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Blood glucose and lipid reducing activities of the oral administration of aqueous leaf extract of *Moringa oleifera* in Wistar rats

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Abstract

The effect of the repeated administration of *Moringa oleifera* on blood glucose and serum lipid profile was investigated in male Wistar rats. Dry leaf powder of *Moringa oleifera* was extracted with water and lyophilized. Twenty four Wistar rats with body weight of 86.2 ± 4.43 g were grouped equally into four (A-D) and distilled water, 250, 500 and 1000 mg/kg body weight of extract were orally administered once daily for 56 days groups respectively. The body weights of rats were reduced ($p<0.05$) at high doses of the extract, while the blood glucose decreased ($p<0.05$) at all the doses. All the lipid profile parameters and atherogenic index were reduced ($p<0.05$) in rats administered the extract, except the high density lipoprotein cholesterol. From the foregoing, the aqueous leaf extract of *Moringa oleifera* has blood glucose and lipid reducing activities, and body weight maintenance capabilities.

Keywords: *Moringa oleifera*, glucose, lipid, reducing, body weight maintenance

1. 0 Introduction

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser reported side effects (Brahmachari, 2001). Herbal medicine, in which plants (dried or in extract form) are used as therapeutic substances, is a practice encompassed by the term "complementary and alternative medicine" (CAM). CAM has gained enormous popularity worldwide over the past 20 years and several studies have reported that alternative/herbal medicine for the prevention and treatment of various illnesses, has brought vast concerns and fears over professionalism of practitioners and the quality, efficacy and safety of the 'natural' formulations available in the market (Saad *et al*., 2006; Mohanty *et al*., 2010). However, alternative therapies have become a significant component of over-the-counter market recently (Martins *et al.*, 2010).

Moringa oleifera Lam (syn. *M. ptreygosperma* Gaertn.) is one of the best known and most widely distributed and naturalized species of a monogeneric family *Moringaceae* (Ramachandran *et al.*, 1980; Nadkarni, 1982). According to the annals of the ayurveda, India's old tradition of medicines, the leaves of the *Moringa oleifera* could treat at least 300 diseases, which include fomentation to relieve spasm, diarrhoea, as diuretic and stimulant in paralytic affliction, epilepsy and hysteria (Quisumbing, 1978). *Moringa* goes by many names, such as: "mother's best friend" or "malunggay" in the Philippines, where the leaves of the *Moringa* are cooked and fed to babies, 'shagara al Rauwaq'in the Nile valley, which means 'tree for purifying'. Other names of *Moringa oleifera* include the benzolive tree (Haiti), Saguna/Sainjna (India), Zogale, Bagaruwar Maka (Hausa) Ewe Ile, Igi Iyaanu (Yoruba) and Okweoyeibe (Igbo) (Farooq *et al.,* 2007).

Moringa oleifera has numerous medicinal uses, which have long been recognized in the Ayurvedic and Unani systems of medicines (Mughal *et al*., 1999). The leaf preparations of *Moringa oleifera* have been reported in the scientific literature as having quite a number of biological uses (Table 2) such as; antiulcer, anti-inflammatory, antimicrobial, anti-herpes simplex virus, diuretic, anthelmintic, hepatoprotective among others (Caceres *et al.,* 1991; Faizi *et al.,* 1994; Gilani *et al.*, 1994; Nwosu and Okafor, 1995; Ghasi and Ofili, 2000; Rastogi and Ram, 2006). In addition, the consumption of the leaf of *Moringa oleifera* in Nigeria has been alleged to balance or boost the energetic, soothing ability, prevent ulcer, inflammation, pain, skin problems, detoxify the blood and gastrointestinal tract, promote wound healing and promote immune functions (Siddhuraju

and Becker, 2003; Carrasco *et al*., 2009). Since the disorders of the immune system are known as the basis of most clinical and pathological conditions and blood glucose and lipid levels have significant roles in an individual's immune status (Digirolamo, 1994; Spelman *et al*., 2006), this study therefore, investigated the effect of the repeated administration of the aqueous leaf extract of *Moringa oleifera* on blood glucose and lipid concentrations in male Wistar rats.

