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ANTIDIABETIC ACTIVITY OF METHANOLIC AERIAL EXTRACT OF *EUPHORBIA MILII* DES MOUL IN STREPTOZOTOCIN–NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS

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Keywords:

Euphorbia milii Des Moul; Euphorbiaceae, Streptozotocin–nicotinamide induced diabetes, Antidiabetic effect

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ABSTRACT: It is an ornamental shrub native to Madagascar and the Philippines, widely distributed in India. *Euphorbia milii* Des Moul is widely used in folk medicine to treat warts (South Brazil), cancer and hepatitis in (China). The present study seeks to evaluate the methanolic aerial extract of *Euphorbia milii* Des Moul (MAEEM) for its anti-diabetic effect against streptozotocin–nicotinamide induced type 2 diabetic model in Albino Wistar rats. Oral glucose tolerance test, Phytochemical screening and Acute toxicity study were carried out. Graded doses of MAEEM (100mg/kg, 200mg/kg and 400mg/kg) were administered to diabetic rats for 21 days. The activity was evaluated by using some biochemical parameters such as blood glucose levels, serum lipid profiles, liver profile markers (AST, ALP, ALT), and renal profile markers (serum creatinine, blood urea). Type-2 diabetes significantly altered these parameters, while oral administration of the MAEEM significantly ameliorated them.

INTRODUCTION: *Euphorbia milii* Des Moul; Euphorbiaceae, commonly known as Crown of thorns, is an ornamental shrub native to Madagascar and Philippines, widely distributed in India however, in Nepal the latex is used for treating strains, while in China it is used for the treatment of hepatitis and abdominal edema [1, 2]. It is a succulent climbing shrub growing to 1.8 m (5 ft 11 in) tall, with densely spiny stems. The leaves are found mainly on new growth and are obovate, up to 3.5 cm long and 1.5 cm broad. The flowers are small, subtended by a pair of conspicuous petal-like bracts, variably red, pink or white, up to 12 mm broad. The sap is moderately poisonous [3].

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It has been reported that Euphorbia possesses antiarthritis, anticancer, anticonvulsant, anti-eczema, anti-eczema, anti-inflammatory, antimicrobial, antioxidant, antispasmodic, antitumor, antitussive properties hormonal and myelopoiesis [4]. The latex of this plant used to control the mollusks by its embryofetal-toxicity is frequently used in traditional medicine against liver fluke, schistosomiasis in sheep, cattle and even humans (Sauza *et al.*, 1997; Schall *et al.*, 2001).

Also, the latex contains many medicinally valuable alkaloids such as β-sitosterol, euphol, euphorbol, euphorbol hexacosanoate and a potent antileukaemic macrolide-lasiodiplodin. However, the latex's proteins and other biochemical constituents have not been investigated in detail [5]. Though there is no scientific evidence to support the antidiabetic effect of *Euphorbia milii* Des Moul tribal men continue to use the plant in the management of diabetes.

The objective of this investigation was to ascertain the scientific basis for using this plant to manage diabetes, using Streptozotocin–Nicotinamide-induced Type 2 Diabetic rats.

MATERIALS AND METHOD:

Plant Material: The aerial part of the plant *Euphorbia milii* Des Moul was collected in January 2021 from Forest Research Institute, Dehradun, Uttarakhand, India. The plant was identified by Scientist S.K Singh, Botanical Survey of India, Northern regional center, Kaulagarh Road, Dehradun, and a voucher specimen (PP 683) has been deposited in the herbarium of the Botanical Survey of India.

Preparation of MAEEM: Shaded dried aerial part of *Euphorbia milii* Des Moul was crushed to produce coarse powder of approximately 60-mesh size. Approximately 150g of dried and coarsely powdered aerial part of *Euphorbia milii* Des Moul was subjected to selected solvent extraction by continuous hot extraction (Soxhlet) with 750ml of methanol. The extract was concentrated by distilling the solvent in a rotatory flask evaporator to obtain the extracts as solid residues (yield 15 g) and was stored in desiccator and used for subsequent experiments.

