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INVESTIGATION OF ANTI-DIABETIC PROPERTIES OF *MORINGA OLEIFERA* BARK EXTRACT IN ALLOXAN INDUCED TYPE-2 DIABETIC RATS.

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Abstract

The present study investigated the antidiabetic properties of *Moringa oleifera* bark (MO) extract in alloxan induced type-2 diabetic rats. Diabetes was induced by a single IP injection of alloxan (150mg/kg) in SD rats. Extract of *Moringa oleifera* (250 and 500mg/kg) and glibenclamide (10mg/kg) were orally administered for 21 days. Blood glucose levels were measured at day 0, 7, 14 and 21 of treatment. At the end, rats were sacrificed and biochemical parameters like-fasting serum glucose (FBG), total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), high density lipoproteins (HDL), ALT, AST and S. creatinine levels were estimated. Results indicated a significant improvement in blood glucose level in both the acute ($p<0.001$) and chronic study ($p<0.001$), MO significantly lowered TC ($p<0.001$), TG ($p<0.001$), LDL-C ($p<0.001$) levels and improved HDL-C insignificantly in hyperglycemic subjects. Administration of MO significantly reduced the SGPT ($p<0.001$), SGOT ($p<0.001$) and creatinine levels ($p<0.01$) when compared to diabetic control group. The investigation showed that the MO bark extract has glucose and lipid lowering capacity in alloxan-induced diabetic rats. The study also provides a scientific rationale for the use of *M. oleifera* in the management of diabetes in traditional use.

Keywords: *Type-2 diabetes mellitus, alloxan induced diabetes, Moringa oleifera, Antidiabetic effect.*

Introduction

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced [1]. Diabetes mellitus is a major public health problem in the developed as well as developing countries. It is ranked seventh among the leading causes of death, and third when it's fatal complications are taken in to account [2]. The International Diabetes Federation (IDF) estimated the global burden of diabetes was 366 million in 2011 and it would rise to 552 million by 2030 [2].

Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for the development as pharmaceutical entities or as simple dietary adjuncts to existing therapies [3]. Herbal treatments are becoming increasing by popular as the herbal preparations have no or least side effects [4]. Many herbal medicines have been recommended for the treatment of diabetes [5]. Bangladesh is a country of great biodiversity of medicinal plants having a long history of use of traditional medicine. As a result, research in medicinal plants has been an area of discovering lead compounds in Bangladesh [6].

Moringa oleifera (MO) belongs to the family moringaceae, is a small or middle sized tree and usually grows 10-12m in height. The plant is indigenous and abundantly seen in India, Pakistan, Bangladesh and Afghanistan. A good number of phytochmicals has been isolated and reported from various parts of *M. oleifera* [7, 8, 9]. *M. oleifera* is traditionally used as abortifacient, antifungal, antibacterial, stimulant, tonic and diuretic and antiseptic [10]. It is also used in fever, diabetes, epilepsy, asthma, enlarged liver & spleen, pain and inflammation [11]. The present study investigated the antidiabetic effect of *Moringa oleifera* bark extract in alloxan induced type-2 diabetic model rats.

Methods

Plant collection and Extraction: The bark of *Moringa oleifera* was collected, taxonomically identified and authenticated by Md. Abdur Rahim, Taxonomist, Department of Botany, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh. The collected materials were shed dried, crushed into coarse powder and was extracted using ethanol.

Experimental Animals: The study was conducted with adult Sprague Dawley (SD) rats (weighing 150-200g) of either sex. All protocols for animal experiment were approved by the institutional animal ethical committee.

Induction of Type 2 Diabetes: Type-2 diabetes was induced by a single IP injection of alloxan monohydrate (150 mg/kg, b.w). The type 2 diabetic rats with blood glucose levels of 8–12 mmol/L under fasting conditions were selected for the experiments [12].

Acute study : *Moringa oleifera* extract (250 and 500mg/kg) was orally administered to 12 h fasted rats. The control animals received an equal volume of distilled water and standard group received glibenclamide (10mg/kg). One hour after oral administration, glucose was administered and thirty minutes after glucose load serum glucose levels were measured at 0, 60 and 120 minutes [13].

