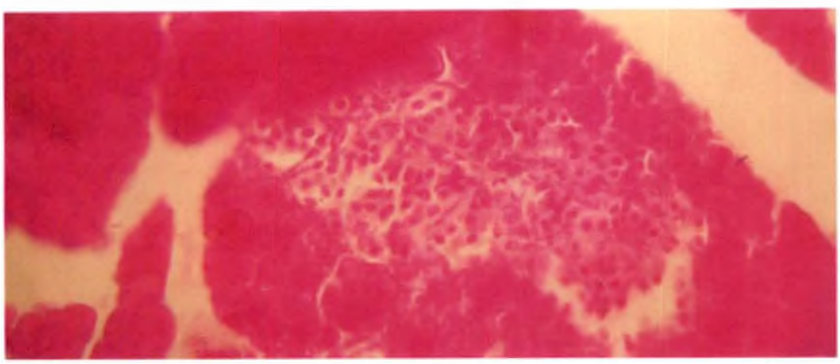
Normal control (Vehicle 1ml/kg)



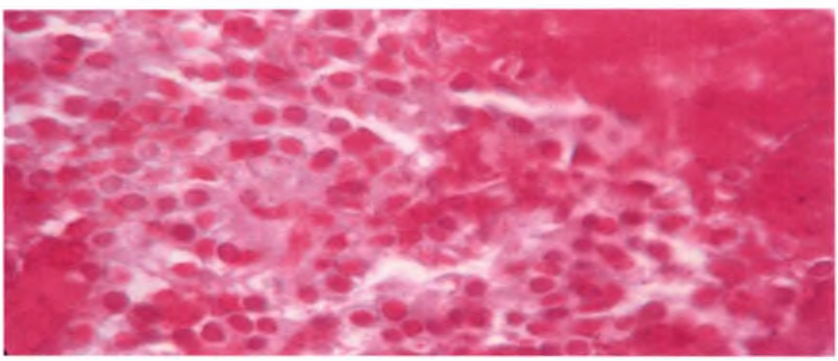
Pancreatectomized control (Vehicle 1ml/kg)



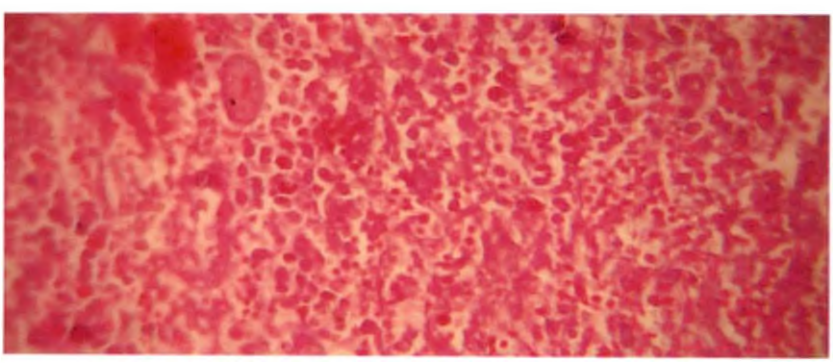
Red lotus seed extract 600 mg/kg for 28 days



White lotus seed extract 600 mg/kg for 28 days



Metformin 200 mg/kg for 28 days



Nicotinamide 500 mg/kg for 28 days

Simvastatin treated group at the dose rate of 20 mg kg^{-1} showed reduction in total cholesterol levels by 29.87, 40.68, 50.81 and 57.84 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 16). It was significant ($P < 0.01$) from 7^{th} day onwards (Table. 15)

The rats treated with ethanolic extract of red lotus seeds at the dose rate of 600 mg kg^{-1} reduced serum LDL-cholesterol levels by 5.58, 26.44, 36.90 and 46.80 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table. 18). On the other hand white lotus seeds at the same dose rate reduced serum LDL-cholesterol levels by 8.93, 30.10, 38.96 and 51.57 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 18). It was significant ($P < 0.01$) from 14^{th} day onwards (Table 17). Simvastatin treated group at the dose rate of 20 mg kg^{-1} showed reduction in LDL-cholesterol levels by 42.46, 56.34, 69.08 and 80.33 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 18). It was significant ($P < 0.01$) from 7^{th} day onwards (Table 17).

The serum HDL-cholesterol levels in red lotus seeds extract treated group increased by 2.51, 5.17, 11.78 and 19.51 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 20) and white lotus seeds treated group increased by 3.58, 7.24, 13.13 and 23.00 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 20). It was significant ($P < 0.01$) on 28^{th} day only (Table 19).

Simvastatin treated group at the dose rate of 20 mg kg^{-1} showed increase in HDL- cholesterol levels by 15.05, 19.31, 22.56 and 29.62 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 20). It was significant on 7 ($P < 0.05$), 14, 21 and 28 days ($P < 0.01$) treatment (Table 19).

Table 15. Effect of ethanolic extract of red and white lotus seeds on total cholesterol levels in hypercholesterolemic rats, mg/dl

		0 day	7th day	14th day	21st day	28th day
Normal control	Mean	82.16	81.91	82.78	82.03	81.07
	S.E	1.67	1.47	1.48	1.88	1.61
Hyper cholesterolemic control	Mean	210.82	221.45	229.44	233.91	227.12
	S.E	2.67	4.50	6.84	6.42	6.52
Red lotus seed extract 600 mg/kg	Mean	213.45	212.40	185.83**	170.72**	152.26**
	S.E	4.61	5.29	2.89	4.04	4.60
White lotus seed extract 600 mg/kg	Mean	211.11	207.24	178.89**	168.12**	145.48**
	S.E	3.93	4.03	4.29	4.20	6.22
Simvastatin 20 mg/kg	Mean	213.74	155.30**	136.11**	115.07**	95.76**
	S.E	4.99	4.05	6.30	6.69	2.36

** P <0.01 , *P <0.05 Compared to hypercholesterolemic control

Table 16. Effect of ethanolic extract of red and white lotus seeds on total cholesterol levels in hypercholesterolemic rats, percentage reduction

Day	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Simvastatin 20 mg/kg
7th	4.08	6.42	29.87
14th	19.01	22.03	40.68
21st	27.01	28.13	50.81
28th	32.96	35.95	57.84

Table 17. Effect of ethanolic extract of red and white lotus seeds on LDL levels in hypercholesterolemic rats, mg/dl

		0 day	7th day	14th day	21st day	28th day
Normal control	Mean	21.40	21.62	21.56	22.10	21.66
	S.E	1.03	0.95	0.90	0.99	0.49
Hypercholesterolemic control	Mean	153.12	163.93	171.92	178.51	171.85
	S.E	3.33	3.94	7.52	7.37	6.41
Red lotus seed extract 600 mg/kg	Mean	153.52	154.78	126.46**	112.64**	91.43**
	S.E	6.46	5.97	2.36	3.83	4.51
White lotus seed extract 600 mg/kg	Mean	152.55	149.29	120.17**	108.96**	83.23**
	S.E	4.43	4.64	4.20	4.02	5.77
Simvastatin 20 mg/kg	Mean	153.03	94.32**	75.07**	55.20**	33.81**
	S.E	8.65	5.07	6.36	7.48	2.90

** P <0.01 , *P <0.05 Compared to hypercholesterolemic control
LDL- Low density lipoprotein

Table 18. Effect of ethanolic extract of red and white lotus seeds on LDL levels in hypercholesterolemic rats, percentage reduction

Day	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Simvastatin 20 mg/kg
7th	5.58	8.93	42.46
14th	26.44	30.10	56.34
21st	36.90	38.96	69.08
28th	46.80	51.57	80.33

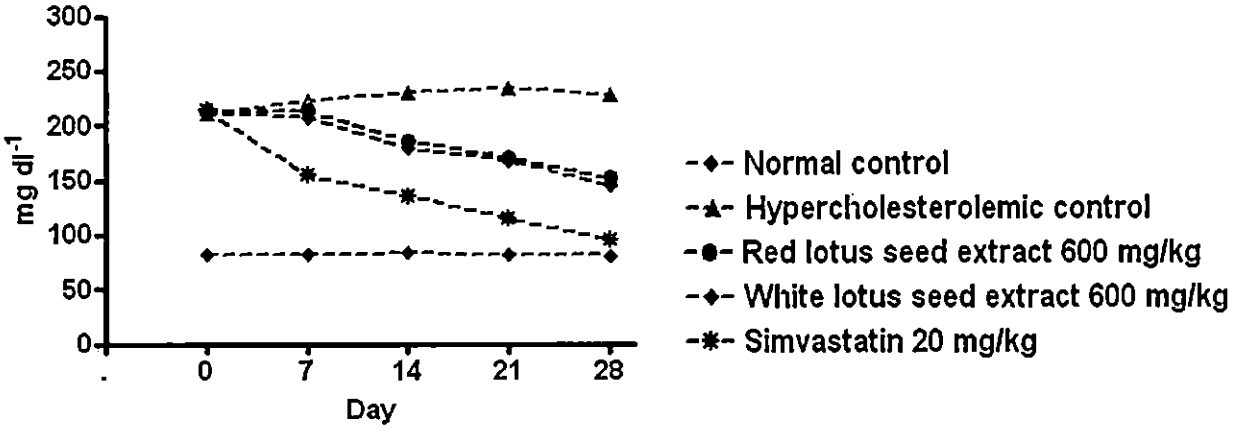


Fig. 13. Effect of ethanolic extract of red and white lotus seeds on total cholesterol levels in hypercholesterolemic rats

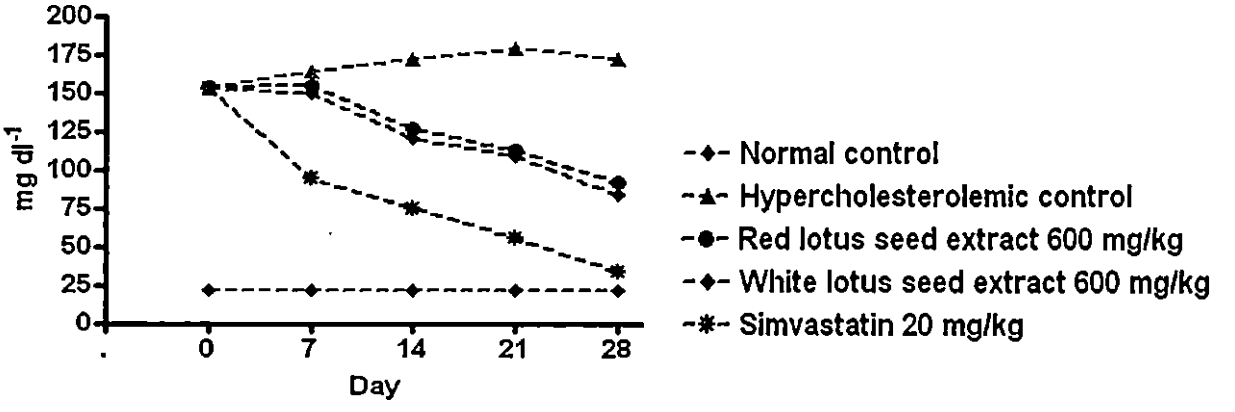


Fig. 14. Effect of ethanolic extract of red and white lotus seeds on LDL levels in hypercholesterolemic rats

Table 19. Effect of ethanolic extract of red and white lotus seeds on HDL levels in hypercholesterolemic rats, mg/dl

		0 day	7th day	14th day	21st day	28th day
Normal control	Mean	45.66	45.17	46.93	46.09	45.24
	S.E	1.50	1.12	1.17	1.47	1.51
Hypercholesterolemic control	Mean	34.98	33.70	34.28	34.14	34.17
	S.E	0.83	1.07	1.60	1.78	1.49
Red lotus seed extract 600 mg/kg	Mean	33.57	34.54	36.05	38.16	40.83**
	S.E	2.55	1.40	0.93	1.33	1.16
White lotus seed extract 600 mg/kg	Mean	34.39	34.90	36.76	38.62	42.02**
	S.E	2.17	1.25	1.49	1.25	1.37
Simvastatin 20 mg/kg	Mean	34.86	38.77*	40.90**	41.84**	44.29**
	S.E	1.33	0.97	1.46	1.43	1.49

** P <0.01 , *P <0.05 Compared to hypercholesterolemic control

HDL- High density lipoprotein

Table 20. Effect of ethanolic extract of red and white lotus seeds on HDL levels in hypercholesterolemic rats, percentage increases

Day	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Simvastatin 20mg/kg
7th	2.51	3.58	15.05
14th	5.17	7.24	19.31
21st	11.78	13.13	22.56
28th	19.51	23.00	29.62

Table 21. Effect of ethanolic extract of red and white lotus seed extracts on liver total lipid levels in hypercholesterolemic rats, mg/100 gm wet tissue

	Normal control	Hyper cholesterolemic control	Red Lotus seed extract 600 mg/kg	White Lotus seed extract 600 mg/kg	Simvastatin 20 mg/kg
Mean	797.77	1654.02	1431.70*	1345.09**	1192.41**
S.E	57.47	51.27	44.87	50.66	76.57

** P <0.01 , *P <0.05 Compared to hypercholesterolemic control

Table 22. Effect of ethanolic extract of red and white lotus seed extracts on liver total lipid levels in hypercholesterolemic rats, percentage reduction

	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Simvastatin 20 mg/kg
Total lipid	13.44	18.68	27.91

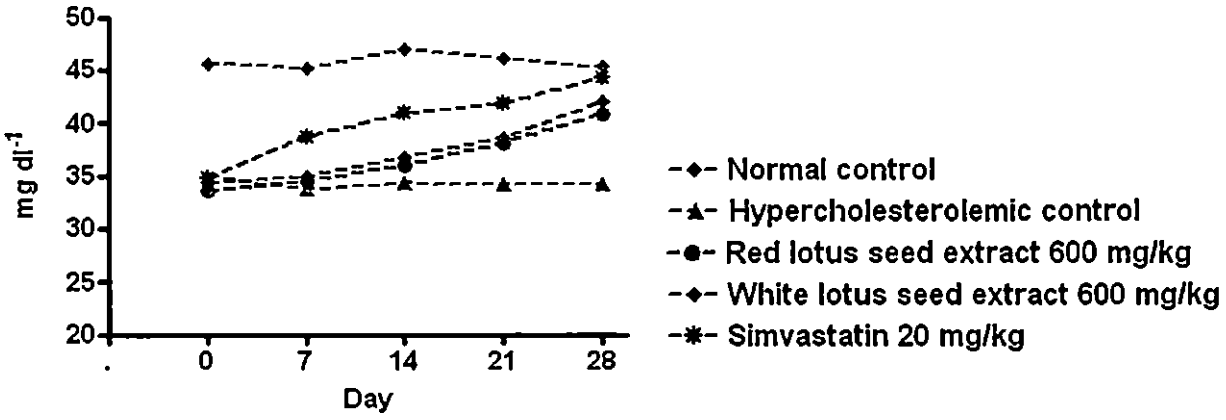
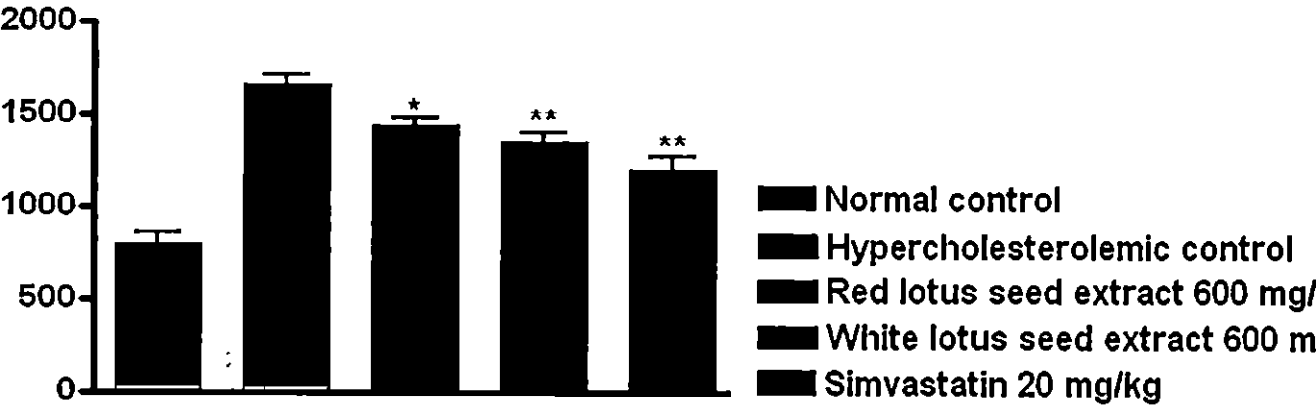


Fig. 15. Effect of ethanolic extract of red and white lotus seeds on HDL levels in hypercholesterolemic rats



*P<0.05, **P<0.01 compared to hypercholesterolemic control

Fig. 16. Effect of ethanolic extract of red and white lotus seeds on liver total lipid levels in hypercholesterolemic rats

The effect on liver total lipids levels is shown in Table 21 and Figure 16. Red and white lotus seeds extract showed significant ($P < 0.05$ and $P < 0.01$) decrease in liver total lipids by 13.44 and 18.68 % respectively. (Table 22). In simvastatin treated group significant ($P < 0.01$) reduction in liver total lipids by 27.91 % was noticed (Table 22).

