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A study on the effects of *Moringa oleifera* lam. pod extract on alloxan induced diabetic rats

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ABSTRACT

The present study was designed to evaluate the effects of Moringa oleifera Lam. pods in the alloxan treated diabetic rats. The rats with body weight 200–250 g of either sex were selected for the study. Alloxan was given by single tail-vein injection (60 mg kg^{-1}). The alloxan treated rats showed significant increase in the blood glucose and impaired glucose tolerance. The metfornin and ethanolic extract of Moringa oleifera Lam. pod (MOP) was administered orally, once a day for 21-days. The blood glucose, oral glucose tolerance, body weight and biochemical parameters like SGOT, SGPT and creatinine was evaluated in alloxan induced diabetic rats for 21-days. The result of the study showed that decrease in blood glucose level by MOP and improvement in the glucose tolerance at the end of 21-days. The MOP also significantly reduced elevated SGPT, SGOT and creatinine in alloxan treated rats indicating reduction in the secondary complications in the diabetic rats. MOP also improves the body weight of alloxan treated rats. The result of study proved the hypoglycemic effects of MOP in alloxan treated diabetic rats. It may be due to the presence of flavonoids, which helps to speed up the natural healing process and may improve the glycemic control in diabetic rats.

Key words: Alloxan, Diabetics, *Moringa oleifera*, OGTT.

INTRODUCTION

Diabetes mellitus appears to have been a death sentence in the ancient era. Hippocrates makes no mention of it, which may indicate that he felt the disease was incurable. Aretaeus did attempt to treat it but could not give a good prognosis; he commented that "life (with diabetes) is short, disgusting and painful [1]. Sushruta (6^{th} century BC) identified diabetes and classified it as *Madhumeha*. He further identified it with obesity and sedentary lifestyle, advising exercises to help "cure" it. The ancient Indians tested for diabetes by observing whether ants were attracted to a person's urine, and called the ailment "sweet urine disease" (*Madhumeha*) [2].

Diabetes is a leading cause of deaths in the world. According to the World Health Organization, at least 171 million people worldwide suffer from diabetes, or 2.8% of the population. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will almost double [3].

Herbal drugs play a role in the diseases; most of them speed up the natural healing process. Numerous medicinal plants and their formulations are used for various disorders in ethno medical practices as well as traditional systems of medicines in India [8]. In India, medicinal plants have been used as natural medicine since the days of Vedic glory. Many of these medicinal plants and herbs are part of our diet as spices, vegetables and fruits.

According to the literature review it is observed that the *Moringa oleifera* Lam. pod contain flavonoids, which helps to speed up the natural healing process and may improve the glycemic control in diabetics, hence selected for present study.

MATERIALS AND METHODS

The fresh *Moringa oleifera* pods were collected from local area of Aurangabad, Maharashtra, India, during July season. The Plant was identified and authenticated at the Botany Department, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India. The Voucher specimen (Number 0773) was deposited in herbarium.

Extraction procedure

The dried powdered pods of *M. oleifera* were defatted with petroleum ether (60-80^0 C) in a Soxhlet apparatus. The defatted powder material (marc) thus obtained was further extracted with ethanol. The solvent was removed by distillation under low pressure and the resulting semisolid mass was vacuum dried by using rotary flash evaporator. The mass was stored in a refrigerator and considered as the extract (MOP).

Animals

Albino (Wistar) rats of either sex (200–250 g) were selected for the study. The experimental design was approved by Institutional Animal Ethical Committee and the study was performed according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the use and care of animals. The animals were distributed at random and housed in poly propylene cages (43 X 23 X 15 cm) according to their sex, so that each cage contains maximum four animals of the same sex. The animals were fed with standard rat feed and allowed water *ad libitum.* All the animals were subjected to a period of observation and acclimatization of at least two weeks between the date of issue and the start of treatment (approval ref: CPCSEA/IAEC/P'COL-04/20).

Experimental Design

The Acute Toxicity of MOP was performed as per OECD guideline no. 425 for toxicity studies, using 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 MOP. Maximum dose of MOP administered was 5000 mg kg^{-1} [4].

Induction of diabetes in rats

Rats were made diabetic by a single intravenous injection of alloxan (hydrate) at a dose of 60 mg/kg. 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 [5].

Treatments

The Normal and diabetic control group was treated with normal saline (0.2 ml). Metformin was administered orally at a dose of 120 mg/kg using sterile oral feeding needle [6]. The extracts (MOP) were administered in the dosage of 50, 100 and 200 mg/kg for 21 days orally using sterile oral feeding needle. The quantity of drug administered to each animal was calculated daily from its body weight.

