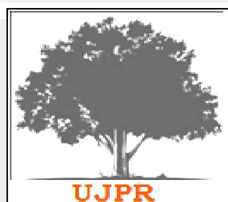

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RESEARCH ARTICLE

EVALUATION OF METHANOLIC EXTRACT OF *EUPHORBIA NERIIFOLIA* STEM BARK ON BLOOD SUGAR LEVELS, SERUM AND TISSUE LIPIDS IN A PRECLINICAL MODEL

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ABSTRACT
The present study is undertaken to evaluate the effect of *Euphorbia neriifolia* stem bark on blood glucose and lipid levels in experimental diabetic rats. Methanolic extract of *Euphorbia neriifolia* stem bark (MEEN) was administered at different doses and its effect on blood glucose, haemoglobin, serum and tissue lipids, hexokinase and glucose-6-phosphatase in streptozotocin-induced diabetic rats were studied. Glibenclamide was used as standard reference drug. *Euphorbia neriifolia* methanolic extract (MEEN), at doses of 100,200 and 400mg/kg body weight for 30 days, suppressed the elevated blood glucose and lipid levels in diabetic rats. *Euphorbia neriifolia* at 400mg/kg was found to be comparable to glibenclamide. The study indicates that the *Euphorbia neriifolia* possess antihyperlipidaemic effect as well as antidiabetic activity.
Keywords: Blood glucose, carbohydrate enzymes, *euphorbia neriifolia*, insulin, lipids.

INTRODUCTION

Diabetes mellitus is a principal cause of morbidity and mortality in human populations[1]. It is a syndrome characterized by hyperglycemia, polydipsia and polyuria and causes complications to the eyes, kidneys, and nerves. It is also associated with an increased incidence of cardiovascular disease[2]. The clinical manifestations and development of diabetes often differ significantly between countries and also between racial groups within a country. For example, diabetes currently affects an estimated 15.1 million people in North America, 18.5 million in Europe, 51.4 million in Asia, and just under 1 million in Oceania[3]. It is estimated that globally, the number of people will rise from 151 million in the year 2000[4], to 221 million by the year 2010, and to 300 million by 2025[5].

The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025[6,7].

The clinical diagnosis of diabetes is often suggested by the presence of hyperglycemic symptoms and glycosuria, sometimes with drowsiness or coma. The World Health Organization (WHO) criteria define diabetes by fasting plasma glucose (FPG) level of 140mg/dL (7m mol/L) or greater, or post-prandial 2h plasma glucose (PG) level of 200mg/dL (11.1m mol/L) or greater during an oral glucose tolerance test[8]. The National Diabetes Data Group of the National Institutes of Health recommends the following criteria for diagnosing diabetes:

- ✓ Fasting (overnight) venous plasma glucose concentration greater than or equal to 140 mg/dL on at least two separate occasions.
- ✓ Venous plasma glucose concentration greater than or equal to 200 mg/dL at 2h post-ingestion of 75 g of glucose and at least one other sample during the 2-h test.

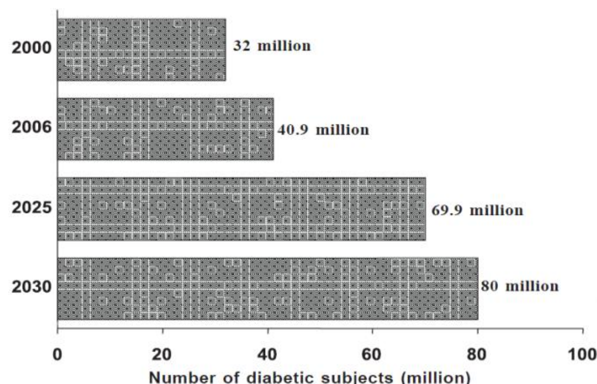

Figure 1: Estimated population of diabetic subjects in India

Euphorbia neriifolia L. (Euphorbiaceae, common name: Indian Spurge Tree) a common plant in India,

has been widely used in traditional medicine as a cure for aphrodisiac, diuretic, cough and cold, and also used in the treatment of bronchitis, bleeding piles, ano-rectal fistula . In addition, roots as antispasmodic, the root mixed with black pepper is applied to cure snake bites. Papiya Bigoniya *et al.* evaluated the hepatoprotective activity of saponin fraction isolated from leaf of *E. neriifolia* on CCl_4 induced heaptotoxicity[9]. CCl_4 (5mg/kg; ip) induces peroxidative degeneration of membrane lipids causing hypo perfusion of membrane. Kalpesh Gaur *et al.* determined the immunomodulatory activity of 70% v/v hydro-alcoholic extract of dried leaves of *E. neriifolia* by oral administration at dose of 400mg/kg/day of body weight to healthy albino rats[10]. This study was thus initiated with the aim of evaluating the effects of methanolic extract of *Euphorbia neriifolia* stem bark (MEEN) on the blood glucose level, serum and tissue lipids in streptozotocin diabetic rats.

