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Evaluation of anti-diabetic activity of euphorbia neriifolin linn. in the experimentally induced diabetic animal model

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Abstract

Ethanolic extract of 400 mg/kg b.wt, *Euphorbia neriifolia Linn*. was administered orally to Wistar albino rats of both sexes. Glibenclamide 2.5 mg/kg was used as a standard drug to compare extract potency. Body weight, Oral Glucose Tolerance Taste (OGTT), serum lipid profile, fasting blood glucose (FBG), hepatic glycogen, serum insulin, and glycated hemoglobin were evaluated. In OGTT, the decline in fasting blood glucose content occurred 60 minutes after administration of the extract. *Euphorbia neriifolia* ethanolic extract (ENEE) produced a significant dose-dependent decrease in FBG. After the end of the treatment, the 15th-day dose of ENEE is 400 mg/kg. After two weeks, the animals were given established repeated oral administration of the ENEE at FBG levels, and after 21 days, ENEE produced a dose-dependent decrease in body weight, FBG, triglycerides, total cholesterol, LDL, VLDL; there was a significant increase in HDL content and liver glycogen as a standard drug glibenclamide 2.5 mg/kg was used to compare the potency of the extract. In OGTT reduction, FBG levels were observed after sixty minutes of extract administration. After treatment, The data concluded that ENEE showed dose-dependent anti-diabetic potential and a potent antihyperlipidemic effect.

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INTRODUCTION

This same aspiration of the present research is to evaluate the diabetic activity of *Euphorbia neriifolin Slinn*. In an experimentally induced diabetic animal model, To induce the diabetic in the experimental animals. To assess the hypertensive complications with diabetes mellitus. In vivo evaluation of anti-diabetic activity. Acquisition of lipids in Diabetes would be lessened through particular deficits; hence, diabetic patients are extra susceptible to hypertriglyceridemia and hypercholesterolemia [1]. One field of study is the pathogenesis of lipid metabolism perturbation throughout hyperglycemia, which seems to be the significant

increase in the fatty acid composition of fat cells and the secondary elevation of the free fatty acid stage in the blood. Dyslipidemia(High cholesterol) seems to be the leading risk factor, such as Congestive Heart Failure (CHF), and this is one of the leading causes of cardiac difficulty somewhere around unstable angina and myocardial ischemia. Worldwide, far too many persons pass away because of Diabetes associated with Dyslipidemia (hyperlipidemia)[2].

With us, Indian medicinal plants have an extensive citation for going to cure one of these severe diseases. The use of medicinal vegetation to treat hyperglycemia related to hyperlipidemia has been bereft of severe side effects. For this purpose, indigenous herbal vegetation has been chosen for the present research. Current studies, such as anti-diabetic and antihyperlipidemic activity, will be evaluated. Euphorbia neriifolia Linn., also known as Common Milk Hedge in English, Sehund, and Thohar in Hindi, belongs to the Euphorbiaceae family. *Euphorbia neriifolia Linn*. has been typically used to treat Diabetes in numerous rural areas of India. Conventional use of vegetation in Diabetes and no scientific research report was produced to cure Diabetes regarding this plant. With us, try to create this same scientific information of all these plants as popular choice anti-diabetic agents[3].

METHODOLOGY:

Euphorbia neriifolia Linn. region of Seshachala forest in the Tirupathi district, Andhra Pradesh, India in July-August-2024. The Botanist, Head, Department of Botany, D K W College, Nellore, Andhra Pradesh, India, has authenticated the plant.

Ethanolic Extraction of leaves of *Euphorbia Neriifolia Linn*:

Freshly collected leaves of *Euphorbia neriifolia Linn*. have been dried and powdered. Coarse powder (1400 gms) must have been derived using 70% ethanol by the soxhlet apparatus. The retrieved filtrate must have been filtered through muslin cloth and vaporized at 400C up to a third of the initial volume[4]. The remaining solvent was evaporated using a hot air oven and subjected to storage for a couple of days. Afterward, it was dried and stored at 50C in an airtight container. Euphorbia neriifolia ethanolic extract (ENEE) was used for further animal experimental studies.

Acute and sub-acute toxicity evaluation of Euphorbia neriifolia Linn.

Experimental animals:

Spargue-Dawley (S.D.) rats of either sex at eight weeks of age, weighing 200-250g Ratnam Institute of Pharmacy, were acclimatized to laboratory animals for ten days before beginning the experiments [5]. Animals were housed in polypropylene cages, and Rats were maintained at 23 ± 3 0C and 45 ± 5% relative humidity on a light and dark cycle of 12 hours. Rats were deprived of experiments. The Animal Ethics Committee (Reg. no.1558/P.O./Re/S/11/CPCSEA) of Ratnam Insitute of Pharmacy, Nellore, approved experimental protocols(Proposal no.09-23/RIPh/CPCSEA) and procedures were performed according to the guidelines of CPCSEA, India.

The LD50 was found to be greater than 4000 mg/kg if three or more rats survived.

SUBACUTE TOXICITY EVALUATION:

According to OECD TG 407, rats (SD 10; five males and five females per group). The weights of the animals were recorded. Was administered the whereas an equal volume of vehicle was administered to the control rats (group I). The toxic signs and mortality observations were monitored daily for 28 days. Animals intraperitoneally sinus into anticoagulant-containing tubes for determination of complete blood count (B.C.), RBC (red blood cell) count, P.T. (platelet) count, and tubes did not contain anticoagulant for determination of blood chemistry and enzyme analysis[6]. All rats were sacrificed by inhalation of carbon dioxide after blood collection. The vital and particular organs like the brain, Kidney, liver, and heart were isolated, allowed to dry, examined for gross lesions, and accurately weighed. The relative organ weight was calculated according to the weight of organ/body weight of the animal on the day of sacrifice X 100. Compared with the control value, organs were kept in 10% phosphate-buffered formalin for histopathological examination of organs.

Determination of hematological and bio chemical-parameters:

A hematological examination was conducted using an automatic hematological analyzer called Nihon

kohdenCelltac alfa. Different parameters include leukocyte (WBC) count, RBC, hemoglobin (Hb), Hematocrit (HCt), lymphocyte, granulocyte, and monocyte platelet count[7].

