HYPOGLYCAEMIC EFFECT OF *Pleurotus ostreatus* IN SPRAGUE-DAWLEY RATS

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

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DECLARATION

I hereby declare that the thesis entitled **"Hypoglycaemic effect of *Pleurotus ostreatus* in Sprague-Dawley rats"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis, entitled **"Hypoglycaemic effect of *Pleurotus ostreatus* in Sprague-Dawley rats"** is a record of research work done independently by **Dr. Saritha Krishna.L.K,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, associateship or fellowship to her.

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SARITHA KRISHNA.L.K

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Introduction

1. INTRODUCTION

Diabetes mellitus is a metabolic disease of greater concern worldwide causing severe complications including blindness, cardiac and kidney diseases. It is ranked seventh among the leading causes of death and third when all its fatal complications are taken into account. The diabetes epidemic is accelerating in the developing world, with an increasing proportion of affected people in younger age groups. The latest WHO Global Burden of Disease estimates the worldwide burden of diabetes in adults to be around 173 million in the year 2002 and around two thirds of these live in developing countries (Orhan *et al.*, 2005). By the year 2010, the total number of people worldwide with diabetes mellitus is projected to reach 239 millions. Regions with greatest potential are Asia and Africa, where diabetes mellitus rates could rise to two to three folds than the present rates (Vats *et al.*, 2002). In India, the prevalence of diabetes is estimated to be one to five per cent. Complications are the major cause of morbidity and mortality in diabetes mellitus.

Diabetes mellitus is a chronic metabolic disorder/syndrome resulting from a variable interaction of hereditary and environmental factors. It is characterized by abnormal insulin secretion or insulin receptor or post-receptor events affecting carbohydrate, protein and fat metabolism and in addition it damages liver, kidney and β-cells of pancreas (Singh *et al.*, 2005). The central feature of this syndrome is hyperglycaemia. Insulin also enhances the transcription of lipoprotein lipase in the capillary endothelium. Thus, in the untreated or under- treated diabetic patient, hyperlipidaemia often occurs (Lino *et al.*, 2004).

In animals, diabetes mellitus occurs most frequently in dogs and cats with an incidence of approximately 0.2-0.5 per cent. The disease in dogs occurs most frequently in the mature or older females, often in association with estrus. Some

breeds like Miniature poodle, Scottish terriers, Rotweiler and Dachshund have a genetic predisposition towards diabetes. In cats, males appear to be more commonly affected than females. It is less frequently reported in ruminants and is often mild.

There are three main types of diabetes, insulin-dependent (IDDM or Type I diabetes), non-insulin-dependent (NIDDM or Type II diabetes) and gestational diabetes mellitus. Insulin -dependent diabetes mellitus which represents only 5 per cent of total diabetes, most often develops in children and young adults. Noninsulin - dependent diabetes is the most prevalent form of diabetes (90–95 per cent) and is common in adults over 40 years old who are usually overweight. Gestational diabetes develops in women during pregnancy (Persaud and Mendoza, 2004).

In modern medicine, satisfactory effective therapy is not available to cure diabetes mellitus. It can be managed by exercise, diet and chemotherapy (Pari and Satheesh, 2004). Insulin and oral hypoglycaemic agents like sulfonylureas and biguanides are still the major drugs in the management of this disease (Ghosh *et al* ., 2004). Although oral hypoglycaemic agents/insulin are the mainstay of treatment of diabetes and are effective in controlling hyperglycaemia, they have side effects and fail to alter the course of diabetic complications.

Long before the use of insulin, indigenous remedies have been used for the treatment of diabetes mellitus. There is an increasing demand by the patients to use the natural products with antidiabetic activity, mainly because of their lesser side effects and oral effectiveness. Moreover, oral hypoglycaemic agents are not always effective in lowering the blood sugar in chronic cases (Ponnachan *et al.*, 1993). This highlights the importance of searching for an alternate therapy with drugs having not only insulinotropic effect but also increase insulin sensitivity.

Aegle marmelos, commonly called as Bael has been used as a herbal medicine for the management of diabetes mellitus in Ayurveda, Unani and Siddha systems of medicine in India, Bangladesh and Srilanka. *A.marmelos* leaves and root bark possess significant blood glucose lowering activity. The crude extract of the leaves and the alkaloid isolated from it also have been found to exhibit hypoglycaemic effect in diabetic rats (Kamalakkannan and Prince, 2003).

Murraya koenigii is popularly known as 'curry leaf' in India. It has been extensively used in Ayurvedic medicine for the treatment of amoebiasis, diabetes and hepatitis. The leaves of *M.koenigii* are used as tonic, stomachic, to promote appetite and digestion, and also to destroy pathogenic organisms (Shah and Juvekar, 2006). Several studies have been done on fresh leaves and its aqueous and methanolic extract indicating its potent hypoglycaemic nature.

Mushrooms are nutritious food and are source of physiologically beneficial medicine. *Pleurotus* species commonly called as Oyster mushrooms are edible and nutritious and rank second among the commercially cultivated mushrooms in the world. They are good source of non starchy carbohydrates, moderate quantities of good quality proteins with most of the essential amino acids, minerals and vitamins. *Pleurotus* species of mushrooms are promising as medicinal mushrooms exhibiting antiviral, antitumor, antibacterial, hypocholesterolic and immunomodulatory activities (Cohen *et al.*, 2002).

It is quite clear from the literature that not much scientific studies have been carried out to elucidate the hypoglycaemic activity of *Pleurotus* species. Therefore it is considered worthwhile to undertake this study to evaluate the hypoglycaemic effect of *Pleurotus* species in alloxan diabetic rats. Moreover, comparative study with already proven hypoglycaemic plants, *A.marmelos* and *M.koenigii* will be of

great use in selecting the most ideal plant for therapeutic purposes. Combination of these plants will help to know whether it is having any synergistic effect or not.

Review of Literature

2. REVIEW OF LITERATURE

2.1 ALLOXAN INDUCED DIABETES

Mishra *et al.* (1995) studied the activities of two enzymes viz:-Na+-K+-ATPase and succinic dehydrogenase in brain and liver of alloxan diabetic swiss albino mice. They found that alloxan diabetes cause a significant decrease in the activity of Na+-K+-ATPase reflecting reduced glucose transport across cell membrane and a significant enhancement in the activity of succinic dehydrogenase.

Chattopadhyay *et al.* (1997) opined that blood glucose level in normal nonfasted animals after alloxan injection fluctuates in a triphasic pattern; there is early marked hyperglycaemia of short duration followed by hypoglycaemia of short duration, followed by hyperglycaemia of long duration.

Soto *et al.* (1998) suggested that induction of diabetes mellitus by alloxan in rats can be prevented by administration of Silymarin, a free radical scavenger. This drug has a favourable effect on free radical induced pancreatic damage produced by alloxan.

Experiments conducted by Kar and Chakraborti (1999) revealed that increased blood glucose in diabetes mellitus stimulated the non-enzymatic glycosylation of haemoglobin yielding free reactive iron which in turn may be responsible for the associated oxidative stress in diabetes.

Szkudelski (2001) reported that alloxan and its reduced form, dialuric acid established a redox cycle with the formation of superoxide radicals which dismutate to hydrogen peroxide and by Fenton reaction, hydroxyl radicals are formed. He also reported that the action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration caused rapid destruction of β cells.

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

The name 'alloxan' given by Wohler and Liebeg, is recorded as being derived from a combination of allantoin (a product of uric acid among others excreted by foetus into the allantois) and oxalsure (Oxaluric acid derived from oxalic acid and urea, found in urine). The remarkable discovery that a single injection of alloxan can produce diabetes mellitus in laboratory animals was first reported by Dunn, J.S. and Mc Letchie,N. in 1942 (Mc Letchie, 2002).

Walde *et al.* (2002) stated that the glucose transporter-2 (GLUT-2) and glucokinase (GK) are the target molecules for alloxan. The mRNA expression of beta-actin was also slightly affected with time after alloxan exposure.

2.2 PHARMACOLOGICAL EFFECTS OF *Pleurotus* SPECIES

Experiments conducted by Bobek and Galbavy (1999) revealed that addition of 10 per cent dried fruiting bodies of Oyster mushroom to the diet containing 1 per

cent cholesterol, reduced serum cholesterol level by 65 per cent and cholesterol content in liver, heart and aorta of Chinchilla male rabbits by 60, 47 and 80 per cent respectively.

Antitumour activity of Oyster mushroom, *Pleurotus sajor-caju* was reported by Jose and Janardhanan (2000). They found that the methanolic extract of the mushroom possessed significant antitumour activity against the solid tumour model induced by Ehrlichs ascites carcinoma cell lines in BALB/c mice. The tumour reducing effect of the extract was comparable to the clinically accepted antitumour drug, cisplatin at a concentration of 1000 mg/kg body weight.

Jose and Janardhanan (2000) assessed the antitumour and antioxidant activity of *Pleurotus florida*. The results revealed that the methanolic extract of *P. florida* was having significant tumour growth inhibition against the solid tumour induced by Ehrlichs ascites carcinoma cell lines at concentrations of 250, 500 and 1000 mg/kg body weight in BALB/c mice. They also found that the ethyl acetate and methanolic extracts of the mushroom had potent hydroxyl radical scavenging and lipid peroxidation inhibition activities.

Cohen *et al.* (2002) opined that *Pleurotus* species are promising as medicinal mushrooms exhibiting antiviral, antitumour, antibiotic, hypocholesterolic and immunomodulatory activities.

Antioxidant, antitumour, and antiinflammatory activities of the methanolic extract of *Pleurotus pulmonarius* in BALB/c mice was evaluated by Jose *et al.* (2002). They found that the extract had significant hydroxyl-radical scavenging and

lipid-peroxidation inhibiting activities and was effective in causing regression of solid tumour at concentrations of 250, 500 and 1000 mg/kg and was equally effective in reducing paw edema at concentrations of 500 and 1000 mg/kg.

The results of the experiment conducted by Hossain *et al.* (2003) revealed that feeding of 5 per cent powder of fruiting bodies of *Pleurotus ostreatus* mushroom to hypercholesterolaemic rats reduced their plasma total cholesterol by approximately 28 per cent with a concurrent increase in plasma high density lipoprotein-cholesterol concentration of greater than 21 per cent.

The study conducted by Jose *et al.* (2004) revealed that the Oyster mushroom extract could protect antioxidant defense system in cisplatin induced nephrotoxicity in mice. Prior administration of methanolic extract of *Pleurotus florida* at a dose of 500 and 1000 mg/kg body weight significantly reduced the elevated serum creatinine and urea level and increased superoxide dismutase, catalase and glutathione peroxidase activities in the kidney consequent to cisplatin treatment, in a dose dependent manner.

The antiinflammatory and platelet aggregation inhibition activities of the methanolic extract of *Pleurotus florida* were investigated by Jose *et al.* (2004). The extract showed significant activity in ameliorating acute inflammation induced by carrageenan and chronic inflammation induced by formalin at 500 and 1000 mg/kg body weight. It also showed significant platelet aggregation inhibition activity of washed human platelets at a concentration of 500µg/mL.

Investigations were carried out to evaluate the antimutagenic activity of the methanolic extract of *Pleurotus ostreatus* strain *florida* and its protective effect

against Benzo[a] pyrene (B[a] P) - induced hepatic damages by Lakshmi *et al.* (2004). The results suggested that the extract at a concentration of 3mg/ plate significantly inhibited the in vitro NaN_3-, MNNG- and NDP- induced mutagenicity. Prior administration of the extract at 500 mg/kg body weight also markedly inhibited the in vivo mutagenicity caused by Benzo[a] pyrene.

2.3 PHARMACOLOGICAL EFFECTS OF *Murraya koenigii*

Narayana and Sastry (1975) reported the hypoglycaemic activity of *Murraya koenigii* (curry leaf) spreng leaves given parenterally and orally in normal and alloxan induced diabetic dogs.

Philip (1981) suggested the following medicinal uses of curry leaves. External application of pulped bark and root of *Murraya koenigii* is useful for skin eruptions, insect bites and poisonous animal bites. The decoction is used orally to check vomiting. The leaves are used as food and condiments. Fresh leaf juice is said to relieve kidney pain and green leaves are useful in dysentery.

Singh (1986) found that the leaves of *Murraya koenigii* are used as the food and condiment of Indian and Fiji food. It is used for treating pimples, rashes, itching and to treat constipation. The infusion of dry seeds is suggested in the treatment of asthma.

Rathnasooriya *et al.* (1994) reported the anxiolytic activity of leaf extracts of *Murraya koenigii* in rats.

Khan *et al.* (1995) studied the effect of *Murraya koenigii* and *Brassica juncea* on carbohydrate metabolism in rats and found that both showed significant hypoglycaemic action. They also found that both the plants exerted hypoglycaemic effect by enhanced glycolysis, glycogenesis and decreased glycogenolysis.

The status of lipid peroxidation in rats fed with *Murraya koenigii* (Curry leaf) and *Brassica juncea* (Mustard) was studied by Khan *et al.* (1996). The levels of malondialdehyde and glutathione in liver, heart and kidney were lowered. Superoxide dismutase and catalase activity increased in liver and heart along with a sharp rise in glutathione reductase, glutathione peroxidase and glutathione S-tranferase activity.

Yadav *et al.* (2002) conducted studies on the hypoglycaemic and antihyperglycaemic activity of *Murraya koenigii* leaves in diabetic rats. . Results revealed that feeding of diet containing variable doses of curry leaves (5, 10 and 15 per cent) to mild and moderate diabetic rats caused a maximal reduction in blood sugar by 13.1, 16.3 and 21.4 per cent and 3.2, 5.58, 8.21 per cent respectively.

Murraya koenigii spreng leaves can be used as stimulant, antidysentric agent and for the management of diabetes mellitus (Adebajo *et al.*, 2004).

The potent larvicidal activity of *Murraya koenigii* used alone and in combination with synthetic larvicidal agents against *Aedes aegypti* was revealed by Harve and Kamath (2004). They found that the petroleum ether extract of *Murraya koenigii* gave 92 per cent mortality against *Aedes aegypti*.

The antidiabetic effect of aqueous extract (600 mg/kg) and methanolic extract (200 mg/kg) of *Murraya koenigii* spreng leaves in normal and alloxan induced diabetic rats was reported by Vinuthan *et al.* (2004). They noticed that plasma insulin increased significantly on 43rd and 58th day of treatment, which suggested that the hypoglycaemic effect might be mediated through stimulating insulin synthesis and/or from secretion from beta cells of pancreas.

The hypoglycaemic activity of aqueous extract of *Murraya koenigii* leaves was evaluated in normal and alloxan induced diabetic rabbits by Kesari *et al.* (2005). They found that a single oral administration of various dose levels (200, 300 and 400mg/kg) led to the lowering of blood glucose level in normal as well as in diabetic rats and concluded that the maximum fall in blood glucose was observed at 300 mg/kg.

The ethanolic extract of *Murraya koenigii* induced a positive inotropic effect on isolated frog heart by increasing the availability of calcium from extracellular sites and the response was not affected by theophylline, imidazole, propranolol and sildenafil (Shah and Juvekar, 2006).

2.4 PHARMACOLOGICAL EFFECTS OF *Aegle marmelos*

Rusia and Srivastava (1988) opined that the ethanolic and various other organic solvent extracts of *Aegle marmelos* exhibited potent antibacterial and antifungal activity.

The extract of *Aegle marmelos* leaves at a dose of 1 g/kg body weight maintained the weight of diabetic rats near to that which were given insulin injection and produced significant lowering of blood glucose level and decrease in blood urea and serum cholesterol. The results indicated that the active principle in *Aegle marmelos* leaf extract had similar hypoglycaemic activity to insulin treatment (Ponnachan *et al.*, 1993).

Rao *et al.* (1995) reported the hypoglycaemic and antihyperglycaemic effects of *Aegle marmelos* leaves in normal and alloxanised rabbits. The highest decrease in blood glucose level was recorded with 1 g equivalent dose after 4 hours of administration in normal rabbits, while in diabetic rabbits, significant antihyperglycaemic effect was observed within 3 days at a dose of 1 g powder/kg/day.

Seema *et al.* (1996) conducted a study on the kinetics of purified malate dehydrogenase in liver of streptozotocin-diabetic rats and demonstrated the effect of leaf extract of *Aegle marmelos* on malate dehydrogenase activity in diabetic rats. They found that leaf extract treatment could reverse the significant increase in Km of crude malate dehydrogenase and purified S-malate dehydrogenase in liver of diabetic rats, but not the increased Vmax of purified liver S-malate dehydrogenase.

The inhibitory effect of *Aegle marmelos* on lipid peroxidation and antioxidant enzymes in blood and tissue in diabetic rats was reported by Sabu and Kuttan (2001). They found that the oral administration of methanolic extract of *Aegle marmelos* (100 mg/kg) could decrease serum lipid peroxidase activity thereby lowering oxidative stress in diabetic rats. There was also an increase in superoxide dismutase

and catalase activity in liver indicating that the treatment might help to lower H_2O_2 concentration and subsequently oxidative stress.

Sachdewa *et al.* (2001) reported the effect of *Aegle marmelos* and *Hibiscus rosa sinensis* leaf extract on glucose tolerance in glucose induced hyperglycaemic rats and found that both given an oral dose of 250 mg/kg showed significant improvement in their ability to utilize external glucose load.

Sur *et al.* (2002) studied the effect of *Aegle marmelos* leaf on rat sperm motility through in vitro study. The sperm motility was observed to be practically nil at 10 per cent concentration of *Aegle marmelos* extract in 30 seconds clearly indicating its antimotility action on spermatozoa in rats.

The hypoglycemic activity of *Aegle marmelos* fruits extract was evaluated in normal and streptozotocin induced diabetic Wistar rats by Kamalakkanan *et al.* (2003). They found an increase in glucose level and glycosylated haemoglobin and a decrease in plasma insulin and liver glycogen in diabetic rats. Treatment with the extract reversed the effect of diabetic to near normal levels.

Daily administration of *Aegle marmelos* extract in alloxan induced diabetic rats via gastric tube at a dose of 500 mg/kg produced a significant reduction in blood glucose level and blood urea level at the end of second week. The extract also produced a significant increase in glutathione level and a decrease in malondialdehyde in erythrocytes of treated animals (Upadhya *et al.*, 2004).

The hepatoprotective effect of aqueous extract of *Azadirachta indica* and *Aegle marmelos* in paracetamol induced hepatotoxicity in rats was studied by Mathew (2005). The results revealed that the extract of *Azadirachta indica* (500 mg/kg) as well as *Aegle marmelos* (1 g/kg) reduced the lipid peroxidation and elevated serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase and increased the reduced levels of superoxide dismutase, catalase, total protein and albumin confirming its strong hepatoprotective activity.

George *et al.* (2006) conducted studies on the immunomodulatory effect of *Aegle marmelos* correa root in BALB/c mice. Adminstration of *Aegle marmelos* extract induced elevation in the cellularity in a dose dependent manner reflecting the enhanced circulation of haematopoietic factors responsible for enhanced immune response. Hence they concluded that antitumour property of *Aegle marmelos* might have been contributed by this enhanced immunomodulation.

2.4 OTHER INDIGENOUS PLANTS WITH HYPOGLYCAEMIC EFFECT

Jose (1974) tested the hypoglycaemic activity of six indigenous plants namely *Salacia reticulata*, *Eugenia jambolana*, *Embilica officinalis*, *Gymnea sylvestre*, *Momordica charantia* and *Curcuma longa* in normal as well as alloxan diabetic dogs. He found that the intensity of hypoglycaemic effect varied with different plants; the seeds of *E. jambolana* were found to have maximum hypoglycaemic effect whereas the roots of *S. reticulata* proved to be least effective.