MATERIALS AND METHODS

Animals

All the experiments were carried out with male Wistar rats aged seven to eight weeks (180-200 g), obtained from the Central Animal House, Y. B. Chavan College of Pharmacy, B.A.M. University, Aurangabad India. The animals were housed in polypropylene cages and provided with water and standard pellet diet ad libitum. The animals used in the present study were approved by the institutional Animal Ethics Committee

Chemicals

Streptozotocin was obtained from Himedia Laboratory Limited, Mumbai, India. All other reagents used were of analytical grade.

Plant Material

Euphorbia neriifolia stem bark collected freshly from Dhule and Nandurbar District, Maharashtra, India. The plant was identified and authenticated at the Herbarium of Botany Department of the University.

Preparation of plant extract

Five hundred g of *Euphorbia neriifolia* stem bark extracted with 1,500 ml of methanol by the method of continuous hot extraction at 60ºC for six hours and evaporated. The residual extract was used in the study[11].

Induction of experimental diabetes

A freshly prepared solution of streptozotocin (45 mg/kg i.p) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1 ml/kg. After 48 hours of streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycaemia (i.e. with a blood glucose of 200-300 mg/dl) were taken for the experiment[12].

Experimental procedure

In this study, a total of 36 rats (30 diabetic surviving rats, six normal rats) were used. The rats were divided into six groups of six rats each.

Group 1: Normal untreated rats.

Group 2: Diabetic control rats given 1 ml of aqueous solution daily using an intragastric tube for 30 days.

Group 3: Diabetic rats given MEEN (100mg/kg body weight) suspended in 0.5% CMC daily using an intragastric tube for 30 days.

Group 4: Diabetic rats given MEEN (200mg/kg body weight) suspended in 0.5% CMC daily using an intragastric tube for 30 days.

Group 5: Diabetic rats given MEEN (400mg/kg body weight) suspended in 0.5% CMC daily using an intragastric tube for 30 days.

Group 6: Diabetic rats given glibenclamide (600µg/ kg body weight) suspended in 0.5% CMC daily using an intragastric tube for 30 days[13].

At the end of 30 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in two different tubes (i.e.,) one with anticoagulant- potassium oxalate and sodium fluoride for plasma and another without anticoagulant for serum separation. Plasma and serum were separated by centrifugation. Liver was immediately dissected out, washed in ice cold saline, patted dry and weighed.

Analytical Procedure

Fasting blood glucose was estimated by O-toluidine method[14]. Plasma insulin level was assayed by Enzyme Linked Immunosorbent Assay (ELISA) kit, using human insulin as standard. Haemoglobin was estimated by the method of Drabkin and Austin[15]. Lipids was extracted from serum and tissues by the method of Folch *et al*[16]. Total cholesterol and triglycerides were estimated by the method of Zlatkis *et al.*[17] and Foster and Dunn[18] respectively. Free fatty acids and phospholipids were analysed by the method of Falholt *et al.*[19] and Zilversmit *et al.*[20].

Hexokinase and glucose-6-phosphatase were assayed by the method of Brandstrup *et al.*[21] and Koida and Oda *et al.*[22].

Statistical analysis

All values were expressed as the mean obtained from a number of experiments (n). Data from all the tables of normal animals, diabetic control animals, reference drug treated and MEEN treated animals were compared by ANOVA followed by Duncan's Multiple Range Test (DMRT)[23].

RESULTS

Blood glucose and Plasma insulin

Table 1 shows the levels of blood glucose, plasma insulin, total haemoglobin, changes in body weight and urine sugar of normal and experimental rats. There was a significant elevation in blood glucose, while the plasma insulin and total haemoglobin levels decreased significantly in streptozotocin diabetic rats when compared with normal rats. Administration of MEEN and glibenclamide tends to bring the parameters significantly towards the normal. The effect of MEEN at a dose of 400mg/kg body weight was more highly significant than 100 and 200mg/kg body weight and therefore the dose was used for further biochemical studies.

