

Effect of Ethanolic Leaves Extract of *Moringa oleifera* on Blood Glucose Levels of Streptozocin-Induced Diabetics and Normoglycemic Wistar Rats

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Abstract: This study was undertaken to determine the hypoglycemic effect of *Moringa oleifera* [family: Moringaceae] ethanolic extract in normal (normoglycemic) and STZ induced diabetic Wistar rats. In one set of experiment, graded doses of the leaves extract (250 and 500 mg/kg i.p.) were separately administered to groups of fasted normal and fasted STZ diabetic rats. The hypoglycemic effect of the ethanolic leaves extract was compared with that of insulin 6 i.u/kg in fasted normal and STZ diabetic rats. Following treatment, relatively moderate to high doses of *Moringa oleifera* (250 and 500 mg/kg i.p.) produced a dose-dependent, significant reduction ($p < 0.05$) in blood glucose levels of fasted STZ diabetic rats only. A significant decrease in the blood glucose levels after 1-7 h of administration with the doses of 250 and 500 mg/kg was observed in the STZ diabetic group when compared to control. As regards to the dose of 250 and 500 mg/kg for the fasted normal rats, there was significant increase in the blood glucose levels when compared to control. In conclusion the ethanolic extract of the leaves of *Moringa oleifera* possesses hypoglycemic activity in STZ induced diabetic Wistar rats only.

Key words: Diabetic, ethanolic extract, *Hypoglycemic*, *Moringa oleifera*, *Normoglycemic*, *Streptozocin*

INTRODUCTION

The underlying goal of all diabetes treatment and management is to maintain an adequate blood glucose concentration. Progress in understanding the metabolic staging of diabetes over the past few years has led to significant advances in regimen for treatment of this devastating disease (Tiwari and Rao, 2002). Diabetes mellitus is a group of metabolic diseases characterized by hypoglycemia resulting from defects in insulin secretion, insulin action or both (Teixeira *et al.*, 2000). According to WHO projection, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 millions diabetics world-wide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetic will rise from 15 million in 1995 to 57 million in the year 2025 making it the country with the highest number of diabetics in the world (King *et al.*, 1998; Boyle *et al.*, 2001). For centuries, people in many countries have used *Moringa* leaves as Traditional medicine for common ailments. (Trees For Life, 2005). Clinical studies have begun to suggest that at least some of these claims are valid. With such great medicinal value being suggested by traditional medicine, further clinical testing is very much needed. (Dolcas Biotech, 2006-2008). Most of the plants prescribed for Diabetes Mellitus (DM) are not edible

(Atta-ur-Rahman and Zaman, 1989) and therefore, the studies on edible plants which have a hypoglycemic effect would be of great value in the dietary management of the disease. The oral hypoglycemic activity of the leaves of *Moringa oleifera* in normoglycemic healthy and STZ induced diabetic Wistar rats become very imperative.

Moringa oleifera belongs to the family of *Moringaceae*, a fast growing drought-resistant tree native to sub-Himalayan tracts of northern India but now distributed world wide in the tropic and sub tropics (Fuglie, 1999). It is traditionally used for the treatment of a number of ailments including, as fomentation to relieve spasm, diarrhea, as diuretic and stimulant in paralytic affliction, epilepsy and hysteria (Quisumbing, 1978). It is the purpose of this experiment to critically evaluate the effect of ethanolic leave extract of *Moringa Oleifera* on blood glucose levels of Streptozocin-Induced diabetics and normoglycemic wistar rats.

MATERIALS AND METHODS

Plant material: A sample of the leaf of *Moringa oleifera* was collected within Main campus, Ahmadu Bello University, Zaria in September, 2008. The plant was identified and authenticated by M. Musa of the herbarium section in the Department of Biological Science, Ahmadu Bello University Zaria, where a herbarium specimen was

prepared and deposited there with a voucher number (0786).

