

ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDAEMIC ACTIVITIES OF *AMARANTHUS SPINOSUS* LINN EXTRACT ON ALLOXAN INDUCED DIABETIC RATS

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The methanol extract of Amaranthus spinosus (Amaranthaceae) stem was investigated for its anti-hyperglycemic and antihyperlipidaemic effects in male Wistar albino rats. Diabetes was induced in the albino rats by administration of a single dose of alloxan monohydrate (150 mg/kg, i.p). The methanol extract of A. spinosus (MEAS) was administered daily at single doses of 250 and 500 mg/kg, p.o to diabetes-induced rats for a period of 15 days. The effect of MEAS on blood glucose level was measured in the diabetic rats. Serum lipid profiles [total cholesterol, triglycerides, phospholipids (low density, very low density and high density lipoprotein)] were also determined. The activities were also compared to the effect produced by a standard anti diabetic agent, glibenclamide. The present investigation established pharmacological evidence to support the folklore claim that MEAS is an anti diabetic agent.

Keywords: Alloxan, *Amaranthus spinosus*, Glibenclamide, Hyperglycemia

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder affecting approximately 10% of the global population. Besides hyperglycemia, several other factors including dislipidaemia or hyperlipidaemia are involved in the development of micro and macro vascular complications of diabetes which are the major causes of morbidity and death (Joslin and Bennet 1998). Currently, the available therapies for diabetes includes insulin and various oral anti diabetic agents such as sulfonylureas and metformin. These drugs are used as monotherapy or in combination to achieve better glycaemic control. Despite considerable progress in the treatment of diabetes using oral hypoglycaemic agents, the search for newer drugs continues because the existing synthetic drugs have limitations. These oral agents are associated with a number of serious adverse effects (Moller 2001).

Plants have played a major role in the search for new therapeutic agents. Research on *Galega officinalis*, a medicinal plant, led to the discovery and synthesis of metformin (Aiman 1970). In recent times there has been a renewed interest in plant based remedies (Ratnakar and Murthy 1996; Puri and Mohapatra 1997). *Amaranthus spinosus* (Amaranthaceae) is a glabrous herb found in the tropical and sub tropical regions of India. The root of this plant is used as a diuretic and febrifuge (Kirtikar and Basu 1993). Previous reports on this plant showed that its extract possesses anti-malarial (Hilou, Nacoulma and Guiguemde 2006), anti diarrhoea (Sawangaroen and Sawangaroen 2005),

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antihyperglycemic, antihyperlipidemic, spermatogenic (Sangameswaran and Jayakar 2008) and anti inflammatory, activities (Sangameswaran *et al.* 2008). It also stimulates the proliferation of β -lymphocytes (Lin, Chiang and Lin 2005). The plant selected for this present work is locally available in Salem district and has been used for a long time by locals for the treatment of diabetes.

METHODS

Preparation of the Extract

The stem of *A. spinosus* was collected from the foot hills of Yercaud, Salem, Tamil Nadu in September 2005. The plant was identified and authenticated by a botanist and a voucher specimen (ASS-09) has been kept in our institute for future reference. The stem was shade-dried at room temperature for 10 days; coarsely ground with the help of a hand-grinding mill and the powder was passed through sieve No. 60 and used for extraction. The powdered material (300 g) was extracted using methanol (900 mL) by the Soxhlet technique (Kokate 1994). The extract was then dried under reduced pressure. The dried extract (20.8 g) was stored in a desiccator.

Preliminary Phytochemical Screening

The methanolic extract was subjected to preliminary screening for various active phytochemical constituents (Evans 1989). The methanol extract of *A. spinosus* (MEAS) stem was subjected to the following test for the identification of its various active constituents by standard methods: triterpenoids and steroids were identified by Libermann-Buchard test; alkaloids were identified by Dragendorff's test; glycosides were identified by Legal's test; saponins were identified by haemolysis test; flavonoids were identified by lead acetate test.

Animals

Male Wister albino rats, 9–12 weeks old with an average weight of 150–180 g were purchased from M/S Venkateshwara Enterprises (P) Ltd, Bangalore and used for this study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycles of 12 h of darkness and light each. Before each experiment, the animals were fasted for at least 18 h. The experimental protocols were approved by the Institutional Animal Ethical Committee (Vinayaka Mission's College of Pharmacy, Government of India, New Delhi, IAEC No: P.col-24).

Drugs and Chemicals

Alloxan monohydrate was purchased from S. D. Fine Chemicals Ltd., Boisar, Mumbai. Glibenclamide was procured from Aventis Pharma, Mumbai, India. All other chemicals were obtained from local sources and were of analytical grade.