Hossain *et al.* (1992) observed the effect of *Coccinia indica* extract on blood glucose level of normal fed and 48 hour starved rats. They concluded that *Coccinia*

indica possessed significant hypoglycaemic effect which might be partly due to the inhibition of the key gluconeogenic enzyme, glucose-6-phosphatase.

In alloxan induced diabetic rats, a significant lowering of urinary and blood sugar was noticed on day 20, 30 and 40 following treatment with a herbal formulation, D-400. The drug also potentiated the hypoglycaemia following tolbutamide treatment (Anturlikar *et al.*, 1995).

Oral administration of the methanolic extract of the aerial parts of *Artemisia pallens* Wall. led to significant blood glucose lowering effect in glucose-fed hyperglycaemic and alloxan-induced diabetic rats. This effect of the extract was dose dependent and significant at 100 mg/kg level in glucose-fed rats and caused a moderate hypoglycaemic effect at a higher dose of 1000 mg/kg in fasted normal rats (Subramoniam *et al.*, 1996).

Abdel-Barry *et al.* (1997) tested the hypoglycaemic activity of the aqueous and alcoholic extract of fenugreek leaf on normal and alloxan induced diabetic rats. Graded amounts (0.06, 0.2, 0.5, 1 g/kg i.p and 1, 2, 8 g/kg p.o.) of the aqueous extract produced a significant reduction of blood glucose concentration, while the ethanolic extract produced no reduction in blood glucose in normal rats.

Administration of Neem seed kernel powder alone (500 mg/kg) as well as the combination of the powder (250 mg/kg) with glibenclamide (0.25 mg/kg) significantly decreased the concentration of blood glucose in alloxan diabetic rabbits (Bopanna *et al.*, 1997). The activities of serum enzymes like alkaline phosphatase, acid phosphatase and lactate dehydrogenase in liver and intestine were also reduced.

A marked fall of serum cholesterol, LDL-cholesterol and VLDL- cholesterol were observed.

An investigation was made on the effect of *Momordica charantia* fruit juice on the distribution and number of α, β and δ cells in the pancreas of streptozotocin - induced diabetic rats by Ahmed *et al.* (1998). The results suggested that oral feeding of *M. charantia* fruit juice had a significant role in the renewal of β cells in streptozotocin diabetic rats or alternately permitted the recovery of partially destroyed β cells.

Joy and Kuttan (1998) suggested that Cogent-DB, a herbal preparation had potent antidiabetic property. They found that the drug was superior to glibenclamide and could also protect the vital organs including pancreas from the injury related damage produced by alloxan.

Continous administration of alcoholic extract of *Picrorrhiza kurroa* significantly reduced the blood sugar in alloxan-induced diabetic rats. The extract was also found to reduce the increased blood urea nitrogen and serum lipid peroxides in diabetic animals; inhibited the weight reduction and leucopaenia induced by alloxan administration (Joy and Kuttan, 1999).

Pari and UmaMaheshwari (1999) evaluated the hypoglycaemic effect of *Musa sapientum* L. in alloxan induced diabetic rats and found that oral administration of 0.15, 0.20, and 0.25 g/kg of chloroform extract of the *Musa sapientum* flowers for 30 days resulted in a significant reduction in blood glucose from 216.56 ± 15.5 to 80.8 ± 4.1mg/dl.

Jafri *et al.* (2000) studied the effect of aqueous ethanolic extract (50 per cent v/v) of *Punica granatum* Linn. (flowers) on blood glucose level in normal and alloxan induced diabetic rats and reported that the extract at 400 mg/kg body weight led to a significant fall in the concentration of blood glucose level in normal (70.83 ± 1.49 to 66.50 ± 3.60 mg/dl), glucose-fed hyperglycaemic (100.66 ± 3.85 to 81.00 ± 4.28 mg/dl) and alloxan induced diabetic (280.40 ± 7.80 to 200.00 ± 3.04 mg/dl) rats.

Khosla *et al.* (2000) were of the opinion that *Azadirachta indica* when given as a leaf extract and seed oil could produce a significant hypoglycaemic effect in normal as well as diabetic rabbits.

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

Chude *et al.* (2001) investigated the hypoglycaemic effect of aqueous extract of *Boerhavia diffusa* leaves on alloxan induced diabetic rats and found that the extract showed non-dose dependent hypoglycaemic activity.

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

Administration of dried leaf powder of mulberry (*Morus indica* L.) along with diet at 25 per cent level to streptozotocin induced diabetic male wistar albino rats for 8 weeks controlled hyperglycaemia, glycosuria, albuminuria and retarded onset of retinopathy (Andallu and Varadacharyullu, 2002).

Babu *et al.* (2002) concluded that the alcoholic extract of *Cassia kleinii* leaf (200 mg/ kg body weight) for 15 days exhibited antihyperglycaemic effect by reducing the fasting plasma glucose value of 420.5 ± 16.8 to 125.7 ± 5.6 mg/dl in alloxan induced diabetic rats.

According to Chakrabarti *et al.* (2002), the administration of ethanolic extract of *Helicteres isora* root at 300 mg/kg dose for 9 days caused significant reduction in plasma glucose, triglyceride and insulin levels in insulin resistant and diabetic C57BL/KsJdb/db mice. In normoglycaemic and mildly hypertriglyceridaemic swiss albino mice, the extract also showed significant reduction in plasma triglyceride and insulin levels, without affecting plasma glucose level.

The influence of chronic treatment with *Enicostemma littorale* in non-insulin-dependent diabetic (NIDDM) rats was studied by Murali *et al.* (2002).They found that the extract produced an increase in insulin sensitivity, normalized dyslipidaemia and provided nephroprotection in diabetic rats.

Puri *et al.* (2002) were of the opinion that the hypoglycaemic effect of fenugreek seeds in alloxan induced subdiabetic and overtly diabetic rabbits may be mediated through stimulating insulin synthesis and/or secretion from the β pancreatic

cells of Langerhans. The effect might also be by increasing sensitivity of tissues to available insulin.

According to Raphael *et al.* (2002), continued administration of the methanolic extract of *Phyllanthus amarus* (200 mg/kg) in alloxan diabetic rats for 15 days showed significant reduction of the elevated glucose level from sixth day ownwards and produced 75.9% reduction on 18th day.

Antidiabetic effect of *Gymnema montanum* leaves and its effect on lipid peroxidation induced oxidative stress in experimental diabetes was evaluated by Ananthan *et al.* (2003).Oral administration of 200 mg/ kg body weight of the alcoholic extract of the leaf for 3 weeks resulted in a significant reduction in blood glucose and an increase in plasma insulin, whereas the effect of 50 and 100 mg/ kg body weight was not significant. The alcoholic extract also resulted in decreased free radical formation in plasma of diabetic rats.

On the basis of the studies conducted by Hu *et al.* (2003) on the effect of Gosha-jinki-gan on insulin resistance in streptozotocin-induced diabetic rats, it was concluded that that a single dose administration of Gosha-jinki-gan could improve the glucose utilization and insulin resistance in streptozotocin-induced diabetic rats, probably via the nitric oxide (NO) pathway.

John (2003) studied the hypoglycaemic activity of seed powders of *Brassica juncea* (mustard) and *Trigonella foenum graecum* (fenugreek) at two different doses (2g/kg and 8g/kg) in alloxan induced diabetic rats. Results indicated that fenugreek seed powder at both doses and mustard seed powder at 8g/kg body weight reduced

blood glucose, serum cholesterol, serum triglyceride and increased liver glycogen whereas animals treated with mustard at 2g/kg body weight showed similar results to that of diabetic control.

Qin *et al.* (2003) concluded that the cinnamon extract treatment would improve insulin action via increasing glucose uptake in vivo, at least in part through enhancing the insulin-signaling pathway in skeletal muscle.

Oral administration of *Aloe arborescens* (200&1000 mg/kg) increased glucose tolerance significantly in normal rats and continued administration of the extract at similar doses produced 26.9 and 42.3 per cent reduction respectively in the elevated serum glucose level produced by alloxan administration (Sabu *et al.*, 2003).

Diatewa *et al.* (2004) studied the hypoglycaemic and antihyperglycaemic effects of diethyl ether fraction isolated from the aqueous extract of the leaves of *Cogniauxia podoleana* in normal and alloxan-induced diabetic rats and found that 100 mg/kg of diethyl ether fraction reduced the blood glucose levels by 40.0 per cent three hours after oral administration to normal rats and decreased the levels of hyperglycaemia by 41.4 per cent and 70.4 per cent respectively, after three and four hours in alloxan-induced diabetic rats.

Eddouks *et al.* (2004) investigated the hypoglycaemic effect of aqueous extract of *Carum carvi* and *Capparis spinosa* fruit in normal and streptozotocin-diabetic rats and found that oral administration of the aqueous *Carum carvi* and *Capparis spinosa* extracts (20 mg/kg) produced a significant decrease in blood glucose level in streptozotocin diabetic rats. Both the extracts decreased the blood

glucose level from 22.5 ± 1.5 to 9.5 ± 0.5 mmol/ l and 21.5 ± 0.5 to 11.5 ± 1.5 mmol/l, respectively after 15 days of treatment.

The results of the experiment conducted by Ghosh *et al.* (2004) demonstrated that the alcoholic extract of bark of *Ficus hispida* showed significant reduction of blood glucose level in both normal and diabetic rats. They concluded that the hypoglycaemic activity might be due to increased glycogenesis and enhanced peripheral uptake of glucose.

Le *et al.* (2004) revealed that in vivo treatment with the petroleum ether extract of *Nigella sativa* exerted an insulin-sensitizing action by enhancing the activity of the two major intracellular signal transduction pathways of the hormone receptor.

The antidiabetic activity of aqueous, ethanolic and hexane extracts of *Bauhinia forficata* was demonstrated in a model of alloxan-induced diabetic rats by Lino *et al.* (2004). Extracts administered daily for seven days at doses of 200 and 400 mg/kg, p.o., 48 hours after alloxan injection (60 mg/kg, i.v.) produced significant reduction in plasma glucose, triglycerides, total cholesterol and HDL-cholesterol as compared to the diabetic controls.

Maiti *et al.* (2004) studied the antidiabetogenic activity of aqueous extract of seed of *Tamarindus indica* in streptozotocin-induced diabetic rats and found that the extract at the dose rate of 80 mg/0.5 ml of distilled water/100 g body weight per day produced significant diminution of fasting blood sugar level from 368.8 ± 12.5 to 87.4 ± 10.6 mg/dl after seven days of treatment.

Mohamad *et al.* (2004) suggested that lower doses of vanadate (0.2 mg/ml) in combination with *Trigonella foenum graecum* seed powder (5% w/w) were able to restore altered carbohydrate metabolism and antioxidant status in alloxan-diabetic rats.

The seed powder of *Datura metel* was tested for its hypoglycaemic activity in normal and alloxan-induced diabetic rats by Murthy *et al.* (2004). A significant reduction in blood glucose by 22.35, 31.89, and 34.26 per cent was observed at the eighth hour with seed powder at 25, 50 and 75 mg/kg body weight, respectively in both normal and diabetic rats. The effect was found to be dose dependent with all treatments at the doses administered.

Prince *et al.* (2004) evaluated the antidiabetic effect of alcoholic extract of *Syzigium cumini* seeds in alloxan diabetic rats. They found that oral administration of alcoholic extract to diabetic rats (100mg/kg body weight) for 42 days resulted in a significant reduction in blood glucose from 265.7 ± 3.9 to 85.7 ± 3.6 mg/dl.

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

Sepici *et al.* (2004) suggested that the hypoglycaemic effect of myrtle oil obtained from the leaves of *Myrtus communis* could be due to the reversible

inhibition of α-glucosidases present in the brush-border of the small intestinal mucosa, higher rate of glycolysis as envisaged by the higher activity of glucokinase, and enhanced rate of glycogenesis as evidenced by the higher amount of liver glycogen present after myrtle oil administration.

Daily treatment of diabetic rats with aqueous extract prepared from fresh leaves of *Cissus sicyoides* for seven days (100 and 200 mg/kg, p.o.) significantly decreased blood glucose levels by 25 and 22 per cent respectively and plasma triglyceride levels as compared to the same groups before treatment (Viana *et al.*, 2004).

Yadav *et al.* (2004) opined that feeding of a fructose rich diet containing 10 per cent *Brassica juncea* seed powder for 30 days significantly prevented the development of insulin resistance and also decreased the fasting serum glucose and cholesterol levels. The results suggested that *Brassica juncea* could therefore play an important role in management of pre-diabetic state of insulin resistance.

Youn *et al.* (2004) reported that the aqueous extract of *Commelina communis* L. showed inhibitory effect on the α-glucosidase activity in a dose-dependent manner, in vitro, which could contribute to the delay in carbohydrate digestion and thereby glucose absorption.

The hypoglycaemic and anti-diabetic effect of *Rehmannia glutinosa* oligosaccharide in glucose-induced hyperglycaemic and alloxan induced diabetic rats was studied by Zhang *et al.* (2004). It was found that pretreatment of the oligosaccharide in normal rats with 100 mg/kg for three days, i.p., induced a partial

prevention of hyperglycaemia caused by glucose (2 g/kg, i.p), while in alloxan-induced diabetic rats, the oligosaccharide (100 mg/kg 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.

Chakrabarti *et al.* (2005) studied the antidiabetic activity of *Caesalpinia bonducella* F. in chronic type 2 diabetic model in Long-Evans rats and evaluated the insulin secretagogue property of its fractions on isolated islets. The results revealed that both the aqueous and ethanolic extracts showed potent hypoglycaemic activity in chronic type 2 diabetic model and two fractions namely BM 169 and BM 170 B could increase secretion of insulin from isolated islets.

Eddouks *et al.* (2005) studied the effect of both a single dose and daily oral administration for 15 days of the aqueous extract of the aerial part of *Chamaemelum nobile* (*C. nobile*) at a dose of 20 mg/kg body weight on blood glucose concentrations and basal insulin levels in normal and streptozotocin-induced diabetic rats. They concluded that the aqueous extract of *C. nobile* exhibited a significant hypoglycaemic effect in normal and streptozotocin diabetic rats without affecting basal plasma insulin concentrations.

The protective effect of *Piper nigrum* and *Vinca rosea* in alloxan induced diabetic rats was observed by Kaleem *et al.* (2005). The results suggested that treatment with *Piper nigrum* and *Vinca rosea* was useful in controlling not only the glucose and lipid profile but also was helpful in strengthening the antioxidant potential.

Antidiabetic, antihyperlipidaemic and antiatherogenic properties of mangiferin in streptozotocin diabetic rats were reported by Muruganandan *et al.* (2005). They found that the chronic intraperitoneal administration of mangiferin (10 and 20 mg/kg) once daily for 28 days exhibited antidiabetic activity by significantly lowering fasting plasma glucose level at different time intervals in streptozotocin-diabetic rats. In addition, the chronic administration of mangiferin also markedly improved oral glucose tolerance in glucose-loaded normal rats suggesting its potent antihyperglycaemic activity.

The acute hypoglycaemic and antioxidant activity of water and ethanolic extracts of three *Viscum album* subspecies, subspecies *album*, subspecies *austriacum*, subspecies *abietis*, were investigated in normoglycaemic and streptozotozocin-induced diabetic rats by Orhan *et al.* (2005). The findings obtained in the experiments demonstrated that European mistletoe (*Viscum album* L.) subspecies possessed potent antihyperglycaemic and antioxidant activity depending on host plant.

Ruzaidi *et al.* (2005) investigated the effect of cocoa extract on serum glucose levels and lipid profiles in streptozotocin-diabetic rats. They reported that supplementation of cocoa extract at one and three per cent to diet significantly lowered the serum glucose level and total cholesterol level in diabetic rats. They also found that the effect of cocoa extract was dose-dependent.

Shirwaikar *et al.* (2005) tested the antidiabetic potential of alcoholic stem extract of *Coscinium fenestratum* in streptozotocin-nicotinamide induced type II

diabetic rats. Graded doses of the alcoholic stem extract (250 &500 mg/kg) produced significant reduction in fasting blood glucose level in normal as well as in treated diabetic rats.

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

Sy *et al.* (2005) studied the hypoglycaemic and antidiabetic activity of acetonic extract of *Vernonia colorata* leaves in normoglycaemic and alloxan induced diabetic rats and found that 100 mg/kg p.o. of the extract for six days produced a significant decrease of blood glucose in alloxan induced diabetic rats from 20.17 ± 0.55 to 5.05 ± 0.05 mmol/l.

Materials and Methods

3. MATERIALS AND METHODS

3.1. EXPERIMENTAL ANIMALS

The study was conducted in adult Sprague- Dawley strain male albino rats weighing 150-200g. The rats were purchased from the Small Animal Breeding Station, Kerala Agricultural University, Mannuthy. They were maintained under identical feeding and management practices in the laboratory for one week before commencement of the study. The experiment was carried out for a period of 45 days.

3.2 EXPERIMENTAL DESIGN

Seventy-two rats were randomly divided into nine groups, each group comprising of eight animals.

Group I (T_0)	Normal control, administered with 5 per cent gum acacia p.o. daily (0.5 ml) from 16^{th} to 45^{th} day.
Group II (T_1)	Diabetic control, administerd with single dose of 10 per cent alloxan at a dose of 130 mg/kg subcutaneously on zero day.
Group III (T_2)	Diabetic rats, administered with ethanolic extract of *Pleurotus ostreatus* at a dose of 250 mg/kg p.o. daily from 16^{th} to 45^{th} day.
Group IV (T_3)	Diabetic rats, administered with ethanolic extract of *Pleurotus ostreatus* at a dose of 500 mg/kg p.o. daily from 16^{th} to 45^{th} day.

Group V (T_4) Diabetic rats, administered with ethanolic extract of *Pleurotus ostreatus* at a dose of 1000 mg/kg p.o. daily from 16^{th} to 45^{th} day.

Group VI (T_5) Diabetic rats, administered with ethanolic extract of *Murraya koenigii* at a dose of 250 mg/kg p.o. daily from 16^{th} to 45^{th} day.

Group VII (T_6) Diabetic rats, administered with ethanolic extract of *Aegle marmelos* at a dose of 250 mg/kg p.o. daily from 16^{th} to 45^{th} day.

Group VIII (T_7) Diabetic rats, administered with suitable dose of *Pleurotus ostreatus* from the above studies, *Aegle marmelos* at a dose of 250 mg/kg and *Murraya koenigi*i at a dose of 250 mg/kg p.o. daily from 16^{th} to 45^{th} day.

Group IX (T_8) Diabetic rats, administered with reference drug, glibenclamide at a dose of 0.25mg/kg p.o. daily from 16^{th} to 45^{th} day.

Blood glucose, serum cholesterol and serum triglyceride were estimated on zeroth, 16^{th}, 30^{th} and 45^{th} day. Body weight was also recorded on these days. On 45^{th} day, animals were sacrificed and liver glycogen was estimated.

3.3 PROCEDURE FOR INDUCTION OF DIABETES

All the animals were fasted overnight and their body weight and blood glucose were estimated on the next day (zeroth day) morning. As a preliminary trial,

alloxan* was given at 100, 130 and 150 mg/kg body weight subcutaneously so as to fix up a convenient dose for the experiment. At 100 mg/kg dose hyperglycemia was not produced. At 130 mg/kg there was a remarkable increase in blood glucose. Dose at 150 mg/kg was found to be toxic and some of the animals died within two to four days after the injection.

