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HYPOGLYCEMIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *MIMUSOPS ELENGI* LINN IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT
The ethanolic extract of *Mimusops elengi* Linn. (Sapotaceae) leaf was used for its anti hyper glycemic effects in wistor albino rats. Diabetes was induced in albino rats by administration of single doses of streptozotocin (STZ) 40mg/kg body weight, i.p. The ethanolic extract *M. elengi* at optimum dose concentration of 100mg/kg body weight was administered as a single dose/day to diabetes induced rats for a period of 30 days. The effect of leaf extract on blood glucose, insulin (in plasma), hemoglobin, glycosylated haemoglobin (HbA1c) and carbohydrate metabolic enzymes such as glucokinase, glucose -6-phospate dehydrogenase and glycogen content in liver and kidney, and gluconeogenic enzymes such as glucose -6 phosphatase, fructose 2,6 bis phosphatase levels has also been studied. Leaf extract elicit significant ($p < 0.05$) reduction of blood glucose levels and lipid profile levels in liver and kidney of diabetic rats except HDL, and carbohydrate metabolic enzymes significantly ($p < 0.05$) increased. The extract also caused significant ($p < 0.05$) increases in plasma insulin levels, increased total proteins, albumin, globulin and A/G ratio. A significantly ($p < 0.05$) decrease in gluconeogenic enzymes in liver and kidney was observed.

INTRODUCTION

Diabetes milletus (DM) is occurs due to abnormality of carbohydrate, fat and protein metabolism, it affects the 10% of the population of world wide. The number of diabetic peoples is expected to rise from 150 million to 230 millions in 2025 (Paul *et al.,* 2001).Impaired glucose tolerance and the metabolic syndrome often lead to development of type-II diabetes. Currently available therapies for Diabetes include insulin and various oral hypoglycemic agents such as sulphonylurea, biguanides, alpha glucosidase inhibitors and glimids, in developing countries as products are expensive and not easily accessible. Since oral hypoglycemic agents cause side effects, there is growing interest in herbal remedies for the treatment of Diabetes milletus (Bailey and Day, 1989). Many plant preparations are used in folk medicine to manage Diabetes milletus. Many Indian plants have been previously investigated for their beneficial use in different types of Diabetes (Pulok *et al.,* 2006). The investigation of antihyperglycemic agents from plant origin in those of great significance because of their effectiveness, minimal side effects and relatively low cost (Dolly *et al.,* 2008). The review of the literature revealed no report concerning the hypoglycemic effect of these ethanolic extract of leaves of *Mimusops elengi* Linn in the albino rats. Therefore, the present study was conducted to investigate the effect of ethanolic extract of leaves of *M. elengi* on the carbohydrate metabolic enzymes and antihyperlipedemic activity in Streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Animals

Male albino rats of wistar strain (150-200g) were used for the study; the animals were maintained in an air conditioned room under controlled temperature and humidity. They were fed standard rat pellets diet supplied by Hindustan Lever Ltd., Bangalore, India. Animals were deprived of food for 16 hrs but allowed free access to water. Ethical clearance for the handling of experimental animals are obtained from the institutional animal ethics committee (IAEC) constituted for the purpose and the care of laboratory animals was taken as for the guidelines of the committee for the purpose of control and Supervision on experiments on animals (CPCSEA).

Collection of plant material and authentication

Fresh leaves of *M. elengi* were collected during the month of May and June from Acharya Nagarjuna University campus. India. The plant was identified by using flora of Madras Presidency (Gamble, 1915-1936) and voucher specimen was deposited at Botany Department in Acharya nagarjuna University (ANU: 1323).

Preparation of plant extract

The plant leaves were shade dried at room temperature (32 ± 2ºC) and the dried leaves were grinded into fine powder using pulverizer. The powdered paste was sieved and kept in deepfreezer. Dry powder of 100 g was suspended in 400 mL of 95 % ethanol for 72 h. The extract was filtered using a muslin cloth and concentrated at 40 ± 5ºC.

