

Effects of *Zizyphus mauritiana* Lam. leaves extract in alloxan induced diabetes and its secondary complications in rats

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ABSTRACT

In the present study we evaluated the effect of 4-week treatment of ethanolic extract of Zizyphus mauritiana Lam. leaves (ZME), on the blood glucose, oral glucose tolerance and biochemical parameters like cholesterol, urea, creatinine and SGPT in alloxan induced diabetic rats. Albino rats (Wistar), weighing between 200-225 g. of either sex were selected for the study. Diabetes was introduced by single tail-vein injection of alloxan (60 mg kg⁻¹). Blood glucose level was detected by glucose oxidase and peroxidase method using commercially available kit. The biochemical parameters (cholesterol, urea, creatinine, bilirubin and SGPT) were analyzed using Transasia Chem-5 Plus v2 auto analyzer using standard kits. Oral glucose tolerance test was performed at the end of 4-weeks. Glucose (2.5 g kg⁻¹) was administered to 12-h fasted rats. Blood sample was collected at various time intervals. The result shows that oral administration of ZME to diabetic animals' for 4 weeks dose dependently reduced the blood glucose level and it is comparable to that of standard drug metformin. The ZME significantly increases oral glucose tolerance. The extract also reduces elevated cholesterol, urea, creatinine and SGPT level and increases hemoglobin count in diabetic rats. The result of present study proved the hypoglycemic effect of ZME and it was also observed that the extract may delay the onset of some secondary complication reported diabetes.

Keywords: *Zizyphus mauritiana*; Alloxan; diabetes; oral glucose tolerance test.

INTRODUCTION

Diabetes mellitus is a chronic systemic disease capable of affecting virtually every organ system in body. It has been shown to be associated with atherosclerosis and cardiovascular diseases with altered metabolism of cholesterol [1]. Insulin deficiency leads to various metabolic alterations in the animals with increase blood glucose, decrease protein content, increased cholesterol, increased alkaline phosphatases and increased level of transaminases [2]. Oral hypoglycemic agents like Metformin, have shown to produce beneficial effects on the lipid profile mainly by correcting abnormal glucose metabolism [3]. Several herbal preparation containing shilajit, gymnema sylvestre, jambolan seeds, polygala arvensis etc. have also shown to decrease the blood glucose level and lipid levels. Co-administration of such herbal preparation with oral hypoglycemic agents has shown to be more effective in regulating the metabolism [4, 5].

The *Z. mauritiana* has been used as a traditional Indian and Chinese medicinal herb and considered to affect various physiological functions in the body for thousands of years.

Hence it decided to study the effect of ethanolic extract of *Z. mauritiana* leaves on the blood glucose, oral glucose tolerance and other biochemical parameters in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant material:

The fresh leaves of *Zizyphus mauritiana* were collected from Aurangabad, Maharashtra, India and air-dried in shade at room temperature. The sample specimen of plant material was deposited at the Herbarium, Dept. of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (Voucher specimen No. 5640). The dried leaves were powdered mechanically and kept separately in airtight container till the time of use.

Preparation of extract:

The powdered leaves were defatted with petroleum ether (60-80 °C) in soxhlet's extractor, and the mark was dried and extracted with ethanol. The ethanolic extract was evaporated to dryness in vacuum. The mass was stored in a refrigerator and considered as the extract.

Animals:

Albino rats (Wistar), weighing between 200-225 g. of either sex were selected for the study. The animals were allowed to acclimatize to laboratory condition for 10 days after their arrival. The animals were housed into group of six under standard housing conditions. The animals were fed with standard rat feed and allowed water *ad libitum*. The experimental procedures were performed in accordance with the protocol approved by Institutional Animal Ethics Committee (IAEC) of Y.B. Chavan college of Pharmacy (CPCSEA/IAEC/P'Col/19/43), constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under Ministry of Animal Welfare Division, Government of India, New Delhi, India.

Experimental Design

Acute toxicity test

The Acute Toxicity of ZME was performed as per OECD guideline no. 425 for toxicity studies, in the Swiss albino mice of either sex (20-25g) maintained under standard dietary conditions. The animals were fasted for 3hr before experiment. Animals were administered with single dose of ZME. Maximum dose of ZME administered was 5000 mg kg⁻¹.

Induction of diabetes:

Diabetes was introduced by single tail-vein injection of alloxan (60 mg kg⁻¹). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (15-20 ml) intraperitoneally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia [6].

Rats showing hyperglycemia with blood glucose > 200 mg dL⁻¹ 48 h after alloxan injection were selected for the experiments.