After fixing the effective dose at 130 mg/kg body weight, alloxan was weighed and 10 per cent (w/v) alloxan solution was prepared in distilled water. All the treatment groups except the normal control (T_0) were made diabetic by the subcutaneous injection of alloxan monohydrate at a rate of 130 mg/kg body weight after the rats have been fasted for 12 hours. After 16 days, blood glucose was estimated using O-toluidine method. Rats showing moderate hyperglycemia (200-295 mg/100ml) only were selected for the study.

3.4 PREPARATION AND ADMINISTRATION OF DRUGS

The leaves of *Aegle marmelos* and *Murraya koenigii* and the fruiting bodies of the Oyster mushroom were collected fresh and dried in the shade at room temperature. The dried leaves and the fruiting bodies were then powdered well in a pulverizer and were further 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 them in a water bath at low temperature so as to obtain a semisolid/ solid residue. The crude extract thus prepared was kept in the refrigerator at 4^0 C for further use. A weighed quantity of the crude extract was homogenized with 5 per cent gum acacia and was administered orally to individual rats for 30 days based on their body weight.

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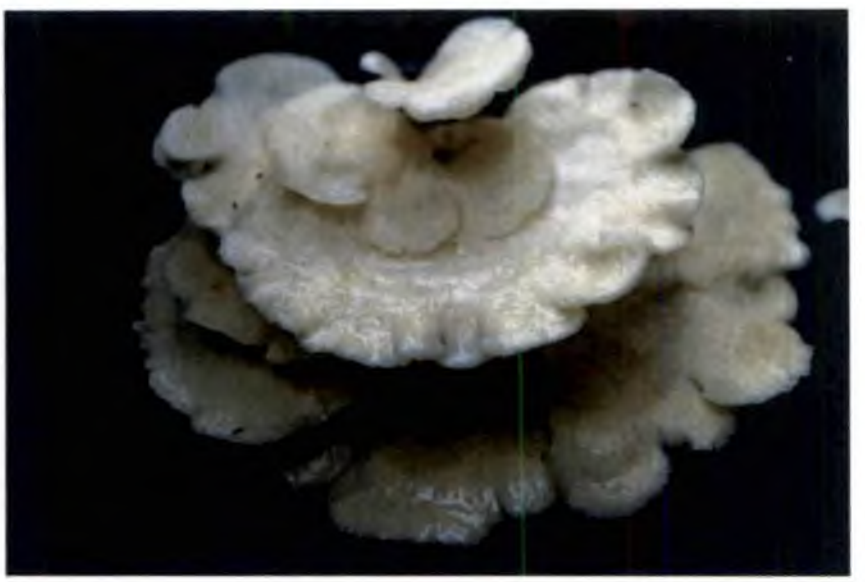

Fig.1. ***Pleurotus ostreatus*** **(Oyster mushroom)**

Fig.2. ***Murraya koenigii*** **(Curry leaf)**

Fig.3. ***Aegle marmelos*** **(Bael)**

Glibenclamide

Tab Daonil* (5 mg) was powdered and suspended in 25 per cent solution of distilled water with the help of gum acacia. It was then given orally at the rate of 1 ml/kg daily for 30 days to treatment group T_8.

3.5 COLLECTION OF BIOLOGICAL SAMPLES

3.5.1 Blood

Blood samples were taken from retro orbital plexus from the inner canthus of eye under light ether anaesthesia using sodium heparinised capillary tubes (microhaematocrit capillaries) and were collected in fresh vials containing disodium salt of Ethylene Diamine Tetra Acetic Acid (EDTA, 1 mg/ml) as anticoagulant. Blood was also collected in fresh vials without any anticoagulant and kept at room temperature for one hour. Then it was centrifuged at 2000 rpm for 20 minutes. Serum was separated and was used for cholesterol and triglyceride estimations.

3.5.2 Liver

Liver samples were collected after sacrificing the animals on 45th day of the experiment and liver glycogen was estimated by the method described by Carroll *et al.* (1956).

3. 6 ESTIMATION OF BIOCHEMICAL PARAMETERS

3. 6. 1. Blood Glucose

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

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*Hoechst Marion Roussel

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

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

3. 6. 1. 2. *Reagents*

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

Preparation of Reagents

1. Tungstic acid reagent

One gram of polyvinyl alcohol was dissolved 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, 2.1 ml of concentrated sulphuric acid was added to about 300 ml of distilled water and mixed. It was then added to the tungstate solution in one litre volumetric flask, mixed well and made up the volume with distilled water. The solution is stable for one year at room temperature.

2. O-toluidine Reagent

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

3. Glucose standard (100mg/100ml)

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

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

Protein free 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, allowed to stand for 5 minutes and centrifuged at 3000 rpm for 10 minutes. Supernatant was collected and 0.5 ml was mixed with 2.5 ml of O-toluidine reagent in a glass stoppered test tube and mixed well. The blank was prepared by adding 0.5 ml of distilled water instead of the deproteinised blood to 2.5 ml of O-toluidine reagent. The standard was set by adding 0.05 ml of the glucose standard (100mg/100ml) to 0.45 ml of distilled water and 2.5 ml of O-toluidine reagent. Mixed well and placed all the loosely stoppered test tubes in a boiling water bath 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.

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

3. 6. 2. Liver Glycogen

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

3. 6. 2.1 *Principle*

The sulphuric acid medium of the anthrone reagent causes dehydration of the sugar to a furfural derivative which presumably condenses with anthrone to form a blue coloured compound, the absorbance of which is measured in a spectrophotometer at 620nm.

3.6.2.2 *Reagents*

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

Preparation of Reagents

1. Anthrone reagent

Placed in a flask 500 mg of purified anthrone, 10 gm of highest purity thiourea and 1 litre of 72 per cent sulphuric acid. (72 per cent sulphuric acid was prepared by adding cautiously 720 ml of concentrated sulphuric acid with a specific gravity of 1.84 to 280 ml of distilled water). Warmed the mixture to 80-90^0, occasionally shaking the flask to mix the contents. Cooled and stored in a refrigerator.

2. Glucose standard

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

(b) Working standard - Pipetted out 0.5 ml of the stock solution to a 10ml volumetric flask and made up the volume with saturated benzoic acid solution. Two ml of this solution, containing 0.1 mg of glucose, was used as the standard.

3. 6. 2. 3. ***Procedure***

1. Two gram of tissue sample was homogenized in a Teflon homogenizer with 5 ml of 5 per cent 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. The tissue was again reextracted by transferring the whole residue to the homogenizer with 5 ml of 5 per cent 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 per cent 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 in 2 ml of distilled water, in a manner that the water added will wash down the sides of the tube.

9. The reagent blank was prepared by pipetting 2 ml of distilled water into a clean test tube. The standard was prepared by pipetting 2 ml of working 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. 6. 3. Total Cholesterol

Cholesterol in serum was estimated by enzymatic CHOP-PAP method (Allain *et al*., 1974) in semi automatic blood analyzer ('Microlab 200')using Ecoline kit from E.Merck India Limited.

3.6.4 Triglyceride

Triglyceride in serum was estimated by GPO-PAP method (Nussel and Arav, 1975) in semi automatic blood analyzer ('Microlab 200') using Ecoline kit from E.Merck India Limited.

3.7 STATISTICAL ANALYSIS OF DATA

The results obtained were statistically analyzed by one way analysis of variance (ANOVA) for comparison among means followed by Duncan's multiple range test for pair wise comparison. All the values are expressed as mean ± standard error (SE).

Results

4. RESULTS

4.1 Evaluation of Hypoglycaemic activity

The present study was carried out in two phases:- i) a preliminary study to find out the suitable effective dose of *Pleurotus ostreatus* in reducing blood glucose level in diabetic rats among different doses of 250, 500 and 1000 mg/kg. ii) Among these doses, the most effective dose of *Pleurotus ostreatus* was then selected for further study.

4.1.1 Preliminary study to find out the most effective dose of *Pleurotus ostreatus*

4.1.1.1 Body Weight

The individual and mean body weight of the rats (Groups I, II, III, IV and V) recorded on zeroth, 16th, 30th and 45th day of the experiment are presented in Tables 1,2,3 and 4 respectively. The values on zeroth day represent the body weight before the commencement of the experiment (before injecting alloxan) and on 16th day represent body weight after 16 days of alloxan administration. The body weight recorded on zeroth day of Groups I, II, III, IV and V were 170.00 ± 4.22, 173.75 ± 4.97, 178.75 ± 3.98, 175.00 ± 3.27 and 171.25 ± 3.98 g respectively.

On 16th day, all groups showed gradual decrease in body weight except group I (normal control). The mean body weight obtained for groups I to V on 16th day were 180.00 ± 3.78, 152.50 ± 3.66, 148.75 ± 2.26, 151.25 ± 3.50 and 146.25 ± 3.24 g respectively.

On 30th day (after 14 days of treatment), groups III to V showed a significant ($p < 0.05$) increase in body weight and the mean body weight recorded on 30th day for Groups II, III, IV and V were 140.00 ± 2.67, 158.75 ± 2.26, 162.50 ± 3.13 and 165.00 ± 2.67 g respectively.

Table 1.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on body weight (g) in diabetic rats-0thday

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	160	180	190	180	160
2	170	160	180	180	170
3	180	150	190	170	170
4	160	190	180	160	160
5	190	170	170	170	180
6	160	170	160	190	160
7	180	190	190	180	190
8	160	180	170	170	180
Mean±SE	170.00±4.22	173.75±4.97	178.75±3.98	175.00±3.27	171.25±3.98

Table 2.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on body weight (g) in diabetic rats-16thday

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	170	150	150	160	130
2	170	150	160	150	150
3	180	140	150	140	150
4	180	160	140	140	140
5	190	150	150	150	150
6	180	140	140	170	140
7	200	170	150	150	150
8	170	160	150	150	160
Mean±SE	180.00±3.78^{A}	152.50±3.66^{B}	148.75±2.26^{BC}	151.25±3.50^{B}	146.25±3.24^{BC}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 3.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on body weight (g) in diabetic rats-30thday

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	180	140	160	170	160
2	190	130	160	160	170
3	200	140	160	150	170
4	190	140	150	160	160
5	200	130	160	160	160
6	200	140	150	180	160
7	200	150	170	160	160
8	180	150	160	160	180
Mean±SE	192.50±3.13^{A}	140.00±2.67^{F}	158.75±2.26^{CDE}	162.50±3.13^{BCD}	165.00±2.67^{BC}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 4.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on body weight (g) in diabetic rats-45thday

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	190	140	170	170	160
2	200	130	160	170	170
3	200	120	160	150	170
4	200	130	150	160	170
5	210	130	160	160	160
6	210	130	160	180	170
7	220	140	170	160	160
8	190	150	160	160	180
Mean±SE	202.50±3.66^{A}	133.75±3.24^{F}	161.25±2.26^{DE}	163.75±3.23^{CDE}	167.50±2.50^{BCD}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

On 45th day, group III to V showed a significant ($p < 0.05$) increase in body weight with respect to group II. The mean body weights obtained are presented in Table 4.

4.1.2 Biochemical parameters

4.1.2.1 Blood Glucose Level

Blood glucose level was estimated before giving alloxan (zeroth day), and on 16th , 30th and 45th day after alloxan administration. The individual and mean values recorded are represented in Tables 5, 6, 7 and 8 respectively.

The blood glucose level of rats before giving alloxan (zeroth day) were 90.57 ± 1.11, 94.62 ± 3.25, 95.43 ±2.85, 94.56 ± 2.28 and 91.54 ± 2.65 mg/dl for groups I to V respectively.

On 16th day, all the groups except normal control showed an increase in blood glucose level with a mean value of 260.69 ± 8.07, 265.10 ± 4.85, 255.12 ± 3.68 and 258.86 ± 4.53 mg/dl respectively for groups II, III, IV and V. These values represents the alloxan induced diabetic blood glucose levels before giving various treatments.

On 30th day, Groups III, IV and V showed significant ($p < 0.05$) reduction in blood glucose level compared to diabetic control. Group V treated with *Pleurotus ostreatus* at the dose rate of 1000 mg/kg showed a much greater decrease of 201.57 ± 4.09 mg/dl than that obtained for groups III and IV. The reduction in blood glucose level produced by groups III and IV were comparable, for which the values obtained were 219.70 ± 4.39 and 215.17 ± 4.53 mg/dl respectively. The mean blood glucose level obtained for different groups on 30th day is presented in Table 7 and Figure 4.

Table 5.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on blood glucose level in diabetic rats- 0th day, mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	93.20	86.33	85.68	88.45	79.84
2	90.51	106.25	89.96	94.01	89.50
3	88.43	80.91	92.45	85.33	95.44
4	94.64	87.42	109.42	89.25	83.25
5	89.53	92.68	93.77	103.77	96.58
6	86.77	98.74	96.66	96.15	99.13
7	94.35	100.15	90.33	100.09	88.39
8	87.15	104.50	105.15	99.41	100.16
Mean±SE	90.57±1.11	94.62±3.25	95.43±2.85	94.56±2.28	91.54±2.65

Table 6.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on blood glucose level in diabetic rats- 16th day, mg/dl

Animal No	Group I	Group II	Group III	Group IV	Group V
1	95.42	274.04	245.59	250.67	255.30
2	86.18	238.32	268.32	246.58	268.16
3	94.36	288.46	257.69	238.75	259.63
4	103.18	295.18	276.44	261.55	274.36
5	96.28	248.30	281.19	258.94	275.22
6	90.64	235.65	279.38	270.27	240.59
7	89.30	245.48	248.46	264.35	247.26
8	100.08	260.12	263.72	249.84	250.34
Mean±SE	94.43±1.99^{B}	260.69±8.07^{A}	265.10±4.85^{A}	255.12±3.68^{A}	258.86±4.53^{A}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 7.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on blood glucose level in diabetic rats- 30th day, mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	98.62	286.50	208.87	214.62	194.03
2	88.40	235.28	219.11	198.45	204.74
3	99.15	290.04	210.91	196.66	207.70
4	108.54	292.52	223.91	215.78	205.36
5	91.35	245.18	233.80	216.01	223.34
6	97.43	240.22	237.47	235.13	184.53
7	95.74	241.35	201.13	226.02	196.27
8	94.68	270.54	222.42	218.66	196.62
Mean±SE	96.74 ± 2.12^{F}	262.70 ± 8.74^{A}	219.70 ± 4.39^{BCD}	215.17 ± 4.53^{CD}	201.57 ± 4.09^{DE}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 8.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on blood glucose level in diabetic rats- 45th day, mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	94.09	284.31	168.84	160.05	160.84
2	91.24	230.64	182.77	150.29	155.94
3	90.58	295.56	170.77	146.88	147.99
4	101.11	290.18	176.92	156.93	157.62
5	85.39	248.45	189.80	166.89	172.15
6	88.60	243.32	195.56	167.76	146.09
7	85.22	248.68	162.12	174.47	145.51
8	94.38	278.26	173.73	162.52	153.08
Mean±SE	91.33 ± 1.86^{F}	264.93 ± 8.77^{A}	177.56 ± 3.95^{C}	160.72 ± 3.26^{D}	154.90 ± 3.16^{D}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

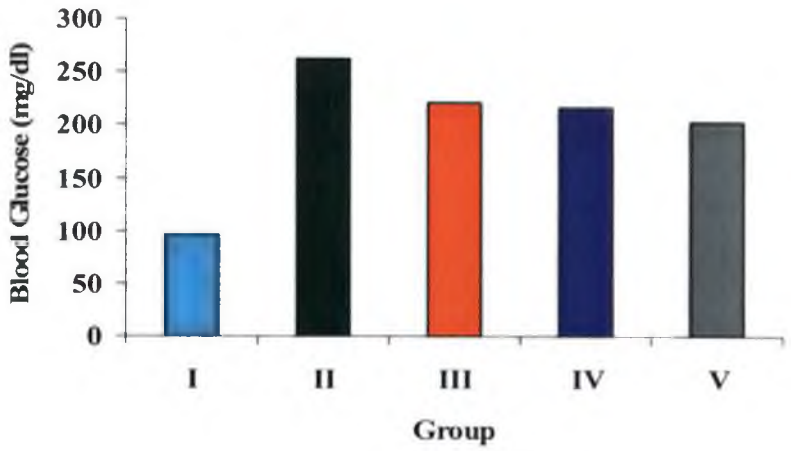

Fig.4.Effect of different doses of *Pleurotus ostreatus* on blood glucose level in diabetic rats-30th day

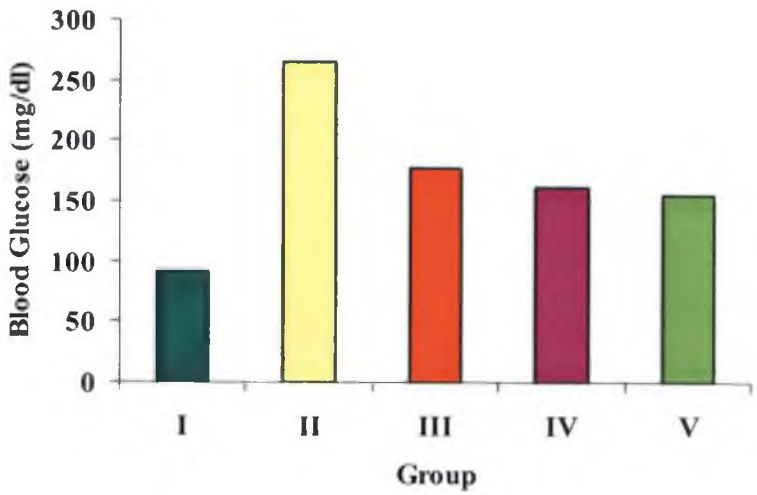

Fig.5.Effect of different doses of *Pleurotus ostreatus* on blood glucose level in diabetic rats-45th day

Group I-Normal Control, Group II- Diabetic Control, Group III- Diabetic + *Pleurotus ostreatus* (250 mg/kg), Group IV- Diabetic + *Pleurotus ostreatus* (500 mg/kg), Group V- Diabetic + *Pleurotus ostreatus* (1000 mg/kg)

At the end of the experiment (on 45th day), the three treatment groups (III, IV and V) showed a significant ($p<0.05$) fall in blood glucose level compared to diabetic control. The most effective reduction in blood sugar level was obtained with *Pleurotus ostreatus* at the dose rate of 1000 mg/kg body weight with a mean value of 154.90 ± 3.16 mg/dl. Among groups III and IV, group IV treated with *Pleurotus ostreatus* at the dose rate of 500 mg/kg showed a much greater decrease in blood glucose value of 160.72 ± 3.26 mg/dl. However the reduction in blood glucose level produced by groups IV and V were comparable. The reduction in blood glucose level produced by different treatments on 45th day is presented in Table 8 and Figure 5.

4.1.2.2 Serum Cholesterol Level

Serum cholesterol level estimated for groups I to V on zeroth day, 16th, 30th and 45th day are presented in Tables 9, 10, 11 and 12 respectively. The serum cholesterol level of rats on zeroth day was 78.13 ± 3.74, 75.00 ± 4.21, 68.13 ± 3.35, 79.38 ± 2.57 and 82.00 ± 2.47 mg/dl for groups I to V respectively.

On 16th day, all groups except normal control showed an increase in cholesterol level with a mean value of 156.75 ± 3.48, 151.75 ± 3.61, 155.75 ± 3.37 and 151.75 ± 3.68 mg/dl respectively for groups II, III, IV and V.

On 30th day, there was a significant ($p< 0.05$) decrease in serum total cholesterol value in groups III, IV and V compared to group II. The mean serum cholesterol values are presented in Table 11.The reduction in serum cholesterol level seen in groups III and IV were almost similar, for which the values obtained were 132.75 ± 3.28 and 131.38 ± 3.05 mg/dl respectively. Group V produced a significant reduction compared to groups III and IV, the value obtained was 124.75 ± 3.07 mg/dl. The mean serum cholesterol level produced by different treatments on 30th day is presented in Table11 and Figure 6.