Drugs and chemicals

Streptozotocin (STZ) was purchased from Sigma St Louisa (MO, USA). All chemicals and solvent used are analytical grade, purchased from SD Fine Chemical Ltd. Mumbai, India. Ranbaxy Laboratories, New Delhi, India. Hi-Media Chemicals, Mumbai, India.

Experimental induction of Diabetes

The animals were rendered diabetes by intraperetonial injection of Streptozotocin (STZ) 40 mg/kg body weight in freshly prepared citrate buffer (0.1 M, pH 4.5). After an over night fast, STZ injected animals were given 20% glucose solution for 24 h to prevent initial drug induced hypoglycemia mortality. STZ injected animals exhibited massive Glycosuria (Determined by Benedict's qualitative test) and hyperglycemia by GOD-POD method (Trinder, 1969) and diabetes in STZ rats was confirmed by measuring the fasting blood glucose concentration, 96 hr after injection with STZ the animals with blood glucose more than 220 mg/dl were considered diabetic and used for the experiment.

Experimental protocol

A total number of 30 rats were randomized into 5 groups of control and experimental animals and each group consist of 6 rats.

Group I – were served as untreated control.

Group II – were served as treated control rats. (Control + Ethanolic extract of *M. elengi* leaf 100 mg/kg bwt).

Group III – Diabetic control rats treated with single intraperitonial injection of STZ dissolved in 0.1M citrate buffer pH-4.5.

Group IV – Diabetic rats treated with leaf extract at optimum concentration of 100 mg/kg body weight for 30 days by gastric gavages after the diabetes state was assessed.

Group V – Diabetic rats treated with standard drug glibenclamide 0.6 mg/kg BW. Diabetic rats received oral administration of glbenclamide (0.6 mg/kg bwt. IP) daily for 30 days by gastric gavage after the diabetes state was assessed.

Collection of blood, liver and kidney

Blood was collected by retro -orbital puncture after 12 hr fasting and 2 h after giving the extract in gum acacia for the estimation of plasma glucose. Auto analyzer was used in this study to minimize sample requirement. After 30 days, the animals were fasted for 12 h, anaesthetized between 8.00-9.00AM using ketamine (24mg/kg Body weight, intramuscular injection) and sacrificed by decapitation. Blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of plasma glucose and estimation of various Bio-chemical parameters. Tissues (liver and kidney) were surgically removed, washed with cold physiological saline, cleared off adherent lipids and immediately transferred to ice cold containers.

Processing of blood and tissue samples

Serum preparation

Blood was collected in fresh test tube and allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 2000 rpm for 10 min.

Plasma preparation

The Blood collected in a heparinized centrifuge tube and centrifuged at 2000 rpm for 10 min and the plasma was separated by aspiration.

Tissue homogenate preparation

Liver and kidney tissues (250mg) were sliced into pieces and homogenized in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate (w/v). The homogenate were centrifuged at 1000 rpm for10 min at 0ºC in cold centrifuge. The supernatant was separated and used for various bio chemical estimations.

Bio chemical parameters analysis

Bio chemical estimations were carried out in blood, plasma, liver and kidney samples of control and experimental rats in each group. Blood glucose estimated by the method of Trinder, (1969) using reagent kit. The plasma insulin was assayed by the solid phase system amplified sensitivity amino sorbent using reagents kit obtained from Medgenex-ins-ELISA, Biosource, Europe S.A, Belgium (Brugi *et al.*, 1988). Hemoglobin and glycosylated hemoglobin levels were measured by the method of Drabkin and Austin (1932) and Sudhakar and Pattabhiraman (1981) respectively. The activities of carbohydrate metabolic enzymes, hexokinase, glucose-6-phosho dehydrogenase and glucose-6-phosphatase, fructose1,6-bis phosphatase were determined in the liver and kidney by the method of Brandstrup *et al.* (1957) Koide and Oda (1959), Bergmeyer (1984); Gancedo and Gancedo (1971). The glycogen content in liver was estimated by the method of Morales *et al.* (1973). The total cholesterol in the plasma, liver and kidney was estimated by the enzymatic method described by Allain *et al.* (1974). Triacyl glycerol in the plasma and tissues were estimated by using the diagnostic kit based on the enzymatic method described by McGowan *et al.* (1983).Free fatty acids in the plasma and tissue were estimated by the method of Falholt *et al.,* (1973).Phospholipids in the plasma and tissues were estimated by the method of Zilversmith and Davies. (1950).

