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Evaluation of Antidiabetic and Antihyperlipidemic Activity of *Euphorbia Neriifolia* Linn. in High Fat Diet- Streptozotocin Induced Type-2 Diabetic Model

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ABSTRACT

Euphorbia neriifolia Linn. (Euphorbiaceae) is traditionally used to treat diabetes mellitus. The extract of *Euphorbia neriifolia* Linn. are having potential in the development of drug for diabetes due to their antidiabetic activity. Purpose of the study was to evaluate the antidiabetic activity of ethanolic extracts of leaves of *Euphorbia neriifolia* Linn. (Euphorbiaceae). The present study was undertaken to evaluate the antidiabetic and antihyperlipidemic effect in high fat diet-streptozotocin (HFD-STZ)induced type-2 diabetic rats. Sprague Dawley rats weighing 200-250 gm were consumed high fat diet (HFD). Two weeks later the animals were given with intraperitonial injection of streptozotocin (STZ) (35mg/kg body weight). The purpose of this study was to examine the effect of repeated oral administration of the ethanolic extract of *Euphorbia neriifolia* Linn at a dose of (200 and 400 mg/kg) on fasting blood glucose levels and lipid metabolism in streptozotocin induced type-2 diabetic rats. After 21 days of repeated oral administration of 400mg of *Euphorbia neriifolia* ethanolic extract (ENEE) produced a significant decrease on fasting blood glucose, triglyceride, total cholesterol, LDL levels in HFD-STZ induced type-2 diabetic rats, on the other hand there was significant increase in HDL levels. Glibenclamide 2.5mg/kg,p.o was used as standard drug. In oral glucose tolerance test, reduction of fasting blood glucose levels took place from 60 min of extract administration. We conclude that the ethanolic extract of *Euphorbia neriifolia* (400mg/kg) exhibits anti diabetic potential along with potent lipid lowering effect after repeated oral administration.

KEYWORDS

Euphorbia neriifolia, high fat diet-streptozotocin.

INTRODUCTION

Diabetes is defined as a state in which the homeostasis of carbohydrate and lipid metabolism is improperly regulated by the pancreatic hormone, insulin, ultimately resulting in increased blood glucose level. It is the world's largest endocrine disorder and is one of the major killers in recent times[1]. According to World Health Organization (WHO), the world wide global population is in the midst of a diabetes epidemic with people in Southeast Asia and Western Pacific being mostly at risk.

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The number of cases for diabetes which is currently at 171 million is predicted to reach 366 million by the end of 2030[2]. Therefore, it is necessary to search for new drugs and interventions that can be used to manage this metabolic disorder. The most prevalent form of diabetes is non-insulin dependent diabetes mellitus (type 2).

Ayurveda, the Indian system of traditional medicine, provides a number of medicinal plants to treat type 2 diabetes. Traditional knowledge and historic literatures on medicine play an important role in the discovery of novel leads from medicinal plants[3]

Euphorbia neriifolia Linn. is also known as Common Milk Hedge in English, Sehund and Thohar in Hindi belonging to family Euphorbiaceae[4]. *Euphorbia neriifolia* is a large succulent shrub, with stipular thorns and is found in throughout the Deccan peninsula of India. It is believed to be a native of India and Deccan peninsula is the country of origin (South India). It is commonly found in rock ground, among rock crevices of hills; extensively cultivated in the Bengal for hedges and elsewhere in native villages[5]. Today, it is widely distributed throughout the world. Pharmacological studies and traditional uses of *Euphorbia Neriifolia* shows medicinal values,such as antibacterial, antifungal, antiviral, antiparasitic, antiarthritic, antidiabetic, anticonvulsant, antioxidant[6,7], wound healing, immuno-modulatory, radioprotective, spasmodic, aphrodisiac, anticancer[8,9], and diuretic properties due to the presence of phytoconstituents like lectin, quercetin, saponin, flavonoids, triterpenes, diterpenes, anthocyanins,alkaloids and glycosides[10,11,12].

In the present investigation, ethanolic extract of leaves of the *Euphorbia* neriifolia was used to evaluate the anti diabetic and antihyperlipidemic activity in normal and HFD-STZ induced diabetic rats.

MATERIALS AND METHODS

Collection of Plant Material

Fresh leaves of *Euphorbia neriifolia* were collected from regions of Sabarkantha district in the month of July and August. The leaves of the plant were identified by the botanist of H.N.S.B. Ltd. science college, Himatnagar of Sabarkantha district.

Preparation of Plant Extracts

Freshly collected *Euphorbia neriifolia* leaves were dried in shade and coarse powder was extracted using soxhlet apparatus in 70% ethanol. The extracted mixture was filtered through muslin cloth and evaporated at 40°C up to one third of initial volume. Remaining solvent was completely evaporated at 40°C, using a hot air oven and kept in dissector for two days. The yield (20% w/w) of the powdered plant material was collected dried and stored at 5°C in air tight container.