4.4. HYPOTRIGLYCERIDEMIC EFFECT OF RED AND WHITE LOTUS SEEDS EXTRACT IN HYPERTRIGLYCERIDEMIC ANIMAL MODEL

The effect of ethanolic extract of red and white lotus seeds on triglyceride (Table 23 and Figure 17) and free fatty acid (Table 25 and Figure 18) levels in high fat diet induced hypertriglyceridemic rats are presented in respective tables and figures.

Hypertriglyceridemic rats treated with ethanolic extract of red lotus seeds at the dose rate of 600 mg kg^{-1} reduced serum triglyceride levels by 3.84, 13.25, 23.01 and 30.96 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 24). On the other hand white lotus seeds at the same dose rate reduced serum triglyceride levels by 6.46, 14.53, 25.52 and 36.21 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 24). It was significant ($P < 0.01$) from 14^{th} day onwards (Table 23). Fenofibrate treated group at the dose rate of 30 mg kg^{-1} showed reduction in triglycerides levels by 21.57, 45.62, 52.56 and 59.48 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 24) It was significant ($P < 0.01$) from 7^{th} day onwards (Table 23)

The rats treated with ethanolic extract of red lotus seeds at the dose rate of 600 mg kg^{-1} reduced plasma free fatty acid levels by 5.14, 9.01, 12.44 and 17.43 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 26). It was significant on 21 ($P < 0.05$) and 28 ($P < 0.01$) days treatment (Table 25). In case of white lotus seeds

treated group at the same dose level it was reduced by 6.09, 11.38, 15.22 and 20.01 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 26). It was significant from 21 ($P < 0.01$) days treatment onwards (Table 25). Fenofibrate treated group at the dose rate of 30 mg kg^{-1} showed reduction in free fatty acids levels by 6.98, 13.57, 17.25 and 23.08 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table. 26). It was significant on 14 ($P < 0.05$), 21 and 28 days ($P < 0.01$) treatment (Table 25).

The effect on liver total lipids levels is shown in Table 27 and Figure 19. Red and white lotus seed extract also showed significant ($P < 0.05$ and $P < 0.01$) reduction in liver total lipids by 27.60 and 33.81 % respectively. (Table 28). In fenofibrate treated group significant ($P < 0.01$) reduction in liver total lipid by 43.04 % was noticed (Table 28).

4.5. CYTOPROTECTIVE EFFECT OF RED AND WHITE LOTUS SEEDS EXTRACT IN REPEATED DOSE ALLOXAN TREATED ANIMAL MODEL

The effect of ethanolic extract of red and white lotus seeds on superoxide dismutase (Table 29 and Figure 20), catalase (Table 31 and Figure 21), glutathione peroxidase (Table 33 and Figure 22), and lipid peroxidation (Table 35 and Figure 23), levels in repeated dose alloxan treated rats are presented in respective tables and figures.

Repeated dose alloxan administered rats treated with ethanolic extract of red lotus seeds at the dose rate of 600 mg kg^{-1} increased superoxide dismutase levels in pancreas by 2.69, 14.57, 28.56 and 39.35 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 30). It was significant on 14 ($P < 0.05$) 21 and 28 ($P < 0.01$) days treatment (Table 29).

Table 23. Effect of ethanolic extract of red and white lotus seeds on triglycerides levels in hypertriglyceridemic rats, mg/dl

		0 day	7th day	14th day	21st day	28th day
Normal control	Mean	80.40	79.79	82.51	82.22	80.25
	S.E	2.91	3.15	3.86	3.10	3.15
Hypertriglyceridemic control	Mean	221.01	238.54	242.18	240.61	243.21
	S.E	4.04	3.39	3.09	4.01	3.43
Red lotus seed extract 600 mg/kg	Mean	219.60	229.38	210.08**	185.25**	167.90**
	S.E	4.70	6.90	4.77	6.25	5.28
White lotu seed extract 600 mg/kg	Mean	224.85	223.13	207.00**	179.19**	155.14**
	S.E	5.68	4.47	3.47	1.52	3.24
Fenofibrate 30 mg/kg	Mean	213.94	187.08**	131.69**	114.14**	98.56**
	S.E	5.15	4.06	2.19	2.42	1.85

** P <0.01 , *P <0.05 Compared to hypertriglyceridemic control

Table 24. Effect of ethanolic extract of red and white lotus seeds on triglycerides levels in hypertriglyceridemic rats, percentage reduction

Day	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Fenofibrate 30 mg/kg
7th	3.84	6.46	21.57
14th	13.25	14.53	45.62
21st	23.01	25.52	52.56
28th	30.96	36.21	59.48

Table 25. Effect of ethanolic extract of red and white lotus seed on free fatty acids levels in hypertriglyceridemic rats, mmole/l

		0 day	7th day	14th day	21st day	28th day
Normal control	Mean	1.20	1.21	1.24	1.22	1.22
	S.E	0.01	0.01	0.02	0.01	0.01
Hypertriglyceridemic control	Mean	1.55	1.57	1.58	1.60	1.63
	S.E	0.05	0.06	0.06	0.05	0.03
Red lotus seed extract 600 mg/kg	Mean	1.53	1.49	1.44	1.40*	1.35**
	S.E	0.05	0.05	0.05	0.06	0.04
White lotus seed extract 600 mg/kg	Mean	1.53	1.48	1.40	1.36**	1.31**
	S.E	0.05	0.05	0.04	0.04	0.03
Fenofibrate 30 mg/kg	Mean	1.56	1.46	1.37*	1.32**	1.26**
	S.E	0.08	0.08	0.08	0.08	0.04

** P <0.01 , *P <0.05 Compared to hypertriglyceridemic control

Table 26. Effect of ethanolic extract of red and white lotus seed on free fatty acids levels in hypertriglyceridemic rats, percentage reduction

Day	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Fenofibrate 30 mg/kg
7th	5.14	6.09	6.98
14th	9.01	11.38	13.57
21st	12.44	15.22	17.25
28th	17.43	20.01	23.08

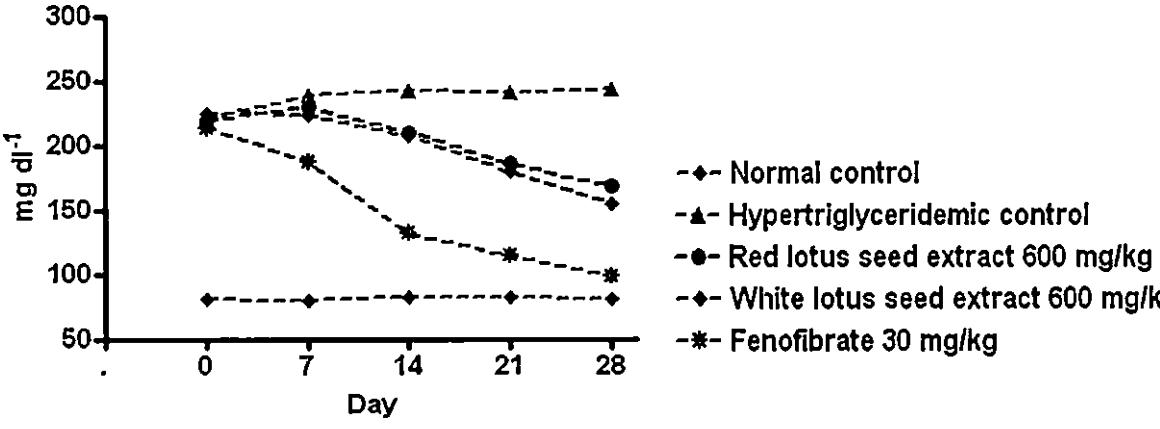


Fig. 17. Effect of ethanolic extract of red and white lotus seeds on triglycerides levels in hypertriglyceridemic rats

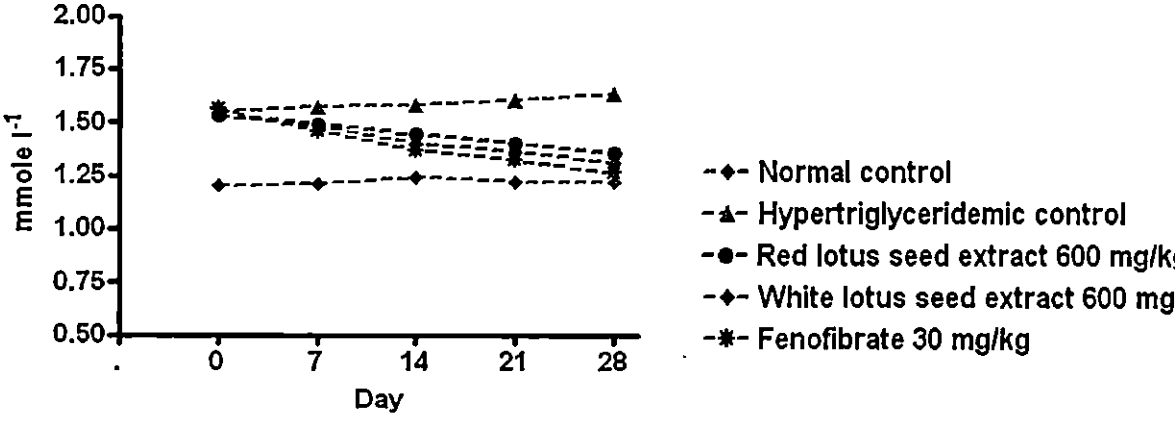


Fig. 18. Effect of ethanolic extract of red and white lotus seeds on free fatty acids levels in hypertriglyceridemic rats

Table 27. Effect of ethanolic extract of red and white lotus seeds on liver total lipid levels in hypertriglyceridemic rats, mg/100 gm wet tissue

	Normal control	Hyper triglyceridemic Control	Red lotus seed extract 600 mg/kg	White lotus seed extract 600 mg/kg	Fenofibrate 30 mg/kg
Mean	770.83	2047.40	1482.29*	1355.21**	1166.15**
S.E	33.27	74.67	83.10	68.56	85.54

** P <0.01 , *P <0.05 Compared to hypertriglyceridemic control

Table 28. Effect of ethanolic extract of red and white lotus seeds on liver total lipid levels in hypertriglyceridemic rats, percentage reduction

	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Fenofibrate 30 mg/kg
Total lipid	27.60	33.81	43.04

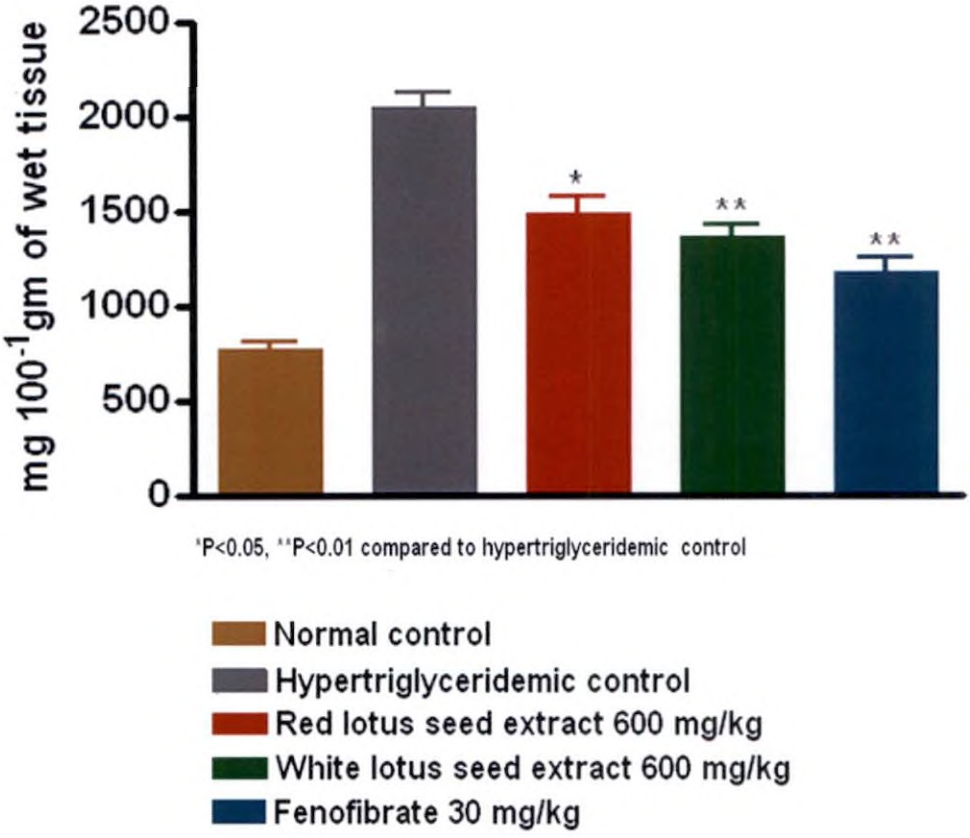


Fig. 19. Effect of ethanolic extract of red and white lotus seeds on liver total lipid levels in hypertriglyceridemic rats

The white lotus seeds at the same dose rate also increased superoxide dismutase levels in pancreas by 12.07, 23.01, 37.06 and 46.59 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 30). It was significant from 14^{th} ($P < 0.01$) day treatment onwards (Table 29). Metformin treated group at the dose rate of 200 mg kg^{-1} showed increase in superoxide dismutase levels by 14.85, 31.82, 44.45 and 53.93 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 30). It was significant ($P < 0.01$) from 14^{th} day onwards. (Table 29).

The rats treated with ethanolic extract of red lotus seeds at the dose rate of 600 mg kg^{-1} increased catalase levels by 4.81, 24.08, 35.69 and 42.89 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 32). In white lotus seeds extract treated group it was increased by 9.43, 28.75, 39.85 and 48.80 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 32). It was significant from 14^{th} ($P < 0.01$)day onwards (Table 31). Metformin treated group at the dose rate of 200 mg kg^{-1} showed increase in catalase levels by 16.23, 36.31, 44.12 and 54.42 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 32). It was significant from 14^{th} ($P < 0.01$) day onwards (Table 31).

The glutathione peroxidase level also increased by 8.40, 21.35, 35.41 and 52.10 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment in red lotus seed extract treated group (Table 34). In white lotus seeds extract treated group also it was increased by 9.91, 25.52, 38.94 and 56.45 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 34). It was significant on 14 ($P < 0.05$), 21 and 28 ($P < 0.01$) days in red and white lotus seeds treated groups (Table 33). Metformin treated group at the dose rate of 200 mg kg^{-1} showed increase glutathione peroxidase levels by 12.44, 31.55, 45.45 and 61.34 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 34). It was significant ($P < 0.01$) from 14^{th} day treatment onwards (Table 33).

The rats treated with ethanolic extract of red lotus seeds at the dose rate of 600 mg kg^{-1} reduced lipid peroxidation levels by 4.05, 12.06, 22.01 and 37.03 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 36). It was significant ($P < 0.01$) from 21^{st} day onwards (Table 35). On the other hand white lotus seeds at the same dose reduced lipid peroxidation levels by 7.99, 17.49, 29.28 and 44.50 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 36). It was significant from 14^{th} day ($P<0.01$) treatment onwards (Table 35). Metformin treated group at the dose rate of 200 mg kg^{-1} showed significant reduction in lipid peroxidation levels by 12.04, 19.62, 35.62 and 49.41 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment (Table 36). It was significant ($P < 0.01$) from 14 day treatment onwards (Table 35).

