COMPARATIVE STUDY OF THE HYPOGLYCEMIC EFFECT OF *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi) AND *Tinospora cordifolia* (Chittamruthu) AND THEIR COMBINATION IN DIABETIC RATS

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

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DECLARATION

I hereby declare that this thesis entitled **"Comparative Study of the Hypoglycemic Effect of *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi) and *Tinospora cordifolia* (Chittamruthu) and their Combination in Diabetic Rats"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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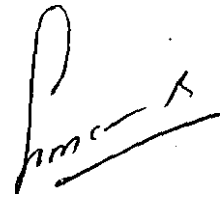
Certified that this thesis, entitled **"Comparative Study of the Hypoglycemic Effect of *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi) and *Tinospora cordifolia* (Chittamruthu) and their Combination in Diabetic Rats"** is a record of research work done independently by **Dr. Archana Sathyan**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



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Introduction

1. INTRODUCTION

Diabetes is possibly the world's fastest growing metabolic disease. Once regarded as a single disease entity, it is now seen as a heterogenous group of diseases characterized by a state of chronic hyperglycemia which causes cardiovascular, renal, neurological and ocular complications (WHO Expert Committee, 1985). The increasing number of ageing population, consumption of calories rich diet, obesity and sedentary lifestyle have led to a tremendous increase in the number of diabetics worldwide. India today leads the world with its largest number of diabetic subjects in any given country. It has been estimated that in 1995, 19.4 million individuals were affected by diabetes in India and these numbers are expected to increase to 57.2 million by the year 2025 (King *et al.*, 1998).

Diabetes mellitus was recorded in the Ebers Papyrus. It was referred to as honey urine and a melting away of flesh in the urine (Krantz and Carr, 1969). There are two main forms of diabetes mellitus, type I diabetes (Insulin Dependent Diabetes Mellitus, IDDM) and type 2 diabetes (Non-Insulin Dependent Diabetes Mellitus, NIDDM). Type I diabetes is primarily due to auto immune mediated destruction of the pancreatic beta cells resulting in absolute insulin deficiency. Type 2 diabetes is characterized by insulin resistance and/or abnormal insulin secretion either of which may predominate. The diabetes epidemic relates particularly to the type 2 diabetes and is prevalent in both developed and developing nations,predominantly due to the changing demography and increased longevity (Pradeepa and Mohan, 2002).

Diabetes is a syndrome resulting from a variable interaction of hereditary and environmental factors and characterized by abnormal insulin secretion or insulin receptor or post receptor events affecting metabolism including carbohydrate, protein and fat, in addition to damaging liver, kidney and beta cells

of pancreas (Ghosh and Suryawanshi, 2001). It is characterized by an elevated blood sugar level and concomitant glycosuria tending towards ketosis. The loss of carbohydrates results in polyphagia and asthenia, disturbed water balance resulting in polyuria and polydypsia. The failure of normal utilization of glucose in the tissue is accompanied by increased production of fatty acid which are formed more rapidly than they are utilized. Acetone and beta-hydroxy butyric acid are formed in excessive amounts and accumulate in the blood and tissues. The presence of these compounds produce acidosis, ketosis, finally coma and death (Krantz and Carr, 1969).

The disease has been reported virtually in all laboratory animals and in horse, cattle, sheep and pigs, but it is most frequently found in dogs and cats. The disease in dogs occurs most frequently in the mature or older females, often in association with estrus and in all breeds. In contrast, male cats appear to be more commonly affected than females. In animals, a third type of diabetes mellitus is also seen, Type 3 diabetes characterized by normal initial insulin levels, a normal or delayed insulin response to the glucose load and a delayed return of insulin to normal levels as in chemical diabetes (Kaniko *et al.*, 1997).

Treatment of diabetes follows three patterns viz, diet and exercise, the use of oral hypoglycemic agents such as sulfonylurea and biguanides and insulin replacement therapy (Ponnachan *et al.*, 1992). The modern drugs control the blood sugar level only as long as they are regularly administered. The current treatments, although provide good glycemic control, do little in preventing complications. Besides these drugs are associated with various side effects. One of the hazards of insulin administration is the production of hypoglycemic shock. Insulin cannot be used orally and continuous insulin injection has many side effects and toxicity (Ponnachan *et al.*, 1993). Prolonged use of sulfonyl urea in diabetes has met with several disadvantages such as hyperlipidemia, greater incidence of myocardial infarction and unsatisfactory control of postprandial blood sugar (Annamala and Augusti, 1980). Hence it is necessary to look for

new and if possible, more efficacious drugs for treatment of diabetes and the vast reserve of phytotherapy may be an ideal target (Vats *et al.*, 2004).

Long before the use of insulin, indigenous remedies have been used for the treatment of diabetes mellitus. Many herbal products have been described for the care of diabetes in the ancient literature. Ayurveda has been the first to give an elaborate description of this disease, its clinical features and patterns and its management by herbal or herbomineral drugs. It is seen that certain resistant cases of diabetes which do not respond well to modern medicines like tolbutamide, chlorpropamide and glibenclamide, respond very well when treated with herbal preparations alone or in combination with other oral hypoglycemic agents (Anturlikar *et al.*, 1995). Plant drugs are frequently considered to be less toxic and comparatively free from side effects than synthetic ones.

In the recent past, many plants of folklore importance used as antidiabetic agents for generations by rural population and forest ethics have been brought to light through ethno botanical field studies and investigated for their bioactive constituents and mechanism of action (Khan and Singh, 1996). The World Health Organisation (WHO) has recommended further investigations in this area. Many plants like *Allium cepa*, *Ficus bengalensis*, *Allium sativum*, *Gymnema sylvestre*, *Pterocarpus marsupium*, *Trigonella foenum graecum*, *Eugenia jambolana* etc. have been shown to possess antidiabetic activity (Shukla *et al.*, 2000).

In the present investigation, three plants namely *Azadirachta indica*, *Ocimum sanctum* and *Tinospora cordifolia* have been selected with the objective to assess and compare the hypoglycemic action of these plants within and with that of glibenclamide and to find out the additive/synergistic effect of these in rats.

Review of Literature

2. REVIEW OF LITERATURE

2.1 ALLOXAN INDUCED DIABETES

House *et al.* (1956) made investigations on the organ changes during the first 24 hours of alloxan diabetes in hamster. They found that during initial hyperglycemia, liver glycogen was almost completely lost and nucleic acids were greatly reduced. Beta-cells lost their ability to synthesize insulin and 95 per cent of them was degenerated in 24 hours. Kidney changes were characteristic of hyperactivity and high pressure but were not degenerative.

Effects of alloxan diabetes on the kinetic attributes of Na+-K+ ATPase were examined in rat kidney, brain and erythrocytic membrane by Kumthekar and Katyare (1992). The enzyme activity decreased significantly in the three membrane systems. Kinetic analysis revealed that Km of the enzyme increased by five and ten fold in kidney and brain membranes, while Vmax decreased by 50 to 60 per cent.

A significant increase in the total lipid contents of the intestinal brush border membrane was observed in alloxan diabetic rats after seven days. A significant increase in sphingomyelin with a parallel decrease in ethanolamine was noticed. The observed changes might be responsible for the increased fluidity of intestinal membrane and thereby enhanced passive transport of various metabolites (Dutt and Sarkar, 1993).

Rajalingam *et al.* (1993) reported that the concentration of total lipids were significantly increased in cortical and medullary zones of kidney in alloxan induced diabetic rats. Concentration of the total cholesterol was also significantly increased in cortical region. The relative wet weight of kidney showed a significant increase.

Szkudelski (2001) reported that the cytotoxic action of alloxan was mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Highly reactive oxygen species are formed by the Fenton reaction. The action of reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, causes rapid destruction of beta cells.

Walde *et al.* (2002) revealed that the glucose transporter 2 (GLUT 2) and glucokinase were 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 AZADIRACHTA INDICA

Decoction of the tender leaves of *Azadirachta indica*, the oil from its seeds and nimbidin, a bitter active principle isolated from the oil were fed to detect their hypoglycemic effect in fasting rabbits by Pillai and Santhakumari (1981). The oil at 2.5ml and nimbidin at 200mg/kg bodyweight exhibited significant hypoglycemic activity by reducing blood sugar level by 24 and 26 per cent respectively. The tender leaf extract failed to show any significant effect.

Nat *et al.* (1987) investigated the interference of an aqueous extract of the stem bark of *A. indica* with different parts of the human immune system. The extract showed strong anticomplementary effects which were dose and time-dependent and most pronounced in the classical complement pathway assay. A dose dependent decrease in the chemiluminescence of polymorphonuclear leukocytes and a dose-dependent increase in the production of migration inhibition factor by lymphocytes were observed.

Activity guided isolation and identification of *A. indica* bark extract constituents which specifically inhibit chemiluminescence production by activated human polymorphonuclear leukocytes was done by Nat *et al.* (1991).

The responsible compounds were found to be gallic acid, gallocattechin, epicatechin, catechin and epigallo catechin.

Chattopadhyay *et al.* (1992) conducted studies on the hepatoprotective activity of *A. indica* leaves on paracetamol induced hepatic damage in rats. *A. indica* (One g /kg, p.o) showed significant reversal effects on the elevated serum levels of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, acid phosphatase and alkaline phosphatase in paracetamol hepatotoxicity.

Sen *et al.* (1992b) evaluated the effect of *A. indica* leaf extract on some biochemical, immunological and visceral parameters in normal and stressed rats. *A. indica* (100mg/kg) lowered blood glucose, triglycerides and Serum Glutamate Oxalo acetate Transaminase (SGOT) levels. The stress induced elevations of cholesterol and urea levels were reduced. An increase in humoral immune response to sheep RBC was also noticed.

Azadirachtin extracted from the seeds of Neem acts as a strong feeding deterrent and growth disruptor in neck ligated post feeding larvae of *Spodoptera mauritia* Boisd. The Azadirachtin induced morphogenetic effects are due to delayed or suppressed haemolymph ecdysteroid titres (Jagannadh and Nair, 1993).

Susha *et al.* (1993) observed a reduction in vitellogenic oocyte number in *Trogoderma granarium* when pupae were topically treated with azadirachtin. Disruption of the hormonal control of oocyte development was hypothesized to be the mode of action of azadirachtin.

Jaiswal *et al.* (1994) noticed that *A. indica* leaf extract exhibited anxiolytic effect comparable to diazepam at low doses (10-200 mg/kg) when tested in rats. Higher doses greater than 400 mg/kg did not show anxiolytic activity. The data substantiates the adaptogenic activity of Azadirachta. Anti 5-HT activity has been implicated in anxiety modulation

Neem leaf alcoholic extract was investigated for its effects on the ECG and blood pressure of rats by Koley and Lal (1994). Intravenous administration (100, 300 and 1000 mg/kg) resulted in initial bradycardia followed by cardiac arrhythmia. A significant and dose-related fall in blood pressure which was immediate, sharp and persistent was seen. Pretreatment with either atropine or mepyramine failed to prevent the hypotensive effect.

Venugopal and Venugopal (1994) tested the *in vitro* activity of ethanolic and aqueous extracts of *A. indica* leaves against 88 clinical isolates of dermatophytes using agar dilution technique. Ethanolic extract showed much more significant antidermatophytic activity compared to the aqueous extract. The MIC_{90} of the ethanolic extract was found to be 100 μg/ml and that of aqueous extract was 500 μg/ml.

Khanna *et al.* (1995) reported the antinociceptive action of *A. indica* in mice. Azadirachta (10, 30, 100 mg/kg) reduced both the incidence and number of writhes in glacial acetic acid induced writhing test in a dose dependent manner. An enhanced tail withdrawal latency in the tail flick test was also noticed with similar dose levels.

Praneem Vilci, a highly purified oil of Azadirachta seed, had a reversible antifertility effect after a single intrauterine instillation in rodents and primates without any adverse effects (Talwar *et al.*, 1995). The ovulatory status remained unaltered and endometrial biopsy was normal. Co-administration with heterospecies dimer birth control vaccine did not prevent antibody response to the vaccine.

Joshi *et al.* (1996) reported a general reduction in the diameter of seminiferous tubule, nuclei of germinal elements and a mass atrophy of the spermatogenic elements in Azadirachta treated rats. Leydig cells were found to be atrophic. A decrease in protein content and acid phosphatase activity and an increase in the total free sugar, glycogen and cholesterol contents were observed.

Neem seed extracts, both mechanically expressed and solvent extracted when given orally to rodents at a dose of 0.4 ml on day eight, nine and ten of pregnancy after confirming implantations revealed complete resorption of embryos. Probable mechanism may be cell mediated immunity (Mukherjee *et al.*, 1996).

Ray *et al.* (1996) demonstrated higher IgG and IgM levels and anti-ovalbumin antibody titres in ovalbumin immunized mice treated with neem leaf extract (100mg/kg). Induction of cell mediated immunity was revealed by the enhancement of macrophage migration inhibition and foot pad thickness after neem leaf extract treatment. There was no appreciable influence on different organs or on body weight of mice.

Singh *et al.* (1996) studied the molluscicidal activity of neem against the snails *Lymnaea acuminata* and *Indoplanorbis exustus*. The leaf, bark, cake, neem oil and neem based pesticides, achook and nimbecidine showed time and dose-dependent molluscicidal activity. The toxic effect of pure azadirachtin against both the snails was greater than the synthetic molluscicides.

Srinivasan and Bajaj (1996) conducted studies on the effect of neem leaf extract on contractile response of rat fundus smooth muscle in Non Insulin Dependent Diabetes Mellitus (NIDDM) rats. The extract prevented the metabolic derangement and down regulation of β-receptors as a result of elevated plasma catecholamines in diabetes. It also restored the reduction in relaxant response and increase in contractile response to norepinephrine.

Neem seed kernel powder (500mg/kg) significantly decreased the concentration of blood glucose level 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 was obtained.

Chattopadhyay (1997) observed a dose-dependent hypotensive effect without altering the amplitude or rate of respiration. At lower doses, no noticeable change in amplitude of contraction or rate of the heart was seen. However, at higher doses, there was temporary cardiac arrest in diastole.

Oral administration of 20,40,60 mg of dry neem leaf powder for 24 days resulted in a decrease in the weight of seminal vesicles and ventral prostrate. A reduction in epithelial height, nuclear diameter and secretory material, was also observed in the lumen. Biochemically, there was a decrease in total protein and acid phosphatase activities. Observations suggest the antiandrogenic property of the neem leaves (Kasturi *et al.*, 1997).

Dhar *et al.* (1998) demonstrated that the extracts of neem seeds and its purified fractions inhibited the *in vitro* growth and development of asexual and sexual stages of *Plasmodium falciparum*. Susceptible target stages were found to be trophozoites/schizonts. All the maturation stages of gametocytes were also observed on parasites resistant to other antimalarial drugs.

Gowda *et al.* (1998) noted that incorporation of neem kernel meal into a standard layer diet at 150 and 200 g/kg resulted in significantly lower food intakes, decreased rate of egg production and egg weight in birds. Fertility and hatchability were adversely affected by higher inclusion rates. A higher level beyond 100g/kg significantly reduced the hemoglobin level, erythrocyte count, packed cell volume, serum calcium, serum uric acid concentrations and Serum Glutamate Pyruvate Transaminase (SGPT) activity.

Methanolic extract of neem leaves inhibited plaque formation in six antigenic types of coxsackie virus B at a concentration of 1000 μg/ml at 96hrs *in vitro* indicating its antiviral activity. The virus inactivation, yield reduction and effect of time of addition assays suggested that the extract was most effective against coxsackie virus B-4 as a virucidal agent (Badam *et al.*, 1999).

Bajaj and Srinivasan (1999) concluded that in Insulin Dependent Diabetes Mellitus (IDDM) models, a fall in blood glucose levels of 45.4 and 38.02 per cent were observed when male Wistar rats were treated with neem leaf extract (One g/kg) and leaf extract and insulin respectively. In NIDDM models, the effect of neem leaf extract was greater than that of glibenclamide.

