HYPOGLYCAEMIC EFFICACY OF *SCOPARIA DULCIS* AND *COSTUS* SPECIES IN ALBINO RATS

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

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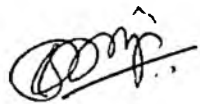
DECLARATION

I hereby declare that this thesis entitled **"HYPOGLYCAEMIC EFFICACY OF *SCOPARIA DULCIS* AND *COSTUS* SPECIES IN ALBINO 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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BALAJI, S.

CERTIFICATE

Certified that the thesis entitled **"HYPOGLYCAEMIC EFFICACY OF *SCOPARIA DULCIS* AND *COSTUS* SPECIES IN ALBINO RATS"** is a record of research work done independently by Mr. **BALAJI, S.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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And at last not the least I would place my sincere thanks for ***Miss.Annie*** *and* ***Miss.Sandhya*** *for their help in taking print out for my thesis.*

With immense pleasure, I am grateful to my beloved ***Parents, Brothers and Sister*** *to whom I am indebted for their everlasting love and without their unstinted support and moral boost I would not have done this Herculean task.*

Above all I bow before ***Almighty*** *for showering His kindness and blessings.*

LIST OF PLATES

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Introduction

1. INTRODUCTION

Diabetes mellitus is a complex metabolic disorder characterized by hyperglycaemia resulting from insufficient insulin secretion, insulin resistance or both, characterized by a state of chronic hyperglycaemia, which causes cardiovascular, renal, neurological and occular complications (World Health Organization (WHO), Expert Committee, 1985).

Diabetes is possibly the world's fastest growing metabolic disease. According to WHO projections, the diabetic population is likely to increase to 300 million or more by the year 2025, and in India it may rise from 19.4 million to 57.2 million by the year 2025 (King *et al.*, 1998). This rise may be due to the increasing number of ageing population, consumption of junk foods, obesity and sedentary life style (Yajinik, 2001). Diabetes mellitus has been recognized for centuries as a debilitating disease characterized by excretion of "sweet urine" (mellituria), polydypsia, polyuria, wasting of tissue, ketoacidosis and ultimately coma and death (Krantz and Carr. 1969).

Diabetes is a syndrome resulting from multifactorial interaction of hereditary and environmental factors affecting the metabolism of carbohydrate, protein and fat, in addition to the damaging effects on liver and kidney. (Ghosh and Suryawanshi, 2001). The failure of normal utilization of glucose in the tissue is accompanied by increased production of fatty acids, which are formed more rapidly than they are utilized (Krantz and Carr, 1969).

Diabetes mellitus is reported in all laboratory animals, horse, cattle, sheep and pigs, but most frequently observed in dogs and cats. In dogs diabetes is most frequently encountered in mature or older females in association with estrus and in

some breeds such as Miniature Poodle, Scottish terrier, Rotweiller and Dachshund. In contrast, male cats appear to be more frequently affected than queen. There are two main forms of diabetes mellitus in animals and human beings, Type1 diabetes (Insulin Dependent Diabetes Mellitus, IDDM) and Type 2 diabetes (Non-Insulin Dependent Diabetes Mellitus, NIDDM). Type 1 diabetes is primarily due to autoimmune mediated destruction of the pancreatic beta cells resulting in absolute deficiency. Type 2 diabetes is characterized by insulin resistance due to receptor abnormality and/or structurally abnormal insulin secretion either of which predominates. In animals, a third type of diabetes mellitus is also seen, Type 3 diabetes characterized by normal initial response to glucose load and a delayed return of insulin to normal levels as in chemical diabetes (Kaneko, *et al.*, 1997).

Treatment of diabetes depends upon proper management of diet and exercise, use of oral hypoglycaemic agents such as sulfonylurea and biguanides and insulin replacement therapy. Though biguanides and sulfonylureas are efficient in treating diabetes mellitus, their use is restricted due to their side effects such as hyperlipidaemia, greater incidence of myocardial infarction and unsatisfactory control of postprandial blood sugar (Annamalai and Augusti, 1980). The major disadvantage of insulin injection is that it may cause hypoglycaemic shock. Hence it is necessary to look for new and if possible, a more efficacious and economical indigenous drugs either administered alone or in combination, for the management of diabetes. (Vats *et al.*, 2004).

Long before the use of insulin, indigenous remedies have been used for the treatment of diabetes. Ayurveda has been the first to give an elaborate description of this disease, its clinical features, pattern and management by herbal or herbomineral drugs. It is seen that certain resistant cases of diabetes which do not respond well to allopathic medicines like tolbutamide, chlorpropamide and glibenclamide respond

very well when treated with herbal preparations. (Anturlikar *et al.*, 1995). Herbal medicines are frequently considered to be safe and comparatively free from side effects than their synthetic counter parts.

Many plants of folklore importance were used as antidiabetic agents for many centuries by rural population. (Khan and Singh, 1996). Some plants like *Allium cepa, Pterocarpus marsupium, Trigonella foenum graecum, Eugenia jambolana,* etc.have been shown to possess antidiabetic activity (Shukla *et al.*, 2000). This study is directed towards investigation on the effectiveness of two medicinal plants namely *Scoparia dulcis* (Sweet Broom weed, Kalluruki) and *Costus pictus* (Insulin plant) commonly used in folklore medicine for the treatment of diabetes mellitus. In the present study the anti-diabetic effect of these plants separately and also in combination in alloxan induced diabetic rats are investigated. The efficiency of these treatments are compared with a standard oral hypoglycaemic agent, glibenclamide.

Review of Literature

2. REVIEW OF LITERATURE

2.1. *SCOPARIA DULCIS* AND ITS MEDICINAL PROPERTIES

Scoparia dulcis (Family: Scrophulariaceae) commonly known as sweet broom weed (Kalluruki) is an erect annual herb with serrated leaves, producing white flower and measuring upto half a meter in height when fully grown. These plants usually grow in tropical climate and is vey abundant in Western Ghats. Bever (1980) analyzed the phytochemical constituents of *Scoparaia dulcis* plant and found that the alcoholic extract contained glycosides, flavanoids and tannins. It is used to cure various ailments such as kidney stones and it has also got antiviral activity (Hayashi, 1990) and antitumor promoting activity (Nishino, 1993).

Freire *et al.* (1993) found out the ether extract of *Scoparia dulcis* L. (0.25-1 g/kg *per os)* reduced the writhing induced by acetic acid in mice. They also found that the ether extract and glutinol-triterpene from the ether extract (30 mg/kg p.o) reduced the paw edema and pleurisy induced by Carrageenin in rats.

Ahmed *et al.* (2001) isolated Scoparinol, a diterpene from *Scoparia dulcis* and showed that it produced a significant anti-inflammatory activity at a dose of 30 mg/kg body weight (b.w) in Swiss albino mice. They also found that it had significant diuretic action at the same dose.

Oral administration of *Scoparia dulcis* powder at the dose of 12.5 mg/kg b.w for four weeks on *Trypanosoma brucei* infected rabbits lowered the higher levels of serum transaminases, alkaline phosphatase and bilirubin content to normal level in treated infected rabbits (Orhue and Nwanze, 2004).

Ratnasooriya *et al.* (2005) studied the antioxidant activity of aqueous extract of *Scoparia dulcis* and showed that the extract had marked antioxidant activity in vitro, which was dose-dependent. At the highest dose tested, the

efficacy of the antioxidant activity was comparable to 0.01 mg/ml of vitamin E or BHT (butylated hydroxytoluene).

2.2. *COSTUS* SPECIES AND ITS MEDICINAL PROPERTIES

Costus pictus (Family: Costaceae) commonly known as insulin plant is a perennial herb, which produces yellow flower and is widely found in Kerala, India, imported from South Africa. These plants grow widely in tropical climate and found in the Western Ghat region. This plant is commonly called as Insulin plant and its leaves are munched by the local folk for treating diabetes mellitus. The leaves of this plant taste slightly sour. The aerial part of the plant appears in cluster arising from the rhizome under ground. The stem of the plant first appears pink when young and turns into green when mature.

Another wild variety of *Costus* genus is found in Kerala, which is called as *Costus speciosus*. This variety produces white coloured flower and its leaves are tasteless. These plants are used for ornamental purposes and can be grown by vegetative propagation either by using stem cutting or by using rhizome.

Tewari (1971) conducted a preliminary phytochemical and pharmacological investigation on *Costus speciosus* and found that the chloroform and alcoholic extracts revealed the presence of saponins.

Singh and Srivastava (1981) investigated the phytochemical constituents present in the alcoholic and chloroform extract of *Costus speciosus* seeds and found that the extracts contained saponins and steroids as main constituent.

Mosihuzzaman *et al.* (1994) found out that the freeze -dried juice of rhizome of *Costus speciosus* produced significant hypoglycaemic effect when fed with simultaneous glucose load in Insulin Dependent Diabetes mellitus (IDDM) model rats.

Kemp (2003) collected information on the use of plant sources for treatment of various ailments from the Rengma tribes of Nagaland and reported that *Costus speciosus* stem after removing the skin/bark is used for jaundice treatment.

Shiva *et al.* (2003) said that *Costus speciosus* plant contains alkaloids, starch and fiber as important phytochemicals.

2.3. EFFECT OF INDIGENOUS PLANTS ON THE BODY WEIGHT

The body weight is reduced during diabetes mellitus due to the increased mobilization of fatty acids from the storage site for meeting out the energy demand.

Medina *et al.* (1994) opined that the administration of the decoction of Juniper berries (125 mg total berries/kg) to streptozotocin induced diabetic rats for 24 days resulted in a significant reduction in the mortality index, as well as the prevention of loss in body weight from 190 ± 10.8 to 205 ± 5.6 g.

Pari and UmaMaheshwari (1999) evaluated the hypoglycaemic effect of *Musa sapientum* in alloxan-induced diabetic rats and found that oral administration of 0.15,0.20 and 0.25 g/kg of chloroform extract of the *Musa sapientum* flowers (MSFEt) for 30 days resulted in preventing the loss in body weight (initial body weight=180.5 ± 10.3 g and final body weight =187.6 ± 12.5g).

Prince and Menon (2000) examined the effect of oral administration of an aqueous *Tinospora cordifolia* root extract (TCREt) @ 0.5 g/kg b.w for 42 days to alloxan diabetic rats and observed an increase in body weight of alloxan diabetic rats from 165.6 ± 3.2 to 186.3 ± 1.4 g.

Vetrichelvan *et al.* (2002) evaluated the anti-diabetic activity of alcoholic extract of *Celosia argentea* L.seeds in diabetic rats and found that the extract @

250 and 500 mg/kg for 15 days produced an increase in body weight by 8.7 ± 2.7 g and 9.1 ± 3.2 g, respectively in alloxan-induced diabetic rats.

Sachdewa and Khemani (2003) studied the effect of ethanolic extract of *Hibiscus rosa sinensis* flowers in streptozotocin induced diabetic rats and found that oral administration of the extract @ 250 mg/kg for 21 days significantly lowered the loss of body weight in streptozotocin induced diabetic rats from 178.2 ± 10.34 to 193.5 ± 5.78 g.

Xie *et al.* (2003) evaluated the body weight reduction activity of *Gymnema yunnanense* extract in obese *ob/ob and* diabetic *db/db* mice and reported that intraperitoneal injection of the extract 100 mg/kg for 12 days decreased fasting blood glucose levels significantly from day zero to day five. They also reported that after 12 days of treatment body weight of *ob/ob* mice reduced significantly from 51.7±1.9 g on day zero to 45.7 ± 1.2 g on day 12 and the body weight in *db/db* mice reduced from 61.8 ± 1.4 g on day zero to 59.8 ± 1.1 g on day 12.

Alarcon-Aguilar *et al.* (2005) studied the acute and chronic hypoglycaemic effect of *Ibervillea sonorae* root extracts in normal and alloxan diabetic mice and rats and showed that the aqueous decoction and the raw extract (juice) from the root resulted in significant reduction of glycemia in healthy mice after intraperitoneal administration at a dose of 600 mg/kg for 41 days. They also showed that Dichloromethane (DCM) extract administered daily p.o to alloxan diabetic rats at 300 mg/kg/day improved glycemia and lowered the body weight reduction due to diabetes from 335.8 ± 27.3 to 246.5 ± 40.4 g.

Chakrabarti *et al.* (2005) studied the antidiabetic activity of *Caesalpinia bonducella* F. in chronic type 2 diabetic model in Long-Evans rats and showed that the aqueous extract of *Caesalpinia bonducella* @ 1.25 mg/kg increased the body weight significantly on day 28 in diabetic treated animals from 152.20 ± 1.21 to 167.22 ± 5.59 g.

Shirwaikar *et al.* (2005) investigated the antidiabetic potential of alcoholic stem extract of *Coscinum fenestratum* in streptozotocin-nicotinamide diabetic rats and found that the extract @ 250 and 500 mg/kg produced a significant increase in the body weight of the treated diabetic animals from 256.1 ± 11.1 to 248.5 ± 15.1 and 218.2 ± 15.6 to 199.3 ± 11.5 g, respectively.

2.4. EFFECT OF INDIGENOUS PLANTS ON HAEMATOLOGICAL PARAMETERS

2.4.1. Effect on Red Blood Cell (RBC) Count and White Blood Cell (WBC) Count

The main function of RBC is to transport oxygen and carbon dioxide to and from the cells, respectively for metabolizing the nutrients. The WBC main role is to prevent the body from any assault from foreign agents. The normal RBC count for rats' ranges from 5.4 to 8.9 million/µl and the normal WBC count ranges from 4.0 to 10.2 thousand/µl (Pritchett and Corning, 2004). The red blood cell count and the white blood cell count did not show any changes during diabetes mellitus.

2.4.2. Effect on Total Haemoglobin

The haemoglobin concentration is very low in diabetes mellitus due to the increased glycosylation of haemoglobin molecule by the excess glucose present in the blood. This makes the available haemoglobin level to be lower than the normal value in the blood during diabetes mellitus.

Pari and UmaMaheshwari (1999) evaluated the effect of chloroform extract of *Musa sapientum* flowers (MSFEt) in alloxan-induced diabetic rats and found that oral administration of 0.15, 0.20 and 0.25 g/kg of the extract for 30 days significantly increased the reduced level of haemoglobin from5.81 ± 0.7 to

8.01 ± 0.8 g/dl in diabetic treated rats. They also found that the dose level of 0.25 g/kg was very effective in bringing back the haemoglobin level to normal from 6.01 ± 0.6 to 12.3 ± 1.6 g/dl.

Prince and Menon (2000) studied the effect of oral administration of aqueous *Tinospora cordifolia* root extract (TCREt) @ 0.5 g/kg b.w in alloxan-induced diabetic rats for 42 days and found that the extract increased the haemoglobin content significantly from10.3 ± 1.9 to 12.1 ± 0.9 g/dl.

Dhandapani *et al.* (2002) investigated the effect of *Cuminum cyminum* supplementation in alloxan-induced diabetic rats and found that oral administration of 0.25 g/kg b.w of *Cuminum cyminum* for six weeks to diabetic rats significantly increased the concentration of total haemoglobin from 11.55 ± 1.69 to 14.73 ± 1.59 g/dl in diabetic rats.

Pari and Latha (2002) reported that *Cassia auriculata* flower extract (CFEt) at doses of 0.15,0.30 and 0.45 g/kg b.w for 30 days increased the lowered levels of total haemoglobin content from 5.60 ± 0.45 to 11.5 ± 0.91 g/dl in diabetic treated animals. They also opined that at the dose rate of 0.45 g/kg was more effective.

Oral administration of *Boerhaavia diffusa* L.leaf extract (BLEt) @ 200 mg/kg for four weeks produced a significant increase in total haemoglobin level from 9.04 ± 0.68 to 11.69 ± 0.51 g/dl in alloxan-induced diabetic rats (Pari and Sathcesh, 2004).

Prince *et al.* (2004) evaluated the effect of alcoholic extract of *Syzigium cumini* seeds (JSEt) in alloxan-induced diabetic rats and found that oral administration of JSEt to diabetic rats @ 100 mg/kg b.w resulted in a significant elevation in the total haemoglobin content from 10.8 ± 0.8 to 14.1 ± 0.9 g/dl.

Degirmenci *et al.* (2005) studied the effects of acarbose and *Rumex patientia* in streptozotocin induced diabetic rats and found that 40 mg

acarbose/100 g feed and 2 percent decoction of *Rumex patientia* grain produced a significant rise in total haemoglobin level from 9.17 ± 1.32 to 12.52 ± 1.49 g/dl after 20 weeks of treatment.

Shirwaikar *et al.* (2005) evaluated the effect of alcoholic stem extract of *Coscinum fenestratum* in streptozotocin-nicotinamide diabetic rats and found that the extract @ 250 and 500 mg/kg for 12 days increased the haemoglobin content significantly from 8.46 ± 0.18 to 11.59 ± 0.43 g/dl.

2.5. EFFECT OF INDIGENOUS PLANTS ON BIOCHEMICAL PARAMETERS

2.5.1. Effect on Glycosylated Haemoglobin

Glycosylated haemoglobin represents stable ketone-amine or aldehyde amine linkages formed by non-enzymatic glycosylation of valine and lysine in the haemoglobin molecule. The glucose molecule when present in excess binds to the lysine and valine residue of the haemoglobin molecule, which represent time averaged values for blood glucose over the preceding two to four months.

Pari and UmaMaheshwari (1999) evaluated the hypoglycaemic effect of *Musa sapientum* L.in alloxan-induced diabetic rats and found that oral administration of 0.15, 0.20 and 0.25 g/kg of chloroform extract of the *Musa sapientum* flowers (MSFEt) for 30 days resulted in a significant reduction in glycosylated haemoglobin from0.9 ± 0.11 to 0.23 ± 0.02 mg/g Hb which is very effective at 0.25 g/kg.

Pari and Saravanan (2002) evaluated the antidiabetic effect of Cogent db, a herbal drug in alloxan-induced diabetic rats and found that 0.45 g/kg b.w of the aqueous solution of Cogent db for 40 days produced a significant reduction in the glycosylated haemoglobin in diabetic rats from 0.87 ± 0.06 to 0.25 ± 0.04 mg/g Hb.

Dhandapani *et al.* (2002) studied the role of *Cuminum cyminum* supplementation in alloxan diabetic rats and observed that oral administration of 0.25 g/kg b.w of *Cuminum cyminum* for six weeks significantly reduced glycosylated haemoglobin from 6.24 ± 0.63 to 2.24 ± 0.14 mg/g Hb in the treated diabetic rats.

Pari and Latha (2002) reported that *Cassia auriculata* flower extract (CFEt), at doses of 0.15,0.30 and 0.45 g/kg b.w for 30 days significantly reduced the glycosylated haemoglobin content in alloxan induced diabetic rats from 0.81 ± 0.07 to 0.37 ± 0.04 mg/g Hb. They also found that the dose of 0.45g/kg b.w was more effective.

Pari and Satheesh (2004) studied the effect of oral administration of aqueous extract of *Boerhaavia diffusa* L. leaf (BLEt) at the dose of 200 mg/kg b.w for four weeks and found that the extract significantly reduced the glycosylated haemoglobin levels from 0.782 ± 0.05 to 0.41 ± 0.03 mg/g Hb.

Prince *et al.* (2004) evaluated the effect of alcoholic extract of *Syzigium cumini* seeds (JSEt) in alloxan diabetic rats and found that oral administration of alcoholic JSEt to diabetic rats at a dose of 100 mg/kg b.w for 42 days resulted in a significant reduction in glycosylated haemoglobin from 0.85 ± 0.06 to 0.46 ± 0.03 mg/g Hb.

Degirmenci *et al.* (2005) performed an experiment to observe the effects of acarbose and *Rumex patientia* in streptozotocin induced diabetic rats and found that 40 mg acarbose /100 g feed and 2 percent decoction of *Rumex patientia* grain produced a significant decrease in glycosylated haemoglobin level from 7.46 ± 1.05 to 4.46 ± 0.13 mg/dl and 5.36 ± 0.10 mg/dl, respectively after 20 weeks.

Shirwaikar *et al.* (2005) evaluated the antidiabetic potential of alcoholic stem extract of *Coscinum fenestratum* in streptozotocin-nicotinamide diabetic rats and found that the extract produced a significant reduction in glycosylated haemoglobin level from 7.1 ± 0.2 to 4.5 ± 0.4 percent and 4.1 ± 0.1 percent,

respectively in treated diabetic rats than in the diabetic control at a dose of 250 and 500 mg/kg for 12 days.

2.5.2. Effect on Plasma Glucose

Blood glucose is the main source of instant energy to the cells and it is increased two to three fold in diabetes mellitus. However the cells are unable to utilize glucose for their energy needs because of the insulin insufficiency and may retort to utilize the fatty acids for energy. The excess glucose present in the blood may also lead to diabetic complications when left untreated.

Abdel-Barry *et al.* (1997) tested the hypoglycaemic activity of the aqueous and alcoholic extracts of fenugreek leaf on normal and alloxan induced diabetic rats. Graded amounts (0.06, 0.2, 0.5,1 g/kg i.p and 1, 2, 8 g/kg p.o) of the aqueous extract produced a significant reduction of blood glucose concentration. But the ethanolic extract produced no reduction in blood glucose level in normal rats. Intraperitoneal administration of 0.8 g/kg of the ethanolic extract to diabetic rats produced a significant reduction of blood glucose concentration from 262 ± 11 to 95 ± 4 mg/dl, six hours after treatment.

Administration of Neem seed kernel Powder (NP) alone (500 mg/kg) as well as the combination of NP (250 mg/kg) with glibenclamide (0.25 mg/kg) significantly decreased the concentration of blood glucose in alloxan diabetic rabbits from 380.32 ± 5.12 to 98.17 ± 2.56 mg/dl after 42 days of treatment (Bopanna *et al.*1997).

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

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

Administration of aqueous extract of *Catharanthus roseus* (*Vinca rosea*) flower (VRF) and leaf (VRL) @ 4 mg/kg b.w for 7 days was found to lower the blood sugar level in alloxan diabetic rats from 343 ± 6.8 to 92.9 ± 6.2 mg/dl and 348.5 ± 5.5 to 90.4 ± 3.8 mg/dl respectively. Its hypoglycaemic activity was through β-cell rejuvenation, regeneration and stimulation (Ghosh and Suryawanshi, 2001).

The study conducted by Babu *et al.* (2002) revealed that alcoholic extract of *Cassia kleinii* leaf @ 200 mg/kg bodyweight for 15 days exhibited anti-hyperglycaemic effect by reducing the fasting plasma glucose value of 420.5 ± 16.8 to 125.7 ± 5.6 mg/dl in alloxan-induced diabetic rats.

Sharma *et al.* (2003) found that ethanolic extract of *Eugenia jambolana* seeds when given orally to severe diabetic rabbits for 15 days significantly lowered the fasting blood glucose level by 31.6 percent.

Yadav *et al.* (2002) evaluated the antihyperglycaemic effect of *Murraya koenigii* in normal and alloxan induced diabetic rats and found that feeding 10 percent and 15 percent diet containing *Murraya koenigii* leaves in normal rats, the reduction in blood glucose level was almost negligible (four percent with 10 and 15 percent diet) and in case of mild and moderate diabetic rats, feeding of five, 10 and 15 percent diet caused a maximal reduction in blood sugar by 13.1, 16.3 and 21.4 percent and 3.2, 5.58, 8.21 percent, respectively.

Daitewa *et al.* (2004) studied the hypoglycaemic and antihyperglycaemic effects of diethyl ether fraction isolated from the aqueous extract of the leaves of *Cogniauxia podoleana* in normal and alloxan-induced diabetic rats and found that 100 mg/kg of diethyl ether fraction reduced the blood glucose level by 40.3 percent three hours after oral administration to normal rats and decreased the level of hyperglycaemia by 41.4 percent and 70.4 percent respectively, after three and four hours in alloxan- induced diabetic rats.

Eddouks *et al.* (2004) investigated the hypoglycaemic effect of aqueous extracts of *Carum carvi* (CC) and *Capparis spinosa* (CS) fruit in normal and streptozotocin-diabetic rats and found that oral administration of the aqueous CC and CS extracts (20 mg/kg) produced a significant decrease in blood glucose level in streptozotocin diabetic rats. Both the extracts of CC and CS decreased the blood glucose level from22.5 ± 1.5 to 9.5 ± 0.5 mmol/l and 21.5 ± 0.5 to 11.5 ± 1.5 mmol, respectively after 15 days of treatment.

Latha *et al.* (2004) studied the effect of *Scoparia dulcis* (Sweet broom weed) on insulin-secretagogue activity and cytoprotective role in streptozotocin diabetic rats and found out that an aqueous extract of *Scoparia dulcis* @ 200 mg/kg b.w significantly decreased the blood glucose from 250.0 ± 13.7 to 112.0 ± 10.0 mg/dl with a significant increase in plasma insulin level at the end of 15 days treatment. They also suggested that the glucose lowering effect of aqueous extract of *Scoparia dulcis* was associated with potentiation of insulin release from the pancreatic islets.

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

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

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

Bhandari *et al.* (2005) evaluated the ethanolic extract of *Zingiber officinale* on hypoglycemia and found that the extract @ 200 mg/kg orally for 20 days produced significant antihyperglycaemic effect in streptozotocin-diabetic rats by reducing the blood sugar level from 573.9 ± 29.6 to 252.9 ± 41.3 mg/dl.

Fuliang *et al.* (2005) investigated the effects of propolis on blood glucose in rats with diabetes mellitus and found that ethanolic extracts of propolis @ 15 mg/kg b.w for eight weeks decreased the level of blood glucose significantly from 21.94 ± 2.01 to 19.44 ± 5.31 mmol/l.

Ojewole (2005) studied the antidiabetic effects of aqueous leaf extract of *Bryophyllum pinnatum* (Crassulaceae) and reported that the extract (400 mg/kg p.o) produced a significant hypoglycaemia in streptozotocin induced diabetic rats by reducing the blood glucose level from 560.20 ± 20.35 to 365.51 ± 18.40 mg/dl 8 hours after treatment. The antidiabetic property was accounted to the different flavanoids, polyphenols, triterpenoids and other chemical constituent in the plant extract.

Singh *et al.* (2005) reported that diabetic rats fed with potato peel powder-supplemented diet (5 percent&10 percent) for four weeks produced a significant

decrease in blood glucose level from 310.5 ± 25.5 to 283.7 ± 10.5 mg/dl and 300.25 ± 20.5 to 245.5 ± 12.5 mg/dl, respectively.

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

2.5.3. Effect on Plasma Lipid Profile

Lipids are the major metabolic nutrients that are used by the cells during diabetes mellitus due to the unavailability of glucose to cells for energy production. In diabetes mellitus the free fatty acids are mobilized from the tissue stores for meeting out the energy demand and thereby increasing the plasma lipids. The concentration of total lipids, total cholesterol, triglycerides, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels are increased. The high density lipoprotein (HDL) level is decreased.

Sharma *et al.* (1997) studied the hypolipidaemic activities of the aqueous and ethanolic extracts of *Caesalpinia bonducella* seeds in streptozotocin diabetic rats @ 100 mg/kg b.w for 10 days and showed that the aqueous extract exhibited significant antihypercholesterolemic and antihypertriglyceridemic effects in streptozotocin diabetic rats by reducing the total cholesterol from 75.5 to 59.5 mg/dl and triglycerides from 135.5 to 119.3 mg/dl.

Administration of Neem seed kernel Powder (NP) alone (500 mg/kg) as well as the combination of NP (250 mg/kg) with glibenclamide (0.25 mg/kg) significantly decreased the concentration of LDL-C in alloxan diabetic rabbits from 126.32 ± 5.12 to 85.17 ± 2.56 mg/dl after 42 days of treatment (Bopanna *et al.*, 1997).