Table 9.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on serum cholesterol level in diabetic rats- 0^{th} day, mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	64	65	64	73	72
2	77	58	57	75	81
3	71	69	62	68	76
4	80	71	74	81	88
5	66	77	59	90	78
6	90	80	77	87	80
7	92	84	84	82	89
8	85	96	68	79	92
Mean±SE	78.13±3.74	75.00±4.21	68.13±3.35	79.38±2.57	82.00±2.47

Table 10.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on serum cholesterol level in diabetic rats-16^{th} day,mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	66	148	150	144	138
2	82	139	136	151	150
3	76	152	148	156	147
4	85	158	157	163	156
5	71	160	162	149	163
6	85	163	165	167	167
7	88	166	140	169	139
8	81	168	156	147	154
Mean±SE	79.25 ± 2.70^{D}	156.75 ± 3.48^{AB}	151.75 ± 3.61^{ABC}	155.75 ± 3.37^{ABC}	151.75 ± 3.68^{DC}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 11.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on serum cholesterol level in diabetic rats-30th day,mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	70	154	134	121	113
2	80	144	118	129	125
3	78	151	127	129	119
4	81	147	137	140	129
5	74	150	141	128	133
6	88	155	145	140	138
7	87	160	123	143	115
8	83	162	137	121	126
Mean±SE	80.13±2.17^{F}	152.88±2.17^{A}	132.75±3.28^{B}	131.38±3.05^{BC}	124.75±3.07^{D}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 12.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on serum cholesterol level in diabetic rats-45th day,mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	69	150	105	99	86
2	84	140	98	96	96
3	73	158	106	110	91
4	86	149	114	109	93
5	70	156	110	104	106
6	84	161	122	113	102
7	89	165	98	112	89
8	90	158	108	94	102
Mean±SE	80.63±3.03^{E}	154.63±2.80^{A}	107.63±2.83^{B}	104.63±2.64^{C}	95.63±2.51^{D}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

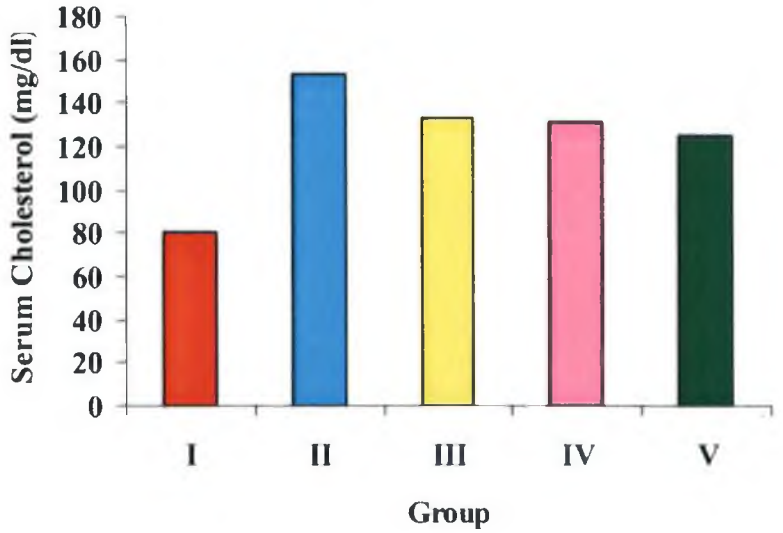

Fig.6.Effect of different doses of *Pleurotus ostreatus* on serum cholesterol level in diabetic rats-30th day

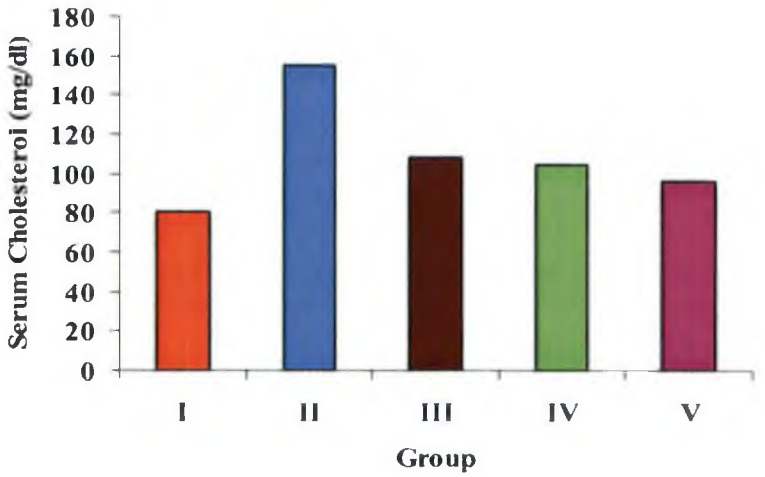

Fig.7.Effect of different doses of *Pleurotus ostreatus* on serum cholesterol level in diabetic rats-45th day

All treatment groups showed significant ($p<0.05$) decrease in serum cholesterol level compared to group II on 45th day. Group II (diabetic control) had a higher cholesterol value of 154.63 ± 2.80 mg/dl. There was much significant difference in the reduction of serum cholesterol level between group III, IV and V. However the lowest serum cholesterol level among these three groups was seen in group V for which the value obtained was 95.63 ± 2.51 mg/dl. Table 12 and Figure 7 represent the mean reduction in serum cholesterol level produced by different treatments on 45th day.

4.1.2.3 *Serum Triglyceride Level*

Serum triglyceride level estimated on zeroth, 16th, 30th and 45th day are presented in Tables 13, 14, 15 and16 respectively. The mean values obtained on zeroth day were 69.63 ± 1.90, 70.25 ± 1.89, 70.38 ± 1.66, 72.75 ± 1.94 and 74.13 ± 1.91 mg/dl respectively for groups I to V which represent the normal serum triglyceride level before giving alloxan.

On 16th day, all the groups with the exception of normal control showed an increase in serum triglyceride level with a mean value of 158.13 ± 1.63, 158.88 ± 2.14, 163.38 ± 2.26 and 165.13 ± 1.88 mg/dl respectively for groups II, III, IV and V.

On 30th day, all the treatment groups (III, IV and V) showed a significant ($p< 0.05$) decrease in serum triglyceride level than group II. There was no significant difference in reduction of serum triglyceride between groups III, IV and V, the values obtained were 137.38 ± 2.17, 138.13 ± 2.01 and 137.00 ± 1.96 mg/dl respectively. The reduction in serum triglyceride level obtained for different groups on 30th day is presented in Table 15 and Figure 8.

Table 13.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on serum triglyceride level in diabetic rats-0thday,mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	64	79	74	79	67
2	69	68	70	74	73
3	67	72	68	82	69
4	78	66	65	73	76
5	62	75	73	69	84
6	72	64	79	66	78
7	75	73	68	71	71
8	70	65	66	68	75
Mean±SE	69.63±1.90	70.25±1.89	70.38±1.66	72.75±1.94	74.13±1.91

Table 14.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on serum triglyceride level in diabetic rats-16thday,mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	69	162	158	170	167
2	73	154	165	158	172
3	76	161	163	163	169
4	74	151	152	164	157
5	68	159	168	158	159
6	69	155	154	154	164
7	71	165	159	168	170
8	76	158	152	172	163
Mean±SE	72.00±1.13^{D}	158.13±1.63^{BC}	158.88±2.14^{BC}	163.38±2.26^{AB}	165.13±1.88^{A}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 15.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on serum triglyceride level in diabetic rats-30thday,mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	66	160	135	141	145
2	75	157	140	133	139
3	71	154	145	136	134
4	72	156	128	141	133
5	63	162	145	135	130
6	74	159	132	131	134
7	66	158	140	139	145
8	73	155	134	149	136
Mean±SE	70.00±1.56^{F}	157.63±0.94^{A}	137.38±2.17^{C}	138.13±2.01^{C}	137.00±1.96^{C}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 16.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on serum triglyceride level in diabetic rats-45thday,mg/dl

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	65	163	113	117	105
2	70	161	120	108	115
3	68	159	113	108	110
4	76	154	105	114	103
5	66	158	124	107	102
6	65	165	111	103	109
7	69	163	112	116	107
8	71	160	110	116	103
Mean±SE	68.75±1.31^{G}	160.38±1.22^{A}	113.50±2.09^{C}	111.13±1.85^{CD}	106.75±1.57^{D}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

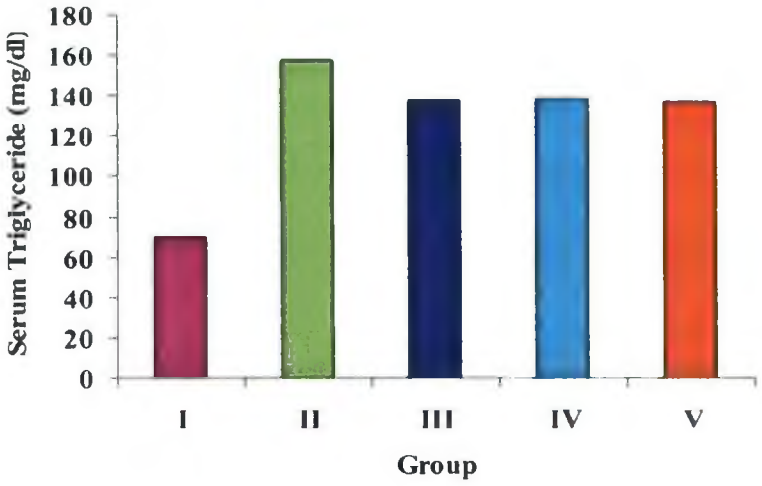

Fig.8.Effect of different doses of *Pleurotus ostreatus* on serum triglyceride level in diabetic rats-30th day

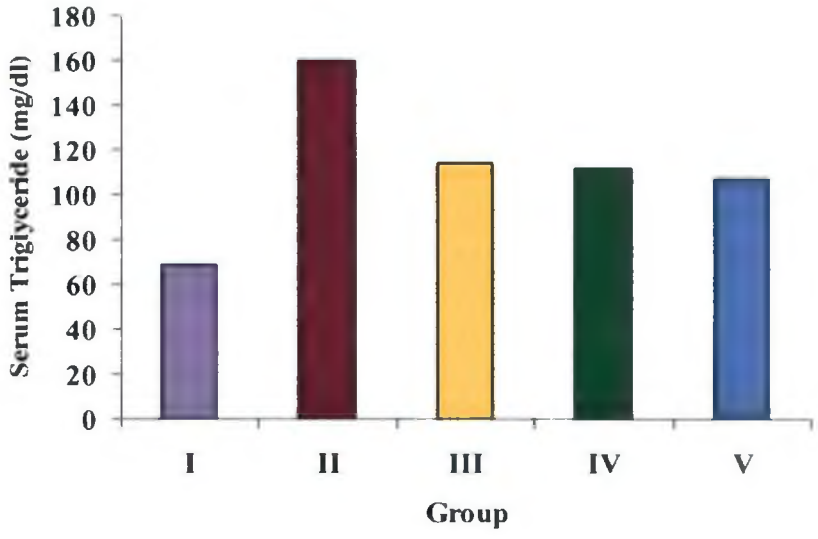

Fig.9.Effect of different doses of *Pleurotus ostreatus* on serum triglyceride level in diabetic rats-45th day

In terms of absolute value obtained on 45th day, group V treated with *Pleurotus ostreatus* at the dose rate of 1000 mg/kg showed the most significant ($p< 0.05$) reduction, the mean value obtained was 106.75 ± 1.57 mg/dl. Group II remained hypertriglyceridaemic with a mean serum triglyceride value of 160.38 ± 1.22 mg/dl. The reduction in serum triglyceride level obtained for Group IV was intermediate to that of group III and V which had a triglyceride level of 111.13 ± 1.85 mg/dl. Effect of various treatments on serum triglyceride level on 45th day is presented in Table 16 and Figure 9.

4.1.2.4 Liver Glycogen Level

Liver glycogen estimated on 45th day of the experiment is presented in Table 17 and Figure 10. Group I (normal control) had a mean liver glycogen value of 70.48 ± 2.33 mg%. There was a significant ($p< 0.05$) reduction in liver glycogen level of group II compared to group I, the value obtained was 33.70 ± 1.95 mg%. All the treatment groups (III, IV and V) showed a significant ($p< 0.05$) increase in liver glycogen level than group II. Increase in liver glycogen produced by groups III and IV were almost similar, the values obtained were 46.62 ± 0.91 and 50.09 ± 0.86 mg% respectively. Group V produced a significant ($p< 0.05$) increase in liver glycogen value compared to group III for which, the value obtained was 53.41 ± 0.99 mg%.

It is quite clear from the results obtained from the above studies that *Pleurotus ostreatus* at the dose rate of 1000 mg/kg was the most effective in reducing the blood glucose, cholesterol and triglyceride level in diabetic rats. Hence this was selected as the most suitable dose for combination with *Aegle marmelos* and *Murraya koenigii* each at a dose of 250 mg/kg body weight.

Table 17.Effect of different doses of ethanolic extract of *Pleurotus ostreatus* on liver glycogen level (mg %) in diabetic rats-45thday

Animal No.	Group I	Group II	Group III	Group IV	Group V
1	58.34	25.47	46.35	48.25	49.75
2	65.47	32.35	45.12	51.50	51.98
3	76.10	39.13	48.91	50.45	55.16
4	68.63	31.50	47.04	52.39	52.05
5	75.22	35.47	49.26	46.55	56.34
6	78.74	36.35	44.22	53.18	50.39
7	69.09	27.69	42.48	51.15	54.15
8	72.28	41.67	49.56	47.28	57.46
Mean±SE	70.48±2.33^{A}	33.70±1.95^{H}	46.62±0.91^{EF}	50.09±0.86^{DE}	53.41±0.99^{CD}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

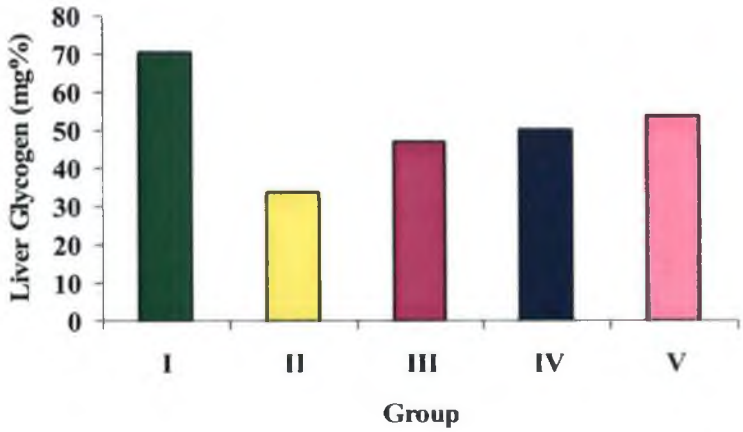

Fig.10.Effect of different doses of *Pleurotus ostreatus* on liver glycogen level in diabetic rats-45th day

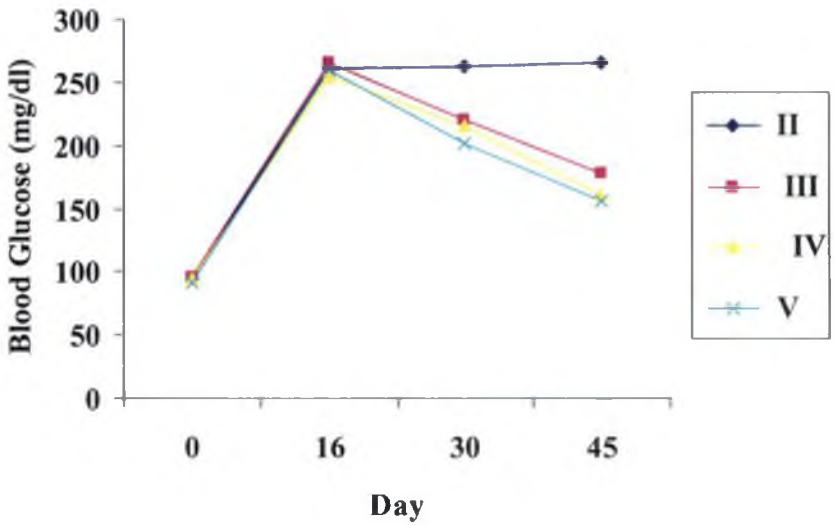

Fig.11. Comparison of the hypoglycaemic effect of different doses of *Pleurotus ostreatus*

4.2.1 Comparative study of *Aegle marmelos*, *Murraya koenigii* with combination of *Pleurotus ostreatus*, *Aegle marmelos* and *Murraya koenigii*

4.2.1.1 Body Weight

The individual and mean body weight of the rats (Groups I, II, VI, VII, VIII, and IX) recorded on zeroth, 16th, 30th and 45th day of the experiment are presented in Tables 18,19,20 and 21 respectively. The mean body weight recorded on zeroth day of Groups I, II, VI, VII, VIII, and IX were 170.00 ± 4.22, 173.75 ± 4.97, 170.00 ± 5.34, 172.50 ± 4.53, 183.75 ± 4.60 and 172.50 ± 5.26 g respectively.

On 16th day, all groups (II, VI, VII, VIII and IX) showed gradual decrease in body weight except group I (normal control). The mean body weight obtained on 16th day were 180.00 ± 3.78, 152.50 ± 3.66, 140.00 ± 3.78, 145.00 ± 4.63, 153.75 ± 4.20 and 153.75 ± 3.75 g respectively for groups I, II , VI , VII, VIII and IX.

On 30th day (after 14 days of treatment), groups VI, VII,VIII and IX showed a significant ($p< 0.05$) increase in body weight and the mean body weight recorded on 30th day were 140.00 ± 2.67, 151.25 ± 2.26, 155.00 ± 2.67, 168.75 ± 2.95 and 170.00 ± 3.27 g respectively for groups II, VI, VII, VIII and IX.

On 45th day, among the groups treated with ethanolic extract , group VIII which received combination produced the maximum gain in body weight compared to diabetic control with a mean value of 172.50 ± 4.12 g. Increase in body weight produced by group VI and VII were almost similar, for which the values obtained were 156.25 ± 2.63 and 160.00 ± 2.67 g respectively. However the most significant regain in body weight was produced by group IX which received glibenclamide, with a mean value of 175.00 ± 4.22 g. The mean body weights obtained are presented in Table 21.

Table 18.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on body weight (g) in diabetic rats- 0^{th} day

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	160	180	160	180	200	180
2	170	160	180	160	190	180
3	180	150	170	150	170	160
4	160	190	200	180	180	190
5	190	170	170	180	190	160
6	160	170	170	170	170	190
7	180	190	150	170	200	170
8	160	180	160	190	170	150
Mean±SE	170.00±4.22	173.75±4.97	170.00±5.34	172.50±4.53	183.75±4.60	172.50±5.26

Table 19.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on body weight (g) in diabetic rats- 16^{th} day

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	170	150	140	140	160	170
2	170	150	140	140	160	160
3	180	140	150	120	140	150
4	180	160	150	160	160	160
5	190	150	150	150	170	140
6	180	140	130	140	140	160
7	200	170	120	150	160	150
8	170	160	140	160	140	140
Mean±SE	180.00 ± 3.78^{A}	152.50 ± 3.66^{B}	140.00 ± 3.78^{C}	145.00 ± 4.63^{BC}	153.75 ± 4.20^{B}	153.75 ± 3.75^{B}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 20.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on body weight (g) in diabetic rats- 30th day

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	180	140	150	160	170	180
2	190	130	150	150	180	170
3	200	140	160	140	160	170
4	190	140	150	160	170	180
5	200	130	160	160	180	160
6	200	140	140	150	160	180
7	200	150	150	160	170	160
8	180	150	150	160	160	160
Mean±SE	192.50±3.13^{A}	140.00±2.67^{F}	151.25±2.26^{E}	155.00±2.67^{DE}	168.75±2.95^{B}	170.00±3.27^{B}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 21.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on body weight (g) in diabetic rats- 45th day

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	190	140	160	160	170	190
2	200	130	150	150	190	180
3	200	120	170	150	160	170
4	200	130	150	160	170	190
5	210	130	160	170	190	170
6	210	130	150	160	170	180
7	220	140	150	160	170	160
8	190	150	160	170	160	160
Mean±SE	202.50±3.66^{A}	133.75±3.24^{F}	156.25±2.63^{E}	160.00±2.67^{DE}	172.50±4.12^{BC}	175.00±4.22^{B}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

4.2.2 Biochemical parameters

4.2.2.1 Blood Glucose Level

Blood glucose level was estimated before giving alloxan (zeroth day), and on 16th, 30th and 45th day after giving alloxan. The individual and mean values recorded are represented in Tables 22, 23, 24 and 25 respectively.

