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Research Article

Co-administeration of Ethanolic Leaf Extract of *Moringa oleifera* and Metformin Improves Glucose, Lipid and Protein Profiles of Diabetic Wistar rats

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ABSTRACT: **Herbs are often co-administered with orthodox drugs, raising the potential for herb-drug interactions. This study investigated the pharmacological interaction between ethanol extract of *Moringa oleifera* (MOE) leaves and metformin co-administered to diabetic Wistar rats. Diabetes was induced in rats by administration of 150 mg alloxan/kg body weight intraperitoneally. A dose-response study for MOE at doses of 100-2000 mg/kg body wt. was carried out. A plot of percentage glycaemic reduction at 4h post-treatment versus log dose was used to estimate the median effective dose (ED_{50}). Nine (9) groups of rats were used for the interaction study. Groups I and II served as normoglycaemic and diabetic controls respectively and received 1ml Normal saline. Diabetic Groups III-V received 375, 750 and 1500 mg/kg MOE respectively. Groups VI-VIII also diabetic received the same doses of MOE respectively but co-administered with a fixed dose of metformin (150 mg/kg). Group IX received metformin (150 mg/kg) alone. Fasting blood sugar (FBS) was monitored weekly and blood samples collected on day 28 for protein and lipid profile assay. The MOE/metformin co-administered groups showed greater antihyperglycaemic activity ($p<0.001$) than the MOE and metformin alone groups. Significant increases in serum levels of cholesterol, TG and LDLC with the decrease in HDLC levels in the alloxan induced diabetic rats were reversed in MOE ($p<0.01$) and MOE/metformin ($p<0.001$) administered groups. These findings indicate that MOE/Metformin co-administration produced additive anti-hyperglycaemic and hypolipidaemic effects compared to either MOE or Metformin alone and may be useful in the therapeutic management of diabetes mellitus that is associated with dyslipidaemia.**

KEYWORDS: **Diabetes, Hyperglycaemia, Pharmacological interaction, *Moringa oleifera*, Metformin.**

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INTRODUCTION

Global use of herbal medicinal products and supplements has increased tremendously over the past three decades (Ekor, 2013). More than 80% of people in Africa and Asia use herbal medicines and an increasing number in the Western world (WHO, 1999). As the global use of herbal medicinal products continues to grow and many new drug products are introduced into the market, there may be an increase in the use of herbs alongside conventional drugs for better therapeutic outcomes (Vidushi, 2013).

Diabetes mellitus is a chronic metabolic disease resulting from defects in insulin secretion, insulin action or both (Wadkar *et al.*, 2008). Diabetes is characterized by hyperglycaemia which can be managed through diet, exercise, oral anti-diabetic agents, insulin replacement therapy and the use of herbs (Adikwu *et al.*, 2010). Improperly managed hyperglycaemia predisposes patients to a number of infections and risk of developing microvascular and macrovascular complications (Claudia *et al.*, 2006). To avert this, some patients co-use orthodox anti-diabetic agents alongside herbs that are used traditionally to decrease blood sugar (Mohammed & Mohammed, 2009).

Moringa oleifera also known as horseradish tree is one of such herbs with antidiabetic activity (Tende *et al.*, 2011). It is a fast growing drought-resistant tree belonging to the Moringaceae family. Various parts (leaves, flower and seed) of *Moringa oleifera* have been reported to possess antidiabetic activity (Jaiswal *et al.*, 2009; Nku-Ekpang *et al.*, 2015). Due to its acclaimed therapeutic properties, availability and acceptability, it is often used with first-line orthodox antidiabetic agents such as metformin in the management of hyperglycaemia (Fasinu *et al.*, 2011).

The interaction between herbal preparations and orthodox medicinal drugs could either be pharmacodynamic or pharmacokinetic in nature. A pharmacodynamic interaction occurs when both the orthodox drug and the herb have affinity for the same receptors or physiological process while pharmacokinetic interaction results when the absorption, distribution, biotransformation or elimination of an orthodox drug is modified by a herbal product or *vice versa* resulting in an altered pharmacological response (Lal *et al.*, 2011). Both pharmacodynamic and pharmacokinetic interactions can lead to additive, synergistic or antagonistic pharmacological activity. This study was undertaken to investigate the effect of co-administered ethanol extract of *Moringa oleifera* leaves and metformin on serum glucose levels, protein and lipid profiles in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant Materials and Preparation of Extracts

Fresh leaves of *M. oleifera* were collected in Samaru, Sabon Gari Local Government Area of Kaduna State, Nigeria in April, 2013. The botanical identity was confirmed by Mallam U. Mohammed an ethno-botanist at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria where a voucher specimen (Voucher No. 571) was deposited for future reference.