Prince *et al.* (1999) evaluated the hypolipidemic effect of an aqueous extract of *Tinospora cordifolia* roots in alloxan diabetic rats and showed that administration of the extract of *T.cordifolia* roots (5.0 g/kg b.w) for 6 weeks resulted in a significant reduction in serum tissue cholesterol (102.2 ± 5.7 vs 148.4 ± 6.2 mg/dl), phospholipids (130.4 ± 4.3 vs 137.0 ± 3.5 mg/dl) and free fatty acids (97.2 ± 12.3 vs 159.4 ± 7.9 mg/dl) than the control rats.

Prince and Menon (2000) evaluated the effect of oral administration of an aqueous *Tinospora cordifolia* root extract (TCREt) @ 0.5 g/kg b.w for 42 days in alloxan diabetic rats and observed a significant reduction in brain lipid level such as cholesterol from 2560.8 ± 110.4 to 1557.4 ± 8.6 mg/100 g wet tissue, phospholipids from 3025.3 ± 146.5 to 2410.4 ± 120.4 mg/100 g wet tissue and free fatty acids from 28.6 ± 3.3 to 14.6 ± 3.1 mg/100 g wet tissue.

Dhandapani *et al.* (2002) studied the role of *Cuminum cyminum* supplementation on the plasma lipids in alloxan diabetic rats and observed that oral administration of 0.25g/kg b.w of *Cuminum cyminum* in diabetic rats for six weeks significantly reduced the plasma cholesterol from 153.43 ± 15.9 to 98.21 ± 10.49 mg/dl, phospholipids from 113.18 ± 14.01 to 61.24 ± 5.19 mg/dl, free fatty acids from 323.56 ± 32.05 to 170.38 ± 46.28 mg/dl and triglycerides from 99.81 ± 9.32 to 54.21 ± 6.34 mg/dl.

Pari and Latha (2002) reported that *Cassia auriculata* flower extract (CFEt), at doses of 0.15, 0.30 and 0.45 g/kg b.w for 30 days suppressed the elevated lipid levels in diabetic rats. They found that the extract effectively lowered the levels of cholesterol from 98.66±4.03 to 83.46 ± 2.18 mg/dl, triglycerides from 62.83 ± 1.50 to 53.93 ± 2.70 mg/dl, free fatty acids from 83.86 ± 6.67 to 75.06 ± 1.55 mg/dl and phospholipids from 98.75 ± 4.28 to 85.50 ± 2.86 mg/dl at 0.45 g/kg very effectively.

Pari and Saravanan (2002) evaluated the antidiabetic effect of Cogent db, an herbal drug in alloxan-induced diabetic rats and found that 0.45g/kg b.w of the

aqueous solution of Cogent db for 40 days produced a significant reduction in levels of total cholesterol from237.3 ± 8.13 to 162.4 ± 5.3 mg/dl, phospholipids from 225.2 ± 8.4 to 179.1 ± 6.5 mg/dl, triglycerides from 160.5 ± 8.2 to 120.5 ± 6.5 and increased from 33.5 ± 2.5 to 49.4 ± 4.1 mg/dl.

John (2003) evaluated the hypolipidaemic effect of *Brassica juncea* and *Trigonella foenum-graecum* seeds in diabetic rats and found that feeding of the powdered seeds @ 8 mg/kg b.w reduced the serum total cholesterol from 167.03 ± 12.34 to 85.19 ± 14.31 mg/dl and from 158.15 ± 17.65 to 78.13 ± 16.81 mg/dl, respectively and also found that the triglyceride level was also reduced from 150.13 ± 11.37 to 87.26 ± 23.92 mg/dl and from 141.26 ± 18.47 to 106.35 ± 15.48 mg/dl, respectively.

Sachdewa and Khemani (2003) studied the effect of ethanolic extract of *Hibiscus rosa sinensis* flowers in streptozotocin induced diabetic rats and found that oral administration of the extract @ 250 mg/kg for 21 days significantly lowered the total cholesterol and triglycerides by 22 and 30 percent, respectively. They also said that the level of HDL-cholesterol level significantly increased by the extract (12 percent) than glibenclamide (1 percent).

Uadia (2003) investigated the hypolipidaemic effect of *Dioscorea dumetorum* tuber in alloxan-induced diabetic rats and found out that the extract @ 400 mg/kg for 7 days significantly reduced elevated blood levels of triacylglycerols from1.77 ± 0.01 to 1.29 ± 0.01 mmol/l, cholesterol from 2.98 ± 0.06 to 2.41 ± 0.02 mmol/l and β-hydroxybutyrate from 2.55 ±0.13 to 2.36 ± 0.16 mmol/l associated with alloxan-induced diabetes mellitus.

Yadav *et al.* (2004) assessed the hypocholesterolemic effect of *Brassica juncea* seeds in rats fed with fructose-enriched diet and showed that feeding of a fructose diet containing 10 percent *Brassica juncea* seed powder for 30 days significantly decreased cholesterol level in treated rats (1.77 ± 0.11 mmol/l) than in fructose control (1.95 ± 0.10 mmol/l) but did not normalize it.

Alarcon-Aguilar *et al.* (2005) studied the effect of oral administration of Dichloromethane (DCM) extract of *Ibervillea sonorae* root in alloxan diabetic rats and found that the DCM extract @ 300 mg/kg/day significantly decreased the total cholesterol from 101.5 ± 1.2 to 91.5 ± 2.3 mg/dl and triglycerides from 261.5 ± 69.8 to 89.0 ± 1.22 mg/dl after 41 days of treatment.

Chakrabarti *et al.* (2005) studied the antidiabetic activity of *Caesalpinia bonducella* F. in chronic type 2 diabetic model in Long-Evans rats and showed that the aqueous extract @ 1.25 mg/kg produced significant reduction in the triglyceride level from day first value of 100 mg/dl to 74.77 mg/dl on day 28th.

Fuliang *et al.* (2005) investigated the effects of propolis on lipid in rats with diabetes mellitus and found that ethanolic extracts of propolis @ 15 mg/kg for 8 weeks decreased the levels of total cholesterol from 2.34 ± 0.44 to 1.97 ± 0.28 mmol/l, triglycerides from 1.31 ± 0.35 to 0.95 ± 0.37 mmol/l, LDL from 0.82 ± 0.39 to 0.34 ± 0.16 mmol/l and VLDL from 1.52 ± 0.24 to 1.14 ± 0.25 mmol/l in serum of fasting rats and increased the HDL level from 0.81 ± 0.09 to 0.84 ± 0.23 mmol/l.

Gupta *et al.* (2005) studied the effect of ethanolic extract of *Annona squamosa* in alloxan-induced diabetic rabbits and found that the extract @ 350 mg/kg produced a significant reduction in the level of total cholesterol from 221.0 ± 6.2 to 112.0 ± 5.8 mg/dl, triglycerides from 146.0 ± 104.0 ±7.8 mg/dl and LDL from 159.4 ± 5.2 to 44.7 ± 4.8 mg/dl and increased the HDL from 32.4 ± 6.2 to 46.5 ± 5.4 mg/dl in severely diabetic rabbits after 15 days of treatment.

Muruganandan *et al.* (2005) investigated the effect of mangiferin on hyperglycemia in streptozotocin diabetic rats and showed that chronic intraperitoneal administration of mangiferin (10&20 mg/kg) once daily for 28 days exhibited hypolipidaemic effect. They also showed that mangiferin (10&20 mg/kg p.o) decreased the plasma total cholesterol (203.46 ± 4.96 to 128.50 ± 7.45 mg/dl @ 10 mg/kg i.p and 215.40 ± 9.13 to 128.50 ± 9.53 mg/dl @ 20 mg/kg i.p)

and triglycerides (126.00 ± 13.60 to 80.88 ± 4.00 mg/dl @ 10 mg/kg i.p and 131.11 ± 16.96 to 82.07 ± 3.23 mg/dl @ 20 mg/kg i.p). They also reported mangiferin reduced LDL concentration (142.40 ± 10.34 to 69.09 ± 15.44 mg/dl @ 10mg/kg i.p and 143.17 ± 3.71 to 64.45 ± 8.36 mg/dl @ 20 mg/kg i.p) and increased HDL concentration (41.65 ± 0.73 to 46.61 ± 1.15 mg/dl @ 10 mg/kg i.p and 42.19 ± 1.21 to 48.90 ± 0.84 mg/dl @ 20 mg/kg i.p) with a diminution of atherogenic index (3.88 ± 0.09 to 1.74 ± 0.09 Units @ 10 mg/kg i.p and 4.05 ± 0.12 to 1.70 ± 0.10 Units @ 20 mg/kg i.p) in diabetic rats.

Ruzaidi *et al.* (2005) studied the effect of Malaysian cocoa extract on lipid profiles in diabetic rats and showed that the cocoa extract (1&3 percent) diets were found to significantly reduce the level of total cholesterol from 2.24 ± 0.11 to 1.32 ± 0.09 mmol/l and 2.45 ± 0.07 to 1.75 ± 0.31 mmol/l, respectively in diabetic rats. In addition, 1,2, &3 percent cocoa extract diets had significantly lowered the total triglycerides from 2.07 ± 0.27 to 0.38 ± 0.07 mmol/l, 3.65 ± 0.38 to 0.40 ± 0.15 mmol/l and 3.72 ± 0.60 to 0.33 ± 0.06 mmol/l, respectively. They also found out that serum levels of HDL-cholesterol had increased significantly in diabetic rats fed with 2 percent cocoa extract from 0.95 ± 0.05 to 1.35 ± 0.18 mmol/l, while the LDL- cholesterol had decreased significantly by one percent in treated group from 0.58 ± 0.09 to 0.31 ± 0.07 mmol/l.

Shirwaikar *et al.* (2005) evaluated the antidiabetic potential of alcoholic stem extract of *Coscinum fenestratum* in streptozotocin-nicotinamide diabetic rats and found that the extract produced a significant reduction in the level of triglycerides from 183.0 ± 13.2 to 105 ± 15.1 and 80.5 ± 6.5 mg/dl and total cholesterol from 123.9 ± 15.2 to 69.3 ± 1.1 and 60.1 ± 4.4 mg/dl. They also reported an increase in the HDL-cholesterol level from 35.2 ± 2.7 to 53.5 ± 5.1 and 56.9 ± 1.5 mg/dl, respectively in treated animals at 250 and 500 mg/kg after 12 days of treatment.

Singh *et al.* (2005) reported that diabetic rats fed with potato peel powder (PP)-supplemented diet (10 percent) for four weeks significantly lowered the total

cholesterol by 20 percent in control and supplementation of PP in diet of diabetic rats markedly decreased the total cholesterol (16-29 percent) and triglyceride concentration (97-100 percent) at five percent and 10 percent levels, respectively.

2.5.4. Effect on Protein Profile

Diabetes mellitus, a metabolic disorder produces hypoproteinemia due to the increased utilization of nutrients such as fatty acids and amino acids rather than glucose for meeting out the energy requirement by the cells.

Wanke and Wong (1991) found the inhibitors of albumin promoter activity in the cell free system in the liver of alloxan induced diabetic rats and when treated with insulin, the albumin promoter activity was found to be normal in hepatonuclear extracts from the diabetic rats.

Ghosh and Suryawanshi (2001) studied the effect of oral administration of aqueous extract of *Catharanthus roseus* (*Vinca rosea*) flower (VRF) and leaf (VRL) @ 4 mg/kg b.w for seven days on protein metabolism and found that the aqueous extract prevented the hypoproteinemia produced by diabetes mellitus. They showed that the total protein level increased from 7.1 ± 0.5 to 7.3 ± 0.6 g/dl and serum albumin increased from 2.9 ± 0.3 to 3.4 ± 0.4 g/dl.

Babu *et al.* (2003) observed that oral feeding of ethanolic extract of *Cassia kleinii* leaf to streptozotocin induced diabetic rats @ 200 mg/kg b.w for 14 days significantly increased the concentration of total protein in treated diabetic rats (6.08 ± 0.74 g/dl) than the diabetic control (5.06 ± 0.89 g/dl).

2.5.5. Effect on Liver Glycogen and Enzymes

The liver glycogen content is decreased in diabetes mellitus due to the increased utilization of glycogen from the liver for energy production. In alloxan

induced diabetes mellitus the liver enzymes were elevated to three fold. The liver plays a major role in utilizing the nutrients effectively and efficiently. The elevated liver enzymes suggest about the important role of liver in mobilizing the nutrients for the efficient utilization of cells.

Ghosh and Suryawanshi (2001) reported that administration of aqueous extract of *Catharanthus roseus* (*Vinca rosea*) flower (VRF) and leaf (VRL) @ 4 mg/kg b.w for seven days lowered the Aspartate amino transferase (AST) level from 342.5 ± 14.2 to 249 ± 5.5 and 351.8 ± 14.6 to 270.3 ± 12.4 U/l, respectively.

Babu *et al.* (2003) opined that the ethanolic extract of *Cassia kleinnii* leaf @ 200 mg/kg in streptozotocin diabetic rats significantly increased the liver glycogen content by 34.5 ± 1.7 g/100 g than the diabetic control of 6.7 ± 0.7 g/100g.

John (2003) studied the hypoglycaemic effect of *Brassica juncea* and *Trigonella foenum-graecum* seeds in diabetic rats and found that the feeding of the powdered seeds @ 8mg/kg b.w increased the liver glycogen content in diabetic rats by 2.16 ± 0.05 g percent and 2.18 ± 0.05 g percent, respectively.

Maiti *et al.* (2004) studied the antidiabetogenic activity of aqueous extract of seed of *Tamarindus indica* in streptozotocin- induced diabetic rats and found that the extract @ 80 mg/0.5ml of dist.water/100 g b.w per day produced significant reduction in liver Aspartate amino transferase (AST) from 27.2 ± 0.8 to 16.0 ± 0.6 units/mg of tissue and Alanine amino transferase (ALT) from 24.8 ± 0.6 to 12.9 ± 0.7 units/mg of tissue.

Experimental diabetes induced by alloxan in rats causes tissue damage in the pancreas, liver, kidney and heart, which can be reflected in the increment of ALT and AST levels (Prince *et al.*, 2004).

Alarcon-Aguilar *et al.* (2005) studied the effect of Dichloro methane (DCM) extract of *Ibervillea sonorae* root in normal and alloxan diabetic rats and

found that DCM extract at the dose of 300 mg/kg/day brought down the increased AST and ALT level to normal (122.1 ± 40.0 to 71.0 ± 5.25 U/l and 149.5 ± 53.5 to 54.5 ± 0.24 U/l).

Chakarabarti *et al.* (2005) showed that the ethanolic extract of *Caesalpinia bonducella* F. @ 1.25 mg/kg b.w for 28 days produced a significant increase in glycogen level by 20.77 ± 2.22 mg/g than in control of 7.06 ± 2.18 mg/g in chronic Type 2 diabetic model of Long-Evans rats.

El-Demadash *et al.* (2005) investigated the effects of onion (*Allium cepa*) and garlic (*Allium sativum*) juices on enzyme activities in alloxan-induced diabetic rats and found that oral administration of one ml of either onion or garlic juices/100 g b.w (equivalent to 0.4 g/100g b.w) restored the elevated levels of serum ALT and AST from 157 ± 5 to 49 ± 4 U/l and 325 ± 8 to 145 ± 7 U/l, respectively in diabetic treated rats.

Musabayane *et al.* (2005) investigated the hypoglycaemic effect of *Syzygium cordatum* leaf extract in non-diabetic and streptozotocin-induced diabetic rats and found that the leaf extract @ 6 mg/100 g b.w for four weeks increased hepatic glycogen content of treated STZ-diabetic rats (28 ± 5 mg/100g b.w) in comparison to untreated STZ-diabetic rats (16 ± 3 mg/100g b.w).

Shirwaikar *et al.* (2005) evaluated the antidiabetic potential of alcoholic stem extract of *Coscinum fenestratum* in streptozotocin-nicotinamide diabetic rats and found that the extract produced a significant increase in the liver glycogen level in treated animals (2.0 ± 0.2 and 2.6 ± 0.3 mg/kg, respectively) than the diabetic control (1.2 ± 0.1 mg/kg).

Singh *et al.* (2005) reported that diabetic rats fed with potato peel powder supplemented diet (10 percent) for four weeks significantly lowered the elevated liver enzymes such as ALT from 112 U/l to 75 U/l and AST from 248 U/l to 198 U/l.

2.5.6. Effect on Trace Minerals

Derewenda *et al.* (1989) suggested that in the presence of zinc within the cell,insulin monomer formed assemble to a dimeric form for storage and release when needed.

Chausmer (1998) suggested that the increased glucose content in the plasma is the main reason for the increased urinary loss of zinc and decreases the total body zinc content.

Shaheen and El-Fattah (1995) opined that diabetogenic doses of alloxan lowered the plasma zinc content in rats and produced zinc deficiency due to increased urinary loss of zinc in chemical diabetes and they also said that oral supplementation of zinc produced a protective effect in diabetic rats.

Fernandez-Real *et al.* (2002) suggested that an increased iron store increases the chances of development of Type 2 diabetes while iron depletion was protective and iron-induced damage might also modulate the development of chronic diabetic complications. They also suggested that iron depletion had been beneficial in coronary artery responses, endothelial dysfunction, insulin secretion, insulin action, and metabolic control in Type 2 diabetes.

Kechrid *et al.* (2002) investigated the effect of low dietary zinc intake in alloxan diabetic rats and found that the low-zinc diabetic and non-diabetic rats were able to maintain a normal zinc level as the control groups.

2.6. ALLOXAN INDUCED DIABETES

Szkudelski (2001) reported that alloxan and its reduced form, dialuric acid established a redox cycle with the formation of superoxide radicals, which

dismutate to hydrogen peroxide and by Fenton reaction, hydroxyl radicals are formed. He also reported that the action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B-cells.

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

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

Materials and Methods

3. MATERIALS AND METHODS

3.1. EXPERIMEMENTAL ANIMALS

Seventy-two male Sprague-Dawley albino rats weighing 150-200 g aged between six to eight weeks, procured from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy were used for conducting the experiment. The rats were reared in cages with adequate aeration and photoperiod. The rats were fed with standard ration and water *ad libitum*. The experiment was carried out for a period of 60 days.

3.2. PREPARATION OF DIABETIC RATS

The rats were fasted overnight 16 hours and their body weight and fasting blood glucose were estimated on next day morning. As a preliminary trial alloxan was given at 90,120,130 and 150 mg/kg b.w intraperitoneally (i.p) to establish a safest dose. At the dose of 130mg/kg b.w i.p for two consecutive days all the animals survived with blood glucose level above 350 mg/dl at the end of 5^{th} day, from the start of the injection (Daitewa *et al.*, 2004). Other doses were found to be either too toxic or inefficient in producing diabetes. After five days from the start of the injection, when the blood glucose level was stabilized animals with blood glucose level above 350 mg/dl were selected for the study.

3.3. PREPARATION AND ADMINISTRATION OF THE TEST SUBSTANCE

3.3.1. Collection of the Plant Material

The plant *Scoparia dulcis* (Sweet Broom weed, Kalluruki) was collected from the farm area in the Veterinary College campus, Mannuthy and the leaves were dried in shade for a week and pulverized (Plate 1). The leaves of *Costus pictus* (Insulin plant) were cut into small pieces and dried in shade for two weeks and then pulverized (Plate 2).

PLATE 1. *SCOPARIA DULCIS* PLANT

PLATE 2. *COSTUS PICTUS* PLANT WITH FLOWER

3.3.2. Preparation of the Aqueous Extract

The powdered leaves were allowed to boil for one hour after mixing it with water at the ratio of 1:10(100g powder per 1000ml water).The supernatant was strained. The remaining was then boiled with 500 ml water and this process was repeated three times. The supernatant so collected was further boiled for one hour in order to make it a thick pasty material. The final yield of the aqueous extract was about 30g (30 percent) in case of *Scoparia dulcis* and 10 g (10 percent) in case of *Costus pictus*. The crude extract thus obtained was stored in a refrigerator at 4°C till used for treatment (Plate 3). A weighed quantity of the crude extract was homogenized with 5 percent Gum acacia and was administered orally to individual rats for 60 days at the dose of 500mg/kg b.w every day at 0900 hours.

3.3.3. Preparation of the Alcoholic Extract

The powdered leaves (100g) were subjected to the extraction using methanol (1000ml) in 1:10 ratio in a Soxhlet apparatus .The liquid extract so obtained was collected in a wide mouthed vessel and the solvent was evaporated by keeping them in a water bath at temperature of 70°C so as to obtain a semisolid residue. The yield of the extract was 12 percent and 5 percent for *Scoparia dulcis* and *Costus pictus*, respectively. The crude extract thus prepared was kept in the refrigerator at 4°C till used for treatment (Plate 3). A weighed quantity of the crude extract was homogenized with 5 percent Gum acacia and was administered orally to the individual rats for 60 days at the dose of 500mg/kg b.w.

3.3.4. Glibenclamide

Tablet Daonil® (5 mg) was powdered and given orally at the rate of 0.5mg/animal/day for 60 days.

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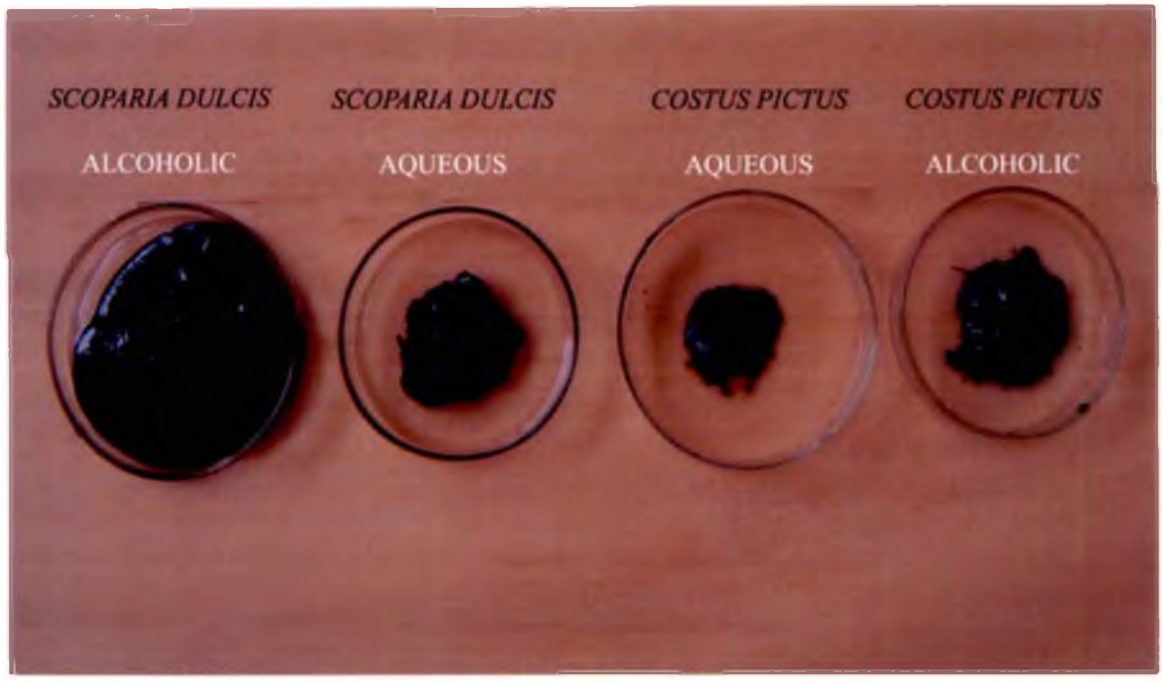

PLATE 3. EXTRACTS OF *SCOPARIA DULCIS* AND *COSTUS PICTUS* LEAVES

3.4. EXPERIMENTAL DESIGN

Diabetes was induced five days before the commencement of the actual experiment on sixty four rats. On the fifth day of induction, the diabetic rats were randomly divided into eight groups of eight rats each.

Group I	Normal rats or control.
Group II	Diabetic control.
Group III	Diabetic, fed with aqueous extract of *Scoparia dulcis* leaves (SDAEt) @ 500mg/kg b.w orally for 60 days.
Group IV	Diabetic, fed with alcoholic extract of *Scoparia dulcis* leaves (SDAlEt) @ 500mg/kg b.w orally for 60 days.
Group V	Diabetic, fed with aqueous extract of *Costus pictus* leaves (CPAEt) @ 500mg/kg b.w orally for 60 days.
GroupVI	Diabetic, fed with alcoholic extract of *Costus pictus* leaves (CPAlEt) @ 500mg/kg b.w orally for 60 days.
Group VII	Diabetic, fed combinedly with aqueous extract of *Scoparia dulcis* (@ 250 mg/kg b.w) and *Costus pictus* (@ 250 mg/kg b.w) leaves (SDAEt + CPAEt).
Group VIII	Diabetic, fed combinedly with alcoholic extract of *Scoparia dulcis* (@ 250 mg/kg b.w) and *Costus pictus* (@ 250 mg/kg b.w) leaves (SDAlEt + CPAlEt).
Group IX	Diabetic, fed normal feed with a standard antidiabetic drug glibenclamide @ 0.5 mg/kg b.w for 60 days.

Body weight was recorded on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day. All the chemicals used in this experiment were of high quality analytical grade. Anthrone reagent was procured from BDH, England.Alloxan monohydrate was obtained from S.D.Fine Chemicals, Mumbai. All other chemicals used in this experiment were from Merck Co, Mumbai.

3.5. COLLECTION OF BIOLOGICAL SAMPLES

Blood was collected from retro orbital plexus of the inner eye canthus under light ether anaesthesia using sodium heparinized capillary tubes (microhaematocrit capillaries). Blood (0.5 ml) was collected in fresh vials containing sodium fluoride (10mg/ml blood) meant for plasma glucose level (PGL). A volume of 2ml blood was also collected in vials containing disodium salt of Ethylene Diamine Tetra Acetic (EDTA, 1mg/ml) as anticoagulant meant for the estimation of total erythrocyte count, total leucocyte count, total haemoglobin and glycosylated haemoglobin (HBA1c) on zero,7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day of the experiment. From the blood collected plasma was separated by centrifugation (3000 rpm for 10 min) and used for estimation of biochemical parameters like alanine amino transferase (ALT) and aspartate amino transferase (AST), Total lipids, Total cholesterol, Triglycerides, HDL-cholesterol, LDL-cholesterol, Total protein, Albumin, Globulin. On 60^{th} day the rats were sacrificed and the liver sample was collected for liver glycogen estimation.

3.6. ESTIMATION OF HAEMATOLOGICAL PARAMETERS

3.6.1. Total Erythrocyte Count

The erythrocytes were counted using standard dilution technique using Hayme's fluid diluent and haemocytometer (Benjamin, 1985).

3.6.2. Total Leucocyte Count

The leucocytes were counted by standard dilution technique using Thomas fluid diluent and haemocytometer (Benjamin, 1985).

3.6.3. Estimation of Haemoglobin

The haemoglobin concentration was estimated using acid haematin method (Benjamin, 1985).

3.7. ESTIMATION OF BIOCHEMICAL PARAMETERS

3.7.1. Estimation of Glycosylated Haemoglobin (HBA $_1$c)

Glycosylated haemoglobin (HBA_1c) was estimated by the method of SudhakarNayak and Pattabiraman (1981) with modifications according to Bannon (1982).

Reagents:

a. Citrate Buffer 0.1M (pH 6.5): 1.92 g of citric acid and 2.3 g of trisodium citrate were dissolved in 100 ml separately and the pH was adjusted to 6.5.
b. 1 M Oxalate in 2 M HCl: 13.2 g of oxalic acid was dissolved in 100ml of water containing 6.75 ml of 2 M HCl.
c. Phenol-80 percent
d. Concentrated Sulphuric acid
e. Trichloroacetic acid (TCA) -40 percent
f. Fructose Stock standard (1mg/ml): 100 mg of fructose was dissolved in 100 ml of water.
g. Fructose working standard (100µg/ml): 10 ml of stock was diluted to 100 ml with distilled water.

