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# Antidiabetic and hypolipidemic activity of *Euphorbia neriifolia* in Streptozotocin induced diabetic rats

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**ABSTRACT**

*The concerned study reveals the experimental investigation of the biological activity of Euphorbia neriifolia (Family: Euphorbiaceae) used as a traditional antidiabetic and hypolipidemic agent in past and present culture. To study the effect of Euphorbia neriifolia in both normal and streptozotocin induced diabetic rats. The methanol stem extract of Euphorbia neriifolia at the dose of 200, 400 mg/kg body weight was administered orally once a day to the groups for 15 days. The fasting blood glucose, cholesterol, HDL cholesterol and serum triglyceride content were estimated in both normal and streptozotocin induced diabetic rats. The fasting blood glucose, cholesterol and serum triglyceride content were found to be significantly reduced ($p < 0.05$) in treated rats whereas the extract also showed the potent elevation in the level of serum HDL cholesterol. The study reveals that Euphorbia neriifolia has significant antidiabetic activity and a hypolipidemic activity in streptozotocin induced and normal fasting rats. The extract seems promising for the development of a phytomedicine for diabetes mellitus.*

**Key words:** Diabetes mellitus, *Euphorbia neriifolia*, Streptozotocin, Hypolipidemic activity

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## INTRODUCTION

Diabetes mellitus is syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting impaired metabolism of glucose and other energy-yielding fuels such as lipids and protein [1]. Diabetes mellitus is a common disorder among the Indian population. It is estimated that diabetes would affect approximately 57 million people by the year 2025 [2]. Therapeutic options for diabetes are diet, exercise, oral hypoglycemic drugs and insulin therapy. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic one [3].

Although, there are numerous traditional plants reported to have antidiabetic and hypoglycemic properties. *Euphorbia neriifolia* Linn (Euphorbiaceae) commonly known as “Sehund or thohar” in Hindi, is found throughout the Deccan Peninsula of India and grows luxuriously around the dry, hilly, rocky areas of North, Central and South India. Ayurveda describes the plant as bitter, pungent, laxative, carminative, improves appetite useful in abdominal troubles, bronchitis, tumors, loss of consciousness, delirium, leucoderma, piles, inflammation, enlargement of spleen, anaemia, ulcers and fever [4, 5]. As far as our literature survey could ascertain, no information was available

on the antidiabetic activities of the stem of *E. neriifolia*. Therefore, the aim of this current investigation was to explore the antidiabetic potential of methanol extract of stem of *E. neriifolia* against streptozotocin indiced diabetic rats.

## MATERIALS AND METHODS

*Plant material*

The stem of *Euphorbia neriifolia* was collected from the rural region of Midnapore, West Bengal, India. The plant was authenticated by the Botanical Survey of India (BSI), Shibpur (W.B), India. Air dried whole stem (500 g) were powdered in a mechanical grinder and the powdered materials was extracted by methanol using Soxhlet extraction apparatus. The solvent was completely removed under reduced pressure in a rotary vacuum evaporator. The concentrated extract (yield 35.42%) was stored in vacuum desiccators for further use.

*Animals*

Male albino rats, 9-12 weeks old with an average weight of 150-175 g were used for the study. They were housed in polypropylene cages and fed with a standard chow diet and water *ad libitum.* The animals were exposed to an alternating 12 h and light cycle. Before each experiment, the animals were fasted for at least 18 h. The experimental protocols were approved by Institutional Animal Ethical Committee (1585/PO/a/11/CPCSEA).

*Drugs and chemicals*

Thiobarbituric acid (TBA), streptozotocin (STZ), 5,5´-dithio bis-2-nitro benzoic acid (DTNB), phenazonium methosulfate (PMS), nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH) from SISCO Research Laboratory, Mumbai, India; potassium dichromate, glacial acetic acid from Ranbaxy, Mumbai; and glibenclamide from Hoechst, India. All the other reagents used were of analytical reagent grade.

*Preliminary phytochemical screening*

The extracts were subjected to preliminary screening for various active phytochemical constituents [6].