HDL-cholesterol (High density lipoproteins) was estimated using the diagnostic kit based on the enzymatic method described by Izzo *et al.* (1981). VLDL (Very low density lipoproteins) and LDL (Low density lipoproteins) cholesterol was estimated by using the method described by Friedewald *et al.* (1972). Total proteins and albumin in the serum were estimated by Biuret method, (Reinhold, 1953, 1980) globulin concentration calculated using formula from the estimation of total protein and albumin.

Globulin = total proteins - albumin

Statistical analysis

The data are expressed as mean ± S.D. statistical comparisons were performed by one-way analysis of variance (ANOVA). Followed by Duncans multiple range test (DMRT).The results

were considered statistically significant if the p values less than 0.05($p < 0.05$).For glucokinase values expressed as μmol of glucose phosphorylated/hr. For glu -6-phosphate dehydrogenase values expressed as nmol of NADPH formed/ minute. For glu-6-phosphatase values expressed as U = μ mol of phosphate liberated/minute. For fru- 1, 6-bis-phosphatase values expressed as U = μmol of phosphate liberated/h.

RESULTS

The levels of blood glucose and glycosylated hemoglobin significantly ($p < 0.05$) increased. Where as, haemoglobin and plasma insulin levels significantly ($p < 0.05$) decreased in diabetic rats as compared to control rats (Table 1). However, the above mentioned biochemical parameters were significantly bought back to near normal range in diabetic rats treated with optimum concentration of ethanolic extract of leaves of *M.elengi* (100mg/kg bwt) and Diabetic rats treated with glibenclamide (0.6mg/kg bwt). In each group hexokinase activity and glucose-6-phosphate-dehydrogenase, and glycogen content in liver was significantly ($p < 0.05$) decreased (Table 2). However, the activities of hexokinase, glucose-6-phosphate-dehydrogenase and glycogen content were returned to near normal range in diabetic rats treated with optimum concentration of leaf extract (100mg/kg bwt) and diabetic rats treated with glibenclamide. Wistor rats treated with leaf extract (100mg/kg bwt) alone showed no significant difference in hexokinase, glucose-6-phosphate-dehydrogenase and glycogen content in liver as compared to normal rats.

Both glucose-6-phosphatase and fructose1, 6-bis phosphatase activity was increased in the liver and kidney of diabetic rats compared to control rats (Table 3). However, activity of glucose-6-phosphatase and fructose1,6-bis phosphatase were returned to near normal range in diabetic rats treated with optimum concentration of leaf extract (100mg/kg bwt) and diabetic rats treated with glibneclamide. Wistar rats treated with leaf extract (100mg/kg bwt) alone showed no significant difference in glucose-6-phosphatase and fructose1, 6-bis phosphatase activities of compared to control rats. Plasma, liver and kidney of diabetic rats showed significantly increased levels of cholesterol triglycerides, Fatty acids, phospholipids, LDL and VLDL-cholesterol in plasma liver and kidney, when compared to normal rat (Tables 4 to 8). Where as, HDL cholesterol significantly decreased in STZ induced diabetic rats ($p < 0.05$) when compared with normal rats (Table 8). In rats treated with optimum concentration of leaf extract (100mg/kg bwt), there was a significant ($p < 0.05$) decrease in the content of triglycerides, fatty acids, phospholipids (Tables 5,6,7) and also LDL and VLDL-cholesterol in the serum as well as tissues(Table 8), when compared with controlled diabetic rats. Where as, HDL- cholesterol significantly ($p < 0.05$) increases and returned

Table 1: Effect of *M.elengi* leaf extract on glucose (plasma), insulin, haemoglobin and glycosylated haemoglobin in normal and STZ induced DM rats after 30 days.