Histopathological examination of pancreas revealed islet hyperplasia with more number of active cells in animals treated with extracts, metformin and nicotinamide. It was duration dependent (Figure 24 A-N).

Table 29. Effect of ethanolic extract of red and white lotus seeds on SOD levels in repeated dose alloxan treated rats, U/mg of protein

		7th day	14th day	21st day	28th day
Normal control	Mean	48.20	48.68	47.83	51.32
	S.E	2.62	2.03	1.08	1.78
Alloxan treated control	Mean	68.24	66.49	63.06	60.38
	S.E	3.85	3.03	2.49	2.96
Red lotus seed extract 600 mg/kg	Mean	70.08	76.19*	81.07**	84.14**
	S.E	2.37	2.22	2.48	2.17
White lotus seed extract 600 mg/kg	Mean	76.48	81.79**	86.43**	88.52**
	S.E	3.79	2.21	3.06	1.17
Metformin 200 mg/kg	Mean	78.37	87.65**	91.09**	92.95**
	S.E	4.36	2.12	2.87	1.26

** P <0.01 , *P <0.05 Compared to alloxan treated control
SOD - Superoxide dismutase

Table 30. Effect of ethanolic extract of red and white lotus seeds on SOD levels in repeated dose alloxan treated rats, percentage increase

Day	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Metformin 200 mg/kg
7th	2.69	12.07	14.85
14th	14.57	23.01	31.82
21st	28.56	37.06	44.45
28th	39.35	46.59	53.93

SOD - Superoxide dismutase

Table 31. Effect of ethanolic extract of red and white lotus seeds on CAT levels in repeated dose alloxan treated rats, U/mg of protein

		7th day	14th day	21st day	28th day
Normal control	Mean	23.25	24.26	25.01	25.26
	S.E	1.15	1.42	0.95	0.89
Alloxan treated control	Mean	34.67	34.25	33.75	32.68
	S.E	1.69	1.74	3.06	3.94
Red lotus seed extract 600 mg/kg	Mean	36.33	42.49**	45.79**	46.70**
	S.E	1.81	1.51	1.84	0.98
White lotus seed extract 600 mg/kg	Mean	37.93	44.09**	47.20**	48.63**
	S.E	2.88	0.98	1.17	0.92
Metformin 200 mg/kg	Mean	40.29	46.68**	48.64**	50.47**
	S.E	2.74	1.47	0.64	0.97

** P <0.01 , *P <0.05 Compared to alloxan treated control
CAT - Catalase

Table 32. Effect of ethanolic extract of red and white lotus seeds on CAT levels in repeated dose alloxan treated rats, percentage increase

Day	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Metformin 200 mg/kg
7th	4.81	9.43	16.23
14th	24.08	28.75	36.31
21st	35.69	39.85	44.12
28th	42.89	48.80	54.42

CAT - Catalase

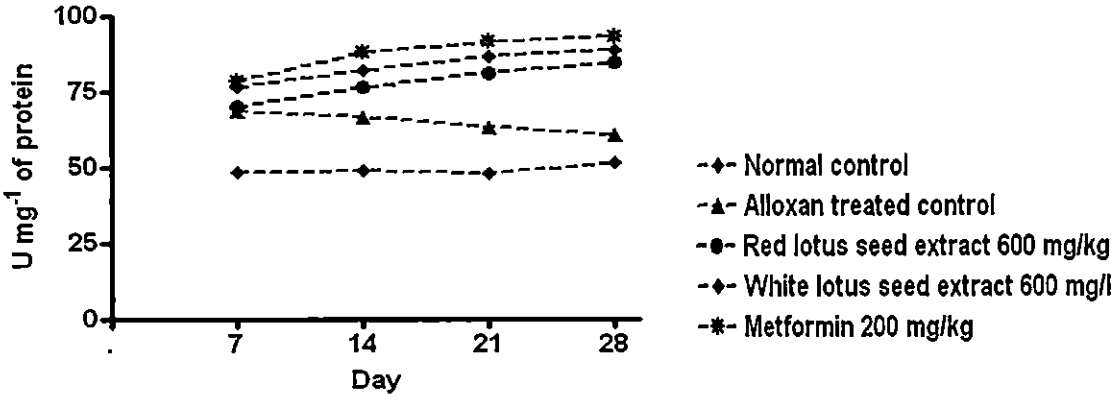


Fig. 20. Effect of ethanolic extract of red and white lotus seeds on SOD levels in repeated dose alloxan treated rats

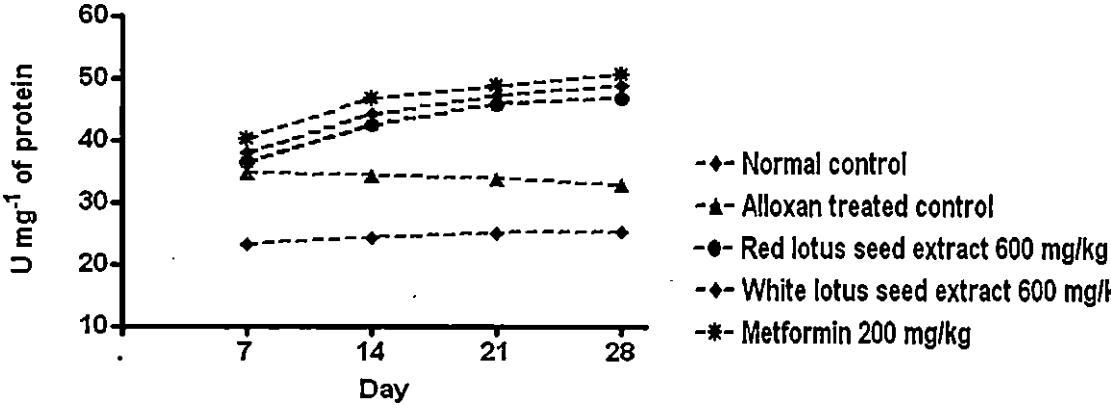


Fig. 21. Effect of ethanolic extract of red and white lotus seeds on CAT levels in repeated dose alloxan treated rats

Table 33. Effect of ethanolic extract of red and white lotus seeds on GPx levels in repeated dose alloxan treated rats, U/mg of protein

		7th day	14th day	21st day	28th day
Normal control	Mean	5.81	6.37	5.76	6.05
	S.E	0.35	0.32	0.34	0.28
Alloxan treated control	Mean	11.84	11.37	10.58	9.92
	S.E	0.55	0.66	0.40	0.45
Red lotus seed extract 600 mg/kg	Mean	12.83	13.79*	14.33**	15.08**
	S.E	0.64	0.75	0.58	0.85
White lotus seed extract 600 mg/kg	Mean	13.01	14.27*	14.70**	15.51**
	S.E	0.48	0.62	0.82	0.54
Metformin 200 mg/kg	Mean	13.31	14.95**	15.39**	16.00**
	S.E	0.65	0.75	1.07	0.94

** P <0.01 , *P <0.05 Compared to alloxan treated control
GPx - Glutathione peroxidase

Table 34. Effect of ethanolic extract of red and white lotus seeds on GPx levels in repeated dose alloxan treated rats, percentage increase

Day	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Metformin 200 mg/kg
7th	8.40	9.91	12.44
14th	21.35	25.52	31.55
21st	35.41	38.94	45.45
28th	52.10	56.45	61.34

GPx - Glutathione peroxidase

Table 35. Effect of ethanolic extract of red and white lotus seeds on LPO levels in repeated dose alloxan treated rats, mmole/100g wet tissue

		7th day	14th day	21st day	28th day
Normal control	Mean	0.91	0.92	0.87	0.83
	S.E	0.09	0.06	0.03	0.05
Alloxan treated control	Mean	2.38	2.20	2.21	2.29
	S.E	0.19	0.12	0.09	0.10
Red lotus seed extract 600 mg/kg	Mean	2.29	1.93	1.73**	1.44**
	S.E	0.09	0.05	0.05	0.04
White lotus seed extract 600 mg/kg	Mean	2.19	1.81**	1.57**	1.27**
	S.E	0.05	0.07	0.08	0.04
Metformin 200 mg/kg	Mean	2.10	1.77**	1.43**	1.16**
	S.E	0.06	0.06	0.06	0.03

** P <0.01 , *P <0.05 Compared to alloxan treated control
LPO - Lipid peroxidation

Table 36. Effect of ethanolic extract of red and white lotus seeds on LPO levels in repeated dose alloxan treated rats, percentage reduction

Day	Red lotus seeds extract 600 mg/kg	White lotus seeds extract 600 mg/kg	Metformin 200 mg/kg
7th	4.05	7.99	12.04
14th	12.06	17.49	19.62
21st	22.01	29.28	35.62
28th	37.03	44.50	49.41

LPO - Lipid peroxidation

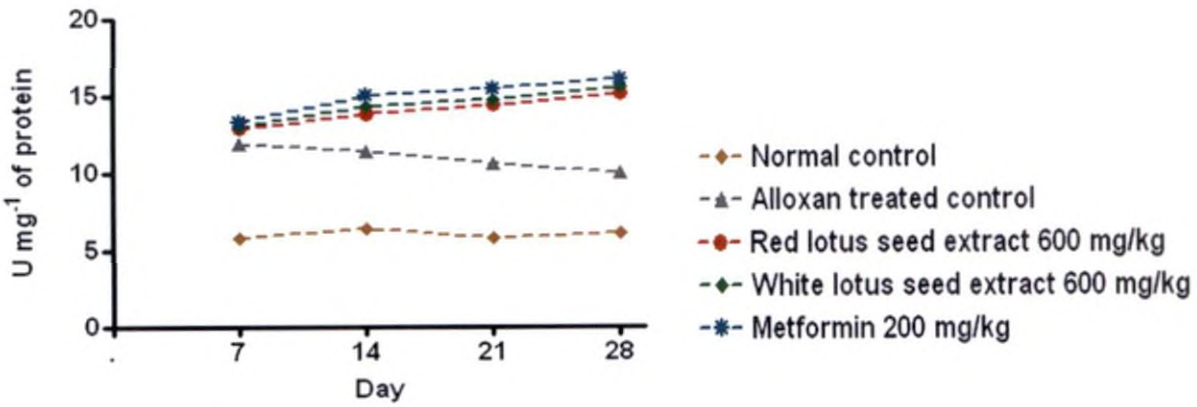


Fig. 22. Effect of ethanolic extract of red and white lotus seeds on GPx levels in repeated dose alloxan treated rats

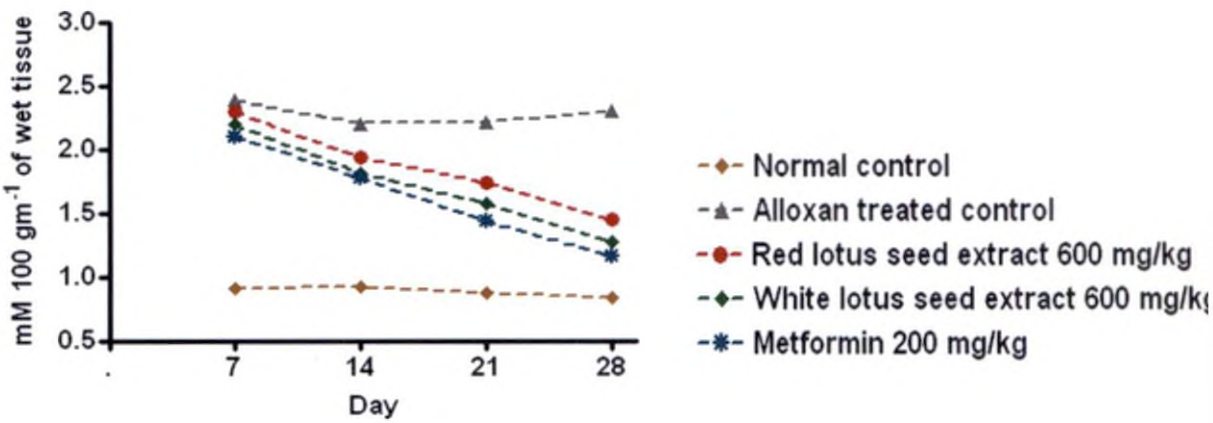
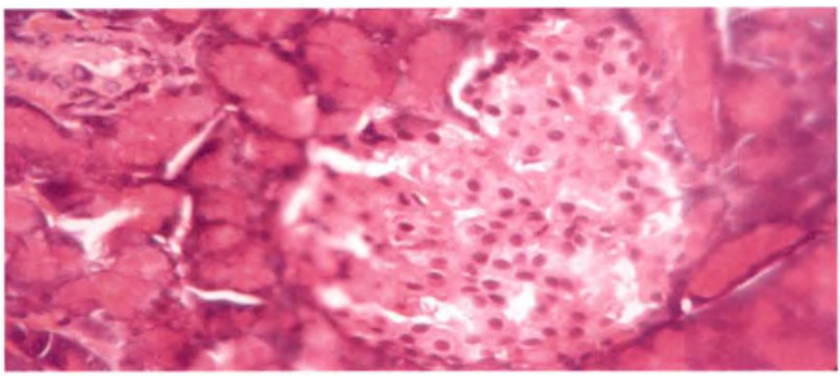
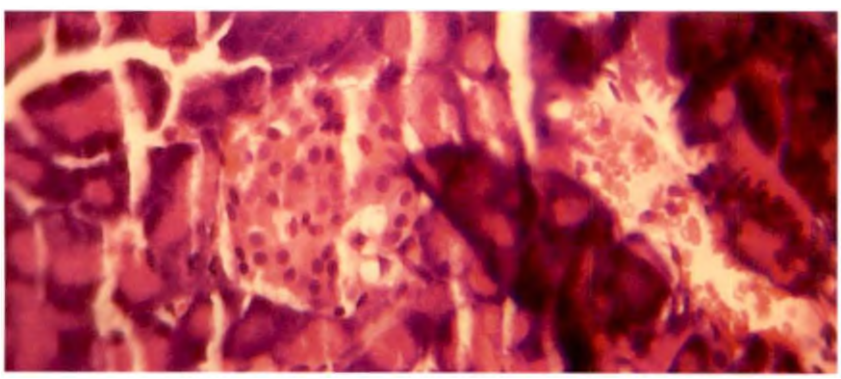


Fig. 23. Effect of ethanolic extract of red and white lotus seeds on LPO levels in repeated dose alloxan treated rats

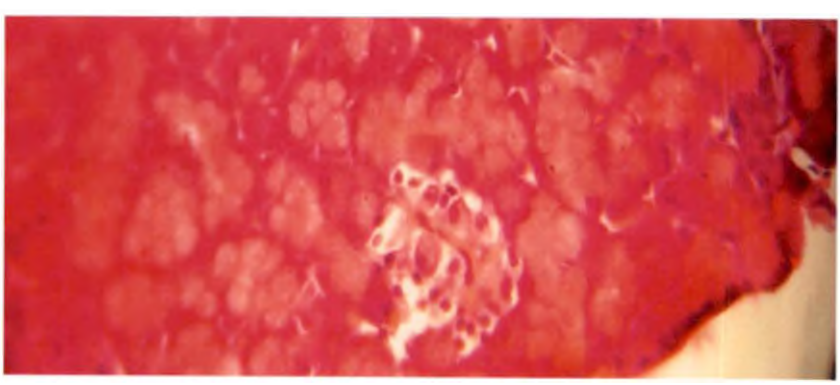
Fig. 24. Effect of ethanolic extract of red and white lotus seeds on histopathological changes in pancreas of repeated dose alloxan treated rats (H&E and Aldehyde fuchsin and Phloxine B, A-N: 160 X)



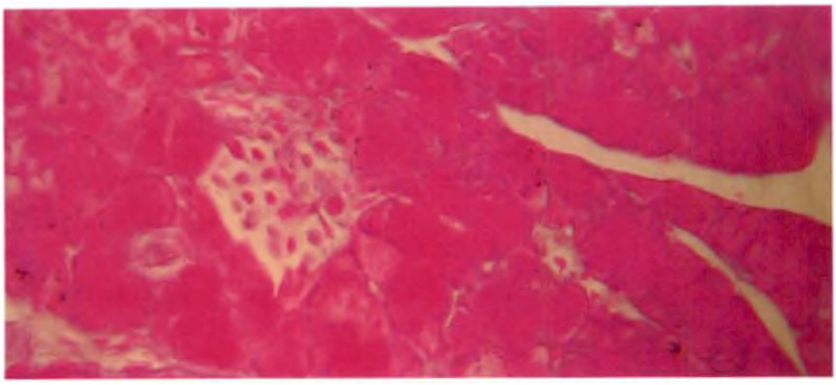
Normal control (Vehicle 1ml/kg)