In diabetic rats, the urine sugar was (+++) but in the case of MEEN treated rats at a dose of 100 and 200mg/kg body weight showed decreased urine sugar (++) and (+) respectively. MEEN at a dose 400mg/kg of body weight, showed urine sugar as seen in normal rats. These effects were compared with glibenclamide.

Serum and tissue lipids

The effect of MEEN on serum and tissue lipids of normal and experimental rats is summarized in Table 2 and Table 3 respectively. A marked increase in the frequency of cholesterol, free fatty acids, triglycerides and phospholipids were observed in diabetic control rats. Treatment with MEEN significantly reduced the lipid levels.

Hepatic hexokinase and glucose-6-phosphatase

The activities of carbohydrate enzymes are represented in Table 4. Activity of hexokinase in liver decreased markedly while the glucose-6-phosphatase activity increased significantly in diabetic control rats. Treatment with MEEN in diabetic rats increased the hexokinase activity and decreased the glucose-6-phosphatase activity.

DISCUSSION

Streptozotocin is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms[24]. Intraperitoneal administration of streptozotocin (45 mg/kg) effectively induced diabetes in normal rats as reflected by glycosuria, hyperglycaemia, polyphagia, polydipsia and body weight loss when compared with normal rats[25.] In our present study we have observed that *Euphorbia neriifolia* stem bark extract of can reverse these effects. The possible mechanism by which MEEN brings about its antihyperglycemic action may be by potentiation of pancreatic secretion of insulin from β-cell of islets or due to enhanced transport of blood glucose to peripheral tissue. This was clearly evidenced by the increased level of insulin in diabetic rats treated with MEEN. In this context a number of other plants have also been reported to have antihyperglycemic and insulin-release stimulatory effect [26, 27].

We have observed a decrease in total haemoglobin during diabetes and this may be due to the formation of glycosylated haemoglobin. Increase in the level of haemoglobin in animals given MEEN may be due to decreased level of blood glucose MEEN administration to streptozotocin dosed animals reversed the weight loss. The ability of MEEN to recover body weight loss seems to be due to its antihyperglycemic effect.

Excess of fatty acids in serum produced by the streptozotocin-induced diabetes promotes conversion of excess fatty acids into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins[28]. The abnormal high concentration of serum lipids in the diabetic subject is due, mainly to increase in the mobilisation of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in streptozotocin diabetic rats[29,30] and significant increase observed in our experiment was in accordance to these studies. The marked hyperlipidaemia that characterise the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots[31].

The antihyperlipidaemic effect of MEEN may be due to the down regulation of NADPH and NADH, a cofactor in the fat metabolism. Higher activity of glucose-6-phosphatase provides H^+ which binds with $NADP^+$ in the form of NADPH and is helpful in the synthesis of fats from carbohydrates. When glycolysis slows down because of cellular activity, the pentose phosphate pathway still remain active in liver to breakdown glucose that continuously provides NADPH which converts acetyl radicals into long fatty acid chains. MEEN may be capable of oxidising NADPH. Enhanced hexokinase activity in MEEN treated rats suggests greater uptake of glucose from blood by the liver cells.

Activities of enzymes suggest that enhanced lipid metabolism during diabetes is shifted towards carbohydrate metabolism and it enhances the utilisation of glucose at the peripheral sites. One of the possible actions of MEEN may be due to its inhibition of endogenous synthesis of lipids.

Metabolic aberrations in streptozotocin diabetic rats suggest a high turnover of triglycerides and phospholipids. MEEN may antagonise the metabolic aberration and thereby restore the normal metabolism by tilting the balance from high lipids to high carbohydrate turnover. Alteration of fatty acid composition by increased lipid levels contribute to lowering the resistance of tissues and higher rate of oxidative stress. Decreased activity of glucose-6-phosphatase through pentose phosphate shunt results in high reduced glutathione to oxidised glutathione ratio (GSH/GSSG)[30,] which is coupled with conversion of NADPH to NADP. MEEN may produce high NADP+ which results in down regulation of lipogenesis and lower risk of the tissues for oxidative stress and high resistance for diabetes.

