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Antidiabetic and Glycogenesis Effects of Different Fractions of Ethanolic Extract of Leaves of *Mangifera indica* (Linn.) in Normal and Alloxan Induced Diabetic Rats

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Diabetes is a major public health problem. The development of new therapies that are able to improve glycemia management and even to cure diabetes is of great interest. The antihyperglycemic activity of leaves of *Mangifera indica* was evaluated with scientific approaches. The study was undertaken to investigate the antihyperglycemic, Oral Glucose Tolerance Test (OGTT) and glycogenesis effects of the different fractions (Petroleum ether, ethyl acetate and chloroform) of ethanolic extract of *Mangifera indica*. The different extracts were administered intraperitoneally as a single dose of 150 mg kg⁻¹ b.wt. to normal, glucose induced and alloxan induced diabetic rats and found to reduce blood glucose level significantly (p<0.05). Beside these, the different fractions of *Mangifera indica* to the alloxan-induced diabetic rats resulted in the significant elevation of liver glycogen content which was decreased by 50.60% in diabetic control. The effects of plant extracts were compared with standard drug metformin. The phytochemical screening tests indicate the different constituents such as triterpenes, alkaloids, flavonoids etc. are present in the plant which have the antidiabetic property. Thus, this investigation paves the way for plant based diabetic treatment and indicates that various fractions (Petroleum ether, ethyl acetate and chloroform) of the ethanolic extract of *Mangifera indica* have favorable effect in bringing down the severity of diabetes as well as increase glycogenesis activity by increasing the cellular uptake of glucose.

Key words: *Mangifera indica*, fasting blood glucose (FBG), liver glycogen, intraperitoneally, glycogenesis, Oral Glucose Tolerance Test (OGTT), alloxan

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disease which now afflicts 3% of the world population. Diabetes mellitus is classified into two types (type 1 and 2) based on individual etiologies. Around 95% of diabetic patients are diagnosed with type 2 diabetes (Attele *et al.*, 2002). A major feature of type 2 diabetes is insulin resistance and/or insulin deficiency which can cause hyperglycemia (Laakso, 2001). The pathogenesis of diabetes mellitus and the possibility of its management by existing therapeutic agents without any side effects have stimulated great interest in recent years (Bailey, 1999).

The number of cases of non-insulin dependent diabetes mellitus (Type 2) has increased dramatically due to the changes in lifestyle, increasing prevalence of obesity and ageing of populations. In the year 2000, the number of diabetic patients was 151 million and is estimated to rise to 300 million by 2025 (King *et al.*, 1998).

According to the World Health Organization, more than 70% of the world's population must use traditional medicine to satisfy their principal health needs. A great number of medicinal plants used in the control of the DM have been reported (Bailey and Day, 1989).

Plants are recognized as a wonderful source for medicines. It is estimated that 1200 species of plants are used as folk medicines for diabetes (Marles and Farnsworth, 1995). Most of them lack of scientific evidence for their alleged benefits.

There are various medicinal plants in the world, which are the potential sources of the drugs. The discovery of the widely used hypoglycemic drug, metformin (N, N-dimethylguanylguanidine) came from the traditional approach through the use of *Galega officinalis* (Grover *et al.*, 2002a). Traditionally various plants are being used to treat diabetic patients. It is believed that, herbal medicine has little side effects as well as it requires no cost in few cases. Thus it can solve the economic problem of the poor. Now a day, the scientists and researchers are looking for natural plant products by research all over the world and a large number of the evidence have shown the immense potential of medicinal plants used traditionally.

Therefore, a key strategy in treating patients with type 2 diabetes is maintenance of blood glucose level. Current oral anti-diabetic agents, which include insulin releasers, insulin sensitizers and glucosidase inhibitors, have modest efficacy and limited modes of action. In addition, current anti-diabetic drugs usually have adverse side effects, decreased efficacy over time, ineffectiveness against some long-term diabetic complications and low

cost-effectiveness (Grover *et al.*, 2002a). Therefore, discovery and development of novel drugs for diabetes is still needed.

