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Phytochemical Screening and Anti Diabetic Activity of Methanolic Extract of Leaves of *Ximenia Americana* in Rats

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ABSTRACT

The hypoglycemic effect of Methanolic extract *Ximenia americana* was evaluated in normal glucose fed and alloxan-induced diabetic rats. Oral administration of extract (200, 400 and 600mg/kg bw) for seven days resulted in a significant reduction in blood glucose level. The effect was compared with 0.5gm/kg (i.p) glibenclamide. Alloxan induced hyperglycemia and glucose fed hyperglycemia rat models were used for the evaluation of anti-diabetic activity. The effect of MEXA of normalglycemic, glucose fed hyperglycemic activity and alloxan induced hyperglycemic activity were showed in dose dependant manner. Activity is more for 600mg/kg bw dose in comparision with 400 and 200mg/kg bw dose.

Keywords: *Ximenia americana Methanolic extract*, Glibenclamide, Alloxan, Antidiabetic Activity.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by insufficient production of insulin by pancreatic glands and decrease in absorption of glucose by the cells in the human systems and caused increase the concentration of glucose in blood. It is also produced due to the hereditary Characters. Due to increase glucose level in blood causes various deficiencies and hampers the normal Physiological effects of the human system like blood vessels and nerves system etc. It is projected that the Diabetic is the main disease which can increase the deaths retain next coming 25 years in Asian countries and Africans.

Now days there are number allopathic drugs are available to treat this disease. But all these agents causing serious side effects after prolong use.

Hence to overcome the adverse effects like Heamatological effects, coma, disturbance of liver and kidney etc (Larmer J. Insulin 1985). Many traditional plants medicines are used throughout the world to treat the Diabetic diseases (Syed Mansoor Ahmed *et al*., 2005).

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When compared with synthetic drugs, the plant drugs have less toxic effects with fever side effects (Moming A, 19873).

Ximenia americana Linn (olacaceae) Commonly known as false sandal wood. In Indian system of Medicine, the various plants parts like leaves, roots, bark, roots, fruits etc are used for the treatment of diabetic, mouth ulcers, Malaria Cancer, diarrhoea fever, inflamation etc.

Hence the present investigation was under taken to evaluate the Antidiabetic activity of methanolic extract of of *Ximenia americana* leaf in alloxan induced diabetic rates to confirm the Pharmacological evidence in support of Folklore claim.

MATERIALS AND METHODS

Plant Meterial

Fresh leaves were collected from Chintala village, Dornala Mandala, Prakasam Dist.Andhra Pradesh India and authentified by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany S.V. University, Tirupathy., Andhra Pradesh, Indian. Voucher Specimen No. 1295 is kept for further future reference at S.v. University, Andhrapradesh, India.

Extraction

The collected leaves were shade dried and Powdered in a grinder mixture to get coarse powder and then passed through 40 mesh sieve. The Powdered leaves (100g) were defatted with hexane and later extracted with methanol. The extract was evaporated to dryness, gave a residue 13% W/W.

Phytochemical screening

A Preliminary phytochemical screening of aqueous extract of MEXA was carried by using standard procedures described by Khandelwal (KL Khandelwal, 2003).

Animals

Wister albino rats (200-250g) of both sexes were purchased from Sri Venkateshwara Enterprises, Bangalore. Before and during the experiment rats were fed with standard diet (Gold Mohr, Lipton India Ltd). After randomization in to various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived off food and water for 16 hours *ad libitum*. Ethical clearance for animal study was obtained from institutional animal ethics committee (IAEC/ACP/1220/ a/08).

Acute Toxicity Studies

An acute toxicity study was carried out to determination of LD_{50} values by using different doses of the extract as per the method described by Ghosh *et al*., 1984 from the toxicity study, it was indicated that the extract is safe up to dose 5.0g/kg body weight. It is very safe for further studies at different doses.