Preparation of Extract, Reference Drug and Alloxan

The extract was administered orally to rats at various doses, as a suspension in 1% carboxy methyl cellulose (CMC). Glibenclamide (500 µg/kg) was suspended in 1% w/v CMC and also administered orally (reference drug). Alloxan monohydrate (S.D. Fine Chemicals Ltd., Boisar, Mumbai) was prepared at a concentration of 150 mg/mL in sterile normal saline.

Experimental Design

Alloxan-induced hyperglycaemia

The rats were administered with alloxan monohydrate at the dose of 150 mg/kg body weight intraperitoneally (i.p). Only animals with blood glucose levels greater than 300 mg/dL, 2 days after alloxan treatment were used in the experiment (Sheweita *et al.* 2002). These rats were divided into 4 groups each containing 6 animals (Groups II to V) as follows: Group II (untreated) was diabetic rats given 0.5 mL of 5% Tween 80; Group III was diabetic rats given 0.5 mL of 5% Tween 80 containing glibenclamide (500 µg/kg); Group IV and V were diabetic rats given MEAS 250 and 500 mg/kg in 0.5 mL 5% Tween 80, respectively. Group I (normal control group) rats were given food and water, orally using a gastric tube. Blood samples (for glucose estimation) were collected from the tail on 0 day (just before the administration of extract) and a days 1, 4, 7, 10 and 15 (1 h after administration of extract).

Antihyperlipidaemic activity

Total cholesterol was estimated according to Liebermann Burchard reaction method (Richterich and Colombo 1981). Low density lipids (LDL) cholesterol was estimated indirectly by Friedwald's method (Friedwald, Levy and Freidrickson 1972). The very low density lipoprotein (VLDL) was also determined using friedwald's method (estimation of VLDL cholesterol = triglycerides/5). Triglycerides (TG) were determined using Hantzsch condensation method. High density lipids (HDL) cholesterol was also estimated by Liebermann Burchard reaction method (Richterich and Colombo 1981).

Estimation of biochemical parameters

Serum lipid profiles like LDL, VLDL, HDL, TG and total cholesterol were determined following standard procedures in an auto analyser using Ecolin kits (E. Merck, Mumbai).

Histopathology studies: Pancreas

At the end of 15 days, pancreas from the control, diabetic, glibenclamide and extract (250 and 500 mg/kg) treated rats were quickly removed for histological studies. Removed pancreatic tissues were washed in saline, fixed in Hollande-Bouin fixative (Humason 1979) for 48 h and processed for paraffin embedding. The sections stained in Ehrlich haemetoxlin, counterstained in eosin and mounted were observed under microscope.

Statistical Evaluation

Data are presented as mean \pm SEM. The differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. $p < 0.01$ was considered to be significant (Woodson 1987).

RESULTS

Preliminary Phytochemical Test

MEAS was found to contain a number of phytochemicals such as alkaloids, glycosides, terpenes and phytosterol (Table 1).

Antihyperglycemic Activity

The effects of MEAS on blood glucose level in diabetic control rats are reported in Table 2. Blood glucose levels of the alloxan treated rats were significantly higher than those in control rats. In alloxan (150 mg/kg) induced rats, blood glucose levels significantly increased from 96.50 ± 2.88 to 328.18 ± 3.84 mg/dL. MEAS at doses of 250 and 500 mg/kg when given up to the 15th day after alloxan treatment, produced significant decrease in blood glucose levels from 327.17 ± 8.42 to 175.2 ± 2.2 and from 325.00 ± 9.10 to 170.6 ± 1.2 mg/dL, respectively. In glibenclamide-treated rats, blood glucose levels decreased from 326.33 ± 9.03 to 198.2 ± 1.2 mg/dL.

Table 1: Qualitative phytochemical analysis in MEAS stem.

Plant constituents	Methanol extract
Carbohydrates	-
Protein	-
Steroids	++
Alkaloids	++
Tannins	-
Glycosides	+
Saponins	++
Flavonoids	++
Fixed oils	-

Note: +ve and -ve symbol indicates the presence and absence respectively, of plant constituent

Table 2: Antihyperglycemic activity of MEAS on alloxan induced diabetic rats.