Collection of Blood samples

The blood was collected initially (basal) and after 7th day of administration of test drug and then 14th and 21th day. Animals were anaesthetized with ether and blood was collected by retro-orbital puncture method. Serum was separated by using centrifuge machine at 5000 rpm for 15 min and stored at 4 - 8˚ C until use.

Biochemical parameters

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 (creatinine, SGOT and SGPT) were analyzed using Transasia Chem-5 Plus v2 auto analyzer using standard kits (Span Diagnostics).

Oral Glucose tolerance test

This study was carried after 21 days. FBG was checked initially, and then BGL was taken after 30 min of treatment and considered as '0' h value. The rats were then orally administered with 2g kg−1 of glucose after 30 min of their respective treatments. The blood sample was collected periodically up to 3 h at regular interval of 1 h each [5].

Statistical Analysis

All the data are expressed as Mean ± SE and analyzed statistically using one way ANOVA followed Dunnett's test. A value of $P < 0.05$ was considered significant.

RESULTS

Acute toxicity studies

No mortality and signs of any toxicity were evidenced after the administration of 2000 mg/kg, 3000 mg/kg and 5000 mg/kg ethanolic extract of *Moringa oleifera* pod (MOP) in acute oral toxicity test.

Blood Glucose

Normal control rats were maintained the blood glucose level ranging from 87.50 ± 5.63 mg/dl to 97.50±5.95 mg/dl during the period of study. Diabetic control rat were showed significant ($p<0.01$) elevation in blood glucose throughout the study compared to normal control. The results are shown in the Table 1. The treatment with metformin showed gradual reduction in the blood glucose in diabetic rats, initial from 301.50 ± 26.73 mg/dl to 222.50 ± 43.01 mg/dl on day-7, 200.25 ± 33.97 mg/dl on day-14 and 174.00 ± 64.03 mg/dl on day-21 respectively. The treatment with MOP 50 mg/kg, 100 mg/kg and 200 mg/kg showed similar type of gradual reduction in the blood glucose level in the diabetic rats for 21-days treatments. Maximum reduction in the blood glucose level was reported with MOP 200 mg/kg on day-21 ($p<0.01$).

Table 1: Effect of *Moringa oleifera* Pod extract on Blood Glucose level in alloxan induced diabetic rats

Sr. No	Groups	Blood Glucose mg/dl			
		Basal	7th Days	14th Days	21st Days
1.	Normal Control	87.50±5.63	74.25±4.34	88.75±8.75	97.50±5.95
2.	Diabetic Control	343.50±52.12**	330.00±16.62	326.75±25.53	295.00±8.67*
3.	Diabetic + Metformin	301.50±26.73**	222.50±43.01**	200.25±33.97**	174.00±64.03**
4.	Diabetic + MOP (50 mg/kg)	309.25±61.13**	256.75±77.11*	216.75±58.86**	175.50±57.22**
5.	Diabetic + MOP (100 mg/kg)	257.75±19.01**	187.00±23.00**	140.75±20.39**	139.75±12.22**
6.	Diabetic + MOP (200 mg/kg)	291.50±16.00**	201.25±11.99**	158.50±19.51**	110.75±6.78**

*n=6, *p<0.05, **p<0.01 vs. respective control (One way ANOVA followed by Dunnett's test)*

Body weight

The change in the body weight of control and diabetic groups of rat treated with MOP 50 mg/kg, 100 mg/kg and 200 mg/kg and metformin is shown in Table 2. Alloxan-induced (60 mg/kg body weight) rat showed decrease in the body weight throughout the study. The body weight of the diabetic control rat showed a gradual decrease from 232.00 ± 2.70 gms. to 175.50 ± 2.10 gms. ($p<0.01$) at the end of 21-days. The diabetic rats treated with MOP and metformin showed significant inhibition in the reduction of body weight. These animals were maintained the body weight like normal animals.