Separation serum was carried out at four 0C and stored at -20 0C for biochemical estimation using an autoanalyzer Merck microlab 300. Different parameters were determined like SGPT, SGOT, creatinine, alkaline phosphatase (ALP), total protein (T.P.), direct Bilirubin (D.B.), total Bilirubin (T.B.), glucose, and urea **Error! R eference source not found.**.

Histopathological examination of tissues

Kidney, heart, and liver specimens were dehydrated by serial ethanolic solution and enclosed with paraffin. Micrometer sections were done (5 µm) and cut with a microtome (Leica RM 2255) were stained with H&E (Hemotoxylin and erosion) dye[8]**.**

Evaluation of the oral glucose tolerance test (OGTT) for *Euphorbia neriifolia* ethanolic extract (ENEE) leaf on glucose-fed hyperglycemia in normal rats:

Experimental design

Group I:

Rats were given distilled water and glucose (3 g/kg) orally for 1 hour as a control to elicit the glucose tolerance curve.

Group II:

Rats were given Glibenclamide (2.5 mg/kg) orally in distilled water 1 hour before the induced glucose tolerance curve.

Group III:

The rats were given the lower dose of Euphorbia neriifolia ethanolic extract (ENEE 200 mg/kg) in distilled water orally 1 hour before the glucose tolerance curve was elicited.

Group IV:

Rats were given a higher dose of Euphorbia neriifolia ethanolic extract (ENEE 400 mg/kg) in distilled water orally 1 hour before eliciting the glucose tolerance curve.

Evaluation of the anti-diabetic and antihyperlipidemic activity of Euphorbia neriifolia ethanolic extract (ENEE).

Experimental design

Animals are divided into five groups. Ethanolic extracts of Euphorbia neriifolia leaves, and Glibenclamide was administered orally for fifteen days [9].

Group I

Saline, 0.9% w/v, was administered to

Group II Saline 0.9% to diabetic control rats

Group III Glibenclamide 2.5 mg per kilogram

Group IV Euphorbia neriifolia ethanolic extract ENEE 400 mg pre kilogram

The administration of the ENEE effect was determined by determining the (FBG) lipid profile of FBG after the day of ENEE. After the end of the experimental period, the rats were anesthetized by an injection, deprived of overnight food and water, and anesthetized by a mixture of xylazine 6. ketamine (60. Samples of the plexus of the sinus were in an anticoagulant anticoagulant-containing tube for the determination of glycosylated hemoglobin and in a plain tube for the measurement of glucose, insulin, and kits. The liver was isolated after inhalation of carbon dioxide gas, patted dry, and afterward used to determine glycogen amount. The pancreas was dissected, isolated, and fixed in 10% phosphate-buffered formalin for histopathological examination.

An evaluation of the ethanolic extract (ENEE) of *Euphorbia neriifolia* anti-diabetic and antihyperlipidemic properties

Adult healthy Sprague-Dawley (S.D.) rats of either sex between the ages of eight to twelve weeks were used for acclimatization for 14 days in our laboratory animal house environment before the study. Animals were kept in polypropylene cages, and each cage contained three animals and cycles at a temperature of 23 ± 3 0C with humidity of 45±5 %. And carried out in the laboratory of Ratnam Institute of Pharmacy.

Adult Sprague Dawley rats of either sex, aged 8-10 weeks and weighing 200–250g. Rats environment with a relative humidity of 55±5% and 12 hours of light and dark cycles. Rats were fed the composition and preparation described above (Srinivasan et al., 2004). After two weeks, the rats fed with HFD were administered with low dose

intraperitoneally to induce diabetes mellitus. Selected for the experimental study, and each group contained six rats. Glibenclamide 2.5 mg/kg was used to compare the potency of ENEE.

Experimental Design:

Rats HFD was also given to animals till the end of the study. Group I Saline 0.9% was administered to control rats. Group II Saline 0.9% was administered to diabsseticcontroll Group III Glibenclamide 2.5 mg per kilogram for 21 days.Group IV Euphorbia neriifolia ethanolic extract ENEE 400 mg per kilogram for 21 days Administration of ENEE effect FBG was determined after day the rat and anesthetized by an injection of a were deprived of overnight food and water and anesthetized by mixture of xylazine Samples of collected from plexus of retro-orbital sinus by in anticoagulant containing tube for the determination of glycosylated hemoglobin and in a plain tube for the measurement of glucose, insulin, kits. The liver was isolated after inhaling carbon dioxide gas and used to determine the glycogen amount. The pancreas was dissected, isolated, and fixed in 10% phosphate-buffered formalin for histopathological examination[10].

Estimation of biochemical parameters

Glucose estimation

Procedure:

Mix thoroughly after 10 minutes of incubation, measure the standard's absorbance at 505 nm, and compare it to the blank reagent. The final reaction mixture's color remained consistent for at least an hour[11].

Calculate

$$Glucose\ (\frac{mg}{dl}) = \frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard} \times 100$$

Total cholesterol estimation

An autospam diagnostic kit based on an enzymatic colorimetric procedure was used to estimate serum cholesterol (CHOD/POD). Cholesterol esterase hydrolyzes cholesterol, gives enzymes, and causes the liberation of cholesterin 3-one and hydrogen peroxide.

Calculation

$$Cholesterol\ in\ \left(\frac{mg}{100ml}\right) = \frac{Absorbance\ of\ sample}{Absorbance\ of\ Standard} \times Conc.\ of\ Std$$

Serum triglycerides estimation

Procedure: Properly combined and then incubated for 10 minutes at 37°C. Within an hour, the absorbance of the sample and standard at 505 nm wavelength was measured compared to the reagent blank.

Calculation

$$Triglycerides\ \left(\frac{mg}{dl}\right) = \frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard} \times 200$$

Serum High-density lipoprotein estimation

Serum HDL was estimated using an autospan diagnostic kit[14].

Methodology

CHOD PAP endpoint colorimetry of polyethylene glycol, two-reagent chemistry with lipid clearing factor

LDL and VLDL have been caused by a standard solution containing Polyethylene glycol 6000, forsaking the High-Density Lipoproteins in the solution. HDL-cholesterol has been guesstimated within the supernatant layer by the sequence of enzyme reactions, and it was started by the oxidation by cholesterol oxidase, which converts cholesterol to cholestenone and causes the formation of H2O2. In the second reaction, this was base-catalyzed by peroxidase, 4-amino antipyrine, and phenol responded with H2O2 to form red-colored quinoneimine. The absorbance of the standard and sample was measured within 1 hour against the reagent blank at 505 nm wavelength, which is directly proportional to HDL cholesterol.