Experimental Design

Effects of MEXA on blood glucose levels in Normoglycemic rats

In this study the entire groups of animals were fasted over night and administered with respective drugs as per the mentioned dosage schedule. Animals were divided in to four groups of six rats in each group. Group–I, II, III and IV.Group-I receives 1% tween80 (3ml/kg) and groupI,II,III andIV receives 200,400 and 600 mg/kg orally of MEXA respectively. Blood glucose levels were determined at O (before drug administration) 60, 120 min, after drug administration.

Effect of MEXA on blood glucose level on glucose fed hyperglycemic rats (Oral Glucose Tolerance Test)

In this study the entire groups of animals were fasted over night and administered with respective drugs as per the mentioned dosage schedule. Animals were divided into five groups of six rats in each group. Group-I,II,III,IV and V receives glucose 2g/kg only, glibenclamide 0.5mg/kg i.p. 200 and 400, 600mg/kg and glucose 2g/kg orally half an hour before administration of standard and test extract respectively. Blood glucose levels were determined at o (before glucose challenge) 30,60,90,120th mins after glucose administration.

Effect of MEXA on blood glucose level in allaxon induced diabetic rats.

Different groups of rats were used to study the effects of MEXA. The rats were divided into six groups each consisting of six rats.

Group-I Normal/control animals received 1% tween80, 3ml /kg body wt. per orally.

Group-II: Alloxan (150mg/kg body wt) induced diabetic animals received in 1% tween80, 3ml/kg body wt. Per orally.

Group-III: Alloxan (150g/kg body wt) induced diabetic animals received glibenclamide 0.5mg/kg body wt. Per orally

Group-IV: Alloxan (150mg/kg body wt) induced diabetic animals received MEXA 200mg/kg, body wt.(P.O)

Group-V: Alloxan (150mg/kg body wt) induced diabetic animals received MEXA 400mg/kg, body wt per orally.

Group-VI: Alloxan (150mg/kg body wt) induced diabetic animals received MEXA 600mg/kg, body wt per orally.

Significant hyperglycemia was achieved within 48 hrs after Allaxon (150mg/kg b.w. i.p.) injection induced diabetic rats with more than 200mg/dl of blood glucose were identified as to be diabetic and used for the study.

In acute study all the surviving diabetic animals and normal animals were fasted over night Blood samples were collected form the fasted animals prior to the treatment with above scheduled and after administration, at each day up to 7 days . For glucose determination, blood was obtained shipping tail with sharp razor [8] then the blood glucose levels were determined by using Haemo-Glukotest (20-800R) glucose strips supplied by M/S Boechringer Mannherim India Limited. These methods which permit the measurement of blood glucose levels with minimum injury to rat, was previously validated by comparison with glucose oxidase method (B. Sangameswaran *et al*., 2007, Aydin E *et al*., 1995, Jayakar B *et al*., 2003).

Statistical Analysis

All values were expressed as mean ± SEM. The data were statistically analyzed by ANOVA Followed by Dunnets't test (Eixeira CC *et al*., 1990).

Results and Discussion

Phytochemical screening

The Preliminary Phytochemical studies MEXA revealed that presence of alkaloids. Tannins flavonoids, proteins, glycosides, flavonoids and carbohydrates saponins, steroids, glycosides as shown Table.1.

Acute toxicity Studies

From the toxicity study it was observed that MEXA is non-toxic and caused no death up to 5 g/kg orally. The results presented in Table.2.

Effect of MEXA leaf on blood glucose in Normoglycemic rats

At dose 200mg/kg and 400mg/kg of MEXA on fasting blood sugars level were determined in normal rats at various time interval is shown in Table.3 the mean blood glucose level decrease from 77.83 to 77.06 mg/dl at dose of 200mg/kg, 77.60 to 76.01 mg/dl bw at a dose 400mg/kg bw and 78.0-77.60mg/dl 600 at dose of 400mg/kg body weight in rats treated with ME XA.