Treatment (mg/kg)	(mg/dL)				
	1 st day	4 th day	7 th day	10 th day	15 th day
Normal (control)	96.50 ± 2.88	96.52 ± 2.60	96.54 ± 2.65	96.60 ± 2.81	98.8 ± 0.02
Diabetic (control)	328.18 ± 3.84*	348.33 ± 3.93*	356.33 ± 3.74*	365.00 ± 3.33*	370.0 ± 2.26
Glibenclamide (500 µg/kg p.o.)	326.33 ± 9.03	314.50 ± 9.07	280.33 ± 8.76**	215.17 ± 11.69**	198.2 ± 1.2**
MEAS (250 mg/kg)	327.17 ± 8.42	318.83 ± 8.34	225.17 ± 8.45**	200.83 ± 8.80**	175.2 ± 2.2**
MEAS (500 mg/kg)	325.00 ± 9.10	314.83 ± 10.00	250.83 ± 8.98**	206.17 ± 20.69**	170.6 ± 1.2**

Notes: Values are mean ± SEM, n = 6, when compared with diabetic control, * $p < 0.05$, ** $p < 0.001$, F = 16.510, $p < 0.0001$, (one way ANOVA followed by Dunnette's multiple comparison test).

Antihyperlipidaemic Activity

The lipid profiles in control and experimental rats are depicted in Table 3. In alloxan induced diabetic control rats there was a significant ($p < 0.001$) increase in total cholesterol, TG, LDL and VLDL cholesterol. In addition, there was a significant ($p < 0.001$) decrease in HDL cholesterol in diabetic control rats compared with normal control. The extract-treated rats had significant ($p < 0.001$) decrease in their total cholesterol, TG, LDL and VLDL cholesterol, and significantly ($p < 0.001$) increased HDL cholesterol.

Table 3: Antihyperlipidaemic effect of MEAS on alloxan induced diabetic rats.

Treatment (mg/kg)	Changes in level (mg/dL)				
	Total cholesterol	TG	HDL	LDL	VLDL
Normal (control)	76.16 ± 2.39	62.30 ± 2.14	43.50 ± 2.52	16.33 ± 1.33	14.83 ± 1.83
Diabetic (control)	94.33 ± 2.38	96.16 ± 2.82	30.32 ± 1.96*	21.50 ± 1.22*	21.16 ± 3.42*
Glibenclamide (500 µg/kg)	80.33 ± 2.59**	72.00 ± 2.59**	50.60 ± 1.45**	20.00 ± 1.56**	14.83 ± 2.16**
MEAS (250 mg/kg)	79.05 ± 2.95**	66.83 ± 2.59***	49.00 ± 3.32***	18.60 ± 2.73***	18.50 ± 1.34***
MEAS (500 mg/kg)	77.03 ± 4.08**	64.50 ± 2.52***	44.89 ± 3.82***	17.66 ± 2.33***	16.24 ± 1.56***

Notes: Values are mean ± SEM, n = 6 (One way ANOVA followed by Dunnette's is multiple comparison test). **, *** denotes statistically significance of $p < 0.05$, $p < 0.01$, $p < 0.001$, when compared with respective normal control.

Body Weight

Body weight slightly increased in the normal control rats compared to initial body weight, whereas in diabetic control rats there was a significant decrease in body weight. Groups that were treated with glibenclamide (500 µg/kg) and MEAS (250 and 500 mg/kg) showed significant reduction in body weights. The final body weights of treated groups were significantly lower than the final weights of normal control group (Table 4).

Table 4: Changes in body weight in the treatment MEAS on alloxan induced diabetic rats.

Treatment (mg/kg)	Change in body weight (gm)		
	Initial	10 th day	15 th day
Normal (control)	152 ± 0.2	177.00 ± 2.14	192.50 ± 2.50
Diabetic (control)	182 ± 0.4	155.00 ± 2.88	131.67 ± 2.10
Glibenclamide (500 µg/kg)	162 ± 0.2	150.67 ± 2.10**	152.33 ± 3.00*
MEAS 250	176 ± 0.4	150.00 ± 2.88**	158.83 ± 3.00**
MEAS 500	178 ± 0.6	159.00 ± 1.53**	169.83 ± 1.53**

Histopathological Investigation: Pancreas

Histopathology of the pancreas in control rats showed normal pancreatic parenchyma cells [Fig. 1 (a)]. In diabetic control [Fig. 1(b)] pancreas section showed severe congestion in pancreatic parenchyma, and mild infiltration of inflammatory cells.

In diabetic rats treated with MEAS 250 and 500 mg/kg [(Fig. 1 (d) and (e)], pancreas secretion showed mild hyperplasia of islet cells and congestion of pancreatic parenchyma. Glibenclamide 500 µg/kg [Fig. 1 (c)] treated rats shows depletion of cells and a mild infiltrate of lymphocytes.