In a comparative evaluation of the blood sugar lowering activity of four medical plants, *Azadirachta indica, O. sanctum, Gymnema sylvestre* and *Catharanthus roseus* by Chattopadhyay (1999), Azadirachta leaf extract was found to have the most potent blood sugar lowering activity followed by *Catharanthus roseus, Gymnema sylvestre* and *O. sanctum.*

Hypoglycemic effect of *A. indica* was observed by Khosla *et al.* (2000) when leaf extract (500 mg/kg) and seed oil (five ml/kg, p.o) were given to normal as well as alloxan diabetic rabbits.

Aladakatti *et al.* (2001) found that *A. indica* treatment for 48 days in albino rats resulted in a decrease in the total sperm count, sperm motility and forward velocity. An increase in the per centage of abnormal sperms and a decrease in the fructose content of caudal semen of the epididymis was observed.

Baswa *et al.* (2001) used fourteen strains of pathogenic bacteria to assess the antibacterial activity of neem seed oil using the tube dilution technique. The activity was bactericidal and independent of temperature and energy. Most of the pathogens were killed more rapidly at four degree centigrade than at 37^0 C. The activity was mainly due to the inhibition of cell membrane synthesise.

Neem leaf extract administered at doses of 40,80 and 160 mg/kg produced dose dependent anti ulcer effect in albino rats. Extract showed significant protection at 80mg/kg and complete protection at 160 mg/kg. Observations clearly suggest that neem leaf extract can be used against peptic ulcer. (Das *et al.*, 2001)

Neem leaf extract showed significant anti-inflammatory effect at 250 and 500mg/kg bodyweight when tested by cotton pellet granuloma method. The effect was comparable with that obtained with standard antiinflammatory drugs (Bhabagrahi *et al.*, 2002).

Kumar *et al.* (2002) conducted studies on the radiosensitizing effects of neem oil using Balbc/373 cells. Neem oil enhanced the radiosensitivity of the cells when applied both during and after X-irradiation under aerobic conditions. Neem oil completely inhibited the repair of sub-lethal damage and potentially lethal damage, thereby converting them into lethal damage by inhibiting the double strand break repair or reducing the G_2 phase of the cell cycle.

Parida *et al.* (2002) observed the *in vitro* antiviral activity of aqueous neem leaf extract in cloned cells of larvae of *Aedes albopictus* employing virus inhibition assay. The maximum non-toxic concentration of 1.897 mg/ml of the extract completely inhibited 100-10,000 $TCID_{50}$ of virus as indicated by the absence of cytopathic effects. The *in vitro* protection studies resulted in inhibition of the virus replication as confirmed by absence of clinical symptoms in suckling mice.

Neem seed oil in doses of 0.5 ml and one ml/kg bodyweight injected intraperitoneally to different groups of rats showed significant dose dependent antipyretic activity in Brewer's yeast induced pyrexia (Sanjay *et al.*, 2002).

2.3 PHARMACOLOGICAL EFFECTS OF OCIMUM SANCTUM

The results of the experiment conducted by Batta and Santhakumari (1970) revealed that the benzene extract of *Ocimum sanctum* showed 80 per cent anti fertility activity in fertile female rats. The Petroleum ether extract was found to be less active (60 per cent) while other extracts were either inactive or less than 50 per cent active.

Mediratta *et al.* (1988) studied the effect of steam distilled extract of Ocimum leaves on the humoral immune response in experimental animals. The study clearly suggested that *O. sanctum* modulate the humoral immune response by acting at various levels in the immune system such as antibody production, release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs. They also found that Ocimum significantly inhibited the antigen induced histamine release from the peritoneal mast cells of sensitised rats *in vitro*. Ocimum also antagonised responses to various spasmogens on isolated guinea pig ileum.

Sarkar and Pant (1989) showed that the dried seed powder of *O. sanctum* (tulsi) at one and two g per cent feeding levels caused approximately 16 and 21 per cent lowering in the fasting blood sugar level at the end of fourth week. A decrease of approximately 16 and 19 per cent was observed with one and two g fresh leaves respectively at the end of the fourth week. Leaves were found to be more effective in lowering the blood sugar levels.

Sarkar *et al.* (1990) established that leaves and seeds of tulsi exert significant hypouricemic and uricosuric effects. The findings justify the traditional use of tulsi for the treatment of gouty arthritis. On administration of leaves as well as seeds, blood urea levels increased resulting in increased urea excretion as well as diuresis.

On the basis of studies conducted by Sen *et al.* (1992a) on the mechanism of antistress activity of *O. sanctum* and eugenol, it was concluded that both Ocimum and eugenol lowered the restraint stress induced cholesterol levels. Stress induced elevations in Lactate dehydrogenase (LDH) and Alkaline phosphatase (ALP)were effectively lowered. Both attenuated the stress induced changes in RBC membrane as increased membrane protein clusterization, increased membrane fluidity and reduced membrane thickness.

The antipyretic effect of *O. sanctum* was examined in rats by Suresh (1992). He found that the benzene extract, essential oil and decoction of *O. sanctum* produced a dose dependent antipyretic effect.

Chattopadhyay (1993) demonstrated that the oral administration of alcoholic extract of leaves of *O. sanctum* (200mg/kg) in normal, glucose fed hyperglycemic and streptozotocin induced diabetic rats led to marked lowering of blood sugar level. Maximum glucose suppression occurred two hours after treatment by the working dose. The extract also potentiated the action of exogenous insulin in normal rats.

Hypolipidemic effect of O. sanctum leaves was reported by Sarkar *et al.* (1994). They administered fresh leaves of Ocimum mixed along with feed (one and two per cent) for four weeks which brought about significant changes in the lipid profile of normal albino rabbits. The treatment resulted in significant lowering in serum total cholesterol. The LDL-cholesterol was progressively decreased and HDL-cholesterol and faecal sterol excretion were found to be progressively increased during the four week experimental period.

Devi and Ganasoundari (1995) noticed that a single intraperitoneal injection of 50 mg/kg bodyweight of aqueous extract of tulsi leaves increased the survival time by 30 per cent in mice when given before a whole body exposure to 11Gy of 60 Co gamma radiation. Aqueous extract was more effective and less toxic compared to alcoholic extract. Intraperitoneal route gave best protection compared to intramuscular, intravenous or oral routes.

Antiarthritic activity of fixed oil of tulsi in doses of one, two and three mg/kg bodyweight intraperitoneally were studied against adjuvant induced and formalin induced arthritic models (Singh and Majumdar, 1995). Results revealed that the fixed oil possessed significant anti arthritic activity in both models.

Butanolic extract of *O. sanctum* at 50-200 mg/kg bodyweight administered to mice immunized with sheep RBC increased the number of plaque forming cells and serum haemagglutination antibody titre. Partial immuno restoration in betamethasone treated and significant restoration in cyclophosphamide treated animals on primary humoral response was observed. The immunorestorative effect may be via stimulation of phagocytic function of the cells of reticuloendothelial system and activation of T-helper lympocytes resulting in enhanced antibody production (Khajuria *et al.*, 1996).

Singh *et al.* (1996) reported that gas liquid chromatography of fixed oil of *O. sanctum* revealed the presence of five fatty acids *viz.* stearic, palmitic, oleic, linoleic and linolenic acids which demonstrated anti-inflammatory activity against PGE_2, leukotriene and arachidonic acid induced paw oedema. The anti-inflammatory activity of linolenic acid present in the fixed oil was probably due to blockade of both the cyclooxygenase and lipooxygenase pathways of arachidonic acid metabolism. The triglyceride fraction of the oil showed higher protection compared to fixed oil against carrageenan induced paw edema and acetic acid induced writhings in rats and mice respectively.

Treatment of animals with ethanolic extract of tulsi leaves (100 mg/kg bodyweight, p.o) prevented the change in plasma level of cortico sterone, induced by exposure to both acute and chronic noise stress, indicating the antistressor property of the plant (Sembulingan *et al.*, 1997)

Devi and Ganasoundari (1999) observed that the aqueous extract of leaves of *O. sanctum* protected mice against radiation lethality and chromosome damage and also against radiation induced lipid peroxidation. Pretreatment with the extract checked the radiation induced depletion of glutathione and the antioxidant enzymes glutathione transferase, reductase, peroxidase and superioxide dismutase and maintained their levels within or above the control range.

Administration of water soluble portion of the alcoholic extract of *O. sanctum* in graded doses produced a significant fall in rectal temperature of rats which started at 60 min and lasted for more than 120 min. The antipyretic activity may be due to the inhibition of prostaglandin synthesis (Lata *et al.*, 1999).

Prakash *et al.* (1999) evaluated the seed oil and fresh aqueous extract of *O. sanctum* for their anti proliferative activity using microculture tetrazolium assay. Significant anti proliferative activity was observed in seed oil at concentrations of 250 μg/ml and 83.33 μg/ml over a period of four days.

Rodrigues *et al.* (1999) examined the effect of *O. sanctum* on learning behaviour in stressed rats by feeding them with fresh plant extract (two ml/kg/day) for three weeks. Spontaneous alteration and rewarded alternation learning tests in a T-maze clearly indicated that the plant can help in improving stress induced learning impairments.

Pretreatment of rats with *O. sanctum* extract (100 mg/kg bodyweight) for seven days prior to the exposure to noise stress prevented leoucopenia and changes in differential count (Sembulingam *et al.*, 1999).

Studies conducted on Wistar rats using incision and excision wound models by Shetty *et al.* (1999) revealed that oral administration of tulsi at 0.1 ml/100 g bodyweight significantly increased the tensile strength in incision wound and promoted epithelization in excision wound. The study clearly suggested that tulsi had promising prohealing action.

Singh (1999) reported that the fixed oil of *Ocimum basilicum* possessed significant antiulcer activity against aspirin, indomethacin, alcohol, histamine, reserpine, serotonin and stress induced ulceration in experimental animal models. Intra peritoneal administration of the fixed oil at the dose levels of one, two and three ml/kg body weight significantly reduced the ulcer index in a dose

dependent manner. The lipooxygenase inhibiting, histamine antagonistic and anti secretory effect of the oil could probably contribute towards the anti ulcer activity.

Rodrigues *et al.* (2000) demonstrated that tulsi leaf extract (two ml/kg/day for three weeks) could reverse the stress induced decrease in the dendritic branching points and intersections in the hippocampal CA_3 neurons in adult rats. Treatment with the extract helped the stressed animals to restore the impaired learning and memory.

Activity of *O. sanctum* against enteric pathogens was investigated by Geeta *et al.* (2001). Aqueous and alcoholic extracts at two concentrations, 30mg and 60 mg, were tried against the enteric pathogens by Agar diffusion method. Wide zones of inhibition were observed at 60mg concentration. Aqueous extracts showed wider zones of inhibition for Klebsiella, *E coli*, Proteus and *Staphylococcus aureus*. Alcoholic extract showed wider zone for *Vibrio cholerae*.

Ahmed *et al.* (2002) revealed that the treatment of albino rats with benzene extract of tulsi leaves (250mg/kg body weight) for 48 days decreased the total sperm count, sperm motility and forward velocity. The percentage of abnormal sperms increased in caudal epididymal fluid and the fructose content decreased in the caudal plasma of the epididymis and the seminal vesicles. The results suggested that such effects are due to androgen deprivation caused by the anti androgenic property of tulsi leaves.

Sharma *et al.* (2002) noted that *O. sanctum* extract (10 mg / kg body weight, p.o) given before and after mercury intoxication showed a significant decrease in lipid peroxide level, SGOT and SGPT activities and increase in serum alkaline phosphatase activity and glutathione content. The results suggested that oral administration of ocimum extract provided protection against mercuric chloride induced toxicity in Swiss albino mice.

Arora *et al.* (2003) investigated the antioxidant activity of aqueous extract of Ocimum leaves in hypercholesterolemic rabbits. Administration of the extract at 100 mg/kg/day for six weeks decreased the serum and platelet rich plasma lipid peroxide levels significantly. The extract may have beneficial roles in atherosclerosis and coronary artery disease through an increase in antioxidant defence and a direct free radical scavenging action.

Hydroalchoholic extract of *O. sanctum* at 25,50,70 and 100 mg/kg bodyweight produced significant reversal of the histopathological changes caused by isoproterenol induced myocardical infarction as myonecrosis, myophagocytosis and lymphocytic infiltration in rats (Arya *et al.*, 2003).

Jena *et al.* (2003) demonstrated that Immu- 21 containing extracts of *O. sanctum, Withania somnifera, Emblica officinalis* and *T. cordifolia* given at 100mg/kg daily over seven days in mice inhibited both cyclophosphamide induced classical and non-classical chromosomal aberrations. A significant reduction in micronuclei in bone marrow erythrocytes was also observed.

In glacial acetic acid induced writhing test, alcoholic extract of tulsi leaves at 500 and 100mg/kg intra peritoneally and orally, reduced the number of writhes. It also increased the tail withdrawal latency in mice. The analgesic action of Ocimum is exerted both centrally as well as peripherally and involves an interplay between various neurotransmitter systems (Khanna and Bhatia, 2003).

According to Vats *et al.* (2004), administration of *O. sanctum* at 200 mg/kg for 30 days led to a significant decrease in plasma blood glucose levels. They suggested that the anti hyperglycemic effect of Ocimum is at least partially dependent upon insulin release from the pancreas as the extract showed a greater hyperglycemic effect in milder form of diabetes and a lower response in moderate forms.

2.4 PHARMACOLOGICAL EFFECTS OF TINOSPORA CORDIFOLIA

Gupta *et al.* (1967) found that oral administration of the aqueous and alcoholic extracts of dried powdered stem of *Tinospora cordifolia* at different dose rates (100,200,400 mg/kg p.o) caused reduction in fasting blood sugar level in rabbits and rats.

Aqueous extract of *T. cordifolia* stem significantly decreased the bronchospasm induced by five per cent histamine aerosol in guinea pigs. It decreased the capillary permeability in mice and reduced the number of disrupted mast cells in rats (Nayampalli *et al.,* 1986).

Rege *et al.* (1989) revealed that the water extract of *T. cordifolia* at a dose rate of 100 mg/kg bodyweight improved the cellular immune functions in immunosuppressed rats with obstructive jaundice cholestasis. The phagocytic activity of macrophages and polymorpho nuclear neutrophils was normalized. There was marked elevation of killing capacity of neutrophils. The mortality rate in treatment groups following *E.coli* infection was significantly reduced to 16.65 per cent.

Benzene extract of *T. cordifolia* at the dose rate of 400mg/kg produced a better reduction in temperature than aspirin. A decoction of *T. cordifolia* reduced the temperature from 39.16^{0}C to 38.5^{0}C after the first hour of its administration (Suresh, 1992).

The aqueous, alcoholic and chloroform extracts of the leaves of *T. cordifolia* administered at doses of 50,100,150 and 200 mg/kg bodyweight to normal and alloxan diabetic rabbits showed a significant hypoglycemic effect (Wadood *et al.*, 1992).

T. cordifolia decreased the hepatic fibrosis induced by carbon tetrachloride injection in rats. It significantly improved the suppressed kupffer

cell function in chronic liver damage produced by heterologous serum. The antifibrotic effect of Tinospora may be mediated through activation of kupffer cells (Nagarkatti *et al.*, 1994).

Thatte *et al.* (1994) reported the colony stimulating activity of *T. cordifolia* in serum. According to them, ten days treatment with Tinospora at 100 mg/kg/day induced a significant increase in the number of Colony Forming Units of the granulocyte-macrophage series. Activation of macrophages by Tinospora led to increase in GM-CSF which in turn led to leucocytosis and improved neutrophil function.

The *in vitro* amoebicidal activity of a crude drug formulation comprising of five medicinal plants namely, *Boerhaavia diffusa*, *Berberis aristata*, *T. cordifolia*, *Terminalia chebula* and *Zingiber officinalis* was studied by Sohni *et al.* (1995). In experimental caecal amoebiasis in rats, the formulation had a curative rate of 89% with the average degree of infection reduced to 0.4 in a group dosed with 500mg/kg/day. There were varying degrees of inhibition of DNAase, RNAse, aldolase, alkaline phosphatase, acid phosphatase, alpha-amylase and protease activity in axenically cultured amoebae.

The effect of *T. cordifolia* on the functions of macrophages obtained from mice treated with the carcinogen, ochratoxin- A was examined by Dhuley (1997). It was found to inhibit the ochratoxin- A induced suppression of chemotactic activity of murine macrophages. Tinospora also restored the decreased production of interleukin-1 and tumor necrosis factor by macrophages, induced by the carcinogen.

