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Research Article

Effect of *Moringa oleifera* leaf extract on serum lipids and glycaemic control in alloxan induced diabetic albino rats

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Abstract

Objective: To determine the possible effects of aqeous extract of *Moringa oleifera* on blood glucose and lipid profile in alloxan induced diabetic albino rats.

Materials and Methods: Forty four (44) adult healthy Albino rats of both sexes divided into three groups (A=23, B=10 and C=11)were used for this study; they were kept under standard conditions in the Animal House of the Department of Biomedical Sciences of Ladoke Akintola University of Technology, Osogbo, Nigeria. Diabetes mellitus was induced in rats in groups A and B with 5-day of alloxan administered intraperitoneally. Only rats in group A were given aqueous extracts of *Moringa oleifera* for a period of nine days. Body weight and fasting blood glucose were checked at 5th and 14th day of the experiment. Parameters of lipid profile were determined in rats at day 14.

Results: Average fasting blood sugar for groups A, B and C were observed to be 10.10mmol/l, 9.84mmol/l and 4.23mmol/l respectively at day 5. There was a reduction in fasting blood glucose in rats in groups A and C while there was an increase in fasting blood glucose in rats in group B when checked at day 14. Rats in group B (alloxan induced DM without *Moringa oleifera* supplement) had lowest value of HDL-Chol while their values of LDL-Chol, TC-Chol and Tg were the highest. There was significant lower value of HDL-Chol in group B than rats in Group A ($p<0.05$) and rats in group C ($p<0,05$).

Conclusion: There was a hypoglycaemic effect of the aqueous extract of *Moringa oleifera.* It also has capacity to ameliorate dyslipidaemia in diabetic rats.

Keywords: *Moringa oleifera*, Diabete Mellitus, fasting blood sugar, plasma lipid profile

1. Introduction

It has been estimated that about 285 million people in the world were suffering from Diabetes Mellitus (DM) as at 2010 and more than 438 million victims are assumed to be having the disease by the year 2030.[1] Diabetes Mellitus is described as a metabolic disorder characterized by hyperglycaemia that may be as a result of inadequate insulin production or insensitivity of body cells to respond to insulin action, or both. This results into not only the disturbance of carbohydrate metabolism but also fat and protein metabolisms[2]. In the absence of early diagnosis and adequate treatment, patients with this disease may suffer from complications like hyperosmolar hyperglycaemic state, kitoacidosis as well as dyslipidaemia[3].

More than 70% of people with Diabetes Mellitus live currently in low and middle income nations, with the proportion in Africa is expected to experience the highest increase[4]. Several attempts have been made to treat and monitor this disease condition. Chemotherapeutic agents, therapeutic life style changes as well as dietary modification have been explored in the past. Despite all these, patients still come down with complications like dyslipideamia[5]. This is one of the reasons why fasting plasma lipid profile is recommended in the course of management of Diabetes Mellitus[5]. Framingham study observed that 13% of men and 24% of women with DM had evidence of dyslipideamia[6].

The use of medicinal plants for the treatment of Diabetes Mellitus is on the increase now, especially in the developing countries. It is becoming a concern to understand the effects of using herbal medicines to control body diseases like Diabetes Mellitus. *Moringa* tree has been described as a multi-functional plant that is cultivated in tropical regions and being used as a medicinal plant based on its high protein, vitamins, mineral and carbohydrate contents[7,8]. It has high nutritional value for both human and livestock.[8]

In view of complications, mortality, increased cost of management and lack of curative treatment for Diabetes Mellitus, the study was designed to look into cost effective and readily available form of management and prevention of complications of DM. Many studies have revealed that the extracts of *Moringa oleifera exhibit* anti-diabetes agents. However, there has been paucity of experimental study using the crude aqueous extracts; the form in which it is often used in the ethnomedical treatment of Diabetes Mellitus. This work also seeks to evaluate the possible blood sugar lowering and lipid adjustment effects of the aqueous extracts of *Moringa oleifera* in the alloxan induced diabetic albino rats