2.0 Materials and methods

2.1 Materials

2.1.1 Leaf material

Fresh mature leaf of *Moringa oleifera* was collected before sun rise in August from the natural habitat around Masifa area, Ogbomoso, Oyo State. The plant was authenticated in the Department of Crop Science, Ladoke Akintola University of Technology, Ogbomoso, Oyo State. The fresh leaf was rinsed thoroughly in distilled water and dried in the shade for 18 days. The dried leaf was ground to fine powder, using a domestic electric grinder and suspended in distilled water at room temperature. The filtrates were pulled together and lyophilized using a freeze dryer. The yield of the aqueous leaf extract of *Moringa oleifera* was 18.22% ($^{w}/_{w}$). The lyophilized extract was stored air tight and kept in the dark till when needed.

2.1.2 Blood glucose glucometer

Accu-chek active glucometer and test strips, products of Roche Diagnostic Gmbh, D-68298 Mannheim, Germany were used for the fasting blood glucose level estimation.

2.1.3 Quantitative assay kits and other reagents

The kits for the determination of Total Cholesterol, Triacylglyceride and High Density Lipoprotein Cholesterol (HDL-C) were products of LABKIT, CHEMELEX, S.A. Pol. Canovelles-Barcelona, Spain. All the chemicals and reagents used in the study were of analytical grade and were purchased from the British Drug House (BDH) Poole England and Sigma Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A.

2.1.4 Laboratory animals

Eight to ten weeks old male Wistar rats of average body weight of 86.2 ± 4.43 g were obtained from the Animal Care Facility, Ladoke Akintola University of Technology, Ogbomos, Oyo State. The rats were fed with rat pellet (product of Bendel Feeds and Flour Mills Ltd, Ewu, Edo State, Nigeria).

2.2 Methods

2.2.1 Experimental animals and procedure

The Twenty four male Wistar rats were randomly grouped into four, comprising of six rats per group. The rats were housed in cages made of wooden frames and metal netting, and were fed *ad libitum* with rat pellet and tap water with 12-hours light/dark cycle. The cages were cleaned every morning and disinfected at intervals of 3 days. The rats were allowed to acclimatize for 14 days before extract administration was commenced. Calculated amount of lyophilized aqueous leaf extracts of *Moringa oleifera* were constituted in distilled water to give doses of 250, 500 and 1000 mg/kg body weight. The various doses were administered as illustrated:

Group A: control, received 1.0 ml distilled water
Group B: received 250 mg/kg body weight of the extract
Group C: received 500 mg/kg body weight of the extract
Group D: received 1000 mg/kg body weight of the extract

The feed intake of the rats were monitored daily and prior to the administration of aqueous leaf extract of *Moringa oleifera* and every interval of 7 days, the fasting blood glucose levels and the body weights of the animals were recorded. Administration of aqueous leaf extract of *Moringa oleifera* was performed orally once daily between 7:20 am ± 30 minutes, using metal cannula attached to a 2 ml syringe. Administration lasted for 56 days, after which the rats were fasted for 12 hours and the blood glucose level and body weights determined. This study was conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (1985).

2.2.2 Blood glucose determination

The blood glucose concentration was determined by glucose oxidase reaction, using Accu-chek active glucometer and test strips. Glucose oxidase chromogen indicators and non-reactive agents are contained in the reagent pad to which about 2µl of whole blood was applied.

2.2.3 Serum lipid profile determination

The concentration of serum total cholesterol level was determined by CHOD-POD enzymatic colourimetric reaction, according to the method as described by Naito (1984[a]); while serum triglyceride level was determined by GPO-POD enzymatic colourimetric reaction, according to the method as described by Fossati *et al*. (1982); and serum HDL-C cholesterol level was determined by precipitation and CHOD-POD enzymatic colourimetric reaction, according to the method as described by Naito (1984[b]). However, serum VLDL-C cholesterol and LDL-C were determined by computation, according to the methods described by Friedewald *et al*. (1972).