Procedure

Table 1 Step-A: High-density lipoprotein cholesterol

Pipette into the centrifuge container	Quality
Sample	0.2 ml
Reagent that precipitates	0.2 ml

Step B was performed after letting the mixture sit for 15 minutes to produce a clear supernatant.

LDL and VLDL estimation

Friedewald formula adequately estimates LDL and VLDL for most fasting specimens but is less reliable as triglyceride concentration increases.

Formula of friedewald

LDL = TC-HDL-TG/5.0 (mg/dl)

VLDL = Triglycerides/5 (mg/dl)

Table 2 Step-B Development of color

Pipette into the tubes marked	Blank	Standard	Test
The step-A supernatant	-	-	100 µl
Standard for HDL cholesterol	-	100 µl	-
Reagent for cholesterol	1000 µl	1000 µl	1000 µl

Liver glycogen estimation

Anthrone reagent was used to quantify liver glycogen.

Anthrone reagent

A mixture of 250 mg anthrone and 500 cc 75% sulphuric acid. It is kept in dark bottles and is photosensitive.

Standard glucose solution

Standard of Work: 100 ml of a saturated benzoic acid solution containing 100 mg of dissolved glucose was mixed with 5 milliliters of the stock solution.

Procedure

The tissue sample was put in an effective homogenizer with the right amount of TCA and homogenized for three minutes. Homogenate was taken into a suitable centrifugation tube. The supernatant fluid was collected by centrifugation and poured on, which was again homogenized for one minute with an appropriate volume of TCA. The mixture was centrifuged, and the supernatant filter was pumped through this filtration system. Two successive extractions have been carried out in the same sort of way.

The desired volume has been obtained by adding 5% TCA and mixing well. There were 10–200 microgrammes of glycogen per milliliter in the final volume.

Pipetted into a 15 ml centrifuge tube (in duplicate) is 1 ml of the trichloroacetic acid filtrate. Five volumes of 95% ethanol and carefully blow it. Capped with rubber stoppers and at 37 to 400C, tubes were kept in an organ bath for three hours. After completion of precipitation tubes, In2 ml of distilled water glycogen was dissolved, and the sides of tubes were washed by pouring water on the edges of tubes. Tubes were agitated to dissolve the glycogen instantly.

Calculation

Glycogen content was committed while using the following equation

(DU/DS) X 0.1 X (Vol of extract/g.of tissue) X 100 X 0.9 = mg of glycogen per 100 g of tissue

Serum glutamic oxaloacetic transaminase (SGOT) estimation

An Accucare Diagnostics kit was used to estimate the SGOT.

Methodology

This was done by kinetic U.V. test

Preparation of reagent

1 ml of enzyme reagent and 4 ml of buffer reagent combined. Incubated for one minute at 370C after well mixed and abs gradually decreased per minute during three minutes, and ΔA/minutes was calculated.

Serum glutamic pyruvate transaminase (SGPT) estimation

SGPT was determined using an accurate diagnostic kit.

Methodology

Kinetic U.V. test

Preparation of reagent

1 ml of enzyme reagent and 4 ml of buffer reagent combined. Incubated for one minute at 370C after well mixed, abs gradually decreased per minute for three minutes, and ΔA/minutes were calculated.

Serum alkaline phosphatase (ALP) estimation

ALP was estimated by using an accurate diagnostic kit

Methodology

Kinetic U.V. test

Serum total protein (T.P.) estimation

T.P. was estimated using an accurate diagnostic kit

Methodology

Colorimetric test: Biuret

Serum urea estimation

Serum urea was estimated using an accurate diagnostic kit[13].

Methodology

Kinetic U.V. test

Serum Creatinine estimation

Serum creatinine was estimated using an accurate diagnostic kit [14].

Methodology

Kinetic U.V. test

Serum insulin estimation

An Abbott AxSYM system kit was used to estimate serum insulin[15].

Methodology

Insulin assays have been based on microsphere enzymatic immunoassay (MEIA) technology.

Principle

The AxSYM insulin reagent and sample were diluted while continuing to follow manners. All the samples must have diluted out the test, and all insulin reactants needed with one test are drawn to investigate different wells of reaction vessels (R.V.) in the sampling center. Into the processing center, R.V. was quickly transmitted. The processing probe pipetted further into the processing center.

Sequences of reaction were to occur in the following manners.

Anti-insulin, sample-encased microparticles, and assay buffer have been diluted with one well about reaction vessel. Upon incubation, insulin binds with anti-insulin microparticles and forms an antigen-antibody complex.

The Aliquot of the reaction mixture was transferred to the matrix cell, and microparticles were bound irreversibly to the matrix of glass fiber. Matrix cells have been washed with water to remove unrestricted unshackled substances. Anti-insulin ALP coupling is distributed to the matrix cell, which is obligated to the antigen-antibody complex. Four methyl umbelliferyl phosphates have been added to a matrix cell, and indeed, the percentage of fluorescent-formed products has been assessed even by the MEIA optical assembly.

Pack of Reagent

Reagent pack of AxSYM insulin

- One 6.8 ml bottle (Reagent bottle 1) containing protein stabilizers and anti-insulin (mouse monoclonal) coated microparticles in a buffer.
- One 11.8 ml anti-insulin bottle containing a buffer containing protein stabilizers and an alkaline phosphatase conjugate (Reagent bottle 2)
- Reagent bottle 3 (one bottle, 14.4 ml assay buffer in calf serum)

Table 3 Insulin master calibrators

Bottle	Insulin Concentration (µU/mL)
Master Cal 1	0.0
Master Cal 2	100.0

AxSYM insulin standard calibrators

Insulin can be prepared in a buffer at the continuing-to-follow concentrations and is contained in six bottles of AxSYM insulin standard calibrators (4 ml each).

Table 4 AxSYM insulin standard calibrators

Bottle	Insulin Concentration(µU/mL)
Standard Cal A	0.0
Standard Cal B	3.0
Standard Cal C	10.0
Standard Cal D	30.0
Standard Cal E	100.0
Standard Cal F	300.0

Controls

AxSYM insulin controls

Three bottles, eight ml, and every one of the AxSYM insulin controls contained insulin that could be prepared in a buffer to yield to follow the concentration range.