Bangladesh is full of medicinal plants, which are used by the people for the treatment of various diseases even at this modern era. In Bangladesh about 5 million people are affected with diabetes for various reasons, in recent years the popularity of complementary medicines has increased. Dietary measures and traditional plant therapies as prescribed by Ayurvedic and other indigenous systems of medicine have been used commonly in India (Warrier *et al.*, 1996).

Mangifera indica (Family: Anacardiaceae) is used medicinally as an astringent, for bronchitis, catarrh, internal hemorrhages, skin disease and toothache. The leaf is claimed to be useful in the treatment of diabetes by local herbalists who led us to investigate its antihyperglycemic properties (Aderibigbe *et al.*, 1999).

The objective of the present study was to make an analysis of the ethno botanical information on the medicinal plants used in diabetes control and of the results obtained in the investigation of the hypoglycemic Activity of such plants.

In the present study the antidiabetic properties of the leaves of *Mangifera indica* was assessed by evaluating the comparative antihyperglycemic, oral glucose tolerance test (OGTT) and glycogenesis activities in normal, alloxan induced and glucose induced diabetic rats.

MATERIALS AND METHODS

Plant materials: The fresh leaves of *Mangifera indica* (Local name- Aam) were collected from medicinal plant garden. The plant parts were dried completely under the mild sun and crushed with an electric grinder in to coarse powder and used for cold extraction. The authenticity of the *Mangifera indica* was identified by Mr. AHM Mahbubur Rahman, Department of Botany, University of Rajshahi.

Preparation of crude extract: In cold extraction the coarse powder was submerged in methanol. Flat bottom 2.5 L reagent bottle were used for this purpose which were kept at room temperature and allowed to stand for several days (7-10) with occasional shaking and stirring. When the solvent become concentrated, the liquid alcohol content were filtered through cotton and then through filter paper (Whatman filter paper No. 1) Then the solvents were allowed to evaporate using rotary evaporator at temperature 40-45°C. Thus the highly concentrated crude extracts were obtained.

Fractionation of crude extract: The crude extract was ready for further fractionation. The crude extract was diluted by addition 150 mL distilled water to obtained aqueous solution. The aqueous solution was then treated with 50 mL petroleum ether for three times. The upper fraction was collected in each time of fraction by using separating funnel. The aqueous fraction was then treated with 50 mL ethyl acetate for three times. The upper fraction was collected in each time of fraction by using separating funnel. The aqueous fraction was then treated with 50 mL chloroform for three times. The lower fraction was collected in each time of fraction by using separating funnel. The fractions were then evaporated by rotary evaporator. The remaining portions of the different fractions were then dried by using desiccators and then mild sunlight. The dried extracts were then preserved in the freezer for the experimental use

Phytochemical screening methods: The following Phytochemical screening methods (Nayak and Pinto Pereira, 2006) were used for the tests:

Test for saponins: Boiled 300 mg of extract with 5 mL water for two minutes. Mixtures was cooled and mixed vigorously and left it for three minutes. The formation of frothing indicates the presence of saponins.

Test for tannins: To an aliquot of the extract added sodium chloride to make to 2% strength. Filtered and mixed with 1% gelatin solution. Precipitation indicates the presence of tannins.

Test for triterpenes: Three hundred milligram extract mixed with 5 mL chloroform and warmed for 30 min. The chloroform solution is then with a small volume of concentrated sulfuric acid and mixed properly. The appearance of red color indicates the presence triterpenes.

Test for alkaloids: Three hundred milligram extract was digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature and examined the alcoholic layer for the pink color indicates the presence of alkaloids.

Test for flavonoids: The presence of flavonoids was determined using 1% aluminum chloride solution in methanol, concentrated HCl magnesium turnings and potassium hydroxide solution.