Procedure:

After separation of plasma from 2ml of blood the sediment was washed with normal saline and centrifuged at 3000 rpm for 30 min to obtain the packed cells. To 0.5 ml of packed cell, 5 ml of citrate buffer was added, mixed and incubated at 37°C for 15 min, centrifuged and the supernatant was discarded. Then 0.5ml of saline was added, mixed and processed for the estimation. To this aliquot, 4.0 ml of oxalate (1M) in HCl (2M) solution was added, mixed and heated at 100°C for 4 hours, cooled and precipitated with 2.0ml of 40 percent TCA. It was then centrifuged and to the supernatant 0.05 ml of 80 percent phenol and 3.0

ml of conc.sulphuric acid was added. A set of standards (10-50mg) was also treated in the similar manner. The optical density (OD) of colour developed was read at 480 nm absorbance after 30 min.

Calculation:

$$\text{Conc. of } HBA_{1}c \text{ (mg/g Hb)} = \frac{\text{OD of the sample}}{\text{OD of the standard}} \times \text{Conc. of Std/ Conc. of Hb}$$

The values were expressed as mg/g haemoglobin.

3.7.2. Plasma Glucose

Plasma glucose was estimated by the GOD-PAP method (Trinder, 1969) using kit from Agape Diagnostics, Maharashtra.

3.7.3. Liver Glycogen

Liver Glycogen was estimated by Anthrone method (Narasimhan, 1971).

Principle:

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

Reagents:

a. **30 percent 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.

b. **95 percent Sulphuric Acid**: Mixed 950 ml of concentrated sulphuric acid with 50 ml of distilled water and cooled.

c. **0.2 percent Anthrone reagent**: The reagent was prepared by dissolving 0.2g anthrone in 100ml of 95 percent sulphuric acid. The reagent was prepared fresh whenever required.

d. **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 500ml with distilled water.

Procedure:

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

Sample: Five ml of digesta prepared at the end of second dilution of 50 ml.
Standard: Five ml of glucose working standard.
Blank: Five ml of distilled water.

The sample, standard and blank (five ml each in labeled 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 min 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.

Calculation:

$$\text{Liver glycogen (g percent)} = \frac{A_u}{A_s} \times \frac{100}{1.11} \times \frac{500}{5} \times \frac{100}{\text{Weight of tissue in g}} \times \frac{1}{1000000}$$

A_u = OD of unknown sample

A_s = OD of standard

Concentration of standard in μg = 100

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

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

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

3.7.4. Plasma Total Lipids

Plasma total lipids was estimated by phosphovainilline method (Zoeliner, 1962) using Labkit® kit (M/S Labkit, Spain).

3.7.5. Plasma Cholesterol

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

3.7.6. Plasma Triglyceride

Triglyceride level in plasma was estimated by GPO-PAP method (Bucolo and David, 1973) using kit from Agappe Diagnostics, Maharashtra.

3.7.7. Plasma HDL-Cholesterol

HDL-cholesterol was estimated by precipitation method using phosphotungstate-Magnesium chloride (Bachorik *et al.*, 1976) using kit from Agappe Diagnostis, Maharashtra.

3.7.8 Plasma LDL-Cholesterol

The LDL-Cholesterol was derived using the Friedewald equation (Friedewald *et al.*1972).

$$\text{LDL-Cholesterol (mg/dl)} = \frac{\text{Total cholesterol} - (\text{HDL-Cholesterol} + \text{Triglycerides})}{5}$$

$$\text{LDL-Cholesterol (mmol/l)} = \frac{\text{Total cholesterol} - (\text{HDL-Cholesterol} + \text{Triglycerides})}{2.2}$$

3.7.9. Plasma VLDL-Cholesterol

Plasma VLDL –cholesterol was derived using the Friedewald equation (Friedewald *et al.*, 1972).

$$\text{VLDL-cholesterol} = \frac{\text{Triglycerides}}{5}$$

3.7.10. Plasma Total Protein

Plasma total protein was estimated by Biuret method (Lowry *et al.*, 1951) using kit from Agappe Diagnostics, Maharashtra.

3.7.11. Plasma Albumin

The albumin concentration in plasma was estimated by the method of Doumas *et al.* (1971) using kit from Agappe Diagnostics, Maharashtra.

3.7.12. Globulin

Globulin concentration was estimated using the following formula:

Globulin (g/dl) = Concentration of total protein (g/dl) – Concentration of Albumin (g/dl)

3.7.13. Albumin/Globulin Ratio

The albumin/globulin ratio was calculated using the following formula:

$$\text{Albumin/Globulin ratio} = \frac{\text{Concentration of Albumin (g/dl)}}{\text{Concentration of Globulin (g/dl)}}$$

3.7.14. Plasma Alanine AminoTransferase (ALT)

The Alanine Amino Transferase concentration was estimated by U-V kinetic method (Bergmeyer, 1974) using kit from Agappe Diagnostics, Maharashtra.

3.7.15. Plasma Aspartate Amino Transferase (AST)

The Aspartate Amino Transferase concentration of the sample was estimated by U-V kinetic method (Bergmeyer, 1974) using kit from Agappe Diagnostics, Maharashtra.

3.7.16. Trace Minerals

The trace minerals such as copper, iron and zinc were estimated using Atomic Absorption Spectrophotometer (Perkin Elmer Model No.3110).Standard conditions set in the atomic absorption spectrophotometer for the estimation of various elements were shown below (Beaty and Kerber, 1993).

Elements	Wavelength (nm)	Slit (nm)	Flame-gases	Sensitivity*check (mg/l)
Cu	324.70	0.70	Air-acetylene	4.0
Fe	248.30	0.20	Air-acetylene	6.0
Zn	213.90	0.70	Air-acetylene	1.0

3.8. STATISTICAL ANALYSIS

The data obtained were statistically analyzed by one-way ANOVA followed by Tukey's multiple comparison test as described by Snedecor and Cochran (1985).

Results

4. RESULTS

The present study was undertaken to evaluate the hypoglycaemic efficacy of *Scoparia dulcis* and *Costus pictus* in male albino rats. The results obtained were statistically analysed and presented in Figures 1 to 20.

4.1. EFFECT OF *SCOPARIA DULCIS* AND *COSTUS PICTUS* ON BODY WEIGHT

The normal control animals (Group I) did not show any significant difference with the diabetic control animals (Group II) until 7th day of treatment. On 14th, 28th, 56th and 60th day the body weight in Group II decreased significantly ($p \leq 0.001$) than Group I. The mean body weight obtained on zero, 7th, 14th, 28th, 56th and 60th day for Group I was 181.50 ± 5.02,187.75 ± 4.96, 197.63 ± 4.10, 207.63 ± 4.85, 221.63 ± 5.44 and 224.63 ± 5.70 g and that of Group II was 185.50 ± 4.87,180.25 ± 5.29, 174.50 ±.5.09, 165.25 ± 4.91, 149.13 ± 5.07, 144.88 ± 4.62 g, respectively.

After 28 days of treatment Groups III, IV, V, VI, VII, VIII and IX showed a significant ($p \leq 0.01$) increase in body weight compared to Group II and the mean body weight obtained on 28th day for Group II, III, IV, V, VI, VII, VIII and IX was 165.25 ± 4.9, 188.63 ± 4.50, 190.13 ± 3.90, 187.75 ± 2.42, 189.63 ± 3.55, 192.00 ± 3.81, 199.75 ± 3.61 and 189.38 ± 1.70 g, respectively. On 56th and 60th day Group III to IX showed a significant ($p \leq 0.001$) increase in body weight with respect to Group II. The mean body weights obtained are presented in Fig.1.

Group IX (treated with glibenclamide) did not show any significant difference when compared with other treatment groups on all treatment days. All the treatment groups (Group III to IX) showed a significant ($p \leq 0.001$) increase in body weight on 60th day over the zero day value (Fig. 1).

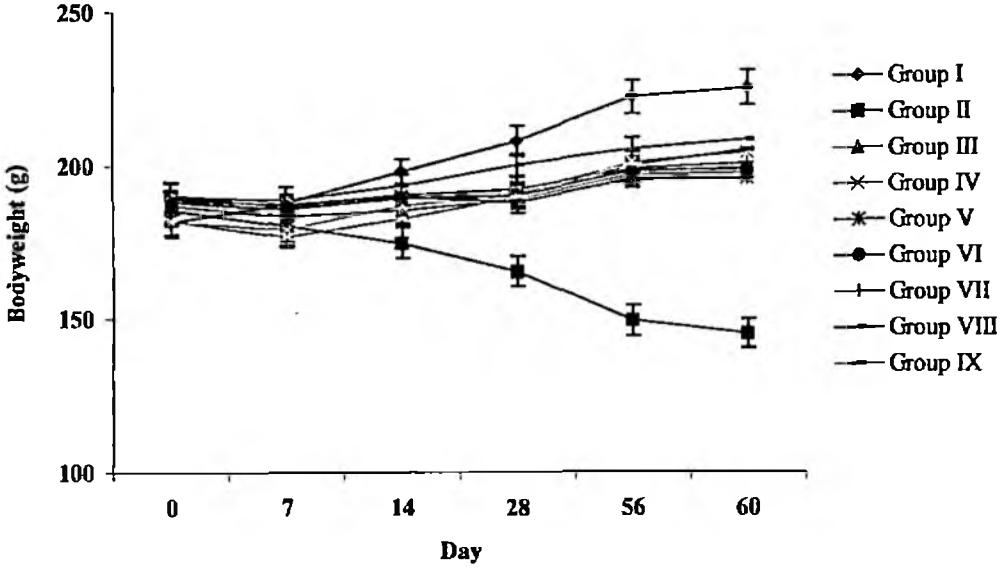

Fig. 1. Effect of *Scoparia dulcis* and *Costus pictus* on body weight (g) in rats

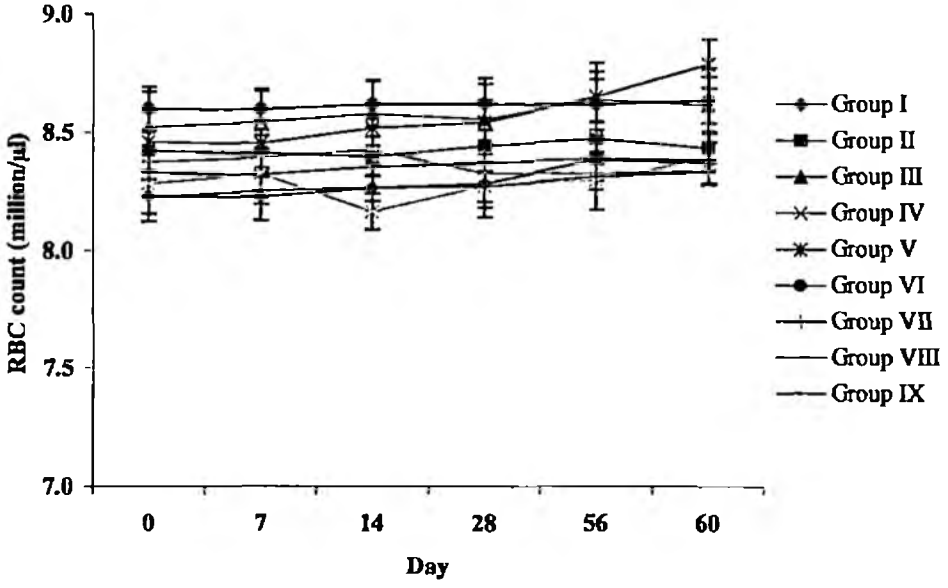

Fig. 2. Effect of *Scoparia dulcis* and *Costus pictus* on RBC count (million/µl) in rats

Mean ± SE, N=8, Group I- Normal control, Group II- Diabetic control, Group III- Diabetic, treated with SDAEt, Group IV- Diabetic, treated with SDAlEt, Group V- Diabetic, treated with CPAEt, Group VI- Diabetic, treated with CPAlEt, Group VII- Diabetic, treated with SDAEt + CPAEt, Group VIII- Diabetic, treated with SDAlEt + CPAlEt, Group IX- Diabetic, treated with glibenclamide.

The mean body weight obtained on 60th day for Group VIII (treated with SDAlEt + CPAlEt) was 208.00 ± 3.70 g, which was the maximum weight gain obtained among the treatment groups followed by Group IX (treated with glibenclamide) with a mean body weight of 204.50 ± 1.86g.The mean body weight obtained in descending order of weight gain for different treatment groups are Group IV (treated with SDAlEt), Group VII (treated with SDAEt + CPAEt), Group VI (treated with CPAlEt), Group III (treated with SDAEt), Group V (treated with CPAEt).The mean body weight of the treatment groups are presented in Fig.1.

4.2. EFFECT OF *SCOPARIA DULCIS* AND *COSTUS PICTUS* ON HAEMATOLOGICAL PARAMETERS

4.2.1. Effect on RBC and WBC Count

The mean RBC count for Group I was 8.23 ± 0.11, 8.23 ± 0.10, 8.26 ± 0.10, 8.28 ± 0.10, 8.38 ± 0.09 and 8.36 ± 0.09 million/µl on zero, 7th, 14th, 28th, 56th and 60th day, respectively. The mean RBC count for the treatment groups are presented in Fig. 2. The mean WBC count for Group I was 8.11 ± 0.07, 8.14 ± 0.06, 8.18 ± 0.07, 8.21 ± 0.06, 8.22 ± 0.07 and 8.23 ± 0.07 thousand/µl, respectively. The mean WBC count for the treatment groups are presented in Fig. 3. The RBC and WBC count did not differ significantly between any of the treatment groups and were maintained within the normal range. (Fig. 2 and 3).

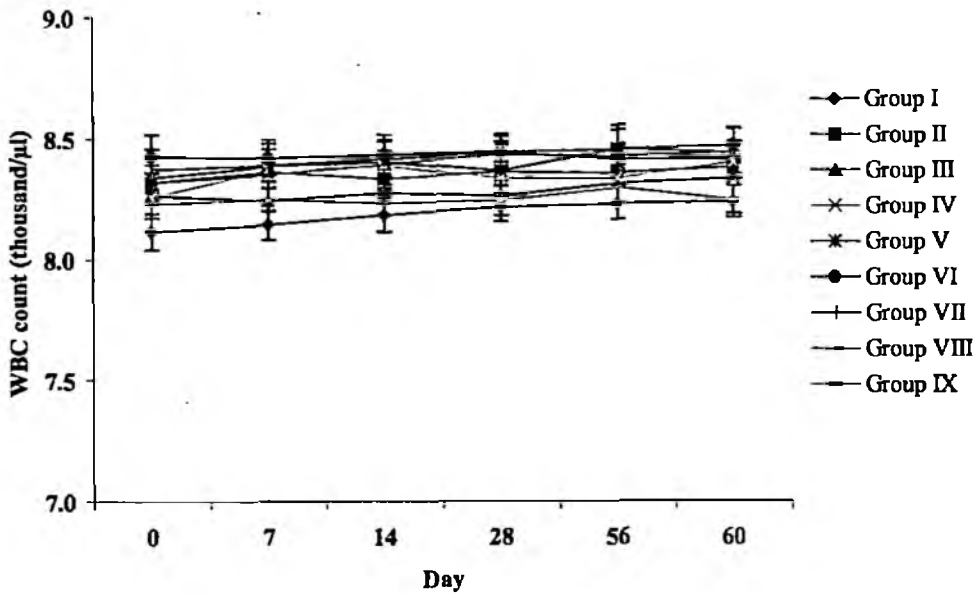

Fig. 3. Effect of *Scoparia dulcis* and *Costus pictus* on WBC count (thousand/µl) in rats

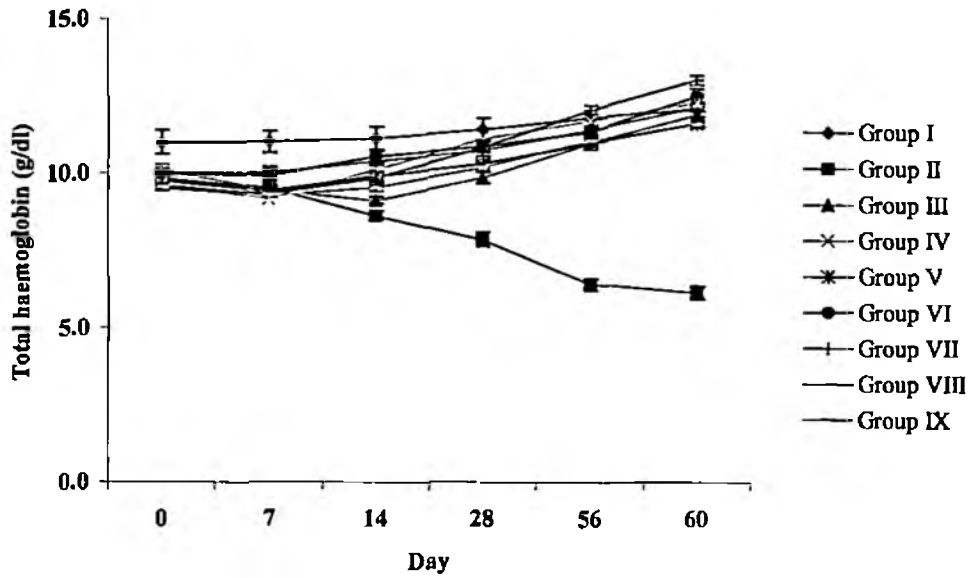

Fig. 4. Effect of *Scoparia dulcis* and *Costus pictus* on total haemoglobin (g/dl) level in rats

Mean ± SE, N=8, Group I- Normal control, Group II- Diabetic control, Group III- Diabetic, treated with SDAEt, Group IV- Diabetic, treated with SDAlEt, Group V- Diabetic, treated with CPAEt, Group VI- Diabetic, treated with CPAlEt, Group VII- Diabetic, treated with SDAEt + CPAEt, Group VIII- Diabetic, treated with SDAlEt + CPAlEt, Group IX- Diabetic, treated with glibenclamide.

4.2.2. Effect on Total Haemoglobin (Hb)

The total haemoglobin content decreased significantly ($p \leq 0.001$) in Group II with respect to Group I during the entire study period. The values obtained for Group I on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 10.99 ± 0.38, 11.01 ± 0.35, 11.11 ± 0.37, 11.39 ± 0.35, 11.74 ± 0.25 and 12.01 ± 0.24 g/dl respectively. The values within Group I did not differ significantly for the entire treatment period and were maintained within the normal range. Within Group II the total Hb values decreased significantly ($P \leq 0.01$) from 14^{th} day onwards with respect to zero day value. The values obtained for Group II on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 9.83 ± 0.13, 9.51 ± 0.11, 8.63 ± 0.15, 7.83 ± 0.23, 6.38 ± 0.21 and 6.13 ± 0.21 g/dl, respectively. There was a gradual decrease in Hb content from the zero day to the 60^{th} day of the experiment (Fig. 4).

The values obtained on zero day for Groups II, III, IV, V, VI, VII, VIII and IX were 9.83 ± 0.13, 10.09 ± 0.18, 9.69 ± 0.11, 9.76 ± 0.13, 10.00 ± 0.14, 10.00 ± 0.16, 9.59 ± 0.12 and 9.53 ± 0.11 g/dl, respectively and that on 7^{th} day were 9.51 ± 0.11, 9.45 ± 0.09, 9.16 ± 0.09, 9.43 ± 0.11, 9.93 ± 0.18, 10.03 ± 0.15, 9.35 ± 0.13 and 9.29 ± 0.10 g/dl, respectively (Fig.4). None of these groups showed any significant change till one week of treatment. Group II did not differ significantly with the treatment groups until 7^{th} day but differed significantly ($P \leq 0.001$) from 14^{th} day onwards. The mean total Hb values for Groups III, IV, V, VI, VII, VIII and IX on 14^{th} day were 9.11 ± 0.09, 10.10 ± 0.15, 9.88 ± 0.15, 10.53 ± 0.20, 10.39 ± 0.14, 9.84 ± 0.14 and 9.51 ± 0.10 g/dl, respectively and they were significantly ($P \leq 0.001$) higher than the Group II (8.63 ± 0.15 g/dl). From two weeks onwards till the end of study all the treatment groups (Group III to IX) showed a significant ($P \leq 0.001$) increase in total Hb value than the diabetic control (Group II). The mean total Hb values are presented in Fig. 4.

Within Group III (treated with *Scoparia dulcis* aqueous extract) the total Hb values differed significantly ($P \leq 0.05$) between zero and 14^{th} day in which there was a gradual reduction upto 14^{th} day from day zero value of 10.09 ± 0.18 g/dl to 9.11 ± 0.09 g/dl, respectively. From 28^{th} day onwards there was an increase in total Hb value but not significant between zero and 28^{th} day value. On 56^{th} day there was a significant ($P \leq 0.05$) increase in total Hb value, which was 10.94 ± 0.19g/dl over the zero day value of 10.09 ± 0.18 g/dl. On 60^{th} day the total Hb content showed a significant ($P \leq 0.001$) increase over the zero day value and the Hb concentration obtained on 60^{th} day was 11.85 ± 0.25 g/dl (Fig. 4)

In Group IV (treated with *Scoparia dulcis* alcoholic extract) there was a significant ($P \leq 0.05$) decrease in total Hb value from zero day to 7^{th} day. The values obtained were 9.69 ± 0.11 and 9.16 ± 0.09g/dl on zero and 7^{th} day, respectively. From 14^{th} day onwards there was a significant increase ($P \leq 0.001$) in total Hb concentration over the zero day. The values obtained were 10.10 ± 0.15, 11.09 ± 0.12, 11.65 ± 0.14 and 12.24 ± 0.13 g/dl on 14^{th}, 28^{th}, 56^{th} and 60^{th} day of treatment (Fig. 4).

In Group V (treated with *Costus pictus* aqueous extract) there was no significant difference upto 14^{th} day of treatment and showed a significant ($P \leq 0.001$) increase in total Hb value from 28^{th} day onwards. The values obtained were 10.29 ± 0.12, 10.93 ± 0.13 and 11.56 ± 0.10 g/dl on 28^{th}, 56^{th} and 60^{th} day respectively (Fig. 4).

Group VI (treated with *Costus pictus* alcoholic extract) showed a significant ($P \leq 0.001$) increase in total Hb concentration from 14^{th} day onwards over the zero day value. On 60^{th} day there was a maximum increase in total Hb value (12.45 ± 0.24 g/dl) when compared with zero day (Fig.4).

In Group VII (treated with aqueous extract of *Scoparia dulcis* and *Costus pictus*) there was a significant ($P \leq 0.001$) increase in total Hb values from 14^{th} day onwards. The values obtained were 10.00 ± 0.16, 10.03 ± 0.15, 10.39 ± 0.14, 10.70 ± 0.16, 11.34 ± 0.11 and 12.09 ± 0.14 g/dl on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day respectively. Group VII also showed a significant ($P \leq 0.001$) increase in total Hb value from 14^{th} day onwards till the end of the experiment over the zero day value (Fig. 4).

In Group VIII (treated with *Scoparia dulcis* and *Costus pictus* alcoholic extract) there was a significant ($P \leq 0.001$) increase in Hb value from 14^{th} day onwards. The total Hb concentration obtained on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 9.59 ± 0.12, 9.35 ± 0.13, 9.84 ± 0.14, 10.81 ± 0.16, 12.00 ± 0.15 and 12.96 ± 0.16 g/dl respectively (Fig. 4).

In Group IX there was a significant ($P \leq 0.001$) increase in total Hb value from 14^{th} day onwards (Fig. 4). The values obtained were 9.53 ± 0.11, 9.29 ± 0.10, 9.51 ± 0.10, 10.18 ± 0.15, 11.03 ± 0.14 and 11.54 ± 0.11 g/dl, respectively on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day. On 14^{th} day only Group VI and VII showed a significant ($P \leq 0.01$) increase in total haemoglobin value than Group IX. On 28^{th} day Group IV showed a significant ($P \leq 0.01$) increase in total Hb value than Group IX. On 56^{th} day only Group VIII showed a significant ($p \leq 0.01$) increase in total Hb with respect to Group IX. Group IX showed a significant ($P \leq 0.01$) decrease in total Hb value than Group VI and VIII on 60^{th} day. The mean value obtained on 60^{th} day for Groups VI, VIII and IX were 12.45 ± 0.24, 12.96 ± 0.16 and 11.54 ± 0.11 g/dl, respectively.

In terms of absolute total Hb values obtained on 60^{th} day Group VIII (treated with SDAlEt + CPAlEt) showed the maximum total Hb content among the treatment groups (III to IX), followed by Group VI (treated with CPAlEt). The descending order of total Hb content for other treatment groups are Group IV (12.24 ± 0.13 g/dl),

Group VII (12.09 ± 0.14 g/dl), Group III (11.85 ± 0.25 g/dl), Group V (11.56 ± 0.10) and Group IX (11.54 ± 0.11 g/dl).

4.3. EFFECT OF *SCOPARIA DULCIS* AND *COSTUS PICTUS* ON BIOCHEMICAL PARAMETERS

4.3.1. Effect on Glycosylated Haemoglobin (HBA1c)

The diabetic control animals (Group II) showed a significant ($p \leq 0.001$) increase in glycosylated haemoglobin on all treatment days than the normal control animals (Group I). The mean values of glycosylated haemoglobin obtained on zero, 7th, 14th, 28th, 56th and 60th day for Group I were 0.17 ± 0.01,0.18 ± 0.02,0.19 ± 0.01, 0.20 ± 0.01, 0.20 ± 0.01, 0.23 ± 0.04 mg/gHb, respectively. Within Group II there was a significant ($P \leq 0.001$) increase in glycosylated haemoglobin on 56th and 60th day when compared with zero day value. The mean value of glycosylated haemoglobin obtained for Group II was 0.39 ± 0.03,0.62 ± 0.14,0.68 ± 0.10, 0.78 ± 0.11,0.88 ± 0.07 and 0.92 ± 0.06 mg/gHb, respectively on zero, 7th, 14th, 28th, 56th and 60th day (Fig.5).