Other reagents

Solution 1 (MUP) contains 4-methylumbelliferyl phosphate, 1.2 Mm in buffer

Solution 2 (Matrix cell wash) contains 0.3 M sodium chloride in TRIS buffer.

Glycated hemoglobin estimation (HbA1c)

An Agappe diagnostics kit was used to estimate HbA1C.

Thoroughly. Left it for 10-15 minutes. In steps 5 and 10, this hemolysate was used

Preparation of column

3. The Upper cap of the column was removed, and then the bottom tip was snapped.

4. The upper disc was pushed down using the flat end of a pipette to touch the resin

surface.

HbA1C separation

5. 50 µL hemolysate was carefully piped on the upper filter.

6. Then 200 µL reagent two was added carefully on the upper filter.

7. 2 ml reagent two was carefully pipetted on the upper filter, and the column was drained to

waste

8. The column was placed over a test tube, 4 ml reagent three was added, and the elute

was collected.

9. Test tubes thoroughly shaken

Calculation

$$\%\ HbA1C = \frac{A\ HbA1C X\ V\ HbA1C}{AHb\ total\ X\ V\ Hbtotal}\ X\ 100$$

V HbA1C – Volume of HbA1C V Hb total – Volume of Hb total A HbA1C – Absorbance of HbA1C (from step 9) A Hb total – Absorbance of Hb total (from step 11)

Table 5 AxSYM insulin controls

Bottle	Insulin concentration (µU/mL)	Range (µU/mL)
Control L	8.0	6.0 – 10.0
Control M	40.0	32.0 – 48.0
Control H	120.0	96.0 – 144.0

RESULTS:

Euphorbia neriifolia Ethanolic extract (ENEE) was determined above 4000mg/kg, and one-tenth of LD50 dose (400 mg/kg) was selected as a higher dose to treat anti-diabetic and antihyperlipidemic activity. One-twentieth of the LD50 dose (200 mg/kg) was chosen as a lower dose to evaluate anti-diabetic and antihyperlipidemic activity in animal models.

Effect of Euphorbia neriifolia Ethanolic extract (ENEE) on relative organ weight of animals.

Table 6 Effect of Euphorbia neriifolia Ethanolic extract (ENEE) on relative organ weight of animals

Relative organ weight (Gram)	Normal Control	Treated Groups	
		ENEE 200 mg/kg	ENEE 400 mg/kg
Heart	1.68 ± 0.05	1.69 ± 0.25	1.72 ± 0.06
Liver	7.65 ± 0.02	7.45 ± 0.98	6.98 ± 0.04
Brain	1.65 ± 0.01	1.65 ± 0.47	1.67 ± 0.05

Every valuation shown there Mean ± SD, where n(number of animals in each group) = 10,

ENEE: *Euphorbia neriifolia*ethnolic extract. Data analysis was done using a one-way analysis of variance (ANOVA) followed by Dunnet's test. Treated groups were compared with the standard control group.

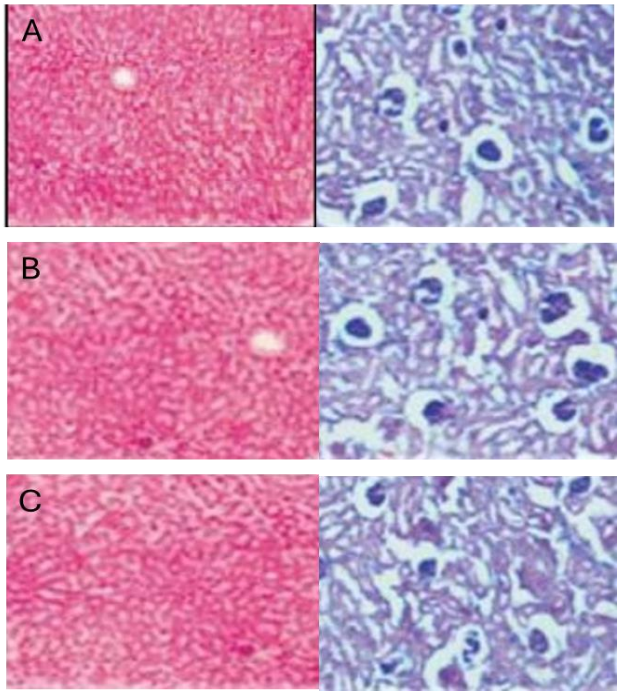

Figure 1 Histological slides of liver& Kidney
A) Normal Control; B) Treated Group ENEE 200 mg/kg; C) Treated Group ENEE 400 mg/kg.

Table 7 Effect of Euphobia nerrifolia Ethanolic extract (ENEE) on hematological parameters of rats in sub-toxicity study.

Parameters	Normal Control	Treated Groups	
		ENEE 200 mg/kg	ENEE 400 mg/kg
WBC (X 10^3/µL)	6.82 ± 1.20	7.23 ± 0.87	6.62 ± 0.78
RBC (X 10^6/µL)	8.25 ± 0.24	8.34 ± 1.67	9.02 ± 1.21
Hb (g/dl)	14.15 ± 0.23	15.23 ± 0.69	15.72 ± 1.02
HCt (%)	50.12 ± 2.12	49.76 ± 2.56	52.21 ± 2.34
MCV (fL)	51.21 ± 3.45	50.23 ± 3.02	52.21 ± 4.21
MCHC (g/dl)	32.26 ± 4.21	31.28 ± 2.10	34.21 ± 3.98
Platelets (X 10^3/µL)	820.21 ± 56.45	798.87 ± 67.42	762.29 ± 67.72
Lymphocytes (%)	68.24 ± 5.43	66.56 ± 61.24	66.43 ± 4.21
Monocytes (%)	1.98 ± 0.21	0.97 ± 0.21	1.23 ± 0.09
Neutrophils (%)	23.25 ± 2.24	25.21 ± 1.43	23.32 ± 1.98
Eosinophils (%)	1.23 ± 0.34	0.96 ± 0.67	1.02 ± 0.42

Every valuation is shown in Mean ± S.D., where n (number of animals in each group) = 10,

ENEE: *Euphorbia neriifolia*ethnolic extract. Dunnet's test was conducted after a one-way analysis of variance (ANOVA) was used to analyze the results. The treatment groups and the typical control group were contrasted.

Effects of Euphorbia nerrifolia ethanolic extract on body weight, fasting blood glucose level, lipid profile, plasma insulin, hepatic glycogen, glycated hemoglobin, and pancreas in rats treated with streptozotocin (STZ) nicotinamide-induced type-II diabetes in rats.