Treatments:

The rats were divided into six groups each having six animals. Group 1 received the vehicle 0.2 ml orally. Group 2 was diabetic control and received the vehicle 0.2 ml orally. The third group includes diabetic rats treated with metformin at a dose of 25 mg kg⁻¹ orally. Group 4, 5 and 6 were diabetic rats treated with ZME at a dose of 50; 100 and 200 mg kg⁻¹, orally, respectively.

Biochemical Parameters:

All the treatments were given once a day for four weeks. At the end of 4 weeks, blood samples were collected from the tail vein into centrifuge tube. Blood glucose level was detected by glucose oxidase and peroxidase method using commercially available kit (Bayer Diagnostic, India). Plasma was separated by centrifuging the sample at 5000 rpm for 10 min and stored in refrigerator until analyzed. The biochemical parameters (cholesterol, urea, creatinine, bilirubin and SGPT) were analyzed using Transasia Chem-5 Plus v2 auto analyzer using standard kits (Span Diagnostics).

Oral glucose tolerance test:

All the rats were subjected to oral GTT at the end of 4-weeks. Glucose (2.5 g kg^{-1}) was administered to 12-h fasted rats after 0.5 h of drug administration. Blood sample were collected at 0, 30, 60, 90, 120 and 180 min respectively [7].

Statistical Analysis:

All the data are expressed as Mean \pm SE and analyzed statistically using ANOVA followed by Tukey-Kramer test or Repeat measure ANOVA and compare with respective control group. A value of $P < 0.05$ was considered significant.

RESULTS

In the present work we have studied the effect 4-week treatment of ethanolic extract of *Z. mauritiana* leaves (ZME) on blood glucose, Glucose tolerance and other biochemical parameters in alloxan induced diabetic rats. The results obtained are illustrated as follows.

Blood Glucose Level: The blood glucose level was significantly ($P < 0.01$) elevated in diabetic rats as compare to normal rats. Metformin significantly ($P < 0.01$) reduced the blood glucose level. Oral administration of ZME dose dependently reduced ($P < 0.01$) the blood glucose level. The dose of ZME (200 mg kg^{-1}) shows decrease in elevated blood glucose level by $52.08 \pm 2.77 \%$ (Table 1).

Table 1: Effect of extracts on blood glucose level in normal and diabetic rats

Treatment	Blood glucose level (mg dl^{-1})		%decrease
	Basal	4-week	
Normal Control	84.40 \pm 3.51	87.90 \pm 3.00	---
Diabetic Control	253.50 \pm 24.37*	343.67 \pm 16.06**	---
Diabetic+Metfor. (25 mg kg^{-1})	220.83 \pm 14.16	132.94 \pm 8.96**	53.80 \pm 4.84
Diabetic + ZME(50 mg kg^{-1})	244.64 \pm 15.03	180.30 \pm 9.61**	38.00 \pm 4.21
Diabetic + ZME (100 mg kg^{-1})	236.78 \pm 15.40	151.79 \pm 8.00**	48.07 \pm 2.54
Diabetic + ZME(200 mg kg^{-1})	225.99 \pm 16.40	139.96 \pm 8.21**	52.08 \pm 2.77
Normal + ZME(100 mg kg^{-1})	80.15 \pm 11.90	78.52 \pm 8.90	10.67 \pm 3.41

*ID*₅₀ dose : ZME = 155 mg kg^{-1}

$n=6$, * $p < 0.05$; ** $p < 0.01$ vs. respective control (ANOVA followed by Tukey-Kramer test)

Table 2: Effect of extracts on oral glucose tolerance test

Time (Min) / Treatment	Mean Glucose Concentration \pm S.E.M. (mg dl^{-1})					
	0	30	60	90	120	180
Normal Control	86.23 \pm 3.98	145.63 \pm 10.15	154.13 \pm 9.87	133.30 \pm 5.37	112.23 \pm 4.65	92.84 \pm 3.63
Diabetic Control	283.67 \pm 10.28*	397.68 \pm 9.73*	420.82 \pm 8.25**	412.25 \pm 8.97*	394.59 \pm 7.40*	384.05 \pm 6.20*
Diabetic + Metformin (25 mg kg^{-1})	141.27 \pm 7.61*	185.57 \pm 7.33*	163.95 \pm 6.18*	152.79 \pm 4.48*	143.36 \pm 5.38*	135.85 \pm 4.47*
Diabetic + ZME(155 mg kg^{-1})	149.63 \pm 9.27*	203.46 \pm 11.14*	230.96 \pm 8.21*	218.16 \pm 7.40*	199.33 \pm 3.12*	166.73 \pm 7.62*