Kapil and Sharma (1997) conducted studies on the immunopotentiating compounds, syringin and cordiol from *T. cordifolia*. The compounds inhibited the *in vitro* immunohaemolysis of antibody-coated sheep erythrocytes by guinea pig serum. The reduced hemolysis was found to be due to the inhibition of the C3-convertase of the classical complement pathway. The compounds also gave

rise to a significant increase in IgG antibodies in serum. Macrophage activation was reported for cordioside, cordiofolioside A and cordiol.

Exposure of HeLa cells to zero, five, 10,25,50 and 100 μg/ml of guduchi extracts (methanol, aqueous and methylene chloride) resulted in a dose dependent significant increase in cell killing. Methylene chloride extract enhanced the cell killing effect by 2.8 and 6.8 fold when compared to methanol or aqueous extract at 50 and 100 microg/ml respectively. The frequency of micronuclei was significantly increased in a concentration-dependent manner in guduchi-treated groups. The results clearly suggested the antineoplastic activity of guduchi (Jagetia *et al.*, 1998).

Noor and Aschcroft (1998) suggested that the hypoglycemic effect of *T. cordifolia* was probably due to stimulation of insulin release via modulation of beta-cell calcium concentration. Insulin release at basal conditions may be mediated by closure of ATP sensitive potassium ion channels to depolarize the beta cell membrane.

T. cordifolia (50 mg/kg body weight, p.o) produced significant anti inflammatory effect in both acute and subacute models of inflammation in albino rats. In acute inflammation, efficacy of Tinospora was more than the acetyl salicylic acid. A reduction of 67.28 per cent in acute inflammation and 40.30 per cent in subacute inflammatory process was observed (Jana *et al.*, 1999).

Prince *et al.* (1999) recorded that administration of the extract of *T. cordifolia* roots (2.5 and five g/kg bodyweight) for six weeks resulted in a significant reduction in serum and tissue cholesterol, phospholipid and free fatty acid in alloxan diabetic rats. The root extract at a dose of five g/kg bodyweight showed the highest hypolipidemic effect. The efficacy at 2.5 and five g/kg body weight was better than glibenclamide.

Experimental studies by Rege *et al.* (1999) showed that the whole, aqueous, standardized extracts of *T. cordifolia* offered protection against various biological, physical and chemical stressors in albino rats. The alterations in gastric emptying and intestinal motility induced by cisplatin were reversed significantly. It was found to normalize the changes in phagocytic activity of peritoneal macrophages after exposure of rats to either carbon tetrachloride or horse serum.

Effect of Tinospora on bodyweight, total peritoneal exudates cell count percentage and on plasma proteins in malnourished rats were reported by Marathe *et al.* (2001). The herb was found to increase the bodyweight which could be due to its appetite stimulating action.

Roopa *et al.* (2001) noted that the alcoholic extract of Tinospora significantly reduced the ulcer index in pyloric ligation, ibuprofen and cold stress induced gastric ulcers in albino rats at a dose of 500 mg/kg bodyweight. The anti ulcer effect was comparable to that of famotidine in pyloric ligation and cold stress models and to misoprostol in ibuprofen induced ulcer model.

According to Vaingankar *et al.* (2001), Tinospora stimulated nitric oxide, production by alvelolar macrophages in a dose dependent manner with optimal stimulation at 200 mg/ml and 400 mg/ml in rats. A significant increase in serum nitric oxide level was detected in human volunteers after treatment with Tinospora for 10 days.

Agarwal *et al.* (2002) used alcoholic and aqueous extracts of Tinospora to study its effect in learning and memory in normal and cognition deficit rats. Both alcoholic (200 mg/kg body weight) and aqueous (100mg/kg bodyweight) extracts significantly enhanced cognition in normal rats as seen in behavioural tests-Hebb William maze and the passive avoidance task. Tinospora along with cyclosporine administration showed an enhancement in locomotor activity in the open field chamber. The probable mechanism of cognitive enhancement could be

by immunostimulation and increasing the synthesis of acetyl choline which is an important neurotransmitter in learning and memory process.

Bishayi *et al.* (2002) found that Tinospora extract (100 mg/kg bodyweight for 15 days) in carbon tetrachloride intoxicated rats protected the liver as indicated by enzyme levels in serum. A significant reduction in serum levels of SGOT, SGPT, ALP and bilirubin were observed. A significant increment in the functional capacities of rat peritoneal macrophages was also observed.

An arabinogalactan polysaccharide isolated from Tinospora showed good protection against iron-mediated lipid peroxidation of rat brain homogenate as revealed by the thiobarbituric acid reactive substances and lipid hydroperoxide assay. Significant protection to protein against gamma-ray induced damage was also noticed. The protective action can possibly be due to the high reactivity of the polysaccharide towards superoxide radicals and hydroxyl radicals (Subramanian *et al.*, 2002).

Oral administration of 70% methanolic extract of Tinospora stem to male rats at a dose level of 100 mg/rat/day for 60 days decreased the weight of testes, epididymis, seminal vesicles and ventral prostate in a significant manner. Sperm motility as well as sperm density were reduced significantly which resulted in reduction of male fertility by 100 per cent (Gupta and Sharma, 2003).

The antiatherogenic effect of a herbal formulation, Caps HT2 containing methanolic extracts of *Commiphora mukul, Allium sativum, Plumbago indica, T. cordifolia, Withania somnifera* and *O. sanctum* was evaluated by Mary *et al.* (2003). The formulation was found to scavenge superoxide and hydroxyl radicals, the IC_{50} required being 55.0 and 610.0 µg/ml respectively. The intravenous administration of the formulation (five mg/kg) enhanced the release of lipoprotein lipase enzyme significantly. Formulation also inhibited ADP induced platelet aggregation *in vitro*. The anti inflammatory action was

significant with acute and chronic inflammations induced by carrageenan and formalin respectively in rats.

Pahadiya and Sharma (2003) evaluated the radio protective effect of an aqueous extract of Tinospora against Cobalt 60 gamma radiation. Oral administration of Tinospora (five mg/kg body weight) to Swiss albino mice 15 days prior to whole body radiation exposure produced significant protection in terms of survival per centage. Oral administration at 10mg/kg bodyweight/day to mice seven days prior to whole body irradiation prevented mortality until day 13 and 50% of the animals survived until day 30.

2.5 OTHER INDIGENOUS PLANTS WITH HYPOGLYCEMIC EFFECT

Jos (1974) conducted investigations on the hypoglycemic effect of selected indigenous plants as *Eugenia jambolana, Momordica charantia, Gymnema sylvestre, Emblica officinalis, Salacia reticulata* and *Curcuma longa.* All the six indigenous plants were found to exert hypoglycemic effect in normal as well as alloxan diabetic dogs. The seeds of *Eugenia jambolana* had the maximum hypoglycemic effect, the roots of *Salacia reticulata* proved to be least effective.

Desai and Bhide (1985) reported that the alcoholic extract of roots of *Hamiltonia suaveolens* caused 45 per cent reduction in blood sugar level in normal rats and 37 per cent reduction in alloxanised rats at the end of fifth hour. Similar reduction was observed in normal rabbits, dogs and monkeys. Ethyl acetate fraction of alcoholic extracts also showed hypoglycemic activity of similar magnitude in normal rats but was inactive in alloxanized rats.

Oral administration of the aqueous extract of *Tephrosea purpurea* Linn. led to marked lowering of blood glucose level in normal and alloxan induced diabetic rabbits. The hypoglycemic effect of the extract was comparable with

that of tolbutamide in normal rabbits. In diabetic rabbits, it exerted 60-70 per cent hypoglycemic effect as compared to tolbutamide (Kashfudduja *et al.*, 1985).

Hikino *et al.* (1989) found that ganoderan B, a glycan isolated from *Ganoderma lucidum,* increased the plasma insulin levels in normal and glucose loaded mice. A significant increase in the activities of hepatic glucokinase, phosphofructokinase and glucose-6-phosphate dehydrogenase was noticed .A reduction in the glycogen content in the liver was also noticed

Sheela and Augusti (1992) observed that S-allyl cysteine sulphoxide, a sulphur containing amino acid of garlic which is the precursor of allicin and garlic oil, showed anti diabetic effect in alloxan diabetic rats. Administration at a dose of 200mg/kg bodyweight significantly decreased the concentration of serum lipids, blood glucose and the activities of various enzymes.

Cherian and Augusti (1993) noted that the glycoside of leucopelargonidin isolated from the bark of *Ficus bengalensis* is having hypoglycemic, hypolipidemic and serum insulin raising effects in moderately diabetic rats with similarities to the effects of a minimal dose of glibenclamide. It also significantly enhanced the faecal excretion of sterols and bile acids resulting in hypocholesterolemia.

Ponnachan *et al.* (1993) used alloxan induced diabetic rats to evaluate the anti diabetic effect of alkaloids extracted from leaves of *Aegle marmelos.* The extract maintained the weight of animals near to that of control ones and produced significant lowering of blood glucose level, but no decrease in blood urea and serum cholesterol were noticed.

Single oral administration of Swerchirin containing hexane fraction of *Swertia chirayita* at 50mg/kg bodyweight to rats resulted in about 60 per cent fall in blood glucose by seven hour post treatment. Swerchirin lowered blood

glucose level by stimulating insulin release from islets of Langerhans (Saxena *et al.*, 1993).

The hypoglycemic effect of orally administered extracts of *M. charantia* L.fruits was examined in normoglycemic and cyproheptadine induced hyperglycemic mice by Cakici *et al.* (1994). The aqueous extract produced significant decrease on the nonfasting blood glucose levels in hyperglycemic mice.

Glombitza *et al.* (1994) investigated the effect of butanol extract of *Zizyphus spinachristi* as well as christinin-A, its principle saponin glycoside in streptozotocin induced diabetic rats. Significant reduction of serum glucose levels, liver phosphorylase and glucose-6-phosphatase activities and increase in liver glycogen content were noticed after four weeks of treatment. Marked improvement in glucose utilization was also observed.

The methanol extract of rhizomes of *Polygonatum officinale* at 800mg/kg bodyweight reduced the blood glucose of normal mice from 170 $\pm$ 3 to 136 $\pm$ 5 mg/dl in four hours after intra peritoneal administration and also significantly lowered the blood glucose of streptozotocin diabetic mice from 696 $\pm$ 60 to 407 $\pm$ 35 mg/dl. The extract also suppressed epinephrine induced hyperglycemia in mice (Kato and Miura, 1994).

Khan *et al.* (1994) observed that alcoholic extract of *Ipomoea pescaprae* stimulated insulin release from islets of Langerhans *in vitro* dose dependently. Oral feeding of the extract to normal rats for seven days caused significant blood glucose lowering effect and increase in liver glycogen levels with decrease in the activity of glucose-6-phosphatase.

Hypoglycemic activity of a decoction of Juniper berries was investigated by Medina *et al.* (1994). Juniper decoction decreased the glycemic levels in normoglycemic rats at a dose of 250 mg/kg bodyweight. Administration to

streptozotocin induced diabetic rats at 125mg/kg for 24 days resulted in a significant reduction in blood glucose levels and in mortality index. It also prevented the loss of bodyweight.

Khan *et al.* (1995) concluded that *Murraya koenigi* and *Brassica juncea* caused significant decrease in fasting blood glucose and an increase in the concentration of hepatic glycogen. The activity of glycogen synthase in the liver was increased and that of glycogen phosphorylase was decreased. Decreased rate of glycogenolysis and gluconeogenesis was also noticed.

Mariam *et al.* (1996) reported that treatment of normoglycemic rats with the aqueous extract of *Orthosiphon stamineus* at a dose of one g/kg produced a significant decrease in blood glucose level. The effect of the extract in streptozotocin diabetic rats was comparable to that of 10mg/kg of glibenclamide.

Trejo-Gonzalez *et al.* (1996) evaluated the hypoglycemic activity of a purified extract from prickly pear cactus (*Opuntia fuliginosa*) on streptozotocin diabetic rats. Blood glucose and glycated haemoglobin levels were reduced to normal levels by a combined treatment of insulin and Opuntia extract. The prickly pear extract alone also maintained normoglycemic state in the diabetic rats.

Experiments conducted by Abdel-Barry *et al.* (1997) showed that graded amounts (0.06, 0.2, 0.5 and one g/kg intraperitoneally and one, two and eight g/kg p.o) of aqueous extract of *Trigonella foenum graecum* leaves caused a significant reduction of blood glucose concentration in alloxan diabetic rats. Ethanolic extract of fenugreek leaves produced no reduction in blood glucose concentration in normal rats. However, a reduction of blood glucose levels was noted in diabetic rats on intra peritoneal administration of 0.8g/kg.

Studies on trans-dehydrocrotonin, a norclerodane diterpene from the bark of *Croton cajucara* by Farias *et al.* (1997) demonstrated a significant

hypoglycemic activity in alloxan induced diabetic rats at doses of 25 and 50mg/kg bodyweight orally. The drug also effectively lowered the blood sugar levels in glucose fed normal rats.

Paeoniflorin isolated from the dried root of *Paeonia lactiflora* produced a significant blood sugar lowering effect in streptozotocin induced diabetic rats and had a maximum effect at 25min after treatment (Hsu *et al.*, 1997).

Sharma *et al.* (1997) concluded that both the aqueous and 50 per cent ethanolic extracts of *Caesalpinia bonducella* exhibited hypoglycemic activity as early as four hour after administration at a dose of 100mg/kg. Hypoglycaemia produced by the aqueous extract was of prolonged duration as compared to the ethanolic extract. In diabetic rats, antihyperglycaemic effect was seen from day five onwards.

Administration of 2.5g almond seed and its proportionate fractions *viz.* 1.22g defatted seed and 1.28g oil to three groups of albino rabbits showed a definite hypoglycemic action during a two months study by Teotia and Singh (1997). Blood sugar levels were decreased by about 28 per cent at the end of 60 days. Defatted seeds possessed comparatively less hypoglycemic activity and oil portion exhibited the least activity.

According to Amalraj and Ignacimuthu (1998), single doses of unroasted seeds of *Cajanus cajan* resulted in a significant reduction in serum glucose levels after one to two hours and a significant rise at three hour. Treatment with roasted seed preparation was devoid of any hypoglycemic activity during the entire period of study.

Prince *et al.* (1998) investigated the hypoglycemic activity of *Syzigium cumini* seeds and found that oral administration of 2.5g and five g/kg bodyweight of the aqueous extract for six weeks resulted in a significant reduction in blood

glucose and an increase in total haemoglobin. It also prevented a decrease in bodyweight.

The flavonoids of *Cuminum nigrum* seeds at a dose range of 0.5 to 1.5 g/kg caused a hypoglycemic effect both in normal and alloxan diabetic rabbits. (Ahmad *et al.*, 2000). The effect started two hr after drug administration, reached a maximum within four to eight hour. The blood glucose levels returned close to normal within 24 hour of drug administration.

The insulin secretory effects of *Citrullus colocynthis* fruit extracts were evaluated *in vitro* in the isolated rat pancreas and isolated rat islets in the presence of 8.3 *mM* glucose by Nmila *et al.* (2000). All tested extracts when perfused for 20 min at 0.1mg/ml immediately and significantly stimulated insulin secretion. The effect was transient. A significant and persistent increase in pancreatic flow rate appeared during perfusions. The insulinotropic effect, at least partially, account for the antidiabetic activities of these fruits.

An alcoholic extract of *Phyllanthus niruri* administered in normal rats at 200 and 1000mg/kg bodyweight reduced the blood sugar by 34.5 per cent and 47.4 per cent respectively. In alloxan diabetic rats, blood sugar was reduced by 6.07 per cent at 200mg/kg and 18.7 per cent at 1000 mg/kg (Raphael *et al.*, 2000).

Srinivas *et al.* (2000) conducted studies on the antidiabetic activity of *Raphanus sativus* Linn. Aqueous extract of the leaves showed marked antidiabetic effect on streptozotocin induced models of both IDDM and NIDDM. A fall in blood glucose levels of 46.3 per cent in IDDM and in NIDDM, the effect was greater than that of glibenclamide.