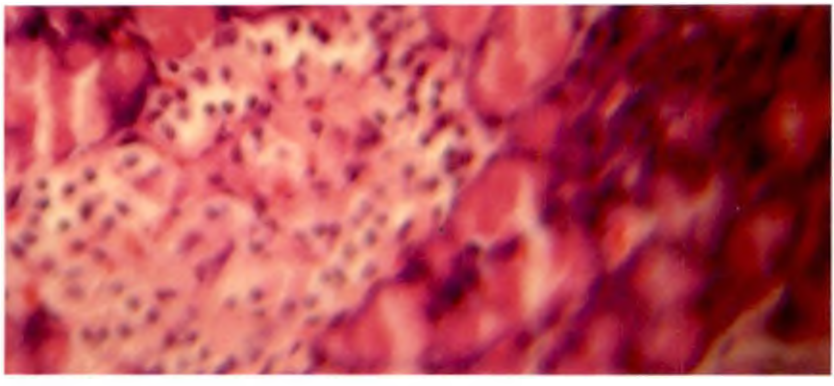
Alloxan treated control (Vehicle 1ml/kg)



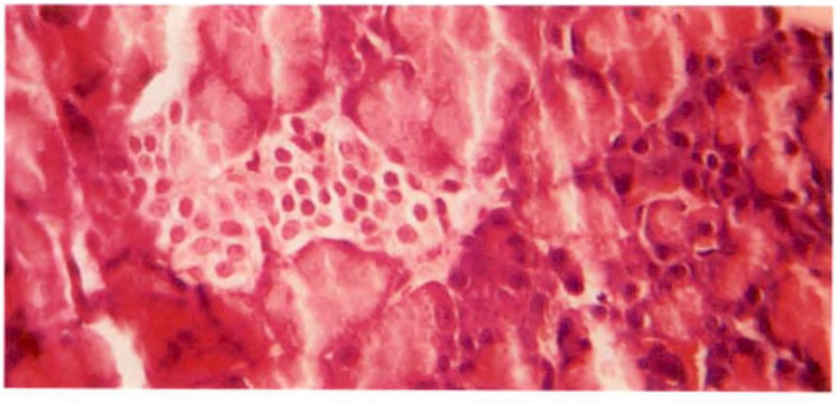
Red lotus seed extract 600 mg/kg for 7 days



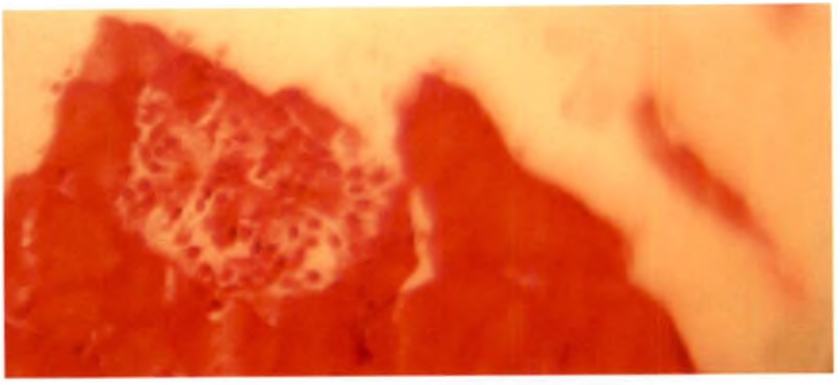
White lotus seed extract 600 mg/kg for 7 days



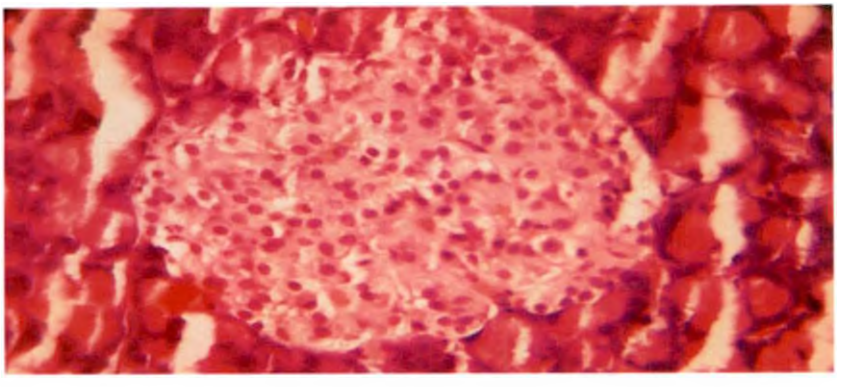
Metformin 200 mg/kg for 7 days



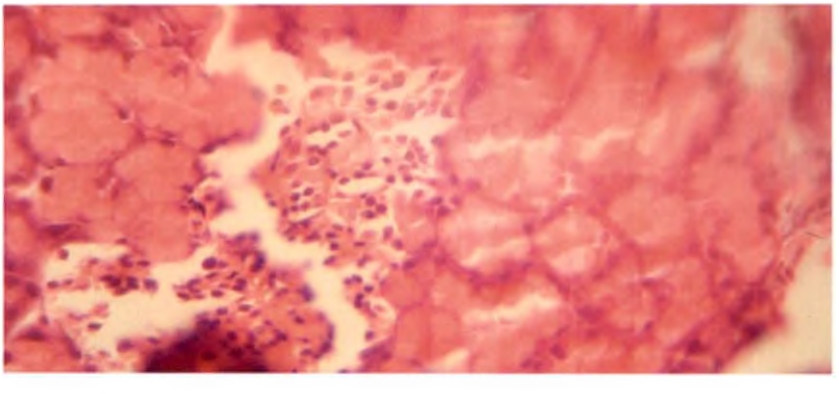
Red lotus seed extract 600 mg/kg for 14 days



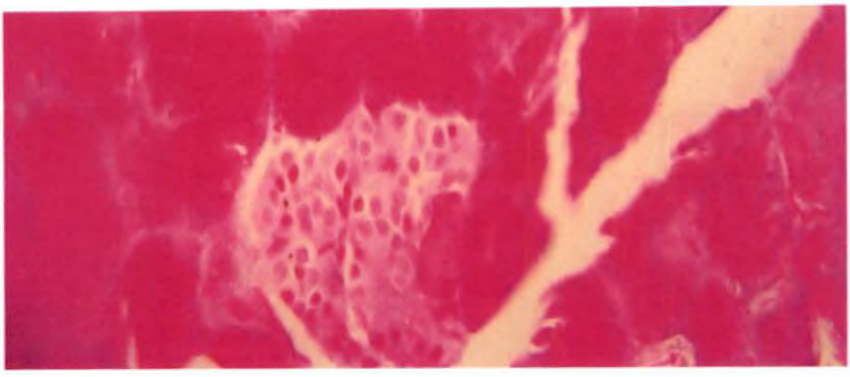
White lotus seed extract 600 mg/kg for 14 days



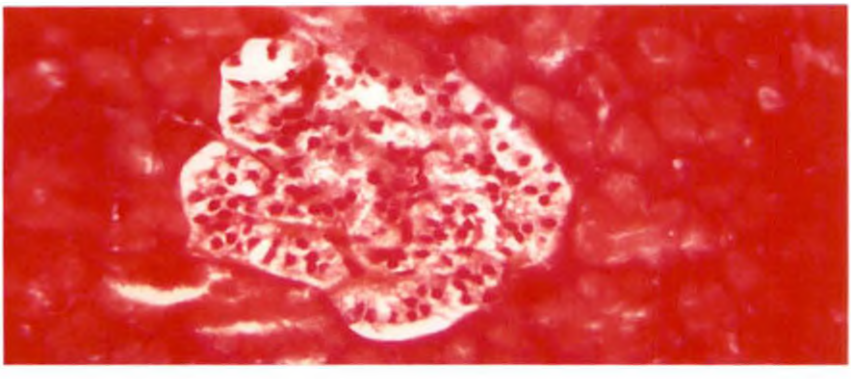
Metformin 200 mg/kg for 14 days



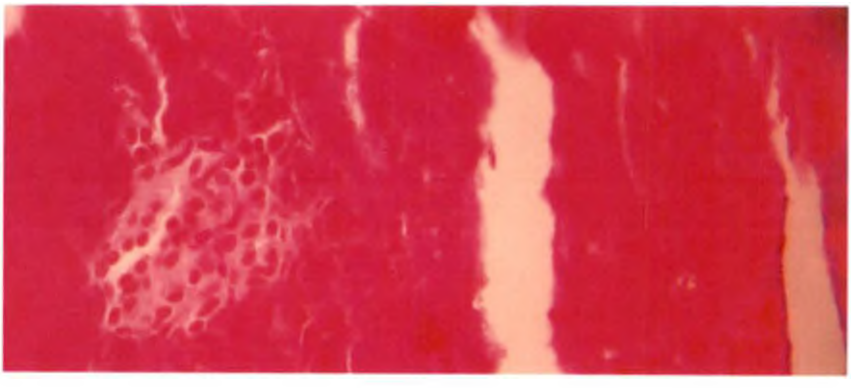
Red lotus seed extract 600 mg/kg for 21 days



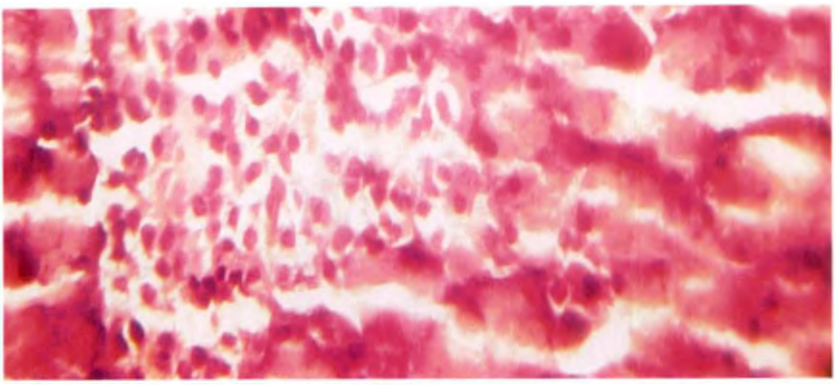
White lotus seed extract 600 mg/kg for 21 days



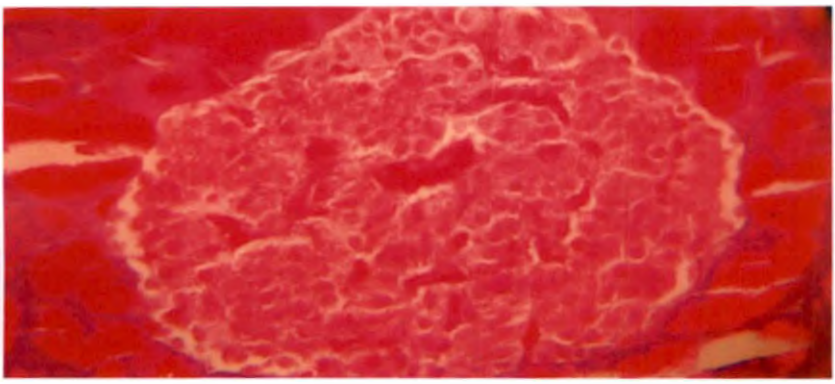
Metformin 200 mg/kg for 21 days



Red lotus seed extract 600 mg/kg for 28 days



White lotus seed extract 600 mg/kg for 28 days



Metformin 200 mg/kg for 28 days

4. 6. PHYTOCHEMICAL ANALYSIS

The active principles present in the red and white lotus seeds extract detected by quantitative tests are presented in the Table 37.

4. 6.1. Steroids

As per the Salkowski test, red colour was obtained and Liberman Burchardt test also gave a reddish ring at the junction in both extracts. Thus it could be concluded that detectable levels of steroids were present in ethanolic extract of red and white lotus seeds.

4. 6. 2. Alkaloids

A creamy white precipitate as per Mayer's test and a reddish brown coloured precipitate as per Wagner's test were obtained. Dragendroff's test yielded reddish brown precipitate and Hager's test produced yellow precipitate in both extracts. Thus, the tests revealed the presence of detectable level of alkaloids in lotus seeds extracts.

4. 6. 3. Phenolic Compounds

A dark blue colour was produced, when lotus seeds extract was mixed with ten per cent ferric chloride indicating the presence of phenolic compounds.

4. 6. 4. Tannins

Intense blue colour was not obtained in ferric chloride test and a white precipitate was not obtained in gelatin test in both lotus seed extracts. These results indicated the absence of tannins in the lotus seeds.

4. 6. 5. Flavonoids

A green colour in the ferric chloride test and a yellow precipitate in lead acetate test obtained indicated the presence of flavonoids in ethanolic extract of red and white lotus seeds.

4. 6. 6. Glycosides

In the Benedict's test, brown colour was obtained indicating the presence of glycosides. Yellow colour was obtained by mixing the extract with sodium hydroxide, which also indicated the presence of glycosides in ethanolic extract of red and white lotus seeds.

4. 6. 7. Diterpenes

Diterpene was detected in the extract of both red and white lotus seeds as indicated by the green colour, when it was mixed with copper acetate solution.

4. 6. 8. Triterpenes

As per Liberman Burchardt test, a deep red ring appeared at the junction of two layers in ethanolic extract of red and white lotus seed. These results indicated the presence of triterpenes in ethanolic extract of red and white lotus seeds.

4. 6. 9. Saponins

In the foam test, foam was persisted for 10 minutes in the white and red lotus seeds extract, which indicated the presence of saponins.

Table 37. Screening of ethanolic extract of red and white lotus (*Nelumbo nucifera)* seeds for active principles

Serial No	Active Principles	Red lotus seed Extract	White lotus seed Extract
1	Steroids	Detected	Detected
2	Alkaloids	Detected	Detected
3	Phenolic compounds	Detected	Detected
4	Tannins	Not detected	Not detected
5	Flavonoids	Detected	Detected
6	Glycosides	Detected	Detected
7	Diterpenes	Detected	Detected
8	Triterpenes	Detected	Detected
9	Saponins	Detected	Detected

Discussion

5. DISCUSSION

Diabetes mellitus is one of the major metabolic disorders affecting millions of people worldwide and is often associated with high frequency of mortality rate besides heart ailments, blindness and gangrene. Despite concerted efforts of medical scientists over the years, the disease still eludes satisfactory cure in modern medicine (Khan and Singh, 1996). Another metabolic disorder is dyslipidaemia which is common in patients with type II diabetes and is associated with increased risk of coronary heart disease (CHD). Dyslipidaemia is characterised by elevated triglyceride levels, excessive low-density lipoprotein (LDL) and lowered high-density lipoprotein (HDL) levels. Evidence indicates that abnormalities in triglycerides, LDL and HDL contribute to increased atherosclerotic risk in patients with diabetes (Taskinen, 2003).

Most of the hypoglycemic and hypolipidaemic agents used in allopathic medicine are reported to have side effects in the long run. Hence there is a need to search for effective and safe alternative drugs for diabetes. Herbal medicines are one such alternative. A great deal of attention has been recently given to the therapeutic use of herbal remedies for reasons of safety, efficacy and economy.

The present study was directed to evaluate the effectiveness of extract from red and white flowered lotus seeds. In this study, the hypoglycemic, hypolipidaemic and cytoprotective effects of ethanolic extract of these seed were assessed.

5. 1. BIOCHEMICAL PARAMETERS

5. 1. 1. Blood Glucose

The fundamental mechanism underlying hyperglycemia involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased

utilization of glucose by the tissues (Shirwaikar *et al.*, 2005). In the present study the hypoglycemic effect of ethanolic extract of seeds from red and white lotus in alloxan-induced diabetic rat was noticed and was in a duration dependent fashion.