Group	Glucose(mg/dL)	Insulin (μU/mL)	Hb (g/dL)	Glycosylated Hb(mg/g Hb)
Control	74.50 ± 03.5^{a}	15.29 ± 1.55^{a}	14.55 ± 1.5^{a}	0.53 ± 0.02^{a}
C + leaf exract (100mg/kg bw)	73.1 ± 03.5^{a}	15.30 ± 1.30^{a}	14.75 ± 1.19^{a}	0.55 ± 0.02^{a}
DM	260.3 ± 11.1^{b}	5.50 ± 0.35^{b}	8.50 ± 0.54^{b}	1.10 ± 0.07^{b}
DM + leaf extract 100mg/kg bw	143.23 ± 09.50^{c}	10.30 ± 0.75^{c}	11.25 ± 0.85^{c}	0.63 ± 0.02^{c}
DM + Glibenaclamide(0.6mg/kg bw)	110.50 ± 10.25^{d}	14.70 ± 1.30^{a}	13.95 ± 0.55^{a}	0.52 ± 0.03^{a}

Values are $\pm$ SD means for 6 rats.; Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

Table 2: Effect of *M.elengi* leaf extract on CHO metabolic enzymes and glycogen content in liver of control and STZ induced DM rats after 30 days

Group	Glucokinase dehydrogenase U/h/mg protein	Glu-6-Phosphate U/mg protein	Glycogen mg/100g tissue
Control	0.180 ± 0.01^{a}	5.50 ± 0.20^{a}	47.00 ± 2.89^{a}
C + leaf extract (100mg/kg bw)	0.183 ± 0.02^{a}	5.65 ± 0.15^{a}	49.20 ± 3.15^{a}
DM	0.100 ± 0.02^{b}	2.12 ± 0.15^{b}	18.55 ± 3.50^{b}
DM + leaf extract100mg/kg bw	0.148 ± 0.01^{c}	3.01 ± 0.10^{c}	31.01 ± 2.12^{c}
DM + Glib(0.6mg/kg bw)	0.175 ± 0.02^{a}	2.97 ± 0.10^{d}	41.05 ± 3.17^{d}

Values are SD $\pm$ mean of 6 rats. Values not sharing a common letters differ significantly at $p < 0.05$ (DMRT)., For glucokinase values expressed as μmol of glucose phosphorylated/h, For glu-6-phosphate dehydrogenase values expressed as nmol of NADPH formed / minute.

Table 3: Effect of *M.elengi* leaf extract on gluconeogenic enzymes and glycogen content in liver and kidney of control and STZ induced DM rats after 30 days

Group	Glu-6-phosphatase U/min/mg protein		Fruc1,6-bisphosphatase U/h/mg protein	
	Liver	Kidney	Liver	Kidney
Control	0.173 ± 0.01^{a}	0.278 ± 0.02^{a}	0.410 ± 0.02^{a}	0.880 ± 0.04^{a}
C + Me (100mg/kg bwt)	0.169 ± 0.02^{a}	0.270 ± 0.03^{a}	0.398 ± 0.03^{a}	0.878 ± 0.03^{a}
DM	0.288 ± 0.04^{b}	0.368 ± 0.04^{b}	0.620 ± 0.03^{b}	1.390 ± 0.15^{b}
DM + Me 100mg/kg bwt)	0.210 ± 0.01^{c}	0.315 ± 0.02^{c}	$0.501 \pm 0.02C$	1.043 ± 0.06^{c}
DM + Glib(0.6mg/kg bwt)	0.179 ± 0.03^{d}	0.270 ± 0.02^{d}	0.455 ± 0.04^{ad}	0.848 ± 0.06^{a}