Selection of animals: A total number of 30 long-Evans female rats weighing about 150-180 mg, age 2 months were purchased from animal's house of International

Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). Prior to commencement of the experiment, all the rats were acclimatized to the new environmental condition for a period of one week. During the experimental period the rats were kept in a well ventilated animal house at room temperature of 25°C and were supplied with standard pellets supplied from ICDDR, B and fresh drinking water. All the rats were kept in cages with wide square mesh at bottom to avoid coprophagy and maintained with natural 12 h light and dark cycle.

Grouping of experimental rats: Fifty long-Evans female rats were randomly assigned in to 10 groups, 5 rats in each group.

Group 1: Normal Control

Group 2: Diabetic Control

Group 3: Diabetic+ Metformin (150 mg kg⁻¹ b.wt.)

Group 4: Diabetic+ Pet-ether fraction MI (150 mg kg⁻¹)

Group 5: Diabetic+ Ethyl acetate fraction MI (150 mg kg⁻¹)

Group 6: Diabetic+ Chloroform fraction MI (150 mg kg⁻¹)

Group 7: Normal+ Glucose

Group 8: Normal+ Glucose+ Metformin (150 mg kg⁻¹)

Group 9: Normal+ Glucose+ Pet-ether fraction MI (150 mg kg⁻¹)

Group 10: Normal+ Glucose+ Ethyl acetate fraction MI (150 mg kg⁻¹)

Drugs and chemicals used: Metformin was the generous gift sample from Square Pharmaceuticals Ltd., Pabna, Bangladesh. DMSO was purchased from Loba Chemie, Bombay, India. DMSO (dimethyl sulfoxide) was used to dissolve metformin and the extracts of *C. roseus*, since these substances are insoluble in water and other available inert solvents (Akhtar *et al.*, 2007). Alloxan was purchased from Loba Chemie, Bombay, India.

Experimental induction of diabetes: Group 2-6 animals were allowed to fast for 12 h were rendered diabetic by injection intraperitoneally a freshly prepared solution of alloxan (110 mg kg⁻¹ b.wt.) in normal saline after base line glucose estimation was done. The alloxan treated animals were allowed to food over night to overcome drug induced hypoglycemia. After 48 h blood glucose content was measured by using Bioland G- 423 test meter (Bioland, Germany) using blood sample from the tail vein of the rats. When the condition of diabetes was established animals with blood glucose levels above 11.1 mmol L⁻¹ was selected for the study.

Preparation of dosage of active drug and plant extract

Metformin: Metformin was in microcrystalline form and freely soluble in water. The dosage was prepared in solution form using sterilized water in such a concentration that, each 0.1 mL of solution contained metformin according to the dose of 150 mg kg⁻¹ b.wt. since metformin is effective in such dose in case humans.

Mangifera indica: The fractionated extracts of *Mangifera indica* were dissolved in 99% DMSO to prepare the solution where each 0.1 mL contained *Mangifera indica* according to the dose of 150 mg kg⁻¹ b.wt. (Gosh *et al.*, 2001). 0.1 mL of each solution was administered intraperitoneally to every 100 g b.wt. of the rats during treatment to achieve required dose of plant extract.

Antihyperglycemic effects of plant extracts: The group 2-6 was prepared for resting antihyperglycemic effect after alloxan induction. All the rats were starved at water for 16 h. All the rats were tested for baseline glucose level. The group 2 was selected for diabetic control group which does not receive extract or metformin. The group 3 stands for metformin control group which is administered metformin intraperitoneally at a dose of 150 mg kg⁻¹ b.wt. The group 4-9 receives the different fractions of extract of the plants. The blood glucose level was then tested by using glucometer (Bioland Glucometer, Germany). In this case, the blood was collected by picking the tail vein in 0, 2, 6, 16 and 24 h after drug and plant extract administration.