The blood glucose level of rats before giving alloxan (zeroth day) were 90.57 ± 1.11, 94.62 ± 3.25, 91.68 ± 1.57, 92.30 ± 1.12, 94.01 ± 1.92 and 88.73 ± 2.12 mg/dl respectively for groups I, II, VI, VII, VIII and IX.

On 16th day, all the groups except normal control showed an increase in blood glucose level with a mean value of 260.69 ± 8.07, 266.71 ± 9.75, 265.49 ± 10.67, 268.92 ± 4.12 and 267.80 ± 9.55 mg/dl respectively for groups II,VI, VII, VIII and IX.

On 30th day, Group VIII showed significantly ($p< 0.05$) higher reduction in blood glucose level , 203.41 ± 3.76 mg/dl compared to the groups VI and VII for which the values obtained were 236.39 ± 9.29 and 231.05 ± 9.45 mg/dl respectively. Group IX (glibenclamide treated) showed a much greater decrease in blood glucose value of 195.45 ± 7.12 mg/dl. The mean blood glucose level obtained for different groups on 30th day is presented in Table 24 and Figure 12.

At the end of the experiment (on 45th day), group VIII showed a significantly ($p< 0.05$) high reduction of 147.23 ± 2.91 mg/dl compared to groups VI and VII for which values obtained were 195.43 ± 7.14 and 185.12 ± 9.05 mg/dl respectively. Group IX had a significantly lower value than all the treatment groups with a mean

Table 22.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on blood glucose level in diabetic rats- 0^{th} day, mg/dl

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	93.20	86.33	98.31	87.75	87.75	88.44
2	90.51	106.25	85.15	90.12	92.18	86.05
3	88.43	80.91	90.26	95.85	88.04	79.09
4	94.64	87.42	87.46	93.48	96.38	96.24
5	89.53	92.68	89.63	94.01	100.06	90.18
6	86.77	98.74	94.68	88.51	95.35	82.34
7	94.35	100.15	95.92	92.64	89.94	92.78
8	87.15	104.50	92.04	96.04	102.28	94.69
Mean±SE	90.57±1.11	94.62±3.25	91.68±1.57	92.30±1.12	94.01±1.92	88.73±2.12

Table 23.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on blood glucose level in diabetic rats- 16^{th} day, mg/dl

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	95.42	274.04	282.28	295.00	251.08	238.02
2	86.18	238.32	285.68	207.55	258.84	244.37
3	94.36	288.46	284.49	256.38	265.63	287.26
4	103.18	295.18	244.56	243.16	279.84	294.12
5	96.28	248.30	283.60	259.68	260.05	297.20
6	90.64	235.65	294.31	279.91	281.91	292.64
7	89.30	245.48	235.76	293.72	273.62	248.00
8	100.08	260.12	222.96	288.48	280.35	240.77
Mean±SE	94.43 ± 1.99^{B}	260.69 ± 8.07^{A}	266.71 ± 9.75^{A}	265.49 ± 10.67^{A}	268.92 ± 4.12^{A}	267.80 ± 9.55^{A}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 24.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on blood glucose level in diabetic rats- 30th day, mg/dl

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	98.62	286.50	239.94	244.85	185.70	170.47
2	88.40	235.28	260.48	181.60	199.39	178.02
3	99.15	290.04	261.73	228.17	201.88	218.46
4	108.54	292.52	222.55	211.55	215.95	208.47
5	91.35	245.18	243.90	220.72	193.95	207.89
6	97.43	240.22	262.35	251.92	216.56	219.83
7	95.74	241.35	205.11	267.29	203.93	181.41
8	94.68	270.54	195.09	242.32	209.93	179.08
Mean±SE	96.74 ± 2.12^{F}	262.70 ± 8.74^{A}	236.39 ± 9.29^{B}	231.05 ± 9.45^{BC}	203.41 ± 3.76^{DE}	195.45 ± 7.12^{E}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 25.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on blood glucose level in diabetic rats- 45th day, mg/dl

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	94.09	284.31	215.21	203.55	138.57	125.48
2	91.24	230.64	214.26	130.76	142.97	123.77
3	90.58	295.56	207.65	186.88	140.31	140.24
4	101.11	290.18	181.46	182.25	158.22	135.30
5	85.39	248.45	202.15	170.94	142.25	148.60
6	88.60	243.32	207.78	190.34	148.31	143.92
7	85.22	248.68	169.50	208.54	146.28	112.47
8	94.38	278.26	165.46	207.71	160.89	113.84
Mean±SE	91.33 ± 1.86^{F}	264.93 ± 8.77^{A}	195.43 ± 7.14^{B}	185.12 ± 9.05^{BC}	147.23 ± 2.91^{D}	130.45 ± 4.81^{E}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

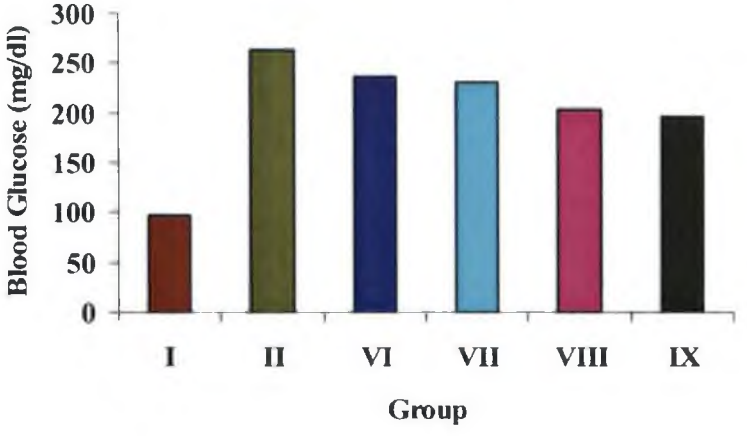

Fig.12.Effect of *Murraya koenigii*, *Aegle marmelos* and their combination with *Pleurotus ostreatus* on blood glucose level in diabetic rats- 30th day

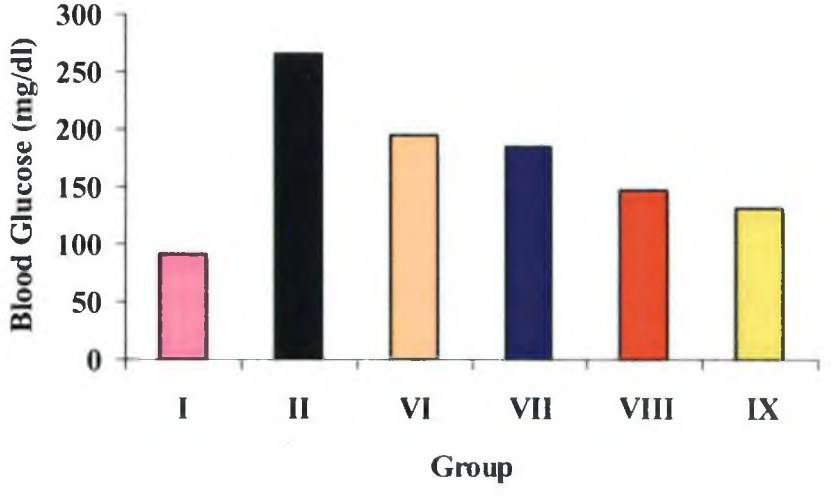

Fig.13.Effect of *Murraya koenigii*, *Aegle marmelos* and their combination with *Pleurotus ostreatus* on blood glucose level in diabetic rats- 45th day

Group I-Normal Control, Group II- Diabetic Control, Group VI- Diabetic + *Murraya koenigii*, Group VII- Diabetic+*Aegle marmelos*, Group VIII-Diabetic + combination of *Pleurotus ostreatus* (1000 mg/kg), *Murraya koenigii* (250 mg/kg) and *Aegle marmelos* (250mg/kg), Group IX- Diabetic + glibenclamide

value of 130.45 ± 4.81 mg/dl. The reduction in blood glucose level produced by different treatments on 45th day is presented in Table 25 and Figure 13.

4.2.2.2 *Serum Cholesterol Level*

Serum cholesterol level estimated for groups I, II, VI, VII, VIII and IX on zeroth day, 16th, 30th and 45th day are presented in Tables 26, 27, 28, and 29 respectively. The serum cholesterol level of rats on zeroth day were 78.13 ± 3.74, 75.00 ± 4.21, 85.75 ± 3.37, 79.50 ± 1.91, 73.13 ± 1.93 and 81.13 ± 2.59 mg/dl for groups I, II, VI, VII, VIII and IX.

On 16th day, all the groups except normal control showed an increase in cholesterol level with a mean value of 156.75 ± 3.48, 155.38 ± 3.15, 157.00 ± 2.14, 156.75 ± 2.60 and 150.63 ± 3.58 mg/dl respectively for groups II,VI, VII, VIII, and IX.

On 30th day, there was a significant ($p< 0.05$) decrease in serum total cholesterol value in groups VI, VII, VIII and IX than group II. The reduction in serum cholesterol level seen in groups VI and VII were almost similar for which the values obtained were 138.00 ± 3.41 and 133.13 ± 3.22 mg/dl respectively. Group VIII which received combination produced a significant decrease in serum cholesterol level compared to other groups, the value obtained was 122.25 ± 2.01 mg/dl. Group IX had the lowest serum cholesterol level of 114.25 ± 2.74 mg/dl. The mean serum cholesterol level produced by different treatments on 30th day is presented in Table 28 and Figure 14.

All treatment groups showed significant ($p< 0.05$) decrease in serum cholesterol level compared to group II on 45th day. Group II (diabetic control) had a

Table 26.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on serum cholesterol level in diabetic rats- 0th day, mg/dl

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	64	65	102	85	73	70
2	77	58	95	88	71	83
3	71	69	86	74	65	78
4	80	71	82	77	67	91
5	66	77	73	80	78	75
6	90	80	90	78	73	86
7	92	84	80	72	77	77
8	85	96	78	82	81	89
Mean±SE	78.13±3.74	75.00±4.21	85.75±3.37	79.50 ±1.91	73.13±1.93	81.13±2.59

Table 27.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on serum cholesterol level in diabetic rats- 16th day, mg/dl

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	66	148	170	165	147	168
2	82	139	163	158	152	159
3	76	152	158	152	149	155
4	85	158	152	155	156	151
5	71	160	142	164	158	138
6	85	163	159	160	165	150
7	88	166	148	147	159	139
8	81	168	151	155	168	145
Mean±SE	79.25±2.70^{D}	156.75±3.48^{AB}	155.38±3.15^{ABC}	157.00±2.14^{ABC}	156.75±2.60^{A}	150.63±3.58^{C}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 28.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on serum cholesterol level in diabetic rats- 30th day, mg/dl

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	70	154	152	140	114	127
2	80	144	142	133	120	122
3	78	151	145	125	117	115
4	81	147	130	130	119	114
5	74	150	128	145	126	102
6	88	155	147	144	125	115
7	87	160	133	120	126	109
8	83	162	127	128	131	110
Mean±SE	80.13 ± 2.17^{F}	152.88 ± 2.17^{A}	138.00 ± 3.41^{B}	133.13 ± 3.22^{B}	122.25 ± 2.01^{D}	114.25 ± 2.74^{E}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 29.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on serum cholesterol level in diabetic rats- 45th day, mg/dl

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	69	150	134	125	85	92
2	84	140	125	107	93	88
3	73	158	117	110	90	88
4	86	149	109	120	88	80
5	70	156	111	112	92	73
6	84	161	123	119	95	89
7	89	165	113	100	95	82
8	90	158	120	115	101	84
Mean±SE	80.63 ± 3.03^{E}	154.63 ± 2.80^{A}	119.00 ± 2.93^{B}	113.50 ± 2.82^{B}	92.38 ± 1.73^{D}	84.50 ± 2.15^{E}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

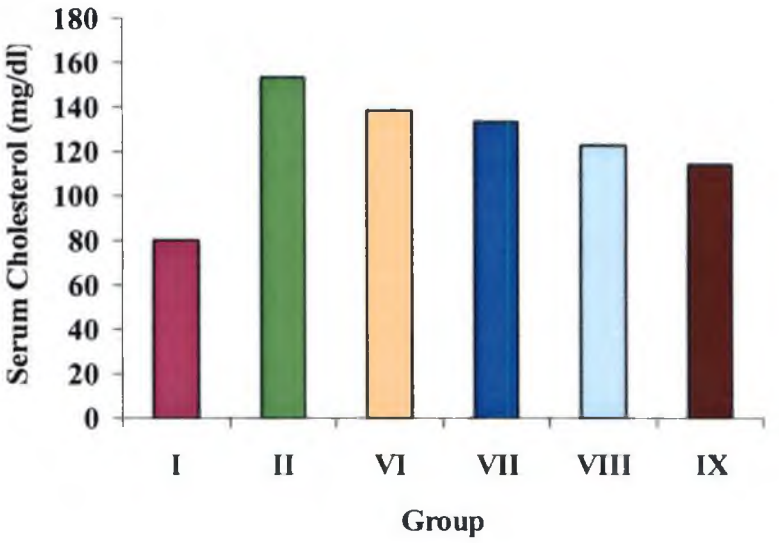

Fig.14.Effect of *Murraya koenigii*, *Aegle marmelos* and their combination with *Pleurotus ostreatus* on serum cholesterol level in diabetic rats- 30th day

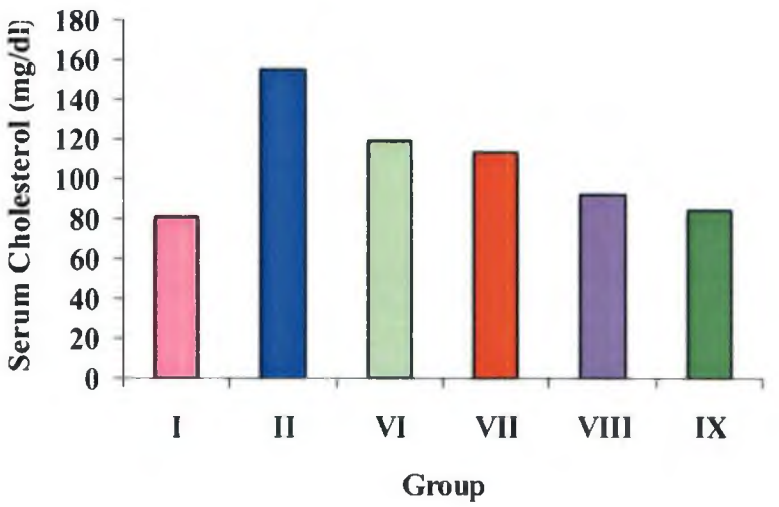

Fig.15.Effect of *Murraya koenigii*, *Aegle marmelos* and their combination with *Pleurotus ostreatus* on serum cholesterol level in diabetic rats- 45th day

higher cholesterol value of 154.63 ± 2.80 mg/dl. Among groups treated with ethanolic extract, group VIII showed the lowest serum cholesterol level and the value obtained was 92.38 ± 1.73 mg/dl. There was not much significant difference in reduction of serum cholesterol level between groups VI and VII, the values obtained were 119.00 ± 2.93 and 113.50 ± 2.82 mg/dl respectively. Group IX which received glibenclamide had the lowest cholesterol level of 84.50 ± 2.15 mg/dl. Table 29 and Figure 15 represent the reduction in serum cholesterol level produced by different treatments on 45th day.

4.2.2.3 *Serum Triglyceride Level*

Serum triglyceride level estimated on zeroth, 16th, 30th and 45th day are presented in Tables 30, 31, 32 and 33 respectively. The mean values obtained on zeroth day were 69.63 ± 1.90, 70.25 ± 1.89, 72.75 ± 1.16, 71.25 ± 1.58, 68.63 ± 1.84 and 69.75 ± 1.71 mg/dl respectively for groups I, II, VI, VII, VIII and IX, which represent the normal serum triglyceride level before giving alloxan.

On 16th day, all the groups with the exception of normal control showed an increase in serum triglyceride level with a mean value of 158.13 ± 1.63, 159.25 ± 1.91, 162.88 ± 1.92, 161.38 ± 1.37 and 156.75 ± 2.16 mg/dl respectively for groups II, VI, VII, VIII, and IX.