Experimental Design of chronic study: The experimental animals were divided into five groups and treated as follows. All doses were continued for 21 days in hyperglyceamic rats. Blood samples were collected from the cut tip of the tail at 0, 7, 14 and 21st day from the respective start of treatments and measured serum glucose. At the final day, animals were sacrificed; serum was prepared and subjected to further analysis of total cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL), creatinine, SGPT and SGOT level [12].

Group Name	Treatment
Normal Control, Normal rats (n=6)	Water, 10 ml/kg bw.
Diabetic Control, Type-2 rats (n=6)	Water, 10 ml/kg bw.
STD, Type-2 rats (n=6)	Glibenclamide, 10mg/kg bw
MO 250mg/kg, Type-2 rats (n=6)	Extract of *Moringa oleifera* at 250 mg/kg bw.
MO 500mg/kg, Type-2 rats (n=6)	Extract of *Moringa oleifera* at 500 mg/kg bw.

Statistical analysis: Graphs were prepared by MS Excel 2007 and data analysis for animal studies were done by SPSS 16.0 using One way ANOVA followed by Bonferroni's post hoc test. All the data were presented as Mean±SEM. *(P<0.05), **(p<0.01) and ***(p<0.001) were counted as significant, highly significant and very highly significant respectively as compared to the vehicle treated diabetic control group [14].

Results

Oral glucose tolerance test was carried out in glucose loaded diabetic animals. Glucose level was at increased level till 60 min after glucose administration in MO and diabetic control groups. At 120 min after glucose administration glucose level was observed to be reduced significantly by MO at 250mg (p<0.05) and 500mg/kg (*P*<0.01) showing antihyperglycemic effect (figure 1).

Treatment with *M. oleifera* for 21 days improved the fasting glucose level. Treatment with MO did not reduced FBG significantly till 7 days. At the day 14, MO 250mg/kg reduced FBG significantly (p<0.05) and MO 500mg/kg reduced highly significantly (p<0.01). At the day 21, the effect was observed highly significant at 250mg/kg (p<0.01) and very highly significant at 500mg/kg (p<0.001) respectively. Glibenclamide (10mg/kg) showed very highly significant effect (p<0.001) as compared to the vehicle treated diabetic control group (figure 2).

The effect of the administration of MO on serum lipids like TC, TG, LDL-C, and HDL-C were presented in figure 3. Serum TC, TG and LDL-C levels were very highly significantly (p<0.001) decreased by the treatment with MO at both the doses and HDL-C level was increased insignificantly as compared to the diabetic control group.

In the present study, we also observed protective capacity of MO against the renal damage of alloxan in diabetes (figure 4). The serum creatinine level was decreased highly significantly (p<0.01) after 21 days treatment of MO at the dose of 500mg/kg.

The effect of *M. oleifera* on SGPT and SGOT were presented in figure 5. IP administration of alloxan increased liver function biomarkers such as SGPT and SGOT in comparison with normal control rats. Both the enzymes level was reduced by treating diabetic rats with MO for 21 days. At MO 250mg/kg, the reduction of SGPT and SGOT were significant (p<0.05) and very highly significant (p<0.01) respectively. At 500mg/kg the effect was highly significant (p<0.01) and very highly significant (p<0.001) for SGPT and SGOT respectively. The standard drug glibenclamide at 10mg/kg dose reduced SGPT and SGOT level very highly significantly (p<0.001) as compared to vehicle treated diabetic control group.

Discussion

The prevalence of diabetes is rising relentlessly around the world. Current estimates suggest that, globally, the number of persons with diabetes will rise from 151 million in the year 2000, to 300 million by 2025 [15, 16]. This rise is predicted to occur in virtually every nation, with the greatest increases expected in developing countries. Diabetes mellitus causes disturbances in the uptake of glucose by cells as well as glucose metabolism. Thus, alloxan induced hyperglycemia is a very useful experimental way of studying and demonstrating the activity of new hypoglycemic agents [17]. Alloxan has been observed to cause a massive reduction of the β-cells of the islets of Langerhans and induce hyperglycaemia in rats [18].