The treatment groups (Group III to IX) did not show any significant difference with Group II until 14th day of treatment. From 28th day onwards Group III, IV, V, VI, VIII and IX showed a significant ($p \leq 0.001$) decrease in glycosylated haemoglobin when compared to Group II. The mean values obtained on 28th day for Groups II, III, IV, V, VI, VIII and IX were 0.78 ± 0.11, 0.48 ± 0.02, 0.43 ± 0.03, 0.49 ± 0.05, 0.47 ± 0.04, 0.39 ± 0.03 and 0.37 ± 0.03 mg/gHb, respectively. On 56th and 60th day the treatment groups (Group III to IX) showed a significant ($p \leq 0.001$) decrease in glycosylated haemoglobin than the Group II. The mean values of glycosylated haemoglobin obtained are presented in Fig. 5. All the treatment groups

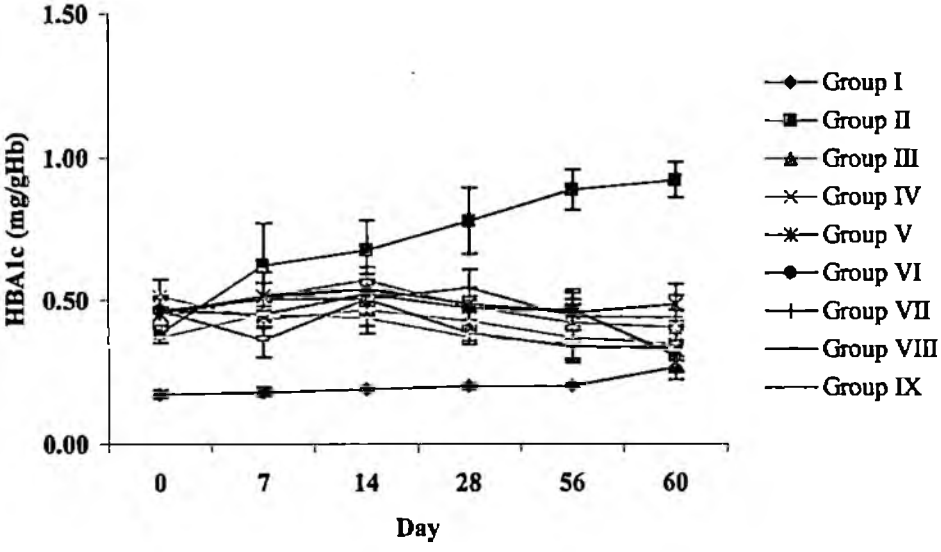

Fig.5. Effect of *Scoparia dulcis* and *Costus pictus* on HBA1c (mg/gHb) in rats

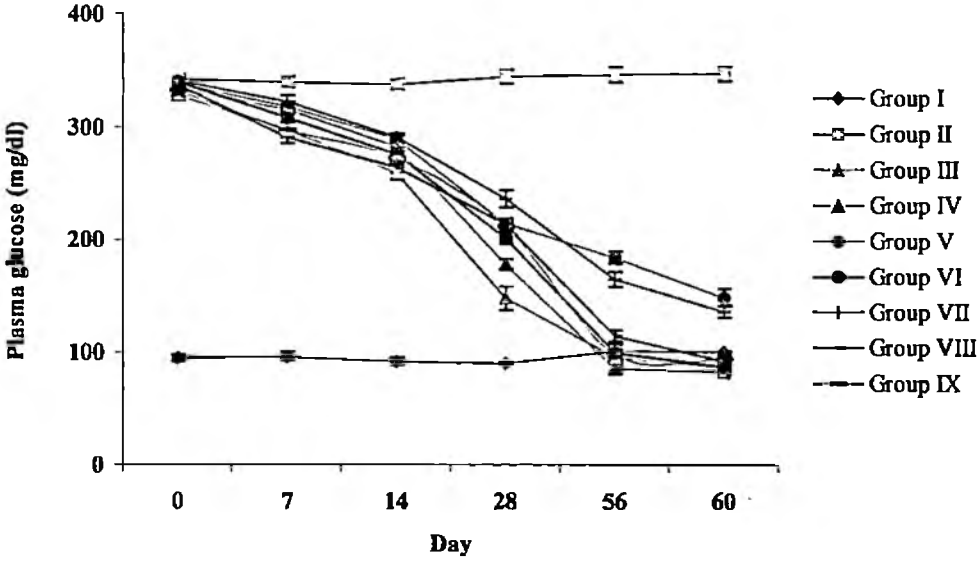

Fig 6. Effect of *Scoparia dulcis* and *Costus pictus* on plasma glucose (mg/dl) level in rats

Mean ± SE, N=8, Group I- Normal control, Group II- Diabetic control, Group III- Diabetic, treated with SDAEt, Group IV- Diabetic, treated with SDAlEt, Group V- Diabetic, treated with CPAEt, Group VI- Diabetic, treated with CPAlEt, Group VII- Diabetic, treated with SDAEt + CPAEt, Group VIII- Diabetic, treated with SDAlEt + CPAlEt, Group IX- Diabetic, treated with glibenclamide.

showed a general tendency of decrease in glycosylated haemoglobin upto 60 days of treatment when compared with zero day level. There was no significant difference between the treatment groups when compared with Group IX (Fig. 5).

After 60 days, Group VI (treated with CPAIEt) showed the minimum level of HBA1c in terms of absolute value followed by Group IX (treated with glibenclamide). The descending order of HBA1c level among other treatment groups are Group VIII (0.34 ± 0.09mg/gHb), Group IV (0.35 ± 0.06 mg/gHb), Group III (0.40 ± 0.06 mg/gHb), Group VII (0.44 ± 0.08 mg/gHb), Group V (0.48 ± 0.07 mg/gHb).

4.3.2. Effect on Plasma Glucose

The plasma glucose level of normal control animals (Group I) did not differ significantly between the treatment days for the entire study and was maintained within the normal range. The diabetic control animals (Group II) showed a significant ($P \leq 0.001$) increase in plasma glucose concentration with respect to Group I for the entire study period (Fig. 6). The mean values of plasma glucose for Group I on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 94.38 ± 2.77, 95.63 ± 3.76, 91.00 ± 3.36, 89.75 ± 1.69, 100.13 ± 2.31, 99.50 ± 1.08 mg/dl, respectively. Group II did not show any significant difference between the treatment days even though there was a steady increase in plasma glucose level. The mean values of plasma glucose for Group II on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 342.00 ± 4.11, 339.63 ± 4.86, 336.88 ± 4.73, 343.75 ± 5.75, 345.13 ± 6.41 and 345.88 ± 6.18 mg/dl, respectively.

The treatment groups (Group III to IX) did not differ significantly with Group II on day zero in plasma glucose level in which all the groups showed an increase in plasma glucose level with a mean value of 342.00 ± 4.11, 328.13 ± 5.09, 332.38 ± 6.45, 337.63 ± 5.02, 339.63 ± 4.98, 339.88 ± 5.62, 337.13 ± 5.33 and 332.25 ± 5.87

mg/dl, respectively for Groups II, III, IV, V, VI, VII, VIII and IX. On 7th day Group III, VI, VIII and IX showed a significant ($p \leq 0.001$) decrease in plasma glucose level than Group II with a mean plasma glucose level of 339.63 ± 4.86, 297.25 ± 6.62, 307.88 ± 4.33, 290.63 ± 5.45 and 297.50 ± 6.09 mg/dl, respectively for Groups II, III, VI, VIII and IX. All the treatment groups showed a significant ($p \leq 0.001$) decrease in plasma glucose from 14th day onwards with respect to Group II, till the end of the study (Fig. 6).

Within Group III there was a significant ($P \leq 0.001$) decrease in plasma glucose level from 7th day till the end of the experimental period (Fig. 6). On 60th day the plasma glucose level was 86.38 ± 2.24, which was brought back from the elevated level of 328.13 ± 5.09 mg/dl on day zero. The values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 328.13 ± 5.09, 297.25 ± 6.62, 259.25 ± 7.31, 147.38 ± 10.10, 94.25 ± 4.13 and 86.38 ± 2.24 mg/dl, respectively.

Within Group IV there was a significant ($P \leq 0.001$) decrease in plasma glucose value from 14th day onwards till the end of the study. On 60th day the plasma glucose level was brought back to the normal level with a mean value of 82.50 ± 2.65 mg/dl. The values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 332.38 ± 6.45, 315.25 ± 4.25, 282.00 ± 4.0.4, 178.00 ± 4.37, 84.88 ± 4.34, 82.50 ± 2.65 mg/dl, respectively (Fig. 6).

Within Group V there was a significant ($P \leq 0.001$) decrease in plasma glucose value from 14th day onwards till the end of the study period. The values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 337.63 ± 5.02, 317.80 ± 4.38, 288.80 ± 3.33, 214.50 ± 4.12, 183.75 ± 5.33 and 148.13 ± 7.96 mg/dl, respectively.

Within Group VI there was a significant ($P \leq 0.001$) decrease in plasma glucose level from 7th day onwards when compared to zero day value. The mean plasma glucose level obtained on zero, 7th, 14th, 28th, 56th and 60th day were 339.63 ±

4.98, 307.88 ± 4.33, 275.75 ± 5.52, 201.13 ± 4.90, 99.13 ± 6.70 and 86.50 ± 3.97mg/dl, respectively (Fig. 6). The value obtained on 60th day was close to the normal plasma glucose level in rats.

Within Group VII the plasma glucose value showed a significant (P ≤0.001) decrease from 14th day onwards when compared to zero day value. The mean plasma glucose values obtained were 339.88 ± 5.62, 321.88 ± 5.53, 290.63 ± 3.38, 235.50 ± 7.51, 164.25 ± 6.35 and 135.63 ± 5.69 mg/dl respectively on zero, 7th, 14th, 28th, 56th and 60th day (Fig.6).

Within Group VIII there was a significant (P ≤0.001) decrease in plasma glucose level from 7th day onwards with respect to zero day value. The mean plasma glucose values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 337.13 ± 5.33, 290.63 ± 5.45, 263.88 ± 5.13, 213.25 ± 3.97, 113.63 ± 5.06 and 90.88 ± 5.01 mg/dl, respectively. The plasma glucose value restored to the normal concentration on 60th day.

Within the Group IX there was a significant (P ≤0.001) decrease in plasma glucose level from 7th day onwards with respect to zero day value. The mean plasma glucose values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 332.25 ± 5.89. 297.50 ± 6.09, 272.38 ± 5.39, 211.75 ± 3.21, 98.25 ± 7.86 and 92.75 ± 1.98 mg/dl respectively. Group IX did not differ significantly until 14th day with other treatment groups but showed a significant (P ≤0.001) increase in plasma glucose value than Group III and IV on 28th day. On 56th day Group IX showed a significant (P≤0.001) reduction in plasma glucose value than the Group V and VII with a mean value of 183.75 ± 5.33 and 164.25 ± 6.35 mg/dl, respectively. On 60th day also Group IX had a significantly (P ≤0.001) lower value than the Group V and VII with a mean plasma glucose level of 148.13 ± 7.96 and 135.63 ± 5.69 mg/dl, respectively. The mean value of plasma glucose for Group IX on 60th day was 92.75 ± 1.98 mg/dl.

Group IV (treated with SDAlEt) showed the minimum plasma glucose level on 60th day when compared to zero day level in terms of absolute value followed by Group III (treated with SDAEt). The mean plasma glucose values are presented in Fig. 6. The values in descending order of minimal increase in plasma glucose level among other treatment groups are Group VI (86.50 ± 3.97 mg/dl), Group VIII (90.88 ± 5.01 mg/dl), Group IX (92.75 ± 1.98 mg/dl), Group VII (135.63 ± 5.69 mg/dl) and Group V (148.13 ± 7.96 mg/dl).

4.3.3. Effect of *Scoparia Dulcis* and *Costus Pictus* on Plasma Lipid Profile

4.3.3.1. Effect on plasma total lipid

The normal control animals (Group I) did not differ significantly between the treatment days and the mean plasma total lipid values obtained were 104.643 ± 4.27, 105.50 ± 3.43, 106.75 ± 2.87, 110.88 ± 3.58, 109.00 ± 2.83 and 112.38 ± 2.02 mg/dl on zero, 7th, 14th, 28th, 56th and 60th day, respectively. The diabetic control animals (Group II) showed a significant ($P \leq 0.001$) increase in plasma total lipids than Group I on all the treatment days. Eventhough in Group II there was no significant difference in plasma total lipid levels between the treatment days, it increased from day zero to 60 days. The values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 156.13 ± 2.87, 161.25 ± 4.17, 165.00 ± 3.93, 166.38 ± 3.41, 169.38 ± 2.98 and 169.25 ± 33.14 mg/dl, respectively (Fig. 7).

The treatment groups did not differ significantly in plasma total lipid value with Group II until 7th day. On 14th day there was a significant ($P < 0.001$) reduction in plasma total lipids in Group V, VII, VIII and IX than the Group II (Diabetic control). The mean plasma total lipid values obtained on 14th day for Group II, V, VII, VIII and IX were 165.00 ± 3.93, 135.88 ± 3.23, 141.38 ± 3.07, 149.00 ± 2.91 and

149.63 ± 3.69 mg/dl. From 28th day onwards the treatment groups (Group III to IX) showed a significant (P ≤0.001) decrease in plasma total lipids than the Group II. The mean value of plasma total lipid obtained for Group II, III, IV, V, VI, VII, VIII and IX on 28th day was 166.38 ± 3.41, 140.75 ± 3.37, 128.63 ± 3.23, 119.50 ± 2.35, 117.88 ± 2.07, 113.50 ± 3.16, 116.63 ± 1.26 and 127.88 ± 3.80 mg/dl, respectively. On 56th and 60th day there was a significant (P ≤0.001) decrease in plasma total lipids in all the treatment groups (Group III to IX) with respect to Group II. The mean plasma total lipid values obtained are represented in Fig. 7.

Within Group III there was a significant (P≤ 0.001) decrease in plasma total lipid from 28th day onwards. On 60th day the mean plasma total lipid value obtained was 114.13 ± 2.81 mg/dl (Fig. 7).

Within Group IV there was a significant (P≤ 0.001) decrease in plasma total lipid level from 28th day onwards. The mean plasma total lipid obtained on 60th day was 96.25 ± 1.97 mg/dl.

Within Group V there was a significant (P ≤0.001) reduction in plasma total lipids from 14th day onwards. The mean plasma total lipid value obtained on 60th day was 98.75 ± 1.90 mg/dl (Fig.7).

Within Group VI, there was a significant (P ≤0.001) decrease in plasma total lipid value from 14th day onwards. The mean plasma total lipid value obtained on 60th day was 86.50 ± 1.87 mg/dl.

Within Group VII there was a significant (P ≤0.001) decrease in plasma lipid from 14th day onwards. The total plasma lipid value obtained on 60th day was 100.38 ± 2.49 mg/dl.

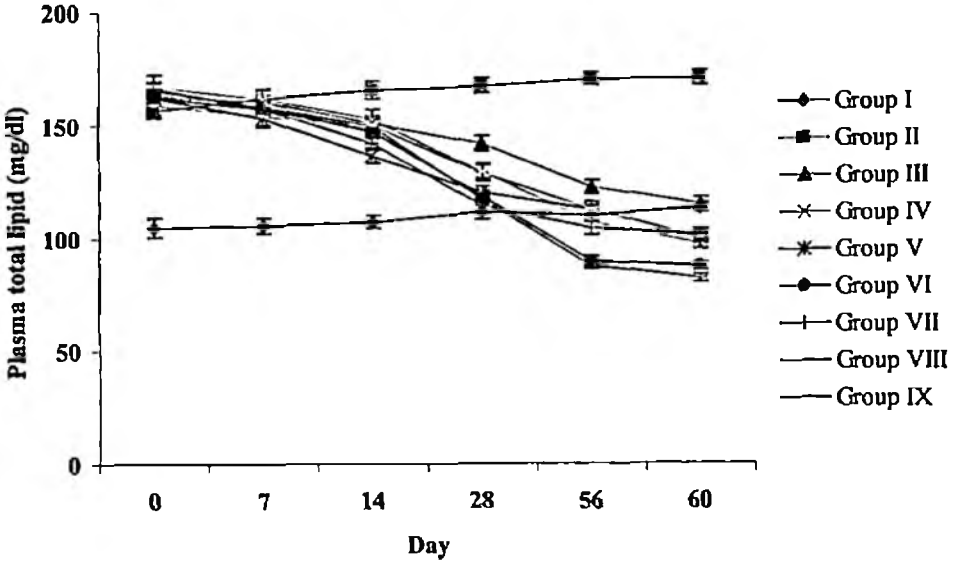

Fig. 7.Effect of *Scoparia dulcis* and *Costus pictus* on plasma total lipid (mg/dl) level in rats

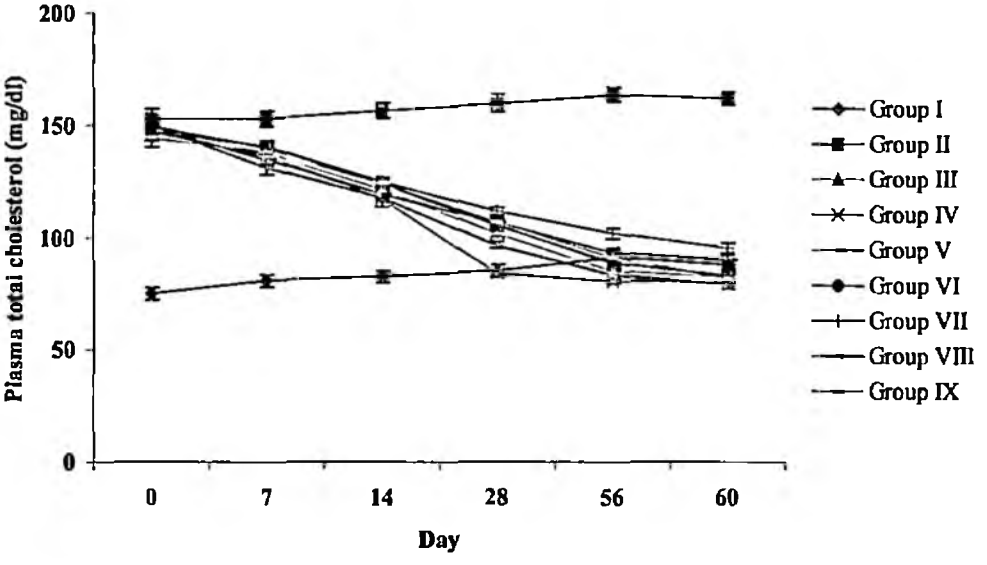

Fig. 8. Effect of *Scoparia dulcis* and *Costus pictus* on plasma total cholesterol (mg/dl) level in rats

Mean ± SE, N=8, Group I- Normal control, Group II- Diabetic control, Group III- Diabetic, treated with SDAEt, Group IV- Diabetic, treated with SDAlEt, Group V- Diabetic, treated with CPAEt, Group VI- Diabetic, treated with CPAlEt, Group VII- Diabetic, treated with SDAEt + CPAEt, Group VIII- Diabetic, treated with SDAlEt + CPAlEt, Group IX- Diabetic, treated with glibenclamide.

Within Group VIII there was a significant (P ≤0.001) decrease in plasma total lipid value from 14th day onwards. The mean value of plasma total lipid obtained on 60th day was 81.50 ± 1.66 mg/dl.

Within the Group IX there was a significant (P ≤0.001) decrease in plasma total lipid from 28th day onwards. The mean plasma total lipid obtained on zero, 7th, 14th, 28th, 56th and 60th day were 158.75±4.01, 153.75 ± 3.96, 149.63 ± 3.659, 127.88 ± 3.80, 111.88 ± 3.16 and 98.75 ± 1.95 mg/dl, respectively (Fig. 7). The Group IX did not differ significantly between the treatment groups in plasma total lipids until 28th day of treatment. On 56th and 60th day Group VI and VIII showed a significant (P ≤0.001) decrease in plasma total lipid than Group IX. The mean plasma total lipid values are represented in Fig.7.

In terms of absolute plasma total lipid value on 60th day Group VIII (treated with SDAIEt + CPAIEt) showed the minimum plasma total lipid value followed by Group VI (treated with CPAIEt). The descending order of plasma total lipid level among other treatment groups on 60th day are Group IV (96.25 ± 1.97 mg/dl), Group IX (98.75 ± 1.95 mg/dl), Group V (98.75 ± 1.90 mg/dl), Group VII (100.38 ± 2.49 mg/dl) and Group III (114.13 ± 2.81 mg/dl).

4.3.3.2. Effect on plasma total cholesterol

The diabetic control animals (Group II) showed a significant (P ≤0.001) increase in plasma total cholesterol than the normal control animals (Group I) on all the treatment days. Within Group I there was no significant difference between the treatment days and was maintained in the normal range. The mean plasma total cholesterol value obtained for Group I was 75.00 ± 2.58, 80.75 ± 2.63, 82.50 ± 2.70, 85.00 ± 2.52, 90.63 ± 2.81 and 87.63 ± 1.63 mg/dl, respectively on zero, 7th, 14th, 28th, 56th and 60th day. Within Group II there was no significant difference in plasma

total cholesterol value between the treatment days though there was an increase in the plasma total cholesterol level from zero to 60th day.

The treatment groups (Group III to IX) did not differ significantly with Group II on day zero. On 7th day the Groups III, IV, V, VIII and IX showed a significant ($P \leq 0.05$) decrease in plasma total cholesterol value than Group II. The mean plasma total cholesterol value obtained on 7th day for Group II, III, IV, V, VIII and IX are represented in Fig.8. From 14th day onwards all the treatment groups (Group III to IX) showed a significant ($P \leq 0.001$) decrease in plasma total cholesterol value than Group II. On 14th day the mean plasma total cholesterol values of Groups III, IV, V, VI, VII, VIII and IX were 121.13 ± 2.63, 117.13 ± 3.61, 119.00 ± 2.06, 124.00 ± 1.76, 124.63 ± 1.94, 117.50 ± 1.79 and 123.88 ± 2.00 mg/dl, respectively. On 28th day there was a significant ($P \leq 0.001$) decrease in plasma total cholesterol value in Groups III, IV, V, VI, VII, VIII and IX than Group II, with a mean plasma total cholesterol value of 101.38 ± 2.00, 85.88 ± 1.72, 106.50 ± 2.13, 105.00 ± 2.58, 111.13 ± 1.74, 96.25 ± 1.50 and 106.25 ± 2.19 mg/dl respectively. On 56th and 60th day also there was a significant ($P \leq 0.001$) decrease in plasma total cholesterol value in all the treatment groups (Group III to IX) than Group II and the mean plasma total cholesterol value are presented in Fig.8.

Within Group III there was a significant ($P \leq 0.001$) decrease in plasma total cholesterol value from 7th day onwards with respect to zero day value. On 60th day the mean plasma total cholesterol value obtained was 82.00 ± 2.66 mg/dl.

Within Group IV there was a significant reduction ($P \leq 0.001$) in plasma total cholesterol values from 14th day onwards. The mean plasma total cholesterol value obtained on 60th day was 79.63 ± 0.97 mg/dl. The mean plasma total cholesterol values obtained for different treatment days for Group IV are represented in Fig.8.

Within Group V there was a significant ($P \leq 0.001$) decrease in plasma total cholesterol level from 7th day onwards. On 28th day there was a maximum reduction plasma total cholesterol level with a mean value of 106.50 ± 2.13 mg/dl. The mean plasma total cholesterol obtained on zero, 7th, 14th, 28th, 56th and 60th days were 149.13 ± 2.97, 134.63 ± 2.65, 119.00 ± 2.06, 106.80 ± 2.13, 92. 63 ± 2.38 and 89.63 ± 1.98 mg/dl (Fig. 8).

Within Group VI there was a significant ($P \leq 0.001$) decrease in plasma total cholesterol value from 14th day onwards. The mean plasma total cholesterol level obtained on zero, 7th, 14th, 28th, 56th and 60th days were 148.88 ± 3.12, 139.88 ± 2.67, 124.00 ± 1.76, 105.00 ± 2.58, 88.50 ± 2.06 and 82.50 ± 1.60 mg/dl, respectively.

Within Group VII there was a significant ($P \leq 0.001$) decrease in plasma total cholesterol value from 14th day onwards. The mean value of plasma total cholesterol obtained on zero, 7th, 14th, 28th, 56th and 60th days were 147.00 ± 2.84, 140.38 ± 2.62, 124.63 ± 1.94, 111.13 ± 1.74, 101.25 ± 2.35 and 94.75 ± 2.19 mg/dl, respectively (Fig.8).

Within Group VIII there was a significant ($P \leq 0.001$) decrease in plasma total cholesterol level from 7th day onwards. The value obtained on 60th day was 78.38 ± 1.53 mg/dl. The mean plasma total cholesterol values obtained for Group VIII are presented in Fig. 8.

Within Group IX there was a significant ($P \leq 0.001$) decrease in plasma total cholesterol from 14th day onwards. The mean plasma total cholesterol value obtained on 60th day was 81.38 ± 2.19 mg/dl. Group IX did not show any significant difference between the treatment groups (Group III to VIII) until 14th day of treatment but showed a significant ($P \leq 0.01$) increase in plasma total cholesterol on 28th day with Group IV. The mean value of total plasma cholesterol obtained on 28th day for Group IV and IX were 83.88 ± 1.72 and 106.25 ± 2.19 mg/dl. On 56th day it did not show

any significant difference with any of the treatment groups. On 60th day it showed a significant ($P \leq 0.001$) decrease in plasma total cholesterol value than Group VII (94.75 ± 2.19 mg/dl).

After 60 days of study, Group VIII (treated with SDAlEt + CPAlEt) showed the minimum plasma total cholesterol level in terms of absolute value, followed by Group IV (treated with SDAlEt). The descending order of plasma total cholesterol level among other treatment groups on 60th day are Group IX (81.38 ± 2.19 mg/dl), Group III (82.00 ± 2.66 mg/dl), Group VI (82.50 ± 1.60 mg/dl), Group V (89.63 ± 1.98 mg/dl) and Group VII (94.75 ± 2.19 mg/dl).

4.3.3.3. Effect on plasma triglyceride

The diabetic control animals (Group II) showed a significant ($P \leq 0.001$) increase in plasma triglyceride than Group I on all treatment days. There was no significant difference within Group I and were within the normal range. The mean plasma triglyceride values obtained for Group I on zero, 7th, 14th, 28th, 56th and 60th day were 58.25 ± 4.13, 59.38 ± 3.64, 64.13 ± 3.38, 59.75 ± 3.96, 66.50 ± 2.74 and 68.13 ± 1.87 mg/dl, respectively. Within Group II there was a significant ($P \leq 0.001$) increase in plasma triglyceride level from 14th day onwards when compared with zero day value. The mean plasma triglyceride values are presented in Fig.9.

All the treatment groups (Group III to IX) showed a significant ($P \leq 0.01$) increase in plasma triglyceride value than Group II on zero and 7th day. The mean plasma triglyceride values of the treatment groups are presented in Fig.9. On 14th day Groups IV and VIII showed a significant ($P \leq 0.001$) decrease in plasma triglyceride level than Group II. On 28th day all the treatment groups (Group III to IX) showed a significant ($P \leq 0.001$) decrease in plasma triglyceride level than Group II. The mean plasma triglyceride level obtained on 28th day were 139.50 ± 3.72, 106.38 ± 1.81, 94.50 ± 1.90, 103.88 ± 1.84, 102.25 ± 1.77, 107.13 ± 1.77, 90.50 ± 2.36 and 99.88 ±

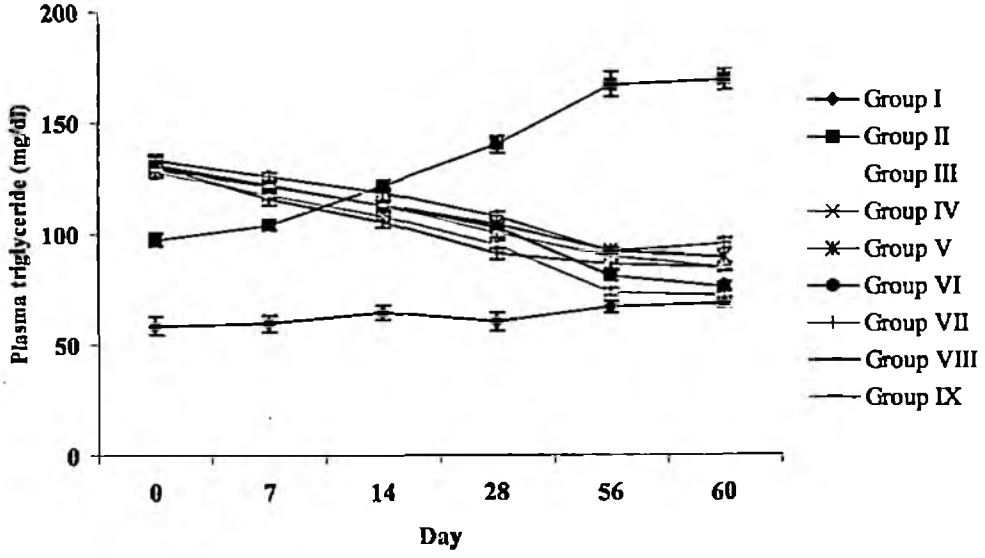

Fig. 9.Effect of *Scoparia dulcis* and *Costus pictus* on plasma triglyceride (mg/dl) level in rats

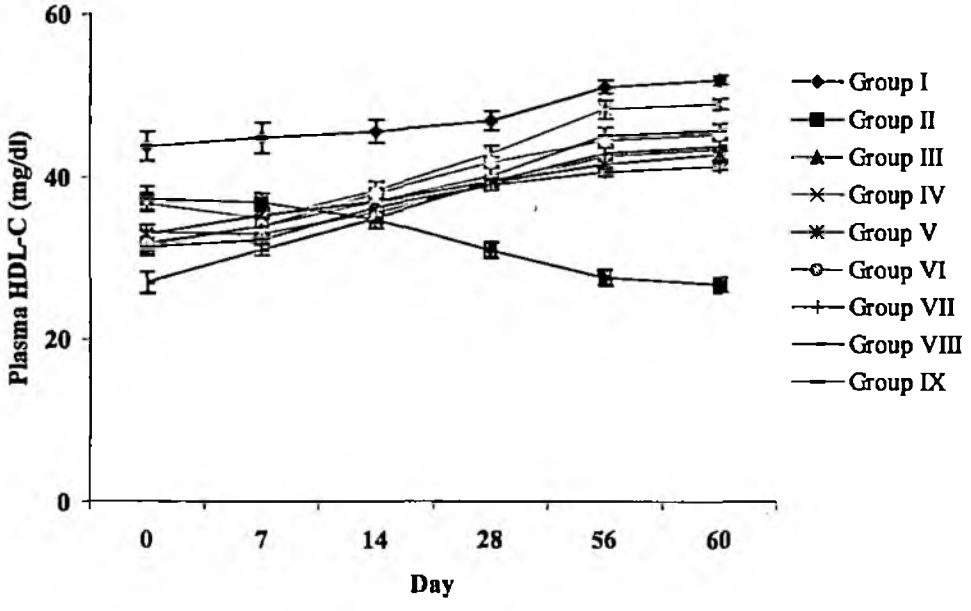

Fig. 10. Effect of *Scoparia dulcis* and *Costus pictus* on plasma HDL-C (mg/dl) level in rats

Mean ± SE, N=8, Group I- Normal control, Group II- Diabetic control, Group III- Diabetic, treated with SDAEt, Group IV- Diabetic, treated with SDAlEt, Group V- Diabetic, treated with CPAEt, Group VI- Diabetic, treated with CPAlEt, Group VII- Diabetic, treated with SDAEt + CPAEt, Group VIII- Diabetic, treated with SDAlEt + CPAlEt, Group IX- Diabetic, treated with glibenclamide.

