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Research Article

EVALUATION OF ANTI DIABETIC ACTIVITY OF *MARSILEA MINUTA* LINN AGAINST ALLOXAN INDUCED DIABETES IN ALBINO RATS

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ABSTRACT

The present study was design with an aim to evaluate the Ethanolic leaf extract of *Marsilea minuta* Linn (EEMM). The study was performed on ethanolic extract of MM leaf in oral glucose tolerance test (OGTT) and alloxan-induced diabetes models in albino rats. Three weeks treatment of diabetic animals with EEMM (250 and 500 mg/kg) showed significant check in rise of blood glucose compared to untreated diabetic rats along with improved complete lipid profile. The fasting blood glucose, cholesterol, HDL cholesterol and serum triglyceride content were estimated in both normal and alloxan induced diabetic rats. The fasting blood glucose, cholesterol and serum triglyceride content were found to be significantly reduced ($p<0.05$) in EEMM treated rats and the extract also showed the potent elevation in the level of serum HDL cholesterol. On the basis of analysis of data obtained during the study, it may be concluded that EEMM leaf is having significant antihyperglycemic potential and can be further fractionated in order to get a responsible constituent for this very action.

Keywords: *Marsilea minuta*, Alloxan, hyperglycemia, Blood glucose.

INTRODUCTION:

Diabetes mellitus (DM), a state of chronic hyperglycaemia, is a common disease affecting over 124 million individuals worldwide[1-2]. DM is associated with high risk of atherosclerosis, renal, nervous system and ocular damage [3]. Uncontrolled hyperglycaemia appears to be the principal biochemical abnormality that underlies the increased oxidative load in DM. Increased oxidative stress may contribute to the pathogenesis of the diabetic complication. In addition, increased oxidative injury has been implicated in the premature age related changes in DM [4]. Multiple studies [5-9] have shown that type II diabetes is accompanied by increased oxidative damage to all bio-molecules, especially lipids. Results of studies in animal models and in humans have demonstrated that diabetes is associated with oxidative stress, which is exhibited by elevated blood levels of lipid per-oxidation products (markers of oxidative stress), especially associated with poor blood glucose control[10-15]. Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or alternatively, when the body cannot effectively use the insulin it produces. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels. *Marsilea minuta* Linn is a weedy, common plant belongs to the family Marsileaceae and widely distributed plant in India. It possesses major constituents like flavonoids and tannins, which possess good antidiabetic activity. Hence the present investigation deals the antidiabetic effect of the *Marsilea minuta Linn*.

MATERIALS AND METHODS:

Collection and Authentication of plant:

The plant was widely cultivated in gardens almost throughout India. For the present work the plant was collected from area of Tamarai Lake, Tiruvannamalai. The plant was identified by Prof.P.Jayaraman, Plant anatomy research centre, Chennai, who authenticated the plant from available literature. The leaves were collected and washed with water and dried in shade. Then it was subjected to pulverization and the powder was passed through the sieve No.60 for powder analysis and the coarse fraction was subjected for phytochemical studies.

Preparation of the leaf extract:

The powdered leaf of *Marsilea minuta* Linn was successfully extracted with ethanol using soxhlet apparatus for 2 days. The obtained extract was concentrated by distillation stored in a desiccator and used for subsequent experiments.

Animals:

Healthy male Wistar strains of albino rats, weighing between 150-250g were used in the present study. Rats were maintained at animal house and fed a standard diet and water ad libitum under strictly controlled pathogen free conditions with room temperature 25±2ºC. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. The experimental protocol has been approved by institutional animal ethical committee, K.P College of Pharmacy, Tiruvannnamalai. (Regd.No.745/03/ac/CPCSEA).

Experimental protocol:

The rats were divided into six groups, comprising of six animals in each group.

Group – I: Received 5% CMC 10ml/kg body weight/p.o. The group served as a normal control.

Group – II: Received Alloxan mono hydrate 140 mg/kg/ i.p, served as diabetic control.

Group-III: Received Glibenclamide 3mg/kg/p.o. (standard drug)

Group –IV: Received ethanolic leaf extract of *Marsilea minuta* Linn, 100mg/Kg/p.o.

Group –V: Received ethanolic leaf extract of *Marsilea minuta* Linn, 250mg/Kg/p.o.

Group –VI: Received ethanolic leaf extract of *Marsilea minuta* Linn, 500mg/Kg/p.o.

140mg/kg of alloxan will be injected intra peritoneally (i.p.) to all groups except normal control to induce diabetes before doing the experiment, after 72 hours activity was started. Duration of the study is 21 days. The blood glucose levels were determined on days of 0,7,14 and 21 by using Glucometer . All the animals were sacrificed on 21st day [16-17].

Estimation of serum biochemical parameters:
On 21st day blood was collected from animals under anaesthesia by cardiac puncture. The collected blood samples were centrifuged at 3500 rpm for 15 mins at room temperature for separation of serum. The clear, non-haemolysed sera was separated using clean dry disposable plastic syringe and stored at -20ºC for measurements of Total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein.

