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Partial elucidation on the remedial effect of stem-bark extract of *Moringa oleifera* on diabetic rats

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Abstract

Diabetes is growing public health. The research investigated the modulatory roles of the aqueous stem-bark extract of *Moringa oleifera* on glucose utilization. A modified Oral Glucose Tolerance Test (OGTT) was used in studying the effect of the extract on glucose absorption, on four groups of six rats and standard methods were used to test the effect of the extract on enzyme activities (hexokinase and glucose 6 phosphatase) on three groups of six rats. In OGTT, rats in group-1(diabetic control) and group-4 (normal control) were administered with the vehicle only. The other groups were administered different concentrations of the extract in the vehicle (group-2 and 3 were 200mg/kg body weight and 300mg/kg respectively). In the enzymes activities, 200mg/kg body weight of the extract was administered to diabetic treated group whereas normal untreated and diabetic untreated received 5ml of water only. Glucose concentrations of OGTT showed increased concentration in the first 30 minutes after administration of the extract and steady time-dependent decreased concentration through 120 minutes. Group-3, showed a significant difference in each 30 minutes interval, compared to the 120 minutes ($p<0.05$). Each interval is significantly different from the preceded 30 minutes interval ($p<0.005$). Group-2 was significantly different in the first and second 30-minutes intervals, compared to the preceded interval ($p<0.005$). The first 30 minute interval was significantly different from the baseline and 120 minutes ($p<0.05$). In enzyme activities, the diabetic treated and normal untreated were significantly different from the diabetic untreated ($p<0.05$). The extract improved glucose utilization.

Keywords: Diabetes; Modulatory role; OGTT; Glucose 6 phosphatase; Hexokinase

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder, caused by the complete or relative insufficiency of insulin secretion and/or action [1, 2]. It is a multifaceted and multifactorial disease, affecting all vital body organs [3].

This disease is a main threat to human health in this modern-day [4]. The central identifying feature of DM is a chronic and substantial elevation in the circulating glucose concentration [5]. Developing countries such as Nigeria have had a maximum increase in the last few years. The management of DM can be achieved by diet, exercise, insulin replacement therapy, and by the use of herbal hypoglycaemic agents [5].

Hypoglycaemic agent forms an important way of treating diabetes though with several demerits [7]. In traditional medicine, it is found that composite extract is most effective than the separate one [8].

Many of the plants used for treatments of DM have not received proper scientific investigation. On that background, this work was aimed at investigating the modulatory role of the aqueous stem-bark extract on glucose utilization. The

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specific objectives were the determination of the effect of the stem-bark extract on the activities of key glucose utilization enzymes and possible impact on intestinal glucose absorption.

2. Material and methods

2.1. Plant collection and identification

Fresh *Moringa* stem barks were obtained from 12.00 pm to 2.00 pm, in December 2015, from Gwarimpa, Abuja, FCT Nigeria. The plant was identified by the botanist at the International Centre for Ethenomedicine and Drug Development, Nsukka.

2.2. Experimental animals

Forty-two albino rats, weighing 100-130 g, aged 10-12 weeks were purchased from National Institute for Trypanosomiasis Research (NITR) Vom, Jos. The animals were housed in well-ventilated metal cages in the research laboratory. They were kept under controlled environmental conditions of temperature (25 ± 5 oC), relative humidity (50 ± 5 %), and 12 hours light / dark cycle. The animals were allowed to acclimatize for two weeks. The animals were maintained on palletized Growers feed and allowed free access to clean drinking water ad libitum. The rats were then divided randomly, into two groups: Normal and alloxan monohydrate-induced diabetic rat.

2.3. Chemicals and reagents

All the chemicals were of analytical grade. Alloxan monohydrate and Assay kits were purchased from Sigma-Aldrich, USA.

2.4. Plant materials

The stem barks were dried under controlled environmental conditions, under shade at room temperature (26±2°C) for ten weeks, and then macerated into a uniform powder, using a high-speed Creston grinder with 1mm2 sieves. The powdered stem-barks were kept in plastic bags in desiccators [9].

2.5. Preparation of crude aqueous extract

The powdered plant materials (25% w/v) were soaked in cold distilled water for 36 hours, after which it was filtered using a piece of clean, sterile, white Muslin cloth. Six ml of the filtrates were then evaporated in a drying cabinet, set at 40 ^{o}C, and used in calculating the prospective equivalent concentration in mg/kg as described by Harborne [10]. In all, the extract was administered orally.