2. Materials and Experimental Design

Forty four (44) male and female adult healthy Albino rats weighing between 145 and 180g were used for this study; they were nursed under standard conditions in the Animal House of the Department of Biomedical Sciences of Ladoke Akintola University of Technology, Osogbo, Nigeria and kept on standard rat feed (rat pellet) with tap water. The rats were grouped into three groups (A=23, B=10 and C=11) and each group was kept in separate rectangular shaped cages with dimension 165cm by 55cm, 130cm by 47cm and 129cm by 50cm respectively . The floor areas of cages A, B and C are 9,075cm^2, 6,110cm^2 and 6,450cm^2 respectively. These cages are 45cm, 43cm and 43cm in height

respectively. The floor of cages is made of plastic material and major parts of the body are made of wire mesh (to allow appropriate illumination and visibility to the animals). The animals in these cages were allowed to acclimatize for two weeks. A single dose of alloxan was injected intraperitoneally to rats in groups A and B after the acclimatization periods at 160mg/Kg in order to induce Diabetes Mellitus, which was later confirmed at the fifth day by checking their fasting blood glucose.

2.1 Preparation of Dose of the Plant Extract

The fresh leaves of *Moringa oleifera* gotten from a local farm in Osogbo, Nigeria rinsed with tap water and later grounded into aqueous form with the aid of an electrical grinder. The crude aqueous extracts were preserved at 8-12ºC throughout the period of the experiment. The prepared aqueous plant extract was orally administered every morning till the last day of the treatment but only the test group A was allowed access to it.

2.2 Induction of Experimental Diabetes and Blood Sample Collection

Experimental diabetes was induced in rats in groups A and B by intraperitoneal injection of 5% alloxan monohydrate at a dose of 160mg/kg using 2ml disposable needles and syringes. Body weight and fasting blood glucose were checked at 5th and 14th day of the experiment. Parameters of lipid profile were determined in rats at day 14.

2.3 Analysis of biochemical Parameters

On the last day of the experiment all the animals were sacrificed and their blood samples were collected into Na^+ EDTA bottle. These were centrifuged at 3000 rpm for 5 minutes while the plasma from each bottle was separated into a new plain specimen bottle and later stored at -20ºC until the time for the analysis. The plasma samples collected from the rats were used for the laboratory analysis of full lipid profile while the fasting blood glucose (FBG) of rats was measured from capillary sample gotten at the tail snips of those rats. The first fasting blood glucose level from the rats was considered as the baseline. The blood glucose levels were measured using ACCU-CHECK (Active), a glucose test meter (Roche Diagnostic GmbH Sandhofer Strasse, Ref: 05234441049, Germany), and all the data were compared with the respective standard values. Parameters of lipid profile were assayed using enzymatic colourimetric methods from commercially manufactured ready to use kits by Randox laboratotry, Aldren, USA.

2.4 Statistical analysis

Variables from the study were expressed as mean ± standard deviation. Biochemical parameters between the test and control groups were compared using student t-test. In addition, one way analysis of variance (ANOVA) was carried out and differences in mean values between experimental groups were analyzed by unpaired t test. A probability value of $p < 0.05$ was considered to be significant.

3. Results

Rats in groups A and B lost an average weight of 11grams and 12 grams respectively after 5-day of alloxan administration while rats in group C gained an average weight of 2grams at 5th day of normal feeding without alloxan administration. This as illustrated in table 1

Table 1: Body Weight of the Experimental Rats in the Course of the Experiments.

Groups	Day 1	Day 5	Day 14
A (gram)	167	156	163
B (gram)	169	157	140
C (gram)	166	168	170

Table 2: Fasting Blood Glucose at day 5 (Post alloxan) and day 14 (Post Moringa oleifera administration)

Group	Day 5	Day 14
A (mmol/l)	10.10	4.69
B (mmol/l)	9.84	11.10
C (mmol/l)	4.23	3.97

Rats in all the groups gained average weight of 7grams, 3grams and 2grams respectively when checked at day 14. This has shown in table 1. Average fasting blood sugar for groups A, B and C were observed to be 10.10mmol/l, 9.84mmol/l and 4.23mmol/l respectively at day 5. There was a reduction in fasting blood glucose in rats in groups A and C. while there was an increase in fasting blood glucose in rats in group B when checked at day 14.