Chen *et al.* (2001) noted that four weeks administration of aqueous extract of rhizome of *Polygonati odorati* reduced fasting blood glucose, decreased glycosylated haemoglobin and improved the glucose tolerance in diabetic mice.

In diabetic rats, complicated with hyperlipema, rhizome of Polygonati prevented and reduced both hyperglycemia and hypertriglyceridemia.

Chude *et al.* (2001) suggested that the aqueous extract of *Boerhaavia diffusa* leaves showed non-dose dependent hypoglycemic activity. 400mg/kg dose caused a maximum reduction of 21.56 per cent in glucose level at six hrs. Lower doses of 100mg/kg and 200mg/kg showed more hypoglycemic effect.

The aqueous extract of *Lantana camara* Linn. was found to produce significant reduction of blood glucose concentration between two to four hours of administration in alloxan induced hyperglycemic rats and normoglycemic rats (Dash *et al.*, 2001).

Ghosh and Suryawanshi (2001) tested the aqueous extract of *Catharanthus roseus* flower and leaf for their antidiabetic activity in alloxan diabetic male rats. The treatment not only produced blood glucose homeostasis but also reversed changes in carbohydrate, protein, lipid metabolism and the metabolic and pathologic changes that took place in pancreatic islet cells, liver and kidney during alloxan diabetes.

Gutierrez *et al.* (2001) found that the intraperitoneal administration of 100,150 and 200 mg/kg of hexane extract of *Cirsium pascuarense* produced a significant hypoglycemic effect in normal as well as diabetic mice. In addition, the extracts altered the glucose tolerance in alloxan diabetic rats.

According to Hussain and Rao (2001), treatment of diabetic rats with four ml (four g/drywt) of aqueous extract of *Abroma augusta* for 16 weeks resulted in gradual but significant fall in fasting blood glucose and improvement in glucose tolerance, serum total and LDL cholesterol and triacylglycerol.

Seedling, seedling parts and callus cultures of onion (*Allium cepa*) were tested by Kelkar *et al.* (2001) for their antidiabetic activity after feeding the tissue

extracts to diabetic rats. The results indicated much higher hypoglycemic activity in callus cultures as compared to natural bulbs of onion.

Rao and Rao (2001) observed that the aqueous extract of *Syzygium alternifolium* at a dosage of 0.75 g/kg bodyweight showed the maximum blood glucose lowering effect in both normal and alloxan diabetic rats. The ethanol and hexane fractions also showed hypoglycemic and antihyperglycemic activity but the effect was significantly less than that of aqueous extract.

Sachdewa *et al.* (2001) evaluated the effect of aqueous extract of *Hibiscus rosasinensis* L. leaves on blood glucose and glucose tolerance in Wistar rats. Administration of the extract daily for seven consecutive days at an oral dose of 250 mg/kg, significantly improved glucose tolerance in rats by 47 per cent. The hypoglycemic activity was comparable to tolbutamide.

In normal rabbits, powdered rhizome and its methanol and aqueous extracts of *Alpinia galanga* significantly lowered the blood glucose levels. However in alloxanized rabbits, no significant reduction in blood glucose was noticed (Akhtar *et al.*, 2002).

Arun and Nalini (2002) conducted studies on the efficacy of turmeric (*Curcuma longa*) on blood sugar and polyol pathway in diabetic albino rats. They noticed that administration of turmeric or curcumin to diabetic rats reduced the blood sugar, haemoglobin and glycosylated haemoglobin levels significantly. Turmeric and curcumin supplementation also reduced the oxidative stress encountered by the diabetic rats.

Babu *et al.* (2002) recorded a concentration dependent antihyperglycemic effect of the leaves as well as alcoholic extract of *Cassia kleinii* in glucose loaded rats. In alloxan induced diabetic rats, the extract (200 mg/kg) showed remarkable efficiency.

Bilbis *et al.* (2002) opined that the aqueous extract of *Arachis hypogea* caused a significant decrease of fasting blood glucose of both normal and alloxan induced diabetic rats. The extract also caused a decrease in serum triglyceride, total cholesterol, HDL-Cholesterol and LDL-Cholesterol.

A study was undertaken by Grover *et al.* (2002) to examine the hypoglycemic and antihyperglycemic effects of various strengths (five,10 and 15%) of *Brassica juncea* seed diet in diabetic albino rats. Brassica diet at 10 and 15 per cent showed significant antihyperglyemic effect in alloxan diabetic rats.

Korkmaz and Gurdal (2002) demonstrated that two ml/kg of aqueous extract of *Artemisia santonicum* L. produced a significantly lower blood glucose levels in normal and diabetic rabbits after six hours of administration which was consistent and time dependent.

Experimental studies by Pandey and Khan (2002) demonstrated that feeding for 21 days of the diets containing 15 per cent powdered *Syzigium cumini* seeds, 15 per cent powdered defatted seeds and six per cent water soluble gummy fibre isolated from Syzigium seeds significantly lowered blood glucose levels and improved oral glucose tolerance.

Vetrichelvan *et al.* (2002) noticed that the alcoholic extract of *Celosia argentia* Linn.seeds reduced the increase of blood glucose in alloxan-induced diabetic rats by 27.8 per cent at 250mg/kg bodyweight. Chronic administration significantly reduced the blood glucose in diabetic rats for two weeks. The extract also presented a decrease in bodyweight in the rats.

Farzami *et al.* (2003) concluded that the active component of *Urtica dioica* leaf extract increased the insulin content of blood sera in normal and streptozotocin diabetic rats that were injected intraperitoneally with the active ingredient of the extract. A simultaneous decrease of blood sugar was detected when similar sera was tested for glucose.

John (2003) concluded that fenugreek seed powder at two and eight g/kg bodyweight reduced blood glucose, serum cholesterol, serum triglyceride and increased liver glycogen levels. But the level of these parameters in animals treated with mustard at two g/kg remained similar to that obtained for diabetic control.

Sharma *et al.* (2003) demonstrated that ethanolic extract of *Eugenia jambolana* seeds (100 mg/kg) given orally to subdiabetic rabbits for one day, moderately diabetic for seven days and severely diabetic for 15 days showed 12 per cent, 18.9 per cent and 29 per cent fall respectively in fasting blood glucose level. Significant fall in glycosylated haemoglobin levels and increase in serum insulin levels and liver and muscle glycogen content were also noticed.

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Wistar strain albino rats of either sex weighing 150-250 g, procured from the Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy were used. They were reared in cages with proper aeration and photoperiod. Animals were maintained on feed and water *ad libitum* for one week before the commencement of the study. The experiment was carried out for a period of 37 days.

3.2 EXPERIMENTAL DESIGN

In the experiment, a total number of forty eight rats were used. Diabetes was induced one week before starting the treatment. On the seventh day, the diabetic rats were randomly divided into six groups of eight animals each.

Group I (T_1) — Diabetic, normal feed plus ethanolic extract of dried powdered *Azadirachta indica* leaves at a dose of 200mg/kg bodyweight orally from day seven to day 37.

Group II (T_2) — Diabetic, normal feed plus ethanolic extract of dried powdered *Ocimum sanctum* leaves at a dose of 200mg/kg bodyweight orally from day seven to day 37.

Group III (T_3) — Diabetic, normal feed plus ethanolic extract of dried powdered vine of *Tinospora cordifolia* at a dose of 200mg/kg bodyweight orally from day seven to day 37.

Group IV (T_4) — Diabetic, normal feed plus a combination of ethanolic extracts of *A. indica*, *O. sanctum* and *T. cordifolia* (1:1:1)

at a dose of 200mg/kg bodyweight orally from day seven to day 37.

Group V (T_5) — Diabetic, normal feed plus 0.5 mg glibenclamide/rat/day from day seven to day 37.

Group VI (T_6) — Alloxan control, normal feed plus vehicle (1% Tween-80)

Bodyweight was recorded at the beginning of the experiment and at every week thereafter. Blood was collected and blood glucose was estimated on 7^{th}, 15^{th}, 21^{st}, 30^{th} and 37^{th} day. On 37^{th} day, the animals were sacrificed and serum total cholesterol and triglyceride and liver glycogen were estimated.

3.3 PREPARATION OF DIABETIC RATS

The rats were fasted overnight and their bodyweight and blood glucose were estimated on next day (zero day) morning. As a preliminary trial, alloxan was given at 90,100,120 and 150 mg/kg bodyweight intraperitoneally to find out a safe and convenient dose. At 90mg/kg bodyweight, 60% of the animals survived with blood glucose levels above 200mg/dl. Other doses were found to be too toxic and survival rate was very low.

Alloxan solution (10%) was prepared in distilled water immediately before use and injected intraperitoneally at the rate of 90mg/kg bodyweight after the rats have been fasted for 12 hours. Since alloxan was capable of producing fatal hypoglycaemia by massive pancreatic insulin release, rats were treated with 20 per cent glucose solution intraperitoneally after four to six hours to prevent mortality. The rats were given five per cent glucose solution for the next 24 hours for drinking. After a week, when the blood glucose level was stabilized, animals with blood glucose level above 200mg/100ml were selected for the study.

Fig.1. *Azadirachta indica* (Neem)

Fig.2. *Ocimum sanctum* (Tulsi)

Fig.3. *Tinospora cordifolia* (Chittamruthu)

3.4 PREPARATION AND ADMINISTRATION OF DRUGS

The leaves of *A. indica, O. sanctum* and the vines of *T. cordifolia* were collected fresh and were dried in the shade at room temperature. The *T. cordifolia* vines were pealed and cut into small pieces before drying. The dried leaves and vines were then powdered well in a pulveriser. The powdered leaves and vines were subjected to extraction using 70% 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 yield of the extract was 12 percent, 10 percent and eight per cent for Azadirachta, Ocimum and Tinospora respectively. The crude extract thus prepared was kept in the refrigerator at 4^0C for further use. A weighed quantity of the crude extract was homogenized with 1% Tween-80 and was administered orally to individual rats for 30 days based on their bodyweight.

Glibenclamide

Tablet Daonil[1] (five mg) was powdered and given orally at the rate of 0.5 mg/animal/day for 30days to treatment group T_6.

3.5 PHYTOCHEMICAL SCREENING

The ethanolic extracts of *A. indica, O. sanctum* and *T. cordifolia* were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure quoted by Harborne (1991).

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[1] Hoechst Marion Roussel, Hoechst House, Andheri, Mumbai

3.5.1 Test for Detection of Steroids

3.5.1.1 Salkowski Test

About five mg of the extract was dissolved in three ml of chloroform and then shaken with three ml concentrated sulphuric acid. Development of a red colour indicates the presence of steroids.

3.5.1.2 *Lieberman Burchardt Test*

About five mg of the extract was dissolved in three ml of chloroform. Then five drops of acetic anhydride and one ml of concentrated sulphuric acid was added to it through the sides. A reddish ring at the junction of the two layers indicates presence of steroids.

3.5.2 Test for Detection of Alkaloids

About 0.5g of the extract was mixed with five ml ammonia and then extracted with equal volume of chloroform. To this, 0.1*N* hydrochloric acid was added. The acid layer obtained was used for chemical test for the alkaloids.

3.5.2.1 Mayer's Test (potassium mercuric iodide)

To one ml of the acid layer obtained few drops of Mayer's reagent were added. If a creamy white precipitate is formed, it indicates the presence of alkaloids.

3.5.2.2 Hager's Test (saturated solution of picric acid)

To one ml of the acid layer, a few drops of Hager's reagent were mixed. A yellow precipitate is formed if alkaloids are present.

3.5.2.3 Dragendroff's Test (solution of potassium bismuth iodide)

Two drops of Dragendroff's reagent was mixed with one ml of acid layer. Presence of alkaloids is indicated if a reddish brown precipitate is seen.

3.5.3 Test for Detection of Tannins

3.5.3.1 Ferric Chloride Test

Two mg of the extract was mixed with three ml of 1% ferric chloride solution. If blue green or brownish green colour is obtained, it indicates the presence of tannins.

3.5.4 Test for Detection of Flavonoids

3.5.4.1 Ferric Chloride Test

To two ml of alcoholic solution of the extract (0.5g extract in 10ml methanol), few drops of neutral ferric chloride solution was mixed. Presence of flavonoids is indicated by green colour.

3.5.4.2 Lead Acetate Test

To two ml of alcoholic solution of the extract, (0.5g extract in 10ml methanol), few drops of 10% lead acetate was mixed. Yellow precipitate indicates the presence of flavonoids.

3.5.5 Test for Presence of Glycosides

3.5.5.1 Benedict's Test

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

3.5.5.2 Sodium Hydroxide Test

Dissolved a small amount of the extract (about five mg) in one ml water and added five to six drops of sodium hydroxide solution. Yellow colour indicates the presence of glycosides.

3.5.6 Test for Presence of Phenolic Compounds

About five mg of the extract was dissolved in one ml of water and five drops of ten per cent ferric chloride solution was added to it. Development of dark brown colour occurs if phenolic compounds are present.

3.5.7 Test for Detection of Diterpenes

About five mg of the extract was mixed with three ml of copper acetate solution. Presence of diterpenes is indicated by development of green colour.

3.5.8 Test for Presence of Triterpenes

3.5.8.1 Salkowski Test

About three mg of extract was dissolved in three ml chloroform and then shaken with concentrated sulphuric acid. Lower layer turning yellow on standing indicates presence of triteripenes.

3.5.8.2 Liebermann Burchardt Test

Few drops of acetic acid and one ml concentrated sulphuric acid was added to 30ml chloroform solution of the extract (about three mg extract in three ml chloroform). Deep red ring at the junction of the two layers indicates presence of triterpenes.

3.5.9 Test for Presence of Saponins

3.5.9.1 Foam Test

A small amount of extract (about five mg) was shaken with three ml of water. If the foam produced persists for 10 minutes, presence of saponins is confirmed.

3.6 COLLECTION OF BIOLOGICAL SAMPLES

3.6.1 Blood

Blood was collected from retro orbital plexus in the inner canthus of the eye under light ether anesthesia using sodium heparinised capillary tubes (microhaematocrit capillaries). Blood was collected in fresh vials containing sodium fluoride (10mg/ml blood) and disodium salt of Ethylene Diamine Tetra Acetic acid (EDTA, 1mg/ml) as anticoagulant.

3.6.2 Serum

Blood was 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. The serum was aspirated into another vial and used for cholesterol and triglyceride estimations.

3.6.3 Liver

On day 37, animals were sacrificed using chloroform after blood collection. The liver samples were collected for glycogen estimation.

3.7 BIOCHEMICAL PARAMETERS

3.7.1 Estimation of Blood Sugar by O-toluidine Method (Hyvarien and Nikila, 1962)

3.7.1.1 Principle

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.

3.7.1.2 Preparation of Reagents

Tungstic acid reagent

Dissolved one g polyvinyl alcohol in about 100ml distilled water with gentle warming. Cooled and transferred into a one litre volumetric flask containing 11.1 g sodium tungstate previously dissolved in about 100ml distilled water and mixed by swirling. In a separate vessel, added 2.1ml concentrated sulphuric acid to about 300 ml distilled water and mixed. It was then added to the tungstate solution in the volumetric flask, mixed well and diluted to one litre with distilled water. The solution is stable for one year at room temperature.

O-toluidine reagent

O-toluidine was redistilled to get a colorless solution. Five g thiourea was added to 90ml 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.

Glucose standard (100mg/100ml)

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

3.7.1.3 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 stabilized tungstic acid reagent. Mixed well and allowed to stand for five minutes and centrifuged at 3000 rpm for 10 minutes. Half an ml of the supernatent was added to 2.5 ml of O-toluidine reagent in a test tube and mixed well. The blank was prepared by adding 0.5ml of distilled water instead of the deproteinised blood to 2.5 ml of O-toluidine reagent. The standard was set by adding 0.05ml of the glucose standard

(100mg/100ml) to 0.45ml distilled water and 2.5ml of O-toluidine reagent. Mixed well and placed all the loosely stoppered test tubes in a boiling water bath and boiled for 10 minutes. Then cooled by placing them in cold water bath. The optical density was measured at 625nm in a spectrophotometer.