$n=6$, * $p < 0.05$; ** $p < 0.01$ vs. respective control (Repeat measure ANOVA)

Table 3: Effect of extracts on blood glucose level in normal and diabetic rats

Treatment	Cholesterol (mg dl^{-1})	Urea (mg dl^{-1})	Creatinine (mg dl^{-1})	Billirubin (mg dl^{-1})	SGPT (U l^{-1})
Normal Control	83.80 \pm 3.73	28.46 \pm 3.48	0.75 \pm 0.05	0.70 \pm 0.03	51.50 \pm 2.67
Diabetic Control	145.02 \pm 9.02**	87.32 \pm 4.02**	1.70 \pm 0.06**	0.78 \pm 0.05*	80.68 \pm 7.01*
Diabetic+Metfor. (25 mg kg^{-1})	106.15 \pm 3.86*	31.89 \pm 2.56**	0.96 \pm 0.07**	0.70 \pm 0.04	63.20 \pm 2.54*
Diabetic + ZME (50 mg kg^{-1})	96.93 \pm 3.88*	37.83 \pm 2.87**	1.37 \pm 0.11*	0.66 \pm 0.35*	72.49 \pm 4.86
Diabetic + ZME(100 mg kg^{-1})	95.84 \pm 4.01*	36.38 \pm 2.70**	1.12 \pm 0.08**	0.72 \pm 0.04	65.32 \pm 2.72*
Diabetic + ZME (200 mg kg^{-1})	92.00 \pm 4.50*	34.60 \pm 4.20**	1.33 \pm 0.08*	0.81 \pm 0.05	64.45 \pm 3.03*

$n=6$, * $p < 0.05$; ** $p < 0.01$ vs. respective control (ANOVA followed by Tukey-Kramer test)

Glucose tolerance: Glucose fed normal group showed elevation of blood glucose level at 30 mins. The peak blood glucose level was observed at 60 mins i.e. $154.13 \pm 9.87 \text{ mg dl}^{-1}$ in normal control group. After 60 mins., gradual fall in blood glucose level was observed and it come to normal at 180 mins. Diabetic control animals showed extreme rise ($p < 0.01$) in blood glucose after oral glucose load i.e. initial from 283.67 ± 10.28 to 420.82 ± 8.25 at 60 mins.

Chronic treatment with metformin and ZME (155 mg kg⁻¹) significantly ($p < 0.05$) increased the tolerance for glucose. The glucose levels observed at 60 min. were 163.95 ± 6.18 and 230.96 ± 8.21 respectively, and it falls to near normal at 180 mins. The observations are shown in **Table 2**.

Cholesterol level: Diabetic animals showed significant increase in the cholesterol level compare to the normal control at the end of 4 weeks. ZME and metformin significantly ($P < 0.01$) reduced the cholesterol level.

Ures and Creatinine: Diabetic animals showed significant ($P < 0.01$) elevation in urea and creatinine level as compare to respective normal control. After the 4 weeks treatment metformin and ZME reduces urea and creatinine significantly ($P < 0.01$) compare to their respective diabetic control.

Billirubin: Increase in billirubin level observed in diabetic control animals. No significant change in billirubin was reported in metformin and ZME treatments.

SGPT: The significant increase in SGPT level in chronic diabetic animals was seen and it was significantly reduced by ZME and metformin at the end of 4 weeks. All observations are shown in **Table 3**.

Hemoglobin: Average hemoglobin of normal control rats was 14.52 ± 0.31 g dl⁻¹ and no significant change was observed throughout the course as shown in Table 4. Reduction in hemoglobin count was observed with diabetic control group compare to normal control and also throughout the 4-weeks duration. Metformin and ZME significantly elevated the hemoglobin count in diabetic rats at the end of 4-weeks.

Table 4: Effect of Extracts on total hemoglobin in diabetic rats

Treatment	Average total Hemoglobin count (g dl ⁻¹)	
	Basal	4-weeks
Normal Control	14.52 ± 0.31	14.43 ± 0.69
Diabetic Control	$13.18 \pm 0.81^*$	$10.27 \pm 0.62^{**}$
Diabetic + Metformin (25 mg kg ⁻¹)	$12.91 \pm 0.21^{**}$	$13.97 \pm 0.54^*$
Diabetic + ZME(155 mg kg ⁻¹)	$12.95 \pm 0.58^{**}$	$13.80 \pm 0.72^*$

$n=6$, $p < 0.05$; $^{**}p < 0.01$ vs. respective control (students *t* test)

DISCUSSION

Alloxan induced diabetes is a well documented model of experimental diabetes. It induces 'chemical diabetes' in a wide verity of animal species including rats by damaging the insulin-secreting β -cells of the pancreas. Alloxan causes time and concentration dependent degradation lesions of the pancreatic β -cells leading to hyperglycemia [7]. In the present study ZME and metformin lowers elevated blood glucose level, it was reported high in diabetic control animals. Maximum reduction in the blood glucose level observed with ZME 200 mg kg⁻¹, which is comparable to metformin. Thus ZME shows hypoglycemic activity in alloxan treated diabetic rats.