Effect of MEXA on blood glucose level in glucose fed hyperglycemic rats

levels decreased from 118.34mg/dl to 98.506mg/dl at 200mg/kg body weight and 117.00mg/dl to 88.16 mg/dl at 400mg/kg body Weight and 116.83 to 83.50mg/dl at a dose of 600mg/kgbw.

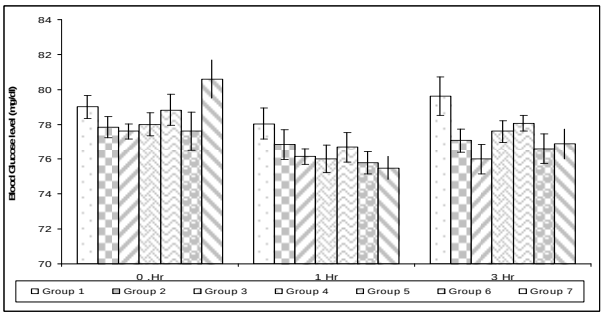
Effect of MEXA and METP on blood glucose in normoglycemic rats

At dose 200,400 and600 mg/kg of MEXA and METP on fasting blood sugar levels were assessed in normal rats at various time intervals. The results were shown in Table.3 and Fig.1.

The effect of MEXA and METP leaf on glucose fed rats was carried out and the results were presented in the Table.4 and Fig 2.

The anithyperglycemic effect of MEXA and METP on the blood sugar levels on alloxan induced diabetic rats and the results were shown in Table.5 and Fig.3.

Fig.1. Effect of MEXA and METP leaf on Blood Glucose in Normoglycemic rats



At dose 200mg/kg and 400mg/kg and 600mg/kg b.w, MEXA blood sugar level were assessed in glucose fed rat at various as shown in Table.4. The blood glucose

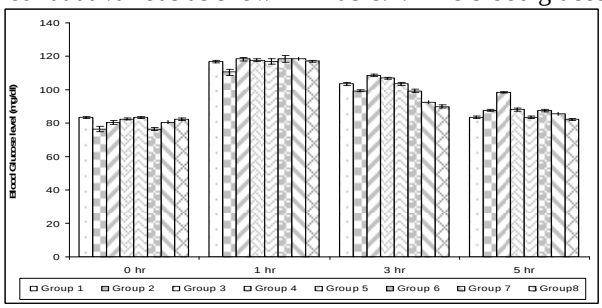


Fig.2 Effect of MEXA and METP leaf on Blood Glucose in glucose fed hyperglycemic rats

Table.1 Phytochemical Screening

S.No.	Tests	AEXMA
1	Alkaloids	++
2	Tannines	++
3	Flavonoids	++
4	Terpenoids	++
5	Proteins	++
6	Aminoacids	++
7	Phenolic Compunds	++
8	Steroids	+
9	Resin	+
10	Glycosids	+

+ + = High Presence + = Less Presence

Table.2 Toxicity Study of AEXA

Treatment	Dose(mg/kg body wt)	No.of animals	No.of Survival	No.of death	Percentage of morality	LD50 value
Control	Tween80	10	10	0	0	-----
MEXA	10	10	10	0	0	-----
MEXA	100	10	10	0	0	-----
MEXA	1000	10	10	0	0	-----
MEXA	2000	10	10	0	0	-----
MEXA	3000	10	10	0	0	-----
MEXA	5000	10	10	0	0	>5.0/kg b. w.

Table.3 Effect of MEXA and METP leaf on Blood Glucose in Normoglycemic rats

Group	Design of treatment	Dose mg/kg	Blood glucose levels (mg/dl)		
			0 Hr	1 Hr	3 Hr
I	N. Saline	3.0 ml	79.0 ±0.66	78.03 ± 0.61	79.60 ± 0.43
II	MEXA	200 mg	77.83 ± 0.89	76.83 ± 0.84	77.06 ± 0.44
III	MEXA	400 mg	77.60 ± 1.10	76.15 ± 0.65	76.01 ± 0.85
IV	MEXA	600 mg	78.0 ± 0.66	76.03 ± 0.61	77.60 ± 0.43
V	METP	200 mg	78.83 ± 0.89	76.68 ± 0.84	78.06 ± 0.44
VI	METP	400 mg	77.60 ± 1.10	75.80 ± 0.65	76.60 ± 0.85
VII	METP	600 mg	80.60 ± 1.10	75.50 ± 0.65	76.87 ± 0.85