3.7.1.4 Calculation

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

3.7.2 Estimation of Liver Glycogen by Anthrones Method (Narasimhan, 1971)

3.7.2.1 Principle

The liver tissue is digested with potassium hydroxide (KOH) solution and the digesta is treated with anthrone reagent[2]. The sulphuric acid medium of anthrone reagent causes dehydration of the sugar to a furfural derivative which presumably condenses with anthrone to form a blue coloured compound. The colour produced is compared with a standard in a spectrophotometer at 620nm.

3.7.2.2 Reagents

1. 30% KOH solution :- Dissolved 300g of reagent grade potassium hydroxide pellets in distilled water in a beaker, cooled and transferred quantitatively into one litre volumetric flask and diluted to one litre with distilled water.

2. 95 % suphuric acid:- mixed 950 ml of concentrated sulphuric acid with 50ml distilled water and cooled.

[2] Central Drug House (P) Ltd., Mumbai

3. 0.2% Anthrone reagent:- The reagent was prepared by dissolving 0.2g anthrone in 100ml, 95% sulphuric acid. The reagent was prepared fresh whenever required.

4. Standard glucose solution (20µg/ml) :- The stock standard was prepared by dissolving one g of highest purity anhydrous glucose in saturated benzoic acid solution and diluted to 100 ml with the same. The working standard was prepared by diluting one ml of stock standard to 500 ml with distilled water.

3.7.2.3 Procedure

Approximately 0.5 g of liver tissue was taken in a test tube containing three ml of 30% KOH solution. The tissue was digested by heating the tube for 20 minutes in a boiling water bath. The sample was then cooled and quantitatively transferred into a 50 ml volumetric flask and diluted to the mark with distilled water. After thorough mixing, five ml of the solution was pipetted into a second 50ml volumetric flask and diluted to the mark. Five ml of this was taken as the unknown sample.

Sample : Five ml of digesta prepared at the end of second dilution of 50ml.

Standard : Five ml of glucose working standard

Blank : Five ml of distilled water.

The sample, standard and blank (Five ml each in labelled test tubes) were kept in a cold water bath and added 10 ml of anthrone reagent to each of the three tubes from a fast flowing burette. Mixed the reactants by swirling the test tubes. After cooling, covered the mouth of test tubes with glass stoppers and heated for 10 minutes in boiling water bath. Then immediately cooled by placing them in cold water bath. The optical density readings were taken against the blank at 620nm in a spectrophotometer.

3.7.2.4 Calculation

$$\text{Liver glycogen (g\%)} = \frac{Ax}{As} \times \frac{100}{1.11} \times \frac{500}{5} \times \frac{100}{\text{Weight of tissue in g}} \times \frac{1}{1000000}$$

Ax = Reading of unknown
As = Reading of standard

Concentration of standard in µg = 100

Correction factor for conversion of glucose to glycogen = $\frac{1}{1.11}$

Dilution factor = $\frac{500}{5}$ = 100

Factor for expressing value in % = $\frac{100}{1000000}$

3.7.3 Estimation of Cholesterol

Cholesterol level in the serum was estimated by enzymatic CHOD-PAP method (Allain *et al.*, 1974)using Kit from Agappe Diagnostics, Maharashtra.

3.7.3.1 Principle

Two thirds of the cholesterol present in the blood is in esterified form. The esterified cholesterol is oxidized by cholesterol esterase to form cholesterol and fatty acid. Cholesterol is again oxidized in the presence of cholesterol oxidase to cholesterol 3-one and hydrogen peroxide. Phenol and 4-amino antipyrene in the reagent then combine with hydrogen peroxide by oxidative condensation in the presence of peroxidases to produce red coloured quinone.

The intensity of colour thus produced is directly proportional to cholesterol concentration.

3.7.3.2 Procedure

Blank, standard and sample were prepared as follows:

	Blank	Standard	Sample
Working reagent	1ml	1ml	1ml
Standard	-	10µl	-
Sample	-	-	10µl

Mixed and read the optical density in a spectrophotometer at 505nm, after five minutes of incubation at 37° c.

3.7.3.3 Calculation

$$\text{Cholesterol concentration (mg \%)} = \frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times 200$$

3.7.4 Estimation of triglyceride

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

3.7.4.1 Principle

Triglycerides are hydrolysed by lipase and liberated glycerol is phosphorylated with the help of glycerol kinase in presence of Adenosine Tri Phosphate to glycerol-3-phosphate. Glycerol-3-phosphate is then oxidized in presence of glycerol phosphate oxidase to dihydroxy acetone phosphate and

hydrogen peroxide. 4-Chlorophenol and 4-amino antipyrene in the reagent then combine with hydrogen peroxide by oxidiative condensation in presence of peroxidase to produce red coloured quineneimine. The intensity of colour thus produced is directly proportional to triglyceride concentration.

3.7.4.2 Procedure

Blank, standard and sample were prepared as follows.

	Blank	Standard	Sample
Reagent	1ml	1ml	1ml
Standard	-	10µl	-
Sample	-	-	10µl

Mixed and read optical density at 505nm in a spectrophotometer after five minutes of incubation at 37°C.

3.7.4.3 Calculation

$$\text{Triglyceride (mg/dl)} = \frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times 200$$

3.8 Statistical Analysis of Data

The results obtained were analyzed for statistical significance by one-way analysis of variance (ANOVA) for comparison between groups and student 't' test for within groups as described by Snedecor and Cochran (1985) using computerized software. All the values were expressed as mean $\pm$ Standard Error (SE).

Results

4. RESULTS

4.1 PHYTOCHEMICAL SCREENING

4.1.1 Steroids

As per Salkowski test, red colour was obtained and Lieberman Burchadt test gave a reddish ring at the junction for Ocimum and Tinospora extracts, but not for Azadirachta alcoholic extract. Thus it could be concluded that steroids are present in alcoholic extracts of Ocimum and Tinospora, but no detectable level of steroids could be obtained in Azadirachta extract.

4.1.2 Alkaloids

A creamy white precipitate as per Mayer's test and an yellow coloured precipitate as per Hager's test was obtained for Azadirachta, Ocimum and Tinospora extracts. Dragendroff's test yielded a reddish brown precipitate for all the three extracts. Thus the tests revealed detectable levels of alkaloids in the alcoholic extracts of Azadirachta, Ocimum and Tinospora.

4.1.3 Tannins

Brownish green colour was obtained in Ferric chloride test for Ocimum extract but not for Azadirachta and Tinospora. The results indicated the presence of tannins in Ocimum alcoholic extract.

4.1.4 Flavonoids

A green colour in the ferric chloride test and an yellow precipitate in lead acetate test indicated presence of flavonoids in Azadirachta and Ocimum extracts. Negative result was obtained for Tinospora.

4.1.5 Glycosides

As per Benedict's test, red colour was obtained indicating the presence of glycosides in all the three extracts. An yellow colour was obtained by mixing the extracts with sodium hydroxide reagent which also indicated the presence of glycosides.

4.1.6 Phenolic compounds

The extract mixed with 10 per cent ferric chloride produced dark brown colour indicating the presence of phenolic compounds for Tinospora alcoholic extracts. No development of colour was seen in the other two extracts.

4.1.7 Diterpenes

Diterpenes were detected in Ocimum and Tinospora extracts as indicated by the green colour when mixed with copper acetate solution. No green colour was obtained for Azadirachta extract.

4.1.8 Triterpenes

For Ocimum and Tinospora extracts, lower layer turned to yellow on standing as per Salkowski test, and by Lieberman Burchadt test, a deep ring appeared at the junction of the two layers. Results indicated the presence of triterpenes in Ocimum and Tinospora extracts and its absence in Azadirachta extract.

4.1.9 Saponins

In the foam test, foam persisted for 10 minutes in the case of Azadirachta and Ocimum extracts and not in case of Tinospora extract indicating the presence of saponins in Azadirachta and Ocimum only.

The results obtained in the above phytochemical study are summarised in the Table1.

Table 1. Results of the phytochemical screening of Azadirachta, Ocimum and Tinospora extracts

Sl. No.	Active principles	Azadirachta	Ocimum	Tinospora
1.	Steroids	Not detected	Present	Present
2.	Alkaloids	Present	Present	Present
3.	Tannins	Not detected	Present	Not detected
4.	Flavonoids	Present	Present	Not detected
5.	Glycosides	Present	Present	Present
6.	Phenolic compounds	Not detected	Not detected	Present
7.	Diterpenes	Not detected	Present	Present
8.	Triterpenes	Not detected	Present	Present
9.	Saponins	Present	Present	Not detected

4.2 Evaluation of Hypoglycemic Activity

4.2.1.1 Body Weight

The individual and mean body weight of the rats (Group I, II, III, IV, V and VI) recorded on zero, 7th, 15th, 21st, 30th and 37th day of the experiment are presented in Tables 2, 3, 4, 5 ,6 and 7 respectively. The values on zero day represented the body weight before the commencement of the experiment (before injecting alloxan) and on 7th day represents body weight after one week of giving alloxan. The blood glucose level of the rats will be stabilized only after seven days of alloxan administration and hence the treatment began only after that. The body weight recorded on zero day of Group I, II, III, IV, V and VI were 205.00 ± 8.86, 207.50 ± 7.01, 205.00 ± 8.24, 202.50 ± 7.01, 217.50 ± 7.26 and 212.50 ± 5.90 g respectively. On seventh day after giving alloxan body weights recorded for Group I, II, III, IV, V and VI were 167.50 ± 12.06, 176.25 ± 6.23, 182.50 ± 7.50, 182.50 ± 8.81, 195.00 ± 8.02 and 190.00 ± 5.01 g respectively.

All the treatment groups (I toV) showed gradual increase in body weight till 37th day, except the control group VI. Gradual decrease in body weight were recorded for control group VI as represented in Table 7. Body weight recorded on day 37 for Group I, II, III, IV and V were 178.75 ± 10.43, 188.75 ± 5.49, 195.00 ± 8.02, 197.50 ± 7.96 and 212.50 ± 6.48 g respectively. A reduction in body weight was shown by Group VI and the weight obtained on day 37 was 172.50 ± 4.53 g.

4.2.2 Biochemical Parameters

4.2.2.1 Blood Glucose Level

Blood glucose level was estimated before giving alloxan (zero day), on 7th, 15th, 21st, 30th and 37th day after giving alloxan. The individual and mean values are represented in Tables 8, 9, 10, 11, 12 and 13 respectively and in

Table 2. Effect of neem leaf extract on body weight (g) of diabetic rats (n=8)

Animal No.	Body weight in g					
	Zero day	7th day	15th day	21st day	30th day	37th day
1	220	180	180	180	190	190
2	240	210	200	210	210	210
3	220	200	210	210	210	210
4	230	200	200	210	210	210
5	180	140	140	150	150	150
6	180	140	150	150	160	160
7	180	120	130	140	140	140
8	190	150	160	160	160	160
Mean ± SE	205 ± 8.86	167.50 ± 12.06	171.25 ± 10.76	176.25 ± 10.68	178.75 ± 10.43	178.75 ± 10.43

Table 3. Effect of tulsi leaf extract on body weight (g) of diabetic rats (n=8)

Animal No.	Body weight in g					
	Zero day	7th day	15th day	21st day	30th day	37th day
1	230	160	160	160	170	170
2	240	210	210	210	210	220
3	210	180	180	190	190	190
4	210	180	190	190	190	190
5	180	170	180	180	190	190
6	200	180	180	180	180	190
7	200	180	180	180	180	190
8	190	150	160	160	160	170
Mean±SE	207.50 ± 7.01	176.25 ± 6.23	180.00 ± 5.67	181.25 ± 5.81	183.75 ± 5.32	188.75 ± 5.49

Table 4. Effect of Tinospora extract on body weight (g) of diabetic rats (n=8)

Animal No.	Body weight in g					
	Zero day	7th day	15th day	21st day	30th day	37th day
1	210	170	170	190	190	190
2	210	210	210	230	220	230
3	230	210	210	220	230	230
4	240	180	180	190	190	190
5	180	160	160	170	170	170
6	210	200	180	190	190	190
7	180	160	160	180	180	180
8	180	170	180	180	190	180
Mean ± SE	205.00 ± 8.24	182.50 ± 7.50	181.25 ± 6.93	193.75 ± 7.30	195.00 ± 7.07	195.00 ± 8.02

Table 5. Effect of combination of neem, tulsi and Tinospora extracts on body weight (g) of diabetic rats (n=8)

Animal No.	Body weight in g					
	Zero day	7th day	15th day	21st day	30th day	37th day
1	210	150	150	170	170	170
2	210	210	210	200	220	220
3	230	200	200	210	210	220
4	180	160	170	170	180	170
5	220	210	210	220	220	220
6	210	200	200	210	200	210
7	180	160	160	180	180	180
8	180	170	180	190	190	190
Mean ± SE	202.50 ± 7.01	182.5 ± 8.81	185.00 ± 8.24	193.75 ± 6.80	196.25 ± 6.80	197.50 ± 7.96

Table 6. Effect of glibenclamide on body weight (g) of diabetic rats (n=8)

Animal No.	Body weight in g					
	Zero day	7th day	15th day	21st day	30th day	37th day
1	240	220	230	230	230	240
2	200	170	180	180	190	190
3	240	220	210	220	220	220
4	220	180	200	220	220	220
5	210	200	200	210	210	210
6	200	180	180	200	200	200
7	190	170	180	180	180	190
8	240	220	220	230	230	230
Mean ± SE	217.50 ± 7.26	195.00 ± 8.02	200.00 ± 6.81	208.75 ± 7.18	210.00 ± 6.55	212.50 ± 6.48

Table 7. Body weight (g) of diabetic rats (control) (n=8)

Animal No.	Body weight in g					
	Zero day	7th day	15th day	21st day	30th day	37th day
1	230	200	170	180	180	170
2	220	180	180	170	170	170
3	220	180	180	170	170	170
4	210	200	180	180	180	170
5	210	200	180	180	180	180
6	200	180	170	160	160	160
7	180	170	170	170	170	160
8	230	210	200	210	200	200
Mean ± SE	212.50 ± 5.90	190.00 ± 5.01	178.75 ± 3.50	177.50 ± 5.26	176.25 ± 4.20	172.50 ± 4.53

Figures 4, 5, 6, 7 and 8 respectively. Table 14 shows the mean blood glucose level of the six treatment groups.

The normal blood glucose levels of rats before giving alloxan showed no significant variation in all groups. The values obtained were 93.87 ± 4.29, 81.52 ± 3.24, 88.98 ± 4.12, 89.34 ± 4.91, 86.70 ± 4.13 and 87.53 ± 4.84 mg/100 ml for Group I, II, III, IV, V and VI respectively. On 7^{th} day, the mean blood sugar level did not differ significantly between the groups. The mean blood sugar values recorded were 285.13 ± 22.29, 287.13 ± 20.35, 292.75± 17.08, 281.00 ± 20.80, 272.13 ± 15.55 and 271.88 ± 16.45 mg/100 ml respectively for Group I, II, III, IV, V and VI.

Group I fed with Azadirachta leaf extract at 200mg/kg body weight showed significant ($P<0.05$) reduction in blood sugar level on 15^{th} day compared to Group II and III. The blood sugar level obtained was 233.88 ± 20.25 mg/100 ml for Group I which was significantly lower when compared to that of Group II and III which were 250.38 ± 18.57 and 254.00 ± 10.46 mg/100ml respectively. Group IV treated with combination had a significantly ($P<0.05$) higher reduction in blood glucose level (201.88 ± 12.50 mg/100 ml) compared to Groups I, II and III. This was comparable to that obtained for Group V (glibenclamide at 0.5 mg/animal/day) which had a mean blood glucose level of 195.75 ± 23.65 mg/100ml on day 15. The diabetic control showed a slight reduction in blood glucose level (252.75 ± 9.78 mg/100 ml) which was not significant.

On day 21, Group I showed significantly ($P<0.01$) higher reduction in blood glucose level (200.25 ± 10.25 mg/100 ml) compared to Group II and III for which values obtained were 225.50 ± 23.91 and 229.25 ± 11.05 mg/100 ml respectively. Group IV which received a combination of the three plants had a much higher reduction in blood glucose level and the value was 188.13 ± 13.37 mg/100 ml. Glibenclamide treated group (Group V) had a blood glucose level of 140.13 ± 10.97 mg/100 ml which was lower than that obtained for Group IV. A

slight reduction in blood glucose level was seen in Group VI and the level obtained was 242.38 ± 8.84 mg/100 ml.