Animals

Sprague Dawley rats weighing 200-250 g were used for the present study. The animals were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum*. The study was approved by Institutional Animal Ethics Committee (Reg. no.811/04/c/CPCSEA), Proposal no.11/APMC/CPCSEA. CPCSEA guidelines were adhered during the maintenance and experiment.

Acute Toxicity Study

Healthy adult Wistar albino rats of either sex, starved overnight were divided into two groups, each consisting of five rats and were orally fed with the extracts in increasing dose levels of 100, 500, 1000, 3000 and 4000 mg/kg body weight. The acute toxicity study was carried out according to OECD guidelines. The animals were observed continuously for 2 h under the following profiles[13].

(I) Behavioral profile. Alertness, restlessness, irritability, and fearfulness.

(II) Neurological profile. Spontaneous activities, reactivity, touch response, pain response and gait.

(III) Autonomic profile. Defecation and urination.

After a period of 24 h, 72 h and 14 days they were observed for any lethality or death

Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test[14] was performed in overnight fasted (18 h) normal rats. Rats divided into four groups, each consisting of six rats were administered 0.9% (w/v) saline, glibenclamide 2.5 mg/kg, *Euphorbia neriifolia* ethanolic extracts (ENEE) 200 and 400 mg/kg, respectively. Glucose (3 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under

ether inhalation at 0, 30, 60 and 120 min of glucose administration and glucose levels were estimated using glucose oxidase–peroxidase reactive strips and a glucometer

Induction of Non Insulin Dependent Diabetes Mellitus (NIDDM)

Experiments were performed in adult Sprague Dawley rats of either sex, aged 6–8 weeks and weighing 200–250g. The animals were housed under standard environmental conditions (23±1°C, with 55±5% humidity and a 12 h light/12 h dark cycle) and the rats were fed with High fat Diet (HFD). The composition (Table 1) and preparation of HFD as were described elsewhere[15].After 2 weeks the animals fed with HFD were administered with low dose of streptozotocin (35 mg/kg, in 0.1 M citrate buffer, pH 4.5) intraperitoneally to induce diabetes mellitus. After injection the animals had free access to food and water and were given 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycemia. The development of diabetes was confirmed after 72 hours of the streptozotocin injection. The animals with fasting blood glucose level more than 200 mg/dl were selected for the experimentation[16]. Five groups of 6 animals were formed and used for the experimentation. Glibenclamide (2.5 mg/kg) was used as the standard drug.

Table 1: Composition of High Fat Diet (HFD)

Ingredients	Diet (g/kg)
Powdered NPD	365
Lard	310
Casein	250
Cholesterol	10
Vitamin and mineral mix	60
dl-Methionine	03
Yeast powder	01
Sodium chloride	01

Anti Diabetic Study

Animals were divided into five groups, each consisting of six rats. The extracts were administered per oral route for 21 days.

Group I: Normal control rats administered saline (0.9%, w/v)

Group II: Diabetic control rats administered saline (0.9%, w/v)

Group III: Diabetic rats administered glibenclamide (2.5 mg/kg) daily for 21 days

Group IV: Diabetic rats administered ENEE 200 mg/kg;

Group V: Diabetic rats administered ENEE 400 mg/kg.

The effects of administration of ENEE in normal and diabetic rats were observed by measuring fasting blood glucose and serum lipid profile. Fasting blood glucose was estimated on days 0, 7, 14, and 21 of extracts administration. The other biochemical parameters were determined on day 21 after the animals were sacrificed by decapitation. Serum lipid profiles were measured by using diagnostic kits.

Statistical Analysis

Values were represented as mean ± S.D. for 6 animals in each group. Data were analyzed using one-way analysis of variance (ANOVA). The values were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Acute Toxicity Study

Acute toxicity study revealed the non-toxic nature of the extracts. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period.

Oral Glucose Tolerance Test (OGTT)

Table 2 depicts the hypoglycemic effects of single oral administration of the extracts at 200 and 400 mg/kg on OGTT of normal rats. The ethanolic extract at the dose of 400 mg/kg produced a maximum fall at 60 min after glucose administration.

Table 2: Effect of ENEE on oral glucose tolerance test (OGTT) in normal rats

Group (n=6)	Treatment	Fasting plasma glucose concentrations mg/dl			
		0 min	30 mins	60 mins	120 mins
I	Normal control	88.6±2.531	92.5±2.341	110.2±3.01	97.5±3.420
II	Glibenclamide (2.5mg/kg)	93.2±2.706	85.7±1.856	81.1±2.617**	68.4±3.623**
III	Normal+ENEE (200mg/kg)	90.1±2.603	89.7±1.964	102.5±2.631	89.1±2.604*
IV	Normal+ENEE (400mg/kg)	92.4±2.812	88.2±3.408	99.6±2.540*	79.3±3.151**

ENEE: *Euphorbia neriifolia* ethanolic extract
Readings are values±S.D. n = no. of animals in each group.
* $p < 0.05$ vs. diabetic control.
** $p < 0.01$ vs. diabetic control.