Values are SD $\pm$ mean of 6 rats. Values not sharing a common letters differ significantly at $p < 0.05$ (DMRT)., For glu-6-phosphatase values expressed as U = μmol of phosphate liberated / minute., For fruc1,6-bisphosphatase values expressed as U = μmol of phosphate liberated / h

Table 4: Effect of *M.elengi* leaf extract on total cholesterol in plasma, liver and kidney of control and STZ induced DM rats after 30 days

Group	Plasma mg/dL	Wet tissue mg/g	
		Liver	Kidney
Control	66.75 ± 2.67^{a}	3.88 ± 0.20^{a}	4.50 ± 0.25^{a}
C+Me (100mg/kg bwt)	64.99 ± 2.10^{a}	4.02 ± 0.23^{a}	4.40 ± 0.25^{a}
DM	150.32 ± 5.23^{b}	5.90 ± 0.40^{b}	9.05 ± 0.50^{b}
DM+Me 100mg/kg bwt)	85.07 ± 4.56^{c}	5.00 ± 0.35^{c}	6.85 ± 0.55^{c}
DM + Glib(0.6mg/kg bwt)	69.00 ± 5.88^{a}	4.6 ± 0.20^{d}	5.75 ± 0.58^{d}

Values are SD ± mean of 6 rats. Values not sharing a common letters differ significantly at $p < 0.05$ (DMRT).

Table 5: Effect of *M.elengi* leaf extract on triacylglycerol in plasma, liver and kidney of control and STZ induced DM rats after 30 days

Group	Plasma mg/dL	Wet tissue mg/g	
		Liver	Kidney
Control	62.15 ± 4.17^{a}	4.88 ± 0.29^{a}	4.20 ± 0.20^{a}
C+Me (100mg/kg bwt)	60.19 ± 2.90^{a}	4.72 ± 0.29^{a}	4.10 ± 0.28^{a}
DM	160.32 ± 9.29^{b}	7.97 ± 0.30^{b}	7.55 ± 0.57^{b}
DM+Me 100mg/kg bwt)	120.07 ± 4.76^{c}	5.35 ± 0.41^{c}	6.35 ± 0.45^{c}
DM + Glib(0.6mg/kg bwt)	65.00 ± 5.00^{a}	4.97 ± 0.36^{a}	4.95 ± 0.58^{d}

Values are SD ± mean of 6 rats. Values not sharing a common letters differ significantly at $p < 0.05$ (DMRT).

Table 6: Effect of *M.elengi* leaf extract on total free fatty acids in plasma, liver and kidney of control and STZ induced DM rats after 30 days

Group	Plasma mg/dL	Wet tissue mg/g	
		Liver	Kidney
Control	52.75 ± 4.57^{a}	6.58 ± 0.29^{a}	4.10 ± 0.20^{a}
C+Me (100mg/kg bwt)	50.29 ± 3.93^{a}	6.32 ± 0.29^{a}	4.10 ± 0.38^{a}
DM	141.32 ± 8.39^{b}	16.00 ± 0.70^{b}	11.65 ± 0.87^{b}
DM+Me 100mg/kg bwt)	91.37 ± 4.46^{c}	10.15 ± 0.45^{c}	9.75 ± 0.65^{c}
DM + Glib(0.6mg/kg bwt)	74.70 ± 5.80^{d}	7.57 ± 0.40^{d}	7.15 ± 0.58^{d}

Values are SD ± mean of 6 rats. Values not sharing a common letters differ significantly at $p < 0.05$ (DMRT).