Table 8 Effect of Euphorbia nerrifolia ethanolic extract (ENEE) in sub-acute toxicity study on biomedical parameters

Parameters	Normal Control	Treated Groups	
		ENEE 200 mg/kg	ENEE 400 mg/kg
Glucose (mg/dl)	74.23 ± 7.21	75.13 ± 3.34	78.12 ± 4.21
Direct Bilirubin (mg/dl)	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
SGOT (U/L)	128.4 ± 8.21	126.5 ± 3.45	113.28 ± 10.21
SGPT (U/L)	42.02 ± 4.56	40.34 ± 2.98	34.21 ± 5.43
ALP (U/L)	82.24 ± 4.32	76.82 ± 3.76	72.28 ± 6.52
Urea (mg/dl)	5.42 ± 2.23	4.98 ± 0.78	5.21 ± 2.41

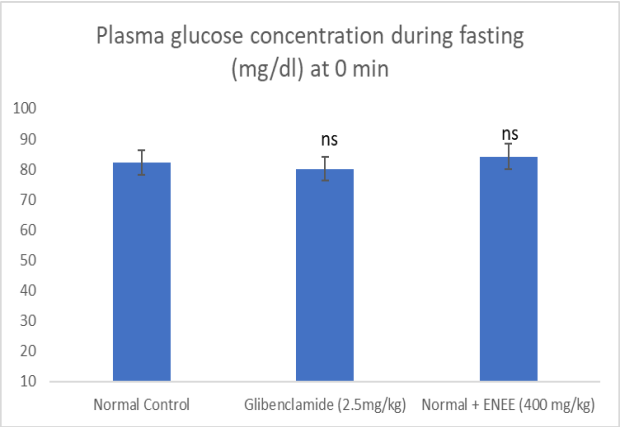

Figure 2 Effect of Euphorbia nerrifolia ethanolic extract ENEE on fasting blood Glucose (FBG) during OGTT) in normal rats at 0 minutes

Table 9 Euphorbia nerrifolia ethanolic extract ENEE affects fasting blood glucose (FBG) levels during oral glucose tolerance in normal rats

Group (n=6)	Treatment	Level of Plasma Glucose During Fasting (mg/dl)			
		0 min	30 min	60 min	120 min
I	Normal Control	82.21 ±1.21	131.21 ±2.12	128.47 ± 1.23	92.36 ± 0.98
II	Glibenclamide (2.5mg/kg)	80.13 ±1.45	92.42 ± 1.98	86.71 ± 1.76	79.32 ± 1.23
III	Normal + ENEE (400 mg/kg)	84.22 ±0.98	100.21 ± 0.72	93.24 ±1.23	83.15 ± 2.01

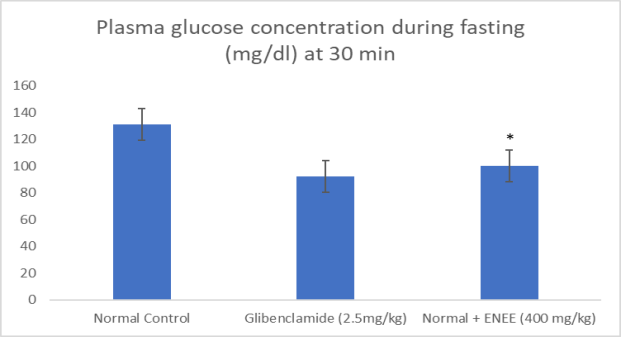

Figure 3 Effect of Euphorbia nerrifolia ethanolic extract ENEE on fasting blood Glucose (FBG) during OGTT) in normal rats at 30 minutes.

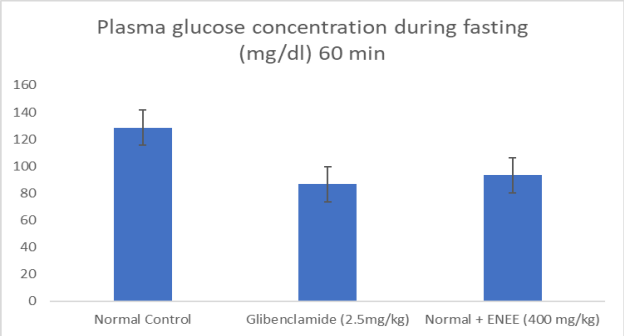

Figure 4 Impact of the ethanolic extract ENEE from Euphorbia nerrifolia on the glucose levels in normal rats during OGTT during a 60-minute fast

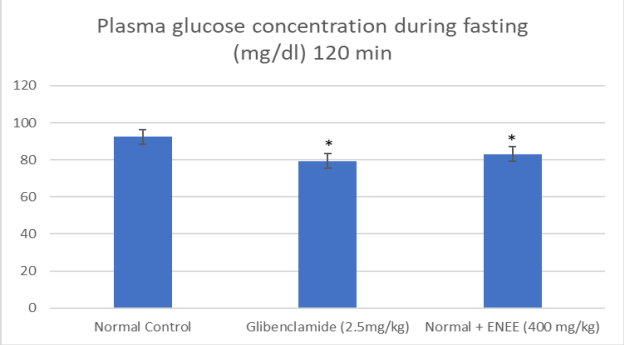

Figure 5 Impact of the ethanolic extract ENEE from Euphorbia nerrifolia on the glucose levels in normal rats during OGTT during a 120-minute fast

The value displayed in mean ± S.D n (number of animals in each group)=6. Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's test. Group II and III (Glibenclamide (2.5mg/kg) & Normal + ENEE (400 mg/kg) respectively compared with Group I Normal Control.

*p<0.05, vs Normal Control.