Table 3: Lipid Profile in Various Study Groups

Dependent Variable	Animal Group	n	Mean ± SD
HDL-Chol(mmol/l)	GROUP A	23	0.36±0.05
	GROUP B	10	0.16±0.04
	GROUP C	11	0.37±0.08
LDL-Chol(mmol/l)	GROUP A	23	0.06±0.03
	GROUP B	10	0.42±0.03
	GROUP C	11	0.08±0.04
TC-Chol(mmol/l)	GROUP A	23	0.65± 0.07
	GROUP B	10	1.52±0.13
	GROUP C	11	0.68±0.07
TG-Chol(mmol/l)	GROUP A	23	0.51±0.07
	GROUP B	10	1.27±0.10
	GROUP C	11	0.53±0.08

Table 4: Comparison of Lipid Profile in Various Study Groups

Variables		Comparison	p-value
HDL-Chol(mmol/l)	A Vs B	0.36±0.05 Vs 0.16±0.04	<0.05
	A Vs C	0.36±0.05 Vs 0.37±0.08	>0.05
	B Vs C	0.16±0.04 Vs 0.37±0.08	<0.05
LDL-Chol(mmol/l)	A Vs B	0.06±0.03 Vs 0.42±0.03	<0.05
	A Vs C	0.06±0.03 Vs 0.08±0.04	>0.05
	B Vs C	0.42±0.03 Vs 0.08	<0.05
TC-Chol(mmol/l)	A Vs B	0.65±0.07 Vs 1.52±0.13	<0.05
	A Vs C	0.65±0.07 Vs 0.68±0.07	>0.05
	B Vs C	1.52±0.13 Vs 0.68±0.07	<0.05
Tg (mmol/l)	A Vs B	0.51±0.07 Vs 1.27±0.1	<0.05
	A Vs C	0.51±0.07 Vs 0.53±0.08	>0.05
	B Vs C	1.27±0.1 Vs 0.53±0.08	<0.05

Key: A= Diabetic Test Group
B= Diabetic Control Group
C= Non Diabetic Control Group
P< 0.05 = significant

Rats in group B (alloxan induced DM without *Moringa oleifera* supplement) had lowest value of HDL-Chol while their values of LDL-Chol, TC-Chol and Tg were the highest. There was significant lower value of HDL-Chol in group B than rats in Group A ($p<0.05$) and rats in group C ($p<0, 05$). There was significant higher value of LDL-Chol in rat in group B than those in group A ($p<0.05$) and C ($p<0.05$). Similar findings were observed in TC and Tg among the study groups when compared

4. Discussion

As illustrated in table i. The decrease in average body weight of rats in group A and B may be associated with weight loss at times experienced in diabetic patients[9]. However, fasting blood glucose level of rats in group A was under control probably because of *Moringa oleifera* administration. This could be explained by a more decrease in average body weight of rats in group B (uncontrolled diabetic rats). Also this can also be substantiated by the increase in the body weight of non diabetic rats, group C. This set of rats experience normal growth processes.

Diabetes mellitus was successfully induced with alloxan administration in groups A and B. Nimenibo *et al*[10] in 2003 reported that alloxan monohydrate has destructive effect on the pancreatic beta cells thus inducing type 1 or Insulin dependent DM. This has been suggested to be associated with toxicity of alloxan on pancreatic beta cells[11]. There was also a report that the metabolic product, dialuric acid of alloxan, establishes a redox cycle which finally results in the formation of a free radical (superoxide radicals)[12]. These radicals attack cell membrane lipid with an increased cytosolic calcium concentration to cause rapid destruction of the beta cells[12]). As a result of these, pancreatic beta cells are not able to produce insulin for proper glucose handling, hence the outcomes of hyperglycaemia and diabetes mellitus