The mean blood glucose level obtained for Group I on 30th day was 167.63 ± 10.61 mg/100 ml which was significantly (P<0.01) lower when compared to that of Group II and III where mean blood glucose level were 187.00 ± 14.44 and 185.38 ± 15.74 mg/100ml respectively. Group IV showed a mean blood glucose level of 148.13 ± 8.75 mg/100 ml which was significantly lower than that of Group I, II and III. Group V which received glibenclamide had a mean blood sugar level of 128.75 ± 9.30 mg/100 ml. The diabetic control had a blood sugar level of 215.00 ± 8.94 mg/100 ml.

At the end of the experiment, on 37th day, Group I showed a significantly (P<0.01) lower blood sugar level of 123.88 ± 2.61 mg/100 ml than Group II and III. Group II and III had no significant difference in blood glucose level, the values obtained being 137.25 ± 4.60 and 144.25 ± 3.28 mg/100ml respectively. Group IV showed comparatively higher reduction in blood glucose level (113.38 ± 6.33 mg/100 ml). The glibenclamide treated group had a mean blood glucose value of 88.88 ± 3.44 mg/100 ml. The diabetic control also showed a slight reduction in blood glucose level and the value obtained was 188.00 ± 6.09 mg/100 ml.

4.2.2.2 Serum Cholesterol Level

Serum cholesterol level estimated on 37th day of the experiment is presented in Table 15 and in Figure 9. There was significant (P<0.01) reduction in serum cholesterol level of Group I, II, III and IV compared to Group VI. Diabetic control (Group VI) had a higher cholesterol level of 143.88 ± 3.92 mg/100ml. The lowest serum cholesterol level among Group I, II and III was seen in Group I and the value obtained was 70.00 ± 5.01 mg/100ml. Group II and III had serum cholesterol level of 102.25 ± 10.51 mg/100ml and 80.50 ± 5.37mg/100ml. Group IV showed a significantly (P<0.01) lower serum

Table 8. Effect of neem leaf extract on blood glucose level (mg/100 ml) of diabetic rats (n=8)

Animal No.	Blood glucose level (mg/100ml)					
	Zero day	7th day	15th day	21st day	30th day	37th day
1	108.37	285	280	240	210	137
2	81.35	327	151	140	120	120
3	107.83	213	210	203	200	122
4	90.76	384	344	189	145	133
5	107.80	348	241	210	151	119
6	88.70	265	225	220	168	118
7	84.78	213	210	195	190	125
8	81.36	246	210	205	157	117
Mean ± SE	93.87 ± 4.29[a]	285.13 ± 22.29[a]	233.88 ± 20.25[ab]	200.25 ± 10.25[bc]	167.63 ± 10.61[bc]	123.88 ± 2.61[c]

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

Table 9. Effect of tulsi leaf extract on blood glucose level (mg/100 ml) of diabetic rats (n=8)

Animal No.	Blood glucose level (mg/100ml)					
	Zero day	7th day	15th day	21st day	30th day	37th day
1	76.67	317	298	232	211	158
2	71.08	215	187	148	125	124
3	72.61	375	271	311	194	143
4	75.77	286	269	218	199	131
5	93.48	223	172	125	124	120
6	95.22	351	323	313	222	138
7	84.78	275	260	249	230	151
8	82.51	255	223	208	191	133
Mean ± SE	81.52 ± 3.24[b]	287.13 ± 20.35[a]	250.38 ± 18.57[a]	225.50 ± 23.91[abc]	187.00 ± 14.44[ab]	137.25 ± 4.60[b]

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

Table 10. Effect of Tinospora extract on blood glucose level (mg/100 ml) of diabetic rats (n=8)

Animal No.	Blood glucose level (mg/100ml)					
	Zero day	7th day	15th day	21st day	30th day	37th day
1	72.36	287	283	229	235	158
2	81.56	313	227	213	180	139
3	76.71	216	214	214	151	131
4	85.96	374	281	283	220	142
5	101.30	278	221	191	140	137
6	102.16	334	281	270	221	156
7	100.43	255	251	213	157	148
8	91.35	285	274	221	179	143
Mean ± SE	88.98 ± 4.12 [b]	292.75 ± 17.08 [a]	254.00 ± 10.46 [a]	229.25 ± 11.05 [ab]	185.38 ± 15.74 [ab]	144.25 ± 3.28 [b]

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

Table 11. Effect of combination of neem, tulsi and Tinospora extracts on blood glucose level (mg/100 ml) of diabetic rats (n=8)

Animal No.	Blood glucose level (mg/100ml)					
	Zero day	7th day	15th day	21st day	30th day	37th day
1	90.43	223	126	106	100	88
2	89.71	368	202	198	156	136
3	65.79	291	228	226	129	119
4	111.30	295	214	196	165	131
5	85.31	215	191	183	162	128
6	102.31	361	206	192	180	110
7	78.36	250	247	227	151	95
8	91.51	245	201	177	142	100
Mean ± SE	89.34 ± 4.91[b]	281.00 ± 20.80 [a]	201.88 ± 12.50[b]	188.13 ± 13.37[c]	148.13 ± 8.75[cd]	113.38 ± 6.33[c]

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

Table 12. Effect of glibenclamide on blood glucose level (mg/100 ml) of diabetic rats (n=8)

Animal No.	Blood glucose level (mg/100ml)					
	Zero day	7th day	15th day	21st day	30th day	37th day
1	103.26	342	261	101	99	79
2	86.09	310	265	151	140	103
3	82.17	230	111	111	108	78
4	98.51	243	203	185	141	85
5	80.95	275	195	168	172	101
6	85.90	230	111	111	101	93
7	75.58	236	149	131	118	81
8	81.12	311	271	163	151	91
Mean ± SE	86.70 ± 4.13 [b]	272.13 ± 15.55 [a]	195.75 ± 23.65 [b]	140.13 ± 10.97 [d]	128.75 ± 9.30 [d]	88.88 ± 3.44 [d]

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

Table 13. Blood glucose level (mg/100 ml) of diabetic rats (n=8)

Animal No.	Blood glucose level (mg/100ml)					
	Zero day	7th day	15th day	21st day	30th day	37th day
1	66.96	272	267	248	209	168
2	82.17	328	264	251	189	164
3	107.49	296	269	260	211	173
4	88.16	215	210	201	198	198
5	98.15	270	262	258	233	201
6	100.43	212	214	210	201	192
7	75.58	247	247	238	210	198
8	81.26	235	289	273	269	210
Mean ± SE	87.53 ± 4.84 [b]	271.88 ± 16.45 [a]	252.75 ± 9.78 [a]	242.38 ± 8.84 [a]	215.00 ± 8.94 [a]	188.00 ± 6.09 [a]

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

Table 14. Mean blood glucose level (mg/100ml) of diabetic rats in the different treatment groups

	Blood glucose level in mg/100 ml					
	Zero day	7th day	15th day	21st day	30th day	37th day
Group I	93.87 ± 4.29[a]	285.13 ± 22.29[a]	233.88 ±20.25[ab]	200.25 ±10.25[bc]	167.63 ±10.61[bc]	123.88 ± 2.61[c]
Group II	81.52 ± 3.24[b]	287.13 ±20.35[a]	250.38 ±18.57[a]	225.50± 23.91[abc]	187.00± 14.44[ab]	137.25 ± 4.60[b]
Group III	88.98 ± 4.12[b]	292.75± 17.08[a]	254.00± 10.46[a]	229.25± 11.05[ab]	185.38± 15.74[ab]	144.25 ± 3.28[b]
Group IV	89.34 ± 4.91[b]	281.00± 20.80[a]	201.88 ± 12.50[b]	188.13 ± 13.37[c]	148.13 ± 8.75[cd]	113.38 ± 6.33[c]
Group V	86.70 ± 4.13[b]	272.13± 15.55[a]	195.75 ± 23.65[b]	140.13± 10.97[d]	128.75 ± 9.30[d]	88.88 ± 3.44[d]
Group VI (Control)	87.53 ± 4.84[b]	271.88± 16.45[a]	252.75 ± 9.78[a]	242.38 ± 8.84[a]	215.00 ± 8.94[a]	188.00 ± 6.09[a]

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

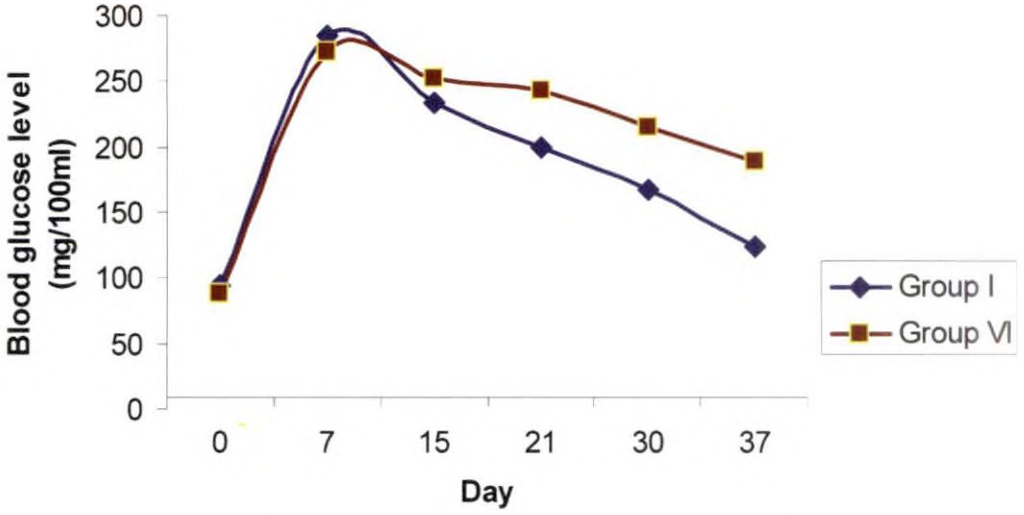

Fig. 4 Effect of alcoholic extract of Azadirachta on blood glucose level in diabetic rats

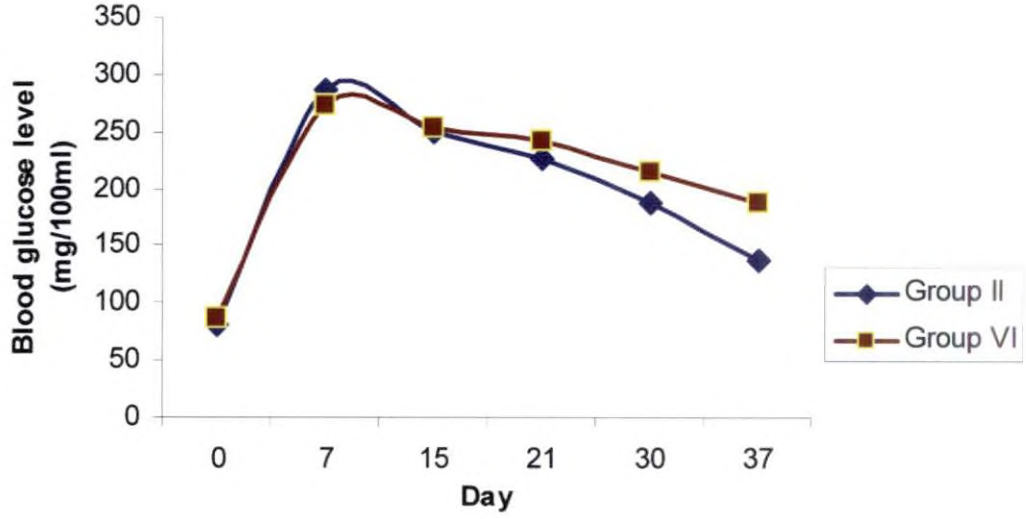

Fig. 5 Effect of alcoholic extract of Ocimum on blood glucose level in diabetic rats

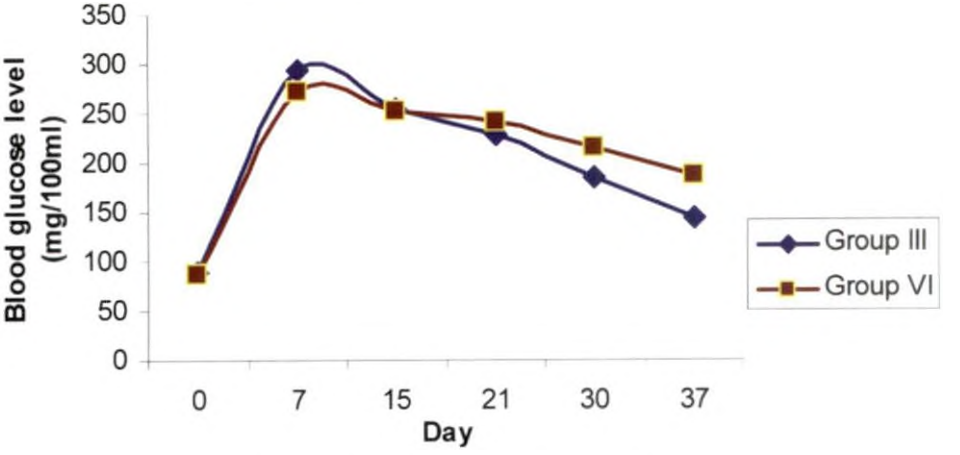

Fig.6 Effect of alcoholic extract of Tinospora on blood glucose level

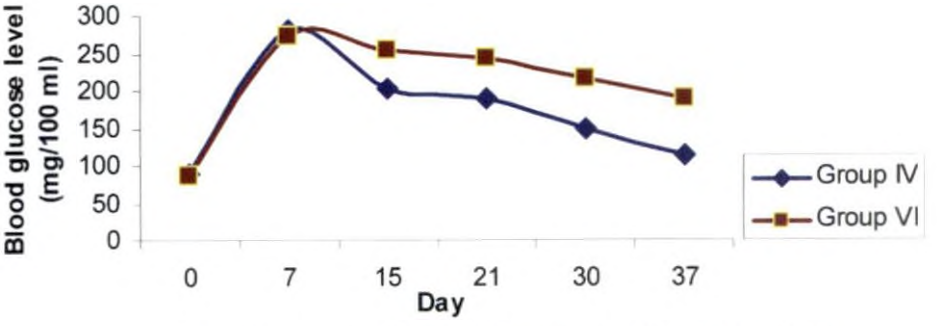

Fig.7 Effect of combination of Azadirachta, Ocimum and Tinospora alcoholic extracts on blood glucose level

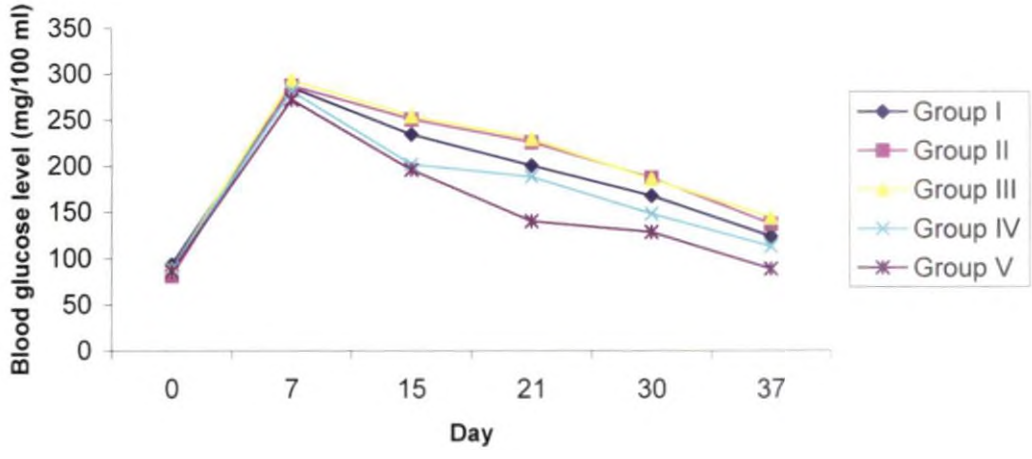

Fig. 8: Comparison of the hypoglycemic effects of Azadirachta, Ocimum, Tinospora and their combination with that of glibenclamide

cholesterol level of 66.88 ± 4.31 mg/100ml compared to Group I, II and III. Group V which received glibenclamide had the lowest cholesterol level of 64.25 ± 6.13 mg/100ml.