The blood glucose levels were estimated on 0, 7th, 14th, 21st and 28th day of treatment. Type I diabetic animals treated with red lotus seed extract showed a reduction of 7.83, 14.63, 17.44, and 22.90 % glucose on 7th, 14th, 21st and 28th day respectively after treatment at the dose rate of 600 mg kg^{-1}. On the other hand white lotus seed extract reduced glucose levels by 9.90, 16.23, 22.23 and 26.60 % on 7th, 14th, 21st and 28th day respectively at the same dose rate. It was significant from 14th day treatment onwards. The red and white lotus seeds extract significantly improved glucose tolerance by 24.64 and 25.78 % respectively..

In case of type II diabetes the rats treated with ethanolic extract of red lotus seed reduced blood glucose levels by 5.66, 9.93, 16.06 and 22.69 % on 7th, 14th, 21st and 28th day respectively after treatment at the dose rate of 600 mg kg^{-1}. On the other hand white lotus seed extract reduced glucose levels by 8.09, 11.07, 19.06 and 26.85 % on 7th, 14th, 21st and 28th day respectively at the same dose rate. It was significant from 21st day treatment onwards. The red and white lotus seeds extract significantly improved glucose tolerance by 24.76 and 25.82 % respectively.

The present findings concur with the earlier findings of various research workers who observed reduction in plasma glucose level in diabetic rats when plant extract was administered. Mukherjee *et al.* (1997) found that ethanolic extract of *N.nucifera* rhizome reduced blood sugar levels *via* improving the glucose tolerance and potentiating action of insulin in normal, glucose fed hyperglycemic and diabetic animals. The reduction of glucose level could be due to decreasing the rate of intestinal glucose absorption in glucose fed hyperglycemic animals and hypoglycemic action like insulin in case of diabetic animals. Rao *et al.* (2001) also reported the antihyperglycemic activity of

Momordica cymbalaria and suggested that the action may be due to its stimulating effect on the remnant beta-cells or improvement in insulin action at cellular level or it could also be due to the insulin like effect of the active principle(s) present in the extract.

Some plants like *Tinospora cordifolia* root extract exert its activity like sulfonylurea which reduces blood glucose by increasing the insulin secretion from pancreatic beta-cells. The aqueous extract of *Tinospora cordifolia* root decreases blood glucose in alloxan diabetic rats and they suggested that it may be by increasing either the pancreatic secretion of insulin from the beta cells of islets of Langerhans or its release from bound form (Stanley *et al.*, 2000). Chakrabarti *et al.* (2005) reported that diabetic rats treated with extracts of *Caesalpinia bonducella* for 28 days increased serum insulin level significantly in both aqueous and ethanolic extracts treated group. This indicated that the test drug might have insulin secretagogue activity. The acetone extract of the leaves of *Vernonia colorata* exhibited antidiabetic properties similar to sulfonylurea. The leaves of vernonia contain alkaloids and flavonoids. Many studies have reported the hypoglycemic activity of flavonoids in diabetic animal models (Sy *et al.*, 2005). In the present study phytochemical analysis revealed the presence of flavonoids in red and white lotus seed extracts. These flavonoids might be the contributory factor for hypoglycemic effect of seeds extract.

Some plants like *Carum carvi* and *Capparis spinosa* L. exert its effect like biguanides which suppress the hepatic glucose production. Eddouks *et al.* (2004) reported the hypoglycemic activity of aqueous extracts of *Carum carvi* and *Capparis spinosa* L. fruits and they have the opinion that it may be due to inhibition of hepatic glucose production and/or stimulation of glucose utilisation by peripheral tissues, especially muscle and adipose tissue. Pari and Satheesh, (2004) found that the diabetic animals treated with *Boerhaavia diffusa* L. leaf extract showed significant reduction in hepatic gluconeogenic hexokinase enzymes like glucose-6-phosphatase and fructose-1,6-bisphosphatase. This may

also be one of the mechanisms of action of hypoglycemic effect of lotus seed extracts. Sepici *et al.* (2004) reported that the hypoglycemic effect of myrtle oil on normoglycemic and diabetic rabbits might due to increased hepatic tissue glycogen level and glucokinase enzyme activity. Muruganandan *et al.* (2005) suggested the mechanism of antidiabetic action of mangiferin which include the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic processes with concomitant decrease in glycogenolysis and gluconeogenesis. It might also reduce blood glucose level by inhibiting the glucose absorption from the intestine.

In the present study lotus seed extract reduced blood glucose levels in type II diabetic rats also. It is in agreement with the earlier finding of Chakrabarti *et al* (2002). They showed that the *db/db* mice treated with ethanolic extract of *Helicteres isora* could significantly reduce the plasma glucose and insulin levels. It might be mediated through an overall insulin sensitization in these animals. Hu *et al.* (2003) reported that single administration of Gosha-jinki-gan (GJG) in STZ induced diabetic rats significantly increased the glucose metabolic clearance rate and insulin sensitivity. It caused an increase in the glucose utilization of peripheral tissues, especially skeletal muscle which is supposed to be NO (Nitric oxide) dependent. The increased glucose transport by NO has also been shown to involve in the translocation of GLUT4 to the plasma membrane.

Aqueous extract of *Commelina communis* L reduced blood glucose levels by inhibiting alfa -glucosidase, a membrane-bound enzyme at the epithelial cells of the small intestine a key enzyme of carbohydrate digestion. Inhibition of this enzyme leads to a delayed and reduced rise in post-prandial blood glucose levels (Youn *et al.*, 2004). Furthermore Ono *et al.* (2006) reported the inhibition of other enzyme alfa- amylase which is important for digestion and absorption of carbohydrates by the phenolic compounds in the *N. nucifera* leaves. The phytochemical analysis of lotus seed extract revealed the presence of phenolic compounds in the present study also.

Jafri *et al.* (2000) also observed an improvement in oral glucose tolerance when they administered the extract of *Punica granatum* Linn flower in normal rats. They suggested that it may be due to inhibition of intestinal absorption of glucose. Thus a possibility exists that retardation of intestinal glucose absorption may also be partly responsible for inhibition of hyperglycaemia in glucose-fed rats. The extract of red and white lotus seeds extract improved oral glucose tolerance in both type I and type II diabetic rats.

5. 1. 2. Liver Glycogen

Insulin is the main regulator of glycogenesis in liver. The decrease in hepatic glycogen contents in diabetic rats have been observed earlier by others (Chakrabarti *et al.*, 2005). In the present study both type I and type II diabetic control animals showed reduction in liver glycogen levels. In type I diabetes red and white lotus seed extract treatment showed significant increase in liver glycogen levels by 25.25 and 32.07 % respectively. In type II diabetes also red and white lotus seed extract showed significant increase in liver glycogen levels by 22.11 and 33.27 % respectively.

The findings of the present study also agreed with the earlier findings of various research workers. Babu *et al.* (2003) reported that the diabetic rats treated with ethanolic extract of *Cassia kleinii* leaf increased liver glycogen. It can be due to increase in glycogenesis and/or a decrease in glycogenolysis. In the present study red and white lotus seed extracts may have similar effects in increasing the liver glycogen levels. John (2003) found an increase in liver glycogen level when the diabetic rats were administered with *Brassica juncea* and *Trigonella foenum-graecum* seeds extract and suggested that these might enhance the rate of glycogenesis in alloxan treated rats. The increase in liver glycogen might be due to the increase in cellular uptake of glucose induced by the extract, which contain phytochemicals, having insulin like properties. Musabayane *et al.* (2005) also

observed that aqueous extract of *Syzygium cordatum* leaves increased the hepatic glycogen content in diabetic rats.

5. 1. 3. GLUT 2 and GLUT 4 Gene Expressions

In hyperglycemia due to diabetes, large amounts of glucose is being presented to the proximal convoluted tubules, inducing an adaptive response to maintain high transtubular glucose flux, through modulation of GLUT2 expression. Even these adaptive changes cannot compensate for the large amount of filtered glucose in diabetics, thus, the final result is still excessive glycosuria. The main inducing factor for modulating glucose transporter expression by tubular cells has been proved to be the intracellular concentration of glucose. High glucose concentrations in the tubular epithelial cells induced by diabetes are the cause for observed increase in GLUT2 expression (Schaan *et al.*, 2005).

Glucose transport determines the rate of glucose utilization in skeletal muscles of streptozotocin induced diabetic rats. Thus, a defect in the expression of GLUT4 in skeletal muscles could explain the *in vivo* insulin-resistant glucose uptake in all diabetes models (Toide *et al.*, 1997). Physiologically the insulin signaling pathway leading to the cellular uptake of glucose begins with the binding of insulin to its receptor (IR). In the sequence, ligand binding to the IR induces autophosphorylation of specific tyrosine residues, which then activates the substrate protein tyrosine kinase of the IR. The activated IR phosphorylates several intracellular proteins, including the IRS- 1(insulin receptor substarte – 1). Tyrosine phosphorylation of the IRS-1 leads to the binding of PI 3-kinase and the activation of its enzymatic activity, a necessary step for the translocation of glucose transporter-4 (GLUT-4) to the plasma membrane, consequently elevating the rate of cellular glucose uptake. Decreased IRS-1 protein expression is involved in the development of insulin resistance (Qin *et al.*, 2003).

In this experiment GLUT2 and GLUT4 gene expression was estimated in type I and type II diabetic rats respectively. In case of type I diabetes GLUT2 gene expression was estimated in kidney. Red and white lotus seed treated group showed significant reduction in gene expression by 25.90 and 34.16 % respectively. In case of type II diabetes GLUT4 gene expression was studied in skeletal muscle. Red and white lotus seed treated group showed significant increase in this gene expression by 19.37 and 40.04 % respectively

In the present study there was an increase in GLUT2 gene expression in alloxan treated diabetic rats. The raised levels of glucose in plasma and tubular fluid stimulate the GLUT2 expression in kidney (Marks *et al.*, 2003). Lotus seed extract reduced GLUT2 expression in kidney and it may be due to reduction in blood and tubular glucose levels.

Type II diabetic animals treated with lotus seed extract showed increased GLUT4 gene expression in skeletal muscle. These results concur with earlier findings of Anandharajan *et al.* (2005). They reported an increase in expression of GLUT 4 in L 6 muscle cell line when treated with *Pterocarpus marsupium* extract and isoflavone at the transcript level. The observation noticed was similar to the effect of insulin and rosiglitazone.

5. 1. 4. Pancreatic Beta - Cells Proliferation

One of the goals of treatment in diabetes is to increase the number of functional insulin-producing beta -cells. Encouraging results have been obtained in this direction in the treatment of patients with type 1 diabetes *via* the transplantation of isolated donor islets of langerhans. This approach, however, has limitation because of the inadequate supply of donor islets available for transplantation. Another more generally applicable method to enhance the functional beta -cell mass is by stimulating the proliferation.

In pancreatectomized rats administered with ethanolic extract of red lotus seed reduced blood glucose levels by 0.06, 0.94, 8.91 and 13.33 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively were observed after treatment at the dose rate of 600 mg kg^{-1}. White lotus seed extract reduce glucose levels by 1.06, 1.19, 10.38 and 14.00 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the same dose rate. It was significant from 21^{st} day treatment onwards. Histopathological study of pancreas showed increasing number of active cell and proliferation of beta cells in red and white lotus seeds extracts treated group.

The present findings concur with the earlier findings of various research workers who also reported proliferative changes in pancreas of diabetic rats when plant extract was administered. Chakravarthy *et al.* (1980) observed an increased in beta-cells population when flavonoid fraction (XE) extracted from the bark of *Pterocarpus marsupium* Roxb. was administered to alloxanised albino rats. In the present study phytochemical analysis showed the presence of flavonoids in both type of seeds. It may be the reason for proliferation of pancreatic cells. Ahmed *et al.* (1998) found significant changes in the pattern of distribution of insulin, glucagon and somatostatin positive cells in the islet of Langerhans of *M. charantia*-treated diabetic rats. Hardikar and Bhonde (1999) noted islet neogenesis, increase in islet cell size and the attainment of normoglycemia in STZ induced diabetic BALB:c mice, when cytosolic factors from regenerating pancreas were administered. List and Habener (2004) found that Zucker diabetic fatty (ZDF) rats treated with glucogon like peptide-1 (GLP-1) increased cellular replication in insulin-positive cells. Regeneration of islets by neogenesis and proliferation of pancreatic beta- cells was noted by Leon *et al.* (2003) also when excentin-4 (Ex-4) was administered in partial pancreatetomized adult rats.

5. 1. 5. Lipids (Serum and Plasma)

Hyperlipidaemia contributes significantly in the manifestation and development of atherosclerosis and coronary heart disease (CHD). Although several factors such as diet high in saturated fats and cholesterol, age, family history, hypertension and life style plays a significant role in causing heart failure, the high levels of cholesterol particularly LDL cholesterol is mainly responsible for the onset of CHD (Choudhary *et al.*, 2005).

In the present study hypocholesterolemic effect of red and white lotus seeds extract was explored in high cholesterol diet fed rats. Red lotus seed extract reduced total cholesterol significantly by 4.08, 19.01, 27.01, and 32.96 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the dose rate of 600 mg kg^{-1}. White lotus seed extract treatment reduced total cholesterol by 6.42, 22.03, 28.13 and 35.95 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the same dose rate. It was significant from 14^{th} day onwards.

In case of LDL- cholesterol red lotus seed extract treatment reduced the levels by 5.58, 26.44, 36.90, and 46.80 % on 7^{th} 14^{th} 21^{st} and 28^{th} day respectively after treatment at the dose rate of 600 mg kg^{-1}, while white lotus seed extract reduced serum LDL cholesterol levels by 8.93, 30.10, 38.96 and 51.57 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the same dose rate. It was significant from 14^{th} day onwards.

Interestingly HDL- cholesterol levels were increased by both red and white lotus seed extracts treatment. The red lotus seed extract treatment increased serum HDL cholesterol level by 2.51, 5.17, 11.78, and 19.51 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the dose rate of 600 mg kg^{-1} and white lotus seed extract increased serum HDL cholesterol levels by 3.58, 7.24, 13.13 and 23.00 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the same dose rate. It was significant on 28^{th} day only.

Hypotriglyceridemic effect of lotus seed extract was studied in high fat diet given rat. Red lotus seed extract reduced triglyceride levels by 3.84, 13.25, 23.01, and 30.96 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the dose rate of 600 mg kg^{-1}. In case of white lotus seed extract the reduction was 6.46, 14.53, 25.52 and 36.21 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the same dose rate. It was significant from 14^{th} day onwards.

The rats administered with ethanolic extract of red lotus seed reduced free fatty acid levels were noticed by 5.14, 9.01, 12.44, and 17.43 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment. On the other hand white lotus seed extract reduced free fatty acid levels by 6.09, 11.38, 15.22 and 20.01 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the same dose rate. It was significant from 21^{st} day onwards.

The present findings also agree with the earlier findings of various research workers who recorded reduction in serum lipid level, when plant extract was administered. Ono *et al.* (2006) also reported the effect of *N. nucifera* leaves extract on lipid metabolism in high fat diet induced obesity in mice. They found that *N. nucifera* leaves extract inhibited intestinal absorption of lipid. The dietary lipid is not directly absorbed from the intestine unless it has been subjected to the action of pancreatic lipase. *N. nucifera* leaves extract has an inhibitory activity on pancreatic lipase. The phenolic compound in the extract is responsible for inhibition of this enzyme. The phytochemical analysis revealed the presence of phenolic compound in red and white lotus seeds. This may be reason for the reduction of serum total cholesterol, triglycerides and LDL-cholesterol and plasma free fatty acids in the present study.

The reduction in the level of lipids may also be due to the lipolytic effect of seed extract. Ono *et al.* (2006) proved the lipolytic effect of *N. nucifera* leaves extract and they suggested that it was mediated by beta – adrenoceptors. 3T3-L1

pre adipocytes are converted to adipocytes by insulin. The intracellular droplets of adipocytes are broken down by nor epinephrine. Catecholamines are powerful regulators of lipid mobilization and are considered to stimulate lipolysis mainly through the B_1-, B_2 - and B_3-adrenoceptors. Stimulation of the B - adrenoceptor in white adipocytes leads to increased lipolysis, primarily through the production of cAMP and activation of hormone-sensitive lipase and other pathways. *N. nucifera* leaves extract was effective in increasing the lipolytic response as B - adrenoceptor agonist.