Estimation of glycogen content in liver: The liver was collected from hearts after sacrificing the rats. Then liver glycogen concentrations were analyzed by taking absorbance by UV spectrophotometer, using o-toluidine reagent diagnostic kits (E PFLUGER Arch. Ges Physiologic 196, 1993). This test utilizes the o-toluidine-glucose coupling reaction E HULTMAN, Nature 183 (1959) 108 for the estimation of glycogen after trichloroacetic acid (TCA) extraction, precipitation by alcohol and hydrolysis.

Oral glucose tolerance test: Groups 7-10 were selected for OGTT test after starving at water for 16 h. The base line glucose level was measured by glucometer (Du-Vigneaud and Karr, 1925). Group 7 stands for normal control group. Group 8 was treated with metformin (150 mg kg⁻¹ b.wt.). The extracts of different fractions were then administered intraperitoneally at the dose of 150 mg kg⁻¹ b.wt. 2 mL glucose (2 g kg⁻¹ b.wt.) solution was administered orally by intra gastric tube. The blood glucose level was then tested by using glucometer

(Bioland Glucometer, Germany). In this case, the blood was collected by picking the tail vein in 0, 30, 60, 90, 150 and 270 min.

Statistical analysis: The results are expressed as Mean±SEM using Graph Pad Prism (version 4.0) computer program (Graph pad Software San Diego, CA, USA). We used a one-way Analysis of Variance (ANOVA), followed by Scheffe's post-hoc test or students paired or unpaired t-test where appropriate. The statistical method applied in each analysis was described in each figure. Results were considered to be significant when p values were less than 0.05 (p<0.05).

RESULTS

The effect of the different extracts of *M. indica* on the Fasting Blood Glucose (FBG) level and glycogen content in liver were investigated in the control and alloxan-induced diabetic rats using metformin as standard antidiabetic agents.

Effect of different fractions of *M. indica* on Fasting Blood Glucose (FBG) level in diabetic rats:

The mean blood glucose concentration of control and different fractions of *M. indica*-treated animals were estimated on the 2, 6, 16 and 24 h, respectively as shown in Fig. 1. Their baseline glucose concentrations was also measured. Ethyl acetate fraction of *M. indica* reduced blood glucose level to 91, 78, 50 and 47 at 2, 6, 16 and 24 h, respectively. Maximum reduction of blood glucose level of 52.81% was observed on 24 h of the experiment. Chloroform fraction of *M. indica* showed reduction of blood glucose level to 75, 63, 37 and 16% at 2, 6, 16 and 24h, respectively. Maximum reduction of blood glucose level of 84% was also observed on 24 h during the 24 h experimental period. Pet-ether fraction reduced blood glucose level to 70, 58, 14 and 11 in 2, 6, 16 and 24 h, respectively. Maximum reduction of blood glucose level of 88.77% was observed for pet-ether fraction in 24 h of experiment.

In case of alloxan induced diabetic rats metformin reduced blood glucose level to 68, 38, 20 and 25% at 2, 6, 16 and 24 h, respectively. So metformin caused maximum reduction of blood glucose level of 80% on 16 hour of the experiment. In case of alloxan induced diabetic rats metformin (Fig. 1) reduced blood glucose level to 66, 38, 20 and 25% in 2, 6, 16 and 24 h, respectively. So, metformin caused maximum reduction of blood glucose level by 80% in 16 h.

Effect of different fractions of *M. indica* on Fasting Blood Glucose (FBG) level in the glucose-induced hyperglycemic rats:

In case of glucose-induced hyperglycemia metformin reduced blood glucose level to