*Acute toxicity*

The acute oral toxicity of Methanol extract of *Euphoria neriifolia* (MEEN) in male Swiss albino mice was studied as per OECD guideline 425. The median lethal dose ($LD_{50}$) value was determined using the method of maximum likelihood.

*Effect on Glucose Tolerance in Rats*

The animals were divided into four groups. Group I served as negative control group (0.9% NaCl, p.o). Group II and III were treated with MEEN at dose levels 200 and 400 mg/ kg b.w. p. o., respectively. Group IV was treated with 0.5 mg/kg of glibenclamide p. o. Zero hour-blood sugar levels were determined on 18 hour fasted animals. After 30 min of drug treatment the animals were fed with glucose (5g/kg) and blood glucose was determined after 30 minutes, 1, 2, and 3 h of the glucose load. The blood sugar level was measured using Accuchek Active" Test strip in Accu-chek Active Test meter.

Experimental design:

*Streptozotocin-induced diabetic rats:*

Streptozotocin (STZ) was dissolved in 0.9% ice-cold saline immediately before use. Diabetes was induced in rats by intra peritoneal (i.p) injection of streptozotocin at a dose of 50 mg/kg, dissolved in saline. 72 hours after streptozotocin administration, blood samples were drawn from tail and glucose levels determined to confirm diabetes. The diabetic rats exhibiting blood glucose levels higher than 225 mg/dl were selected for the studies.

*Treatment schedule and estimation of fasting blood glucose (FBG) level:*

The rats were divided into five groups (n=6). Except for group I, which served as normal non-diabetic control, all other groups were comprised of diabetic rats. Group II served as diabetic (STZ) control. Groups III and IV received MEEN (200 and 400 mg/kg body weight, p.o., respectively), and group-V received reference drug glibenclamide (0.5 mg/kg b.w., p.o.) daily for 15 days. Fasting blood glucose was measured on days 0, 5, 10 and 15 by using a one-touch glucometer (Accucheck®). At 24 h of the last dose, blood was collected from overnight fasted rats from each group by cardiac puncture for estimation of serum biochemical parameters. Then the rats were sacrificed by cervical dislocation for the study of liver and kidney biochemical parameters.

**Table 1: Treatments schedule**

| Groups | Treatments |
|---|---|
| Group I | Normal saline (5 ml/kg b.w., orally) |
| Group II | Diabetic rats-Streptozotocin (50 mg/kg b.w, I.P) |
| Group III | Diabetic rats treated with 200 mg/kg MEEN |
| Group IV | Diabetic rats treated with 400 mg/kg MEEN |
| Group V | Diabetic rats treated with 0.5 mg/kg orally of Glibenclamide |

*Biochemical and antioxidant estimation:*
All animals were sacrificed after the 24 hrs of last dose, blood samples were collected and liver and kidney tissue were excised, washed in ice cold phosphate buffered saline, blotted and weighed. 10% w/v of liver and kidney homogenate was prepared in 0.15 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 2000×g for 20 minute at 4°C to remove the cell debris and then the supernatant was centrifuged for 1 hours at 4°C. The supernatant obtained used for the determination of Lipid peroxidation [7], Glutathione (GSH) [8] and Catalase [9]. Serum was separated and analyzed for SGOT (Serum glutamate oxaloacetate transaminase), SGPT (Serum glutamate pyruvate transaminase), and Serum alkaline phosphatase were estimated by commercially available kits (Span Diagnostic Pvt. Ltd. Surat, India). Total cholesterol level, HDL-cholesterol level and triglyceride level (TG) were estimated by commercially available kit (Span Diagnostic Pvt. Ltd. Surat, India).

Statistical analysis
All the values of body weight, fasting blood sugar, and biochemical parameters were expressed as mean ± standard error of mean (SEM) and were analysed for ANOVA and Dunnett's t test. The values of $p< 0.001$were considered statistically very significant and $p< 0.05$ were considered as significant.