The anti-obesity effect of the *N. nucifera* leaves extract is due to increased thermo genesis by the up-regulation of UCP_3 (Un coupling protein 3) expression. Un coupling protein 3 is expressed in skeletal muscle and brown adipose tissue, and is responsible for the regulation of energy expenditure, thermo genesis and fatty acid metabolism. The up regulation of UCP_3 in skeletal muscle provokes up-regulation of thermo genesis and fatty acid metabolism. This also may be the reason for the reduction in plasma free fatty acid levels in red and white lotus seeds extract treated group.

Chakrabarti *et al.* (2002) found reduction in plasma lipids when ethanolic extract of *Helicteres isora* was administered to high fat diet fed hamsters. They also found reduction in plasma triglyceride level in both *db/db* mice and swiss albino mice. Visavadiya and Narasimhacharya (2005) reported reduction in plasma total cholesterol, LDL and increased HDL in high cholesterol fed rats treated with *Asparagus racemosus* root powder

5. 1. 6. Liver Total Lipid

In the present study both high fat and high cholesterol fed rats showed increased levels of liver total lipid. A significant reduction in liver total lipid levels by 13.44 and 18.68 % respectively were noted in high cholesterol fed animals treated with red and white lotus seed extract. High fat fed animals treated

with red and white lotus seed extracts showed significant reduction in liver lipid levels by 27.60 and 33.81 % respectively. Visavadiya and Narasimhacharya (2005) reported an increase in hepatic lipids in animals fed with high cholesterol diet and significant reduction was noticed when rats were treated with *Asparagus racemosus* root powder. It may be due to decreased absorption of dietary cholesterol. In the present study also this may be the reason for the reduction of liver total lipid levels. Ono *et al.* (2006) found an increase in liver triglycerol levels when mice were fed with high fat diet. A reduction in liver triglycerol levels was noted in mice treated with *N. nucifera* leaves extract. It may due to inhibition of absorption of lipids and increased lipolysis by the active principle present in the extract.

5. 1. 7. Cytoprotective Effect

Superoxide dismutase (SOD) and catalase (CAT) are the two scavenging enzymes that remove the toxic free radicals. In the enzymatic antioxidant defence system, SOD is one of the most important enzymes and scavenges O_2^- anion (which is the first product of O_2 radicals) to form H_2O_2 and hence diminishes the toxic effects due to this radical or other free radicals derived from secondary reactions. The O_2^- anion is known to inactivate CAT and glutathione peroxidase (GPx). These enzyme activities were inactivated by reactive oxygen species ROS during diabetes (Manonmani *et al.*, 2005).

The rats administered with repeated dose of alloxan and treated with red lotus seed extract showed increase in superoxide dismutase levels in pancreas by 2.69, 14.57, 28.56 and 39.35 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the dose rate of 600 mg kg^{-1}. On the other hand white lotus seed extract increased superoxide dismutase levels by 12.07, 23.01, 37.06 and 46.59 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the same dose rate. It was significant from 14^{th} day onwards.

The catalase levels also increased by 4.81, 24.08, 35.69, and 42.89 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment in rat treated with ethanolic extract of red lotus seed. White lotus seed extract increased catalase levels by 9.43, 28.75, 39.85 and 48.80 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the same dose rate. It was significant from 14^{th} day onwards.

The animals treated with ethanolic extract of red lotus seed extract the glutathione peroxidase increased by 8.40, 21.35, 35.41 and 52.10 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment. White lotus seed extract increased glutathione peroxidase levels by 9.91, 25.52, 38.94 and 56.45 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the same dose rate. It was significant from 14^{th} day onwards.

In the present study, there was a duration dependent increase in anti oxidant enzymes SOD, CAT and GPx levels in pancreas of lotus seed extracts treated animals. Initially there was an increase in these enzymes in alloxan alone treated animals which then started to decrease as diabetes progressed. These results concur with findings of Kakkar *et al.* (1998). They reported an increase in SOD, CAT and GPx activity initially because of increased production of superoxide, oxidative stress and higher organic and inorganic peroxides respectively and gradual reduction later as diabetes progressed. The decreasing levels of SOD is due to glycation of enzymes which occur as diabetes progress. Decline in catalase is due to overproduction of $\cdot O_2$ in diabetes.

The increased levels of antioxidant enzymes by administration of plant extract have been observed by other research workers also. Rai *et al.* (2006) reported that administration of hydro alcoholic extract of *N. nucifera* seeds increased the SOD and CAT levels in liver and kidney of rats previously administered with CCL_4. They opined that phenolic compound of the extract may be responsible for the free radical scavenging activity. They also stated that alkaloids, saponins and phenolics present in *N. nucifera* seeds possess free radical

scavenging and antioxidant activity. In the present study also phytochemical screening of lotus seeds extract revealed the presence of phenolic compound. It may be the reason for the increased levels of antioxidant enzymes.

The present observations are further supported by the results obtained by Wang *et al.* (2003) who found that methanolic extracts of lotus plumule and blossom were strong inhibitors of oxidative damage of DNA because it protected DNA from fenton mediated DNA degradation even at concentration as low as 1 μg ml^{-1}. Ananthan *et al.* (2004) who showed increased glutathione (GSH), SOD and CAT levels in the liver and kidney of diabetic rats treated with *Gymnema montanum* leaf extract (GLEt). This may be by removing O2 $^{\bullet}$ and $OH^{\bullet}$ and is probably one of the most effective defense mechanism against diabetes. Furthermore Montilla *et al.* (2004) observed increased levels of GSH, SOD and CAT levels in the liver and kidney of diabetic rats treated with Montilla-Moriles appellation red wine. Okutan *et al.* (2005) also observed similar result when they administered caffeic acid phenethyl ester (CAPE), an active component of propolis in streptozotocin induced diabetic rats.

In diabetes, hypoinsulinaemia increases the activity of the enzyme fatty acyl coenzyme A oxidase, which initiates beta-oxidation of fatty acids, resulting in an increased lipid peroxidation. Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (Orhan *et al.*, 2005). In present study administration of alloxan alone caused increase lipid peroxidation in pancreas. When the animals were treated with red lotus seed reduced lipid peroxidation by 4.05, 12.06, 22.01 and 37.03 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the dose rate of 600 mg kg^{-1} was noticed. It was significant from 21^{st} day onwards. On the other hand white lotus seed extract reduced lipid peroxidation levels by 7.99, 17.49, 29.28 and 44.50 % on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively after treatment at the same dose rate. It was significant from 14^{th} day onwards.

The present findings were supported by the earlier observation of various research workers who recorded reduction in lipid peroxidation level in diabetic rats when plant extract was administered. Rai *et al.* (2006) found that the animals treated with alcoholic extract of *N. nucifera* seeds showed significant decrease in the level of thiobarbituric acid reactive substances (TBARS) in liver and kidney when compared to CCl_4 administered control. They suggested that presence of alkaloids, saponins and phenolics compounds in *N. nucifera* seeds are responsible for free radical scavenging and antioxidant activity. Phytochemical analysis of lotus seed in the present study also revealed the presence of alkaloids, saponins and phenolic compounds and these may be responsible for the reduction of lipid peroxidation in pancreas.

Ananthan *et al.* (2003) found that administration of *Gymnema montanum* leaf extract in diabetic rats reduces the intensity of lipid peroxidation. Similar observation was made by Montilla *et al.* (2004) while administrating of Montilla-Moriles appellation red wine. Okutan *et al.* (2005) also found that administration of caffeic acid phenethyl ester (CAPE), an active component of propolis in streptozotocin induced diabetic rats decreased the level of malondialdehyde (MDA), an end product of LPO (lipid peroxidation) significantly.

Histopathological study of pancreas showed more number of active cells and islet hyperplasia in a duration dependent fashion in red and white lotus seed extracts treated group. Ahmed *et al.* (1998) also found significant changes in the pattern of distribution of insulin, glucagon and somatostatin positive cells in the islet of Langerhans of *M. charantia* treated diabetic rats. It was also supported by the observation made by Hardikar and Bhonde (1999). They observed cytosolic factor(s) from the regenerating pancreas have the potential to induce islet neogenesis, increase in islet cell size and the attainment of normoglycemia in STZ induced diabetic animals. These neo-islets were then seen to grow into larger mature islets.

5.2. ANALYSIS OF LOTUS (*Nelumbo nucifera)* SEEDS EXTRACT FOR ACTIVE PRINCIPLES.

The phytochemical analysis of lotus seeds revealed the presence of alkaloids, flavonoids, glycosides, steroids, saponins, phenolic compounds, diterpenes and triterpenes in them. Mukherjee *et al.* (1997a) analysed and found that *N. nucifera* rhizome contains betulinic acid, a steroidal triterpenoid.

Alkaloids liensinine and its analogues, isoliensinine and neferine from embryo of the seed of lotus were isolated by Wu *et al.* (2004) employing preoperative counter current chromatography. Kashiwada *et al.* (2005) also isolated alkaloids, benzylisoquinoline, aporphine, and bisbenzylisoquinoline, including liensinine, negferine, and isoliensinine from leaves and embryo of *Nelumbo nucifera.*

Jung *et al.* (2003) isolated seven known flavonoids [kaempferol , kaempferol 3-O-beta-D-glucuronopyranosyl methylester, kaempferol 3-O-beta-D-glucopyranoside, kaempferol 3-O-beta-D-galactopyranoside, myricetin 3',5'-dimethylether 3-O-beta-D-glucopyranoside, kaempferol 3-O-alpha-L-rhamnopyranosyl beta-D-glucopyranoside and kaempferol 3-O-beta-D-glucuronopyranoside], along with beta-sitosterol glucopyranoside, from extract of the stamens of *N. nucifera.*

Ling *et al.* (2005) isolated procyanidins from lotus seedpod. Ono *et al.* (2006) found that *N. nucifera* leaves contaning phenolic compound and it inhibit the alfa – amylase and pancreatic lipase.

The active principles of *N.nucifera* seeds were also studied by Rai *et al.* (2006) and found that the seeds contain alkaloids, saponins, and phenolic compound. Phenolic compounds comprise a wide range of plant substances ranging from simple monocyclic compound to complex polyphenolic compounds.

Summary

6. SUMMARY

The present study was undertaken to assess the hypoglycemic, hypolipidaemic and cytoprotective effects of ethanolic extract of red and white lotus seed. The study was carried out in different models and compared with the respective reference drug.

The experiment was conducted in two hundred and seventy six male Sprague-Dawley rats weighing 150-200 gm. Overall six experiments were carried out in the present investigation. For the first five experiments there were six animals per group and for the 6^{th} experiment four large groups of 30 animals each, which was subdivided into five groups of six animals each. In all the experiments Group I and Group II served as normal and experimental control respectively. Group III and Group IV were experimental groups and were administered with red and white lotus seeds extract at the rate of 600 mg kg^{-1} respectively. Group V served as positive control and was administered with respective reference drug. In case of pancreatic beta cells proliferation study additionally one more group was treated with reference drug.

Thirty adult Sprague-Dawley rats were used for the hypoglycemic study. Type I diabetes was induced by administrating alloxan at the rate of 130 mg kg^{-1} i. p. to adult rats and type II diabetes was induced by administrating alloxan at the rate of 200 mg kg^{-1} i. p. to 6 days old rat pups. At the age of 8 weeks the pups developed type II diabetes. In type I and type II diabetes, animals were treated with red and white lotus seed extract for 28 days. Blood glucose was estimated on 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of experiment.

In the case of type I diabetes red and white lotus seeds extract treated groups showed significant reduction in blood glucose levels from 14 days treatment onwards. The metformin treated group showed significant reduction in blood glucose level

from 7th day treatment onwards. There was significant increase in liver glycogen, glucose tolerance and significant reduction in GLUT2 expression in all treated groups. In the case of type II diabetes the extracts showed significant reduction in blood glucose from 21 days treatment onwards. The metformin treated group showed significant reduction in blood glucose level from 7th day treatment onwards. There was significant increase in liver glycogen, glucose tolerance and GLUT4 in all treated groups.

To explore the pancreatic *beta* cells proliferation effect lotus seed extract was administered to pancreatectomized rats. Approximately 70% of the pancreas was removed surgically. Pancreatectomized animals were treated with red and white lotus seed extracts for 28 days. Blood glucose was estimated on 0, 7th, 14th, 21st and 28th day of experiment. All treated groups showed significant reduction in blood glucose levels from 21st day of treatment onwards. Histopathological examination of pancreas showed proliferative changes in all the treated groups.

To find out the hypocholesterolemic effect of lotus seeds, extract was administered to hypercholesterolemic rat. Hypercholesterolemia was induced by providing high cholesterol diet for 7 days before and through out the period of experiment. Hypercholesterolemic animals were treated with red and white lotus seed extract for 28 days. Total cholesterol and HDL-cholesterol was estimated on 0, 7th, 14th, 21st and 28th days treatment. LDL cholesterol was calculated from the standard formula. Significant reduction in total cholesterol and LDL-cholesterol was observed from 14 days of treatment onwards in extract treated group and in the case of simvastatin treated group significant reduction was observed from 7 days onwards. Both the extract treated groups showed an increase in HDL-cholesterol level and it was significant on 28 days treatment. In the case of simvastatin treated group significant increase was observed from 7 days treatment onwards. A significant reduction in liver total lipid was observed in all treated groups.

The hypotriglyceridemic effect of lotus seed extract was explored by administration of extract to hypertriglyceridemic rats. Hypertriglyceridemia was induced by feeding high fat diet for 21 days before and through out the period of experiment. Hypertriglyceridemic animals were treated with red and white lotus seed extract for 28 days. Triglyceride and free fatty acids was estimated on 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} days of treatment. A significant reduction in triglycerides was observed from 14 days of treatment onwards. In case of fenofibrate treated group significant reduction of triglycerides was observed from 7 days onwards. The free fatty acid levels were significantly reduced from 21 days of treatment onwards in red and white lotus seed treated animals. In the case of fenofibrate treated group significant reduction was observed from 14 days of treatment onwards. A significant reduction in liver total lipid was observed in all treated groups.

Cytoprotective effect of red and white lotus seed extract was studied in repeated dose alloxan treated pancreatitis rats. They were divided into four large groups (A, B, C and D) of thirty animals each and each group was further divided into five small groups of six animals each. Animals in group A, B, C and D of each large groups were sacrificed on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively. Animals were sacrificed and pancreas was collected for estimation of superoxide dismutase, catalase, glutathione peroxidase and lipid peroxidation.

Superoxide dismutase, catalase and glutathione peroxidase levels increased in all the treated groups and it was significant from 14 days of treatment onwards. The lipid peroxidation levels decreased in all the treated group and it was significant from 21 and 14 days treatment onwards in red and white lotus seed extract treated groups respectively. In the case of metformin treated group significant reduction was observed from 14 days of treatment onwards. Histopathological study of pancreas

showed more number of active cells and islet hyperplasia in a duration dependent fashion in all treated group.

Phytochemical analysis of the extract of red and white lotus seeds was carried out. Steroids, alkaloids, flavonoids, diterpenes, phenolic compounds, saponins, triterpenes and glycosides were detected in the extract

From the present study it is concluded that the ethanolic extract of lotus seeds have hypoglycemic, hypolipidaemic and cytoprotective effect in rats. However the effects are less than that of respective reference drug. To elucidate the exact mechanism of action of all these effects further detailed investigation is needed.