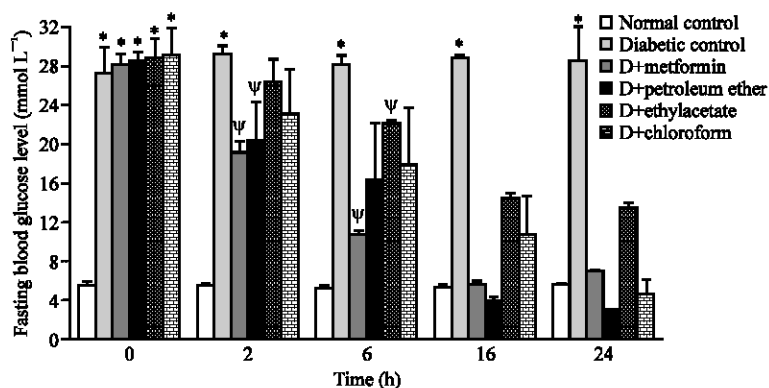


Fig. 1: Effect of different fractions of *M. indica* on the FBG level on diabetic rats compared to normal rats. *Indicates significant change in blood glucose level compared with normal control group ($p < 0.05$). Ψ Indicates significant changes in FBG level in diabetic rats after treatment ($p < 0.05$). The results are expressed as Mean \pm SEM

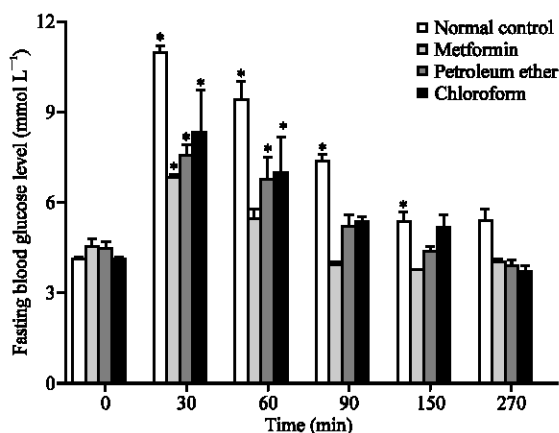


Fig. 2: Effect of different fractions of *M. indica* on the glucose-induced hyperglycemia in normal rats. *Indicates significant changes in FBG level compared to normal rats after treatment ($p < 0.05$). The results are expressed as Means \pm SEM

62, 58 and 53% in 30, 60 and 90 min, respectively. So, metformin caused maximum reduction of blood glucose level of 53% in 90 min. Pet-ether fraction reduced blood glucose level to 69, 72 and 71% in 30, 60 and 90 min, respectively. CHCl₃ fraction reduced blood glucose level to 76, 74 and 74% in 30, 60 and 90 min, respectively (Fig. 2). Here the maximum reduction of 31% was observed by pet-ether fraction in 30 min.

Effect of experimental plant fractions on the level of glycogen in diabetic rats: In this study it is found that the level of glycogen in liver is reduced to 49% in diabetic rats as compared to the normal control group. Treatment of diabetic rats with metformin standard, Pet-ether, Et-Ac and CHCl₃ fractions of *M. indica* the level of glycogen

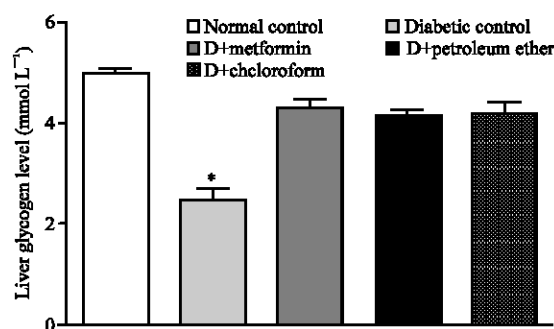


Fig. 3: Effect of different fractions of *M. indica* on the glycogen level in liver on diabetic rats compared to normal rats. *Indicates significant difference ($p < 0.05$) from without alloxan treated time control. The results were expressed as Means \pm SEM

Table 1: The phytochemical constituents of the experimental plant fractions obtained by phytochemical screening tests

Partitionates	Saponins	Tannins	Triterpines	Alkaloids	Flavonoids
Chloroform	+	+	-	+	+
Ethyl acetate	-	+	+	+	+
Petroleum ether	+	-	-	+	+

+: Present, -: Absent

content was improved to 86, 83 and 84%, respectively as shown in the Fig. 3. In this case chloroform fraction of *M. indica* had more significant activity in glycogen synthesis.