The results obtained from the alloxan– induced diabetic rats showed that in the diabetic untreated group, the fasting blood glucose concentration remained very high. Uninterrupted prolong effect of alloxan may be an evidence and this may confirm further destruction of pancreatic beta cells despite the stoppage of alloxan administration at the 5th day. However, significant reduction in fasting blood glucose in *Moringa oleifera* treated rats may suggest hypoglycaemic effect of this leave extract. This finding is similar to the reports of Aderibigbe *et al*[13]. The reason for this finding may be as a result of a selective inhibitory effect of *Moringa oleifera* on glucose absorption. It has also been suggested that it stimulates glycolysis in peripheral tissues, reduces gluconeogenesis in the liver as well as reduces plasma glucagon levels[14,15]. All these biochemical processes lead to metabolic use and less production of glucose in the body. The hyperglycaemic feature of diabetes mellitus also involves highly protein glycation leading to the production of an advanced glycation end products (AGEs). These bimolecules are however, deplete the endogenous pool of lysine and *Moringa oleifera* is rich in lysine production. It is therefore conceivable that another mechanism in which *Moringa oleifera* exerts its hypoglycaemic effects is by restoring some of the lysine lost via the formation of advanced glycation end products in diabetes mellitus. The hypoglycaemic control of *Moringa oleifera* may also have an indirect or direct effect on plasma lipid levels. That uncontrolled Diabete Mellitus has been associated with dyslipidaemia[6].

There is a complex biochemistry to explain the metabolism of lipid in the system and this makes also the explanation of movement of lipids inside the vessels complex. In discussing cholesterol two carrier lipoproteins are mostly considered for it transportation within the vessels, these are high density lipoproteins (HDL) and the low density lipoprotein (LDL). Low density lipoprotein carries cholesterol to the cells where it is deposited whether needed by those cells or not. If deposited in cells where it is not required, it may predispose those vessels to atherosclerosis. The work of HDL is beneficial to the system in which it transports cholesterol to the liver where it can be eleminated from the body.

Evidence of dyslipidaemia observed in diabetes rats not on *Moringa oleifera* supports findings in the literature[6,16]. It has been observed that dyslipidaemia could come as a complication to DM[6]. Rats with *Moringa oleifera* administration had better lipid parameters than those without *Moringa oleifera* administration. This may be due to lipid parameters modification effect of this supplement. The work of Sangkitikomol et al[17] has demonstrated similar finding. It could also be due to its effect on blood glucose itself. Fasting plasma glucose was well controlled in rats with *Moringa oleifera* administration. This is in support of findings from many studies around the world[6,16]. Patient with well controlled DM may be free of complications that may be associated with it.

The pathogenesis of triglyceride-rich lipoproteins like very low density lipoprotein (VLDL) and chylomicron may be able to explain atherosclerosis observed as a result of dyslipidemia in subjects with Diabetes Mellitus. This dyslipidaemia may be inform of an increased hepatic secretion of VLDL and impaired elimination of VLDL. Also there may be an increased chylomicrons derived from the intestine. The above mechanisms importantly, prolong plasma retention of both VLDL and postprandial chylomicrons reminants. These chylomicron remnants are involved in the production of an intermediate density lipoprotein (IDL) which on the average is rich in cholesterol hence atherogenic in nature[18]. It should also be noted that dangerous LDL is also produced from VLDL inform of a small dense LDL particles. This explains the increased fasting plasma level of LDL-Chol observed in our diabetic experimental animals not on *Moringa oleifera* administration. The finding has been supported by previous study[19].

Fasting plasma HDL-chol was fund to be lower in uncontrolled diabetic rats. There has been a multifactorial association between a reduction in HDL in the subjects with type 2 DM and insulin resistance. Furthermore, an increased transfer of cholesterol from HDL to triglyceride-rich lipoproteins, with reciprocal transfer of triglyceride to HDL is a major and leading factor.[20]

5. Conclusion

The results obtained from this study have confirmed the hypoglycaemic effects of the aqueous extract of *Moringa oleifera*. The plant extract was also observed to have the ability to prevent or ameliorate diabetic complications like dyslipidaemia. Fractionation of the leaf extract will help to further know the active component(s). The likely mechanism of action/pathogenesis of the plant extracts as antidyslipidaemia may be postulated.

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