Animals: Wistar rats of either sex weighing 150-200g were employed for assessing the anti-diabetic activity. The animals were maintained in the Institute animal house under standard laboratory conditions of light and temperature (25±2°C with 12-h-light/12-h-dark cycle; 35–60% humidity). Food pellets and water ad *libitum* were provided. The Institutional Animal Ethical Committee meeting held at Shri Guru Ram Rai institute of technology and sciences, Dehradun, India (264/CPCSEA/IAEC/2021/08), approved the study.

Phytochemical Screening: The MAEEM was subjected to qualitative analysis of various phytochemical studies, which revealed the presence of Flavonoids, Terpenoids, Tannins, and Glycosides as described in the literature [6].

Acute Toxicity Studies: The acute oral toxicity study was carried out per OECD 423 guidelines (OECD, 2001). The study was approved by the Institutional Animal Ethics Committee (IAEC). No mortality and no signs of toxicity were found even after administration of a limited dose of 100 mg/kg body weight of extract; hence 1/10th of the dose was taken as an effective dose. Three doses 100, 200 and 400mg/kg were selected for the present study to evaluate anti-diabetic activity [7].

Induction of Non-insulin-dependent Diabetes Mellitus (NIDDM): Diabetes was experimentally induced through intra-peritoneal injection in twelve hours fasted experimental animals with Streptozotocin (STZ) (55 mg/kg body weight) dissolved in 100 Mm citrate buffer (pH 4.5); followed by Nicotinamide (120 mg/kg) after15 min.

After 6 hr of STZ administration, a 10% glucose solution was provided to all the rats to prevent hypoglycemic shock. On completion of 72 h testing and monitoring, rats above 200 mg/dL of blood glucose concentration were noticed to be diabetic, and this conceptualization could be used for further study [8].

Experimental design: Animals were divided into six groups of six rats each. The extract was administered for 21 days. Group I: Normal control rats administered drinking water daily for 21 days; Group II: Diabetic control rats administered drinking water daily for 21 days; Group III: Diabetic rats administered MAEEM (100 mg/kg); Group IV: Diabetic rats administered MAEEM (200 mg/kg); Group V: Diabetic rats administered MAEEM (400 mg/kg); Group VI: Diabetic rats administered standard drug Glibenclamide (0.25 mg/kg) for 21 days.

The effects of administration of MAEEM to normal and diabetic rats were determined by measuring blood glucose levels, serum lipid profiles, liver profile markers, and renal profile (Nicholas, 1956). Blood glucose level was estimated on days 0, 3,7,14 and 21 of extract administration. All other biochemical parameters were determined on day 21. Serum lipid profiles were measured by an autoanalyzer (Hitachi 912).

Statistical Analysis: Data were statistically evaluated using two-way ANOVA, followed by Bonferroni test using GraphPad prism 9 computer software. The significance levels were taken as $p <$ 0 05, $p <$ 0 01 and $p <$ 0 001. The values are expressed as mean ±SEM.

RESULTS:

Acute Toxicity Study of MAEEM: The acute oral toxicity of MAEEM showed no toxic signs even after 24 h and 72 h of extract administration.

Further, no oral toxicity or mortality was detected even after oral administration of higher doses (up to 2000 mg/kg) of MAEEM for 21 days (one dose per day). This indicates the safety of the extract for prolonged use.

Phytochemical Screening of MAEEM: The phytochemical screening detected the presence of alkaloids, glycosides, carbohydrates, tannins, terpenoids, flavonoids and phenols in the MAEEM **Table 1.**