The leaves were cleansed with distilled water to remove debris and dust particles. They were air dried to a constant weight and pulverized with a manual blender. A portion (800 g) of the powdered leaves was cold macerated with ethanol for 24 hours and filtered with Whatman filter paper (Size No1) to obtain *M. oleifera* ethanolic extract (MOE). The extract was evaporated to dryness over a water bath maintained at 40^{o}C and stored in a refrigerator until required for use.

Materials

Metformin 500mg tablets (Glucophage®, Merke Sante s.a.s France) was purchased locally, Alloxan monohydrate (powder) and absolute ethanol were purchased from the country representative of Sigma Chemical, St. Loius USA while a digital glucometer and corresponding test strips (Fine Test®, Infopia Co., Ltd. USA) was purchased from a pharmacy store. All other chemicals used were of analar grade and obtained commercially.

Experimental Animals

Healthy Wistar rats of either sex weighing 100-150g, purchased from the Animal Facility Centre (AFC), Department of Pharmacology and Toxicology, of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria were used for the study. They were kept in stainless steel cages under 12/ 12 hour light/ dark cycles at standard laboratory conditions of temperature 22±1^{o}C, relative humidity 14±1% and 12h light /dark cycle. They were maintained on clean water and standard rodent chow. All experiments were performed according to the "Principles of Laboratory Animal Care" (NIH and NIPRD Standard Operating Procedures (SOPs) for pharmacological studies involving whole animals.

Acute toxicity study

The oral median lethal dose (LD_{50}) of the extract was determined in rats according to the method described by Lorke (1983) but with slight modifications. The study was carried out in two phases. In the first phase, nine rats were randomized into three groups of three rats which were given 10, 100, and 1000mg extract/kg body weight. The rats were kept under the same conditions and observed for signs of toxicity which included but were not limited to paw-licking, stretching, respiratory distress and mortality for the first 4h and thereafter daily for two weeks. Based on the results of the initial phase, the following doses- 1600, 1900 and 5000mg extract/kg body weight were administered to another set of three groups of three rats in the second phase. These rats were also monitored closely for the first 4 h after treatment and subsequently daily for 14 days for signs of toxicity and/or mortality. The results obtained in the second phase were used to calculate the LD_{50}.

Induction of hyperglycaemia

The method described by Stein (1987) was adopted. Diabetes was induced in rats fasted overnight by administering freshly prepared 150 mg alloxan/kg intraperitoneally. The animals were thereafter allowed food and water *ad libitum*. After 72 hours, the blood glucose levels in the rats were determined following an overnight fast, using a Fine Test® digital glucometer and the corresponding test strip. Rats having fasting blood glucose (FBS) levels ≥ 200 mg/dl were considered hyperglycaemic.

Dose-response study

Forty-eight (48) rats having FBS level ≥ 200 mg/dl were randomized into 8 groups of 6 rats each. The rats in group 1 served as diabetic control and received 10 ml/kg body weight of distilled water orally, groups 2, 3, 4, 5, 6 and 7 rats were administered 100, 200, 400, 800, 1000 and 2000 mg extract/kg body weight respectively while group 8 received metformin at a dose of 150 mg/kg. Post-treatment blood glucose concentration (BGC) was measured hourly with a glucometer using the blood samples collected from the tail tip of rats. The blood glucose level monitoring was done for 4h and a plot of percentage glycaemic reduction at 4 h versus log dose was used to estimate the ED_{50} (dose required to produce 50% glycaemic reduction in diabetic rats) of the extract.

Pharmacological interaction studies

Fifty-four (54) rats having FBS level ≥ 200 mg/dl were randomized into 9 groups of 6 rats each and treated as follows. Groups I and II served as normoglycaemic and diabetic controls respectively. Groups III, IV and V were given 375, 750 and 1500 mg/kg of MOE alone orally while Groups VI, VII and VIII rats received 150 mg/kg of Metformin with 375, 750 and 1500 mg/kg of MOE respectively while Group IX rats received150mg of Metformin/kg body weight alone for 28 days.

Determination of Biochemical Parameters

Fasting blood sugar determination: Blood samples were obtained from the tail vein of the rats and glucose level was estimated on 0, 7, 14, 21 and 28th day of the study by FineTest® glucometer

Lipid profile assay: On the last day of the experiment, overnight fasted rats were euthanized by chloroform inhalation. Blood samples were collected via cardiac puncture into plain sera tubes and allowed to clot. Serum was separated by centrifugation [using DenleyBS400 centrifuge (England) with swing-out rotor system, ×1500g at 20 ^{o}C, for 10 min and then assayed for levels of total protein, albumin, total cholesterol (Tchol.), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C).