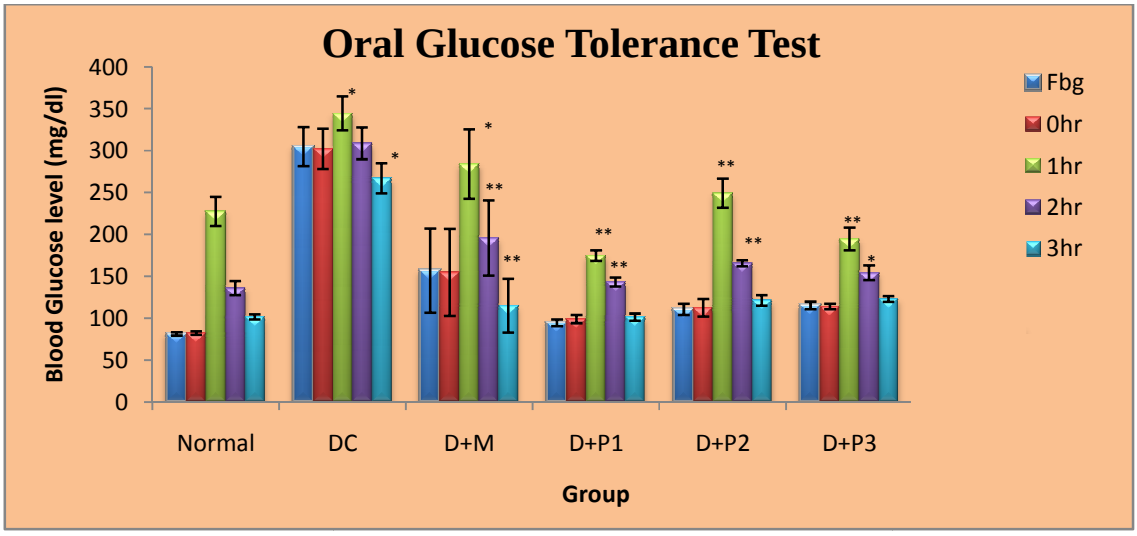
Table 2: Effect of *Moringa oleifera* Pod extract on Body Weight in alloxan induced diabetic rats

Sr. No	Groups	Body weight (grm.)			
		0 Days	7th Days	14th Days	21st Days
1.	Normal	210.00±4.08	215.50±2.46	217.75±1.25	227.50±1.55
2.	Diabetic Control	232.00±2.70*	198.75±4.27**	180.25±1.93**	175.50±2.10**
3.	Diabetic + Metformin	213.75±2.39	216.00±0.70	220.50±2.64	225.75±1.10
4.	Diabetic + MOP (50 mg/kg)	205.00±2.88	210.00±2.10	218.00±1.08*	223.50±1.93*
5.	Diabetic + MOP (100 mg/kg)	206.00±3.75	212.00±3.90	220.25±6.04*	225.50±5.80*
6.	Diabetic + MOP (200 mg/kg)	212.00±6.29	216.00±3.66	224.00±3.10*	231.50±2.63*

*n=6, *p<0.05, **p<0.01 vs. respective control (One way ANOVA followed by Dunnett's test)*

Oral glucose tolerance test

The animals in each group were fasted for 12-14 hrs and then the mean blood glucose level was evaluated before and after oral administration of glucose (2 g/kg body weight). The mean blood glucose value in the normal control rat rose to a peak value 0 hr. after glucose load and decreased to near normal level at 3 hr. The diabetic control rat, however, peak increase in mean blood glucose concentration was observed after 0 hr. and remained high over the next 3 hr. (i.e. 305.00±23.28 to 267.25±18.02 mg/dl) indicating impaired glucose tolerance. The diabetic rats treated with MOP 50 mg/kg, 100 mg/kg and 200 mg/kg and metformin showed significant ($p<0.01$) improvement in the glucose tolerance compare to the diabetic control groups. Results are shown in the figure-1.



Group	Treatment	Group	Treatment
Normal	Normal	D+P1	Diabetic + MOP (50 mg/kg)
DC	Diabetic Control	D+P2	Diabetic + MOP (100 mg/kg)
D+M	Diabetic + Metformin	D+P3	Diabetic + MOP (200 mg/kg)

n=6, $^{*}p<0.05$, $^{**}p<0.01$ vs. respective control (One way ANOVA followed by Dunnett's test)

Figure 1: Effect of *Moringa oleifera* Pod extract on Oral glucose tolerance test in alloxan induced diabetic rats

SGOT, SGPT and Creatinine

Diabetic Rats showed increased SGOT level (360.00±21.60 IU/l) on day 21. This was significantly higher ($P<0.05$) when compared to serum SGOT levels of normal control rats (282.00±21.74 IU/l). The diabetic animal treated with MOP 50 mg/kg, 100 mg/kg and 200 mg/kg and metformin reports the decrease in SGOT on day-21.

SGPT levels in the control diabetic animals were reported elevated compared to the normal and MOP and metformin treated diabetic rats.

The craetinine level in the normal rats was 0.70±0.03 mg/dl and in the diabetic control rats was 0.83±0.04 mg/dl. The MOP and metfornin treated diabetic rats showed reduced serum creatinine. All the observations are shown in the table 3.