It can be concluded from the data that MEEN significantly reduces the levels of serum and tissue lipids, which are actively raised in streptozotocin diabetes rats. MEEN has beneficial effect on plasma insulin and hexokinase activity. Moreover its antihyperlipidaemic effect and antidiabetic could represent a protective mechanism against the development of diabesity.

CONFLICT OF INTEREST

The author has declared that there is no conflict of interest associated with this paper.

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Table 1: Blood glucose, plasma insulin, total haemoglobin, glycosylated haemoglobin, changes in body weight and urine sugar of normal and experimental animals.

Groups	Body Weight(g)		FBG (mg/dl)	Plasma Insulin (IU/ml)	Hgb(g/dl)	Urine Sugar
	Initial	Final				
Normal	201±10.40	213± 8.90	87.12±1.24^a	7.74±0.41^a	13.01±0.71^a	Nil
Diabetic Control	205±15.70	155±11.54* **	296.56±4.87^b	3.50±0.73^b	5.96±0.56^b	+++
Diabetic+MEEN(100mg/kg)	203±17.70	209±13.49* **	150.65±3.72^b	3.95±0.15$^{\beta}$	6.84±0.66^c	++
Diabetic+MEEN(200mg/kg)	204±18.30	214±11.33* **	135.82±2.12^c	3.01±0.36^c	9.55±0.93^d	+
Diabetic+MEEN(400mg/kg)	206±19.68	218±12.74* **	115.32±1.76^{ad}	5.476±0.30^d	11.78±0.89^e	Nil
Diabetic+Glibenclamide (600µg/kg)	200±11.80	211±11.34* **	89.21±0.87^d	7.58±0.72^e	10.24±1.01^d	Trace

FBG-Fasting Blood Glucose
Values are given as mean ± S.D. for six rats in each group.
Values not sharing a common superscript letter differ significantly at $p < 0.05$(DMRT).
Diabetic control was compared with normal, *** $p<0.001$.
Experimental groups were compared with diabetic control, *** $p<0.001$.
A - Indicates 0.25% sugar and (+ + +) indicates more than 1% sugar

Table 2: Changes in levels of cholesterol, free fatty acids, triglycerides and phospholipids in serum of normal and experimental animals.

Groups	Cholesterol mg/100ml	Free Fatty Acids mg/100ml	Triglycerides mg/100ml	Phospholipids mg/100ml
Normal	78.25 ±4.56[a]	67.43±4.06[a]	43.96±3.27[a]	75.27±1.56 [a]
Diabetic Control	97.66 ±4.03[b]	81.86±6.68[b]	61.83±1.50[b]	95.58±3.45[b]
Diabetic+MEEN(400mg /kg)	85.34 ±5.43[c]	73.05±1.45[c]	52.87±2.70[c]	80.53±2.86 [c]
Diabetic+Glibenclamide (600µg/kg)	91.00±4.27[d]	76.51±0.88[d]	57.46±1.70[d]	86.02±2.12[d]

Values are given as mean±S.D for six rats in each group.
Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).

Table 3: Changes in levels of cholesterol, free fatty acids, triglycerides and phospholipids in liver of normal and experimental animals.

Groups	Cholesterol mg/100gm wet tissue	Free Fatty Acids mg/100gm wet tissue	Triglycerides mg/100gm wet tissue	Phospholipids g/100gm wet tissue
Normal	345.04 ±2.55	646.50±30.66	358.79±11.90	1.05±0.66
Diabetic Control	522.70±5.88	895.34±50.49	615.87±7.86	2.34±0.07
Diabetic+MEEN(400mg/kg)	418.54±4.30	792.09±47.35	440.76±12.57	2.00±0.05
Diabetic+Glibenclamide(600µg/kg)	457.89±5.36	801.56±24.30	534.81±11.43	2.30±0.10

Values are given as mean±S.D for six rats in each group.
Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).
Duncan procedure, Range for the level 2.95, 3.09, 3.20.

Table 4: Changes in activities of hexokinase and glucose-6-phosphatase in liver of normal and experimental animals.

Groups	Hexokinase (units[A]/g protein)	Glucose- 6-phosphatase (units[B]/mg protein)
Normal	139.31±5.27	0.159±0.014
Diabetic Control	101.48±4.85	0.257±0.025
Diabetic+MEEN (400mg/kg)	130.01±7.69	0.189±0.011
Diabetic+Glibenclamide (600µg/kg)	126.56±4.94	0.204±0.006

Values are given as mean±S.D for six rats in each group.
Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).
Duncan procedure range for the level

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