Oral glucose tolerance tests were used to analyze blood glucose levels taken at different regular intervals after repeated treatments with *M. oleifera* bark extracts. Results of the oral glucose tolerance test, using MO extract indicated remarkable decrease in fasting blood glucose levels after 21 days treatment. This suggests that the MO extract enhanced glucose tolerance in diabetic rats. It also ensures that glucose tolerance test (GTT) is a suitable measure for the ability of cells to utilize glucose [19].

The abnormal high concentration of serum lipids is mainly due to increase in the mobilization of free fatty acids from the peripheral fat deposits, because insulin inhibits the hormone sensitive lipase production. The elevated level of serum lipids in DM causes the risk of coronary heart disease [20]. However, administration of MO to diabetic rats brought the values to near normal. Therefore, MO demonstrated hypolipidemic effects while at the same time increasing the HDL-C and this may reduce the susceptibility of oxidative stress.

In this study, *M. oleifera* positively regulated glycaemia, restored the desirable lipids while not adversely affecting centers of metabolism and excretion, the liver and kidneys, as observed by the renal function markers, creatinine. Treatment with MO showed improved effect on creatinine level which indicated the efficacy of the extract in improving renal function in diabetes.

The activities of SGPT and SGOT in serum were altered in DM. In diabetic animals, the changes in the levels of SGPT and SGOT are directly related to changes in metabolism in which the enzymes are involved. The increased activities of transaminases, which are active in the absence of insulin due to the availability of amino acids in the blood of DM and are also responsible for the increased gluconeogenesis and ketogenesis [21, 22]. SGPT and SGOT levels also act as an indicator of liver function hence restoration of normal level of these enzymes indicates that the normal functioning of liver [23]. The restoration of SGPT and SGOT to their respective normal level was observed in the MO treated groups which indicated the ability of the test sample to restore the liver function in diabetic subjects.

Conclusion

Results of the above observation confirmed the antidiabetic activity of *M. oleifera* bark extract in type-2 diabetic rats on both acute and chronic study. Triglycerides, total cholesterol and LDL were also reduced significantly where HDL was increased. The test extract also improved other biochemical parameters for diabetes like- creatinine, SGPT and SGOT. Therefore, it can be concluded that the extract acts as antidiabetic agent. These studies could provide a rationale for the use of this plant extract in type-2 DM traditionally and further studies are necessary to assess the exact mechanism for this effect.

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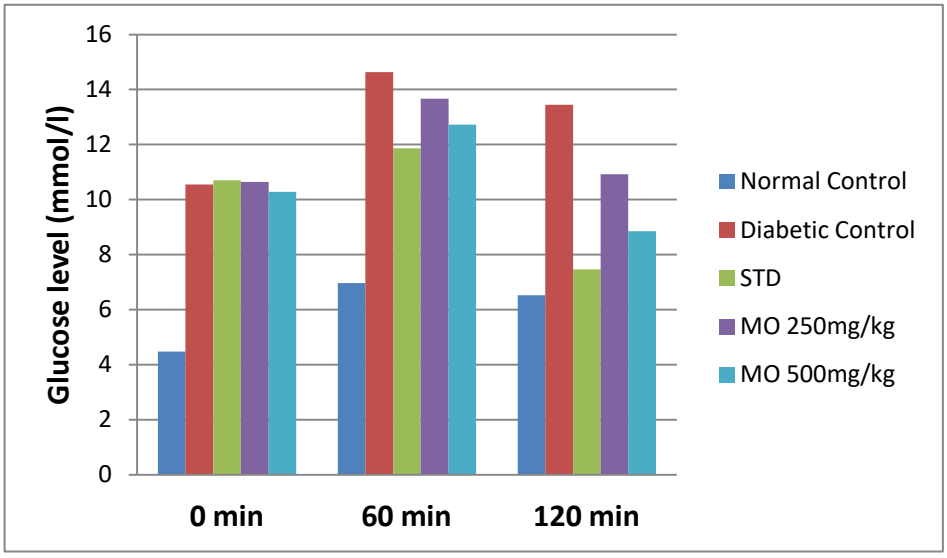

Figure 1: Effect of *M. oleifera* bark extract on the oral glucose tolerance test (OGTT) in alloxan induced type-2 diabetic rats.