4.2.2.3 Serum Triglyceride Level

The effect of treatments on serum triglyceride level estimated on 37th day of the experiment is shown in Table 16 and in Figure 10. All the groups showed significant (P<0.01) reduction in serum triglyceride level when compared to control group VI. Group I showed a significant (P<0.01) reduction in serum triglyceride level (105.38 ± 8.24 mg/100ml) when compared to Group II and III for which values obtained were 114.50 ± 6.14 mg/100ml and 124.38 ± 6.72 mg/100ml respectively. Among all the groups treated with unknown drugs, Group IV (which received combination) showed the highest reduction in serum triglyceride level i.e., 84.50 ± 8.29 mg/100ml. Group V had a serum triglyceride value of 69.13 ± 6.02 mg/100ml. Group VI remained hypertriglyceremic with a serum triglyceride value of 155.25 ± 8.34 mg/100ml.

4.2.2.4 Liver Glycogen Level

Liver glycogen level estimated on day 37 of the treatment are presented in Table 17 and in Figure 11. Liver glycogen level of the three treatment groups I, II and III did not differ significantly from that of control Group VI. The values obtained were 1.90 ± 0.03, 1.88 ± 0.04 and 1.82 ± 0.04 g% respectively. The treatment group IV had a liver glycogen level of 2.07 ± 0.03 g%. Group V had a significantly (P<0.01) higher level of liver glycogen (2.27 ± 0.05 g%). The diabetic control had a liver glycogen level of 1.83 ± 0.02 g%.

Table 15. Effect of treatments on serum cholesterol level, mg/100ml (n=8)

Animal No.	Serum cholesterol level,mg/100ml					
	Group I	Group II	Group III	Group IV	Group V	Group VI
1	89	116	66	81	50	161
2	48	134	100	79	68	152
3	58	124	66	69	45	148
4	62	122	100	56	59	150
5	68	62	64	53	57	142
6	85	68	75	51	61	139
7	81	121	81	78	101	131
8	69	71	92	68	73	128
Mean ± SE	70.00 ± 5.01^{c}	102.25 ± 10.51^{b}	80.50 ± 5.37^{c}	66.88 ± 4.31^{c}	64.25 ± 6.13^{c}	143.88 ± 3.92^{a}

Means bearing the same superscript do not differ significantly at $p<0.01$

Table 16. Effect of treatments on serum triglyceride level, mg/100ml (n=8)

Animal No.	Serum triglyceride level,mg/100ml					
	Group I	Group II	Group III	Group IV	Group V	Group VI
1	129	136	142	94	96	171
2	113	141	121	98	89	132
3	115	121	92	119	53	141
4	119	98	113	92	49	128
5	67	91	132	96	78	162
6	71	108	112	57	68	167
7	108	112	131	71	61	198
8	121	109	152	49	59	143
Mean ± SE	105.38 ± 8.24^{bc}	114.50 ± 6.14^{b}	124.38 ± 6.72^{b}	84.50 ± 8.29^{cd}	69.13 ± 6.02^{d}	155.25 ± 8.34^{a}

Means bearing the same superscript do not differ significantly at $p<0.01$

Table 17. Effect of treatments on liver glycogen level (g%)(n=8)

Animal No.	Liver glycogen level,g%					
	Group I	Group II	Group III	Group IV	Group V	Group VI
1	1.81	1.77	1.86	1.98	2.08	1.79
2	2.06	1.99	1.75	2.11	1.98	1.89
3	1.90	1.66	1.81	2.05	2.31	1.83
4	1.80	1.93	1.69	1.95	2.28	1.78
5	1.91	1.99	1.90	2.12	2.19	1.81
6	1.89	1.86	1.79	2.20	2.09	1.90
7	2.03	1.99	2.01	1.99	2.91	1.85
8	1.85	1.89	1.73	2.16	2.25	1.83
Mean ± SE	1.90 ± 0.03^{b}	1.88 ± 0.04^{b}	1.82 ± 0.04^{b}	2.07 ± 0.03^{b}	2.27 ± 0.05^{a}	1.83 ± 0.02^{c}

Means bearing the same superscript do not differ significantly at $p<0.01$

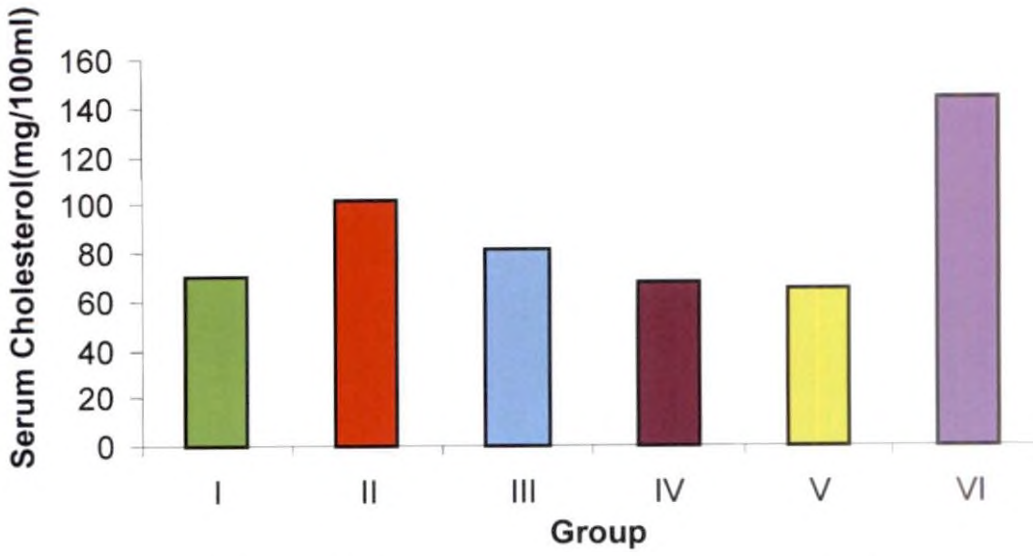

Fig. 9 Effect of treatments on Serum Cholesterol level (mg/100ml)

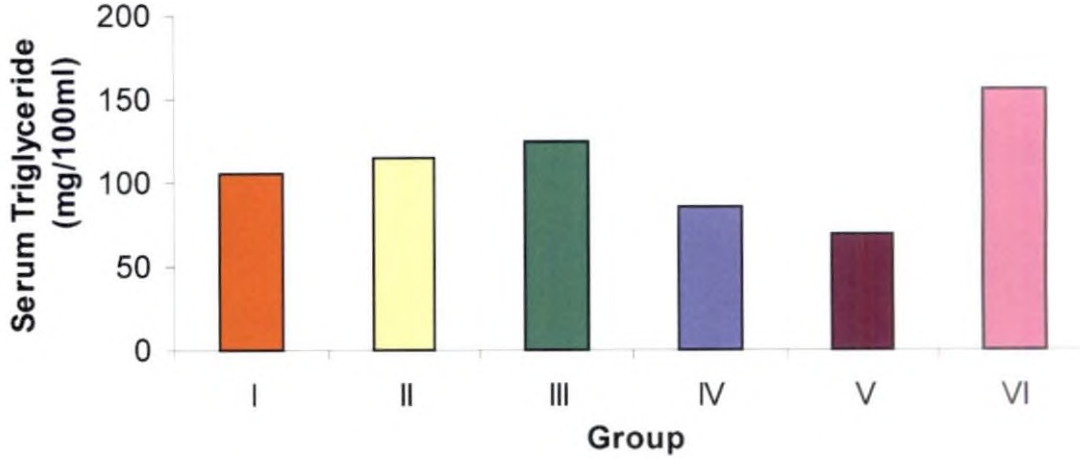

Fig.10 Effect of treatments on Serum Triglyceride level (mg/100ml)

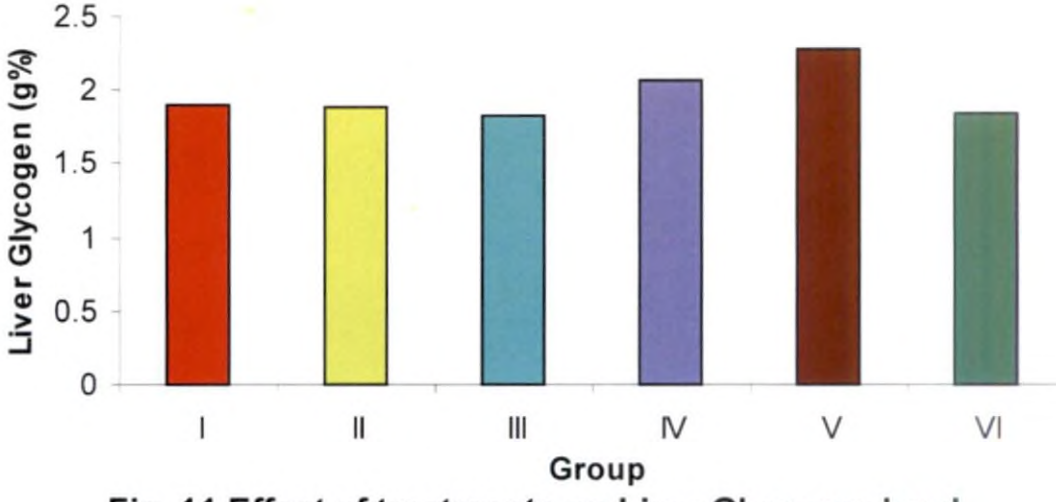

Fig. 11 Effect of treatments on Liver Glycogen level (g%)

Discussion

5. DISCUSSION

Diabetes mellitus is one of the metabolic disease affecting people all over the world. The disease is often associated with high frequency of mortality rates besides heart ailments, blindness and gangrene. Despite concerted efforts of medical scientists over the years, the disease still eludes satisfactory cure in modern medicine (Khan and Singh, 1996). Most of the hypoglycemic agents used in allopathic medicine are reported to have side effects in the long run. Hence there is a need to search for effective and safe alternative drugs for diabetes, herbal medicine is one such alternative. A great deal of attention has been recently given to the therapeutic use of herbal remedies for safety, efficacy and economy.

The present study was directed to evaluate the effectiveness of three medicinal plants namely *Azadirachta indica, Ocimum sanctum* and *Tinospora cordifolia* commonly used in the Ayurvedic and folklore medicine for the treatment of diabetes. In the study, the hypoglycemic effect of the alcoholic extracts of these three plants and their combination were assessed and compared among them and also with the standard oral hypoglycemic agent, glibenclamide.

5.1 PHYTOCHEMICAL SCREENING

Phytochemical screening of alcoholic extract of *Azadirachta indica* for active principles revealed the presence of alkaloids, flavonoids, glycosides and saponins, but no detectable levels of steroids, tannins, phenolic compounds, diterpenes and triterpenes were noticed. Chromatographic separation of *Azadirachta indica* leaf extract by Chattopadhyay *et al.* (1992) also revealed the presence of six flavonol-0-glycosides responsible for the blood sugar lowering activity in diabetic rats. Vallachira (1998) reported that the dried bark and leaves of *Melia azadirachta* contained a bitter amorphous resin, an alkaloid margosine and margosic acid. The present study also revealed the presence of alkaloids.

Screening for active principles of *Ocimum sanctum* alcoholic extract gave positive tests for alkaloids, flavonoids, tannins, steroids, diterpenes, triterpenes and saponins. However, no detectable levels of phenolic compounds were found. Jaggi *et al.* (2003) also reported the presence of saponins, sterols, triterpenoids, carbohydrates, tannins and proteins in the stem callus, stem and leaves of *Ocimum sanctum,* while flavonoids were detected in stem and leaves only. Kelm *et al.* (2000) observed the presence of cirsilineol, cirsimarin, isothymosin, apigenin, rosamarinic acid and eugenol in Ocimum leaves. Numerous other compounds viz., acids like linoleic acid, ursolic acid, gallic acid, vanillic acid, caffeic acid and flavonoids like luteolin, orientin and vicenin were also reported.

Phytochemical screening of *Tinospora cordifolia* extract revealed the presence of alkaloids, glycosides, steroids, diterpenes, triterpenes and phenolic compounds. Negative tests were seen for tannins, flavonoids and phenolic compounds. The present study is in agreement with the findings of Singh *et al.* (2003) who observed the presence of alkaloids as berberine, palmatine, tembetarine, magnoflorine and glycosides as 18-norclerodane glucoside, furanoid diterpene, glucoside, tinocordiside, tinocordifolioside, cordioside, cordiofolioside-A, cordiofolioside-B etc. in *Tinospora cordifolia* stem. Presence of diterpenoid lactones, steroids, sesquiterpenoid and various aliphatic compounds were also observed.

5.2 PHYSIOLOGICAL PARAMETERS

5.2.1 Body Weight

All the treated groups (Groups I, II, III, IV and V) showed gradual increase in body weight. Diabetic control (Group VI) did not show any significant gain in weight during the 30day experimental period. Conversely, the body weight was reduced by17.5g. Though the body weight of Group I, II, 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 *Azadirachta indica* leaf extract (Group I) showed a mean weight gain of 11.25g in 30 days time. This is in agreement with the observations made by Mahidi *et al.* (2003). They reported an increase in body weight of 4.5 ± 1.05 g in streptozotocin induced diabetic rats when treated with *Azadirachta indica* aqueous extract at 500 mg/kg once daily for 30 days.

A mean weight gain of 12.50g was observed in *Ocimum sanctum* treated group (Group II) in the present study. Similar results were obtained by Vats *et al.* (2004). According to them, *Ocimum sanctum* (200 mg/kg) treated rats showed a significant gain in body weight in comparison to the diabetic controls but was lower than the normal controls.

Oral administration of an aqueous extract of *Tinospora cordifolia* roots caused an increase in body weight of alloxan-induced diabetic rats as observed by Stanely *et al.* (2000). The present study also revealed a mean body weight gain of 12.50g in group III treated with *Tinospora cordifolia* stem extract.

5.3 BIOCHEMICAL PARAMETERS

5.3.1 Blood Glucose Level

Initial mean blood glucose level prior to alloxan administration were 93.87±4.29, 81.52±3.24, 88.98±4.12, 89.34±4.91, 86.70±4.13 and 87.53±4.84 for GroupI, II, III, IV, V and VI respectively. After seven days of alloxan administration, the blood glucose level significantly increased to 285.13±22.29, 287.13±20.35, 292.75±17.08, 281.00±20.8, 272.13±15.55 and 271.88±16.45 mg/100ml respectively for Group I, II, III, IV, V and VI. Administration of alloxan led to about three fold elevation of blood glucose level. The blood glucose level of diabetic controls were maintained at higher levels throughout the period of study, even though there was a gradual reduction in blood glucose level indicating the tendancy of the body for natural recovery from alloxan induced

diabetes. Among Groups I, II and III, reduction in blood glucose level was significantly higher for Group I which received alcoholic extract of *Azadirachta indica* at 200mg/kg. Pillai and Santhakumari (1981) also reported hypoglycemic action of a decoction of the tender leaves of *Melia azadirachta*, the oil from its seeds at 2.5 ml/animal and nimbidin, a bitter active principle isolated from the oil at 200 mg/kg body weight in fasting rabbits. Chattopadhyay *et al.* (1992) reported the isolation of six flavonol-0- glycosides from leaf extract of Azadirachta which are known to be responsible for the blood sugar lowering action in streptozotocin diabetic rats.

Neem seed kernel powder (500mg/kg) also significantly decreased the concentration of blood glucose level 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. Bajaj and Srinivasan (1999) found that treatment with aqueous neem leaf extract at one g/kg body weight resulted in a fall in blood glucose level by 45.4 per cent in streptozotocin induced diabetic rats. The extract caused a reduction in blood sugar level probably by increasing the uptake of glucose peripherally.

Hypoglycemic effect of *Azadirachta indica* was also observed in rabbits by Khosla *et al.* (2000) when leaf extract (500 mg/kg) and seed oil (five ml/kg, p.o) were given to normal as well as alloxan diabetic rabbits.