On 30th day, all the treatment groups (VI, VII, VIII and IX) showed a significant ($p< 0.05$) decrease in serum triglyceride level than group II. Group VIII which received combination showed a significant ($p< 0.05$) reduction in serum triglyceride level when compared to other treatment groups for which the value obtained was 128.25 ± 1.77 mg/dl. There was no significant difference in reduction of serum triglyceride between groups VI and VII, the values obtained were 141.13 ± 1.78 and 145.50 ± 2.61 mg/dl respectively. Group IX which received glibenclamide

Table 30.Effect of ethanolic extract of *M.koenigii, A. marmelos* and their combination with *P. ostreatus* on serum triglyceride level in diabetic rats- 0th day, mg/dl

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	64	79	76	72	63	72
2	69	68	74	76	69	76
3	67	72	77	71	67	68
4	78	66	71	78	68	70
5	62	75	68	68	73	66
6	72	64	75	65	79	62
7	75	73	69	73	66	68
8	70	65	72	67	64	76
Mean±SE	69.63±1.90	70.25±1.89	72.75±1.16	71.25±1.58	68.63±1.84	69.75±1.71

Table 31.Effect of ethanolic extract of *M.koenigii, A. marmelos* and their combination with *P. ostreatus* on serum triglyceride level in diabetic rats- 16th day, mg/dl

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	69	162	163	165	157	157
2	73	154	156	169	163	161
3	76	161	165	164	160	168
4	74	151	159	170	166	154
5	68	159	151	159	159	150
6	69	155	155	155	165	150
7	71	165	158	164	156	154
8	76	158	167	157	165	160
Mean±SE	72.00±1.13^{D}	158.13±1.63^{BC}	159.25±1.91^{BC}	162.88±1.92^{AB}	161.38±1.37^{ABC}	156.75±2.16^{C}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 32.Effect of ethanolic extract of *M.koenigii, A. marmelos* and their combination with *P. ostreatus* on serum triglyceride level in diabetic rats- 30th day, mg/dl

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	66	160	145	145	131	123
2	75	157	136	152	131	124
3	71	154	144	148	132	129
4	72	156	140	156	126	116
5	63	162	132	139	123	114
6	74	159	141	134	132	123
7	66	158	144	150	119	125
8	73	155	147	140	132	126
Mean±SE	70.00 ± 1.56^{F}	157.63 ± 0.94^{A}	141.13 ± 1.78^{BC}	145.50 ± 2.61^{B}	128.25 ± 1.77^{D}	122.50 ± 1.78^{E}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

Table 33.Effect of ethanolic extract of *M.koenigii, A. marmelos* and their combination with *P. ostreatus* on serum triglyceride level in diabetic rats- 45th day, mg/dl

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	65	163	135	125	100	92
2	70	161	120	130	99	100
3	68	159	133	119	100	100
4	76	154	123	128	103	90
5	66	158	116	124	101	86
6	65	165	118	110	102	91
7	69	163	128	122	94	92
8	71	160	130	116	103	89
Mean±SE	68.75 ± 1.31^{G}	160.38 ± 1.22^{A}	125.38 ± 2.52^{B}	121.75 ± 2.32^{B}	100.25 ± 1.03^{E}	92.50 ± 1.77^{F}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

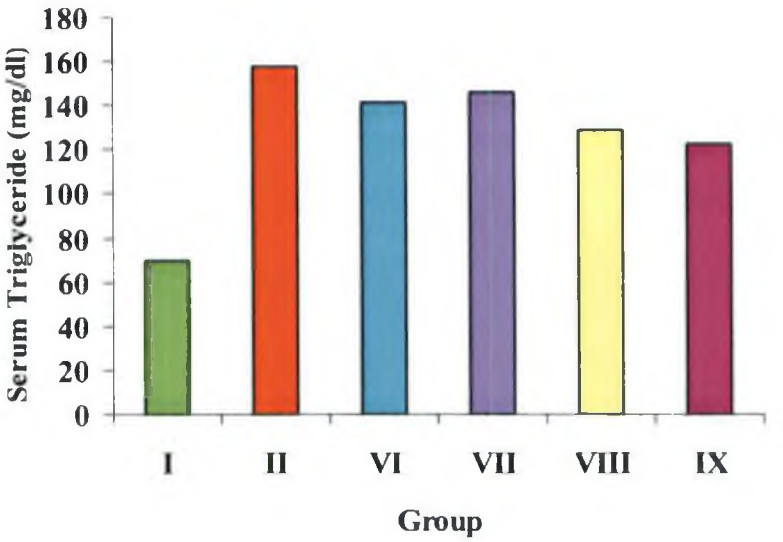

Fig.16.Effect of *Murraya koenigii*, *Aegle marmelos* and their combination with *Pleurotus ostreatus* on serum triglyceride level in diabetic rats- 30th day

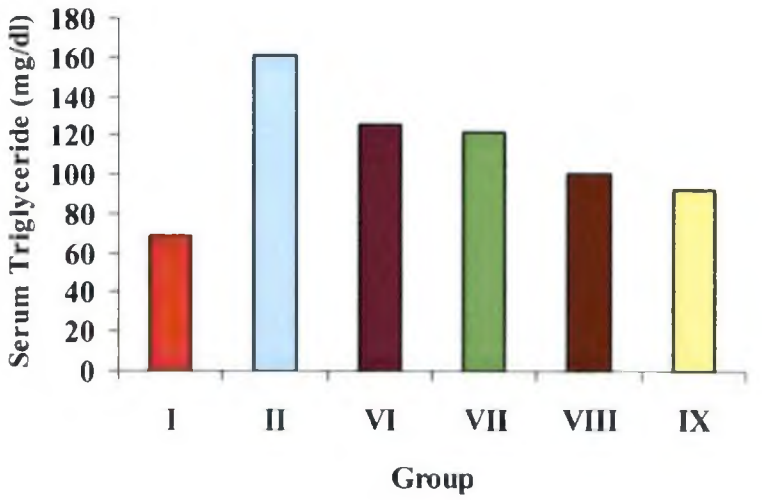

Fig.17.Effect of *Murraya koenigii*, *Aegle marmelos* and their combination with *Pleurotus ostreatus* on serum triglyceride level in diabetic rats- 45th day

had a much lower triglyceride value of 122.50 ± 1.78 mg/dl. The reduction in serum triglyceride value obtained for different groups on 30th day is presented in Table 32 and Figure16.

On 45th day, group IX treated with glibenclamide showed the most significant ($p<0.05$) reduction followed by group VIII, the mean value obtained were 92.50 ± 1.77 and 100.25 ± 1.03 mg/dl respectively. Groups VI and VII produced almost similar reduction in triglyceride value compared to group II, for which the values obtained were 125.38 ± 2.52 and 121.75 ± 2.32 mg/dl respectively. Effect of various treatments on serum cholesterol level on 45th day is presented in Table 33 and Figure 17.

4.2.2.4 *Liver Glycogen Level*

Liver glycogen estimated on 45th day of the experiment is presented in Table 34 and Figure18. Group I (normal control) had a mean liver glycogen value of 70.48 ± 2.33 mg%. There was a significant ($p< 0.05$) reduction in liver glycogen level of group II compared to group I, the value obtained was 33.70 ± 1.95 mg%. All the treatment groups showed a significant ($p< 0.05$) increase in liver glycogen level than group II. There was significant difference in elevation of liver glycogen between groups VI and VII. The highest liver glycogen level among groups VI and VII was seen in group VII, the value obtained was 44.16 ± 1.51 mg%. Among all the groups treated with ethanolic extract, group VIII which received combination showed the highest increase in liver glycogen level, for which the value obtained was 56.94 ± 0.87 mg%. The increase in liver glycogen level produced by group VIII was comparable to that produced by group IX, which had a liver glycogen value of 60.61 ± 0.71 mg%.

Table 34.Effect of ethanolic extract of *M.koenigii*, *A. marmelos* and their combination with *P. ostreatus* on liver glycogen (mg%) in diabetic rats- 45th day

Animal No.	Group I	Group II	Group VI	Group VII	Group VIII	Group IX
1	58.34	25.47	36.59	38.40	56.53	61.38
2	65.47	32.35	40.12	41.27	58.10	60.57
3	76.10	39.13	42.38	44.67	53.38	57.41
4	68.63	31.50	33.68	49.23	59.90	63.49
5	75.22	35.47	46.17	50.34	57.35	61.17
6	78.74	36.35	38.55	39.92	54.42	59.50
7	69.09	27.69	41.23	46.15	60.29	62.71
8	72.28	41.67	35.47	43.32	55.55	58.64
Mean±SE	70.48±2.33^{A}	33.70±1.95^{H}	39.37±1.43^{G}	44.16±1.51^{F}	56.94±0.87^{BC}	60.61±0.71^{B}

(Means bearing same superscript do not differ significantly at $p < 0.05$)

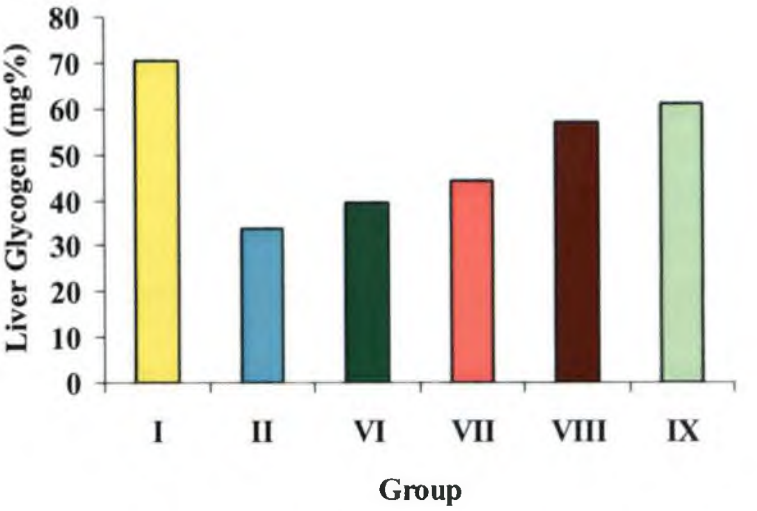

Fig.18.Effect of *Murraya koenigii*, *Aegle marmelos* and their combination with *Pleurotus ostreatus* on liver glycogen level in diabetic rats- 45th day

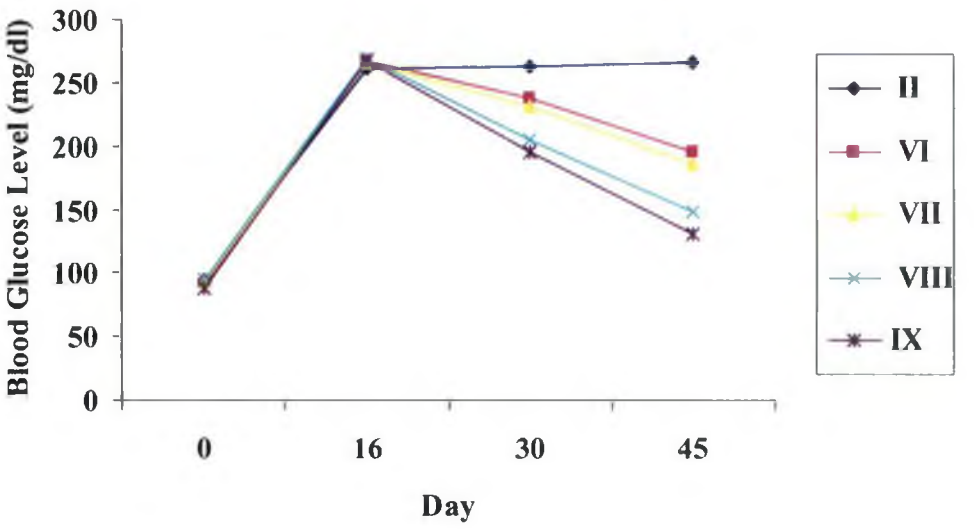

Fig.19. Comparison of the hypoglycaemic effect of *Murraya koenigii*, *Aegle marmelos* and their combination with *Pleurotus ostreatus*

Discussion

5. DISCUSSION

The present study was undertaken to assess the hypoglycaemic effect of *Pleurotus ostreatus* and to evaluate the effect of combination of this plant with *Murraya koenigii* and *Aegle marmelos*. A preliminary study was first conducted to assess the hypoglycaemic effect of the ethanolic extract of *Pleurotus ostreatus* at three different doses (250, 500 and 1000 mg/kg) and the most suitable dose among these was then used for the further study. In the second phase, the effect of combination of the most suitable dose of *Pleurotus ostreatus* with *Murraya koenigii* and *Aegle marmelos* were assessed and compared with *Murraya koenigii* and *Aegle marmelos* each at a dose of 250 mg/kg and also with the reference drug, glibenclamide.

5.1.1 Preliminary study to find out the most effective dose of *Pleurotus ostreatus*

5.1.1.1 *Body Weight*

All the treated groups (III, IV and V) showed gradual increase in body weight from 16th to 45th day of experiment. Diabetic control (group II) did not show any gain in mean body weight during the 45 day experimental period. Conversely, a reduction was noticed from 152.50 ± 3.66g to 133.75 ± 3.24g. This agrees with the findings of Xie *et al.* (2003) who reported that the body weight gets reduced in diabetic patients due the increased mobilization of fatty acids from the storage site to meet the energy demand. Though the mean body weight of groups III, IV and V were progressively increased during the experiment, they never attained the weight at the beginning of the experiment before inducing diabetes.

In the present study, animals treated with *Pleurotus ostreatus* at the dose rate of 250 mg/kg showed a mean body weight of 158.75± 2.26g after 14 days of treatment (on 30th day) which was significantly higher than that of the diabetic control. Group IV treated at the dose rate of 500 mg/kg produced a much greater increase in body weight. The highest increase in body weight (165.00 ± 2.67 g) was obtained with group V which received *Pleurotus ostreatus* at the dose rate of 1000 mg/kg.

After 30 days of treatment (on 45th day) also, the highest increase in body weight compared to diabetic control was obtained in group V. They showed a mean body weight of 167.50 ± 2.50g. The results of the present study indicate that the diabetic animals treated with the plant extract showed a dose dependent gain in body weight. Similar increase in body weight was also observed by Alarcon- Aguilar *et al.* (2005) by the administration of dichloromethane extract of *Ibervillea sonorae* for 41 days at a dose of 300 mg/kg/day in alloxan diabetic rats.

5.1.2 BIOCHEMICAL PARAMETERS

5.1.2.1 ***Blood Glucose Level***

The mean blood glucose levels prior to alloxan administration were similar among all the five groups. After sixteen days of alloxan administration, about three fold elevation of blood glucose level was observed. The blood glucose level in diabetic control animals increased steadily throughout the study period. The mean blood glucose value increased from 94.62 ± 3.25 mg/dl to 264.93 ± 8.77 mg/dl after 45 days which concurs with the findings of Babu *et al.* (2002) who reported a substantial increase in blood glucose level in alloxan induced diabetic animals. The blood glucose level was found to be decreased from the pretreatment value in all the treatment groups.

After 14 days of treatment, animals treated with *Pleurotus ostreatus* at different doses showed a significant reduction in blood glucose levels compared to diabetic control (group II). The highest reduction in blood glucose level was observed with group V. The reduction in blood glucose level shown by group IV was intermediate to that of group III and V.

A dose dependent decrease in blood glucose level was obtained with Pleurotus *ostreatus* at doses of 250,500 and 1000 mg/kg at the end of the experimental period. Group III showed a blood glucose level of 177.56 ± 3.95 mg/dl which was significantly lower than that of diabetic control (264.93 ± 8.77 mg/dl). The highest decrease in blood glucose level of 154.90 ± 3.16 mg/dl was obtained with group V after 30 days of treatment. Similar dose dependent decrease in blood glucose level was reported by Raphael *et al.* (2002), who found that the blood sugar level in alloxan diabetic rats reduced by six per cent at a dose of 200 mg/kg body weight and 18.70 per cent at a concentration of 1000 mg/kg body weight at the fourth hour of administration of *Phyllanthus amarus* extract. Sabu *et al.* (2003) also obtained a higher reduction in blood glucose level (42.30 per cent) with the oral administration of *Aloe arborescens* extract at 1000 mg/kg body weight compared to 26.90 per cent reduction in blood glucose level at 200 mg/kg body weight.

5.1.2.2 *Serum Cholesterol Level*

In the present study, animals of group II (diabetic control) showed a substantial increase in total serum cholesterol level with a mean value of 154.63 ± 2.80 mg/dl after 45 days of study period. This is in agreement with Lino *et al.* (2004), who suggested that hyperlipidaemia often occurs in the untreated or under treated diabetic patients because of the altered metabolism of carbohydrates, lipids,

ketones and amino acids. These abnormalities could be due to a decrease in the circulating concentration of insulin (insulin deficiency) and a decrease in the response of the peripheral tissues to insulin (insulin resistance).

After 14 days of treatment, diabetic rats of group V which received *Pleurotus ostreatus* at the dose rate of 1000 mg/kg body weight had a lower serum cholesterol level of 124.75 ± 3.07 mg/dl compared to that of group III (132.75 ± 3.28 mg/dl) and group IV (131.38 ± 3.05 mg/dl).

After 30 days of treatment, there was substantial reduction in serum cholesterol level in all the treated groups. *Pleurotus ostreatus* at the dose rate of 1000 mg/kg body weight showed the lowest cholesterol level of 95.63 ± 2.51 mg/dl. There was significant difference in reduction of serum cholesterol level between groups III (107.63 ± 2.83 mg/dl) and IV (104.63 ± 2.64 mg/dl).

The results of the present study agree with the findings of Bopanna *et al.* (1997). They found that the administration of neem kernel powder alone at the dose rate of 500 mg/kg as well as in combination with glibenclamide significantly decreased the concentration of serum lipid in alloxan diabetic rabbits. Possible mechanism may be due to the down regulation of NADPH and NADH, a co-factor in fat metabolism.

5.1.2.3 *Serum Triglyceride Level*

In the present study, there was a significant increase in serum triglyceride level in all the four groups except normal control after sixteen days of alloxan administration. Shirwaikar *et al.* (2005) suggested that the most common lipid

abnormalities in diabetes are hypertriglyceridaemia and hypercholesterolaemia. Hypertriglyceridaemia is also associated with metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and insulin tolerance.

After 14 days of treatment, groups III, IV and V showed a similar trend of decrease with serum triglyceride profile. The reduction in serum triglyceride level produced by *Pleurotus ostreatus* at the dose rate of 250, 500 and 1000 mg/kg body weight were 137.38 ± 2.17, 138.13 ± 2.01 and 137.00 ± 1.96 mg/dl respectively.

At the end of the experimental period, groups III, IV and V produced a substantial reduction in serum triglyceride level. Group V showed the lowest serum triglyceride level of 106.75 ± 1.57 mg/dl. Between groups III and IV, group IV had a lower serum triglyceride level of 111.13 ± 1.85 mg/dl.

Significant lowering of serum triglyceride was also observed by Chakrabarti *et al.* (2005) by the administration of aqueous extract of *Caesalpinia bonducella* for a period of 28 days. Serum triglyceride level decreased significantly from its day 1 value of 100.00 mg/dl to 74.77 mg/dl on day 28.

5.1.2.4 *Liver Glycogen Level*

The liver glycogen content was decreased markedly in diabetic animals (group II). The decrease in hepatic and skeletal muscle glycogen content in diabetic rats has been observed by Maiti *et al.* (2004).They suggested that the decrease in

muscle and hepatic glycogen may be due to lack of insulin and also due to inactivation of glycogen synthetase system.

Treatment with *Pleurotus ostreatus* at the dose rate of 250, 500 and 1000 mg/kg produced significant increase in the reduced liver glycogen level compared to diabetic control. The highest liver glycogen value of 53.41 ± 0.99 mg% was noticed in group V. The increase in liver glycogen obtained for groups III and IV were almost similar (46.62 ± 0.91 and 50.09 ± 0.86 mg %).

The findings of the present study concur with the observation of John (2003). She found an increase in liver glycogen level when diabetic rats were administered with *Brassica juncea* and *Trigonella foenum graecum* seeds extract and suggested that this might be due to enhancement of the rate of glycogenesis in alloxan treated rats.

From the results of the preliminary study, it is quite evident that the dose rate of 1000 mg/kg is the most suitable one in reducing blood glucose and lipid level among the three doses of ethanolic extract of *Pleurotus ostreatus*.

5.2.1 Comparative study of *Aegle marmelos*, *Murraya koenigii* with combination of *Pleurotus ostreatus*, *Aegle marmelos* and *Murraya koenigii*

5.2.1.1 *Body Weight*

Body weight of alloxan treated groups markedly reduced after 16 days of its administration. All the treatment groups (VI, VII, VIII and IX) showed gradual increase in body weight after 30 days of treatment. Though the body weight of

treatment groups were progressively increased during the experiment, they never attained the weight at the beginning of the experiment before inducing diabetes.

In the present study, animals treated with ethanolic extract of *Murraya koenigii* (group VI) showed a mean body weight of 151.25 ± 2.26g after 14 days of treatment which was significantly higher than that of diabetic control. Group VII which received *Aegle marmelos* produced a much greater increase in mean body weight of 155.00 ± 2.67g than group VI. Group IX which received glibenclamide produced the highest increase in the mean body weight (170.00 ± 3.27g) after 14 days of treatment. Animals treated with combination of *Aegle marmelos* and *Murraya koenigii* each at the dose rate of 250 mg/kg and *Pleurotus ostreatus* at the dose rate of 1000 mg/kg body weight also showed a similar increase in mean body weight of 168.75 ± 2.95g.

After 30 days of treatment, Group VII treated with *Aegle marmelos* produced a significant increase in body weight (160.00 ± 2.67g) compared to diabetic control. Similar observation was recorded by Seema *et al.*(1996) who found that oral administration of aqueous extract of *Aegle marmelos* at the dose rate of 1g/kg reversed the body weight to near normal levels in streptozotocin induced diabetic rats.