2.2.4 Statistical analysis

This research work used a completely randomised design (CRD) model. The results were expressed as mean of 5 replicates ± standard error of mean (SEM). Results were analyzed using statistical package for social sciences (SPSS) 16.0 for Window software and were subjected to one way analysis of variance (ANOVA) to test the effect of each dose level on the parameter under investigation at 95% level of confidence. The Duncan Multiple Range Test (DMRT) was conducted for the pair-wise mean comparisons, to determine the significant treatment dose at 95% level of confidence. Values were considered statistically significant at ($p<0.05$) and denoted by different alphabets (Mahajan, 1997).

3.0 Results

3.1 Feed intake

The effect of the administration of the aqueous leaf extract on the feed intake (a measure of appetite) of rats is presented in Table 1. The administration of the leaf extract resulted in significant decreases ($p<0.05$) in the feed intake of the rats, in an almost dose dependent manner.

3.2 Body weight

The pattern of the weekly average body weights of rats following repeated administration of the aqueous leaf extract of *Moringa oleifera* is shown in Figure 1. The body weights of control rats and rats administered 250 mg/kg body weight of the extract increased steadily throughout the duration of the experiment with no significant differences ($p>0.05$). However, the body weights of the rats administered 500 and 1000 mg/kg body weight the extract appeared to decrease significantly ($p<0.05$) with increasing concentration of the aqueous leaf extract.

3.3 Blood glucose

The trends presented following the administration of the aqueous leaf extract of *Moringa oleifera* on weekly fasting blood glucose is presented in Figure 2. All concentrations of the extract resulted in significant lowering of blood glucose concentrations ($p<0.05$) in a pattern not dose dependent.

3.4 Serum lipid profile

The effect of the administration of *Moringa oleifera* leaf extract on serum lipid profile is shown in Table 2. The total cholesterol and triacylglycerol (TAG) concentrations were significantly decreased ($p<0.05$) at high doses of the extract, while high density lipoprotein cholesterol (HDL-C) concentration increased significantly ($p<0.05$) with no significant changes ($p<0.05$) with increasing doses. Very low density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol (LDL- C) concentrations and the atherogenic index were reduced significantly ($p<0.05$) in an almost dose dependent manner (Table 2) in rats administered the aqueous extract.

4.0 Discussion

The reduction in the feed intake (Table 1) might be due to slow metabolism of ingested substances in the gastrointestinal tract, which could be caused by the reported level of saponins in the extract (Oyewo *et al*., 2012[b]) or decreased appetite. High concentrations of saponins have been reported to cause marked reduction in the absorption of dietary nutrients in the gastrointestinal tract due to 'autointoxication' or 'leaky gut' (Evers, 2008). However, the administration of the aqueous leaf extract even at high doses did not suggest the possibility of chronic weight loss (catechia) that could arise from systemic inflammatory responses, caused by leaky gut. In addition, Oyewo *et al*., (2012[b]) reported that the administration of the extract did not increase serum interleukin - 6 (IL-6) and tumour necrosis factor-alpha (TNF-α) concentrations in rats, therefore, the extract might have decreased the appetite in the rats, which was responsible for the decrease in the feed intake (Table 1). This is has to be so because the administration of the extract to rats did not reduce the body weight of the rats to the initial weight, but rather resulted in a slow gradual gain in weight (Figure 1) supporting the possibility of depression of

appetite. The trends presented in the body weight of the rats administered the aqueous leaf extract of *Moringa oleifera* suggested that the extract could be a potential candidate as a regime for the maintenance of body weight in conditions like weight regulation, over-weight etc. Furthermore, the reduction in the body weight of rats following the administration of the aqueous leaf extract of *Moringa oleifera* at high doses (Figure 1) supported the reported immune modulating activities of *Moringa oleifera* leaf (Oyewo *et al*., 2012[b]); since immune modulating plants regime have been reported to possess body weight reducing or maintenance properties, while immune disorders are frequently reported in over weight individuals (Pond, 2005; Oyewo and Akanji, 2011; Oyewo *et al*., 2012[a]).

