Original Article

Effect of Pre-treatment with Moringa oleifera (Drumstick) Leaves on Diabetogenesis Produced by Alloxan in Rats

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Abstract

Background: *Medicinal plants constitute an important source of potential therapeutic agents for diabetes.* ***Objective:*** *In the study, we aimed to investigate the pre-treatment effect or preventive effects of Moringa oleifera (MO) leaves on blood sugar of rats.* ***Materials and method:*** *This experimental study was carried out in the department of Pharmacology and Therapeutics of Sir Salimullah Medical College in collaboration with Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. A total 24 long Evans rats were included in this study and divided in to four groups. Hyperglycemia was induced on rats using alloxan (100 mg/kg body weight, intraperitioneally). Blood sample was collected from tail vein by tail tipping method. Pre-treatment effect or preventive role of Moringa oleifera (drumstick) leaf powder on diabetogenesis produced by Alloxan in rats was tested by giving 50 mg/rat/day Moringa oleifera leaf powder for 14 days orally as pre-treatment along with standard rat feed. Then alloxan was administered intraperitoneally on 15th day of the experiment and 50mg/rat/day Moringa oleifera leaf powder was given for 7 days as post-treatment.* ***Results:*** *No significant effect of MO on blood glucose level was observed on normal rats and non significant hypoglycaemic effect was found in rats that were pretreated with MO.* ***Conclusion:*** *The present study suggests that Moringa oleifera leaf powder did not produce any significant protective effect in diabetogenesis produced by alloxan though it has hypoglycaemic effect.*

Keywords: *Plants; oleifera leaf; diabetogenesis.*

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Introduction

Diabetes affects nearly 10% of the population all over the world. The chief reason is urbanization and life style, besides heredity, race, age, nutritional status, stress, altered immune function, altered physiological and metabolic status, drugs and hormones.[1] Diabetes is the leading cause of an array of problems including kidney failure, heart disease, amputation, destructive periodontitis and blindness.[2] Many oral synthetic antidiabetic agents have been developed.[3] However, these synthetic agents produce some serious side effects and are relatively expensive for developing countries.[4] Dietary modification, weight control and regular exercise are the main approaches in the management of diabetes, diet being the sheet anchor.[5] Currently, the challenge is to identify hypoglycaemic diet supplements to control blood glucose level and delay onset of diabetes by some protective means. Moringa oleifera (MO) have been reported to have hypoglycaemic properties

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in animal as well as human studies.[6] Therefore the present study was conducted to evaluate the protective effect of MO using 50 mg/rat/day Moringa oleifera leaf powder orally for 14 days as pre-treatment.

Moringa oleifera (drumstick tree) belongs to Moringacea family which accounts 14 species. MO has anti-cancer, anti-inflammatory and thyroid status regulator efficacies and researchers reported its hypoglycemic potential.[7-10]

Materials and method

This experimental study was carried out in the department of Pharmacology and Therapeutics of Sir Salimullah Medical College, Dhaka, Bangladesh in collaboration with Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh during the period of January 2009 to December 2009.

Preparation of Moringa oleifera leaf powder and computation dose

Moringa oleifera leaves were collected in large quantities from BCSIR campus and shade-dried by spreading over a sheet paper under a ceiling fan for five days. The dried leaves were ground in an electric mixer into fine powder and stored in clean sterile glass container. Fifty mg of MO leaf powder was weighed accurately to compute the dose and was administered daily per rat with laboratory diet and water for 14 days as pre-treatment and 7 days as post-treatment after alloxan induction.

Animal and diet

Total 24 adult long Evans male rats of 150-180 gm were included in this study. All rats were kept in cages in room maintained at 26-29^{o}C with a 12 hours light-dark cycle and were allowed free access to food and water *ad libitum*.

Induction of diabetes in rats

Diabetes was induced by intraperitoneal injection of alloxan monohydrate (Loba Chemicals, India) to overnight fasted animals at a dose of 100 mg/kg by partially destroying pancreatic β cells.[10] The diabetic state was confirmed on fourth day by blood glucose determination.

Investigation of the protective effect of Moringa oleifera leaf powder

The animals were fasted for 12 hours but were allowed access to water before and during the experiment. The blood glucose level was monitored before and after alloxanization by withdrawing blood from the tail vein by tail tipping method.[11]

Experimental design

Twenty four (24) rats were divided into four groups of six rats each -

Group I: Control, received vehicle (distilled water & laboratory diet).

Group II: Received laboratory diet and distilled water and 50 mg/rat/day MO leaf powder orally for 21 consecutive days.

Group III: Diabetic control - received laboratory diet and distilled water without MO. Alloxan was administered on day 1 of experiment and received normal diet for 21 days. Blood glucose level was estimated on day 1, 4, 14 and 22.

Group IV: Pre-treated with 50 mg/rat/day MO leaf powder for 14 days orally and as post-treatment for 7 days after alloxan induction. In this group alloxan was administered on 15th day of the experiment and fasting blood glucose level was estimated on 1st, 15th day, 18th and 22nd day of the experiment.