Table 7: Effect of *M.elengi* leaf extract on phospholipids in plasma, liver and kidney of control and STZ induced DM rats after 30 days

Group	Plasma mg/dL	Wet tissue mg/g	
		Liver	Kidney
Control	82.55 ± 5.57^{a}	22.48 ± 1.29^{a}	14.18 ± 1.22^{a}
C+Me (100mg/kg bwt)	83.89 ± 4.90^{a}	24.02 ± 0.30^{a}	14.20 ± 1.18^{a}
DM	161.22 ± 8.00^{b}	60.10 ± 5.70^{b}	29.55 ± 1.87^{b}
DM+Me 100mg/kg bwt)	106.37 ± 7.56^{c}	40.45 ± 4.44^{c}	21.77 ± 1.50^{c}
DM + Glib(0.6mg/kg bwt)	79.88 ± 5.45^{a}	30.97 ± 3.40^{d}	17.10 ± 1.48^{d}

Values are SD ± mean of 6 rats. Values not sharing a common letters differ significantly at $p < 0.05$ (DMRT)

Table 8: Effect of *M. elengi* leaf extract on HDL,LDL and VLDL Cholesterols in plasma of control and STZ induced DM rats after 30 days

Group	Plasma mg/dL		
	HDL-CHO	LDL-CHO	VLDL-CHO
Control	50.22 ± 2.88^{a}	21.60 ± 1.87^{a}	11.55 ± 0.51^{a}
C+Me (100mg/kg bwt)	51.18 ± 3.13^{a}	22.57 ± 1.65^{a}	10.99 ± 0.40^{a}
DM	33.33 ± 2.18^{b}	88.89 ± 5.89^{b}	32.11 ± 2.22^{b}
DM+Me 100mg/kg bwt)	44.38 ± 3.14^{c}	32.15 ± 3.28^{c}	24.81 ± 1.89^{c}
DM + Glib(0.6mg/kg bwt)	49.44 ± 3.10^{a}	29.29 ± 3.10^{d}	19.19 ± 1.22^{a}

Values are SD ± mean of 6 rats. Values not sharing a common letters differ significantly at $p < 0.05$ (DMRT).

Table 9: Effect of *M.elengi* leaf extract on total protein, albumin, globulin and A/G ratio in plasma of control and STZ induced DM rats after 30 days

Group	Plasma			
	Total protein g/dL	Albumin g/dL	Globulin g/dL	A / G ratio
Control	8.69 ± 0.57^{a}	4.31 ± 0.29^{a}	4.18 ± 0.22^{a}	1.15 ± 1.10^{a}
C+Me (100mg/kg bwt)	8.69 ± 0.40^{a}	4.22 ± 0.30^{a}	4.20 ± 0.18^{a}	1.22 ± 1.05^{a}
DM	4.92 ± 0.20^{b}	1.80 ± 0.11^{b}	2.55 ± 0.10^{b}	0.75 ± 0.42^{b}
DM+Me 100mg/kg bwt)	6.88 ± 0.16^{c}	3.95 ± 0.14^{c}	3.37 ± 0.15^{c}	1.01 ± 0.94^{c}
DM + Glib(0.6mg/kg bwt)	8.03 ± 0.45^{d}	4.17 ± 0.20^{a}	3.60 ± 0.18^{d}	1.11 ± 1.09^{a}

Values are SD ± mean of 6 rats. Values not sharing a common letters differ significantly at $p < 0.05$ (DMRT).

to normal level after treatment with leaf extract (100mg/kg bwt), when compared to normal rats (Table 8). Wistar rats treated with leaf extract alone showed no significant difference in lipid profile levels as compared to control rats. This indicates that the leaf extract showed favorable effects on lipid metabolism of diabetic rats. A significant ($p < 0.05$) decrease in total protein, albumin, globulin, and A/G ratio levels were observed in plasma of STZ-induced diabetic rats compared with control rats (Table 9). However, the levels of total protein, albumin, globulin and A/G ratio were returned to near normal range in diabetic rats treated with optimum concentration of leaf extract (100mg/kg bwt) and diabetic rats treated with glibneclamide (0.6 mg/kg bwt). Wistar rats treated with leaf extract alone showed no significant difference in protein levels as compared to control rats.