TABLE 1: PHYTOCHEMICAL SCREENING OF THE METHANOLIC EXTRACT OF *EUPHORBIA MILII* DES MOUL AERIAL PART

Chemical Test	Observation	Inference
Alkaloids		
Dragendorff test	Reddish precipitate was observed	Alkaloids present
Hager test	Yellow precipitate was observed	Alkaloids present
Mayer test	A milky coloration was observed	Alkaloids present
Glycosides		
Baljet test	Formation of yellow colour	Glycoside present
Carbohydrates		
Molisch test	Formation of red colour at the interphase of two layers	Carbohydrates present
Flavonoids		
Shinoda test	Crimson red color was observed	Flavonoids present
Lead acetate test	Yellow precipitate was observed	Flavonoids present
Tannins		
Bromine water test	The bromine water was decolorized	Tannins present
Lead acetate test	Cream precipitate was observed	Tannins present
Triterpenoids		
Salkowski's test	Reddish brown color at the interface	Triterpenoids present
Saponins		
Froth test	No significant frothing was obtained	Saponins absent
Phenols		
Ferric chloride test	Bluish black color observed	Phenols present
Libermann test	Deep blue color observed	Phenols present

Effect of MAEEM on Blood Glucose Levels: The blood glucose level increased significantly in STZ and nicotinamide-treated group when compared to the control group. The STZ and nicotinamide-induced rats were treated with the MAEEM 100mg/kg/p.o, 200mg/kg/p.o and 400mg/kg/p.o for the duration of 21 days. Treatment with MAEEMat the dose of 100mg/kg and 200 mg/kg/p.o shows a marginal reduction in the blood glucose level at the second week. Treatment with MAEEM at the dose of 400mg/kg/p.o. showed a significant decrease in the blood glucose level at the first week ($p<0.05$), which further reduced in the second, third weeks ($p<0.01$), respectively. Treatment with glibenclamide (0.25mg/kg b.w/ p.o) produced a significant ($p<0.001$) decrease in blood glucose level from the first week to the third week **Table 2.**

TABLE 2: EFFECT OF MAEEM ON BLOOD GLUCOSE LEVELS

Groups Treatment/ Dose	0 day (mg/dL)	After 3 days (mg/dL)	After 7 days (mg/dL)	After 14 days (mg/dL)	After 21days (mg/dL)
Normal control	94.35± 3.13	97.23± 2.94	95.56± 2.45	96.65± 3.12	98.01± 3.93
Diabetic control	268.18± 2.64	272.06± 3.97^{a***}	283.12± 2.47^{a***}	296.37± 2.33^{a***}	304.14± 4.87^{a***}
MAEEM (100mg/kg	272.17±3.21	269.16±2.14 ^{b*}	254.87±2.56 ^{b*}	241.63±2.69 ^{b*}	223.49±2.16 ^{b*}
MAEEM (200mg/kg b.w.)	276.16± 2.67	253.27± 2.23^{b*}	239.13± 3.68^{b*}	179.43± 3.27^{b**}	121.42± 2.09^{b**}
MAEEM (400mg/kg b.w.)	254.26± 2.97	222.24± 3.41^{b**}	169.75± 2.88^{b**}	131.23± 2.74^{b**}	109.33± 3.73^{b***}
Standard Glibenclamide (0.25mg/kg b.w.)	261.93± 3.23	219.22± 2.94^{b***}	162.23± 3.82^{b***}	125.74± 2.65^{b***}	95.16± 3.67^{b***}

Values are expressed as mean ± SEM (n=6), *P<0.05, **P<0.01 & ***P<0.001 Vs control. [a] represents statistically significant versus normal control group, [b] represents statistically significant versus diabetic control group.