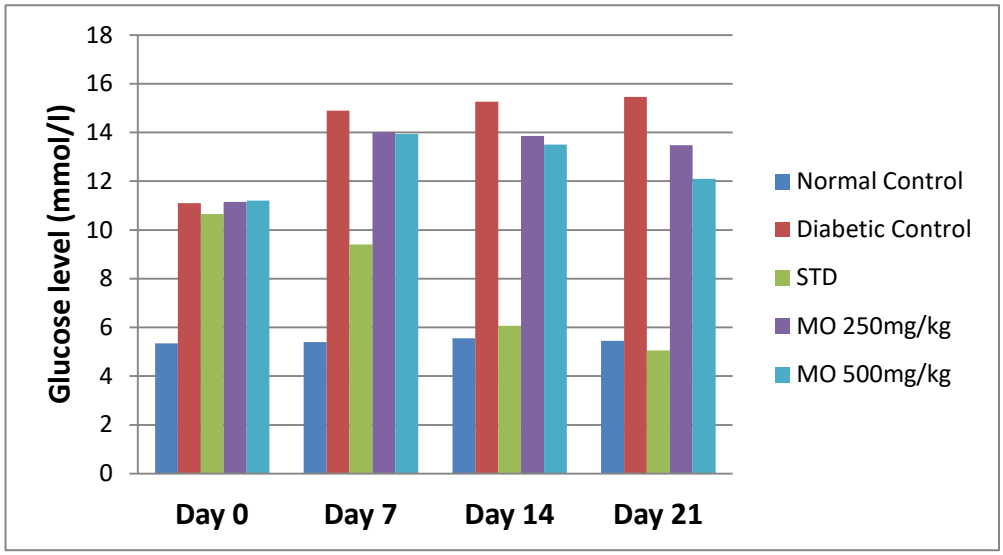

Figure 2: Showing the effect of ethanolic extract of M. oleifera bark on the fasting blood glucose level after 21 days feeding in type-2 diabetic rats.

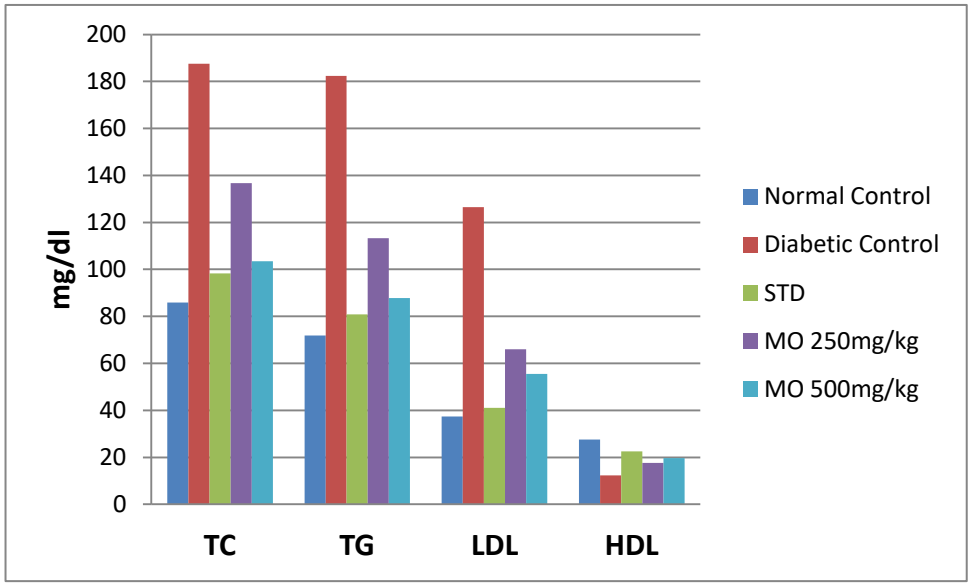

Figure 3: Effect of the ethanolic extract of M. oleifera bark on total cholesterol (TC), triglycerides (TG), HDL and LDL level after 21 days feeding in type-2 diabetic rats.