Effects of ENEE on body weights

Table 10 Effects of ENEE ON body weights

Groups (n=6)	Treatment	Body Weight (g)	
		Before Treatment	After Treatment
I	Normal Control	195.23 ± 5.21	200.21 ± 2.13
II	Diabetic Control	186.92 ± 4.65	164.24 ± 0.98
III	Diabetic with Glibenclamide (2.5mg/kg)	175.25 ± 3.12	182.87 ± 1.23
IV	Diabetic with + ENEE (400 mg/kg)	182.62 ± 2.13	186.12 ± 1.35

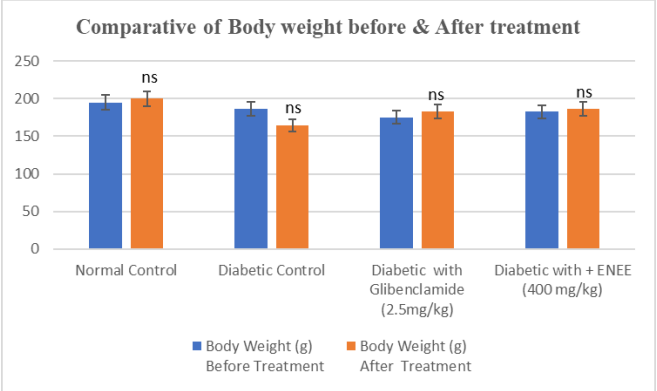

Figure 6 Effect of Euphorbia nerrifolia ethanolic extract ENEE on body weight before and after treatment in streptozotocin - Nicotinamide induced type-II diabetic rats

Effects of ENEE ON FBS

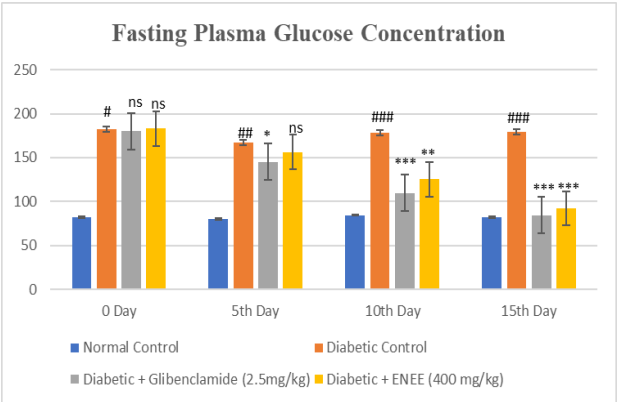

Figure 7 Effect of Euphorbia neffifolia ethanolic extract ENEE on fasting blood glucose FBG in streptozotocin-nicotinamide induced type II diabetic rats on 0, 5, 10 & 15 days respectively

Table 11 Effect of Euphorbia nerrifolia ethanolic extract ENEE on Fasting Blood Glucose (FBG) in Streptozotocin-nicotinamide induced type-II diabetic rats

Group (n=6)	Treatment	Plasma glucose concentration during fasting (mg/dl)			
		0 day	5th Day	10th Day	15th Day
I	Normal Control	82.21 ±1.21	80.23. ±1.12	84.43 ± 0.83	82.36 ± 0.88
II	Diabetic Control	182.12 ±0.96	167.24 ±1.24	178.23 ±2.01	179.53 ±0.81
III	Diabetic + Glibenclamide (2.5mg/kg)	180.13 ±0.45	145.12 ± 0.93	109.71 ± 1.26	84.32 ± 0.93
IV	Diabetic + ENEE (400 mg/kg)	183.22 ±1.98	156.21 ± 1.82	125.24 ±2.23	92.15 ± 2.01

Table 12 Impact of ethanolic extract (ENEE) from Euphorbia neffifolia on the lipid profile in type II diabetic rats induced with streptozotocin and Nicotinamide.

Group (n=6)	Treatment	Serum Lipid Profile (mg/dl)				
		TG	TC	HDL	LDL	VLDL
I	Normal Control	88.11 ±2.21	56.13. ±2.12	22.34 ± 2.83	15.34 ± 0.88	17.42 ± 1.28
II	Diabetic Control	142.42 ±4.96	89.24 ±1.24	13.22 ±1.01	68.14 ±5.81	30.21 ± 2.98
III	Diabetic + Glibenclamide (2.5mg/kg)	93.73 ±2.47	57.16 ± 3.93	16.72 ± 0.46	17.42 ± 1.93	19.02± 0.98
IV	Diabetic + ENEE (400 mg/kg)	99.21 ±2.98	61.25 ± 2.82	18.54 ±3.23	21.25 ± 1.42	22.46 ± 2.86

Several animals in each group (n) = 6 is the mean ± standard deviation of each value displayed. ENEE Euphorbia nerrifolia ethanolic extract. Data had been examined by using a one-way analysis of variance (ANOVA) able to follow Dunnet's test.

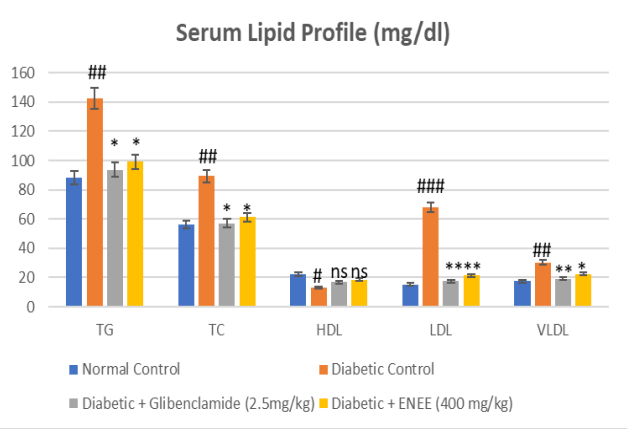

Figure 8 : Effect of Euphorbia neriifolia ethanolic extract (ENEE) on the serum lipid profile of rats with type II diabetes caused by streptozotocin and nicotine

Data from n = 6 were analyzed using one-way ANOVA and then Dunnet's test; values are displayed in Mean ±S.D. Group II was diabetic control was compared with Group I, and Group III & IV were Diabetic + Glibenclamide 2.5 mg/kg and Diabetic+ ENEE 400 mg/kg, respectively, were compared with Group II.