## RESULTS

*Preliminary phytochemical test*
Phytochemical studies indicated that extracts of roots of *E. neriifolia* contains alkaloids, flavanoids, glycosides, saponins and terpenes.

*Acute toxicity studies*
The oral $LD_{50}$ value of MEEN in mice was 2000 mg/kg body weight.

*Antihyperglycemic activity*
The effects of extracts on blood glucose levels in diabetic rats are reported in Table 3. Blood glucose levels of the STZ treated rats were significantly higher than those in normal rats. In STZ (50 mg/kg) induced rats, the blood glucose level increased from 91.56±2.12 of normal group to 252.31±10.3 mg/dl. Methanol extracts (200 mg/kg and 400 mg/kg) given up-to 15 days. After extracts treatment, the blood glucose levels were decreased from 294.41±13.7 to 115.5±3.5 and 283.83 ± 27.8 to 100.3 ± 3.1 mg/dl respectively, whereas in glibenclamide treated rats, blood glucose levels were decreased from 291.12 ± 17.1to 91.50 ± 1.7 mg/dl.

*Effect of body weight*
Streptozotocin administration brought about marked reduction in body weight of rats. This reduction was found to be statistically significant ($p<0.05$) when compared with normal control group. These reduced body weights were found to be increased when compared to their respective diabetic control group and this increase was found to be statistically significant in rats treated with MEEN ($p<0.05$) (Table 2).

*Effect of TBARS, GSH and catalase:*
The effect of MEEN on TBARS, GSH and catalase in experimental diabetic rats was shown in Table 4. There was a significant rise of lipid peroxides in liver and kidney during diabetes when compared to the saline control group. It was found that administration of MEEN significantly ($p<0.05$, $p <0.001$ ) decrease TBARS level in liver and kidney. There was a significant decrease ($p<0.001$) in the level of GSH in STZ-control group when compared with the saline control group. The catalase level in liver and kidney of normal and experimental rats exhibited significant ($p<0.001$) reduction in the activity of catalase in diabetic control.

*Effect of SGOT, SGPT and alkaline phosphatase:*
Hepatic damage induced by STZ caused significant rise in enzymes SGOT, SGPT, alkaline phosphates, administration of MEEN at 200 and 400 mg/kg body weight was observed to lower the enzyme level significantly ($p$<0.01) which is shown in table-5.

The change in the cholesterol, HDL-cholesterol and triglyceride level was measured and observed on potent reduction in serum cholesterol, triglycerides and effective elevation in HDL-cholesterol level over diabetic control when the rats fed MEEN of both doses. The level of serum cholesterol was lower in normal rats that were not treated with STZ and elevations were found in diabetic control (Table 6). In respect to HDL-cholesterol, it showed decrement in normal rats (Table 6) but maximum elevation was found on 15 day due to high dose of MEEN. However, similar trends of HDL-cholesterol elevation were observed at all doses of treatments with given time periods. Rats fed MEEN (both doses) were showed inhibition in serum triglyceride content (Table 6).

**Table 2: The measured body weight of normal and MEEN treated diabetic rats**

| Groups | Initial body weight (gm) | Final body weight (gm) |
|---|---|---|
| Saline control | 175.1±5.5 | 182.0±4.5 |
| Diabetic control | 171.3±7.5 | 132.3±5.8* |
| MEEN 200 mg/kg | 172.5±5.1 | 155.6±5.3 |
| MEEN 400 mg/kg | 172.6±7.8 | 163.3±6.3 |
| Glibenclamide 0.5 mg/kg | 168.3±9.5 | 175.5±6.4** |

*Values are expressed as mean ±SEM from n = 6,* p< 0.001 compared with saline Control group, ** p <0.05 compared with STZ-control group.*