The pattern obtained in the blood glucose concentration in rats administered the aqueous leaf extract of *Moringa oleifera* (Figure 2) may be attributed to the reported levels of alkaloids and polyphenols in the extract (Oyewo *et al*., 2012[b]). The reduction in the blood glucose levels was accompanied by reduction in the body weights of rats (Figure 1). Therefore, the probable mechanism of the reduction in blood glucose level-body weight loss; could be through the prevention of absorption of glucose in the gut and/ or increased insulin secretion by pancreatic stimulation (Borhanduddlin *et al*., 1994). However, the reported levels of alkaloids, saponins and flavonoids (Oyewo *et al*., 2012[b]) might prevent the absorption of dietary glucose in the gastrointestinal tract. Khanna *et al*. (2002) and Oyewo *et al*. (2012[a]) reported that saponins in diets interfere with the absorption of glucose in the small intestine. Thus, the high content of saponins in the leaf extract (Oyewo *et al*., 2012[b]) could have permeabilize the plasma membranes of the small intestine, thereby causing irreversible disorder and disruption of the plasma membrane resulting in marked reduction in the absorption of dietary glucose in the gastrointestinal tract due to 'autointoxication' or "leaky gut' (Choi *et* al., 2001; Evers, 2008).

In addition, IL-6 mediates the absorption of dietary glucose in the gastrointestinal tract by enhancing or inhibiting the intake of glucose by GLUT-2. Serum IL-6 concentration mediates glucose absorption in the small intestine, thereby regulating the exocytosis of insulin by the pancreas and the oxidation and uptake/storage of glucose at the muscles by GLUT-4 (Hardardottir *et al*., 1994). However, since there was reduction in the feed intake of the rats administered with the leaf extract (Table 1), body weight (Figure 1) and serum IL-6 concentration (Oyewo *et al*. (2012[b]) in rats administered the extract, the probable explanation for the trend obtained in the blood glucose levels is by the prevention of the absorption of glucose in the gastrointestinal tract by the reported levels of alkaloids, saponins and flavonoids in the aqueous leaf extract (Oyewo *et al*., 2012[b]). In line with this, the reduction in the blood glucose concentrations in rats administered with the aqueous leaf extract supported the reported immune modulating activities of the aqueous leaf extract of *Moringa oleifera* (Oyewo *et al*., 2012[b]), as some immune modulating regimes are reported to possess blood glucose reducing or maintenance properties (Spleman *et al*., 2006; Oyewo and Akanji, 2011; 2012[a]). Volk *et al*. (1993) reported that increase in blood glucose levels reduced the phagocytic index of macrophage and neutrophils by 75%. In addition, Langley-Evans and Carrington (2006) reported that increased concentrations of glucose in the blood proportionally reduce the ability of cell-mediated immune cells to capture bacteria and increased the incidence of degenerative diseases (cancer). Therefore, the trend obtained in the blood glucose levels in rats administered the aqueous leaf extract of *Moringa oleifera* suggest could be recommended in clinical conditions where the reduction/maintenance of blood glucose level is required.

The trends obtained in the total cholesterol and triacylglycerol concentrations in serum of male rats administered high doses of the aqueous leaf extract of *Moringa oleifera* (Table 2), might have resulted from the levels of alkaloids, saponins and flavonoids that were reported in the extract (Oyewo *et al*., 2012[b]). Saponins are known to inhibit the absorption of dietary lipid in the small intestine through the formation of complexes with cholesterol in diet (Belles *et al*., 2005; Evers, 2008). Furthermore, flavonoids are implicated in the inhibition of cholesterol biosynthesis in the liver (Sinclair *et al*., 2001) and/ or inhibiting the production of apo B, needed for LDL-C production, transport and binding, thereby enhance the liver functions by facilitating reverse cholesterol transport and bile acid excretion (Renaud *et al*., 1999; Turner *et al*., 2004). In addition, the reductions in the serum total cholesterol and triacylglycerol concentrations could be due also to 'autointoxication' or "leaky gut' (Choi *et* al., 2001; Evers, 2008), or the reported level of saponins in the aqueous leaf extract (Oyewo *et al*., 2012[b]). This has to be so, since the reduction in serum cholesterol concentration in this study was not accompanied by lipolysis, as seen in the serum triacylglycerol concentrations (Table 2).