Phytochemical screening: The phytochemical screening tests indicate the different constituents such as saponins, tanins, triterpines, alkaloids, flavonoids are present in the plant *M. indica* which have the antidiabetic property. The results are summarized in Table 1.

DISCUSSION

Diabetes mellitus is a metabolic disorder featured by hyperglycemia and alterations in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion and/or insulin action (Rao *et al.*, 2003). It is one of the oldest diseases affecting millions of people all over the world (Andallu and Varadacharyulu, 2002). Although numerous oral hypoglycemic drugs exist alongside insulin, still there is no promising therapy to cure diabetes (Sumana and Suryawanshi, 2001). Over the last few decades the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety. In recent years, numerous traditional medicinal plants were tested for their antidiabetic potential in the experimental animals (Srivastava *et al.*, 1993). In the present investigation, the study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future.

In the light of the literature on *Mangifera indica* we made an attempt for the first time to study the effect of *Mangifera indica* ethanolic extract partitionates in normoglycemic and hyperglycemic rats. The significant anti diabetic activity of pet-ether, ethyl acetate and chloroform fraction of *Mangifera indica* as shown in Fig. 1 may be due to the presence of hypoglycemic saponins, tanins, triterpenes, alkaloids, flavonoids (Table 1). It could be conceived that the plant extracts may also contain some biomolecules that may sensitize the insulin receptor to insulin or stimulates the β -cells of islets of langerhans to release insulin which may finally lead to improvement of carbohydrate metabolizing enzymes towards the re-establishment of normal blood glucose level.

Oral Glucose Tolerance Test (OGTT) measures the body's ability to use glucose, the body's main source of energy (Du-Vigneaud and Karr, 1925). It can be used to diagnose prediabetes and diabetes. In our study, it is found that various fractions have also hypoglycemic effect in glucose induced hyperglycemic rats. The extracts enhanced glucose utilization. So, the blood glucose level was significantly reduced in the glucose loaded rats (Fig. 2). This may be due to the presence of hypoglycemic flavonoids, alkaloids, triterpenes or saponin glycosides. In diabetes, the glycogen content of the skeletal muscles and liver, markedly depleted (Grover *et al.*, 2002b) and the reduced level of hepatic glycogen is due to inadequate insulin secretion, which results in the inactivation of glycogen synthetase system (Sumana and Suryawanshi, 2001).

In the present study decreased levels of glycogen and glycogen synthase were observed in diabetic control

rats. It may be due to insufficient secretion of insulin in the diabetic state as stated earlier. It was reported that (Fig. 3) the treatment with *Mangifera indica* the accumulation of glycogen and its content rises to of normal level. This may be due to activation of glycogen synthetase system by the modulatory effects of constituents of the fractions through induction of insulin secretion. Decreased in the activities of the enzymes involved in glucose homeostasis in liver and kidney such as hexokinase has been reported in diabetic animals resulting in depletion of liver and muscle glycogen content (Grover *et al.*, 2002b). Treatment with plant extracts might increase the level of enzyme to the control level indicating an over-all increase in glucose influx. The exact mechanism of action needs further investigation.

In conclusion the administrations of plant fractions of *Mangifera indica* leaf extracts produced significant restoration of blood glucose level and also have some beneficial effects such as improvement of oral glucose tolerance and increase liver glycogen synthesis activity in alloxan-induced diabetic rats. Thus, in the light of our pharmacological studies the extracts can be useful in liver glycogen synthesis and cellular utilization of glucose. Further comprehensive pharmacological investigations are needed to elucidate the exact chemical compounds responsible for antidiabetic activity, improvement of oral glucose tolerance as well as liver glycogen synthesis activity and also their exact mechanism of actions.

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