DISCUSSION

The present study focused first time, the scientific explanation about the remedial effects of the ethanolic extract of leaves of *M.elengi* for the management of STZ-induced diabetes. *M. elengi* leaf extract exhibited significant antihyperglycaemic effect in STZ-induced diabetic rats. This was evident by significant decrease in blood glucose level, glycosylated haemoglobin and increase in plasma insulin and total hemoglobin and increase liver glycogen content in diabetic rats after treatment with leaf extract (100mg/kg bwt). An increase in total haemoglobin and decrease in glycosylated haemoglobin in diabetic rats after treatment with leaf extract indicated its ability to prevent the glycosylation process between blood glucose and protein moiety of total haemoglobin during hyperglycemic condition. The antidiabetic potential of *M.elengi* leaves is probably due to the presence of one or more bioactive antihyperglycemic principles such as flavonoids and their synergistic effects. Increased plasma insulin levels in treated diabetic rats suggested that the leaf extract might have stimulated insulin secretion from surviving pancreatic β-cells. In this context number of other plants has also been reported to have anti hyperglycemic and insulin stimulatory effects (Rajagopal and Sasikala, 2008, Pari and Maheswari, 1999). The possible mechanism of action of *M.elengi* leaf extract could be correlated with the reminiscent hypoglycemic effect of sulphonylureas, that promote insulin secretion by closer of k+-ATP channels, membrane depolarization, and stimulation of calcium influx, and these are initial key steps in insulin secretion.

The liver is regarded as one of the central metabolic organs in the body. It regulates and maintaining homeostasis. It performs most of the reactions involved in the synthesis and utilization of glucose. The balance between glucose production and its utilization in liver is regulated primarily by insulin. Liver is an insulin dependent tissue and is severely affected during diabetes. In experimental diabetes, enzymes of glucose metabolism are markedly altered. Persistent hyperglycemia is a major contribution to such metabolic alterations that lead to pathogenesis of diabetic complication (Gupta *et al.,* 1997).

Diabetes results in decrease in glucose utilization and an increase glucose production in insulin dependent tissue such as liver (Seifter and England, 1982). Decreased glycolysis impaired glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver (Baquer, 1998). Hexokinase is insulin dependent and insulin sensitive enzyme are almost completely inhibited or inactivated in diabetic rat liver in the absence of insulin (Gupta *et al.,* 1997). Decreased enzymatic activity of hexokinase has also been reported in diabetic animals, resulting in depletion of liver and muscle glycogen (Laasako *et al.,* 1995). In our study we also have observed decrease in hepatic as well as renal hexokinase activity in STZ-induced diabetic rats. Administration of leaf extract (100mg/kg bwt) to STZ treated rats resulted in a significant ($p < 0.05$) increased activity of hexokinase in liver and kidney. This increase activity of hexokinase can cause the increase utilization of glucose for energy production. The decrease in the concentration of glucose in STZ treated rats given leaf extract (100mg/kg bwt) may be as a result of increased in glycolysis. The shunt enzyme glucose6-phosphate dehydrogenase is regulated by the NADP+/NADPH ratio, a high ratio favoring activation of the enzyme, where as low ratio is deactivating the Enzyme. Two gluconeogenic enzymes such as glucose-6-phoshatase and fructose 1,6-bis phosphatase have been measured in the liver and kidney of diabetic animals and those treated with *M.elengi* leaf extract (100mg/kg bwt). The increased hepatic as well as renal fructos1,6-bis phosphatase activity may be due to the changes in the allosteric effects of the enzymes namely, fructose-2,6 bisphosphate, ATP, AMP and citrate. In diabetic state, there is more lipolysis than lipogenesis, especially in liver, which will results in the formation of more AMP and lower utilization of citrate for lipogenesis leading to high energy state in the cell, *i.e.,* higher concentration of ATP is more favorable for fructose 1,6- bis phosphatase activation. The reduction in the activities of these gluconegenic enzymes can result in decreased concentration of blood glucose. Administration of leaf extract has increased the activity of hexokinase and decreased the activities of both glucose6-phospatase and fructose 1, 6-bisphosphatase in STZ treated rats. It is now well established that the glucogenic effect of pituitary hormones (somatotrophin and corticotrophin) is mediated through an elevation of intracellular effect of extract could be due to lowering of intracellular cyclic AMP, which could be effected in a variety of ways. That activation of the fructose 1, 6-bis phosphatase by the gluconeogenic pancreatic glucagons is linked to an elevation of intra cellular cyclic AMP is now well established (Shibib *et al.,* 1993). Whether the depressing effect of leaf extract on fructose 1.6-bis phosphatase reported here is secondary to a lowering of intracellular cyclic AMP and it is yet to be studied.