DISCUSSION

The aim of the study was to determine the antihyperglycemic activity of *A. spinosus* stem on alloxan induced diabetic rats. Alloxan produces marked and permanent diabetes mellitus in experimental animals (Renold 1990). The results showed that intraperitoneal administration of alloxan (150 mg/kg) effectively induced diabetes in normal animals. The administration of 250 and 500 mg/kg body weight of MEAS stem for 15 days significantly inhibited hyperglycemic action of alloxan. In a previous study by Sangameswaran and Jayakar (2008), significant antihyperglycemic activity was observed in MEAS (250 and 500 mg/kg) in streptozotocin (STZ) induced rats. Our present study showed significant antihyperglycemic and antihyperlipidaemic activities in alloxan induced diabetic rats by the same plant extract. This indicates that *A. spinosus* has significant antihyperglycemic and antihyperlipidaemic effects in both STZ and alloxan induced diabetic rats.

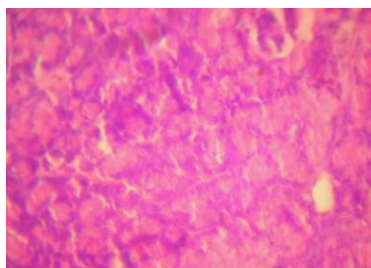


Fig. 1 (a): Normal rats show normal islets. The structure of pancreas is preserved. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei.

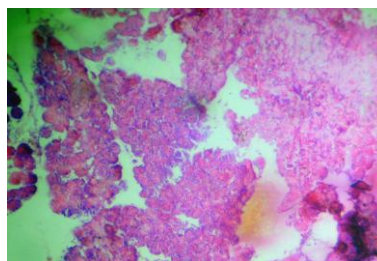


Fig. 1 (b): Alloxan (150 mg 1 kg) induced diabetic rats show depletion of cells and mg/kg. the acini cells are abnormal.

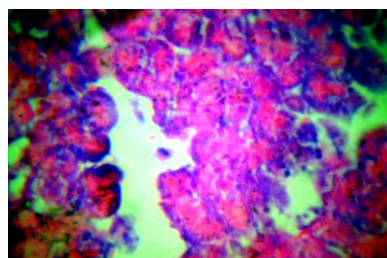


Fig. 1 (c): Glibenclamide (500 µg/kg) treated rats islets shows depletion of cells. There is a mild infiltrate of lymphocytes

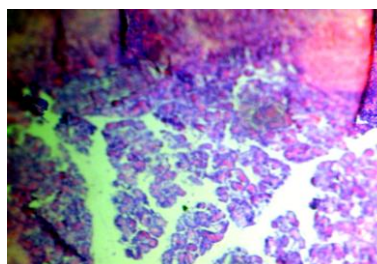


Fig. 1 (d): MEAS 250 mg/kg treated rats. The structure of pancreas is partially effaced. The islets are normal. The acini cells are normal.

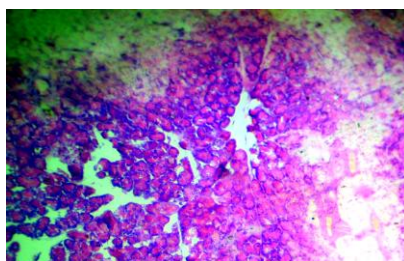


Fig. 1 (e): MEAS 500 mg/kg treated rats. The islets are normal. The structure of pancreas is preserved. The acini are lined by round to oval cells with moderate cytoplasm and small to oval nuclei

It has been established that diabetes mellitus alters the normal metabolism of lipids in diabetic rats (Stanely, Prince and Menon 2000). It is seen that cholesterol and TG are elevated in diabetic condition (Shanmugasundaram *et al.* 1990) and such an elevation represents a risk factor for coronary heart disease (Davidson and Iyer 2002). There was a significant reduction in the cholesterol and TG level of diabetic rats after *A. spinosus* treatment for 15 days. Significant lowering of total cholesterol and increase in HDL cholesterol is a very desirable biochemical state for the prevention of atherosclerosis and chemically conditions (Luc and Fruchart 1991).

CONCLUSION

In conclusion, the findings provide pharmacological evidence and validate the folklore use of *A. spinosus* as an antihyperglycemic and antihyperlipidemic agent. However, further studies are necessary to find out the active phytochemicals as well as the exact mechanisms of hypoglycaemic and hypolipidemic action of *A. spinosus*. Isolation of active compounds and elucidation of exact mechanism may contribute to development of drugs for the treatment of diabetes.

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