The values are expressed as mean ± SEM n=6 as animals in each group

Table.4 Effect of MEXA and METP on Blood Glucose level in glucose Fed Hyperglycemic (Rats Oral Glucose tolerance test)

Group	Design of Treatment	Dose mg/kg	0hr	1hour	3 hours	5 hours
I	Glucose	2000	83.34 ± 0.46	116.83 ± 1.64	103.34 ± 1.11	83.50 ± 0.66
II	Glibenclamide	5	76.50 ± 0.81	110.50 ± 1.84*	99.34 ± 1.05*	87.50 ± 0.79*
III	MEXA	200	80.50 ± 0.99	118.34 ± 0.71*	108.60 ± 0.84*	98.50 ± 0.62*
IV	MEXA	400	82.50 ± 0.94	117.50 ± 0.63*	107.00 ± 0.34*	88.16 ±0.96*
V	MEXA	600	83.34 ± 0.46	116.83 ± 1.64	103.34 ± 1.11	83.50 ± 0.66
VI	METP	200	76.50 ± 0.81	118.50 ± 1.84*	99.34 ± 1.05*	87.50 ± 0.79*
VII	METP	400	80.50 ± 0.99	118.34 ± 0.71*	92.60 ± 0.84*	85.50 ± 0.62*
VIII	METP	600	82.50 ± 0.94	117.12 ± 0.63*	90.00 ± 0.34*	82.16 ± 0.96*

The values are expressed as mean ± SEM n=6 as animals in each group

Fig.4: Effect of MEXA and METP on Blood Glucose level in allaxon induced Diabetic Rats

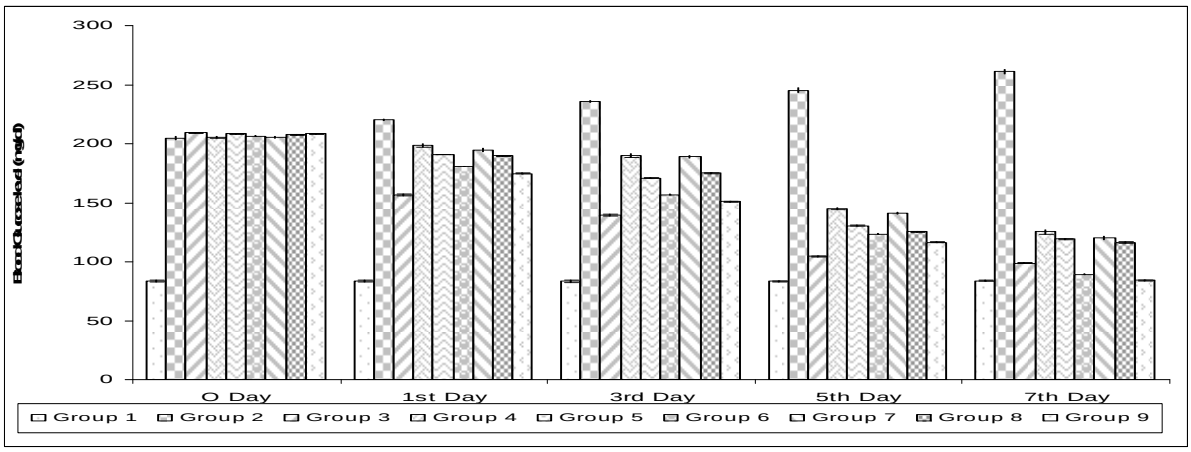


Table: 5 Effect of MEXA and METP on Blood Glucose level in allaxon Induced Diabetic Rats