**p<0.01 vs diabetic control, ##p<0.01 vs Normal control

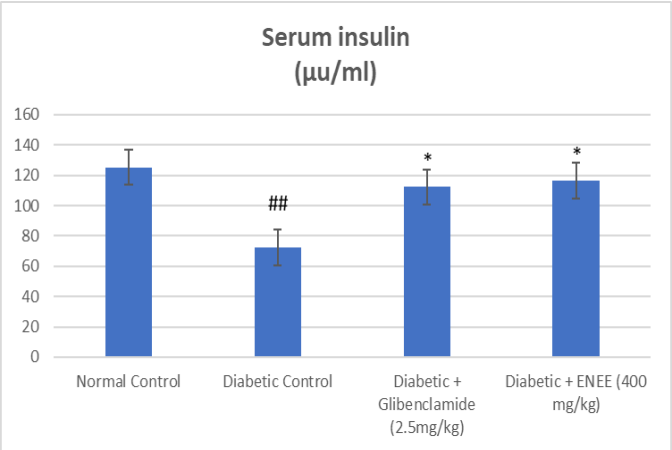

Figure 9 Effect of Euphorbia neriifolia ethanolic extract (ENEE) on Glycated

Table 13 Effect on Serum insulin, Glycated hemoglobin (%) & Liver glycogen (mg/g)

Group (n=6)	Treatment	Serum insulin (µu/ml)	Glycated hemoglobin (%)	Liver glycogen (mg/g)
I	Normal Control	125.24 ± 3.23	1.98 ± 0.97	14.29 ± 1.58
II	Diabetic Control	72.32 ± 4.12	8.42 ± 2.12	4.32 ± 3.53
III	Diabetic + Glibenclamide (2.5mg/kg)	112.23 ± 3.45	2.87 ± 1.25	12.28 ± 2.46
IV	Diabetic + ENEE (400 mg/kg)	116.38 ± 4.72	4.28 ± 2.10	9.97 ± 2.48

Every value displayed in mean ± S.D n (number of animals in each group)=6 ENEE Euphorbia nerrifolia ethanolic extract. Data was analyzed using one-way variance analysis (ANOVA) followed by Dunnet's test.

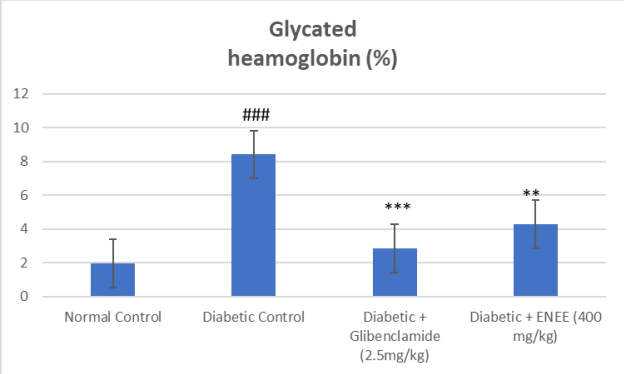

Figure 10 Effect of Euphorbia neriifolia ethanolic extract (ENEE) on Glycated

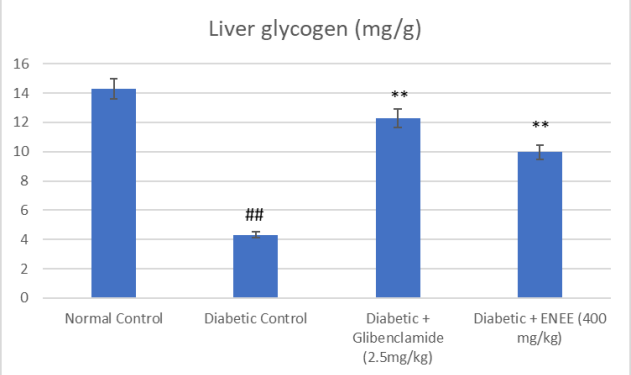

Figure 11 Effect of Euphorbia neriifolia ethanolic extract (ENEE) on liver glycogen in Streptozotocin-nicotinamide induced type II diabetic rats

Values shown in Mean ±S.D where, n=6, data had been analyzed using one-way ANOVA followed by Dunnet's test. Group II was diabetic control has been compared with Group I, and Group III & IV was Diabetic + Glibenclamide 2.5 mg/kg and Diabetic+ ENEE 400 mg/kg, respectively, were compared with Group II

**p<0.01 vs diabetic control, ##p<0.01 vs Normal control

Histopathology

Effect of Euphorbia neriifolia Ethanolic extract (ENEE) on islets of the pancreas

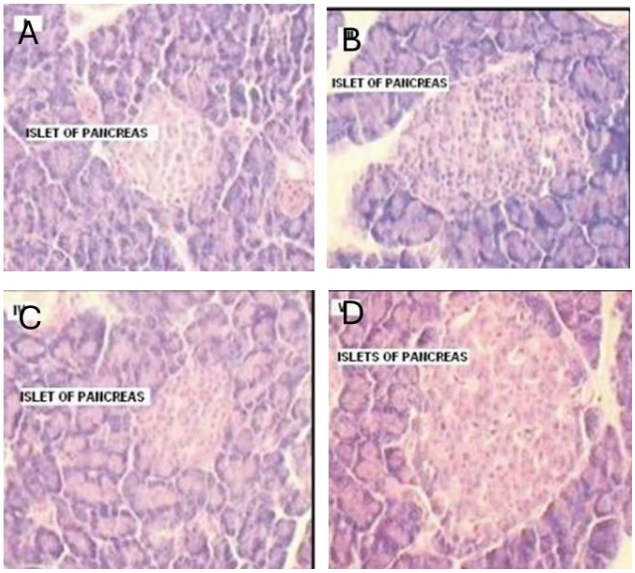

Figure 12 Effect of Euphorbia neriifolia Ethanolic extract (ENEE) on islets of the pancreas A) Histological slide of regular control group showing the typical architecture of islets of the pancreas; B) Histological slide of diabetic control group showing degeneration of islets of the pancreas as compared to standard control group; C) Histological slide of Diabetic+Glibenclamide 2.5 mg/kg group showing recovered islets of pancreas close to the standard control group; E) Histological slide of

Diabetic+ENEE 400 mg/kg group showing recovered islets of the pancreas;

DISCUSSION:

The primary objective of this project work was to evaluate the anti-diabetic and antihyperlipidemic activity of *Euphorbia neriifolia Linn*.

Type 2 diabetes mellitus seems to be a metabolic disorder primarily characterized by relative insulin deficiency, developing insulin resistance, and increased blood glucose levels (hyperglycemia), which is also associated with hyperlipidemia, obesity, and oxidative damage Predominantly 90% of people are suffering from Diabetes decreases the life span due to micro and macro vascular complications which are also associated with morbidity and mortality. Retinopathy, nephropathy, arteriosclerosis, and hypertension are some of these complications.

So many allopathic anti-diabetic contraindications and serious have marred their choice. Traditional medicinal plants have gained popularity.