Extract preparation: The leaves of *Moringa oleifera* were collected and dried under shade and ground into powder. The powder (500 g) was macerated in 70% ethanol at room temperature for 24 h. It was then filtered using a filtered paper (Whatmann size no.1) and the filtrate evaporated to dryness in water bath at 60°C. A brownish residue weighing 30.5 g was obtained. This was kept in air tight bottle in a refrigerator until used.

Chemicals used: All chemicals and drugs used were obtained commercially and of analytical grade.

Phytochemical screening: A preliminary phytochemical screening of the leaves extract of *Moringa oleifera* was also done using standard methods of analysis (Trease and Evans, 1989; Sofowora, 1992).

Animals used: Twenty (20) Wister rats weighing between (120-150 g) of about 20-25 weeks of age of both sexes were used. They were obtained from the Animal House of the Department of Pharmacology and Clinical Pharmacy, A.B.U. Zaria. They were kept in plastic cages under laboratory condition of temperature and humidity and placed on standard feed and allow free access to water with 12 h light/dark cycle. The animals were fasted for 12-18 h with free access to water prior to the administration of the extract.

After 12-18 h, the blood glucose levels was measured using the glucose-oxidase principle and the fasting blood glucose greater than 50 mg/dL was included in the study for the normoglycemic group and fasting blood glucose level greater than 150 mg/dL for the diabetic group (Stanley and Venugopal, 2001).

The normoglycemic rats were randomly assigned into five groups (1-4) of five rats (n = 5) each as follows, namely:

- Group 1: Normal, treated Wistar rats (were given normal saline, 5 mL/kg bodyweight i.p.
- Group 2: Normal treated with 250 mg/kg extract i.p.
- Group 3: Normal treated with 500 mg/kg extract i.p.
- Group 4: Normal treated with 6 i.u/kg insulin i.p.

The streptozocine-induced diabetic rats were randomly assigned into five groups (1-4) of five rats (n = 5) each as follows, namely:

- Group 1: Normal, treated wistar rats (were given normal saline, 5 mL/kg bodyweight i.p.
- Group 2: Normal treated with 250 mg/kg extract i.p.
- Group 3: Normal treated with 500 mg/kg extract i.p.
- Group 4: Normal treated with 6 i.u/kg insulin i.p.

Determination of blood glucose levels: All blood samples were collected from the tail artery of the rats at interval of 0, 1, 3, 5 and 7 h. Determination of the blood glucose levels was done by the glucose-oxidase principle (Beach and Turner, 1958). ONE TOUCH BASIC meter (LIFESCAN, Inc., 2001 Milpitas, CA 95035, USA) was used for the determination of the blood glucose levels of the animals and results were expressed as mg/dl (Rheny and Kirk, 2000).

Statistical analysis: Blood glucose levels were expressed in mg/dL as mean±SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dunnett's method. The values of p<0.05 were taken as significant.

RESULTS

Photochemical screening: Result of the preliminary phytochemical screening of *Moringa oleifera* extract revealed the presence of flavinoids, tannin, anthraquinone, cardiac glycosides alkaloids, triterpenoids, saponins, and reducing sugars.

Blood glucose levels of normoglycemic and Streptozocin-induced diabetic Wistar rats: Table 1 showed the results of the effect of three doses (250 and 500 mg/kg) of ethanolic leaves extract of *Moringa oleifera*, Insulin and control group in normoglycemic Wistar rats. The dose of Insulin did show a significant decrease in blood glucose levels when compared to the normal treated control group. However, the doses of 250 and 500 mg/kg of the extract did not show significant (p<0.05) decrease in the blood glucose levels after 1-7 h, while there was a significant (p>0.05) decrease in blood glucose levels after 1-7 h for the streptozocin-induced treated group (Table 2).