**Table 3: Effect of MEEN on Fasting Blood Sugar levels (FBS)**

| Groups | Blood glucose level mg/dl | | | |
|---|---|---|---|---|
| | 0 day | 5th day | 10thday | 15th day |
| Saline control | 90.56 ±2.1 | 94.33 ±2.3 | 94.16 ± 1.3 | 92.12 ±1.3 |
| Diabetic control | 262.31 ±10.3* | 300.21 ±11.2* | 284.83±10.8* | 283.50±10.7* |
| MEEN 200 mg/kg | 284.41±13.7 | 205.12 ± 1.6** | 156.16±4.7** | 115.51±3.5** |
| MEEN 400 mg/kg | 276.83 ± 27.8 | 198.50 ±9.1** | 148.16±2.8** | 105.3 ± 3.1** |
| Glibenclamide 0.5 mg/kg | 290.11 ± 16.1 | 160.11 ± 4.9** | 120.66±3.5** | 95.50 ± 1.7** |

*Values are expressed as mean ±SEM (n=6), *p< 0.001 compared with saline control group and ** p< 0.001 compared with STZ-control group.*

**Table 4. The level of TBARS, GSH, and catalase in tissues of normal and MEEN treated diabetic rats**

| Group | TBARS (mM/100 g tissue) | | Glutathione (mg/100 g tissue) | | Catalase (U/mg protein) | |
|---|---|---|---|---|---|---|
| | Liver | Kidney | Liver | Kidney | Liver | Kidney |
| Saline control | 1.00 ± 0.05 | 1.53 ± 1.1 | 44.55±2.2 | 21.44±1.8 | 83.23±2.2 | 37.21±3.3 |
| Diabetic control | 1.82 ± 0.06* | 2.66 ± 0.3** | 20.36±1.8* | 6.76±2.2* | 42.15±2.4* | 21.2±1.59** |
| MEEN 200 mg/kg | 1.52 ± 0.08# | 1.85 ±0.11*** | 23.31±2.3 | 10.73±1.3 | 52.34±2.5*** | 26.94±2.6 |
| MEEN 400 mg/kg | 1.12 ± 0.07 | 1.65 ± 03*** | 28.44±2.2** | 12.73±1.2 | 65.12±2.2*** | 29.15±2.4 |
| Glibenclamide 0.5 mg/kg | 1.06± 0.09## | 1.56± 0.07*** | 32.58±1.6# | 14.51±1.8 | 72.58±2.4*** | 35.58±1.7# |

*Values are expressed as mean ±SEM (n=6), *p< 0.01, **p< 0.001 compared with saline control group. #p<0.05, ***p< 0.001 compared with STZ-control group. ##p< 0.01 compared with STZ-control group.*

**Table 5. Effect of SGOT, SGPT and ALP in serum of normal and MEEN treated diabetic rats**

| Groups | SGOT | SGPT | ALP |
|---|---|---|---|
| Saline control | 27.8±2.96 | 26.2±2.53 | 118.16±1.9 |
| Diabetic control | 39.3±2.55 | 43.0±2.54 | 258.50±6.0 |
| MEEN 200 mg/kg | 35.0±2.51 | 33.5±2.27 | 142.16±5.1 |
| MEEN 400 mg/kg | 29.89±1.56 | 23.5±2.11 | 154.12±11.8 |
| Glibenclamide 0.5 mg/kg | 27.2±1.76 | 21.5±2.17 | 132.66±5.5 |

*Values are expressed as means ±SEM (n=6), p<0.01, diabetic control was compared with the vehicle control and treated groups were compared with the diabetic control.*

**Table 6: Effect of serum total cholesterol, HDL and triglyceride level of normal and MEEN treated diabetic rats**

| Groups | Total cholesterol (mg/dl) | HDL (mg/dl) | Triglyceride (mg/dl) |
|---|---|---|---|
| Saline control | 127.8±2.9 | 40.23±2.53 | 78.16±1.9 |
| Diabetic control | 239.3±5.6 | 52.0±2.54 | 158.23±5.2 |
| MEEN 200 mg/kg | 185.0±4.5 | 43.5±2.27 | 102.1±5.2 |
| MEEN 400 mg/kg | 162.8±3.5 | 48.5±2.11 | 100.2±3.8 |
| Glibenclamide 0.5 mg/kg | 141.2±2.7 | 51.5±2.17 | 92.63±4.5 |