2.6. Intestinal glucose absorption

A modified oral glucose tolerance test was used for the estimation of intestinal glucose absorption. They were four groups of six rats each. The administration was as follows;

Group I (normal untreated) were given normal saline simultaneously [(NaCl solution (8.5 g/l) + glucose Solution (54 g/l)];

Group 2 diabetic treated [(NaCl solution (8.5 g/l) + glucose solution (54 g/l) + extract (200 mg/kg body weight)],

Group 3 diabetic treated [(NaCl solution (8.5 g/l) + glucose solution (54 g/l) + extract (200 mg/kg body weight],

Group 4 diabetic untreated (NaCl solution (8.5 g/l) + glucose solution (54 g/l)].

The rats in each group fasted for 12 h (8pm to 8 am.) before treatment. The blood glucose was measured using one-touch ultra-glucometer. Blood glucose sugar was monitored at 30-minute intervals for 2 hr.

2.7. Glucose metabolizing enzymes

For the determination of hexokinase (Hk) and glucose 6 phosphatase (G6Pase) activities, the animal was grouped into three groups of six rats each:

Group 1 normal untreated (5 ml/kg clean tape water),
Group 2: Diabetic control (5 ml/kg clean tape water),
Group 3: Diabetic treated (200 mg/kg extract).

Glucose 6 phosphatase and hexokinase activity was estimated based on the method of Baginsky *et al*., [11] and Brandstrup *et al*., [12] respectively.

2.8. Statistical analysis

Data were analyzed for statistical significance by ANOVA. It was further subjected to the Fischer LSD post hoc test using the SPSS.

3. Results

The results are presented in Tables 1, 2, and 3. The result of intestinal glucose absorption (Table 1), depicts initial rise in glucose level during the first thirty-minute of post-treatment and a steady decline in the subsequent time intervals among the normal and extract-treated groups. These increases in glucose levels were continuous in the diabetic control. There was no significant difference in both controls (diabetic and normal control) with their respective intervals in glucose concentrations, from the baseline glucose concentration. There was a significant difference ($p < 0.05$) in each interval in the 300 mg/kg body weight of the extract. In the 200 mg/kg body weight of the extract, there was a significant difference in the preceded 30 and 60 minutes intervals at $p < 0.05$ and the 30-minute interval was significantly different ($p < 0.05$) from both the baseline and120-minute post-treatment.

Table 1 Effect of crude aqueous extract of *Moringa oliefer*a stem-bark on intestinal Glucose (mg/dl) absorption.

Treatment Group	O minute (baseline)	30 minute	60 minute	90 minute	120 minute
Group-1	220.00 ±8.67	222.33 ±10.84	229.00 ±9.19	231.83 ±3.49	231.00 ±3.16
Group-2	214.00 ±5.22	230.33 ±13.35^{ab*}	210.67±8.80^{b}	208.67 ±7.53	203.33 ±3.27
Group-3	217.33 ±7.69^{a}	235.50 ±7.37 ^{ab*}	215.50 ±4.46 ^{ba}	213.167 ±10.40^{ba}	186.00 ±15.17^{b*}
Group-4	88.67 ±12.11	87.00 ±5.18	86.33 ±6.25	80.33 ±7.17	83.50 ±11.38

Values are mean ± standard deviation of mean of 6 animals; ^{a} Significance compared with the diabetic untreated ($p < 0.05$)
^{b} Significance compared with the preceded value ($P<0.05$); *Significance compared with the base line ($p < 0.05$);
^{ba} Significance compared with the 120 minutes ($P<0.05$),

Table 2 Effect of repeated administrations (21 days) of the extract on the activity of hepatic hexokinase.

Parameter	Hexokinase activity (nmoles of glucose phosphate formed /min/mg/protein)
Normal Untreated	390.09±17.26*
Diabetic untreated	81.39±6.90
Diabetic treated with 200 mg/kg body weight	375.28±30.95*

*Significance compared with the diabetic untreated ($p < 0.05$).

As compared with the diabetic control values, the mean serum levels of the enzymes HK (Table, 2) and G6Pase activities (Table, 3) increased and decreased respectively. In the diabetic treated, the HK and G6Pase were significantly different at $p < 0.05$ from the diabetic control group. The treatment with the extract elicited a rise in the activities of hexokinase when compared with diabetic untreated. On the other hand, the extract repressed the activities of G6Pase when compared with the diabetic untreated. Both the HK and G6Pase activities of the normal untreated rats were significantly different from the diabetic untreated rats at $p < 0.05$.

Table 3 Effect of *Moringa oleifera* on repeated administration (21 days) of the extract on the activity of hepatic Glucose - 6- phosphatase.