Diabetes is associated with hyperlipidemia (Maiti *et al.,* 2005). It is well documented that there is elevation of serum lipid concentration in diabetes (Chase and Glasgow, 1976). Lipids play an important role in the pathogenesis of complications involved with diabetes milletus. The elevated levels of serum cholesterol and reduced levels of serum HDL-cholestrol in diabetic condition possess to be a rise of developing micro vascular complications leading to atherosclerosis and further leads to cardiovascular disease like coronary heart diseases. The abnormal high concentration of serum lipids in diabetes mainly due to increase in mobilization of free fatty acids from

peripheral fat deposit from adipose tissue, when the insulin inhibits the hormone sensitive lipase. The various reports have been studied; the insulin resistance may be responsible for dyslipidemia (Daisy *et al.*, 2008). The present study showed STZ treated diabetic rats has abnormal lipid profile, where as the leaf extract(100mg/kg bwt) treated group showed significant improvement in the lipid profile comparable to glibenclamide treatment group. This effect not only due to better glycemic control but could also been due to inhibition of the pathway of cholesterol synthesis and increased HDL/LDL ratio may be due to the activation of LDL receptors in hepatocytes, which are responsible for taken up LDL into the liver and reduce the serum LDL level (Khosla *et al.,* 1995). Hypolipidemic effect could represent a protective mechanism against the development of atherosclerosis. It is well known that hyperlipidemia as an association with atherosclerosis, and it is vastly increased in diabetes.

Insulin is the main regulator of glycogenesis in muscle and liver. The decrease of liver glycogen level observed in this study may be due to lack of insulin in diabetic condition or oxidative stress by diabetes may inactivate the enzyme glycogen synthase. The marked reduction in liver glycogen level is observed (30 days) in STZ induced diabetic animals. Treatment with leaf extract (100mg/kg bwt) remarkably increased the glycogen content levels in liver. In the view of glycogen level there may be three possible ways of antidiabetic action, one possible way may be increased insulin level by preventing the inactivation glycogen synthase by synthesize the glycogen synthase protein (Selvan *et al.,* 2008). Herman *et al*. (1999) reported that streptozotocin progressively decreased the volume of hepatocytes and their nuclei, as a result of cyctoplasmic changes, and the basal insulin level is also necessary to maintain the state of aggregation of the endoplasmic reticulum-bound polysomes for secretary protein synthesis. In insulin deficient animals, loss of endoplasmic reticulum, reduced amino acid incorporation into proteins, decrease in rough endoplasmic reticulum-bound ribosomes (Lenk *et al.,* 1992).

The decrease in protein and albumin may be due to microproteinurea and albunuria, which are important clinical markers of diabetic nephropathy (Mauer *et al.,* 1981) and/or may be due to increase protein catabolism (Almdal and Vilstrup, 1988). The result of the present study demonstrated that the treatment of diabetic rats with the optimum concentration of leaf extract (100mg/kg bwt) caused a remarkable elevation in the plasma total protein and albumin, globulin and A/G ratio has compared to with their normal levels. Such improvement of serum proteins and albumins was previously observed after the oral administration of Balanites aegyptacaea to experimentally diabetic rats (Mansour and Newairy, 2000).

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