2.48 mg/dl, respectively for Groups II, III, IV, V, VI, VII, VIII and IX. On 56^{th} and 60^{th} day all the treatment (Group III to IX) showed a significant ($P \leq 0.001$) decrease in plasma triglyceride level than Group II. The mean plasma triglyceride level obtained on 56^{th} and 60^{th} day for the treatment groups (Group III to IX) are presented in Fig.9.

Within Group III there was a significant ($P \leq 0.001$) decrease in plasma triglyceride level from 14^{th} day onwards. The mean plasma triglyceride value obtained on 60^{th} day was 86.13 ± 1.30 mg/dl. The mean plasma triglyceride value obtained for Group III on various treatment days was presented in Fig 9.

Within Group IV there was a significant ($P \leq 0.001$) fall in plasma triglyceride level from 7^{th} day onwards. The mean plasma triglyceride value obtained on 60^{th} day was 71.88 ± 1.32mg/dl.

Within Group V there was a significant ($P \leq 0.001$) decrease in plasma triglyceride level from 7^{th} day onwards. The mean plasma triglyceride value obtained on 60^{th} day was 88.63 ± 1.85mg/dl (Fig.9).

Within Group VI there was a significant ($P \leq 0.001$) decrease in plasma triglyceride level from 14^{th} day onwards. The mean plasma triglyceride level obtained on 28^{th} day was 102.25 ± 1.77 mg/dl. The mean plasma triglyceride value obtained on 60^{th} day was 75.75 ± 1.63 mg/dl. The mean plasma triglyceride values for Group VI are represented in Fig. 9.

Within Group VII, there was a significant ($P \leq 0.001$) fall in plasma triglyceride level from 14^{th} day onwards. The mean plasma triglyceride value obtained on 60^{th} day was 94.75 ± 2.19 mg/dl.

Within Group VIII there was a significant (P ≤0.001) decrease in plasma triglyceride level from 7th day onwards. The mean plasma triglyceride value obtained on 60th day was 84.25 ± 1.81 mg/dl. The mean plasma triglyceride value obtained for Group VIII on various treatment days are represented graphically in Fig 9.

Within Group IX there was a significant (P ≤0.001) decrease in plasma triglyceride level from 14th day onwards. The mean plasma triglyceride value obtained on 60th day was 84.50 ± 1.61 mg/dl. Group IX did not show any significant difference in plasma triglyceride level until 28th day of treatment with any of the treatment groups (Fig.9). On 56th day it showed a significant (P ≤0.001) increase in plasma triglyceride value than Group IV, (89.13 ± 1.57 mg/dl). On 60th day it showed a significant (P ≤0.05) increase in plasma triglyceride level than the Group IV, (71.88 ± 1.32 mg/dl).

In terms of absolute value obtained on 60th day Group IV (treated with SDAIEt) showed a minimum plasma triglyceride value followed by Group VI (treated with CPAIEt). The mean values are presented in Fig.9.The descending order of plasma triglyceride level among other treatment groups on 60th day are Group VIII (84.25 ± 1.81 mg/dl), Group IX (84.50 ± 1.61 mg/dl), Group III (86.13 ± 1.30 mg/dl), Group V (88.63 ± 1.85 mg/dl) and Group VII (94.75 ± 2.19 mg/dl).

4.3.3.4. Effect on plasma HDL-C

The diabetic control animals (Group II) showed a significant (P≤ 0.001) decrease in plasma HDL-C level than the normal control animals (Group I) from the 14th day of study. Group I did not differ significantly between the treatment days and the mean value of plasma HDL-C obtained for Group I on zero, 7th, 14th, 28th, 56th and 60th days were 43.75 ± 1.81, 44.75 ± 1.85, 45.50 ± 1.45, 46.75 ± 1.21, 50.88 ± 0.82 and 51.63 ± 0.58 mg/dl, respectively. Within Group II there was a significant (P ≤0.001) decrease in plasma HDL-C from 28th day onwards. The mean

plasma HDL-C value obtained for Group II on different treatment days are represented in Fig 10.

The treatment groups (Group III to IX) did not differ significantly with Group II until 14th day except with Group VIII on zero and 7th day, where a significant ($P \leq 0.01$) decrease in plasma HDL-C (27.00 ± 1.25 mg/dl) was observed in Group VIII. From 28th day onwards all the treatment groups (Group III to IX) showed a significant ($P \leq 0.001$) increase in plasma HDL-C value than Group II. The mean values of plasma HDL-C on 28th day for Group II, III, IV, V, VI, VII, VIII and IX were 30.88 ± 0.98, 38.88 ± 0.98, 42.75 ± 0.93, 39.25 ± 0.66, 41.63 ± 0.66, 38.88 ± 0.57, 39.25 ± 0.34 and 40.00 ± 1.16 mg/dl, respectively. On 56th and 60th day also all the treatment groups showed a significant ($P \leq 0.001$) increase in plasma HDL-C than Group II. The mean plasma HDL-C values obtained are represented in Fig.10.

Within Group III there was a significant ($P \leq 0.001$) increase in plasma HDL-C level from 28th day onwards. On 60th day the mean plasma HDL-C value obtained was 43.25 ± 0.66 mg/dl.

Within Group IV there was a significant ($P \leq 0.001$) increase in plasma HDL-C level from 28th day onwards. The mean plasma HDL-C value obtained on 60th day was 48.75 ± 0.68 mg/dl.

Within Group V there was significant ($P \leq 0.001$) increase in plasma HDL-C level from 14th day onwards. The mean plasma HDL-C value obtained on 60th day was 42.63 ± 0.79 mg/dl.

Within Group VI there was a significant ($P \leq 0.001$) increase in plasma HDL-C level from 14th day of treatment. The mean plasma HDL-C level of Group VI on zero, 7th, 14th, 28th, 56th and 60th day were 31.63 ± 0.83, 33.88 ± 0.86, 37.88 ± 0.60, 41.63 ± 0.66, 44.25 ± 0.46 and 45.00 ± 0.61 mg/dl, respectively (Fig.10).

Within Group VII there was a significant ($P \leq 0.001$) increase in plasma HDL-C value from 14th day onwards. The mean plasma HDL-C value obtained on 60th day was41.13 ± 0.45 mg/dl. The mean plasma HDL-C value obtained on zero, 7th, 14th, 28th, 56th and 60th day was 31.38 ± 1.12, 32.25 ± 0.61, 36.25 ± 0.55, 38.88 ± 0.57, 40.50 ± 0.59 and 41.13 ± 0.45 mg/dl, respectively (Fig.10).

Within Group VIII there was a significant ($P \leq 0.001$) increase in plasma HDL-C level from 7th day onwards. The mean plasma HDL-C level obtained for Group VIII on zero, 7th, 14th, 28th, 56th and 60th day was 27.00 ± 1.25, 31.00 ± 0.71, 34.88 ± 0.67, 39.25 ± 0.34, 42.75 ± 0.66 and 43.63 ± 0.66 mg/dl, respectively (Fig. 10).

Within Group IX, there was a significant ($P \leq 0.001$) increase in plasma HDL-C level from 28th day onwards with a mean plasma HDL-C value of 40.00 ± 1.16 mg/dl. The mean plasma HDL-C value obtained on zero, 7th, 14th, 28th, 56th and 60th days were 31.88 ± 1.49, 33.88 ± 1.35, 37.00 ± 1.25, 40.00 ± 1.16, 44.88 ± 1.01 and 45.50 ± 0.90 mg/dl, respectively. Group IX did not differ significantly with any of the treatment groups (Group III to VIII) until 28th day. It showed a significant ($P \leq 0.05$) increase in plasma HDL-C than Group VII on 56th day (40.50 ± 0.59 mg/dl). Plasma HDL-C level on 60th day of Group VII (41.13 ± 0.45 mg/dl) was also significantly ($P \leq 0.001$) lower than Group IX.

Group IV (treated with SDAlEt) showed the maximum plasma HDL-C level in terms of absolute value on 60th day followed by Group IX (treated with glibenclamide) and the mean values are presented in Fig.10.The descending orders of increase in plasma HDL-C level obtained for other treatment groups on 60th day are Group VI (45.00 ± 0.61 mg/dl), Group VIII (43.63 ± 0.66 mg/dl), Group III (43.25 ± 0.66 mg/dl), Group V (42.63 ± 0.79 mg/dl) and Group VII (41.13 ± 0.45 mg/dl).

4.3.3.5. Effect on plasma LDL-C

The diabetic control animals (Group II) showed a significant ($P \leq 0.001$) increase in plasma LDL-C value than the normal control animals (Group I) on all the treatment days. The Group I did not differ significantly between the treatment days and the plasma LDL-C level were maintained within the normal range. The mean plasma LDL-C values obtained for Group I were 19.60 ± 1.90, 24.13 ± 2.98, 24.18 ± 3.25, 26.30 ± 3.04, 26.45 ± 2.77 and 22.38 ± 1.84 mg/dl on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day, respectively. Within Group II there was no significant difference between the treatment days though there was a steady increase in plasma LDL-C value from day zero to 60^{th} day.

Any of the treatment groups did not differ significantly with Group II on zero day. On 7^{th} day Groups III, IV, V, VI, VIII and IX showed a significant ($P \leq 0.05$) decrease in plasma LDL-C value than Group II. The mean plasma LDL-C value of Groups III, IV, V, VI, VIII and IX was 78.98 ± 3.20, 78.03 ± 4.07, 75.03 ± 2.48, 81.65 ± 2.43, 76.93 ± 2.28 and 79.90 ± 3.78 mg/dl, respectively. From 14^{th} day onwards till the end of the study the treatment groups (Group III to IX) showed a significant ($P \leq 0.001$) decrease in plasma LDL-C level than Group II. The mean plasma LDL-C value obtained on 14^{th} day for Groups II, III, IV, V, VI, VII, VIII and IX was 97.10 ± 4.02, 62.10 ± 3.05, 57.33 ± 3.75, 59.48 ± 1.74, 63.65 ± 1.31, 64.73 ± 1.63, 61.65 ± 0.93 and 64.38 ± 2.29 mg/dl, respectively. The mean plasma LDL-C value obtained for the treatment groups from day zero to 60^{th} day are represented in Fig.11.

Within Group III there was a significant ($P \leq 0.001$) decrease in plasma LDL-C level from 14^{th} day onwards. The mean plasma LDL-C value obtained on 60^{th} day was 21.53 ± 2.55 mg/dl. The mean plasma LDL-C values obtained on zero, 7^{th}, 14^{th},

28^{th}, 56^{th} and 60^{th} day were 90.55 ± 4.08, 78.98 ± 3.20, 62.10 ± 3.05, 41.23 ± 2.06, 24.43 ± 2.49 and 21.53 ± 2.55 mg/dl, respectively (Fig.11).

Within Group IV there was a significant ($P \leq 0.001$) decrease in plasma LDL-C level from 14^{th} day onwards. The mean plasma LDL-C value obtained on 60^{th} day was 16.50 ± 1.02 mg/dl. The mean plasma LDL-C values obtained on zero, 7^{th}, 14^{th}, 28^{th}, 50^{th} and 60^{th} day were 84.68 ± 4.33, 78.03 ± 4.07, 57.33 ± 3.75, 22.23 ± 1.76, 17.38 ± 1.47 and 16.50 ± 1.02 mg/dl, respectively.

Within Group V there was a significant ($P \leq 0.001$) decrease in plasma LDL-C level from 7^{th} day onwards when compared to zero day value. The mean plasma LDL-C value obtained on 60^{th} day was 29.28 ± 1.59 mg/dl. The mean plasma LDL-C values obtained on zero, 7^{th} 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 90.23 ± 2.13, 75.03 ± 2.48, 59.48 ± 1.74, 46.48 ± 1.61, 32.90 ± 1.91 and 29.28 ± 1.59 mg/dl, respectively (Fig.11).

Within Group VI there was a significant ($P \leq 0.001$) decrease in plasma LDL-C level from 7^{th} day onwards. The mean plasma LDL-C values obtained on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 91.10 ± 1.98, 81.65 ± 2.43, 63.65 ± 1.31, 42.93 ± 1.89, 28.15 ± 1.84 and 22.35 ± 1.58 mg/dl, respectively.

Within Group VII there was a significant ($P \leq 0.001$) decrease in plasma LDL-C level from 14^{th} day onwards when compared to zero day value. The mean plasma LDL-C values obtained on zero, 7^{th}, 14^{th}, 28gth, 56^{th} and 60^{th} day were 89.05 ± 2.96, 83.00 ± 2.63, 64.73 ± 1.63, 50.83 ± 1.98, 42.48 ± 2.54 and 34.68 ± 1.79 mg/dl, respectively (Fig.11).

Within Group VIII there was a significant ($P \leq 0.001$) decrease in plasma LDL-C level from 7^{th} day onwards. The mean plasma values obtained on zero, 7^{th},

14^{th}, 28^{th}, 56^{th} and 60^{th} day were 96.73 ± 1.71, 76.93 ± 2.28, 61.65 ± 0.93. 38.90 ± 1.50, 22.98 ± 2.29 and 17.90 ± 1.82 mg/dl, respectively (Fig.11).

Within Group IX there was a significant ($P \leq 0.001$) decrease in plasma LDL-C level from 14^{th} day onwards. The mean plasma LDL-C values obtained on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 86.40 ± 4.17, 79.90 ± 3.78, 64.38 ± 2.79, 46.28 ± 2.49, 28.05 ± 2.44 and 18.98 ± 2.17 mg/dl, respectively (Fig. 11). Group IX did not differ significantly with any of the treatment groups until 14^{th} day. On 28^{th} day it showed a significant ($P \leq 0.001$) increase in plasma LDL-C value than Group IV (22.23 ± 1.76 mg/dl). On 56^{th} day it showed a significant ($P \leq 0.001$) decrease in plasma LDL-C value than Group VII and on 60^{th} day it showed a significant ($P \leq 0.05$) decrease in plasma LDL-C level than Group V and VII (Fig.11).

In terms of absolute value Group IV (treated with SDAIEt) showed a minimum level of plasma LDL-C on 60^{th} day followed by Group VIII (treated with SDAIEt + CPAIEt) and the mean values are presented in Fig.11. The descending order of plasma LDL-C level among other treated groups on 60^{th} day are Group IX (18.98 ± 2.17 mg/dl), Group III (21.53 ± 2.55 mg/dl), Group VI (22.35 ± 1.58 mg/dl), Group V (29.28 ± 1.59 mg/dl) and Group VII (34.68 ± 1.79 mg/dl).

4.3.3.6. Effect on plasma VLDL-C

Group II (diabetic control animals) showed a significant ($P \leq 0.01$) increase in plasma VLDL-C level with respect to Group I on all treatment days. Group I did not differ significantly between the treatment days in plasma LDL-C level and was maintained within the normal range. The mean plasma VLDL-C values obtained for Group I were 11.65 ± 0.83, 11.88 ± 0.73, 12.83 ± 0.68, 11.95 ± 0.79, 13.30 ± 0.55 and 13.63 ± 0.37 mg/dl, respectively on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day. The mean values of VLDL-C obtained for different groups are presented in Fig.12.

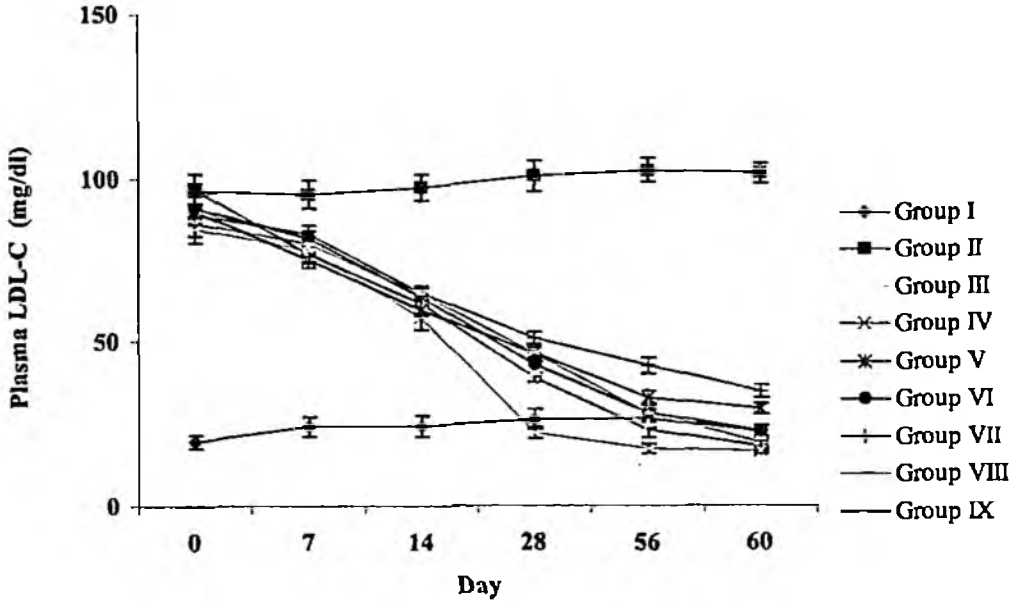

Fig. 11. Effect of *Scoparia dulcis* and *Costus pictus* on plasma LDL-C (mg/dl) level in rats

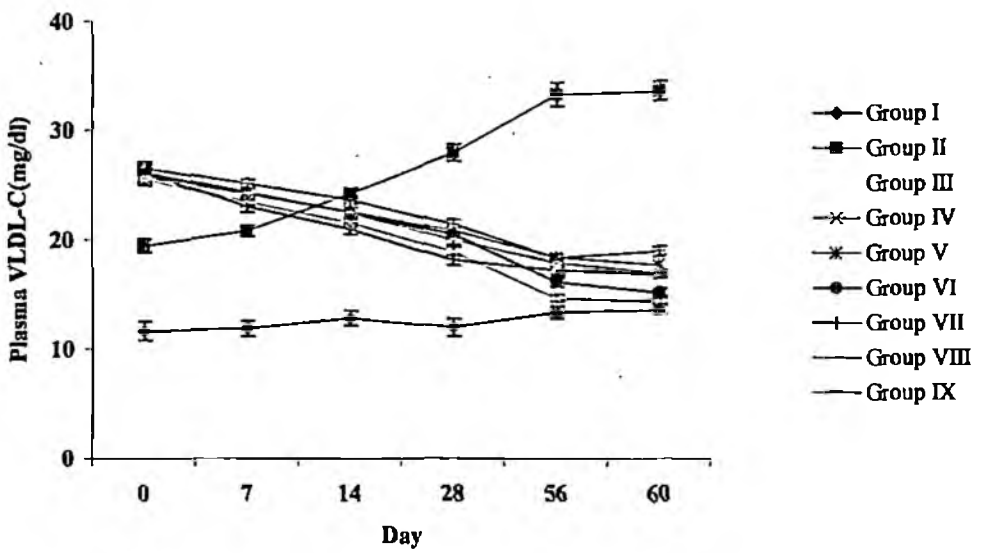

Fig. 12. Effect of *Scoparia dulcis* and *Costus pictus* on plasma VLDL-C (mg/dl) level in rats

Mean ± SE, N=8, Group I- Normal control, Group II- Diabetic control, Group III- Diabetic, treated with SDAEt, Group IV- Diabetic, treated with SDAlEt, Group V- Diabetic, treated with CPAEt, Group VI- Diabetic, treated with CPAlEt, Group VII- Diabetic, treated with SDAEt + CPAEt, Group VIII- Diabetic, treated with SDAlEt + CPAlEt, Group IX- Diabetic, treated with glibenclamide.

Within Group II there was a significant (P ≤0.001) increase in plasma VLDL-C level from 14th day onwards. The mean plasma VLDL-C value obtained on zero, 7th, 14th, 28th, 56th and 60th days were 19.45 ± 0.50, 20.78 ± 0.50, 24.28 ± 0.40, 27.90 ± 0.74, 33.20 ± 1.07 and 33.58 ± 0.91 mg/dl, respectively (Fig.12).

All the treatment groups (Group III to IX) showed a significant (P≤0.01) increase in plasma VLDL-C level compared to Group II on zero and 7th day. On 14th day Group IV and VIII showed a significant (P≤ 0.01) decrease in plasma VLDL-C than Group II. The mean plasma VLDL-C value for Group IV and VIII was 21.55 ± 0.37 and 20.98 ± 0.53 mg/dl. On 28th and 56th day all the treatment groups (Group III to IX) showed a significant (P ≤0.001) reduction in plasma VLDL-C than Group II and the mean plasma VLDL-C value are represented in Fig.12. On 60th day also all the treatment groups showed a significant (P ≤0.001) decrease in plasma VLDL-C than Group II and the mean plasma VLDL-C value obtained for Groups II, III, IV, V, VI, VII, VIII and IX were 33.58 ± 0.91, 17.23 ± 0.26, 14.38 ± 0.26, 17.73 ± 0.37, 15.15 ± 0.33, 18.95 ± 0.44, 16.85 ± 0.36 and 16.90 ± 0.32 mg/dl, respectively (Fig.12).

Within Group III there was a significant (P ≤0.001) decrease in plasma VLDL-C from 14th day onwards. The mean plasma VLDL-C values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 26.45 ± 0.57, 25.15 ± 0.50, 23.40 ± 0.29, 21.28 ± 0.36, 17.70 ± 0.29 and 17.23 ± 0.26 mg/dl, respectively.

Within Group IV there was a significant (P ≤0.001) decrease in plasma VLDL-C level from 7th day onwards till the end of the study. The mean plasma VLDL-C values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 25.58 ± 0.50, 23.48 ± 0.37, 21.55 ± 0.37, 18.90 ± 0.38, 14.63 ± 0.30 and 14.38 ± 0.26 mg/dl, respectively (Fig.12).

Within Group V there was a significant ($P \leq 0.001$) decrease in plasma VLDL-C level from 7^{th} day onwards till the end of the experimental period. The value obtained on 60^{th} day was 17.73 ± 0.37 mg/dl. The mean plasma VLDL-C values obtained on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 25.90 ± 0.33, 24.35 ± 0.30, 22.53 ± 0.30, 20.78 ± 0.37, 18.35 ± 0.32 and 17.73 ± 0.37 mg/dl.

Within Group VI, there was a significant ($P \leq 0.001$) decrease in plasma VLDL-C level from 14^{th} day onwards. The mean plasma VLDL-C level obtained on 60^{th} day was 15.15 ± 0.33 mg/dl. The mean plasma VLDL-C values obtained on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 26.15 ± 0.80, 24.35 ± 0.34, 22.48 ± 0.40, 20.45 ± 0.35, 16.10 ± 0.43 and 15.15 ± 0.33 mg/dl, respectively (Fig.12).

Within Group VII there was a significant ($P \leq 0.001$) decrease in plasma VLDL-C level from 14^{th} day onwards when compared to zero day value. The mean plasma VLDL-C value obtained on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 26.58 ± 0.58, 25.13 ± 0.34, 23.65 ± 0.35, 21.43 ± 0.35, 18.28 ± 0.34 and 18.95 ± 0.44 mg/dl, respectively (Fig.12).

Within Group VIII there was a significant ($P \leq 0.001$) decrease in plasma VLDL-C level from 7^{th} day onwards when compared to zero day value. The mean plasma VLDL-C values obtained on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 26.15 ± 0.49, 23.08 ± 0.60, 20.98 ± 0.53, 18.10 ± 0.47, 17.15 ± 0.40 and 16.85 ± 0.36 mg/dl, respectively.

Within Group IX there was a significant ($P \leq 0.001$) decrease in plasma VLDL-C level from 14^{th} day onwards when compared to zero day value. The mean plasma VLDL-C values obtained on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 25.73 ± 0.58, 24.23 ± 0.56, 22.50 ± 0.42, 19.98 ± 0.50, 17.83 ± 0.31 and 16.90 ± 0.32 mg/dl, respectively (Fig.12). Group IX did not differ significantly until 28^{th} day of

treatment between the treatment groups. On 56th day it showed a significant (P ≤0.001) increase in plasma VLDL-C level than Group IV (14.63 ± 0.30 mg/dl). On 60th day also it showed a significant (P ≤0.05) increase in plasma VLDL-C level than Group IV (14.38 ± 0.26 mg/dl).

Group IV (treated with SDAIEt) with a mean plasma VLDL-C level of 14.38 ± 0.26 mg/dl on 60th day showed the minimum plasma VLDL-C level followed by Group VI (treated with CPAIEt) with a mean plasma VLDL-C value of 15.15 ± 0.33 mg/dl, in terms of absolute value. The descending order of plasma VLDL-C value obtained on 60th day for other treatment groups are Group VIII (16.85 ± 0.36 mg/dl), Group IX (16.90 ± 0.32 mg/dl), Group III (17.23 ± 0.26 mg/dl), Group V (17.73 ± 0.37 mg/dl) and Group VII (18.95 ± 0.44 mg/dl).

4.3.4. Effect of *Scoparia dulcis* and *Costus pictus* on Plasma Protein Profile

4.3.4.1. Effect on plasma total protein

The diabetic control animals (Group II) showed a significant (P≤0.01) decrease in plasma total protein value than the normal control animals on all treatment days. The mean plasma total protein values obtained for Group I were 7.71 ± 0.28, 8.05 ± 0.36, 8.00 ± 0.24, 8.15 ± 0.15,8.36 ± 0.21 and 8.53 ± 0.20 g/dl on zero, 7th, 14th, 28th, 56th and 60th day, respectively. Within Group II, there was a significant (P≤0.01) decrease in plasma total protein level from 56th day onwards. The mean plasma total protein values of all the treatment groups are presented in Fig.13.