References

REFERENCES

Aguilar, F.J.A., Bermejo, F.C., Galicia, E.H., Angeles, C.R and Ramos, R.R. 2005. Acute and chronic hypoglycemic effect of *Ibervillea sonorae* root extracts-II. *J. Ethnopharmacol.* 97: 447–452

Ahmed, I., Adeghate, E., Sharma, A.K., Pallot, D.J and Singh, J. 1998. Effects of *Momordica charantia* fruit juice on islet morphology in the pancreas of the Streptozotocin-diabetic rat. *Diabetes Res. Clin. Prac.* 40: 145–151

Akiyama, T., Tachibana, I., Shirohara, H., Watanabe, N and Otsuki, M. 1996. High-fat hypercaloric diet induces obesity, glucose intolerance and hyperlipidemia in normal adult male Wistar rat. *Diabetes Res. Clin. Prac.* 31: 27-35

Allain, C.C., Poon, J.S and Chan, C.S.G. 1974. Enzymatic determination of serum cholesterol. *Clin.Chem.* 20: 470-475

Anandharajan, R., Pathmanathan, K., Shankernarayanan, N.P., Vishwakarma, R.A and Balakrishnan, A. 2005. Upregulation of Glut-4 and PPARγ by an isoflavone from *Pterocarpus marsupium* on L6 myotubes: a possible mechanism of action. *J. Ethnopharmacol.* 97: 253–260

Ananthan, R., Baskar, C., Narmathabai, V., Pari, L., Latha, M and Ramkumar, K.M. 2003. Antidiabetic effect of *Gymnema montanum* leaves: effect on lipid peroxidation induced oxidative stress in experimental diabetes. *Pharmacol. Res.* 48: 551–556

Ananthan, R., Latha, M., Ramkumar, M., Pari, L., Baskar, C and Narmathabai, V. 2004. Modulatory effects of *Gymnema montanum* leaf extract on alloxan-induced oxidative stress in Wistar rats. *Basic Nutri. Invest.* 20: 280-285

Babu, V., Gangadevi, T and Subramoniam, A. 2002. Anti-hyperglycaemic activity of *Cassia kleinii* leaf extract in glucose fed normal rats and alloxan-induced diabetic rats. *Indian J. Pharmacol.* 34: 409-415

Babu, V., Gangadevi, T and Subramoniam, A. 2003. Antidiabetic activity of ethanol extract of *Cassia kleinii* leaf in streptozotocin-induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. *Indian J. Pharmacol.* 35: 290-296

Bachorik, P.S., Wood, P.D., Albers, J.J., Steiner, P., Dempsey, M., Kuba, K., Warnick, R and Karlsson, L. 1976. Plasma high-density lipoprotein cholesterol concentrations determined after removal of other lipoproteins by heparin/manganese precipitation or by ultracentrifugation. *Clin. Chem.* 22: 1828 –1834

Bancroft, J.D and Cook, H.C. 1984. *Manual of Histological Techniques and their Diagnostic Application.* Second edition. Churchill Livingstone, Edinburgh, 457 p.

Beppu, H., Koike, T., Shimpo, K., Chihara, T., Hoshino, M., Ida, C and Kuzuya, H. 2003. Radical-scavenging effects of *Aloe arborescens* Miller on prevention of pancreatic islet B-cell destruction in rats. *J. Ethnopharmacol.* 89: 37–45

Bhandari, U., Kanojia, R and Pillai, K.K. 2005. Effect of ethanolic extract of *Zingiber officinale* on dyslipidaemia in diabetic rats. *J. Ethnopharmacol.* 97: 227–230

Bopanna, K.N., Kannan, J., Gadgil, S., Balaraman, R and Rathod, S.P. 1997. Antidiabetic and antihyperlipaemic effects of neem seed Kernel powder on alloxan diabetic rabbits. *Indian J. Pharmacol.* 29: 162-167

Bucolo, G and David, H. 1973. Quantitative determination of serum triglyceride by the use of enzyme. *Clin. Chem.* 19: 476-482

Carroll, N.V., Longley, R.W and Roe, J.H. 1956. The determination of glycogen in liver and muscle by use of anthrone reagent. *J. Biol. Chem.* 200: 583-593

Chakrabarti, S., Biswas, K.T., Seal, T., Rokeya, B., Ali, L., Khan, A.K.A., Nahar, N., Mosihuzzamand, M and Mukherjee, B. 2005. Antidiabetic activity of *Caesalpinia bonducella* F. in chronic type 2 diabetic model in Long-Evans rats and evaluation of insulin secretagogue property of its fractions on isolated islets. *J. Ethnopharmacol.* 97: 117–122

Chakrabarti, R., Vikramadithyan, R.K., Mullangi, R., Sharma, V.M., Jagadheshan, H., Rao, Y.N., Sairam, P and Rajagopalan, R. 2002. Antidiabetic and hypolipidemic activity of *Helicteres isora* in animal Models. *J. Ethnopharmacol.* 81: 343-349

Chakravarthy, B.K., Gupta, S., Gambhir, S.S and Gode, K.D. 1980. Pancreatic beta-cell regeneration - a novel antidiabetic mechanism of *Pterocarpus marsupium,* Roxb. *Indian J. Pharmacol.* 12: 123-127

Chomczynski, P and Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162 (1): 156-9

Choudhary, M.I., Naheed, S., Jalil, S., Alam, J.M and Rahman, A. 2005. Effects of ethanolic extract of *Iris germanica* on lipid profile of rats on a high-fat diet. *J. Ehanopharmacol.* 98(1-2): 217-220

Colivicchi, M.A., Raimondi, L., Bianchi, L., Tipton, K.F., Pirisino, R and Corte, L.D. 2004. Taurine prevents streptozotocin impairment of hormone-stimulated glucose uptake in rat adipocytes. *European J. Pharmacol.* 495: 209–215

Coskun, O., Kanter, M., Korkmaz, A and Oter, S. 2005. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta cell damage in rat pancreas. *Pharmacol. Res.* 51: 117–123

Cour, L.B., Molgaard, P and Yi, Z. 1995. Traditional Chinese medicine in treatment of hyperlipidaemia. *J.Ethnopharmacol.* 46 (2): 125-129

Degirmenci, I., Ustuner, M.C., Kalender, Y., Kalender, S and Gunes, H.V. 2005. The effects of acarbose and *Rumex patientia* L. on ultrastructural and biochemical changes of pancreatic B cells in streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* 97: 555–559

Dhandapani, S., Subramanian, V.R., Rajagopal, S and Namasivayam, N. 2002. Hypolipidemic effect of *Cuminum cyminum* l. on alloxan-induced diabetic rats *Pharmacol. Res.* 46: 251-255

Diatewa, M., Samba, C.B., Assah, T.C.H and Abena, A.A. 2004. Hypoglycemic and antihyperglycemic effects of diethyl ether fraction isolated from the aqueous extract of the leaves of *Cogniauxia podoleana* Baillon in normal and alloxan-induced diabetic rats. *J. Ethnopharmacol.* 92: 229–232

Eddouks, M., Lemhadri, A and Michel, J.B. 2004. Caraway and caper: potential anti- hyperglycaemic plants in diabetic rats. *J. Ethnopharmacol.* 94: 143–148

Eddouks, M., Lemhadri, A., Zeggwagh, N.A and Michel, J.B. 2005. Potent hypoglycaemic activity of the aqueous extract of *Chamaemelum nobile* in normal and streptozotocin-induced diabetic rats. *Diabetes Res. Clin. Prac.* 67: 189–195

Folch, J., Less, M., Solane, S.G. 1957. A simple method for isolation and purification of total lipids from animal tissue. *J. Biol .Chem.* 26: 497-509

Fraga, C.G., Leibovitz, B.E and Toppel, A.L. 1988. Lipid peroxidation measured as TBARS in tissue slices. Characterisation and comparison with homogenates and microsomes. *Free Radic. Biol. Med.* 4: 155

Fuliang, H.U., Hepburn, H.R., Xuan, H., Chen, M., Daya, S and Radloff, S.E. 2005. Effects of propolis on blood glucose, blood lipid and free radicals in rats with diabetes mellitus. *Pharmacol. Res.* 51: 147–152

Guerra, S.D., Lupi, R., Marselli, L., Masini, M., Bugliani, M., Sbrana, S., Torri, S., Pollera, M., Boggi, U., Mosca, F., Prato, S.D and Marchetti, P. 2005. Functional and molecular defects of pancreatic islets in human type 2 diabetes. *Diabetes* 54: 727-735

Gupta, M., Mazumdar, U.K., Mukhopadhyay, R.K and Sarkar, S. 1996. Antisteroidogenic effect of seed extract of *N .nucifera* in the testis and ovary of the rat. *Indian J. Pharm. Sci.* 58 (6): 236-242

Harborne, J.B. 1991. *Phytochemical methods- Guide to modern techniques of plant analysis*. Second edition. Chapman and Hall, India, 653 p.

Hardikar, A.A and Bhonde, R.R. 1999. Modulating experimental diabetes by treatment with cytosolic extract from the regenerating pancreas. *Diabetes Res. Clin. Prac.* 46: 203-211

Hron, W and Menahan, L.A. 1981. A sensitive method for the determination of free fatty acids in plasma. *J. lipid Res.* 22: 377-381

Hu, X., Sato, J., Oshida, Y., Xu, M., Bajotto, G and Sato, Y. 2003. Effect of Gosha-jinki-gan (Chinese herbal medicine: Niu-Che-Sen-Qi-Wan) on insulin resistance in streptozotocin-induced diabetic rats. *Diabetes Res. Clin. Prac.* 59: 103-111

Hyvarien, A and Nikila, E.A. 1962. *O*-Toluidine method of determination of blood glucose level. *Clin. Chem. Acta*. 7: 140

Jafri, M.A., Aslam, M., Javed, K and Singh, S. 2000. Effect of *Punica granatum* Linn. (flowers) on blood glucose level in normal and alloxan-induced diabetic rats. *J. Ethnopharmacol.* 70: 309–314

John, P. 2003. The hypoglycaemic effect of *Brassica juncea* (Mustard) and Trigonella *foenum-graecum* (Fenugreek) in albino rats. M.V.Sc thesis, Kerala Agricultural University, Trichur, 96 p.

Ju, J.S., Smith, J.L., Oppelt, P.J and Fisher, J.S. 2005. Creatine feeding increases GLUT4 expression in rat skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 288: E347–E352

*Jung, H.A., Kim J.E., Chung H.Y and Choi, J.S. 2003. Antioxidant principles of *Nelumbo nucifera* stamens. *Arch Pharm Res.* 26(4): 279-85

Kakkar, R., Mantha, S.V., Radhi, J and Prasad, K. 1998. Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. *Clin. Science*. 94: 623-632

*Kashiwada, Y., Aoshima, A., Ikeshiro, Y., Chen, Y.P., Furukawa, H., Itoigawa, M., Fujioka, T., Mihashi, K., Cosentino, L.M., Natschke, S.L and Lee, K.H. 2005. Anti-HIV benzylisoquinoline alkaloids and flavonoids from the leaves of *Nelumbo nucifera*, and structure-activity correlations with related alkaloids. *Bioorg. Med. Chem.* 13 (2): 443-8

Kesari, A.N., Gupta, R.K and Watal, G. 2005. Hypoglycemic effects of *Murraya koenigii* on normal and alloxan-diabetic rabbits. *J. Ethnopharmacol.* 97: 247–251

Khan, M.A and Singh, V. K. 1996. A folklore survey of some plants of Bhopal District forests, Madhya Pradesh, India, described as antidiabetic. *Fitoterapia* 5: 416-421

Khosla, P., Gupta, D.D and Nagpal, R.K. 1995. Effect of *Trigonella foenum graecum* (Fenugreek) lipids in normal and diabetic rats on serum. *Indian J. Pharmacol.* 27: 89 – 93

Kritikar, K.R and Basu, B.D. 1975. Indian Medicinal Plants. Second edition. Periodical Experts, Delhi, pp116-120

Kodama, T., Iwase, M., Nunoi, K., Maki, Y., Yoshinari, M and Fujishima, M. 1993. A new diabetes model induced by neonatal alloxan treatment in rats. *Diabetes Res. Clin. Prac.* 20 (3): 183-189

Le, P.M., Andaloussi, A.B., Elimadi, A., Settaf, A., Cherrah, Y and Haddad, P.S. 2004. The petroleum ether extract of *Nigella sativa* exerts lipid-lowering and insulin-sensitizing actions in the rat. *J. Ethnopharmacol.* 94: 251–259

Lee, M.K., Kim, M.J., Cho, S.Y., Park, S.A., Park, K.K., Jung, U.J., Park, H.M and Choi, M.S. 2005. Hypoglycemic effect of Du-zhong (*Eucommia ulmoides* Oliv) leaves in streptozotocin-induced diabetic rats. *Diabetes Res. Clin. Prac.* 67: 22–28

Leon, D.D.D., Deng, S., Madani, R., Ashima, R.S and Drucker, D.J. 2003. Role of endogenous glucagon- like peptide-1 in islet regeneration after partial pancreatectomy. *Diabetes* 62: 365-371

*Ling, Z.Q., Xie, B.J and Yang, E.L. 2005. Isolation, characterization and determination of antioxidative activity of oligomeric procyanidins from the seedpod of *Nelumbo nucifera* gaertn. *J. Agric. Food Chem.* 53 (7): 2441-2445

List, J.F and Habener, J.F. 2004. Glucagon-like peptide 1 agonists and the development and growth of pancreatic beta-cells. *Am. J. Physiol. Endocrinol. Metab.* 286: 875– 881

Liu, C.P., Tsai, W.J., Lin, Y.L., Liao, J.F., Chen, C.H., Kuo, Y.C. 2004. The extracts from *Nelumbo nucifera* suppress cell cycle progression, cytokine genes expression, and cell proliferation in human peripheral blood mononuclear cells. *Life Sci.* 75(6): 699-716

Lowry, O.H., Rosenberg, N.J., Fair, A.L and Randall, R.J. 1951. Protein measurement with folin-phenol reagent. *J. Biol. Chem.* 193: 265-275

Maghrani, M., Zeggwagh, N.A., Lemhadri, A., Amraoui, M.E., Michel, J.B and Eddouks, M. 2004. Study of the hypoglycaemic activity of *Fraxinus excelsior* and *Silybum marianum* in an animal model of type 1 diabetes mellitus. *J. Ethnopharmacol.* 91: 309–316

Maiti, R., Jana, D., Das, U.K., Ghosh, U.K. 2004. Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* 92: 85–91

Manonmani, G., Bhavapriya, V., Kalpana, S., Govindasamy, S and Apparanantham, T. 2005. Antioxidant activity of *Cassia fistula* (Linn.) flowers in alloxan induced diabetic rats. *J. Ethnopharmacol.* 97: 39–42

Marks, J., Carvou, N.J.C., Debnam, E.S., Srai, S.K and Unwin, R.J. 2003. Diabetes increases facilitated glucose uptake and GLUT2 expression at the rat proximal tubule brush border membrane. *J. Physiol.* 553: 137-145

Maroo, J., Vihas, T., Vasu, V.T., Aalinkeel, R and Gupta, S. 2002. Glucose lowering effect of aqueous extract of *Enicostemma littorale* Blume in diabetes: a possible mechanism of action. *J. Ethnopharmacol.* 81: 317-320

Mazumder, U.K., Gupta, M., Pramanick, G., Muchopadhyay, R.K and Sarkar, S. 1992. Antifertility activity of seed of *Nelumbo nucifera* in mice. *Indian J. Exp. Biol.* 30(6): 533-534

Mimami, M and Yoshikawa, H. 1979. A simplified assay method of superoxide dismutase activity for clinical use. *Clin. Chem. Acta.* 92: 337-342

Mini, K.P. 1992. Hypolipidaemic effect of *Allium sativum* and *Emblica officinalis* in rabbits. M.V.Sc thesis, Kerala Agricultural University, Trichur, 64 p.