Table 1: Effect of ethanolic leaves extract of *Moringa oleifera* on blood glucose levels of Normoglycemic Wistar rats

Treatment	Blood glucose levels (mg/dL)				
	0 h	1 h	3 h	5 h	7 h
Control(normal saline)	57.0±7.8	71.2±3.6	72.2±2.9	67.2±4.8	69.2±2.4
Insulin 6 i.u/kg	42.2±2.2	28.2±2.3	27.8±4.4	26.4±6.6	27.2±7.2
250 mg/kg	47.0±4.4	63.4±5.5 ^{ns}	65.2±7.2 ^{ns}	101±6.3 ^a	108±8.4 ^a
500 mg/kg	43.0±3.9	78.0±5.8 ^{ns}	63.6±8.9 ^{ns}	76.0±10.0 ^{ns}	68.4±10.3 ^{ns}

^a: p<0.05; a = significant; ns = not significant

Table 2: Effect of ethanolic leaves extract of *Moringa oleifera* on blood glucose levels of Streptozocin induced diabetes in Wistar rats

Treatment	Blood glucose levels (mg/dL)				
	0 h	1 h	3 h	5 h	7 h
Streptozocin (60 mg/kg)	306.0±27.3	341.2±22.5	376.0±9.3	395.6±2.3	403±1.8
Insulin 6 i.u/ kg	378.6±4.4	350.6±37.2	328.2±33.5	308.2±24.7	289.2±26.2
250 mg/kg	155.6±5.9	183.8±8.4 ^{ns}	117.8±17.8 ^a	104.6±8.5 ^a	100±7.0 ^a
500 mg/kg	163.0±8.0	195.6±4.4 ^{ns}	87.2±6.2 ^a	80.2±6.1 ^a	74.0±6.6 ^a

^a: p<0.05; a = significant; ns = not significant

DISCUSSION

The major classes of synthetic oral hypoglycemic agents currently available for the management and/or control of adult-onset, NIDDM, type-2 diabetes mellitus include the sulphonyureas, biguanides, thiazolidinediones, and alpha-glucosidase inhibitors and so on.

Moringa oleifera have been shown to have glucose lowering effect only in hyperglycemic i.e. streptozocin induced diabetic rats and not in hypoglycemia by possibly stimulating the β -cells and or due to its insulin-like activity. In relation to the normal rats that received 250 mg/kg body weight of the extract of *Moringa oleifera* there was a significant increase in the blood glucose levels when compared to the control. In regard to the dose of 500, there was no significant change in the blood glucose level when compared to control after 1-7 h of extract administration. A number of investigations have shown that flavonoids and a host of other secondary plant metabolites including arginine and glutamic acid possess hypoglycemic effect in various animal models (Akah and Okafor, 1992; Ross, 1999, 2001; Ojowole, 2002). Although the hypoglycemic effect of terpenoids appear to involve stimulation of pancreatic B-cells and subsequent secretion of preformed insulin. Acute treatment of the 'control' Wistar rats with distilled water alone did not produced any change in the blood glucose concentration of either the fasted normal or the fasted STZ- treated diabetic rats. However, ethanolic plant extract, unlike insulin- an hypoglycemic agent, produced significant reduction in the blood glucose levels of fasted STZ treated diabetic rats. The plant extract examined in this study appears to act promptly and markedly only in fasted STZ treated diabetic rats. The hypoglycemic effect of *Moringa oleifera* appears to be most probably exerted via a mechanism that is different to that of insulin.

A number of investigators have shown that coumarin, flavonoid, terpenoid and a host of other secondary plant metabolites including arginine and glutamic acids possess hypoglycemic effects in various experimental animals model. (Akah and Okafor, 1992; Marles and Farnsworth, 1995).

Hypoglycemic activity of the leaves of *Moringa oleifera* may probably be due to terpenoid present, which appears to be involve in the stimulation of the β -cells and

the subsequent secretion of preformed insulin. One or more of the other chemical constituents of the plant especially flavonoid is also likely to have played a crucial role in the hypoglycemic action of the plant extract.

CONCLUSION

The present study showed that ethanolic leaves extract of *Moringa oleifera* possessed hypoglycemic properties in Streptozocin diabetic Wistar rats only, which suggest the presence of biologically active components which may be worth further investigation and elucidation. The effective hypoglycemic dose was found to be 500mg/kg weight. Further studies are currently under way to isolate and characterized the active components of the crude extract of this plant.

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