In a comparative evaluation of the blood sugar lowering activity of four medical plants, *Azadirachta indica, Ocimum sanctum, Gymnema sylvestre* and *Catharanthus roseus* by Chattopadhyay (1999), Azadirachta leaf extract was found to have the most potent blood sugar lowering activity followed by *Catharanthus roseus, Gymnema sylvestre* and *Ocimum sanctum*. In the present study also among the three plants (Azadirachta, Ocimum and Tinospora) Azadirachta showed the highest blood sugar lowering effect.

In the present study, administration of *Ocimum sanctum* alcoholic extract lowered the blood glucose level from 287.13±20.35 to 137.25±4.60 mg/100ml during a period of 30 days. The hypoglycemic effect of alcoholic extract of *Ocimum sanctum* leaves at 50, 100, 200 and 400 mg/kg was also reported by Chattopadhyay (1993). He found that the extract reduced the blood glucose level in normal, glucose fed hyperglycemic and diabetic rats. Maximum glucose suppression occurred after two hours of treatment by the leaf extract at the dose of 200 mg/kg. According to Vats *et al.* (2004) also administration of *Ocimum sanctum* at 200 mg/kg for 30 days led to a significant decrease in plasma blood glucose levels. They suggested that the antihyperglycemic effect of Ocimum is at least partially dependent upon insulin release from the pancreas as the extract showed a greater hypoglycemic effect in milder form of diabetes and a lower response in moderate forms.

In the present study, *Tinospora cordifolia* extract given at 200mg/kg reduced the blood glucose level from 292.75±17.08 to 144.25±3.28. The effect was comparable to that of Group11 (Ocimum at 200mg/kg) but lower than that of GroupI (Azadirachta at 200mg/kg). Gupta *et al.* (1967) also found that oral administration of the aqueous and alcoholic extracts of dried powdered stem of *Tinospora cordifolia* at different dose rates (100,200,400 mg/kg p.o) caused reduction in fasting blood sugar level in rabbits and rats. They noticed that the hypoglycemic effect of 100 and 200 mg/kg doses was not proportionate to the dosage indicating some indirect influence of the drug on carbohydrate metabolism. The aqueous, alcoholic and chloroform extracts of Tinospora leaves also showed significant hypoglycemic effect at doses of 50, 100, 150 and 200 mg/kg body weight in normal and alloxan diabetic rabbits (Wadood *et al.*, 1992). The extract was found to have a direct insulin like action and also indirectly stimulated the release of insulin from pancreatic β-cells.

Yu Pan *et al.* (2003) reported that berberine, an alkaloid found in Tinospora stem acted as an α-glucosidase inhibitor similar to acarbose and

miglitol, thus delaying absorption of complex carbohydrates in small intestine resulting in a decrease in post prandial glucose content and a reduction in long term diabetic complications.

5.3.2 Serum Cholesterol Level

The abnormally high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots. Insulin inhibits the hormone sensitive lipase. On the other hand, glucagons, catecholamines and other hormones enhance lipolysis. The marked hyperlipidemia that characterize the diabetic state may therefore be regarded as a consequence of uninhibited actions of lipolytic hormones on the fat depots (Al-Shamony *et al.*, 1994). In alloxan diabetes also, higher levels of cholesterol, phospholipids and free fatty acids in liver, kidney and heart were observed.

A decrease in serum cholesterol level from 143.88±3.92mg/100ml of control to 70.00±5.01mg/100ml was observed in Group I given *Azadirachta indica* alcoholic extract at 200mg/kg. The effect was significantly higher than that of Group II (Ocimum at 200mg/kg). Bopanna *et al.* (1997) also got similar results when they fed neem seed kernel powder at 500 mg/kg. They reported a marked fall of serum cholesterol, LDL-cholesterol and VLDL-cholesterol and more effect was observed on the LDL-cholesterol. Possible mechanism suggested was an increase in the liver LDL receptor activity. Anti hyperlipidemic effect may be due to the down regulation of NADPH and NADH, acting as cofactors in fat metabolism. Another mechanism of action may be decreased synthesis or increased excretion of lipids through the intestinal tract.

In the present study, *Ocimum sanctum* produced a significant lowering of serum cholesterol level and the value obtained was 102.25±10.51mg/100ml. Hypolipidemic effect of Ocimum sanctum leaves was also reported by Sarkar *et al.* (1994). They administered fresh leaves of Ocimum mixed along with feed (one and two per cent) for four weeks which brought about significant changes in

the lipid profile of normal albino rabbits. The treatment resulted in significant lowering in serum total cholesterol. The LDL-cholesterol was progressively decreased and HDL-cholesterol and faecal sterol excretion were found to be progressively increased during the four week experimental period.

The hypolipidemic effect of rats in Group III which received *Tinospora cordifolia* had a lower serum cholesterol level of 80.50±5.37mg/100ml compared to that of Group II which received Ocimum (102.25+10.51mg/100ml). The value obtained for Group III was not significantly different from that of Group I which received Azadirachta leaf extract. Hypolipidemic effect of aqueous extract of *Tinospora cordifolia* roots at 2.5 and five g /kg body weight was also reported by Prince *et al.* (1999) when they administered the extract for six weeks to alloxan diabetic rats. However, the hypolipidemic effect of group I, II, III and IV were lower than that of group V which received glibenclamide.

5.3.3 Serum Triglyceride Level

Hypertriglyceridemia is often associated with diabetes, it is one of the risk factors in coronary heart diseases. In the study, all the treatment groups except the control group showed a decrease in serum triglyceride level. Group I (*Azadirachta indica*) had a significantly higher reduction in triglyceride level compared to Groups II and III. Bopanna *et al.* (1997) observed the same effect when they administered neem seed kernel powder (500 mg/kg, p.o) in alloxan diabetic rabbits. Possible mechanism may be due to an increase in the liver LDL-receptor activity and decreased triglyceride synthesise.

Group II which received *Ocimum sanctum* had a lower serum triglyceride level of 114.50±6.14mg/100ml compared to that of control (155.25±8.34 mg/100ml) indicating its hypotriglyceridemic effect. Significant lowering of serum triglyceride level was also observed by Sarkar *et al.* (1994) by the administration of fresh leaves of *Ocimum sanctum* for four weeks at a concentration of one and two per cent in feed. Serum triglyceride level lowered

from 138.89 ± 10.95 mg/dl to 99.28 ± 10.58 mg/dl in the four week period. Hypolipidemic effect could be associated with the essential oil, eugenol present in Ocimum leaf powder.

In *Tinospora cordifolia* treated group (Group III) also, a significant lowering of serum triglyceride level was observed (124.38±6.72mg/100ml). The effect was however lower than that of group I. Group IV given combination had a comparatively more hypotriglyceridemic effect than Group I, II and III. The value obtained was 84.50±8.29mg/100ml. However, it was seen that the serum triglyceride level of group I, II, III and IV were higher than that of group V which received glibenclamide.

5.3.4 Liver Glycogen Level

Glycogen is the primary intracellular storage form of glucose and its level in various tissues are the direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase (Vats *et al.*, 2004).

Alloxan causes selective destruction of β-cells of islets of Langerhans resulting in marked decrease in insulin levels and a depletion of the glycogen content in tissues (liver and skeletal muscles), because insulin is essential for the influx of glucose into the tissues for glycogen synthesis.

The present study revealed that *Azadirachta indica* at 200mg/kg dose rate produced no significant alteration in the reduced liver glycogen level. Chattopadhyay *et al.* (1993) also could not find any significant alteration in the glycogen content when alcoholic extract of *Azadirachta indica* (One g/kg, p.o) was administered to normal rats as compared to control group. In glucose fed hyperglycemic rats, Azadirachta extract substantially lowered the hepatic glycogen level rather than increasing it. The reason for depletion of hepatic glycogen by the extract in presence of glucose load is not very clear.

In the present study, *Ocimum sanctum* also failed to produce any increase in the liver glycogen level. Similar results were obtained by Vats *et al.* (2004) when ethanolic extract of Ocimum leaves at 200 mg/kg was administered to alloxan diabetic rats orally. They could not observe any effect on glycogen content of brain, kidney, heart, muscle or liver. Since the Ocimum extract prevented the loss of body weight in diabetic controls, it is possible that it may have increased the glycogen content in muscle and liver, but the same was utilized for energy expenditure instead of being stored.

Group III which received *Tinospora cordifolia* alcoholic extract at 200mg/kg had no effect on the liver glycogen level. The findings are in agreement with the observations of Grover *et al.* (2000). They found that though the aqueous extract of Tinospora at 400 mg/kg caused significant reduction in glucose in moderate diabetes, it did not significantly affect the hepatic and skeletal muscle glycogen content and hepatic glucokinase, hexokinase, glucose-6-phosphatase and phosphofructokinase levels in diabetic mice.

The combination of Azadirachta, Ocimum and Tinospora extracts at 200mg/kg also failed to produce any significant increase in the liver glycogen levels. A significantly higher level of liver glycogen was observed in Group IV which received glibenclamide at 0.5mg/animal/day.

Results of the present study confirms the glucose lowering and hypolipidemic effects of *Azadirachta indica*, *Ocimum sanctum* and *Tinospora cordifolia* alcoholic extracts at 200 mg/kg dose rate. The hypoglycemic effect of alcoholic extract of *Azadirachta indica* was significantly higher when compared to that of Ocimum and Tinospora at the same dose levels. But the hypoglycemic effects of the three plants were lower than that of the standard oral hypoglycemic drug, glibenclamide. The combination of Azadirachta, Ocimum and Tinospora extracts had a still higher blood glucose lowering effect than the individual drugs, suggesting a synergistic action of the three drugs.

Summary

6. SUMMARY

The present study was undertaken to assess and compare the hypoglycemic effects of alcoholic extracts of *Azadirachta indica* and *Ocimum sanctum* leaves and *Tinospora cordifolia* vines and their combination at 200 mg/kg dose rates in alloxan induced diabetic rats and to compare their efficacy with the established oral antidiabetic drug, glibenclamide. The study was also directed to find out whether the combination had a synergistic/additive effect.

The experiment was conducted in 48 albino rats of either sex weighing 150-250 g. All the animals were induced diabetes by giving alloxan at a dose of 90 mg/kg intraperitoneally. Those rats having blood glucose levels above 200 mg/dl were selected for further study. They were randomly divided into six groups of eight animals each. Group I received alcoholic extract of *Azadirachta indica* leaves at 200mg/kg body weight from day seven to day 37. Group II received alcoholic extract of *Ocimum sanctum* at 200 mg/kg and Group III was given Tinospora alcoholic extract at the same dose rate. A combination of the alcoholic extracts of Azadirachta, Ocimum and Tinospora at 200 mg/kg was given to Group IV from day seven to day 37. Group V received glibenclamide at 0.5 mg/animal/day for 30 days. Group VI served as diabetic control, receiving no drugs.

Different parameters like body weight, the level of blood glucose, serum cholesterol and triglyceride and liver glycogen were studied. Blood glucose was estimated on zero day, 7^{th}, 15^{th}, 21^{st}, 30^{th} and 37^{th} day. Body weight was also taken at weekly intervals. Parameters like serum cholesterol and triglyceride and liver glycogen were estimated on the 37^{th} day after sacrificing the animals.

All the treatment groups except control showed a gradual increase in body weight during the experimental period, but it was seen that the body weight never returned to their original weight noted before the commencement of the experiment.

All the treatment groups (Group I, II, III and IV) showed significant reduction in blood glucose level. Among groups I, II and III, Group I which received Azadirachta at 200 mg/kg showed the maximum decrease in blood glucose level on 37th day. There is no significant difference between Group II and III. Group IV treated with combination of Azadirachta, Ocimum and Tinospora showed a comparatively more reduction in blood glucose level than Group I, II and III which suggested a synergistic effect of the three drugs in producing hypoglycemia.

Oral administration of alcoholic extract of Azadirachta at 200 mg/kg produced significant reduction in serum cholesterol level. Group III also showed a similar effect. Group II was having the least reduction in serum cholesterol level among the treatment Groups I, II, III and IV. Group IV had the lowest serum cholesterol level compared to the other groups.

Serum triglyceride level was highest in Group II and III and lowest in Group IV treated with combination. Among the three treatment groups (Group I, II and III), Group I showed the highest hypolipidemic effect.

Treatment with all the three drugs resulted in no significant change in the liver glycogen level.

Phytochemical study on the alcoholic extract of Azadirachta revealed that various active principles like alkaloids, flavonoids, glycosides and saponins were present in the extracts while there was no noticeable levels of steroids, tannins, phenolic compounds, diterpenes and triterpenes. *Ocimum sanctum* alcoholic extract gave positive tests for steroids, alkaloids, tannins, phenolic compounds, diterpenes and triterpenes and in *Tinospora codifolia* alcoholic extract, steroids, alkaloids, glycosides, phenolic compounds, diterpenes and triterpenes were detected.

From the present study, it was concluded that among the three hypoglycemic plants selected for study, *Azadirachta indica* was found to possess the highest effect. Ocimum and Tinospora alcoholic extracts at the same dose rates had a comparatively lower hypoglycemic effect. A combination of the extracts of the three plants in equal proportion showed a synergistic effect on lowering the blood sugar level. However, it was seen that the hypoclycemic effect of the three plants were lower than that of the standard oral hypoglycemic drug, glibenclamide.

The results of the present study have confirmed the hypoglycemic effect of the three plants and a comparatively better effect for its combination. The plants are widely distributed and their wide use by people implies a relative lack of toxicity. The study helps to validate the folklore use of these plants.

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COMPARATIVE STUDY OF THE HYPOGLYCEMIC EFFECT OF *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi) AND *Tinospora cordifolia* (Chittamruthu) AND THEIR COMBINATION IN DIABETIC RATS

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ABSTRACT

The present study was undertaken to assess and compare the hypoglycemic effects of alcoholic extracts of *Azadirachta indica* and *Ocimum sanctum* leaves and *Tinospora cordifolia* vines and their combination at 200 mg/kg dose rate in alloxan diabetic rats and to compare their efficacy with standard oral hypoglycemic drug, glibenclamide.

The experiment was conducted in forty eight alloxan induced diabetic rats of either sexes for a period of 37 days. Rats were divided into six groups of eight each. Group I was administered alcoholic extract of *Azadirachta indica* at 200mg/kg for 30 days from 7th day onwards, Group II received *Ocimum sanctum* alcoholic extract at the same dose rate and Group III was given Tinospora extract at 200 mg/kg. A combination of alcoholic extracts of Azadirachta, Ocimum and Tinospora was fed to Group IV at 200 mg/kg body weight. Group V received glibenclamide at 0.5 mg/animal/day for 30 days and Group VI served as diabetic control. Blood glucose level was estimated on zero day, 7th, 15th, 21st, 30th and 37th day. Body weight was taken at weekly intervals. Serum cholesterol, serum triglyceride and liver glycogen were estimated at 37th day of the experiment.

All the treatment groups (I, II, III and IV) showed a gradual increase in body weight during the experimental period, but it was seen that the body weights never returned to their original weights before the commencement of the experiment.

Among the treatment Groups I, II and III, Group I given Azadirachta extract had the maximum decrease in blood glucose level. Group IV which received a combination of the three drugs had a higher reduction compared to Group I, II and III. Results suggested a synergistic effect of the combination. However, the effect was comparatively lower than that of glibenclamide.

Significant reduction in serum cholesterol level was seen in Group I, II and III. Highest cholesterol level was seen in Group II and the lowest serum cholesterol level was seen in Group IV. Serum triglyceride level was highest in Group II and III. Group I had a comparatively lower serum triglyceride level. Lowest triglyceride level was shown by Group IV treated with combination.

Treatment with all the three drugs resulted in no significant change in the liver glycogen levels. Combination treatment also failed to produce any significant alteration in the reduced liver glycogen levels. Group I, II and III had a liver glycogen levels comparable to that of the control Group VI.

From the study, it can be concluded that among the three drugs, Azadirachta has the highest hypoglycemic and hypolipidemic effect. The combination treatment produced a comparatively higher effect than that of the three drugs, suggesting a synergistic action. However, the effects of the three drugs and their combination are less when compared to that of glibenclamide and the mechanism of action of the three plants are different from that of glibenclamide.