The treatment Groups III, IV, V, VI and VIII showed a significant (P≤ 0.01) decrease in plasma total protein value than the Group II on zero day. The mean plasma total protein value obtained for Groups II, III, IV, V, VI, VII, VIII and IX were 6.79 ± 0.23, 5.99 ± 0.10, 5.48 ± 0.11, 5.99 ± 0.08, 6.03 ± 0.08, 5.90 ± 0.08 and 6.51 ± 0.28 g/dl. On 28th day, the treatment Groups III, V and VI showed a

significant (P≤ 0.01) increase in plasma total protein value than Group II. From 56th day onwards there was a significant (P≤0.01) increase in plasma total protein value in the treatment groups (Group III to IX) compared to Group II. The mean plasma total protein values are presented in Fig. 13.

Within Group III there was a significant increase in plasma total protein level from 14th day onwards. On 60th day the mean plasma total protein value was 7.23 ± 0.14 g/dl. The mean plasma total protein values are presented in Fig.13.

Within Group IV there was a significant (P≤0.001) increase in plasma total protein level from 28th day onwards. The mean plasma total protein values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 5.89 ± 0.08, 5.93 ± 0.08, 6.19 ± 0.07, 6.41 ± 0.08, 7.05 ± 0.07 and 7.30 ± 0.09 g/dl, respectively.

Within Group V there was a significant (P≤0.001) increase in plasma total protein from 14th day onwards. The mean plasma total protein value obtained on 60th day was 7.34 ± 0.13 g/dl. The mean plasma total protein value obtained on zero, 7th, 14th, 28th, 56th and 60th day were 5.48 ± 0.11, 5.88 ± 0.14, 6.46 ± 0.15, 6.98 ± 0.17, 7.31 ± 0.14 and 7.34 ± 0.13 g/dl respectively.

Within Group VI there was a significant (P≤0.001) increase in plasma total protein level from 28th day onwards. The mean plasma total protein values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 5.99 ± 0.08, 5.84 ± 0.07, 6.06 ± 0.08, 6.88 ± 0.09, 7.46 ± 0.11 and 7.55 ± 0.11 g/dl, respectively (Fig.13).

Within Group VII, there was a significant (P≤ 0.001) increase in plasma total protein value from 28th day onwards when compared to zero day value. The mean plasma total protein values are presented in Fig.13.

Within Group VIII there was a significant ($P \leq 0.001$) increase in plasma total protein value from 28th day onwards. The mean plasma total protein values are presented in Fig.13.

Group IX did not differ significantly between the treatment days, though it showed a gradual increase in plasma total protein from zero day to 60th day. Group IX showed a significant ($P \leq 0.01$) increase in plasma total protein than Group V on zero day. During the remaining treatment period Group IX did not differ significantly with any of the treatment groups (Fig.13).

In terms of absolute value Group VI (treated with CPAIEt) showed the maximum plasma total protein value followed by Group VII (treated with SDAIEt + CPAIEt), at the end of the study (60 days). The mean values are presented in Fig.13. The descending orders of improvement in plasma total protein value on 60th day for other treatment groups are Group VIII (7.38 ± 0.08 g/dl), Group V (7.34 ± 0.13 g/dl), Group IV (7.30 ± 0.09 g/dl), Group III (7.23 ± 0.14 g/dl) and Group IX (7.13 ± 0.10 g/dl).

4.3.4.2. Effect on plasma albumin

The diabetic control animals (Group II) showed a significant ($P \leq 0.01$) decrease in plasma albumin content on all treatment days with Group I (normal control). Group I did not differ significantly between the treatment days and were maintained in the normal range. The mean plasma albumin values obtained for Group I on zero, 7th, 14th, 28th, 56th and 60th day were 3.05 ± 0.08, 3.06 ± 0.06, 3.09 ± 0.06, 3.00 ± 0.07, 3.09 ± 0.06 and 3.09 ± 0.05 g/dl, respectively. Within Group II there was no significant difference between the treatment days and the mean plasma albumin values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 2.43 ± 0.15, 2.43 ± 0.11, 2.23 ± 0.06, 2.39 ± 0.12, 2.33 ± 0.11 and 2.24 ± 0.07 g/dl, respectively.

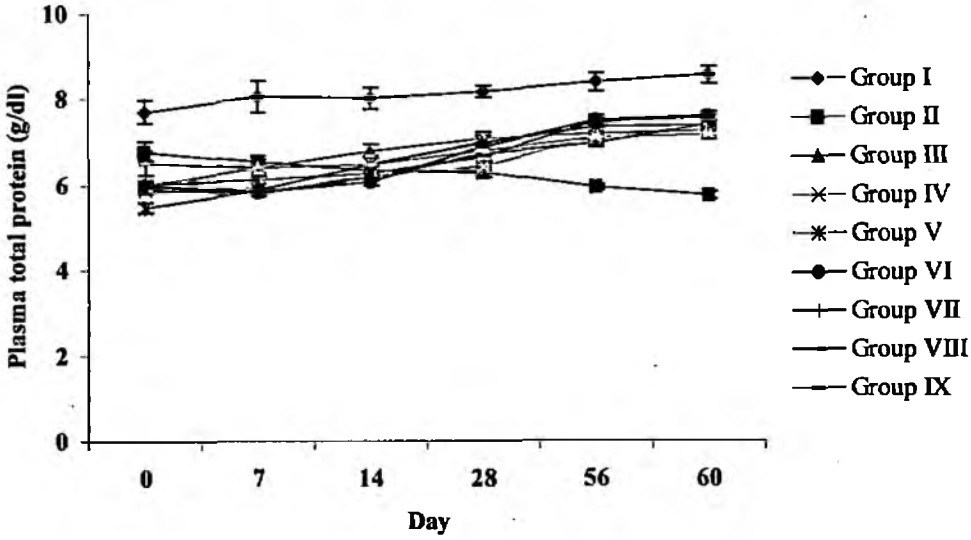

Fig. 13. Effect of *Scoparia dulcis* and *Costus pictus* on plasma total protein (g/dl) level in rats

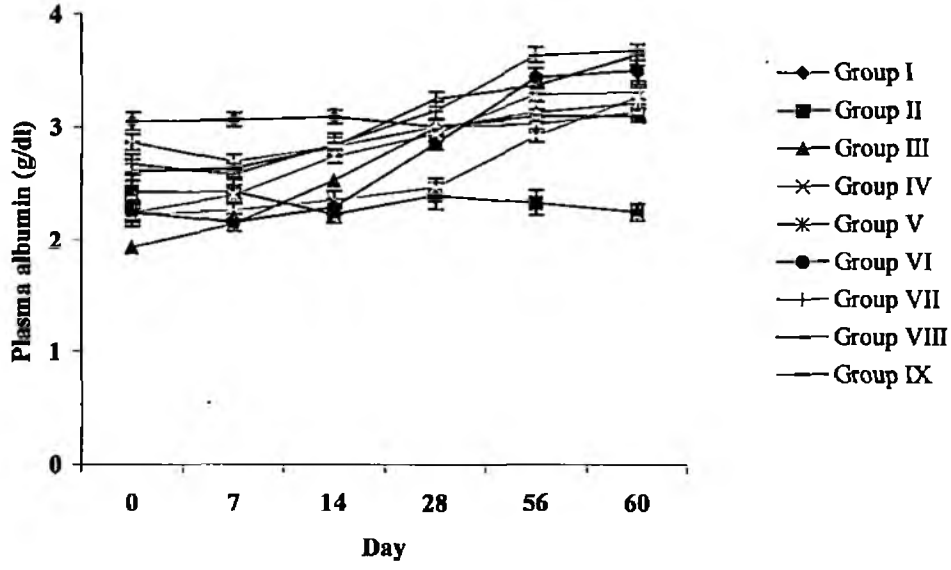

Fig. 14. Effect of *Scoparia dulcis* and *Costus picttus* on plasma albumin (g/dl) level in rats

Mean ± SE, N=8, Group I- Normal control, Group II- Diabetic control, Group III- Diabetic, treated with SDAEt, Group IV- Diabetic, treated with SDAlEt, Group V- Diabetic, treated with CPAEt, Group VI- Diabetic, treated with CPAlEt, Group VII- Diabetic, treated with SDAEt + CPAEt, Group VIII- Diabetic, treated with SDAlEt + CPAlEt, Group IX- Diabetic, treated with glibenclamide.

Group III showed a significant ($P \leq 0.05$) decrease in plasma albumin than Group II on day zero. On day 14th Groups V, VII, VIII and IX showed a significant ($P \leq 0.001$) increase in plasma albumin content than Group II, with a mean plasma albumin content of 2.74 ± 0.06, 2.84 ± 0.10, 2.83 ± 0.03 and 2.83 ± 0.07 g/dl, respectively. On 28th day the treatment Groups III, V, VI, VII, VIII and IX showed a significant ($P \leq 0.001$) increase in plasma albumin content than Group II. The mean plasma albumin value obtained for these groups were 2.99 ± 0.06, 2.94 ± 0.07, 2.85 ± 0.06, 3.34 ± 0.08 and 3.41 ± 0.07 g/dl, respectively. On 56th and 60th day all the treatment groups showed a significant ($P \leq 0.001$) increase in plasma total albumin content than Group II (Fig.14).

Within Group III there was a significant ($P \leq 0.001$) increase in plasma albumin content from 14 day onwards. The mean value obtained on 60th day was 3.20 ± 0.05 g/dl. The mean plasma albumin content obtained on zero, 7th, 14th, 28th, 56 and 60th days were 1.94 ± 0.09, 2.14 ± 0.05, 2.53 ± 0.06, 2.99 ± 0.06, 3.13 ± 0.03 and 3.20 ± 0.05 g/dl, respectively (Fig. 14).

Within Group IV there was a significant ($P \leq 0.001$) increase in plasma albumin content from 56th day onwards. The mean plasma albumin values of Group IV on zero, 7th, 14th, 28th, 56th and 60th day were 2.23 ± 0.10, 2.26 ± 0.10, 2.35 ± 0.08, 2.46 ± 0.08, 2.91 ± 0.06 and 3.25 ± 0.09 g/dl, respectively (Fig.14).

Within Group V there was a significant ($P \leq 0.001$) increase in plasma albumin content from 14th day onwards. The mean plasma albumin values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 2.24 ± 0.06, 2.39 ± 0.06, 2.74 ± 0.06, 2.94 ± 0.07, 3.28 ± 0.06 and 3.30 ± 0.07 g/dl, respectively.

Within Group VI there was a significant ($P \leq 0.001$) increase in plasma albumin content from 28th day onwards. The mean plasma albumin value obtained on

zero, 7th, 14th, 28th, 56th and 60th day were 2.25 ± 0.10, 2.15 ± 0.08, 2.29 ± 0.07, 2.85 ± 0.06, 3.43 ± 0.06 and 3.49 ± 0.09 g/dl, respectively.

Within Group VII there was a significant (P≤0.001) increase in plasma albumin content from 28th day onwards. The mean plasma albumin content obtained on zero, 7th, 14th, 28th, 56th and 60th days were 2.68 ± 0.08, 2.58 ± 0.10, 2.84 ± 0.10, 3.34 ± 0.08, 3.63 ± 0.07 and 3.66 ± 0.05 g/dl, respectively (Fig. 14).

Within Group VIII there was a significant (P≤0.001) increase in plasma albumin content from 28th day onwards. The mean plasma albumin content obtained on zero, 7th, 14th, 28th, 56th and 60th days were 2.61 ± 0.09, 2.63 ± 0.08, 2.83 ± 0.03, 3.41 ± 0.07, 3.36 ± 0.08 and 3.63 ± 0.09 g/dl, respectively.

Within Group IX there was no significant difference in plasma albumin content between the treatment days but was significantly (P≤ 0.001) higher than the diabetic control (Group II). The mean plasma albumin content on zero, 7th 14th, 28th, 56th and 60th day were 2.86 ± 0.07, 2.69 ± 0.07, 2.83 ± 0.07, 3.0 ± 0.06, 3.01 ± 0.06 and 3.13 ± 0.07 g/dl, respectively (Fig. 14).

Group IX showed a significant (P≤0.01) increase in plasma albumin content than Group III, IV, V and VI with a mean plasma albumin content of 1.94 ± 0.09, 2.23 ± 0.10, 2.24 ± 0.06 and 2.25 ± 0.10 g/dl on day zero. On 7th day it showed a significant (P≤0.001) increase in plasma albumin content than Groups III, IV and VI with a mean plasma albumin content of 2.14 ± 0.05, 2.26 ± 0.10 and 2.15 ± 0.06 g/dl, respectively. On 14th day it showed a significant (P≤0.001) increase in plasma albumin content than Group IV and VI with a mean plasma albumin value of 2.35 ± 0.08 and 2.29 ± 0.07 g/dl (Fig.14). On 28th day it showed a significant (P≤0.001) increase in plasma albumin content than Group IV and VIII with a mean plasma albumin value of 2.46 ± 0.08 and 3.41 ± 0.07 g/dl. On 56th day it showed a significant (P≤0.01) decrease in plasma albumin content than Groups VI, VII and VIII, with a

mean plasma albumin value of 3.43 ± 0.08, 3.63 ± 0.07 and 3.36 ± 0.08 g/dl. respectively. On 60th day also it showed a significant (P≤0.001) decrease in plasma albumin content than Groups VI, VII and VIII (3.49 ± 0.09, 3.66 ± 0.05 and 3.63 ± 0.09 g/dl, respectively).

At the end of the study, Group VII (treated with SDAEt + CPAEt) with a mean plasma albumin value of 3.66 ± 0.05 g/dl on 60th day showed the maximum increase in plasma albumin content followed by Group VIII (treated with SDAIEt + CPAIEt) with a mean plasma albumin content of 3.63 ± 0.09 g/dl. The descending orders of increase in plasma albumin content in terms of absolute value for other treatment groups are Group VI (3.49 ± 0.09 g/dl), Group V (3.30 ± 0.07 g/dl), Group IV (3.25 ± 0.09 g/dl), Group III (3.20 ± 0.05 g/dl) and Group IX (3.13 ± 0.07 g/dl).

4.3.4.3. Effect on plasma globulin

The diabetic control animals (Group II) showed a significant (P≤0.01) decrease in plasma globulin content than Group I on all treatment days. Group I did not differ significantly between the treatment days and the values are maintained in the normal range. The mean plasma globulin values obtained for Group I on zero, 7th, 14th, 28th, 56th and 60th day were 4.66 ± 0.26, 4.99 ± 0.36, 4.91 ± 0.23, 5.15 ± 0.13, 5.28 ± 0.17 and 5.44 ± 0.22 g/dl, respectively. Within Group II there was a significant (P≤0.001) decrease in plasma globulin content on 56th and 60th day over the zero day level (Fig.15).

Groups IV, V, VII, VIII and IX showed significant (P≤0.05) decrease in plasma globulin content than the Group II on zero day. On 14th day Group VII and VIII showed a significant (P≤ 0.01) decrease in plasma globulin content than Group II, with a mean plasma globulin content of 3.41 ± 0.06 and 3.33 ± 0.07 g/dl. On 28th day Group VII and VIII showed a significant (P≤0.01) decrease in plasma

globulin content than Group II, with a mean plasma globulin content of 3.29 ± 0.03 and 3.30 ± 0.10 g/dl. On 60th day the treatment Groups III, IV, V, VI and IX showed a significant ($P \leq 0.05$) increase in plasma globulin content than Group II, with a mean plasma globulin content of 4.03 ± 0.12, 4.05 ± 0.04, 4.04 ± 0.06, 4.06 ± 0.05 and 4.00 ± 0.07 g/dl (Fig.15)

Within Group III there was no significant difference between the treatment days and the values are represented in Fig.15.

Within Group IV there was a significant ($P \leq 0.01$) increase in plasma globulin from 14th day onwards. The mean plasma globulin content obtained on zero, 7th, 14th, 28th, 56th and 60th days were 3.66 ± 0.05, 3.66 ± 0.09, 3.84 ± 0.07, 3.95 ± 0.05, 4.14 ± 0.05 and 4.05 ± 0.04 g/dl, respectively.

Within Group V there was a significant ($P \leq 0.001$) increase in plasma globulin from 14th day onwards. The mean plasma globulin values obtained on zero, 7th, 14th, 28th, 56th and 60th days were 3.24 ± 0.06 3.49 ± 0.10, 3.73 ± 0.10, 4.04 ± 0.11, 4.04 ± 0.10 and 4.04 ± 0.06 g/dl, respectively (Fig.15).

Within Group VI there was a significant ($P \leq 0.05$) increase in plasma globulin content from 28th day onwards. The mean plasma globulin content obtained for Group VI on zero, 7th, 14th, 28th, 56th and 60th days were 3.74 ± 0.04, 3.69 ± 0.08, 3.78 ± 0.07, 4.03 ± 0.07, 4.04 ± 0.05 and 4.06 ± 0.05 g/dl, respectively.

Within Group VII there was a significant ($P \leq 0.01$) increase in plasma globulin content from 56th day onwards. The mean plasma globulin contents obtained are presented in Fig.15.

Within Group VIII there was a significant ($P \leq 0.001$) increase in plasma globulin content from 56th day onwards. The mean plasma globulin content obtained

on zero, 7th 14th, 28th, 56th and 60th days were 3.29 ± 0.05, 3.20 ± 0.04, 3.33 ± 0.07,3.30 ± 0.10, 4.01 ± 0.07 and 3.25 ± 0.04 g/dl (Fig. 15).

Within Group IX there was no significant difference between the treatment days, though there was a general increase in plasma globulin content towards the 60th day. Group IX did not differ significantly between the treatment groups for any of the treatment days. The mean plasma globulin values for Group IX are represented in Fig.15.

In terms of absolute value Group VI (treated with CPAIEt) showed the maximum plasma globulin value on 60th day with a mean plasma globulin level of 4.06 ± 0.05 mg/dl followed by Group IV (treated with SDAIEt) with a mean plasma globulin level of 4.05 ± 0.04 mg/dl. The descending orders of increase in plasma globulin value for other treatment groups on 60th day are Group V (4.04 ± 0.06 mg/dl), Group III (4.03 ± 0.12 mg/dl), Group IX (4.00 ± 0.07 mg/dl). Group VII (3.86 ± 0.05 mg/dl) and Group VIII (3.75 ± 0.04 mg/dl).

4.3.4.4. Effect on plasma A/G ratio

The Group II (diabetic control) did not show any significant difference in plasma A/G ratio when compared to Group I. Group I did not differ significantly between the treatment days and was maintained within the normal range. The mean plasma A/G ratio obtained for Group I on zero, 7th, 14th, 28th, 56th and 60th days were 0.65 ± 0.04, 0.61 ± 0.04, 0.64 ± 0.04, 0.58 ± 0.03, 0.59 ± 0.02 and 0.58 ± 0.03, respectively. Within Group II there was no significant difference between the treatment days (Fig.16).

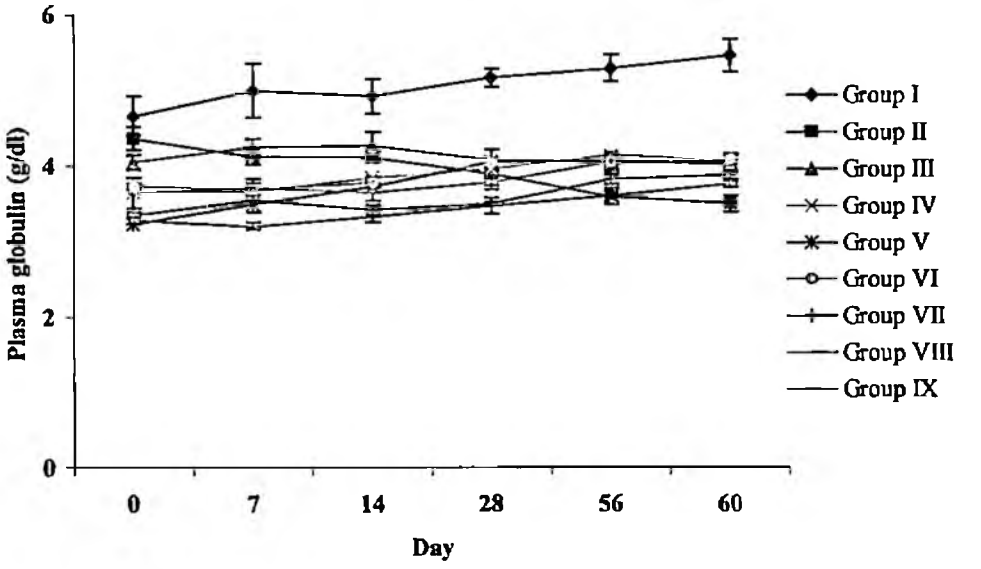

Fig. 15. Effect of *Scoparia dulcis* and *Costus pictus* on plasma globulin (g/dl) level in rats

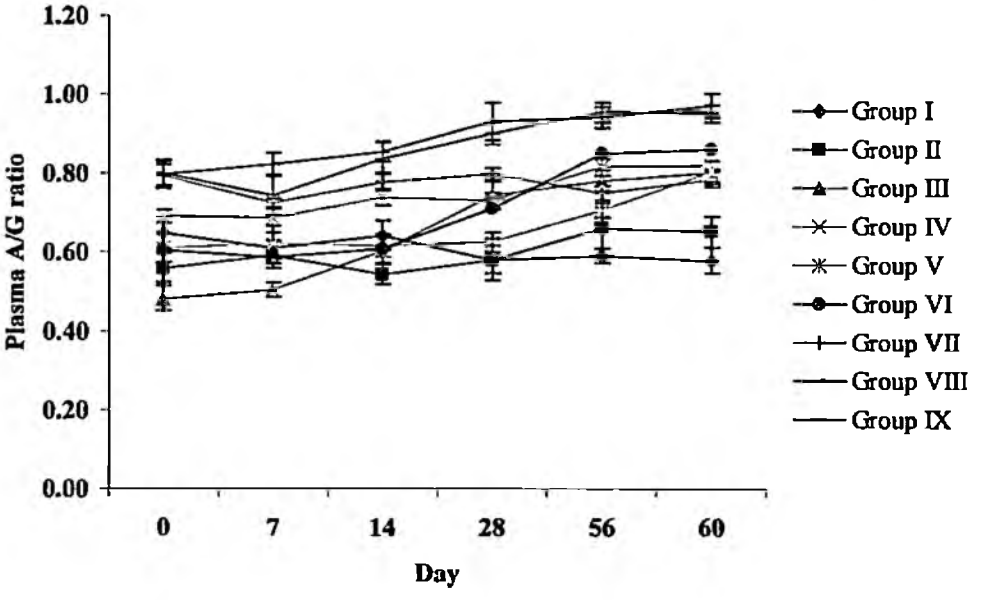

Fig.16. Effect of *Scoparia dulcis* and *Costus pictus* on plasma A/G ratio in rats

Mean ± SE, N=8, Group I- Normal control, Group II- Diabetic control, Group III- Diabetic, treated with SDAEt, Group IV- Diabetic, treated with SDAlEt, Group V- Diabetic, treated with CPAEt, Group VI- Diabetic, treated with CPAlEt, Group VII- Diabetic, treated with SDAEt + CPAEt, Group VIII- Diabetic, treated with SDAlEt + CPAlEt, Group IX- Diabetic, treated with glibenclamide.

Groups VII, VIII and IX showed a significant (P≤ 0.01) increase in plasma A/G ratio on day zero than Group II with a mean plasma A/G ratio of 0.80 ± 0.03, 0.80 ± 0.04 and 0.80 ± 0.03.On 7^{th} day Group VIII showed a significant (P≤0.001) increase in plasma A/G ratio than Group II, with a mean plasma A/G ratio of 0.82 ± 0.03. On 14^{th} day the Groups V, VII, VIII and IX showed a significant (P≤0.001) increase in plasma A/G ratio than Group II with a mean plasma A/G ratio of 0.74 ± 0.02, 0.84 ± 0.04, 0.85 ± 0.03 and 0.78 ± 0.02. On 28^{th} day Group VII and VIII showed a significant (P≤0.001) increase in plasma A/G ratio than Group II with a mean plasma A/G ratio of 1.02 ± 0.03 and 1.05 ± 0.05. On 56^{th} day Groups V, VI, VII and VIII showed a significant (P≤0.001) increase in plasma A/G ratio than Group II, with a mean plasma A/G ratio of 0.81 ± 0.02, 0.85 ± 0.02, 0.95 ± 0.02 and 0.84 ± 0.03. On 60^{th} day all the treatment groups showed a significant (P≤0.001) increase in plasma A/G ratio than the Group II and the mean values are represented in Fig.16.

Within Group III there was a significant (P≤0.001) increase in plasma A/G ratio from 28^{th} day onwards. The mean plasma A/G ratio obtained on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} days were 0.48 ± 0.03, 0.51 ± 0.02, 0.60 ± 0.03, 0.74 ± 0.03, 0.78 ± 0.03 and 0.80 ± 0.03.

Group IV showed a significant (P≤0.01) increase in plasma A/G ratio only on 60^{th} day than the zero day level (Fig. 16).

Within Group V there was a significant (P≤0.001) increase in plasma A/G ratio from 56^{th} day onwards. The mean plasma A/G ratios are presented in Fig.16.

Within Group VI there was a significant (P≤0.001) increase in plasma A/G ratio from 56^{th} day onwards. The mean plasma A/G ratios are represented in Fig.16.

Within Group VII there was a significant (P≤0.05) increase in plasma A/G ratio from 28^{th} day onwards. The value obtained on 60^{th} day was 0.95 ± 0.02.

Within Group VIII there was a significant ($P \leq 0.05$) increase in plasma A/G ratio on 28th day over the zero day level. The mean value obtained on 60^{th} day was 0.97 ± 0.03.

Within the treatment Group IX there was no significant difference between the treatment days (Fig.16). Group IX showed a significant ($P \leq 0.01$) increase in plasma A/G ratio than Groups III, IV and VI on zero day. It also showed significant ($P \leq 0.01$) increase in plasma A/G ratio than Groups III, IV and VI on 14^{th} day with a mean plasma A/G ratio of 0.60 ± 0.03, 0.62 ± 0.04 and 0.61 ± 0.02. On 28^{th} day Group IX showed a significant ($P \leq 0.05$) increase over Group IV and a significant ($P \leq 0.01$) decrease in plasma A/G ratio than Group VII and VIII. On 56^{th} day there was a significant ($P \leq 0.001$) decrease in plasma A/G ratio than the Group VII and on 60^{th} day there was also a significant ($P \leq 0.001$) decrease in plasma A/G ratio than the Group VII and VIII (Fig.16).

After 60^{th} day, Group VIII (treated with SDAlEt + CPAlEt) showed the maximum plasma A/G ratio in terms of absolute value on 60^{th} day followed by Group VII (treated with SDAEt + CPAEt). The mean values are presented in Fig.16. The descending orders of increase in plasma A/G ratio for other treatment groups on 60^{th} day are Group VI (0.86 ± 0.03), Group V (0.82 ± 0.01), Group IV (0.80 ± 0.03), Group III (0.80 ± 0.03) and Group IX (0.78 ± 0.02).

4.3.5. Effect of *Scoparia dulcis* and *Costus pictus* on Liver Glycogen and Liver Enzymes

4.3.5.1. Effect on liver glycogen

The liver glycogen content of Group II showed a significant ($P \leq 0.01$) decrease than Group I. The mean liver glycogen percentage of Group I and II was

2.49 ± 0.04 and 1.82 ± 0.03 g percent, respectively. Liver glycogen level of Group IV and VI did not differ significantly with Group I. The rest of the treatment groups showed a significant decrease ($P \leq 0.01$) in liver glycogen content than Group I (Fig. 19)

The treatment Groups IV, VI, VII, VIII and IX showed a significant ($P \leq 0.01$) increase in liver glycogen content when compared to Group II. The mean liver glycogen content of Group IV, VI, VII, VIII and IX was 2.31 ± 0.04, 2.39 ± 0.06, 2.02 ± 0.02, 2.07 ± 0.05 and 2.14 ± 0.02 g percent. The mean liver glycogen content of Group III and V was 1.98 ± 0.02 and 1.89 ± 0.03 g percent, respectively and was similar to the level in Group II (Fig.19).