Group	Dose mg/kg	Blood Glucose Levels (mg/dl)				
		O Day	1st Day	3rd Day	5th Day	7th Day
Normal	3.0ml	83.65± 0.6	83.53± 0.6	83.53±1.3	83.57± 0.3	83.84± 0.2
Diabetic Control	150	204.54±1.4	220.23± 0.8	235.7 ± 0.9	245.1±1.8	260.89± 1.8
glibenclamide	5	209.1± 0.4	156.90± 0.8*	139.41± 0.6*	104.49± 0.3*	98.74± 0.2*
MEXA	200	205.42±1.0	198.45± 1.3*	189.76± 1.4*	144.95± 0.8*	125.28± 1.8*
MEXA	400	208.37±0.3	190.68± 0.2*	170.87± 0.4*	130.57± 0.4*	119.25± 0.4*
MEXA	600	206.37±0.3	180.68± 0.2*	156.87± 0.4*	123.57± 0.3*	89.25± 0.4**
METP	200	205.42± 1.0	194.45± 1.3*	188.76± 1.4*	140.95± 0.8*	120.28± 1.8*
METP	400	207.37± 0.3	189.68± 0.2*	174.87± 0.4*	125.57± 0.3*	116.25± 0.4*
METP	600	208.37± 0.3	174.68± 0.2*	150.87± 0.4*	116.57± 0.3*	84.25± 0.4**

The values are expressed as mean ± SEM. n = 6 animals in each group Statistical significant test for comparison was done by ANOVA, followed by Dennett's test. The blood glucose values of groups III, IV, V and VI are compared with control animals, values ** P<0.001, *P<0.01, #P<0.05

Discussion

In the recent times many traditionally used medicinally important plants were tested for their anti-diabetic potential by various investigators in experimental animals. These properties were attributed to different formulations, extracts and active principles. Working on the same line, we have undertaken a study on *Ximenia americana* for its anti-diabetic Property.

The MEXA at a dose of 200mg/kg body wt. per orally did not significantly suppress blood glucose levels in over night fasted Normoglycemic animals. The same effect was observed at a higher dose level of 400mg/kg body wt. per Normoglycemic animals after 1st, 2nd and 3rd hour of oral administration, when compared with control group of animals.

The XMAE showed significant improvement in glucose tolerance in glucose fed hyperglycemic normal rats. Such an effect may be accounted for, in part, by a decrease in the rate of intestinal glucose absorption, achieved by an extra pancreatic action including the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process with concomitant decrease in glycogenolysis and glyconeogenesis (Porchezhian E *et al*., 2000). However, the effect was less significant when compared to standard drug glibenclamide.

Alloxan is the most commonly employed agent for the induction of experimental diabetic animal models of human insulin-dependent diabetes mellitus. There is increasing evidence that alloxan caused diabetes by rapid depletion of a cells, by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. It leads to a reduction in insulin release there by a drastic reduction in plasma insulin concentration leading to stable hyperglycemic states (Yasodha Krishna janapati *et al*., 2008). In this study significant hyperglycemia was achieved within 48 hours after alloxan (150mg/kg b.w. i.p) injection. Alloxan induced diabetic rats with more than 200mgdl of blood glucose were considered to be diabetic and used for the study.

The Studies on Antidiabetic activity in alloxanised rats, significant reduction of blood glucose was observed from the 2nd day of the study. The comparable effect of the extract with glibenclamide may suggest similar mode of action since alloxan permanently destroys the pancreatic B cells and the extract lowered blood sugar level in alloxanised rats, indicating that the extent possesses extra pancreatic effect. From the Phytochemical analysis it was found that the major chemical constituents of the extract and some of this active principle including flavonoids are known to be used for the treatments of diabetes (SzkudeskiT 2001, Meiselman HL *et al*., 1976) on the basis of the above evidences it is possible that the presence of flavonoids and tannins are responsible for the observed Antidiabetic activity (Suba V *et al*., 2004).

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