Metformin promotes insulin binding to its receptor and increases the uptake of glucose in the peripheral tissue. It also inhibits hepatic and renal gluconeogenesis [8]. Metformin is less likely to lower blood glucose in normal persons [9]. In the present study ZME and metformin treatment reverses the elevated blood glucose level. ZME showed non-significant decrease in blood glucose level in normal rats. This would indicate that mechanism of hypoglycemia produced by ZME might be by releasing insulin or by promoting its binding to the receptor/s.

Maintenance of normal glucose tolerance is dependent on the finely tuned balance between insulin secretion and insulin action [10, 11]. Among subjects with normal glucose tolerance (NGT), insulin sensitivity varies over a 6- to 7-fold range [12]. Nonetheless, glucose tolerance remains normal because the β -cell is able to adjust its secretion of insulin to compensate for the existing level of tissue insensitivity to the hormone. Numerous studies, using a variety of techniques, have demonstrated that individuals with impaired glucose tolerance (IGT) and overt type 2 diabetes (T2DM) are characterized by moderate to severe insulin resistance [13]. Compared with controls, both IGT and diabetic subjects manifested a slower rise in insulin secretion as a function of rising plasma glucose concentration, with a greater defect in the diabetic compared with IGT subjects.

In this study normal control animals showed elevation, Peak and then decline in blood glucose and hence, indicates the normal glucose metabolism. Alloxan treated diabetic rats showed increase in glucose level peak at 60 min.

Increased glucose level was maintained till 180 min. No significant decrease in glucose level was seen up to 180 min. because of slow or less availability of insulin due to pancreatic destruction by alloxan and insulin resistance.

Metformin, which is known to act exclusively by extrapancreatic mechanism, caused significant lowering of plasma glucose level.

The ZME treated rat shows peak glucose concentration at 60 min and then it starts reducing. Decrease in glucose level was observed with each time interval after 60 min. and may restore glucose metabolism in diabetic rats. It may be due to restoration of delayed insulin response. It may also modify the peripheral uptake of glucose.

Padminikedar and Chakrabarthy [5] have shown that the cholesterol and triglyceride levels are increased in hyperglycemia. Deficiency of insulin causes the increase in the level of enzyme in liver and serum of diabetic animals. Metformin has also shown to be beneficial in improving the lipid profile mainly by correcting the abnormal glucose metabolism [14]. The result of chronic treatment with ZME and Metformin showed significant decrease in the serum cholesterol level. also reduced the serum cholesterol level. Many researchers have reported increase in transaminase activity in the liver and serum of diabetic animals. The increase levels of transaminase which are active in the absence of insulin because of increase activity of amino-acid in diabetes are responsible for increased gluconeogenesis and ketogenesis observed in diabetes [4]. In the present study, chronic diabetic animals showed elevated level of SGPT. ZME and metformin reduced it significantly. There is no significant change in bilirubin level was observed. This might suggest the protective action of extract and metformin reversing any organ damage due to induction of experimental diabetes that is manifested by elevated level of SGPT.

Kidney maintains optimum chemical composition of body fluids by acidification of urine and removal of metabolic wastes such as urea, uric acid, creatinine and ions. During renal diseases, the concentration of these metabolites increases in blood [15]. In this study it was observed that, ZME and metformin, reduces increased level of urea and creatinine. The result obtained is comparable to the lowering effect observed with metformin. This indicates the prevention of any significant kidney damage, which may be possible in diabetic animals.

CONCLUSION

The present study indicates that oral administration of ethanolic extract of *Zizyphus mauritiana* leaves dose dependently reduces the blood glucose level in diabetic rats. ZME also increases Glucose Tolerance in diabetic rats. Further the extract lowers cholesterol level without producing toxicity to liver and kidney up to a period of 4 weeks. Thus the extract proves hypoglycemic effects in alloxan induced diabetic rats and ZME may also delay the onset of secondary complications in diabetes.

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