4.3.5.2. Effect on plasma ALT

The diabetic control animals (Group II) showed a significant ($P \leq 0.001$) increase in plasma ALT value on all treatment days when compared to normal control animals (Group I). Group I did not differ between the treatment days and the ALT level was maintained within the normal range. The mean plasma ALT values obtained for Group I on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 45.00 ± 0.85, 45.13 ± 0.78, 45.13 ± 0.81, 44.88 ± 0.83, 48.63 ± 0.84 and 49.88 ± 0.92 U/L, respectively. Further within Group II there was a significant ($P \leq 0.001$) increase in plasma ALT level from 56^{th} day onwards and the mean plasma ALT values obtained on zero, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day were 151.88 ± 7.61, 159.63 ± 7.20, 166.63 ± 7.77. 166.75 ± 6.41, 173.13 ± 6.97 and 176.63 ± 6.78 U/L respectively (Fig.17).

On comparison with Group II on zero day all the treatment groups (Group III to IX) did not differ significantly in plasma ALT value except Group IV, which showed a significant increase in plasma ALT. On 7^{th} day the Groups IV, VII and VIII showed a significant ($P \leq 0.001$) decrease in plasma ALT than Group II with a mean plasma ALT value of 115.13 ± 18.64, 111.00 ± 22.46 and 131.00 ± 18.55 U/L,

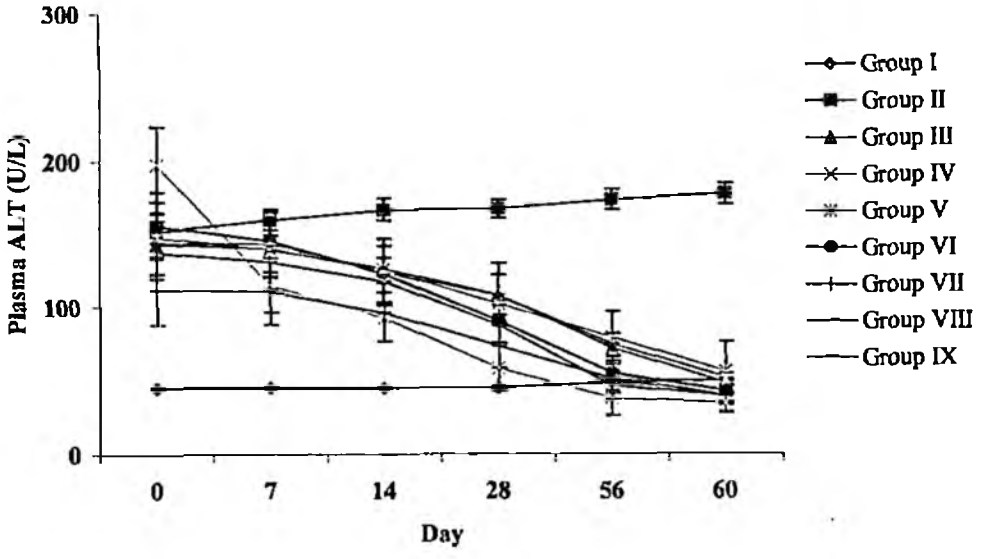

Fig. 17. Effect of *Scoparia dulcis* and *Costus pictus* on plasma ALT (U/L) level in rats

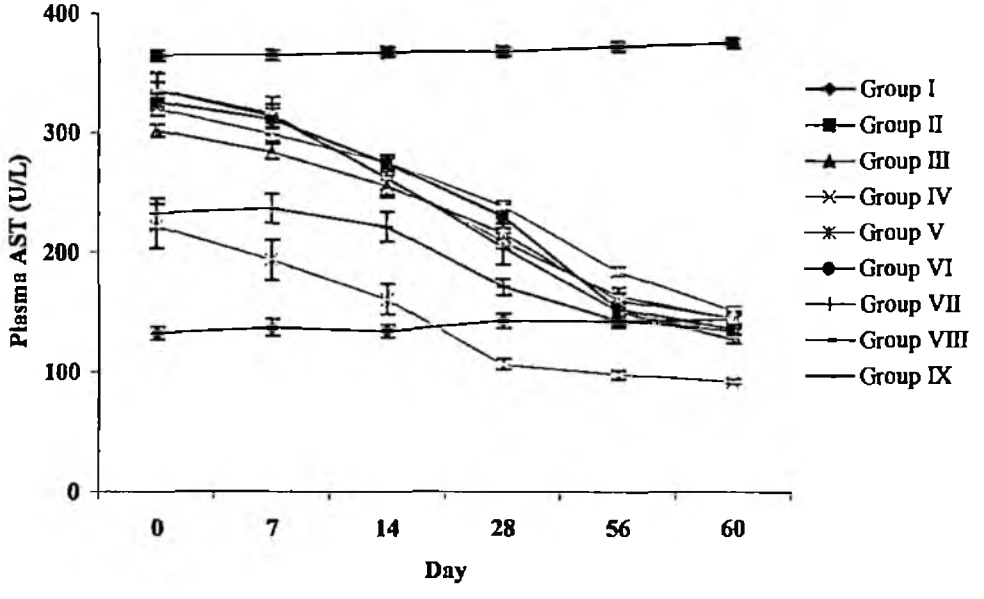

Fig. 18. Effect of *Scoparia dulcis* and *Costus pictus* on plasma AST (U/L) level in rats

Mean ± SE, N=8, Group I- Normal control, Group II- Diabetic control, Group III- Diabetic, treated with SDAEt, Group IV- Diabetic, treated with SDAlEt, Group V- Diabetic, treated with CPAEt, Group VI- Diabetic, treated with CPAlEt, Group VII- Diabetic, treated with SDAEt + CPAEt, Group VIII- Diabetic, treated with SDAlEt + CPAlEt, Group IX- Diabetic, treated with glibenclamide.

respectively. From 14th day onwards all the treatment groups (Group III to IX) showed a significant ($P \leq 0.001$) decrease in plasma ALT value than Group II. On 28th day all the treatment groups showed a significant ($P \leq 0.001$) decrease in plasma ALT than Group II. The mean plasma ALT level obtained on 28th day for Groups III, IV, V, VI, VII, VIII and IX were 108.00 ± 13.55, 58.00 ± 15.52, 102.38 ± 10.01, 90.13 ± 14.96, 73.63 ± 15.38, 88.00 ± 12.91 and 108.38 ± 21.35 U/L. On 56th and 60th day also all the treatment groups showed a significant ($P \leq 0.001$) decrease in plasma ALT value than the Group II. The mean plasma ALT values obtained are presented in Fig.17.

Within Group III there was a significant ($P \leq 0.001$) decrease in plasma ALT from 28th day onwards. The mean plasma ALT values for Group III on zero, 7th, 14th, 28th, 56th and 60th day were 143.25 ± 21.05, 140.25 ± 19.81, 126.13 ± 16.21, 108.00 ± 13.55, 72.38 ± 9.43 and 47.63 ± 6.50 U/L, respectively.

Within Group IV there was a significant ($P \leq 0.001$) decrease in plasma ALT value from 7th day onwards. The mean plasma ALT values for Group IV on zero, 7th, 14th, 28th, 56th and 60th day were 197.75 ± 25.78, 115.13 ± 18.64, 93.25 ± 16.53, 58.00 ± 15.52, 37.75 ± 11.30 and 34.38 ± 6.19 U/L. respectively (Fig.17).

Within Group V, there was a significant ($P \leq 0.001$) decrease in plasma ALT level from 14th day onwards. The mean plasma ALT values obtained on zero, 7th, 14th, 28th, 56th and 60th day were148.00 ± 37.33, 139.50 ± 18.05, 125.63 ± 14.56, 102.38 ± 10.01, 79.38 ± 6.78 and 55.38 ± 5.80 U/L, respectively.

Within Group VI, there was a significant ($P \leq 0.001$) decrease in plasma ALT value from 14th day onwards. The mean values obtained on zero, 7th, 14th, 28th, 56th

and 60th day were 156.13 ± 22.54, 145.13 ± 21.37, 122.88 ± 19.23, 90.13 ± 14.96, 54.88 ± 11.55 and 42.38 ± 7.80 U/L, respectively (Fig.17).

Within Group VII there was a significant (P≤0.001) decrease in plasma ALT level from 28th day onwards. The mean plasma ALT values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 111.88 ± 23.28, 111.00 ± 22.46, 96.50 ± 19.83, 73.63 ± 15.38, 51.38 ± 9.84 and 39.50 ± 7.01 U/L, respectively.

Within the Group VIII there was a significant (P≤0.001) decrease in plasma ALT level from 14th day onwards. The mean plasma ALT values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 137.75 ± 18.13, 131.00 ± 18.55, 118.00 ± 15.78, 88.00 ± 12.91, 46.25 ± 9.61 and 39.50 ± 6.35 U/L., respectively.

Within Group IX there was a significant (P≤0.001) decrease in plasma ALT level from 14th day onwards. The mean value obtained on 60th day was 51.88 ± 24.07 U/L (Fig.17). Group IX showed a significant (P≤0.01) decrease in plasma ALT level than Group IV on zero day (197.75 ± 25.78 U/L). On 7th day it showed a significant (P≤0.001) increase in plasma ALT level than Group IV and VII with a mean plasma ALT value of 115.13 ± 18.64 and 111.00 ± 22.46 U/L, respectively. On 14th day also it showed a significant (P≤0.001) increase in plasma ALT value than Group IV and VII. On 28th day it showed a significant (P≤0.001) increase in plasma ALT value than Groups IV, VI, VII and VIII with a mean value of 58.00 ± 15.52, 90.13 ± 14.96, 73.63 ± 15.38 and 88.00 ± 12.91 U/L. On 56th day it showed a significant (P≤0.001) increase in plasma ALT level than Groups IV, VI, VII and VIII with a mean plasma ALT value of 37.75 ± 11.30, 54.88 ± 11.55, 51.38 ± 9.84 and 46.25 ± 9.61 U/L, respectively. On 60th day it showed a significant (P≤0.01) increase in plasma ALT level than Group IV with a mean plasma ALT value of 34.38 ± 6.19 U/L (Fig. 17).

Group IV (treated with SDAlEt) with a mean plasma ALT value of 34.38 ± 6.19 U/L showed the minimum plasma ALT level in terms of absolute value followed by Group VIII (treated with SDAlEt + CPAlEt) with a mean plasma ALT value of 39.50 ± 6.35 U/L. The descending order of plasma ALT level for other treatment groups on 60th day are Group VII (39.50 ± 7.01 U/ L), Group VI (42.38 ± 7.80 U/L). Group III (47.63 ± 6.50 U/L), Group IX (51.88 ± 24.07 U/L) and Group V (55.38 ± 5.80 U/L).

4.3.5.3. Effect on plasma AST

The diabetic control animals (Group II) showed a significant (P≤ 0.001) increase in plasma AST value than the normal control animals (Group I). Group I did not differ significantly between the treatment days and the AST values were maintained within the normal range. The mean plasma AST values obtained for Group I on zero, 7th, 14th, 28th, 56th and 60th day were 132.13 ± 5.73, 137.00 ± 6.97, 133.50 ± 5.29, 141.88 ± 5.82, 141.63 ± 4.94 and 143.13 ± 4.80 U/L, respectively. Within Group II there was no significant difference between the treatment days though there was an increase in plasma AST value throughout the entire study period (Fig.18).

The treatment Groups III, IV and VII showed a significant (P≤0.001) decrease in plasma AST level than Group II on zero day, with a mean plasma AST value of 301.50 ± 5.14, 221.25 ± 18.86 and 232.25 ± 12.62 U/L, respectively. From 7th day onwards all the treatment groups (Group III to IX) showed a significant (P≤ 0.01) decrease in plasma AST level than Group II. The mean plasma AST value obtained on 7th day for Groups III, IV, V, VI, VII, VIII and IX were 283.88 ± 6.71, 193.00 ± 17.10, 298.50 ± 5.92, 311.13 ± 8.37, 236.38 ± 12.14, 313.63 ± 15.91 and 315.75 ± 7.41 U/L, respectively. The mean plasma AST value obtained for the treatment groups are presented in Fig. 18.

Within Group III there was a significant ($P \leq 0.001$) decrease in plasma AST value from 7th day onwards. The mean plasma ALT values are presented in Fig.18.

In Group IV there was a significant ($P \leq 0.001$) reduction in plasma AST level from 7th day onwards. The mean plasma AST values obtained on zero, 7th, 14th, 28th, 56th and 60th day were 221.25 ± 18.86, 193.00 ± 17.10, 160.63 ± 12.52, 105.63 ± 4.44, 97.25 ± 3.43 and 91.38 ± 2.15 U/L, respectively.

Within Group V there was a significant ($P \leq 0.001$) reduction in plasma AST level from 7th day onwards and the mean plasma AST values are represented in Fig.18.

Within Group VI there was a significant ($P \leq 0.001$) reduction in plasma AST level from 14th day onwards and the mean plasma AST values are presented in Fig.18.

Within Group VII there was a significant ($P \leq 0.001$) decrease in plasma AST level from 28th day onwards and the mean values are represented in the Fig.18.

Within Group VIII there was a significant ($P \leq 0.001$) decrease in plasma AST value from 14th day onwards and the mean plasma ALT values are presented in Fig.18.

Within Group IX there was a significant ($P \leq 0.001$) reduction in plasma AST level from 14th day onwards. The mean values of plasma AST are represented in Fig. 18. Group IX showed a significant ($P \leq 0.001$) increase in plasma AST level than Group IV and VII on zero and 7th day. On 14th day it showed a significant ($P \leq 0.001$) increase in plasma AST level than Group IV. On 28th day Group IX showed a significant ($P \leq 0.001$) increase in plasma AST value than Group IV and VII. On 56th and 60th day it showed a significant ($P \leq 0.001$) increase in plasma

AST level than Group IV with a mean value of 97.25 ± 3.43 and 91.38 ± 2.15 U/L. The mean plasma AST values for the treatment groups are presented in Fig.18.

In terms of absolute value Group IV (treated with SDAIEt) showed minimum plasma AST level with a mean value of 91.38 ± 2.15 U/L on 60th day followed by Group VIII (treated with SDAIEt + CPAIEt) with a mean plasma AST value of 126.63 ± 3.50 U/L. The descending order of plasma AST value for other treatment groups on 60th day are Group VII (133.38 ± 2.71 U/L), Group VI (135.13 ± 3.89 U/L), Group IX (143.00 ± 6.18 U/L), Group III (144.88 ± 5.09 U/L) and Group V (149.88 ± 3.87 U/L).

4.3.6. Effect of *Scoparia dulcis* and *Costus pictus* on Trace Minerals

4.3.6.1. Effect on plasma copper content

The Group II did not differ significantly with Group I. The copper content on the 60th day of treatment did not show any significant difference in any of the treated groups, when compared with Group II. The mean plasma copper content for Groups I, II, III, IV, V, VI, VII, VIII and IX on 60th day were 0.24 ±0.02, 0.26 ± 0.02, 0.23 ± 0.02, 0.30 ± 0.02, 0.31 ± 0.62, 0.31 ± 0.02, 0.31 ± 0.02, 0.32 ± 0.02 and 0.28 ± 0.02 ppm, respectively (Fig. 20).

4.3.6.2. Effect on plasma iron content

The plasma iron content on the 60th day of study in Group II showed a significant ($P \leq 0.01$) increase in iron content than Group I. All the treatment groups (Group III to IX) also showed a significant ($P \leq 0.01$) increase in plasma iron content when compared to Group II. The mean values were 0.19 ± 0.02, 0.22 ± 0.02, 0.19 ± 0.02, 0.18 ± 0.02, 0.22 ± 0.02, 0.24 ± 0.02, 0.24 ± 0.01 and 0.22 ± 0.02 ppm, respectively (Fig. 20).

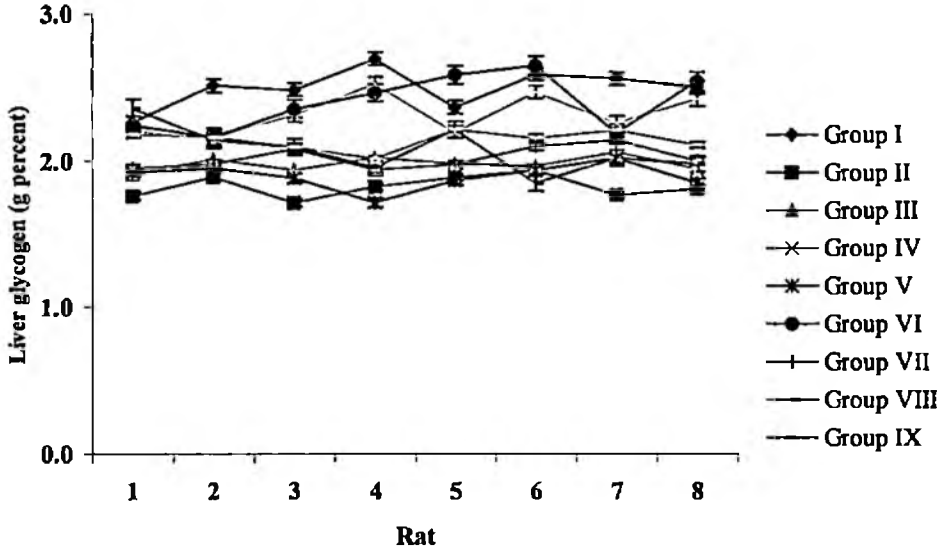

Fig. 19. Effect of *Scoparia dulcis* and *Costus pictus* on liver glycogen (g percent) level in rats

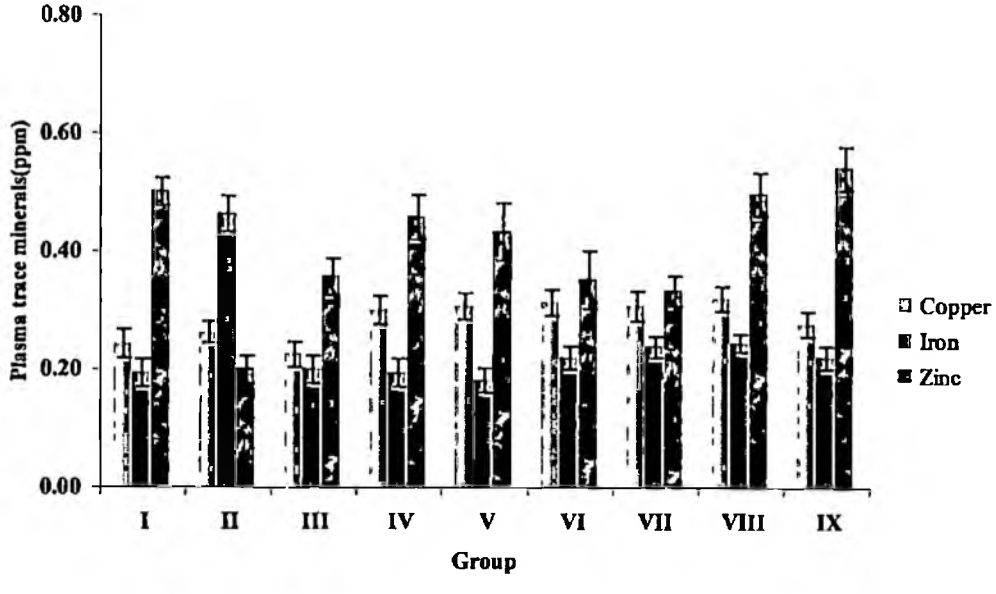

Fig. 20. Effect of *Scoparia dulcis* and *Costus pictus* on trace mineral (ppm) level in rats

Mean ± SE, N=8, Group I- Normal control, Group II- Diabetic control, Group III- Diabetic, treated with SDAEt, Group IV- Diabetic, treated with SDAlEt, Group V- Diabetic, treated with CPAEt, Group VI- Diabetic, treated with CPAlEt, Group VII- Diabetic, treated with SDAEt + CPAEt, Group VIII- Diabetic, treated with SDAlEt + CPAlEt, Group IX- Diabetic, treated with glibenclamide.

4.3.6.3. Effect on plasma zinc content

The Group II showed a significant ($P \leq 0.01$) decrease in plasma zinc content than Group I at the end of the study (60^{th} day). Groups IV, VIII and IX showed significant ($p \leq 0.05$) increase in plasma zinc content in comparison to the diabetic group (Group II) at the end of 60 days treatment. The mean values were 0.46 ± 0.04, 0.49 ± 0.04 and 0.54 ± 0.04 ppm, respectively. The plasma zinc content of Groups III, VI and VII did not differ significantly with Group II at the end of the study (Fig.20).

Discussion

5. DISCUSSION

Diabetes mellitus is one of the major metabolic disorders affecting millions of people worldwide. This disorder is often associated with high frequency of mortality rate besides heart ailments, blindness and gangrene. Despite concerted efforts of medical scientists over the years, the disease still eludes satisfactory cure in modern medicine (Khan and Singh, 1996). Most of the hypoglycaemic 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 and herbal medicines are one such alternative. A great deal of attention has been recently given to the therapeutic use of herbal remedies for reasons of safety, efficacy and economy.

The present study was directed to evaluate the effectiveness of two plants namely *Scoparia dulcis* and *Costus pictus* mentioned in Ayurveda and folklore for the treatment of diabetes. In this study, the hypoglycaemic, hypolipidaemic and hepatoprotective effects of aqueous and alcoholic extract of these plants and their combination were assessed and compared with a standard oral hypoglycaemic agent, glibenclamide.

5.1. BODY WEIGHT

The body weight gets reduced in diabetic patients due to the increased mobilization of fatty acids from the storage site for meeting out the energy demand (Xie *et al.*, 2003).

The animals which were induced to become diabetic (Group II) showed substantial reduction in the body weight after 14^{th} day of study and it was continued even after two months of study. All the treatment groups showed an increase in body weight with respect to the diabetic control animals after 28 days of treatment. Among the treatment groups, the animals treated with glibenclamide

(Group IX) showed the maximum increase of 12.36 percent in body weight after 60 days of treatment. The animals treated with alcoholic leaf extract of *Scoparia dulcis* also showed a similar increase in the body weight (12.03 per cent) on 60th day of treatment with respect to initial weight. The alcoholic and aqueous leaf extract either alone or with their respective combination not only prevented the loss in body weight but also produced a substantial increase in body weight at the end of the treatment period. These findings concur with earlier findings of Prince and Menon (2000) and Shirwaikar *et al.* (2005). In the present study the alcoholic extract of *Scoparia dulcis* was found to be superior over other form of extract tested as far a the weight gain of diabetic rats were concerned. These findings confer the ability of plant extracts not only in preventing loss of body weight but also in increasing the body weight, which might be due to their antidiabetic effect (Vetrichelvan *et al.*, 2002, Sachdewa and Khemani, (2003) and Chakrabarti *et al.*, 2005). The results in the present study indicates that the animals treated with the plant extract showed a substantial gain in body weight, which suggest that the hypoglycaemic activity of the plants could be explained by an improvement in the sensitivity of insulin and/or by the reduction of insulin resistance (Medina *et al.*, 1994 and Alarcon-Aguilar *et al.*, 2005).

5.2. HAEMATOLOGICAL PARAMETERS

5.2.1. Red Blood Cell Count and White Blood Cell Count

The normal RBC count for rats ranges from 5.4 to 8.9 million/µl and the normal WBC count ranges from 4.0 to 10.2 thousand/µl (Pritchett and Corning, 2004). The RBC and WBC values for the experimental groups did not differ significantly for the entire study period and they were maintained within the normal level. The literature survey also showed no changes in the RBC and WBC count during diabetes mellitus.

5.2.2. Total Haemoglobin Content

The total haemoglobin content in diabetes gets reduced due to increased glycosylation of haemoglobin (Dhandapani *et al.*, 2002, Pari and Saravanan, 2002 and Prince *et al.*, 2004).

The total haemoglobin content of diabetic control animals showed substantial decrease from 14th day onwards till the end of the study period. The total haemoglobin content of the diabetic control animals was reduced by 37.63 percent after two months of study. Among the treatment groups, animals treated with either combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* (Group VIII) or *Scoparia dulcis* alone showed a substantial gain in total haemoglobin content with 35.14 and 26.31 percent, respectively compared to the pretreatment values. The animals treated with glibenclamide (Group IX) also showed significant improvement (21.09 percent) in total haemoglobin content after 60 days of treatment. These findings agree with the earlier reports of Pari and UmaMaheshwari (1999) that chloroform extract of *Musa sapientum* flowers increased the total haemoglobin content in diabetic rats. These findings also corroborates with earlier findings of Prince and Menon (2000), who showed that the aqueous root extract of *Tinospora cordifolia* increased the total haemoglobin content by reducing the glycosylation of haemoglobin in diabetic rats. The increase in total haemoglobin content might be due to the good glycaemic control produced by the active principle of the plants which are both water and alcohol soluble and also due to decreased glycosylation of haemoglobin (Prince *et al.*, 2004).

5.3. BIOCHEMICAL PARAMETERS

5.3.1. Glycosylated haemoglobin

Glycosylated haemoglobin represents stable ketone-amine or aldehyde amine linkages formed by non-enzymatic glycosylation of valine and lysine in the

haemoglobin molecule. The glucose molecule when present in excess binds to the lysine and valine residue of the haemoglobin molecule, which represent time averaged values for blood glucose over the preceding 2 to 4 months (Shirwaikar *et al.*, 2005).

The glycosylated haemoglobin value for normal rats was found to be 0.25 ± 0.02 mg/gHb (Pari and UmaMaheswari, 1999). In the present study, the glycosylated haemoglobin value was high in diabetic control animals (0.92 ± 0.06 mg/gHb) compared to other groups, which showed an increase of 135.89 percent in glycosylated haemoglobin after two months of study. Pari and UmaMaheshwari (1999) also obtained a similar result in diabetic control rats where the glycosylated haemoglobin content increased after 30 days. All the treatment groups showed a substantial reduction in glycosylated haemoglobin after 28 days of treatment. Among the treatment groups the animals treated with alcoholic leaf extract of *Costus pictus* (Group VI) showed the maximum reduction (34.04 percent) in glycosylated haemoglobin followed by animals treated with alcoholic leaf extract of *Scoparia dulcis* (32.69 percent). The animals treated with combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* (Group VIII) also showed reduction in glycosylated haemoglobin content (27.65 percent), while the group treated with glibenclamide showed only 10.81 percent reduction in glycosylated haemoglobin level after two months of treatment. Pari and Satheesh (2004) got a similar result of substantial reduction in glycosylated haemoglobin in diabetic rats, while studying the hypoglycaemic effect of aqueous leaf extract of *Boerhaavia diffusa*. The results obtained in the present study clearly indicates that active constituents in the leaf extract which are soluble in both the water and alcohol medium could help in controlling the hyperglycaemia thereby reducing the glycosylation of haemoglobin. These findings concur with the earlier findings of Degirmenci *et al.* (2005) who found that the decoction of *Rumex patientia* grain showed a significant reduction in glycosylated haemoglobin level through its hypoglycaemic property.

5.3.2. Plasma Glucose

Blood glucose is the main source of instant energy to the cells. Diabetes mellitus is a metabolic disorder in which the plasma glucose level is increased, where by the cells are deprived of glucose for their energy needs and may resort to utilize the fatty acids for energy. The excess glucose present in the blood also leads to complications when left untreated (Abdel-Barry *et al.*, 1997).