Estimation of antioxidant parameters:
The liver was perfused with 0.86% cold saline to completely remove all the red blood cells. Then it was suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) cut into small pieces, and the required quantity was weighed and homogenized using a homogenizer. The homogenate was centrifuged at 3000 rpm for 20 min to remove the cell debris. The supernatant was used for the estimation of Catalase, Sodium dismutase, Lipid peroxidase and Glutathione peroxidase.

Statistical analysis:
All the results were expressed as mean ± standard error of mean and were analyzed by Analysis of variance (ANOVA) and groups were compared by Tukey-Kramer multiple comparison test. Differences between groups were considered significant at $P<0.05$ level.

RESULTS

Effect of Ethanolic leaf extract of *Marsilea minuta* Linn and Glibenclamide on glucose tolerance of diabetic rats

In OGTT, the doses of ethanolic leaf extract of *Marsilea minuta* Linn (EEMM) 100mg, 250mg and 500mg shows that increased the tolerance for glucose suggesting increased peripheral utilization of glucose. EEMM at dose, 500 mg/kg possessed significant ($p<0.01$) reduction on blood glucose level when compared to EEMM at dose of 100 mg/kg. Hence, the reduction in blood glucose level was dose dependent. [Table.1]

Effect of EEMM and Glibenclamide on blood glucose level in rats

The effects of EEMM at three dose levels (100,250 and 500 mg/kg, p.o.) on blood glucose level in Alloxan induced diabetes are shown in Table 1 .Diabetes induced by Alloxan caused significant rise in blood glucose level . Administration of EEMM at three different dose levels attenuated the decrease level of glucose, produced by alloxan .EEMM did not affect the blood glucose in normal rats. [Table.2]

Effect of EEMM and Glibenclamide on lipid parameters

Significantly decreases the total cholesterol and triglycerides level in EEMM, when compare to alloxan induced diabetic rats. On administration of EEMM, there was a decrease in Total cholesterol and triglycerides levels, which was statistically significant. [Table.3]

Effect of EEMM on antioxidant enzymes in Alloxan induced diabetic rats

The effects of EEMM at three dose levels (100,250 and 500 mg/kg, p.o.) on liver antioxidant enzymes in Alloxan induced diabetes are shown in Table 2. Diabetes induced by Alloxan caused significant increases in liver antioxidant enzymes such as SOD, Catalase and Glutathione peroxidase. Administration of ethanolic leaf extract of EEMM at different dose levels shown significant dose-dependent decreases, when compared with diseased control animals. Results were shown in [Table.4]

DISCUSSION:

Alloxan is commonly used for inducing pancreatic damage. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. One study suggests that alloxan does not cause diabetes in humans[18-20] .The antioxidant enzymes which are the defence systems of the body which protect the cell membrane and other cellular constituents against oxidative damage by free radical Species (ROS)[21]. Decreased serum concentration of total antioxidant enzymes in alloxan treated diabetic rats were observed due to their utilization during inhibition or destruction of free radical species which also indicates an imbalanced ROS production and antioxidant scavenging systems. *Marsilea minuta* Linn shows significant antidiabetic activity when compared with standard drug Glibenclamide. Reports of earlier studies suggested that various plants was proved to possessing wide variety of natural antioxidant constituents such as tannins, saponoids, alkaloids, flavonoids, phenolic acids and poly phenols etc. which enhances free radical scavenging activities and responsible to ameliorate change in antioxidant enzymes which may be helpful for treatment of diabetic related complications. The qualitative phytochemical analysis on the ethanolic leaf extract of *Marsilea minuta* Linn shows the presence of flavonoids. Flavonoids constituent of plant possess antioxidant[22] and Antidiabetic properties. The present study suggests that ethanolic leaf extract of *Marsilea minuta* Linn have significant Antidiabetic activity.

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TABLE-1: Effect of EEMM and Glibenclamide on Oral glucose tolerance of diabetic rats.

Groups	Treatment	Blood glucose level			
		Fasting	After 30 mins	After 60 mins	After 90 mins
I.	Control	69.83 ± 3.76	154.33 ± 4.86	143.83±3.72	132.14 ± 5.77
II.	Glibenclamide (3mg/kg/p.o)	67.16 ± 4.56	102.5 ± 5.75[a]	94.16 ± 7.18[a]	85.33 ± 4.18[c]
III.	Alloxan (140mg/kg/i.p), EEMM (100mg/kg/p.o)	72.33 ± 4.95	134.83 ± 2.30[b]	125.5±8.62[c]	113.17 ± 5.75[c]
IV.	Alloxan (140mg/kg/i.p), EEMM (250mg/kg/p.o)	70.33 ± 4.45	112.83 ± 2.84[a]	102.83±5.85[a]	100.23± 8.65[a]
V.	Alloxan (140mg/kg/i.p), EEMM (500mg/kg/p.o)	68.12±3.58	101.13±6.12	95.17±3.42	80.63±4.31[a]

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at *p<0.05. ([a] = p<0.001; [b] = p<0.01; [c] = p<0.05) EEMM treated diabetic groups (III, IV, and V) and standard group (II) were compared with control (I) group.