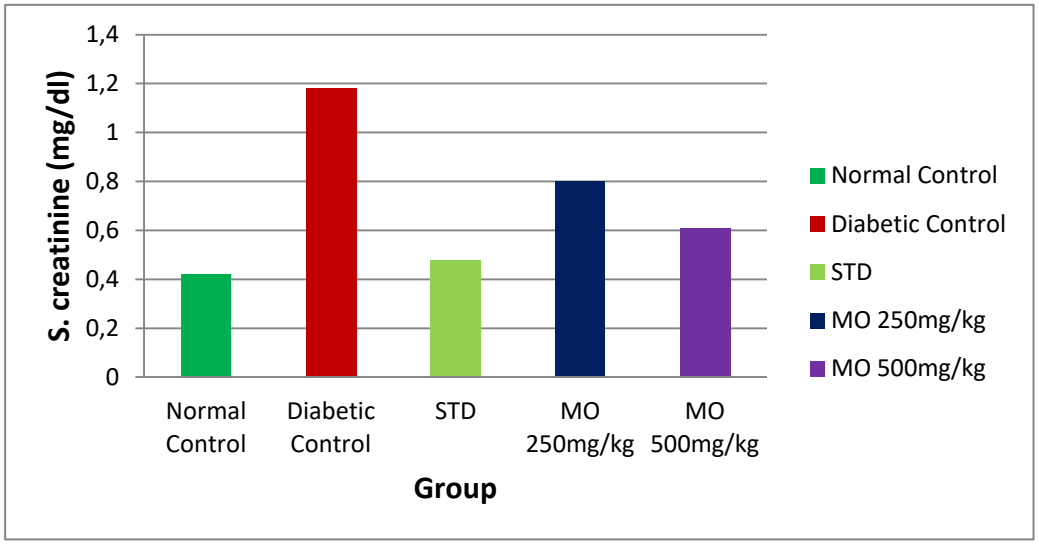

Figure 4: Effect of ethanolic extract of *M. oleifera* bark on the serum creatinine level after 21 days feeding in type-2 diabetic rats.

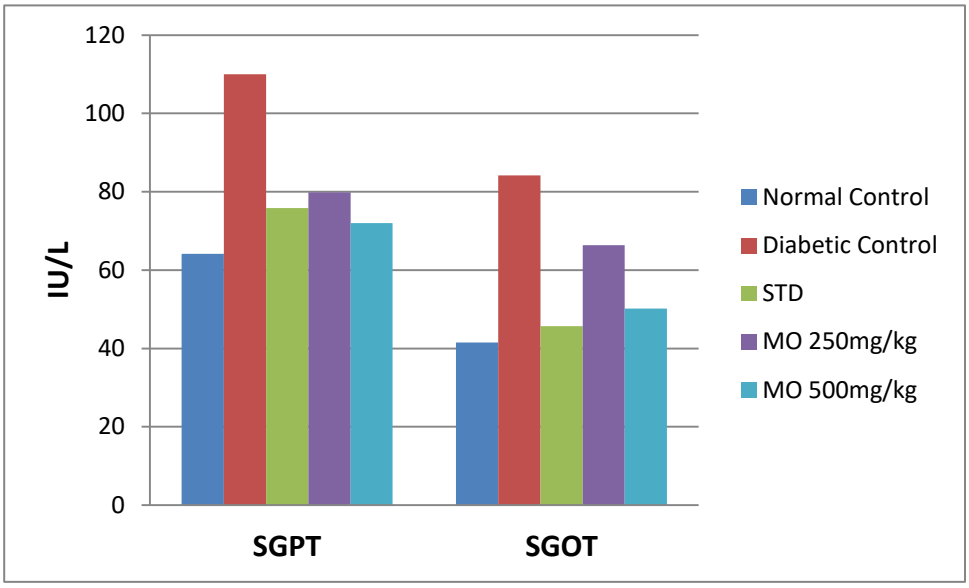

Figure 5: Effect of ethanolic extract of *M. oleifera* bark on the SGPT and SGOT level after 21 days feeding in type-2 diabetic rats.