Table 3: Effect of ENEE on fasting blood glucose level in diabetic rats

Group (n=6)	Treatment	Fasting plasma glucose concentrations mg/dl			
		Day 0	Day 7	Day 14	Day 21
I	Normal control	77.0±1.92***	83.34±1.52***	86.23±1.32***	91.0±0.96***
II	Diabetic control	288.56±3.48	294.26±3.52	297.39±3.34	307.79±3.24
III	Diabetic+Glibenclamide2.5mg/kg	282.0±3.69	194.56±2.73**	147.5±1.62**	94.17±1.15***
IV	Diabetic+ ENEE 200mg/kg	277.35±2.88	236.79±3.08	189.88±2.20	137.76±2.39*
V	Diabetic+ ENEE 400mg/kg	278.73±2.86	212.46±2.32*	164.06±2.24**	127.26±1.43**

ENEE: *Euphorbia neriifolia* ethanolic extract
Readings are values±S.D. n = no. of animals in each group.
* $p < 0.05$ vs. diabetic control.
** $p < 0.01$ vs. diabetic control.
*** $p < 0.001$ vs. diabetic control.

Antidiabetic Study

Table 3 describes the effect of treatment of the extracts on fasting blood glucose levels. Significant reduction was observed in the extract treated rats.

Serum Lipid Profile

Table 4 describes the effect of extracts on serum lipid profile. A decrease in the serum triglycerides, total cholesterol, LDL (low density lipids) and VLDL (very low density lipids) levels, and an increase in the HDL (high density lipids) cholesterol levels were observed.

The present study is the preliminary assessment of the antidiabetic and antihyperlipidemic activity of the ethanolic extracts of *Euphorbia neriifolia*. The extracts showed a dose-dependent fall in FBG in HFD-STZ induced

diabetic rats. Streptozotacin induces diabetes by pancreatic cell damage mediated through generation of cytotoxic oxygen free radicals. The primary target of these radicals is the DNA of pancreatic cells causing DNA fragmentation [17,18].

When ENEE were administered to glucose loaded normal rats (OGTT) fasted for 18 h, reduction in blood glucose levels was observed after 60 min. The decline reached its maximum at 2 h. In the present study, the difference observed between the initial and final fasting blood glucose levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control group at the end of the 15-day experimental period. Administration of extracts to diabetic rats showed a significant decrease in the fasting blood glucose. Hence, the possible mechanism by which ENEE brings about its hypoglycemic action may be by potentiating the insulin effects of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.

Another possible mechanism may be attributed to the rich fiber content of ENEE. Dietary fibers play a major role in lowering the blood glucose level by slowing the rate of carbohydrate absorption from intestine and are hence beneficial for diabetics, especially type II diabetics[19]. Under normal conditions, the enzyme lipoprotein lipase hydrolyses triglycerides. Diabetes mellitus results in failure to activate this enzyme thereby causing hypertriglyceridemia. Dietary fibers lower the cholesterol and triglyceride levels[20]. Therefore, the significant control of levels of serum lipids in the treated groups may be attributed to the rich fiber content in *Euphorbia neriifolia*. Induction of diabetes with HFD-STZ is associated with a characteristic loss of body weight, which is due to increased muscle wasting[21] and due to loss of tissue proteins[22,23,24].

Table 4: Effect of ENEE on serum lipid profile in diabetic rats

Groups (n=6)	Treatment	Serum lipid profile mg/dl				
		TG	TC	HDL	LDL	VLDL
I	Normal control	96.85±1.25***	62.84±1.67***	52.85±2.22 ***	34.03±1.84***	25.73±1.39***
II	Diabetic control	142.33±1.54	87.85±2.96	25.86±1.27	88.83±2.25	46.76±1.79
III	Diabetic+Glibenclamide 2.5mg/kg	105.46±1.16**	68.67±1.35***	50.50±1.05***	47.40±1.46***	31.13±1.32***
IV	Diabetic+ ENEE 200mg/kg	121.90±1.21*	78.43±2.13*	35.67±1.49**	61.97±1.75***	42.07±1.24*
V	Diabetic+ ENEE 400mg/kg	111.16±1.15**	73.0±1.57***	44.07±1.17***	53.56±1.26***	35.54±0.99***

ENEE: *Euphorbia neriifolia* ethanolic extract
Readings are values±S.D. n = no. of animals in each group.
* $p < 0.05$ vs. diabetic control.
** $p < 0.01$ vs. diabetic control.
***$p < 0.001$ vs. diabetic control

CONCLUSION

In conclusion, the sub-acute treatment of ENEE on HFD- STZ induced diabetic animals showed signicant increase in serum HDL cholesterol levels, whereas significant decrease in serum total cholesterol, LDL-cholesterol, VLDL-cholesterol and creatinine levels were observed. Based on the results, ethanolic extract of *Euphorbia neriifolia* at the dose of 400 mg/kg were effective and exhibit potent anti diabetic antihyperlipidemic activity as compared to 200 mg/kg and it may prove to be effective for the treatment of NIDDM. However, longer duration studies on chronic models are necessary to elucidate the exact mechanism of action so as to develop it as a potent antidiabetic drug.

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