In many rural areas of India, Euphorbia nerifolia is used in traditional medicine to cure Diabetes. Conventional medicinal applications for *Euphorbia nerifolia* include wound healing, anticancer, antifungal, antibacterial, antiparasitic, antiviral, anti-diabetic, antiarthritic, antioxidant, and radioprotective properties.

No reports have been found that describe the safety and toxicity profile of Euphorbia neriifolia ethanolic extract (ENEE); hence, it is extracted from the leaves of Euphorbia neriifolia Linn to expand the benefits of the plant. During the acute toxicity study, a single dose was found to be a higher dose (400 mg/kg), and one-twentieth of the lethal dose was selected as a lower dose (200 mg/kg). At selected doses, ENEE did not produce any significant changes in relative organ body weight, hematological parameters, and biochemical parameters, which regulate the functions of the hemopoietic system and vital organs of the body, like the liver, when treated groups compared with standard control groups.

Blood parameters analysis is related to predicting the study of animals. An increase in levels of liver enzymes in blood is associated with functional and structural dysfunction of hepatocyte membrane damage. Examination of the heart, liver, and kidney histopathology showed a standard architecture of cells without any fatty infiltration or necrosis in the treated groups compared with the standard control group, which gives certainty. *Euphorbia neriifolia ethanolic* extract ENEE.

When ENEE (400 mg/kg) hours. After 60 mins, a reduction in blood glucose levels groups and a decline were observed after 120 mins. Changes in blood glucose were observed in different groups' initial and final FBG, showing a significant increase in the study. The hypoglycemic effect of ENEE might Other behind it might be from diet to blood. This action is specifically beneficial to patients with type 2 diabetes. Commonly, LPL (lipoprotein lipase enzyme) causes hydrolysis of triglycerides. In diabetes mellitus, failure to activate LPL occurs, which causes hypertriglyceridemia. The content of dietary fibers lowers T.G. (triglyceride) and cholesterol. Deficiency of insulin leads to various derangements in metabolic and regulatory processes. Increased glucose in the blood is responsible for the higher FFAs. Higher free fatty acid levels cause an increase in coagulation, insulin resistance, endothelial dysfunction, and lipid deposition in various organs, and they also affect the movement of cholesterol content. Diabetic rats showed significant elevation in T.G. (Triglycerides), T.C. group. To diabetic rats shown in T.G., This effect might be due to stimulation of lipoprotein lipase enzyme and antioxidant effect of ENEE. Normalization of lipid levels has a beneficial impact on beta cell function. Hence, a histopathological examination of the pancreas was done herein. Differences in body weights were observed in treated groups with ENEE, which was lower than in diabetic control groups. (gluconeogenesis due to proper glycemic control).

Previous studies showed a decrease in glycogen content, and the Diabetes lead level was observed in treated groups, might be. The anti-diabetic action of ENEE might be due to improvement in the glycogenesis process. The present study showed increased glycosylated hemoglobin (HbA1c) group content. An increase in auto-oxidative and nonenzymatic is one of the possible mechanisms linked with vascular complications and hyperglycemia. Treatment with ENEE (400 mg/kg) and Glibenclamide 2.5 mg/kg STZ produces the destruction of beta cells of the pancreas. Histopathological micrographs of the

pancreas in the diabetic control group showed severe destruction of the beta cells of the pancreas compared to the standard control group. Treatment with the ENEE showed the start of recovery and recovery of beta cells in the pancreas compared to the diabetic control group.

reduction in blood glucose levels was observed after 60min in Glibenclamide 2.5 mg/kg and after 120 min in ENEE 400 mg/kg.. After 60 mins, a reduction in blood glucose levels was observed in treated groups compared with standard control groups, and a decline was observed after 120 minutes. Adipocity enhancement is linked. Leptin plays. Body weights were recorded before and after the treatment in rats. Before treatment, there was a significant increase. After treatment, the significant above enzyme (in vivo) causes a decrease in blood glucose levels. Fasting blood glucose (FBG) levels were determined on days 0, 5,10, and 15 in standard control, diabetic control, and treated groups. On day 0 of treatment, a significant elevation of FBG was observed in diabetic (HFD-STZ) rats compared to the standard control group. Administration of ENEE (200 mg/kg and 400 mg/kg) and Glibenclamide 2.5 mg/kg showed a significant decrease in FBG gradually on days 5, 10, and 15 compared to a diabetic control group. Consumption of high-fat or high. This may indicate the over-activation of glycogen synthase in HFD-STZ diabetic rats.

Degeneration and deterioration of beta cells are associated with impaired actions of the incretin hormones, which could be aiding the mass of beta cells and beta cell's function. Glucagon Peptide (GLP)-1 is high in concentration of beta cells of the pancreas and has many functions like an increase in beta cell proliferation, neogenesis, and beta cell mass. Diabetic patients exert a shift towards an increased rate of beta cell apoptosis compared to the new proliferation of beta cells. To understand the pathological damage to the pancreas, histological photomicrographs of the pancreas were taken in normal, diabetic, and treated rats. Histological slides of the pancreas in the diabetic (HFD-STZ) control group showed significant damage to islets of the pancreas compared to a standard control group. Histological slides of the pancreas in treated groups like ENEE showed recovery in pancreatic beta cells and established standard architecture of islet of the pancreas compared to diabetic (HFD-STZ) control rats. This action might be due to increased activity of GLP-1 and GLP-1 receptor sensitivity, which causes regeneration, proliferation, and a decrease in the apoptosis rate of beta cells of the pancreas. In the present study, Glibenclamide compares the potency of ENEE in treating Diabetes. Overall data showed that ENEE 400 mg/kg had a dose-dependent effect on FBG, lipid profile, insulin, liver glycogen, and Glycated hemoglobin.

CONCLUSION

The present research, it is concluded that *Euphorbia neriifolia* ethanolic extract possesses significant dose-dependent anti-diabetic and antihyperlipidemic activity in streptozotocin nicotinamide and with a large margin of safety when there are no outward indications of toxicity. Laboratory results from this study support using Euphorbia neriifolia Linn. to treat Diabetes, as the extracts demonstrated anti-diabetic and antihyperlipidemic activity. However, these results also indicate the necessity of identifying and assessing the active ingredients responsible for biological activity. Besides the exact mechanism of action, very chronic models need to be developed as a potent requirement to elucidate diabetic drugs for a longer duration of studies on *Euphorbia neriifolia Linn* and antihyperlipidemic activity.

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Conflict of Interest: The Author declares that there is no conflict of interest.

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