TABLE-2: Effect of Ethanolic leaf extract of *Marsilea minuta* Linn and Glibenclamide on blood glucose levels of diabetic rats.

Group	Treatment	Blood glucose level			
		0 day	7 th day	14 th day	21 st day
I.	Normal Control	93.83 ± 3.13	94.66 ± 4.50	90.16 ± 8.60	98.14 ± 3.63
II.	Diabetic control Alloxan (140mg/kg/i.p)	285.66 ±15.24	299 ± 23.84	330 ± 18.32	342.83 ±15.68
III.	Glibenclamide(3mg/kg/p.o)	298 ± 5.57	261 ± 4.40	232.5 ± 5.48	216.33 ± 2.69
IV.	Alloxan (140mg/kg/i.p), EEMM (100mg/kg/p.o)	284.5 ± 6.73	261.5 ± 4.95	254 ± 3.13	242 ± 6.56
V.	Alloxan (140mg/kg/i.p), EEMM (250mg/kg/p.o)	271.67 ± 4.78	254.5 ± 6.62	242.5 ± 4.04	228.17 ± 6.91
VI.	Alloxan (140mg/kg/i.p), EEMM (500mg/kg/p.o)	322.66±11.43[a]	283.83 ± 5.97[a]	260.33 ± 4.35[b]	243.5 ± 5.86[a]

Each value represents mean ± S.E (n=6) and was analysed by ANOVA Tukey-Kramer multiple comparison test.[*] P<0.05, [**]P<0.01, [***]*P*<0.001. Diabetic control group (II) compared with normal control group (I). EEMM treated diabetic groups (IV-VI) and standard group (III) were compared with diabetic control group (II).

TABLE-3: Effect of EEMM on Lipid profiles

Groups	Treatment	Lipid profiles			
		HDL mg/dl	LDL mg/dl	Total cholesterol mg/dl	Triglycerides mg/dl
I.	Normal Control	25.6 ± 1.32	41.98 ± 0.30	136.24 ± 4.76	78.40 ± 3.42
II.	Diabetic control Alloxan (140mg/kg/i.p)	74.93 ± 1.65[***]	136.07 ± 0.23[***]	256.31 ± 7.84[***]	198.72 ± 3.23[***]
III.	Glibenclamide (3mg/kg/p.o)	33.82 ± 2.54[***]	33.9 ± 1.33[***]	139.46 ± 5.73[***]	92.39 ± 6.22[***]
IV.	Alloxan(140mg/kg/i.p), EEMM(100mg/kg/p.o)	52.41 ± 0.72	81.46 ± 0.52	250.22 ± 6.33	181.78 ± 7.44
V.	Alloxan(140mg/kg/i.p), EEMM(250mg/kg/p.o)	41.52 ± 3.24[*]	69.28 ± 0.46[**]	165.42 ± 5.24[***]	119.28 ± 5.11[***]
VI.	Alloxan(140mg/kg/i.p), EEMM(500mg/kg/p.o)	35.84 ± 2.13[***]	47.92 ± 0.28[**]	140.65 ± 7.36[***]	93.61 ± 3.16[***]

Each value represents mean ± S.E (n=6) and was analysed by ANOVA Tukey-Kramer multiple comparison test.[*] P<0.05, [**]P<0.01, [***]P<0.001. Diabetic control group (II) compared with normal control group (I). EEMM treated diabetic groups (IV-VI) and standard group (III) were compared with diabetic control group (II).

TABLE-4: Effect of EEMM on antioxidant enzymes in Alloxan induced diabetes in rats.

Group	Treatment	Lipid peroxidation (µ mole /g of protein)	Catalase (k/mg liver protein)	Superoxide dismutase (U/mg liver protein)	Glutathione peroxidase (U/mg liver protein)
I	Control	0.33±0.06	5.32±0.14**	15.54±0.14	62.6±9.8
II	Diabetic control Alloxan (140mg/kg/i.p)	1.89±0.12*	1.67±0.18	10.32±0.17**	27.3±2.7
III	Glibenclamide (3mg/kg/p.o)	0.35±0.01**	4.22±0.13	14.14±.32	52.7±1.1**
IV	Alloxan(140mg/kg/i.p), EEMM(100mg/kg/p.o)	0.19±0.02	3.54±0.15***	12.85±0.20	37.2±4.7
V	Alloxan(140mg/kg/i.p), EEMM(250mg/kg/p.o)	0.23±0.01**	4.04±0.16**	13.38±0.12**	41.7±2.8**
VI	Alloxan(140mg/kg/i.p), EEMM(500mg/kg/p.o)	0.29±0.03***	4.65±0.13**	13.97±0.21***	49.5±3.6**

Values are given as mean ± Standard error mean (S.E.M) for six groups of six animals each. Values are statistically significant at [*] p<0.05, [**]p<0.01, [***]p<0.001. Group II compared with group I and Groups IV ,V &VI were compared with group II.

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