Table 3: Effect of *Moringa oleifera* Pod extract on biochemical parameters in alloxan induced diabetic rats

Sr. No.	Groups	SGOT (IU/l)	SGPT (IU/l)	CREATININE (mg/dl)
1	Normal	282.00±21.74	65.00±2.55	0.70±0.03
2	Diabetic Control	360.00±21.60*	96.00±5.11*	0.83±0.04*
3	Diabetic + Metformin	261.25±20.65**	65.00±6.57*	0.65±0.02*
4	Diabetic + MOP (50 mg/kg)	310.00±4.08*	92.00±16.26	0.84±0.05
5	Diabetic + MOP (100 mg/kg)	280.00±4.08*	78.75±3.14*	0.63±0.00**
6	Diabetic + MOP (200 mg/kg)	252.50±20.15**	64.50±2.06*	0.64±0.01**

n=6, $^{*}p<0.05$, $^{**}p<0.01$ vs. respective control (One way ANOVA followed by Dunnett's test)

DISCUSSION

Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. It is the most common endocrine disorder [7, 8]. India has more than doubled diabetic patients from 19 million in 1995 to 40.9 million in 2007. Every 3rd person in India is potential diabetic.

The aim of the present study was to investigate the effect of *Moringa oleifera* Lam. pod extract in alloxan treated diabetic rats, as it reported to contain flavonoids, which may helps to speed-up the natural healing process and may improve the glycemic control in diabetics.

Acute toxicity study revealed the non-toxic nature of the extract (MOP) at maximum dose of 5000 mg/kg indicating the dosage selected 50, 100 and 200 mg/kg are safe.

The treatment aim in diabetic patients should to reduce the elevated blood glucose to near normal level. The result of present investigation indicate that MOP produce a significant fall in elevated blood glucose levels in alloxan treated diabetic rats. Earlier report states that insulin deficiency occurs in alloxan induced diabetic rats leading to alterations in the carbohydrate metabolism such as elevated blood glucose and reduced level of insulin. In our study, it was observed that MOP reversed these effects and tends to bring the parameters significantly towards the normal and comparable to the observation found with hypoglycemic drug (Metformin) in diabetic rats.

Alloxan causes massive reduction in insulin release, through the destruction of ß-cells of the islets of Langerhans. The chemical constituents of the various plants containing carbohydrates, phenolics, flavonoids, alkaloids, saponins and glycosides gives recovery of islet which gives increasing the pancreatic secretion of insulin from islet of Langerhans [9, 10]. The possible mechanism by which *Moringa oleifera* pod brings about its hypoglycaemic action may be by potentiating the insulin effect by increasing either the pancreatic secretion of insulin from cells of islets of Langerhans or its release from the bound form.

We have registered a decrease in body weight in alloxan diabetic rats. When MOP was administered to animals treated with alloxan, the weight loss was reversed. The ability of *Moringa oleifera* Pod to protect body weight loss seems to be as a result of its ability to reduce hyperglycaemia [11].

Glucose tolerance remains normal because the ß-cell is able to adjust its secretion of insulin to compensate for the existing level of tissue insensitivity to the hormone. The result of diabetic control animals showed impaired glucose tolerance. The additional load of glucose was found to impair the tolerance further as evident from readings after 3 h in vehicle-treated diabetic animals. The result revealed that *Moringa oleifera* Pod and metformin administration increased the utilization of glucose.

In diabetic animals the change in level of serum enzyme are directly related to change in the metabolism in which the enzyme are involved. Many researchers have reported increased transaminase activity in liver and serum of diabetic animals. The increase level of transaminase which are active in the absences of insulin because of increase activity of amino acid in diabetes are responsible for increased gluconeogenesis and ketogenesis observed in diabetes [11]. In present study the MOP and metformin was effective in reducing the SGOT, SGPT level.

Kidneys maintain optimum chemical composition of body fluids by acidification of urine and removal of metabolite wastes such as creatinine. During renal disease, the concentrations of these metabolites increase in blood. In this study, the levels of creatinine did not appear to increase in any of the treatment groups. This indicates the absence of any significant kidney damage [12].

CONCLUSION

The result of study proved the hypoglycemic effects of MOP in allaoxan treated diabetic rats. MOP also improved the glucose tolerance in diabetic rats. Further MOP lower the elevated SGOT, SGPT and creatinie in diabetic rats. The effects may be due to the presence of flavonoids in MOP, which helps to speed up the natural healing process and may improve the glycemic control in diabetic rats.

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