*Values are expressed as means ±SEM (n=6), p<0.01, diabetic control was compared with the vehicle control and treated groups were compared with the diabetic control.*

## DISCUSSION

In the present experiment it was found that the administration of methanol extract of *Euphorbia neriifolia* stem at the doses of 200 and 400 mg/kg body weight to diabetic rats produced strong antihyperglycemic activity. Induction of diabetes with STZ is associated with a characteristic loss of body weight, which is due to increased muscle wasting [11] and loss of tissue proteins [10]. Diabetic rats treated with the MEEN showed an increase in body weight as compared to the diabetic control, which may be due to its in controlling muscle wasting, *i.e.*, by reversal of antagonizing [12] hitton *et al*., 1975).

As we know that those STZ-induced diabetes mellitus and insulin deficiencies lead to increased blood glucose [13]. Administration of MEEN decreased the elevated blood glucose level, by motivating β-cells of islets of langerhans to produce insulin. From the result it is assumed that the rhizome could be responsible for stimulation of insulin release and the observed restoration of metabolic activities.

From the phytochemical analysis of MEEN, indicates the presence of flavonoids and tannins are well known natural antioxidants due to their electron donating property which either scavenge the principal propagating radicals or halt the radical chain [14].

Since the finding that STZ possesses diabetogenic properties mediated by pancreatic beta cell destruction, this compound has been widely used to induce diabetes in experimental animals [15].

In physiological conditions, our human body can balance for a mild degree of oxidative stress and remove oxidative damaged molecules by activating antioxidant enzymes like catalase, glutathione s-transferase (GST), glutathione peroxidase (GP) *etc* [16].The antioxidant which either able to prevent the excess of oxidative chemical species or stimulate the endogenous antioxidant repairing mechanism are the best scavenging agent [17].

A noticeable increase in the concentration of TBARS indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanism to prevent the formation of excess free radicals due to the consequences of increased production, and liberation into the circulation of lipid peroxide due to pathologic changes [18]. Treatments of MESR significantly lower the TBARS in STZ-induced diabetic rats and improve the pathological condition of diabetes by inhibition of lipid peroxidation.

Significant decrease in the levels of GSH has been observed in STZ-induced diabetic rats when compared to MEEN treated rats [19] suggested that the decrease in hepatic GSH could be the result of decreased synthesis, or increased degradation of GSH by oxidative stress in diabetes. Catalase enzyme catalyses the decomposition of $H_2O_2$ to water and oxygen and thus protecting the cell from oxidative damage by $H_2O_2$ and $OH^-$ [20], MEEN treatment showed normalization of catalase in liver and kidney.

The elevated enzymatic activity of SGOT and SGPT suggests cardiac and liver damage (Scott *et al.*, 1984). In the present study, there was a significant increase in SGOT and SGPT activities of STZ diabetic rats when compared with that of nondiabetic rats, whereas MEEN treated group showed a progressive dose-dependent decrease in SGOT and SGPT activities which was significant only with 400 mg/kg doses after 15 d of treatment.

In this study, the feeding of MEEN resulted in significantly decreased total cholesterol and serum triglycerides and significantly increased HDL-cholesterol level; these findings are correlated with the experiment. Ingestion of MEEN produced a significant lowering of cholesterol in a hypertension model. Insulin is potent inhibitor of lipolysis, since it inhibits the activity of the hormones sensitive lipases in adipose tissue and suppresses the release of triglycerides

[21]. The increase in HDL-cholesterol levels may be beneficial awing to the negative correlation between HDL-cholesterol levels and cardiovascular decrease. This could be due to the presence of other hypolipedemic agents such as alkaloids, triterpenes in methanol extract [22].

## CONCLUSION

It can be concluded from the results that the MEEN stem is beneficial in controlling the blood glucose level and restores body weight, serum enzymes, and prevent lipid peroxidation associated complication with STZ- induced experimental diabetic rats. Further pharmacological and biochemical investigations are underway to find out the active constituent responsible for the antidiabetic activity and to elucidate its mechanism of action.

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