Parameter	**Glucose -6-phosphatase activities (unit/mg of protein)**
Normal Untreated	9.10±0.37*
Diabetic untreated	14.07±0.43
Diabetic treated with 200 mg/kg body weight	13.32±0.34*

*Significance compared with the diabetic untreated ($p < 0.05$).

4. Discussion

The preliminary results on the elucidation of the partial mechanism of action, presented in Tables 1, 2, and 3, indicated that the extract affects intestinal glucose absorption, Hk and G6Pase activities. The maintenance of a normal plasma glucose concentration requires precise matching of glucose utilization with endogenous glucose production / dietary glucose delivery. Plasma glucose concentration is a function of the rate of glucose entering the circulation (glucose appearance) balanced by the rate of glucose removal from the circulation (glucose disappearance).

The transporter GLUT2 participates in glucose transfer across the intestinal membrane [13, 14]. The inhibition of this transporter is paramount to achieving normal postprandial glucose concentration. The blocking tendency of the extract used in the present study on the intestinal glucose absorption may partly underline the mechanism in the observed decrease in glucose concentration treated rats (Group 2 and Group 3) in the OGTT (Table 1). This finding is in agreement with the works on the leaves of the same plant [15, 16].

In the fasting condition, plasma glucose concentration is relatively stable. This is depicted in the normal control untreated (Table, 1)**,** which indicated that glucose production commensurate with utilization.

The rapid absorption of glucose challenges the regulatory mechanisms of glucose homeostasis, the result of intestinal glucose absorption showed that the extract has decreased intestinal glucose absorption/inhibited the excessive absorption of glucose from the intestines, in the treated groups as compared to diabetic control. Hence glucose entered the bloodstream much slowly suggesting improved glucose tolerance. It was significant in every 30 minutes in 300 mg/kg body weight. It is likely to be expected that the aqueous extract of stem bark has some direct effect by increasing the tissue utilization of glucose [17], by inhibiting hepatic gluconeogenesis or, increasing absorption of glucose into the muscles and adipose tissues [18].

G6Pase has been reported to play an important role in glucose homeostasis in the liver and kidney by activating the gluconeogenic pathway. Numerous studies performed in animal models of diabetes have demonstrated an increase in G6Pase activity among others [19, 20]. In nearly all of these studies, the increase in G6Pase was associated with a reduction in insulin secretion and normalization of plasma glucose by insulin treatment also normalized G6Pase activity. On this premise, it may be that the high G6Pase activity in the diabetic untreated group in table 3, was a result of diabetes and its attendant consequence of reduced insulin secretion. In this study, the G6Pase activity in the liver was elevated in alloxan-induced diabetic rats and this was in agreement with earlier reports in diabetic animals [21]. The administration of *M. oleifera* stem-bark extract resulted in a significant reduction of G6Pase activity, thus making way for proposing another mode of anti-diabetic activity of this plant extract. This result is similar to that previously reported where several plant extracts have been known to improve the diabetic condition [22].

HK is found in all cells that utilize glucose for energy and is involved in glucose homeostasis in liver and kidney tissues through the phosphorylation of hexose, a six-carbon sugar. Decreases in the activities of the HK have been reported in diabetic rats resulting in depletion of liver and muscle glycogen content [22]. To understand more, the biochemical mechanism of action of the *M. oleifera* stem-bark extract as an anti-diabetogenic agent, the study further assayed the hepatic Hk activities in diabetic rats after treatment with an aqueous extract of *M oleifera* stem-bark. Treatment with *M oleifera* stem-bark aqueous crude extract caused an increase in the activity of HK as compared with diabetic untreated, indicating an overal increase in glucose influx. The decline in hexokinase activity in alloxan-induced diabetic rats (Table 3) could be due to deficiency of insulin [23]. The increase in HK activity in alloxan-induced diabetic treated rats (Table 3), on the other hand, could be due to activation of HK potential of stem bark of *Moringa oleifera*.

5. Conclusion

In diabetes, enzymes of glucose metabolism are markedly altered. The stem-bark extract of *Moringa oleifera*, appears to exert hypoglycemia, partly by improving the activities of the altered enzymes and lowering the rate of glucose absorption from the intestine. In this way, the extract has modulated the plasma glucose concentration by improving glucose utilization in diabetic treated rats.

Recommendation

Other enzymes of glucose metabolism should be investigated with the extract.

Compliance with ethical standards

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Disclosure of conflict of interest

Statement of ethical approval

5.1. Ethical statement

There are no potential sources of conflict of interest. This study was conducted following the Declaration of Helsinki and all animal protocols were following the guideline in Nigeria Police Academy, ethical committee. The experiments with animals were performed following the legislation for the protection of animals used for scientific purposes.

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Author's short Biography

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