Increase in body weight produced by *Murraya koenigii* was comparatively lower than that obtained with *Aegle marmelos*. In the present study group VIII which received combination was found to be superior over other groups which received individual plant extract. Group VIII showed an increase in body weight of 172.50 ± 4.12g which was significantly higher than the body weight of the diabetic control (133.75 ± 3.24g). Joy and Kuttan (1999) also could find a significant increase in

body weight when alcoholic extract of *Picrorrhiza kurroa* was administered to diabetic rats and found that the body weight was maintained near to normal levels.

5.2.2 BIOCHEMICAL PARAMETERS

5.2.2.1 *Blood Glucose Level*

The blood glucose levels before inducing diabetes were almost similar in all the six groups (I, II, VI, VII, VIII and IX). After 16 days of alloxan administration, the blood glucose level markedly increased in all the groups except normal control which were not administered with alloxan. This concurs with the findings of Anturlikar *et al.* (1995), Puri *et al.* (2002) and Shirwaikar *et al.* (2005) that blood glucose level in alloxan induced diabetic animals increased substantially. The fundamental mechanism underlying hyperglycaemia might be due to over production of glucose by excessive hepatic glycogenolysis and gluconeogenesis and its decreased utilization by the tissues.

Administration of ethanolic extract of *Murraya koenigii* significantly reduced the blood glucose level (236.39 ± 9.29 mg/dl) compared to diabetic control (262.70 ± 8.74 mg/dl) after 14 days of treatment. The reduction in blood glucose level produced by *Aegle marmelos* (231.05 ± 9.45 mg/dl) was comparatively higher than that produced by *Murraya koenigii* after 14 days of treatment. Group VIII which received combination had the lowest blood glucose level (203.41 ± 3.76 mg/dl) than groups treated with individual plant extract. The highest reduction in blood glucose level was seen in group IX treated with glibenclamide which had a blood glucose level of 195.45 ± 7.12 mg/dl.

At the end of the experimental period (after 30 days of treatment), all the treatment groups showed significant decrease in blood glucose level compared to diabetic control. The reduction in blood glucose level after oral administration of ethanolic extract of *Aegle marmelos* and *Murraya koenigii* each at a dose of 250 mg/kg was almost similar after 30 days of treatment.

Murraya koenigii treated group showed a lower blood glucose level of 195.43 ± 7.14 mg/dl compared to diabetic control which had a blood glucose level of 264.93 ± 8.77 mg/dl. Significant lowering of blood glucose level was also observed by Khan *et al.* (1995) when they fed 10 per cent each of *Murraya koenigii* (curry leaf) and *Brassica juncea* (mustard) to diet. They suggested that these spices, curry leaf and mustard exerted their hypoglycaemic activity by enhanced glycolysis, glycogenesis and decreased glycogenolysis. Similar observation was recorded by Narayana and Sastry (1975) who found that oral administration of aqueous extract of leaves of *Murraya koenigii* at the dose rate of 160 mg/kg body weight produced significant hypoglycaemia in normal and alloxan diabetic dogs. The hypoglycaemic effect in normal dogs could be due to the release of endogenous insulin from β cells whereas in diabetic dogs, the reduction could only be due to a decrease in gluconeogenesis and output of hepatic glucose.

Vinuthan *et al.* (2004) reported that the aqueous and methanolic extracts of *Murraya koenigii* leaf powder decreased blood glucose in alloxan diabetic rats. They suggested that it may be either due to increased glycogenesis or decreased glycogenolysis or gluconeogenesis and /or due to insulin secretagogue effect of *Murraya koenigii* which causes an increased glucose uptake and its utilization by cells. A single oral administration of various dose levels (200, 300 and 400 mg/kg) of aqueous extract of *Murraya koenigii* led to lowering of blood glucose level in

normal as well as in diabetic rabbits. The maximum fall of 14.68 per cent in normal and 27.96 per cent in mild diabetic rabbits were observed after 4 hours of oral administration of 300 mg/kg (Kesari *et al.*, 2005).

Group VII treated with *Aegle marmelos* produced a significant reduction in blood glucose level (185.12 ± 9.05 mg/dl) which was comparatively higher than that of group VI after 30 days of treatment. This is in agreement with the observation of many research workers. Ponnachan *et al.* (1993) observed the same effect when he administered aqueous extract of *Aegle marmelos* leaf at 1g/kg body weight. They found that the extract reduced the blood glucose level in diabetic rats near to that of control ones and suggested that the active principle in leaf extract had similar hypoglycaemic activity to insulin treatment. According to Sabu and Kuttan (2004), oral administration of methanolic extract of *Aegle marmelos* at the dose rate of 100 mg/kg body weight for 12 days produced a significant reduction in blood glucose when compared to diabetic control from sixth day onwards and reduced to 54 per cent of the initial value of day 12. Rao *et al.* (1995) suggested that administration of *Aegle marmelos* leaf extract in normal rabbits produced hypoglycaemic effect similar to phenformin by stimulating β cells to release insulin.

In the present study, group VIII treated with combination produced a much greater reduction in blood glucose level (147.23 ± 2.91 mg/dl) than groups treated with individual plant extract. Sathyan (2004) has got similar reduction in plasma glucose level when diabetic rats were fed with combination of *Azadirachta indica, Ocimum sanctum* and *Tinospora cordifolia* leaf extracts and suggested that the extract could delay the absorption of complex carbohydrates in small intestine like that of acarbose resulting in a decreased post prandial glucose content and a reduction in long term diabetic complications.

However, the most effective reduction in blood glucose level was brought about by glibenclamide treated group in which the rats showed a blood glucose level of 130.45 ± 4.81 mg/dl. This concurs with the findings of Bajaj and Srinivasan (1999), Khosla *et al.* (2000) and Ghosh *et al.* (2004). Ananthan *et al.* (2003) has got similar reduction in blood glucose level from 245.58 ± 13.99 to 118.22 ± 4.48 mg/dl when the diabetic rats were administered with glibenclamide for a period of 3 weeks.

Chakrabarti *et al.* (2005) reported the antidiabetic activity of *Caesalpinia bonducella* in chronic type 2 diabetic model in Long- Evans rats and were of the opinion that the hypoglycaemic effect may be at least partly due to insulin secretagogue activity along with its positive effect on liver glycogen synthesis and the insulin secretagogue activity is independent of glucose.

Similar observation was made by Maiti *et al.* (2004) who found that the aqueous extract of seed of *Tamarindus indica* possess potent antidiabetogenic activity which could be attributed to some biomolecules that may sensitize the insulin receptor to insulin or stimulate the β stem cell of islets of Langerhans in pancreas in streptozotocin diabetic rats that may restore the plasma glucose level. Some plants like *Carum carvi* and *Capparis spinosa* L. exert its effect like biguanides which suppress the hepatic glucose production. Edoukks *et al.* (2004) reported the hypoglycaemic activity of aqueous extracts of *Carum carvi* and *Capparis spinosa* L. fruits and they had the opinion that it may be due to inhibition of hepatic glucose production and/or stimulation of glucose utilization by peripheral tissues especially muscle and adipose tissue. The extract could also act as inhibitor of tubular renal glucose reabsorption.

Aqueous extract of *Commelina communis* L. reduced blood glucose levels by inhibiting alpha-glucosidase, a membrane bound enzyme in 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). According to Randle's glucose fatty acid cycle, increased supply of plasma triglyceride per se could constitute a source of increased free fatty acid availability and oxidation that can impair insulin action, glucose metabolism and utilization leading to development of hyperglycaemia. Therefore the reduction of triglyceride following the treatment with leaf extract could also facilitate the glucose oxidation and utilization and subsequently the reduction of hyperglycaemia (Muruganandan *et al.*, 2005).

5.2.2.2 Serum *Cholesterol Level*

On zeroth day, the serum cholesterol levels observed in all the groups were almost similar. On 16th day, all groups (II, VI, VII, VIII and IX) except normal control showed a substantial increase in serum cholesterol level. Bopanna *et al.* (1997) reported that the excess fatty acid in plasma produced by alloxan induced diabetes promotes the liver conversion of some fatty acids into phospholipids and cholesterol. These two substances along with the triglyceride formed at the same time in the liver may be discharged into blood. The plasma lipoproteins increase as much as three fold in alloxan induced diabetes giving a total concentration of plasma lipids of several per cent rather than normal 0.6 per cent. This high lipid concentration may lead to the rapid development of atherosclerosis in diabetic patients.

After 14 days of treatment, both the groups VI and VII showed a significant reduction in serum cholesterol level compared to diabetic control. However the

hypocholesterolaemic effect of both the extracts was almost similar. The elevated serum cholesterol level also decreased in group VIII which was significantly different from that obtained for group VI and VII. Group IX which received glibenclamide showed the highest reduction after 14 days of treatment.

After 30 days of treatment, there was substantial reduction in serum cholesterol level in all the treated groups. Group VII which received *Aegle marmelos* had a lower serum cholesterol level of 113.50 ± 2.82 mg/dl compared to that of group VI which received *Murraya koenigii* (119.00 ± 2.93 mg/dl). Similar observation was recorded by Ponnachan *et al.* (1993), who found that oral administration of aqueous extract of *Aegle marmelos* leaf at the dose rate of 1g/kg in alloxan induced diabetic rats produced a significant decrease in serum cholesterol level to 99.20 ± 8.43 mg/dl compared to diabetic control which had a cholesterol level of 192.67 ± 13.64 mg/dl.

Group VIII treated with combination had a serum cholesterol level of 92.38 ± 1.73 mg/dl which was significantly lower than groups VI and VII. However, the hypocholesterolaemic effect seen in rats of group VIII was not as effective as glibenclamide which had a serum cholesterol level of 84.50 ± 2.15 mg/dl.

The present findings agree with the findings of Alarcon-Aguilar *et al.* (2005), who got a similar decrease in total cholesterol level by the oral administration of dichloromethane extract of *Ibervillea sonorae* root in alloxan diabetic rats.

5.2.2.3 *Serum Triglyceride Level*

In the present study, all the groups except normal control showed a significant increase in serum triglyceride level after 16 days of alloxan administration. Chakrabarti *et al.* (2002) got a significant increase in serum triglyceride level in diabetic mice and suggested that hypertriglyceridaemia that often develops in diabetes may contribute to coronary heart disease.

After 14 days of treatment, group VI (141.13 ± 1.78 mg/dl) and group VII (145.50 ± 2.61 mg/dl) showed a significant reduction in serum triglyceride level compared to the diabetic control (157.63 ± 0.94 mg/dl). The animals of group VIII which received combination showed the lowest serum triglyceride level among the groups treated with ethanolic extract. Group IX treated with glibenclamide showed the highest reduction in serum triglyceride level.

At the end of the experimental period also, significant reduction in serum triglyceride level was maintained with groups VI and VII compared to diabetic control. The hypotriglyceridaemic effect seen in both the groups was almost similar. The animals treated with combination for one month showed significant lowering in serum cholesterol level of 100.25 ± 1.03 mg/dl which was comparatively lower than that of group IX (92.50 ± 1.77 mg/dl). John (2003) observed a similar decrease in serum cholesterol and triglyceride contents in diabetic rats when treated with *Brassica juncea* and *Trigonella foenum graecum* seeds and attributed to the rich fibre content of these seeds, which reduces the intestinal absorption of fat molecules.

5.2.2.4 *Liver Glycogen Level*

In the present study, group II (diabetic control) showed a significant decrease in liver glycogen level. Vats *et al.* (2004) reported that alloxan causes selective destruction of β cells of islet of Langerhans resulting in marked decrease in insulin levels and a deposition of glycogen in tissues especially liver and skeletal muscles.

In all the treatment groups liver glycogen was found to be increased which is in agreement with the findings of Shirwaikar *et al.* (2005). They reported that administration of alcoholic extract of *Coscinium fenestratum* stem significantly increased liver glycogen content compared to diabetic control which may be due to the activation of glycogen synthase system by the extract.

After 30 days of treatment, there was significant difference in increase of liver glycogen level produced between groups VI and VII. Animals treated with *Aegle marmelos* extract (group VII) had a higher liver glycogen value than those treated with *Murraya koenigii*. Ponnachan *et al.* (1993) found that liver glycogen level decreased significantly in diabetic rats whereas those treated with leaf powder extract of *Aegle marmelos* maintained glycogen at par with control rats. Khan *et al.* (1995) also observed an increase in hepatic glycogen when they administered *Murraya koenigii* and *Brassica juncea* in normal rats. They suggested that it may be due to increased activity of glycogen synthetase and decrease in glycogenolysis and gluconeogenesis as evident from the decreased activity of glycogen phosphorylation and gluconeogenic enzymes.

Treatment with the combination produced significant regain in liver glycogen level (56.94 ± 0.87 mg %), but was lower than group IX (glibenclamide treated)

which produced the highest increase in liver glycogen (60.61 ± 0.71 mg %). However, the increment produced by both groups was comparable.

Results of the present study confirm the hypoglycaemic and hypolipidaemic effects of *Pleurotus ostreatus, Murraya koenigii* and *Aegle marmelos*. The combination of *Pleurotus ostreatus* with *Aegle marmelos* and *Murraya koenigii* had a still higher blood glucose and lipid lowering effect than individual drugs suggesting a synergistic action of the three drugs.

Summary

6. SUMMARY

The present study was conducted to assess the hypoglycaemic effect of *Pleurotus ostreatus* and its combination with *Murraya koenigii* and *Aegle marmelos* in a model of alloxan induced diabetic rats. In the initial phase, a preliminary study was carried out to evaluate the hypoglycaemic effect of *Pleurotus ostreatus* at three different doses (250,500 and 1000 mg/kg body weight) and the most suitable dose among these was then selected for further study. In the second phase, the suitable dose of *Pleurotus ostreatus* derived from the first phase was combined with *Murraya koenigii* and *Aegle marmelos*. This combination was then compared with *Murraya koenigii* and *Aegle marmelos* each at a dose rate of 250 mg/kg and also with the established oral antidiabetic drug, glibenclamide.

Seventy two adult Sprague-Dawley male rats weighing 150-200 grams were randomly divided into nine groups of eight animals each. Group I served as normal control. All the groups except normal control were made diabetic by the subcutaneous injection of alloxan at the dose rate of 130 mg/kg body weight. Only those rats showing moderate hyperglycaemia (200-295 mg/dl) were selected for further study. Group II was kept as the diabetic control.

In the initial phase of study, the rats of group III, IV and V were administered orally with ethanolic extract of *Pleurotus ostreatus* at the dose rate of 250,500 and 1000 mg/kg body weight respectively from day 16 to day 45. Parameters like body weight, blood glucose, serum cholesterol and serum triglyceride were recorded on zeroth day, 16^{th}, 30^{th} and 45^{th} day. Liver glycogen was estimated on 45^{th} day after sacrificing the animals.

All the three treatment groups except diabetic control showed gradual increase in body weight by the end of the experimental period, but it was seen that the body weight never returned to their initial value noticed before the commencement of the experiment.

Administration of the ethanolic extract of *Pleurotus ostreatus* at different doses produced a dose dependent reduction in blood glucose level. The highest decrease in blood glucose level was observed at the dose rate of 1000 mg/kg (group V). There was also significant difference in blood glucose level between groups III and IV. However, the blood glucose levels were similar in groups IV and V.

Oral administration of the ethanolic extract of *Pleurotus ostreatus* at different doses produced significant reduction in serum cholesterol level. Group V showed the lowest serum cholesterol level among the three groups. There was significant difference in serum cholesterol levels between groups III and IV.

Serum triglyceride level was found to be highest in group II (diabetic control). All the treated groups showed a significant reduction in serum triglyceride level compared to group II. The highest decrease in serum triglyceride level was obtained with group V. The reduction in serum triglyceride level obtained for group IV was intermediate to that of groups III and V.

Group II showed the lowest liver glycogen level at the end of the experiment. Increase in liver glycogen produced by groups III and IV were almost similar. Among the treated groups, group V showed the highest increase in liver glycogen level.

The results of the present study clearly demonstrated that among the three doses of *Pleurotus ostreatus* chosen, the dose rate of 1000 mg/kg was found to possess

the highest glucose and lipid lowering effect. Hence this was selected as the suitable dose for combination with *Murraya koenigii* and *Aegle marmelos*.

In the second phase of study, group VI received ethanolic extract of *Murraya koenigii* at the dose rate of 250 mg/kg and group VII was given ethanolic extract of *Aegle marmelos* at the same dose rate from day 16 to day 45. A combination of the ethanolic extract of *Pleurotus ostreatus* at the dose rate of 1000 mg/kg with *Murraya koenigii* and *Aegle marmelos* each at the dose rate of 250 mg/kg was given to group VIII for the same period. Group IX received glibenclamide at the dose rate of 0.25 mg/kg/day for 30 days. Group I and II served as normal and diabetic control respectively.

Parameters like body weight, blood glucose, serum cholesterol and serum triglyceride were recorded on zeroth day, 16^{th}, 30^{th} and 45^{th} day. Liver glycogen was estimated on 45^{th} day after sacrificing the animals.

Body weight of all the groups except normal control markedly reduced after sixteen days of alloxan administration. All the treated groups (VI, VII, VIII and IX) showed gradual increase in body weight after 30 days of treatment. Increase in body weight shown by group VI was comparatively lower than that of group VII. The increase in body weight shown by group VIII which received combination was found to be superior over groups VI and VII and the effect was comparable to that produced by glibenclamide.

All treatment groups showed significant reduction in blood glucose level compared to diabetic control. The reduction in blood glucose level after the oral administration of ethanolic extract of *Murraya koenigii* and *Aegle marmelos* each at the dose rate of 250 mg/kg was almost similar. Group VIII which received the

combination showed a significant reduction in blood glucose level than groups VI and VII suggesting a synergistic effect of the three drugs in producing hypoglycaemia. However, the most effective reduction in blood glucose level was produced by glibenclamide.

A significant decrease in serum cholesterol level was seen in all the treated groups compared to diabetic control. Among groups treated with ethanolic extract, group VIII showed the lowest serum cholesterol level. There was not much significant difference in reduction of serum cholesterol level between groups VI and VII. Group IX which received glibenclamide showed the highest reduction in serum cholesterol level.

With respect to serum triglyceride level, group IX treated with glibenclamide showed the highest reduction followed by group VIII. Groups VI and VII produced almost similar reduction in triglyceride values compared to group II.

All the treated groups showed significant increase in liver glycogen level compared to group II. Animals treated with *Aegle marmelos* extract (group VII) had a higher liver glycogen value than those treated with *Murraya koenigii* (group VI). Treatment with the combination produced significant regain in liver glycogen level but was lower than that obtained with group IX.

The results of the present study have confirmed the hypoglycaemic and hypolipidaemic effect of *Pleurotus ostreatus, Murraya koenigii* and *Aegle marmelos*. The combination of *Pleurotus ostreatus* with *Murraya koenigii* and *Aegle marmelos* showed a much better effect in reducing blood glucose and lipid level suggesting a synergistic action of the combination.