The plasma glucose level in diabetic control animals increased steadily throughout the study period. The mean plasma glucose value obtained after two months for diabetic control animals was 345.88 ± 6.18 mg/dl, which concurs with the earlier findings of Babu *et al.* (2002) who showed that the plasma glucose level in diabetic animals would increase substantially. In the present study the plasma glucose was found to be decreased from the pretreatment value in all the treatment groups. Among the treated animals, those treated with alcoholic leaf extract of *Scoparia dulcis* (Group IV) showed 15.15 percent decrease in plasma glucose after two weeks of treatment and it continued to produce a substantial reduction (46.44 percent) in plasma glucose even after one month of treatment. It produced 75.17 percent decrease in plasma glucose level after two months of treatment. The alcoholic leaf extract of *Costus pictus* was also equally effective in reducing plasma glucose (74.53 per cent). Similar observation was made by Jafri *et al.* (2000) who found that the aqueous-ethanolic flower extract of *Punica granatum* reduced the plasma glucose level significantly via better peripheral glucose utilization. The present findings of the study also concur with the earlier findings of various researchers that the leaf extract of antidiabetic plants produce reduction in plasma glucose level in diabetic treated rats (Yadav *et al.*, 2002, Daitewa *et al.*, 2004, Ojewole (2005) and Eddouks *et al.*, 2004). The animals treated with combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* (Group VIII) also showed similar reduction in plasma glucose, which might be due to restoration of delayed insulin response or due to inhibition of intestinal absorption of glucose thereby preventing the rise in blood sugar level as suggested by Prince *et al.* (1999) and Fuliang *et al.* (2005).

The animals treated with aqueous leaf extract of *Scoparia dulcis* also showed reduction (7.41 percent) in plasma glucose level after one week of treatment. After one month of treatment these animals showed a substantial reduction of 20.99 percent and this trend continued and after two months of treatment the reduction in plasma glucose level was 73.67 per cent. The animals treated with combination of aqueous leaf extract of *Scoparia dulcis* and *Costus pictus* showed 60.09 percent decrease and the animals treated with aqueous leaf extract of *Costus pictus* showed 56.12 percent reduction in plasma glucose after two months of treatment. Different mechanisms of action to reduce the blood glucose levels have been attributed to plant extracts. Some plant exhibit properties similar to the well known sulfonylurea drugs like glibencalmide, which reduce the blood glucose in normoglycaemic animals by increasing the insulin secretion from β-cells. Some of the plants act like biguanides such as metformin, suppressing the hepatic glucose production, which is an antihyperglycaemic compound and do not affect blood glucose in normal state (Sy *et al.*, 2005).

In the present study the alcoholic leaf extract of *Scoparia dulcis* alone could produce 75.17 percent reduction in plasma glucose. The alcoholic leaf extract combination could produce 73.04 percent reduction in plasma glucose level and is comparable to glibenclamide, which produced 72.08 percent reduction in plasma glucose level. Sathyan (2004) has got similar reduction in plasma glucose level, when the diabetic rats were fed with *Azadirachta indica*, *Ocimum sanctum* and *Tinospora cordifolia* leaf extracts and suggested that the extracts could delay the absorption of complex carbohydrates in small intestine like that of acarboses resulting in a decreased post prandial glucose content and a reduction in long term diabetic complications. The antidiabetic property of plant extracts involve one or more compounds to decrease blood glucose suggesting that the natural constituents could act separately or synergistically to induce hypoglycaemic effect. (Sy *et al.*, 2005). The glucose lowering action of the leaf extracts could be due to some of the active constituents that are soluble in both water and alcohol solvent. The glucose lowering action of the leaf extract could

also be due to the consequence of an improved lipid metabolism apart from the direct interaction with glucose homeostasis. According to Randle's glucose –fatty acid cycle, increased supply of plasma triglycerides per se could constitute a source of increased free fatty acid availability and oxidation that can impair insulin action, glucose metabolism and utilization leading to development of hyperglycaemia. Therefore the reduction of triglyceride following treatment with the leaf extract could also facilitate the glucose oxidation and utilization and subsequently the reduction of hyperglycaemia (Muruganandan *et al.*, 2005).

5.3.3. Plasma Lipid Profile

The β-cells of the islets of Langherhans in alloxan induced diabetes are destroyed resulting in insulin deficiency. Insulin has an inhibitory effect on the lipase enzyme, which breaks down the fat depots as there is elevated lipolysis resulting in high plasma lipid level in diabetes (Uadia, 2003). Besides in insulin deficiency, glucagon, catecholamines and other hormones enhance lipolysis resulting in hyperlipidaemia (Prince *et al.*, 1999). Changes in plasma lipid concentrations are a frequent complication in patients with diabetes, which contributes to the development of vascular disease (Singh *et al.*, 2005)

In the present study the animals, which were induced to become diabetic, showed a substantial increase in total plasma lipid level with a mean value of 169.25 ± 3.15 mg/dl after two months of study period, which agrees with the findings of Dhandapani *et al.* (2002) that the total plasma lipid content was increased in diabetic rats. It has been reported that the rise in blood sugar level in diabetes mellitus is accompanied with an increase in plasma concentration of total lipids, cholesterol, triglycerides, LDL-C, VLDL-C and a fall in HDL-C (Sharma *et al.*, 2003). This concurs with the earlier observations that hyperlipidaemia in diabetes is regarded as a consequence of unlimited action of lipolytic hormones on the fat depots. In all the treatment groups (III to IX) the plasma total lipid, total cholesterol, triglycerides, LDL-C, VLDL-C were found to be increased on zero day itself, while the HDL-C level was seen decreased. The animals treated with

combination of alcoholic leaf extracts of *Scoparia dulcis* and *Costus pictus* (Group VIII) showed a reduction of 50.82 percent in plasma total lipid level while the animals treated with alcoholic leaf extract of *Scoparia dulcis* (Group IV) alone showed a reduction of 42.41 percent after two months of treatment. These results are comparable with findings of Bhandari *et al.* (2005) where diabetic rats treated with *Zingiber officinale* produced a reduction in lipid profile. Group III, V and VI also showed a reduction in hyperlipidaemic condition, which is comparable to that of Group IX, treated with glibencalmide. Dyslipidaemia is proved to be the most important modifiable risk factor contributing to atherosclerosis (Bhandari *et al.*, 2005). The results of the study indicate that both aqueous and alcoholic leaf extracts of these plants possess active principles that are soluble in both water and alcohol and possessing hypolipidaemic property.

Diabetes mellitus is often associated with blood lipid abnormalities, mainly increased levels of total cholesterol, triglyceride, LDL-C and decreased levels of HDL-C, which leads to high blood pressure, atherosclerosis and coronary heart disease (Fuliang *et al.*, 2005). The elevated plasma total cholesterol level decreased in Group VIII, which received a combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* by 47.70 per cent after two months of treatment. This treatment also produced 35.78 percent reduction in plasma total cholesterol after one month of treatment and the value was much lower than other treated groups. These results suggest that atleast one month is needed to produce a substantial reduction in plasma total cholesterol level and it may take two months of treatment to produce a remarkable reduction. Groups III, IV, V, VI and VII also showed substantial reduction in plasma total cholesterol level which is comparable to this property exhibited by glibenclamide (Group IX). Alcoholic leaf extract of *Scoparia dulcis* alone also expressed an equally effective property as that of Group VIII. Similar observation was recorded by Alarcon-Aguilar *et al.* (2005) who found that oral administration of Dichloromethane (DCM) extract of *Ibervillea sonorae* root in alloxan diabetic rats produced a significant decrease in total cholesterol level. The plasma total lipid and cholesterol lowering effects of

the extracts observed in diabetic treated rats may in part be due to the presence of antinutrients. Antinutrients commonly found in plant foods are phytic acid, lectin, flavonoids, phenolic compounds, amylase inhibitors and saponins and they have been reported to reduce blood glucose and insulin response to starchy foods and /or reduce plasma cholesterol and triglycerides. Saponins are known to possess blood cholesterol lowering activity. The proposed mechanism of plasma cholesterol lowering mechanism of saponin is that, it binds cholesterol in the intestinal lumen and makes less available for absorption. In addition it may also bind with bile acids, causing a reduction in its enterohepatic circulation, increasing their faecal excretion. Increased bile acid excretion is offset by enhanced synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol (Uadia, 2003). In the present study the plants contained saponins (Tewari, 1971 and Singh and Srivastava, 1981), flavonoids, glycosides and tannins (Bever, 1980), which might have produced the hypoglycaemic and hypolipidaemic effects.

The animals treated with alcoholic leaf extract of *Costus pictus* (Group VI) and *Scoparia dulcis* (Group IV) produced a substantial reduction in plasma triglyceride level by 42.06 percent and 41.44 percent, respectively at the end of the experimental period. The Group III (34.87 percent) and VIII (35.56 percent) also showed a reduction in plasma triglyceride level, which are comparable to Group IX, treated with glibenclamide, which induced 34.30 per cent reduction in plasma triglyceride. Other treated groups also showed similar trend with plasma triglyceride profile. These findings suggest that to produce a substantial decrease in plasma triglyceride level the treatment of leaf extracts of *Scoparia dulcis* and *Costus pictus* requires nearly two months. John (2003) observed that serum cholesterol and triglyceride contents were reduced in diabetic rats when treated with *Brassica juncea* and *Trigonella foenum-graecum* seeds and attributed to the rich fiber content of these seeds, which reduces the intestinal absorption of fat molecules. In the present study the rich fiber content of the extracts (Shiva *et al.*,

2003) reduced the intestinal absorption of fat molecules and produced the hypolipidaemic effect.

Decreased HDL-C level in diabetic state, increases the complications of diabetes mellitus by inducing atherosclerosis (Sharma *et al.*, 2003, Chakrabarti *et al.*, 2005, Ruzaidi *et al.*, 2005 and Shirwaikar *et al.*, 2005). In the investigation Group II, which served as diabetic control showed a reduction in HDL-C compared to normal animals. The animals treated with combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* (Group VIII) showed a maximum increment in HDL-C level (61.50 percent). The animals treated with alcoholic leaf extract of *Costus pictus* (Group VI) could also induce an increase in plasma HDL-C level as effectively as glibenclamide (Group IX). The other treatment groups such as Group III, IV, V and VII also showed a significant regain in plasma HDL-C level. These observations agree with the findings of Pari and Saravanan (2002), Sachdewa and Khemani (2003), Sharma *et al.* (2003), Alarcon-Aguilar *et al.* (2005) and Gupta *et al.* (2005) that the treatment of diabetic rats with hypoglycaemic plants increased the HDL-C content. A normal high-density lipoprotein (HDL) level is necessary for the good health of the individuals as it picks up the cholesterol content of the body to the liver for metabolism and excretion through bile salts and thereby reducing the incidence of plaque formation in arteries and thus prevents atherosclerosis, a major complication in diabetes mellitus. In the present study the findings reveals that the alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* both produce a noticeable improvement in HDL-C level and can be used in the management of diabetes mellitus

The elevated plasma LDL-C and VLDL-C levels were decreased in all the treatment groups with a higher reduction in Group VIII treated with combination of alcoholic extract of *Scoparia dulcis* and *Costus pictus* by 81.49 percent followed by animals treated with alcoholic leaf extract of *Scoparia dulcis* by 80.51 percent, which was comparable to that of the Group IX treated with glibenclamide (78.03 percent) reduction. These findings corroborate with the

earlier findings of Bopanna *et al.* (1997) that the neem seed administration reduced the LDL –C and VLDL –C level in rabbits and suggested that it could be due to an increase in the liver LDL receptor activity. Antihyperlipidaemic effect may also be due to the down regulation of NADPH and NADH, which act as cofactors in fat metabolism. Another mechanism of action may be decreased synthesis or increased excretion of lipids through intestinal tract.

The levels of plasma total lipids, total cholesterol, triglyceride, LDL-C, VLDL-C were lowered and HDL-C level was elevated in all the treatment groups than the diabetic control group. So it is reasonable to conclude that both the leaf extracts possess phytochemicals that have a potential hypolipidaemic effect.

5.3.4. Plasma Protein Profile

Hypoproteinemia is a common problem in diabetic animals and is generally attributed to the presence of diabetic nephropathy. The rate of protein synthesis gets diminished in the liver of diabetic rats (Ghosh and Suryawanshi, 2001). The animal induced to become diabetic, recorded a low protein level of 5.73 ± 0.08 g/dl when compared to the normal control (8.55 ± 0.20 g/dl) on 60^{th} day. All the treated groups showed an enhancement in plasma protein content over 60 days of treatment. From the depressed initial value the total protein was increased in animals treated with combination of aqueous leaf extract of *Costus pictus* by 33.94 percent. The albumin concentration was also increased in all the treated groups and the highest value observed in animals treated with aqueous leaf extract of *Scoparia dulcis* was 64.94 percent increment. The protein sparing action of the extracts as shown in the results might be due to the increased glucose availability offered by the plant extracts through its antidiabetic effect. A similar finding was also observed by Ghosh and Suryawasnshi (2001) where feeding aqueous leaf extract of *Catharanthus roseus* to diabetic rats increased the plasma protein profile.

Wanke and Wong (1991) found the inhibitors of albumin gene expression in the cell free system in the liver of alloxan induced diabetic rats. When treated with insulin, the albumin promoter activity was found to be normal in hepatonuclear extracts from the diabetic rats. Since extracts used in the present study also improved the protein profile it could be worthwhile to investigate whether the albumin promoter activity in the liver cells was increased to synthesize more proteins. Similar findings were reported by Babu *et al.* (2003) who found that oral feeding of ethanolic leaf extract of *Cassia kleinii* to diabetic rats increased the albumin content.

The globulin content of all the treated groups substantially increased compared to the diabetic control animals. The animals fed with aqueous leaf extract of *Costus pictus* recorded an increase in the amount of globulin by 24.69 percent more when compared to the pretreatment value. The A/G ratio was also increased in all the treated groups and the highest being seen in animals treated with aqueous extract of *Scoparia dulcis*. These findings concur with the earlier reports of Ghosh and Suryawanshi (2001) where oral administration of the aqueous leaf extract of *Catharanthus roseus* to diabetic rats increased the globulin content. The plasma A/G ratio was also increased in diabetic rats when aqueous leaf extract of *Catharanthus roseus* was fed to diabetic rats (Ghosh and Suryawanshi, 2001).

5.3.5. Liver Glycogen and Enzymes

Glycogen is the primary intracellular storage form of glucose and its level in various tissues is indicative of insulin activity as it promotes intracellular deposition of glucose by stimulating glycogen synthase and inhibiting glycogen phosphorylation. Alloxan cause selective destruction of β- cells of islets of Langherhans resulting in marked decrease in the insulin levels and a deposition of the glycogen in tissue especially liver and skeletal muscles (Vats *et al.*, 2004). The liver glycogen is decreased in diabetes mellitus due to the increased

utilization for energy production and the associated liver enzyme activities are elevated (Maiti *et al.*, 2004).

The liver glycogen was increased remarkably in animals treated with alcoholic leaf extract of *Costus pictus* at the end of 60 days of treatment and it was in par with glycogen content of animals treated with alcoholic leaf extract of *Scoparia dulcis*. The animals treated with glibenclamide also produced significant increase in liver glycogen. All other treated groups produced a moderate increase in liver glycogen than the diabetic control animals, which recorded the lowest glycogen level. Similar finding was also observed by John (2003) when the diabetic rats were fed with *Brassica juncea* and *Trigonella foenum-graecum* seeds, liver glycogen content was increased and suggested that these seeds might enhance the rate of glycogenesis in alloxan treated rats. The increase in liver glycogen might be due to the increase in cellular uptake of glucose induced by the leaf extract, which might contain phytochemical, which has insulin like properties. The increase in hepatic glycogen content observed in all the treated groups could be the result of active phytochemicals responsible for glucose regulation more soluble alcoholic than aqueous medium. These findings concur with the earlier reports of Musabayne *et al.* (2005) who found that aqueous leaf extract of *Syzygium cordatum* increased the hepatic glycogen content in diabetic rats.

Abnormal levels of ALT and AST are of clinical and toxicological importance, being indicative of tissue/organ damage by toxicants or disease condition (Singh *et al.*, 2005). Experimental diabetes induced by alloxan in rats causes tissue damage in the pancreas, liver, kidney and heart, which can be reflected as the increment in the activities of ALT and AST levels (Prince *et al.*, 2004).

The liver enzyme ALT was elevated on zero day and by the treatment with the plant extracts it was brought down towards the normal levels. The animals treated with alcoholic leaf extract of *Scoparia dulcis* produced a significant reduction in plasma ALT level by 82.61 per cent. The elevated AST level was

reduced substantially when the animals were treated with combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* (62.25 per cent). All the other treatment groups also produced significant reduction in level of plasma liver enzymes. Similar findings was also observed by Alarcon-Aguilar *et al.* (2005) that by feeding Dichloromethane extract of *Ibervillea sonorae* root to diabetic rats reduced the increased activity of liver enzymes. These findings also concur with similar findings of Ghosh and Suryawanshi (2001) who found that feeding aqueous leaf extract of *Catharanthus roseus* to diabetic rats reduced the elevated levels of liver enzymes. In diabetes mellitus there is increased gluconeogenesis and ketogenesis and the activities of the transaminases increased. (Maiti *et al.*, 2004). The restoration of the ALT and AST activities towards their respective normal levels after treatment further strengthens the hepatoprotective and antidiabetogenic effect of these plant extracts.

5.3.6. Trace Minerals

There was no significant difference in the level of plasma copper and iron in the treatment groups except for zinc level, which increased in comparison to diabetic control. Shaheen and El-Fattah (1995) observed that diabetogenic doses of alloxan lowered the plasma zinc content in rats and produced zinc deficiency in chemical diabetes. Kechrid *et al.* (2002) also observed a lower level of pancreatic zinc in diabetic animals. The highest increase of zinc content was encountered in animals treated with glibenclamide followed by animals treated with combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus*. The feeding of alcoholic leaf extract of *Scoparia dulcis* alone also showed increase in the plasma zinc level when compared to diabetic control. These finding suggest that the zinc would have potentiated the storage and release of insulin as zinc was essential for dimeric assembly of insulin content within the cell and release (Derewenda *et al.*,1989). A similar finding was observed by Chausmer (1998) where diabetic rats supplemented with oral zinc prevented the hyperzincuria resulting from urinary loss due to hyperglycaemia. In the present study the interrelationship between iron

and zinc was negative. In diabetic control animals the iron content was increased while it was lower in case of treated animals. In contrast the zinc content of diabetic control animals was lower and for that of treated animals it was increased. Fernandez-Real *et al.* (2002) found that an increased iron store increases the chances of development of Type 2 diabetes, while iron depletion was protective and iron-induced damage might also modulate the development of chronic diabetic complications. They also suggested that iron depletion had been beneficial in coronary artery responses, endothelial dysfunction, insulin secretion, insulin action, and metabolic control in Type 2 diabetes. The treatment with these plant extracts increased the plasma zinc content and decreased the plasma iron content, which indicates that these extracts could supplement zinc.

To conclude both the plants, *Scoparia dulcis* and *Costus pictus* used in the present study possessed hypoglycaemic, hypolipidaemic and hepatoprotective actions and it was more pronounced in case of alcoholic extracts which indicates the active phytochemicals responsible for bringing about above actions are more efficiently soluble in alcohol medium than in the aqueous medium. Further phytochemical analysis should be done to evaluate the hypoglycaemic property of these plants for effective treatment of diabetes.

Summary

6. SUMMARY

The present study was undertaken to assess and compare the hypoglycaemic effect of aqueous and alcoholic extracts of *Scoparia dulcis* and *Costus pictus* and their combination @ 500 mg/kg body weight orally in alloxan induced diabetic rats and to compare their efficacy with the established oral hypoglycaemic agent, glibenclamide. The study was also directed to find out whether the combination has a synergistic / additive effect.

The experiment was conducted in seventy-two male Sprague-Dawley rats weighing 150-250 g. All the animals were induced Diabetes by giving alloxan @130 mg/kg intraperitoneally for two consecutive days and those rats showing glucose levels above 300mg/dl after 5 days were selected for the study. They were randomly divided into nine groups of eight animals each. Group I served as normal control. Group II was maintained as diabetic control. Group III received aqueous leaf extract of *Scoparia dulcis* @ 500 mg/kg body weight orally for 60 days and Group IV was given alcoholic leaf extract of *Scoparia dulcis* at the same dose for 60 days. Group V received aqueous leaf extract of *Costus pictus* @ 500 mg/kg body weight orally for 60 days and Group VI was given alcoholic leaf extract of *Costus pictus* at the same dose for 60 days.

A combination of aqueous leaf extracts of *Scoparia dulcis* and *Costus pictus* was given to the animals of Group VII each @ 250 mg/kg body weight and Group VIII received a combination of alcoholic leaf extracts of *Scoparia dulcis* and *Costus pictus* each @ 250 mg/kg for 60 days orally. Group IX received glibenclamide @ 0.5 mg/animal/day orally for 60 days.

Body weight and haematological parameters like RBC, WBC and total haemoglobin content were estimated on zero day, 7^{th}, 14^{th}, 28^{th}, 56^{th} and 60^{th} day of treatments. Biochemical parameters such as glycosylated haemoglobin, plasma

glucose, plasma total lipids, total cholesterol, tirglycerides, HDL-C, total plasma protein, albumin, and activities of enzymes like ALT and AST were also measured. Liver glycogen and plasma trace minerals such as copper, zinc and iron were estimated on the 60th day after sacrificing the animal.

All the treatment groups except the diabetic control showed a gradual increase in body weight during the experimental period, while animals treated with glibenclamide showed the maximum (12.36 percent) increase in body weight than other treated groups. The RBC and WBC count did not show any significant difference between the groups and it maintained normal range.

The total haemoglobin value was increased during the two months study in all the treatment groups. Among the treated groups the animals fed with combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* showed the maximum (35.14 percent) increase in total Hb concentration followed by animals fed with alcoholic leaf extract of *Scoparia dulcis* alone (26.31 percent) over the other treatment groups after two months of the experiment. In diabetic control animals there was a reduction in total Hb content even at the end of two months of study with a mean value of 6.13 ± 0.21g/dl.

The glycosylated haemoglobin value for all the treatment groups was significantly ($p \leq 0.001$) reduced than the diabetic control animals at the end of study. The animals treated with alcoholic leaf extract of *Costus pictus* showed a decrease (34.04 percent) in glycosylated haemoglobin content over the other treated groups followed by animals treated with alcoholic leaf extract of *Scoparia dulcis* (32.69 percent decrease) in glycosylated haemoglobin content after two months of treatment.

All the treatment groups showed significant reduction in plasma glucose level. The animals which received alcoholic leaf extract of *Scoparia dulcis* showed the maximum reduction in plasma glucose level (75.17 percent decrease) with respect to

pretreatment value followed by animals treated with alcoholic leaf extract of *Costus pictus* (74.53 percent decrease).

Oral administration of combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* brought about a significant reduction in plasma total lipids and total cholesterol when compared to other groups, which might be due the synergistic effect of the plant extracts in utilizing glucose for energy production efficiently rather than mobilizing the fatty acids from fat depots.

Administration of alcoholic leaf extract of *Scoparia dulcis* @ 500 mg/kg orally reduced the plasma triglyceride level substantially when compared to other treatment groups. Administration of glibenclamide substantially increased the HDL-C level. The LDL-C level was substantially decreased in diabetic rats treated with combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus*. The VLDL-C level decreased markedly in diabetic rats treated with combination alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* than the other treated groups.

The total protein level in all the treatment groups was increased by two months of treatment. Animals treated with aqueous leaf extract of *Costus pictus* produced a marked increase in total protein content by 25.34 percent which is comparable to the values of animals, which received alcoholic leaf extract of *Costus pictus*. The animals, which received a combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* was also increased substantially.

The albumin content was increased in all the treatment groups and was more pronounced in animals treated with alcoholic leaf extract of *Costus pictus*. Animals treated with alcoholic leaf extract of *Scoparia dulcis* also produced an increase in plasma albumin content with respect to the pretreatment values. Animals, which received a combination of aqueous and alcoholic leaf, extract of *Scoparia dulcis* and

Costus pictus produced substantial increase in albumin content, which might be due to the synergistic effect of the plant extracts used

The activity of liver enzymes such as ALT and AST were decreased in all the treatment group when compared to the diabetic group by the administration of the plant extracts. Animals, which received alcoholic leaf, extract of *Scoparia dulcis* showed marked decrease in these enzymes activity.

The liver glycogen content was substantially increased in most of the treatment groups. Animals, which received alcoholic leaf, extract of *Costus pictus* showed marked increase in liver glycogen, which might be due to the homeostasis of blood glucose level and proper utilization of glucose for energy production.

There was no significant difference in the plasma copper, and iron content among the treated group but the zinc level was increased in all treatment groups. Diabetic control animals showed significant reduction in zinc content when compared to other treatment groups. There was a negative correlation between zinc and iron. The plant extracts treatment might have effectively controlled the urinary loss of zinc and thereby the plasma zinc content was increased in all treatment groups. The iron molecules have a damaging effect on pancreas when it is in excess and the treatment with plant extracts controlled the iron content within the normal level

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HYPOGLYCAEMIC EFFICACY OF *SCOPARIA DULCIS* AND *COSTUS* SPECIES IN ALBINO RATS

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ABSTRACT

The present study was undertaken to assess the hypoglycaemic activity of aqueous and alcoholic extract of *Scoparia dulcis* and *Costus pictus* @ 500mg/kg body weight orally in alloxan induced diabetic rats and also in combination and to compare their efficacy with a standard oral hypoglycaemic drug, glibenclamide.

The experiment was conducted in seventy-two male Sprague-Dawley rats for a period of 60days, with eight animals in each group (Group I to IX). Group I served as normal control and Group II is diabetic control. Aqueous leaf extract of *Scoparia dulcis* and *Costus pictus* @ 500 mg/kg b.w orally were given to Group III and IV, respectively for 60 days. Group V and VI were given alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* @ 500 mg/kg b.w orally, respectively for 60 days. Group VII and VIII received combination of aqueous and alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* @ 500 mg/kg b.w orally, respectively for 60 days. Glibenclamide @ 0.5mg/animal/day was fed to Group IX.

Body weight was recorded and RBC, WBC, total haemoglobin and glycosylated haemoglobin content were estimated on zero day, 7^{th}, 14^{th}, 28^{th}, 56^{th}, and 60^{th} day of the experiment. Plasma glucose, plasma total lipids, plasma total cholesterol, triglycerides, plasma HDL-C, plasma total protein, plasma albumin, plasma ALT and AST were also estimated. The liver glycogen and plasma copper, iron and zinc content were estimated at the end of the experimental period.

Body weight was gradually increased during the experimental period in all treated group except the diabetic control, which showed a significant ($p \leq 0.001$) reduction in body weight. The RBC and WBC values did not show any significant change during the entire course of the experiment and maintained a normal level. The total haemoglobin content was increased in the animals treated with combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* by two months of experiment. Glycosylated haemoglobin level also significantly

decreased in all the treatment groups, which is comparable to that of the animals treated with glibenclamide.

The animals treated with alcoholic leaf extract of *Scoparia dulcis* produced a marked reduction in plasma glucose level, which was higher than the reduction produced by the animals treated with glibenclamide at the end of the experiment.

The plasma total lipids and plasma total cholesterol content were markedly reduced in the animals treated with a combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus*, which is comparable to that produced by glibenclamide treated group. The plasma triglyceride, plasma LDL-C and VLDL-C level were markedly reduced in the animals treated with alcoholic leaf extract of *Scoparia dulcis*.. The HDL-C level was increased in the animals treated with combination of alcoholic leaf extract of *Scoparia dulcis* and *Costus pictus* than the animals treated with glibenclamide.

The plasma total protein and albumin content was increased in the animals treated with aqueous leaf extract of *Costus pictus*. The globulin content and A/G ratio was increased in the animals treated with a combination of aqueous leaf extract of *Scoparia dulcis* and *Costus pictus*.

The liver enzymes such as ALT and AST were reduced in the animals treated with alcoholic leaf extract of *Scoparia dulcis*. The liver glycogen content was increased in animals treated with alcoholic leaf extract of *Costus pictus*, which is comparable to that of animals treated with alcoholic leaf extract of *Scoparia dulcis*.

The plasma copper and iron content did not show any change but the zinc content was increased in all the treated groups than the diabetic control animals.