Mitra, R., Mehrotra, S., Kapoor, L.D. 1973. Medicinal Plants. *Indian J. Pharm.* 35: 207

Mohamad, S., Taha, A., Bamezai, R.N., Basir, S.F and Baquer, N.Z. 2004. Lower doses of vanadate in combination with *Trigonella* restore altered carbohydrate metabolism and antioxidant status in alloxan-diabetic rats. *Clin. Chim. Acta.* 342: 105– 114

Montilla, P., Barcos, M., Munoz, M.C., Castaneda, J.R., Bujalance, I and Tunez, I. 2004. Protective effect of Montilla-Moriles appellation red wine on oxidative stress induced by streptozotocin in the rat. *J. Nutri. Biochem.* 15: 688–693

Mukherjee, P.K., Balasubramanian, R., Pal, M and Saha, B.P. 1996. Studies on psycho-pharmacological effects of *N.nucifera* rhizome extract. *J. Ethnopharmacol.* 54: 63-67

Mukherjee, P.K., Das, J., Bala, R., Saha, K., Pal, M and Saha, B.P. 1995a. Antidiarrhoeal evaluation of *N.nucifera* rhizome extract. *Indian J. Pharmacol.* 27(4): 262- 264

Mukherjee, P.K., Das, J., Saha, K., Giri, S.N., Pal, M and Saha, B.P. 1996a. Antipyretic activity of *N.nucifera* rhizome extract. *Indian J. Exp. Biol.* 34: 275-276

Mukherjee, P.K., Giri, S.N., Saha, K., Pal, M and Saha, B.P. 1995. Antifungal screening of *N.nucifera* rhizome extract. *Indian J. Microbio.* 35(4): 327-330

Mukherjee, P.K., Saha, K., Das, J., Pal, M., Saha, B.P. 1997a. Studies on the anti-inflammatory activity of rhizomes of *Nelumbo nucifera. Planta Med.* 63(4): 367-369

Mukherjee, P.K., Saha, K., Pal, M., Saha, B.P. 1997. Effect of *Nelumbo nucifera* rhizome extract on blood sugar level in rats. *J. Ethnopharmacol.* 58(3): 207-213

Murali, B., Upadhyaya, U.M and Goyal, R.K. 2002. Effect of chronic treatment with *Enicostemma littorale* in non-insulindependent diabetic (NIDDM) rats. *J. Ethnopharmacol.* 81: 199-204

Murthy, B.K., Nammi, S., Kota, M.K., Rao, R.V.K., Rao, N.K and Annapurna, A. 2004. Evaluation of hypoglycemic and antihyperglycemic effects of *Datura metel* (Linn.) seeds in normal and alloxan-induced diabetic rats. *J. Ethnopharmacol.* 91: 95–98

Muruganandan, S., Srinivasan, K., Gupta, S., Gupta, P.K and Lal, J. 2005. Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. *J. Ethnopharmacol.* 97: 497–501

Musabayane, C.T., Mahlalela, N., Shode, F.O and Ojewole, J.A.O. 2005. Effects of *Syzygium cordatum* (Hochst.) [Myrtaceae] leaf extract on plasma glucose and hepatic glycogen in streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* 97: 485–490

Nascimento, N.R.F., Forti, A.C., Peter, A.A and Fonteles, M.C. 2003. Free radical scavengers improve the impaired endothelium-dependent responses in aorta and kidneys of diabetic rabbits. *Diabetes Res. Clin. Prac.* 16: 145-153

Noda, Y., Kneyuki, T., Igarashi, K., Mori, A and Packer, L. 2000. Antioxidant activity of nasunin, an anthocyanin in eggplant peels. *Toxicology* 148: 119–123

Ogneva, V and Martinova, Y. 2002. The effect of in vitro fibroblast growth factors on cell proliferation in pancreas from normal and streptozotocin-treated rats. *Diabetes Res. Clin. Prac.* 57: 11–16

Okutan, H., Ozcelik, N., Yilmaz, H.R and Uz, E. 2005. Effects of caffeic acid phenethyl ester on lipid peroxidation and antioxidant enzymes in diabetic rat heart. *Clin. Biochemi.* 38: 191– 196

Ono, Y., Hattori, E., Fukaya, Y., Imai, S and Ohizumi, Y. 2006. Anti-obesity effect of *Nelumbo nucifera* leaves extract in mice and rats. *J. Ethnopharmacol.* 106: 238–244

Orhan, D.D., Aslan, M., Sendogdu, N.U., Ergun, F and Yesilada, E. 2005. Evaluation of the hypoglycemic effect and antioxidant activity of three *Viscum album* subspecies (European mistletoe) in streptozotocin-diabetic rats. *J. Ethnopharmacol.* 98: 95–102

Pari, L and Latha, M. 2004a. Protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipid peroxidation in STZ diabetic male Wistar rats. *BMC Complementary and Alternative Med.* 4:1-8

Pari, L and Maheswari, J.U. 1999. Hypoglycaemic effect of *Musa sapientum* L. in alloxan-induced diabetic rats. *J. Ethnopharmacol.* 68: 321–325

Pari, L and Satheesh, M.A. 2004. Antidiabetic activity of *Boerhaavia diffusa* L.: effect on hepatic key enzymes in experimental diabetes. *J. Ethnopharmacol.* 91: 109–113

Prince, P.S.M., Kamalakkannan, N and Menon, V.P. 2004. Antidiabetic and antihyperlipidaemic effect of alcoholic *Syzigium cumini* seeds in alloxan induced diabetic albino rats. *J. Ethnopharmacol.* 91: 209–213

Prince, P.S.M., Menon, V.P and Gunasekaran, G. 1999. Hypolipidaemic action of *Tinospora cordifolia* roots in alloxan diabetic rats. *J. Ethnopharmacol.* 64: 53–57

*Qian, J.Q. 2002. Cardiovascular pharmacological effects of bisbenzylisoquinoline alkaloid derivatives. *Acta Pharmacol. Sin.* 23(12): 1086-92

Qin, B., Nagasaki, M., Ren, M., Bajotto, G., Oshida, Y and Sato, Y. 2003. Cinnamon extract (traditional herb) potentiates in vivo insulin regulated glucose utilization via enhancing insulin signaling in rats. 62: 139-148

Rai, S., Wahile, A., Mukherjee, K., Saha, B.P and Mukherjee, P.K. 2006. Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds. *J. Ethnopharmacol.* 104 (3): 322-7

Rao, B.K., Kesavulu, M.M and Apparao, C. 2001. Antihyperglycemic activity of *Momordica cymbalaria* in alloxan diabetic rats. *J. Ethnopharmacol.* 78: 67–71

Risbud, M.V and Bhonde, R.R. 2002. Models of pancreatic regeneration in diabetes. *Diabetes Res.Clin. Prac.* 58: 155-165

Rotruck, J.T., Pope, A.L., Ganther, H.E and Swanson, A.B. 1973. Selinium: biochemical role as a componenet of glutathione peroxidase. *Science* 179: 588

Ruzaidi, A., Amin, I., Nawalyah, A.G., Hamid, M and Faizul, H. A. 2005. The effect of Malaysian cocoa extract on glucose levels and lipid profiles in diabetic rats. *J. Ethnopharmacol.* 98: 55–60

Saiki, R. K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A and Arnheim, N. 1985. Enzymatic amplification of betaglobin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* (Wash. DC). 230:1350-1354.

Sambrook, J., Fritsch, E.F and Maniatis, T. 1989. *Molecular cloning - a laboratory manual.* Second edition. Cold spring harbor laboratory press, New York, 14.5 p.

Sathyan, A. 2004. Comparative study of *Azhardiracta indica* (Neem), *Ocimum sanctum* (Tulsi) and *Tinospora cordifolia* (Chittamruthu) and their combination in diabetic rats. M.V.Sc thesis, Kerala Agricultural University, Trichur, 50 p.

Schaan, B.D.A., Irigoyen, M.C., Lacchini, S., Moreira, E.D., Schmid, H and Machado, U.F. 2005. Sympathetic modulation of the renal glucose transporter GLUT2 in diabetic rats. *Auton. Neurosci.* 117 (1): 54-61

Sepici, A., Gurbuz, I., Cevik, C and Yesilada, E. 2004. Hypoglycaemic effects of myrtle oil in normal and alloxan-diabetic rabbits. *J. Ethnopharmacol.* 93: 311-318

Sheehan, D.C and Hrapchak, B.B. 1980. *Theory and Practice of Histotechnology.* Second edition. C.V. Monsby Co., St. Louis, Toronto, London, 481p.

Shirwaikar, A., Rajendran, K and Punitha, I.S.R. 2005. Antidiabetic activity of alcoholic stem extract of *Coscinium fenestratum* in streptozotocin-nicotinamide induced type 2 diabetic rats. *J. Ethnopharmacol.* 97: 369–374

Singh, N., Kamath, V and Rajini, P.S. 2005. Attenuation of hyperglycemia and associated biochemical parameters in STZ-induced diabetic rats by dietary supplementation of potato peel powder. *Clin. Chim Acta.* 353: 165–175

Sinha, K.A. 1972. Colorimetric assay of catalase. *Anal Biochem.* 47: 389

Sinha, S., Mukherjee, P.K., Mukherjee, K., Pal, M., Mandal, S.C and Saha, B.P. 2000. Evaluation of antipyretic potencial of *Nelumbo nucifera* stalk extract. *Phytother. Res.* P: 424-425

Snedecor, G. W and Cochran, W. G. 1985. *Statistical Methods.* Eighth Edition, Oxford and IBH Publishing Company, Calcutta, 534 p.

Sohn, D.H., Kim, Y.C., Oh, S.H., Park, E.J., Li, X and Lee, B.H. 2003. Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera.* *Phytomedicine* 10(2-3): 165-169

Soto, C.P., Perez, B.L., Liliana P. Favari L.P and Reyes, J.L. 1998. Prevention of alloxan-induced diabetes mellitus in the rat by silymarin. *Comp. Biochem. Physiol.* 119: 125–129

Stanely, P., Prince, M and Menon, V.P. 2000. Hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats. *J. Ethnopharmacol.* 70: 9–15

Subramoniam, A., Pushpangadan, P., Rajasekharan, S., Evans, D.A., Latha, P.G and Valsaraj, R. 1996. Effects of *Artemisia pallens* Wall on blood glucose levels in normal and alloxan-induced diabetic rats. *J. Ethnopharmacol.* 50: 13-17

Sy, G.Y., Ciss, A., Nongonierma, R.B., Sarr, M., Mbodj, N.A., Faye, B. 2005 Hypoglycaemic and antidiabetic activity of acetonic extract of *Vernonia colorata* leaves in normoglycaemic and alloxan-induced diabetic rats. *J. Ethnopharmacol.* 98: 171–175

Talukder, M.J and Nessa, J. 1998. Effect of *Nelumbo nucifera* rhizome extract on the gastrointestinal tract of rat. *Bangladesh Med. Res. Counc. Bull.* 24(1) :6-9

Taskinen, M.R. 2003. LDL-cholesterol, HDL-cholesterol or triglycerides which is the culprit? *Diabetes Res. Clin. Prac.* 61: S19-S26

Toide, K., Man, Z.W., Asahi, Y., Sato, T., Nakayama, N., Noma, Y., Oka, Y and Shima, K. 1997. Glucose transporter levels in a male spontaneous non-insulin-dependent diabetes mellitus rat of the Otsuka Long–Evans Tokushima Fatty strain. *Diabetes Res. Clin. Prac.* 38: 151–160

Vats, V., Grover, J.K and Rathi, S.S. 2002. Evaluation of anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum* Linn, *Ocimum sanctum* Linn and *Pterocarpus marsupium* Linn in normal and alloxanized diabetic rats. *J. Ethnopharmacol.* 79: 95–100

Viana, G., Medeiros, A., Michelle, A., Lacerda, R., Kalyne, L., Leal, A.M., Vale, T.G and Matos, F. 2004. Hypoglycemic and anti-lipemic effects of the aqueous extract from *Cissus sicyoides*. *BMC. Pharmacol.* 4: 9

Visavadiya, N and Narasimhacharya, A.R. 2005. Hypolipidemic and antioxidant activities of *Asparagus racemosus* in hypercholesterimic rats. *Indian J. Pharmacol.* 37(6): 376-380

Wang, J.L., Nong, Y and Jing M.X. 1992. Effects of liensinine on haemodynamics in rats and the physiologic properties of isolated rabbit atria. *Yao Xue Xue Bao.* 27(12): 881-5

Wang, L., Yen, J.H., Liang, H.L and Wu, M.J. 2003. Antioxidant effect of methanol extracts from lotus plumule and blossom (*Nelumbo nucifera* Gertn.). *J. Food and Drug Anal.*11: 60-66

Wu, S., Sun, C., Cao, X., Zhou, H., Hong, Z and Pan, Y. 2004. Preparative counter-current chromatography isolation of liensinine and its analogues from embryo of the seed of *Nelumbo nucifera* Gaertn. using upright coil planet centrifuge with four multilayer coils connected in series. *J Chromatogr A.* 1041(1-2):153-62

Xie, J.T., Wang, A., Mehendale, S., Wu, J., Aung, H.H., Dey, L., Qiu, S and Yuan, C.S. 2003. Anti-diabetic effects of *Gymnema yunnanense* extract. *Pharmacol. Res.* 47: 323–329

Yadav, S.P., Vats,V., Ammini, A.C and Grover, J.K. 2004. *Brassica juncea* (Rai) significantly prevented the development of insulin resistance in rats fed fructose-enriched diet. *J. Ethnopharmacol.* 93: 113–116

Youn, J.Y., Park, H.Y., Cho, K.H. 2004. Anti-hyperglycemic activity of *Commelina communis* L.: inhibition of alfa – glucosidase. *Diabetes Res. Clin. Prac* 66S: S149 – S155

Yu, J and Hu, W.S. 1997. Effects of neferine on platelet aggregation in rabbits. *Yao Xue Xue Bao*. 32(1): 1-4

Zhang, R., Zhou, J., Jia, Z., Zhang, Y and Gu, G. 2004. Hypoglycemic effect of *Rehmannia glutinosa* oligosaccharide in hyperglycemic and alloxan-induced diabetic rats and its mechanism. *J. Ethnopharmacol.* 90: 39–43

Zoeliner, N. Z. 1962. Colorimetric estimation of total lipids. *Gen. Exp. Med.* 135: 545

*Originals not consulted

HYPOGLYCEMIC, HYPOLIPIDAEMIC AND CYTOPROTECTIVE EFFECTS OF LOTUS SEEDS (*Nelumbo nucifera*) IN SPRAGUE-DAWLEY RATS

JEYAMURUGAN. M.

Abstract of the thesis submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2007

Department of Pharmacology and Toxicology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

ABSTRACT

A study was undertaken to assess the hypoglycemic, hypolipidaemic and cytoprotective effects of ethanolic extract of red and white lotus seeds in male Sprague-Dawley rats. It was carried out in different animal models and compared with respective reference drug. The extracts of red and white lotus seeds were administered at the rate of 600 mg kg^{-1} for 28 days.

The experiment was carried out in two hundred and seventy six rats. The blood glucose, serum total cholesterol, serum HDL, serum triglycerides and plasma free fatty acids was estimated on 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment in the respective animal models. The LDL cholesterol was calculated with help of formula.

Type I diabetic rats treated with lotus seeds (red and white) extract showed significant reduction in blood glucose from 14^{th} day onwards and in case of metformin from 7^{th} day onwards. On 28^{th} day oral glucose tolerance test and on 29^{th} day liver glycogen and GLUT2 gene expressions were carried out. A significant increase in liver glycogen and reduction in GLUT2 gene expression was observed in all the treated groups. Glucose tolerance also showed significant improvement in all the treated group.

In case of type II diabetic rats there was significant reduction in glucose levels from 21^{st} day of treatment in extracts treated groups. Metformin treated group showed significant reduction from 7^{th} day onwards. There was significant increase in liver glycogen, GLUT4 gene expression and glucose tolerance in all the treated groups.

The pancreatectomized rats showed significant reduction in blood glucose levels from 21^{st} day onwards in all the treated groups. Histopathological examination of pancreas revealed that there was significant proliferation of beta cells and increasing number of active cells was seen in all the treated groups.

Hypercholesterolemic animals treated with lotus seed extract showed significant reduction in total cholesterol and LDL levels from 14th day treatment onwards. In case of simvastatin treated group significant reduction was noticed from 7th day treatment onwards. A significant increase in HDL level was observed on 28th day and from 7th day in extract and simvastatin treated groups respectively. A significant reduction in liver total lipid was noticed in all the treated group on 29th day.

Hypertriglyceridemic animals treated with the extract showed significant reduction in triglycerides from 14th day treatment onwards. In case of fenofibrate significant reduction was observed from 7th day treatment onwards. The free fatty acid levels reduced significantly from 21st and 14th day onwards for extract and fenofibrate treated groups respectively. Liver total lipid level showed significant reduction in all the treated groups on 29th day.

Pancreatitis was induced by repeated administration of alloxan in rats. They were treated with lotus seed extracts for 28 days. Pancreas was collected after sacrificing the animals for estimation of superoxide dismutase, catalase, and glutathione peroxidase and lipid peroxidation. The superoxide dismutase, catalase and glutathione peroxidase levels increased in pancreas of extract and metformin treated group. It was significant from 14th day treatment onwards. There was a significant reduction in lipid peroxidation level in red and white lotus seed extract treated group from 21st and 14th day treatment respectively. In case of metformin treated group significant reduction was noticed from 14th day of treatment. Histopathological examination of pancreas revealed islet hyperplasia with more number of active cells in a duration dependent fashion in all the treated groups.

From the study, it can be concluded that both red and white lotus seed extract have hypoglycemic, hypolipidaemic and cytoprotective effects. However, the effects are less than that of respective reference drug.