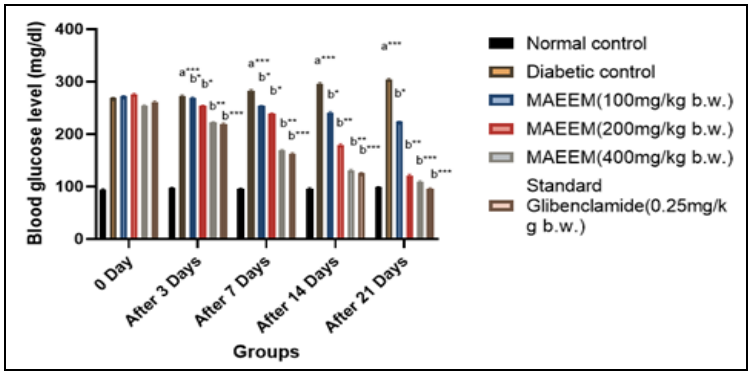

FIG. 1: EFFECT OF MAEEM ON BLOOD GLUCOSE LEVELS

Effects of MAEEM on Lipid Profiles: The serum total cholesterol, triglyceride, LDL, VLDL level was significantly increased, whereas HDL was significantly decreased in STZ-nicotinamide-induced diabetic rat when compared to control rats. Serum total cholesterol, triglyceride, LDL, VLDL level of diabetic animals treated with MAEEM at a dose of 100mg/kg/p.o, 200mg/kg/p.o and 400mg/kg/p.o showed a significant decrease ($p<0.05$, $p<0.05$, $p<0.01$) respectively and HDL level of diabetic animals treated with MAEEM showed a significant increase ($p<0.05$, $p<0.05$ and $p<0.01$) respectively when compared to STZ-nicotinamide induced diabetic animals. Glibenclamide (0.25mg/kg/p.o) also showed a significant decrease ($p<0.001$) in serum total cholesterol, triglyceride, LDL, VLDL level and HDL was significantly increased ($p<0.001$) when compared to STZ-nicotinamide induced diabetic rats **Table 3.**

TABLE 3: EFFECT OF MAEEM ON LIPID PROFILE

Groups Treatment/ Dose	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal control	65.68± 1.95	62.32± 4.74	32.78± 2.82	59.65± 3.16	6.85± 1.22
Diabetic control	117.61± 1.56^{a***}	103.11± 2.16^{a***}	21.23± 3.95^{a***}	74.27± 2.93^{a***}	11.49± 1.30^{a***}
MAEEM (100mg/kg b.w.)	88.43±2.14 ^{b*}	95.13±3.31 ^{b*}	25.39±4.19 ^{b*}	72.13±3.17 ^{b*}	9.77±2.01 ^{b*}
MAEEM (200mg/kg b.w.)	77.74± 3.63^{b*}	84.83± 3.45^{b*}	26.42± 3.17^{b*}	69.28± 3.21^{b*}	8.91± 2.82^{b**}
MAEEM (400mg/kg b.w.)	70.18± 2.38^{b**}	66.51± 2.77^{b**}	28.09± 4.11^{b**}	66.04± 2.64^{b**}	7.89± 2.19^{b***}
Standard Glibencla mide (0.25mg/kg b.w.)	65.41± 3.12^{b***}	58.62± 4.78^{b***}	31.78± 3.16^{b***}	62.27± 3.21^{b***}	7.10± 3.38^{b***}

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01 & ***P<0.001 Vs control. [a] represents statistically significant versus normal control group, [b] represents statistically significant versus diabetic control group. HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very-low-density lipoprotein.

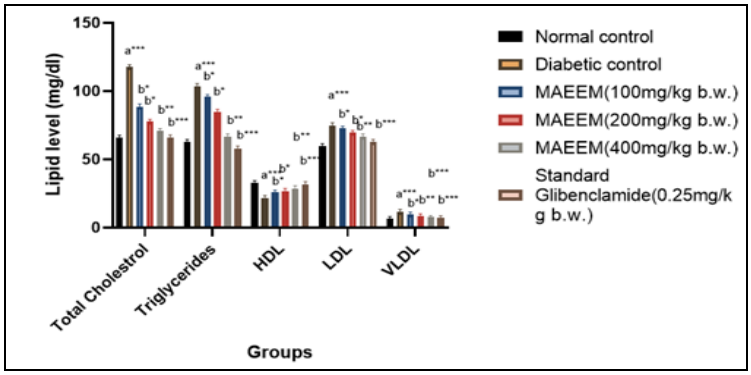

FIG. 2: EFFECT OF MAEEM ON LIPID PROFILE

Effects of MAEEM on Liver Profile: MAEEMat doses of 100, 200 & 400 mg/kg b.w. on liver enzymes (ALP, AST and ALT). It was found that the MAEEM showed significant ($p<0.05$, $p<0.05$ and $p<0.01$) protection on liver parameters, respectively. The standard drug glibenclamide (0.25mg/kg/p.o) showed significant ($p<0.001$) protection in ALP, AST, ALT when compared to STZ-nicotinamide-induced diabetic animals **Table 4.**