The result of the serum HDL concentration in rats administered high dose of the leaf extract suggested a possible boost of HDL-C biosynthesis in the liver, which could have been promoted by the presence of flavonoids in the aqueous leaf extract (Oyewo *et al*., 2012[b]). Renaud *et al*. (1999) reported that flavonoids enhanced the biosynthesis of HDL-C in the liver. Therefore, more cholesterol would be transported from peripheral tissues to the liver for excretion and this could be the reason for the reported trends in the serum cholesterol concentration in rats administered the leaf extract (Table 2). The reduced serum levels of VLDL-C is consistent with the reported decrease in serum TAG, meaning that less TAG was exported from the liver to extra-

hepatic tissues. In addition, the trend obtained in serum LDL-C concentration is consistent with the serum cholesterol-lowering capability of the aqueous leaf extract of *Moringa oleifera*, which possibly enhanced reverse cholesterol transport and bile acid excretion, through the inhibition of production apo B, needed for LDL-C production, transport and binding (Renaud *et al*., 1999; Turner *et al.*, 2004; Oyewo *et al*., 2012[a]). The result of the atherogenic index (Table 2) suggested that the administration of the extract reduced the risk of lipid related inflammatory diseases. In addition, the trend obtained in the body weight (Figure 1) of rats administered the aqueous leaf extract supported the results of the feed intake (Table 1) and the serum lipid concentrations (Table 2), all suggested the depression of appetite and the prevention of the absorption of nutrients via the gut by *Moringa oleifera* leaf.

The overall trends obtained in the blood glucose concentrations, lipid parameters and body weight in rats administered the aqueous leaf extract of *Moringa oleifera* supported the immune modulating activities of the extract as reported by (Oyewo et al., 2012[b]).

5.0 Conclusion

The findings of the study indicated that the aqueous leaf extract of *Moringa oleifera* possessed blood glucose and lipid reducing properties and maintenance of body weight, thereby supporting the reported immunodulatory activities of the extract. The toxicity of the aqueous leaf extract in male Wistar rat is currently being investigated.

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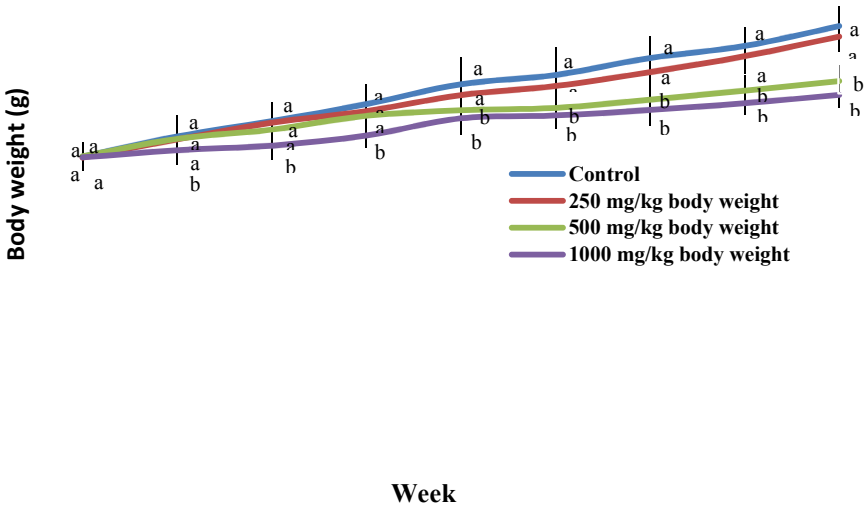

Figure 1. Body weights of rats administered aqueous leaf extract of *Moringa oleifera*
Values are means ± SEM; n=5. *Values bearing different alphabets are significantly different ($p<0.05$).