References

REFERENCES

Abdel-Barry.,Jamal–Ahmed.,Abdel-Hassan,I.A.,Mohammed,H.H.andAl-Hakeim. 1997. Hypoglycaemic and antihyperglycaemic effects of *Trigonella foenum graecum* leaf in normal and alloxan induced diabetic rats. *J. Ethnopharmacol.* **58**: 149-155

Adebajo,A.C., Olayiwola,G., Verspohl,J.E., Iwalewa,E.O., Omisore,N.O.A., Bergenthal,D., Kumar,V. and Adesina,S.K. 2004. Evaluation of Ethnomedical claims of *Murraya koenigii. Pharmaceutical.Biol.* **42**(8): 610-620

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. Pract.* **40**:145-151

Alarcon-Aguilar, F.J., Calzada-Bermejo,F., Hernandez-Galicia,E., Ruiz-Angeles, C. and Roman-Ramos, R. 2005. Acute and chronic hypoglycemic effect of *Ibervillea sonorae* root extracts-II. . *J. Ethnopharmacol.* **97**: 447-452

Aleeva,G.N., Kiyasov, A.P., Minniebave, M.M., Burykin, I.M. and Kbatizyanova, R.K.H. 2002. Change in the count of pancreatic beta and alpha cells and blood glucose level in rats with alloxan induced diabetes. *Bull. Exp. Biol. Med.* **133**(2): 127-129

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

Ananthan,R., Baskar,C., Bai, V.N., 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

Andallu,B. and Varadacharyulu,N.C. 2002. Control of hyperglycemia and retardation of cataract by mulberry (*Morus indica* L.) leaves in streptozotocin diabetic rats. *Indian J.Exp.Biol.* **40**: 791-795

Anturlikar,S.D., Gopumadhavan,S., Chauhan,B.L. and Mitra,S.K. 1995. Effect of D-400, a herbal formulation on blood sugar of normal and alloxan induced diabetic rats. *Indian J. Physiol. Pharmacol.* **39**(2): 95-100

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

Bajaj,S. and Srinivasan, B.P. 1999. Investigations into the antidiabetic activity of *Azadirachta indica*. *Indian J.Pharmacol.* **31**: 138-141

*Bobek, P. and Galbavy,S. 1999. Hypocholesterolaemic and antiatherogenic effect of oyster mushroom (*Pleurotus ostreatus*) in rabbits. *Nahrung.* **43** (5): 339-342

Bopanna,K.N., Kannan,J., Gadgil,S., Balaraman,R. and Rathod,S.P. 1997. Antidiabetic and antihyperlipemic effects of Neem seed kernel powder on alloxan diabetic rabbits. *Indian J.Pharmacol.* **29**:166-167

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. Bio. Chem.* **220**: 583-593

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

Chakrabarti,S., Biswas,T.K., Seal,T.,Rokeya,B. and Ali,L. 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

Chattopadhyay,S., Ramanathan,M., Das,G. and Bhattacharya,S.K. 1997. Animal models in experimental diabetes mellitus. *Indian J.Exp.Biol.* **35**: 1141-1145

Chude,M.A., Orisakwe,O.E., Afonne,O.J., Gamaniel,K.S., Vongtau,O.H. and Obi,E. 2001. Hypoglycemic effect of the aqueous extract of *Boerhavia diffusa* leaves. *Indian J.Pharmacol.* **33**: 215-216

Cohen,R., Persky,L. and Hadar,Y. 2002. Biotechnological applications and potential of wood degrading mushrooms of the genus *Pleurotus*. *Appl. Microbiol. Biotech.* **58** (5): 582-594

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. Pract.* **67**: 189–195

George,S.K., Rajesh,R., Sulekha,B. and Balaram,P. 2006. Dose responsive immunomodulatory study of *Aegle marmelos* (L.) correa root in BALB/c mice. *Amala Res. Bull.* **26**: 40-46

Ghosh,R., Sharatchandra,K., Rita,S. and Thokchum,I.S. 2004. Hypoglycemic activity of *Ficus hispida* (bark) in normal and diabetic albino rats. *Indian J. Pharmacol.* **36**(4): 222-225

Harve, G. and Kamath,V. 2004. Larvicidal activity of plant extracts used alone and in combination with known synthetic larvicidal agents against *Aedes aegypti*. *Indian J.Exp.Biol.* **42**: 1216-1219

Hossain,M.Z., Shibib,B.A. and Rahman,R. 1992. Hypoglycemic effects of *Coccinia indica*: Inhibition of key gluconeogenic enzyme, glucose-6-phosphatase. *Indian J.Exp.Biol.* **30**: 418-420

Hossain,S., Hashimoto,M., Choudhury,E.K., Alam,N., Hussain,S., Hasan,M., Choudhury,S.K. and Mahmud,I. 2003. Dietary mushroom (*Pleurotus ostreatus*) ameliorates atherogenic lipid in hypercholesterolaemic rats. *Clin. Exp. Pharmacol. Physiol.* **30(7)**: 470-475

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. Pract* . **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, Thrissur, 62 p.

Jose, M.P. 1974. Studies on the effect of selected indigenous drugs on the blood sugar level in dogs. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, 75 p.

Jose,N., Ajith,T.A. and Janardhanan, K.K. 2002. Antioxidant, anti-inflammatory and antitumour activities of culinary medicinal mushroom, *Pleurotus pulmonarius*. *Int. J. Med. Mushr.* **4**: 329-335

Jose,N., Ajith,T.A. and Janardhanan, K.K. 2004. Methanol extract of the oyster mushroom, *Pleurotus florida,* inhibits inflammation and platelet aggregation. *Phytother. Res.* **18**: 43-46

Jose,N., Ajith,T.A. and Janardhanan, K.K. 2004. Oyster mushroom extract protects antioxidant defence system in cisplatin induced nephrotoxicity in mice. *Oriental Pharm. Exp. Med.* **4**(1): 22-27

Jose, N. and Janardhanan, K.K. 2000. Antioxidant and antitumour activity of *Pleurotus florida*. *Curr. Sci.* **79**(7): 941-943

Jose,N. and Janardhanan,K.K. 2000. Antitumour activity of an oyster mushroom, *Pleurotus sajor-caju*. *Amala Res. Bull.* **20**: 67-69

Joy,K.L. and Kuttan,R. 1998. Antidiabetic activity of Cogent-DB, a herbal preparation. *Amala Res. Bull.* **18**: 109-114

Joy,K.L. and Kuttan,R. 1999. Antidiabetic activity of *Picrorrhiza kurroa* extract. *J. Ethnopharmacol.* **167**: 143-148

Kaleem,M., Sheema,H., Sarmad. and Bano,B. 2005. Protective effects of *Piper nigrum* and *Vinca rosea* in alloxan induced diabetic rats. *Indian J. Physiol. Pharmacol.* **49**(1): 65-71

Kamalakkannan,N. and Prince,P.S.M. 2003. Effect of *Aegle marmelos* Correa. (Bael) fruit extract on tissue antioxidants in streptozotocin diabetic rats. *Indian J.Exp.Biol.* **41**: 1285-1288

Kamalakkanan, N., Rajadurai, M. and Prince, P.S. 2003. Effect of *Aegle marmelos* fruits on normal and streptozotocin induced diabetic Wistar rats. *J.Med. Food.* 6(2): 93-98

Kar,M. and Chakraborti,A.S. 1999. Release of iron from haemoglobin- A possible source of free radicals in diabetes mellitus. *Indian J.Exp.Biol.* **37**: 190-192

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

Khan,B.A., Abraham,A. and Leelamma,S. 1995. Hypoglycemic action of *Murraya koenigii*(curry leaf) and *Brassica juncea* (mustard): Mechanism of action. *Indian J.Biochem.Biophys.* **32**: 106-108

Khan,B.A., Abraham,A. and Leelamma,S. 1996. Role of *Murraya koenigii* (curry leaf) and *Brassica juncea* (mustard) in lipid peroxidation. *Indian J. Physiol. Pharmacol.* **40**(2): 155-158

Khosla,P., Bhanwra,S., Singh,J., Seth,S. and Srivastava,R.K. 2000. A study of hypoglycemic effects of *Azadirachta indica* (Neem) in normal and alloxan diabetic rabbits. *Indian J. Physiol. Pharmacol.* **44**(1): 69-74

Lakshmi,B., Jose,N., Ajith,T.A. and Janardhanan, K.K. 2004. Antimutagenic activity of methanolic extract of culinary -medicinal oyster mushroom, *Pleurotus ostreatus* and its protective effect against benzo [a] pyrene induced hepatic damages. *Int. J. Med. Mushr.* **6**: 139-149

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

Lino,C.D., Diogenes,J.P.L., Pereira,B.A., Faria,R.A.P. and Viana,G.S.B. 2004. Antidiabetic activity of *Bauhinia forficata* extracts in alloxan-diabetic rats. *Biol. Pharm. Bull.* **27**(1): 125—127

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

Mathew,A. 2005. Hepatoprotective effect of *Aegle marmelos* (Indian Bael) and *Azadirachta indica* (Neem) aqueous leaf extract on paracetamol induced toxicity in rats. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, 76 p.

*Mc Letchie, N.G. 2002. Alloxan diabetes: a discovery, albeit a minor one. *J. R. Coll. Physicians. Edinb.* **32** (2): 134-142

Mishra,G., Routray,R., Das,S.R. and Behera,H.N. 1995. Alloxan diabetes in Swiss mice: activity of Na+-K+ ATP-ase and succinic dehydrogenase. *Indian J. Physiol. Pharmacol.* **39**(3): 271-274

Mohamad,S., Taha, A., Bamezai, R.N.K., 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

Murali,B., Upadhyaya,U.M. and Goyal,R.K. 2002. Effect of chronic treatment with *Enicostemma littorale* in non-insulin-dependent 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

Narayana,K. and Sastry,K.N.V. 1975. The Hypoglycemic effect of *Murraya koenigii* (spreng) in normal and alloxan diabetic dogs. *Mysore J.agric.Sci.* **9**: 132-136

Nussel, S. G. and Arav, E. 1975. Determination of serum triglyceride by enzymatic method. *Medicine* **10**: 25-26

Orhan, D.D., Aslan, M., Sendogdu,N., 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 Satheesh,M.A. 2004. Antidiabetic activity of *Boerhaavia diffusa* L.: effect on hepatic key enzymes in experimental diabetes. *J. Ethnopharmacol.* **91**: 109-113

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

Persaud,D.R. and Mendoza,A.B. 2004. Bovine serum albumin and insulin-dependent diabetes mellitus: is cow's milk still a possible toxicological causative agent of diabetes. *Food Chem. Toxicol* . **42**: 707–714

*Philip,J. 1981. Curry leaf and uses. *World crops.* **1**: 125-126

Ponnachan,P.T.C., Poulose,C.S. and Panikkar,K.R. 1993. Effect of leaf extract of *Aegle marmelos* in diabetic rats. *Indian J.Exp.Biol.* **31**: 345-347

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

Puri,D., Prabhu,K.M. and Murthy,P.S. 2002. Mechanism of action of hypoglycemic principle isolated from fenugreek seeds. *Indian J. Physiol. Pharmacol.* **46**(4): 457-462

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. *Diabetes Res. Clin. Pract.* **62**: 139- 148

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

Rao,V.V., Dwivedi,S.K., Swarup,D.and Sharma,S.R. 1995. Hypoglycemic and antihyperglycemic effects of *Aegle marmelos* leaves in rabbits. *Curr. Sci.* 69(11): 932-933

Raphael,K.R., Sabu,M.C. and Kuttan,R. 2002. Hypoglycemic effect of methanol extract of *Phyllanthus amarus* Schum & Thonn on alloxan induced diabetes mellitus in rats and its relation with antioxidant potential. *Indian J.Exp.Biol.* 40: 905-909

Ratnasooriya, W.D., Banduwardene, H.R. and Amarasekara, A.S. 1994. Anxiolytic effect of *Murraya koenigii* leaf in rats. *Med. Sci. Res.* **22**: 427-428

Rusia,K. and Srivastava,S.K. 1988. Antimicrobial activity of some Indian medicinal plants. *Indian J.Pharmaceutical .Sci.* 1: 57-58

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

Sabu,M.C. and Kuttan,R. 2001. Inhibitory effect of *Aegle marmelos* on lipid peroxidation and antioxidant enzymes in blood and tissue in diabetic rats. *Amala Res. Bull.* **21**: 21-27

Sabu,M.C. and Kuttan,R. 2004. Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. *Indian J. Physiol. Pharmacol.* 48(1): 81-88

Sabu,M.C., Sabitha,P. and Kuttan,R. 2003. Antidiabetic activity of *Aloe arborescens* miller var. *Amala Res. Bull.* **23**: 166-177

Sachdewa,A., Raina,D., Srivastava,A.K. and Khemani,L.D. 2001. Effect of *Aegle marmelos* and *Hibiscus rosa sinensis* leaf extract on glucose tolerance in glucose induced hyperglycemic rats (Charles foster). *J.Environ.Biol.* **22**(1): 53-57

Sathyan,A. 2004. Comparative study on the hypoglycaemic effect of *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi) and *Tinospora cordifolia* (Chittamruthu) and their combination in diabetic rats. M.V.Sc. thesis, Kerala Agricultural University, Thrissur, 100 p.

Seema,P.V., Sudha,B., Padayatti,P.S., Abraham,A., Raghu,K.G. and Paulose,C.S. 1996. Kinetic studies of purified malate dehydrogenase in liver of streptozotocin diabetic rats and the effect of leaf extract of *Aegle marmelos* (L.) Correa ex Roxb. *Indian J.Exp.Biol.* **34**: 600-602

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

Shah, K.J. and Juvekar,A.R. 2006. Positive inotropic effect of *Murraya koenigii* (Linn.) spreng extract on an isolated perfused frog heart. *Indian J.Exp.Biol.* **44**: 481-484

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

Singh,Y.N. 1986. Traditional medicine in Fiji: Some herbal folk cures used by Fiji Indians. *J. Ethnopharmacol.* **15**: 57-88

Soto, C.P., Perez, B.L., Favari, L.P. and Reyes, J.L. 1998. Prevention of alloxan-induced diabetes mellitus in the rat by Silymarin. *Comp. Biochem. Physiol.* **119** (2): 125–129

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

Sur,T.K., Pandit,S., Pramanik,T. and Bhattacharya,D. 2002. Effect of *Aegle marmelos* leaf on rat sperm motility: an in vitro study. *Indian J. Pharmacol.* **34**: 276-277

Sy,G.Y., Cisse,A., Nongonierma,R.B., Sarr,M., Mbodj,N.A. and 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

Szkudelski,T. 2001. The mechanism of alloxan and streptozotocin action in beta cells of the rat pancreas. *Physiol. Res.* **50**: 537-546

Upadhya,S., Shanbhag,K.K., Suneetha,G., Naidu,B.M. and Upadhya,S. 2004. A study of hypoglycemic and antioxidant activity of *Aegle marmelos* in alloxan induced diabetic rats. *Indian J. Physiol. Pharmacol.* **48**(4): 476-480

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

Vats,V., Yadav,S,P. and Grover, J.K. 2004. Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin induced alterations in glycogen content and carbohydrate metabolism in rats. *J. Ethnopharmacol.* 90: 155-160

Viana,G.S.B., Medeiros, A.C.C., Lacerda, A.M.R., Leal, L.K.A.M., Vale,T.G.and Matos, F.J.A. 2004. Hypoglycemic and antilipaemic effects of the aqueous extract from *Cissus sicyoides*. BMC *Pharmacol.* **4**: 400-407

Vinuthan,M.K., Kumar, G.V., Ravindra,J.P., Jayaprakash and Narayana,K . 2004. Effect of extracts of *Murraya koenigii* on the levels of blood glucose and plasma insulin in alloxan-induced diabetic rats. *Indian J. Physiol. Pharmacol.* **48**(3): 348-352

Walde,S.S., Dolhe, C., Schott- Ohly,P. and Gleichmann,H. 2002. Molecular target structures in alloxan induced diabetes in mice. *Life Sci.* 71 (14): 1681-1694

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

Yadav,S., Vats,V., Dhunnoo,Y. and Grover,J.K. 2002. Hypoglycemic and antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats. *J. Ethnopharmacol.* **82**(2-3): 111-116

Youn,J.Y., Park,H.Y. and Cho, K.H. 2004. Anti-hyperglycemic activity of *Commelina communis* L.:inhibition of α-glucosidase. *Diabetes Res. Clin. Pract.* **66**: 149–155

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

•Originals not consulted

HYPOGLYCAEMIC EFFECT OF *Pleurotus ostreatus* IN SPRAGUE-DAWLEY RATS

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ABSTRACT

The present study was undertaken to assess the antidiabetic activity of *Pleurotus ostreatus* and its combination with *Murraya koenigii* and *Aegle marmelos* in a model of alloxan induced diabetic rats.

The experiment was conducted in seventy two adult Sprague-Dawley male rats which were randomly divided into nine groups of eight animals each. Group I served as normal control. All the groups except normal control were made diabetic by the subcutaneous injection of alloxan at the dose rate of 130 mg/kg body weight. Group II was kept as the diabetic control.

In the initial phase, rats of group III, IV and V were administered orally with ethanolic extract of *Pleurotus ostreatus* at the dose rate of 250,500 and 1000 mg/kg body weight respectively from day 16 to day 45. Parameters like blood glucose, serum cholesterol and serum triglyceride were estimated on zeroth day, 16^{th}, 30^{th} and 45^{th} day. Body weight was also recorded on these days. Liver glycogen was estimated on 45^{th} day after sacrificing the animals.

All the three treatment groups except diabetic control showed gradual increase in body weight after 30 days of treatment. Among the treatment groups, the maximum regain in body weight was observed in rats treated with *Pleurotus ostreatus* at the dose rate of 1000 mg/kg (group V). The highest decrease in blood glucose level was also observed with the same group. Administration of the extract also produced significant reduction in serum cholesterol level. Group V showed the lowest serum cholesterol level and serum triglyceride level. The liver glycogen level was found to be highest in group V.

The results of the present study clearly demonstrated the hypoglycaemic effect of *Pleurotus ostreatus*. It was also quite evident that among the three doses of *Pleurotus ostreatus* chosen, the dose rate of 1000 mg/kg was found to possess the highest hypoglycaemic and hypolipidaemic effect. Hence this was selected as the suitable dose for combination with *Murraya koenigii* and *Aegle marmelos*.

In the second phase of study, group VI received ethanolic extract of *Murraya koenigii* at the dose rate of 250 mg/kg and group VII was given ethanolic extract of *Aegle marmelos* at the same dose rate from day 16 to day 45. A combination of the ethanolic extract of *Pleurotus ostreatus* at the dose rate of 1000 mg/kg with *Murraya koenigii* and *Aegle marmelos* each at the dose rate of 250 mg/kg was given to group VIII for the same period. Group IX received glibenclamide at the dose rate of 0.25 mg/kg/day for 30 days. Group I and II served as normal and diabetic control respectively.

Parameters like body weight, blood glucose, serum cholesterol and serum triglyceride were recorded on zeroth day, 16^{th}, 30^{th} and 45^{th} day. Liver glycogen was estimated on 45^{th} day after sacrificing the animals.

After 30 days of treatment, a significant gain in body weight was observed in animals which underwent combination therapy compared to group VI and VII and the effect was comparable to that produced by glibenclamide. The animals which received *Murraya koenigii* and *Aegle marmelos* (groups VI and VII) showed similar reduction in blood glucose level. Group VIII which received combination showed a significant reduction in blood glucose level than groups VI and VII suggesting a synergistic effect of the three drugs in producing hypoglycaemia. However, the most effective reduction in blood glucose level was produced by glibenclamide.

A significant decrease in serum cholesterol level was seen in all treated groups compared to diabetic control. Among the groups treated with ethanolic extract, group VIII showed the lowest serum cholesterol level. Group IX which received glibenclamide showed the highest reduction in serum cholesterol level. The highest reduction in serum triglyceride level was also shown by group IX followed by group VIII. Animals treated with *Aegle marmelos* extract (group VII) had a higher liver glycogen value than those treated with *Murraya koenigii* (group VI). Treatment with the combination produced significant regain in liver glycogen level but was lower than that obtained with group IX.

From the study, it can be concluded that the combination of *Pleurotus ostreatus* with *Murraya koenigii* and *Aegle marmelos* has the highest hypoglycaemic and hypolipidaemic effect than the individual effect of *Murraya koenigii* and *Aegle marmelos* suggesting a synergistic action of the three.