TABLE 4: EFFECT OF MAEEM ON LIVER PROFILE

Groups Treatment/Dose	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Normal control	41.33± 7.12	49.75± 7.36	26.52± 1.55
Diabetic control	79.95± 9.01^{a***}	81.92± 9.07^{a***}	54.37± 7.92^{a***}
MAEEM (100mg/kg b.w.)	71.42± 6.28 ^{b*}	75.73± 5.72 ^{b*}	52.18± 4.09 ^{b*}
MAEEM (200mg/kg b.w.)	55.31± 7.12^{b*}	63.17± 6.03^{b*}	44.41± 3.29^{b*}
MAEEM (400mg/kg b.w.)	51.06± 8.02^{b**}	57.42± 7.18^{b**}	31.25± 2.12^{b**}
Standard Glibenclamide (0.25mg/kg b.w.)	43.61± 7.81^{b***}	52.73± 6.17^{b***}	27.16± 2.82^{b***}

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01 & ***P<0.001 vs control. [a] represents statistically significant versus normal control group, [b] represents statistically significant versus diabetic control group, ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphate.

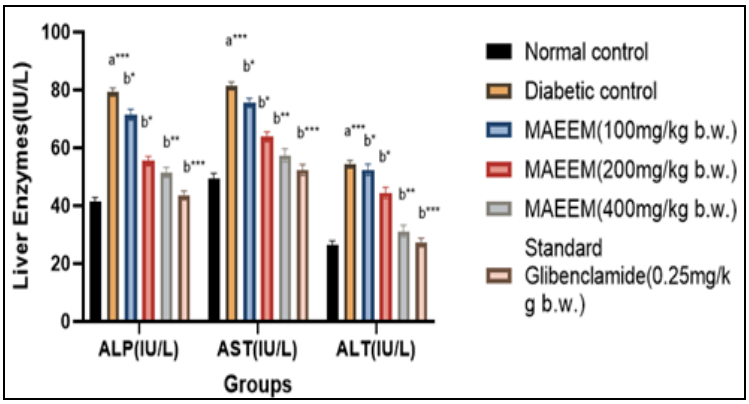

FIG. 3: EFFECT OF MAEEM ON LIVER PROFILE

Effects of MAEEM on Renal Profile: The serum urea level was significantly (p<0.001) increased in STZ-nicotinamide-induced diabetic rats when compared to control rats. Serum urea level of diabetic rat treated with MAEEM 100mg/kg/p.o, 200mg/kg/p.o showed marginal decrease (p<0.05) and 400mg/kg/p.o showed a significant decrease (p<0.01) in serum urea level compared to STZ-nicotinamide-induced diabetic rats. Glibenclamide (0.25mg/kg b.w/p.o) treatment showed a significant (p<0.001) decrease in serum urea when compared to STZ-nicotinamide-induced diabetic animals **Table 5.**

TABLE 5: EFFECT OF MAEEM ON RENAL PROFILE

Groups Treatment/Dose	Serum Creatinine (mg/dl)	Blood Urea (mg/dl)
Normal control	0.77± 0.08	29.72± 2.22
Diabetic control	2.56± 0.16^{a***}	55.11± 2.51^{a***}
MAEEM (100mg/kg b.w)	2.18± 0.11 ^{b*}	45.35±2.79 ^{b*}
MAEEM (200mg/kg b.w.)	1.81± 0.42^{b**}	36.29± 1.13^{b**}
MAEEM (400mg/kg b.w.)	1.62± 0.15^{b**}	31.53± 1.48^{b**}
Standard Glibenclamide (0.25mg/kg b.w.)	1.23± 0.20^{b***}	28.17± 2.53^{b***}

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01 & ***P<0.001 vs control. [a] represents statistically significant versus normal control group, [b] represents statistically significant versus diabetic control group.