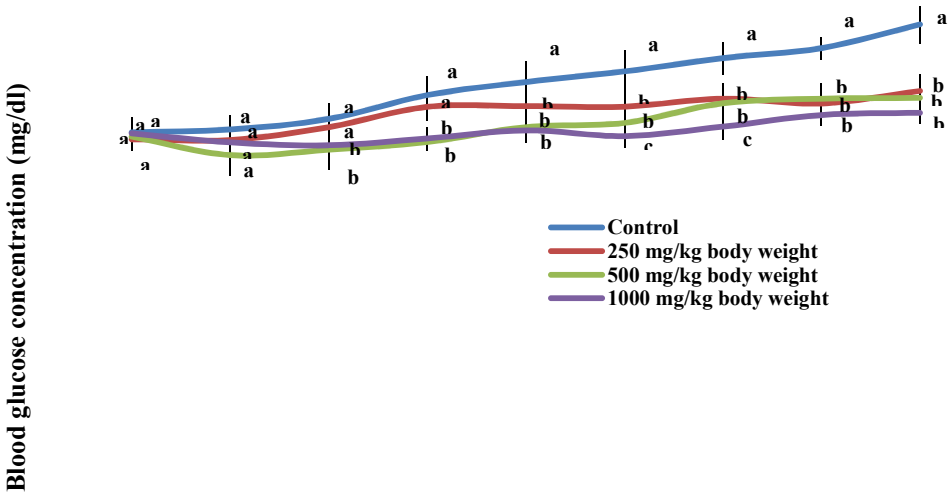

Figure 2. Effect of aqueous leaf extract of *Moringa oleifera* on fasting blood glucose
Values are means ± SEM; n=5. *Values bearing different alphabets are significantly different ($p<0.05$).

Table 1. Feed intake of the rats following the administration of *Moringa oleifera*

		Doses (BW)		
Week (g)	**Control**	**250**	**500**	**1000**
0	100.71 ± 5.55[a]	103.97 ± 4.87[b]	104.17 ± 4.73[b]	97.96 ± 3.69[b]
1	107.40 ± 4.75[a]	102.56 ± 3.99[b]	100.47 ± 6.21[c]	93.61 ± 3.08[b]
2	112.33 ± 3.89[a]	103.18 ± 3.41[b]	98.91 ± 4.36[b]	90.53 ± 4.17[b]
3	109.26 ± 5.07[a]	98.44 ± 4.75[b]	99.61 ± 3.38[b]	85.33 ± 3.37[b]
4	114.53 ± 7.22[a]	93.22 ± 4.15[b]	90.69 ± 4.02[c]	82.88 ± 4.22[b]
5	110.59 ± 4.19[a]	95.73 ± 3.66[b]	84.55 ± 4.31[b]	76.98 ± 4.11[b]
6	102.99 ± 6.22[a]	97.44 ± 3.83[b]	88.43 ± 5.28[b]	77.63 ± 3.26[b]
7	118.65 ± 4.92[a]	96.27 ± 5.02[b]	82.19 ± 3.94[c]	72.89 ± 5.13[b]
8	122.44 ± 5.38[a]	92.31 ± 4.32[b]	82.05 ± 4.61[b]	70.16 ± 4.91[b]

Values are means ± SEM; n=5. Values bearing different alphabets are significantly different ($p<0.05$).
Key: BW (mg/kg body weight)

Table 2. Serum lipids in rat following the administration of the leaf extract of *M. oleifera*

		Doses (BW)		
Conc. (mg/ml)	**Control**	**250**	**500**	**1000**
T. cholesterol	210.42 ± 4.08[a]	202.57 ± 5.05[a]	171.72 ± 5.27[b]	169.76 ± 7.05[b]
Triacylglyceride	218.54 ± 5.05[a]	202.85 ± 8.72[a]	181.33 ± 6.21[b]	168.99 ± 5.85[c]
HDL-C	52.21 ± 4.42[a]	57.24 ± 4.04[b]	59.52 ± 3.95[b]	59.31 ± 4.38[b]
VLDL-C	47.36 ± 2.31[a]	44.11 ± 2.01[a]	38.34 ± 3.11[c]	36.82 ± 2.23[c]
LDL-C	146.22 ± 6.05[a]	121.98 ± 7.27[b]	110.55 ± 6.15[c]	102.01 ± 5.87[c]
LDL-C/HDL-C	2.77 ± 0.32[a]	2.18 ± 0.24[b]	1.89 ± 0.30[b]	1.51 ± 0.21[c]

Values are means ± SEM; n=5. Values bearing different alphabets are significantly different ($p<0.05$).
Key: BW (mg/kg body weight), T. (Total)