Only rats with fasting blood glucose >10 mmol/L were considered diabetic and used for experiment.[12] Body weights of rats were recorded before and after treatment.

Statistical processing of data

The collected data were processed and statistical analyses were done by unpaired Student's t-test. The level of significance was set at p value of 0.05 to 0.001. All statistical analyses were done by using the SPSS 11.5 version for Windows.

Results

Effcts of M. oleifera on fasting blood glucose level on normal healthy rats

The effect of chronic administration of 50 mg/rat/day of MO leaf powder for 21 days on blood glucose level of normal rats were observed and it was found that MO did not produce any significant change in blood glucose level in comparison to vehicle (laboratory diet+distilled water fed) as shown in table I.

Table I: Effect of M. oleifera on blood glucose level on normal rats

Group	Fasting blood glucose (mmol/L) Mean±SD			p value
	Day 1	Day 14	Day 22	
I	4.9±0.08	5.08±0.14	5.08±0.15	>0.05
II	4.9±0.07	5.05±0.06	4.9±0.0 5	

Effects of M. oleifera on fasting blood glucose level on M. oleifera pre-treated rat

Blood glucose level of Group III was estimated on day 1, 4, 14 and day 22. Alloxan was administered in this group on 1st day of the experiment. In case of Group IV blood glucose level was estimated on day 1, 15, 18 and day 22. Alloxan was administered on day 15 of the experiment on the rats of this group. In each case blood sugar was measured after 3 days of alloxan administration as it requires 3 days for diabetes induction (Table II).

Table II: Distribution of blood glucose levels between Group III and Group IV

Group	Test days	Fasting blood glucose (mmol/L) Mean±SD
III	Day 1	5.08±0.15
	Day 4*	12.01±0.54
	Day14	12.03±0.58
IV	Day 1	4.6±0.13
	Day 15	4.5±0.12
	Day18*	10.98±0.38

*** 3 days after alloxan administration**

Administration of MO leaf powder as pre-treatment for 14 days did not produce any significant effect (p>0.10) on diabetogenesis produced by alloxan shown on Table III.

Table III: Comparison of blood glucose levels of Group III and Group IV 3 days after alloxan administration

Group	Test days	Fasting blood glucose (mmol/L) Mean±SD	p value
III	Day 4*	12.01±0.54	> 0.10
IV	Day18*	10.98±0.38	

*** 3 days after alloxan administration**

Post treatment effect of M. oleifera for 7 days

In Group IV blood glucose level reduces from 10.98±0.38 to 9.76±0.32 (mmol/L) after treated with MO for 7 days that is on 22nd day.

Discussion

From the obtained results, we can conclude that Moringa olejfera has hypoglycemic effect in alloxan induced diabetic rat but no significant preventive role on diabetogenesis produced by alloxan. It was observed by giving MO leaf powder 50 mg/rat/day orally as pre treatment for 14 days and for 7 days as post treatment after alloxan induction. The study revealed that MO did not produce any significant (p>0.10) effect in diabetogenesis produced by alloxan. It can be said that MO leaf has therapeutic effect in diabetic rats but no significant protective effect which is revealed by pre-treatment study. It can be assumed that MO might only restore the diminished beta cell numbers but cannot prevent beta cell destruction produced by alloxan. Similar observation was made by other observers.[13,14]

The reason why pretreatment with MO leaves in diabetic rats resulted in slight reduction of hyperglycaemia but not significant is unclear. It reduces blood glucose from 12.01±0.54 mmol/L to 10.98±0.38 mmol/L. Though this difference is not statistically significant but reduction in blood glucose level is evident in short extent. Pretreatment of MO before induction of diabetes might cause a gradual accumulation of active ingredients which may not have been enough to act against alloxan induced β cell damage by increasing islets superoxide dismutase activity.[15]

The concept of pretreatment with MO was taken from the study of white Ocimum Sanctum Linnean leaves by Suanarunsawat and Songsak.[16] In their study they showed, pretreatment with white Ocimum Sanctum Linnean for 3 weeks before induction of diabetes reduced alloxan induced hyperglycaemia. The present study doesn't correspond with the study conducted by them.

Findings from the study indicate that alloxan induced hyperglycaemia can be ameliorated by pretreatment with MO leaf powder. Based on this result, MO leaf powder can be recommended in the daily diet of NIDDM subjects for effective management of diabetes. It has been suggested that MO is at least partially dependent on insulin release from β cell for its antihyperglycaemic activity. Besides this, the major bioactive compounds of dried MO were found to be flavonoid compounds which have antioxidant activity.[17] Supplementation of antioxidants may be a protective factor against free radical induced β cell damage[18] thus preventing or ameliorating diabetes mellitus. On the other hand, flavonoids inhibit cAMP phosphodiesterase which is a modulator of insulin secretion.[19,20]

It could be concluded that, MO leaf is safe and rich in many constituents that are pharmacologically active and can be used as dietary supplement in improving diabetic mellitus. Further studies can be done to identify the active principles responsible for the hypoglycemic effect.

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