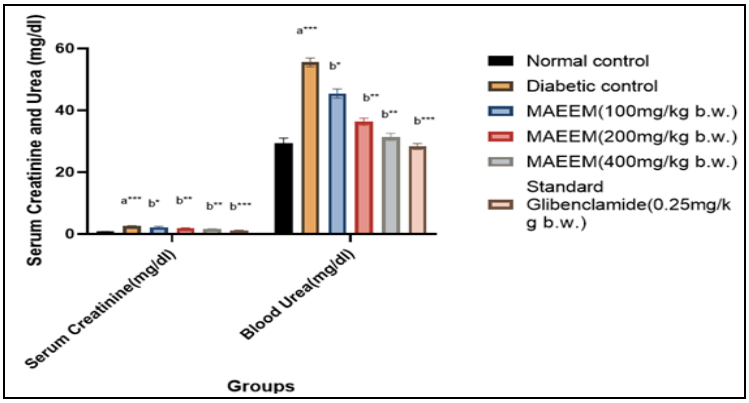

FIG. 4: EFFECT OF MAEEM ON RENAL PROFILE

DISCUSSION AND CONCLUSION: Plant extracts are thought to have anti-diabetic properties due to a combination of phytochemicals or single components. Alkaloids, phenolics, flavonoids, glycosides, and tannins are the phytochemicals responsible for antidiabetic effects. However, some of the medicinal plant's traditional uses are undocumented, resulting in a loss of knowledge and making it unreliable. As a result, it has become vital to document and disseminate all of the knowledge to assure its quality and preservation. The present study is the introductory evaluation of the antidiabetic potential of the MAEEM.

Streptozotocin is commonly used in medical research to create a type II diabetic animal model. STZ functions as a nitric oxide donor in pancreatic cells and is a powerful DNA methylating agent. Because of their low quantities of free radical scavenging enzymes, β-cells are particularly vulnerable to nitric oxide and free radical damage.

Streptozotocin (STZ) and nicotinamide (NA) have been offered to produce experimental diabetes in rats. STZ is well known for causing pancreatic β-cell destruction, whereas NA is given to rats to protect insulin-secreting cells from STZ. STZ is carried into β-cells via the glucose transporter GLUT2, where it induces DNA damage and increases the activity of the DNA repair enzyme poly (ADP-ribose) polymerase (PARP-1).

MAEEM appears significantly diminished the fasting blood glucose level in STZ-nicotinamide-induced diabetic rats in a dose-dependent manner. This is apparent from the analysis that treatment with methanolic aerial extract was effective in the management of blood glucose levels when compared to the diabetes control group at the end of the 21 days. It has been observed that hyperlipidemia is a complication associated with hyperglycemia. Lipid plays an important role in the pathogenesis of complications involved with diabetes mellitus. During the study, it was observed that an increase in total cholesterol, triglycerides, LDL, VLDL, and a decrease in the level of HDL in STZ-nicotinamide-induced diabetic rats as compared to normal control rats. The MAEEM showed a significant decrease in total cholesterol, triglycerides, LDL, VLDL and a significant increase in HDL level compared to the diabetic control group. The antidiabetic activity of MAEEM was compared with Glibenclamide, a standard hypo-glycemia drug. The possible mechanism by which brings about its antidiabetic action in diabetic rats may be by potentiating the insulin effect of plasma by increasing glucokinase activity. The potent antidiabetic effect of MAEEM suggests the presence of potent antidiabetic active constituents, which produced an antihyperglycemic effect in diabetic rats.

The blood glucose levels and lipid profile results confirmed the potent antidiabetic activity of MAEEM. So, the MAEEM showed antidiabetic activity. The result also showed significant decrease in the liver Alkaline Phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), along with serum urea level and serum creatinine level when compared to the control group.

The MAEEM showed significant antidiabetic activity in a dose-dependent manner. Hence, it was concluded that MAEEM is more effective at a 400mg/kg dose by improving antidiabetic symptoms, which can be used to treat diabetes. Further studies are necessary to examine the hypoglycaemic effect's underlying mechanism and isolate the active compounds responsible for antidiabetic activities. This work will be useful for further diabetes mellitus and its related diseases research worker to develop a new entity for treating diabetes mellitus.

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