Assay for serum total cholesterol: The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by the method of Stein (1987).

Assay for serum triglyceride: The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by the method of Tietz (1990).

Assay for serum high density lipoprotein cholesterol:The serum level of HDL-C was estimated by the method of Wacnic and Alber (1978).

Determination of serum low-density lipoprotein cholesterol: The serum level of (LDL-C) was calculated according to the method of Friedewald *et al.* (1972) using the equation below:

LDL-C = TG/5-HDL-C. The value was expressed in mg/dl.

Serum protein and serum albumin: Serum protein and serum albumin were estimated by Biuret method and Bromocresol Green (BCG) binding methods respectively using a commercial assay kit from Randox Laboratories Ltd.

Statistical analysis

All data were expressed as mean ± Standard Error of Mean (SEM) and statistical differences between means were determined by one-way ANOVA followed by Dunnett's *post – hoc* test for multiple comparison tests using GraphPad version 5.0. *P-values* less than 0.05 were considered significant.

RESULTS

Yield of Extract

The yield of the ethanol extract was calculated to be 12.07% $^{w}/_{w.}$

Acute toxicity study

In both phases of the experiment, there were no signs of toxicity or mortality during the acute monitoring and 14 day monitoring periods. The oral LD_{50} value was estimated to be greater than 5000 mg/kg body weight in rats.

Dose-response study

Moringa oleifera ethanol leaf extract produced significant ($p<0.05$), dose- and time-dependent reduction in FBS when compared to the control (Table 1).

Table 1: Effect of Ethanol Extract of *Moringa oleifera* Leaves (MOE) and Metformin on Fasting Blood Sugar of Alloxan-induced Hyperglycaemic Rats at different timed Intervals.

	Post Treatment Time (hours)				
Treatment (mg/kg)	0	1	2	3	4
Control	529.3±48.02	403.5±39.62	393.0±29.90	456.0±48.41	431.5±36.49
MOE 100	501.8±54.20	499.8±24.74	471.0±23.67	415.8±15.20	349.7±42.62
		(-23.87%)	(-19.84%)	(8.82%)	(18.96%)
MOE 200	454.2±63.44	428.3±28.72	387.2±27.56	361.3±18.44	297.3±3.97*
		(-6.14%)	(1.48%)	(20.77%)	(31.56%)
MOE 400	474.2±55.54	497.04±45.09	393.3±46.53	320.5±19.14*	250.8±57.97**
		(-23.18%)	(-0.08%)	(29.71%)	(41.88%)
MOE 800	486.5±38.08	391.7±35.20	242.2±25.28**	216.0±21.59***	193.6±15.92***
		(2.92%)	(38.37%)	(52.63%)	(55.13%)
MOE 1000	469.2±46.88	335.0±24.98	209.7±14.89**	108.0±16.79***	167.2±18.10***
		(16.98%)	(46.64%)	(60.53%)	(61.25%)
MOE 2000	454.7±33.70	335.8±43.02	215.0±16.76**	153.3±8.54***	148.3±8.79***
		(16.78%)	(45.29%)	(66.38%)	(65.63%)
Metformin 150	457.2±49.87	364.5±33.67	231.7±47.44**	202.2±38.69***	199.2±34.00***
		(9.67%)	(41.04%)	(55.66%)	(53.84%)

N=6, *p < 0.05, **p <0.01, ***p< 0.001 compared with the control. Values in parenthesis represent percentage glycaemic reduction at the various hours.

Estimation of Effective Median Dose (ED_{50}) of Ethanol Extract of M. oleifera Leaves.

From the dose-response data, a plot of percentage glycaemic reduction at 4h against log dose, the ED_{50} of MOE was estimated to be approximately 750 mg/kg. (Figure 1)

Effect of Ethanolic Extract of M. oleifera Leaves (MOE), Metformin and MOE/ Metformin Co-administration on FBS of Rats.

When compared to diabetic control, MOE (375 mg/kg) produced a significant (p< 0.05) reduction in FBS only on day 28. On days 21 and 28, MOE (750 mg/kg) produced highly significant (p<0.01) reduction in FBS while MOE (1500mg/kg) produced a significant dose-dependent reduction in FBS on day 7 (p<0.05), days 14, 21 and 28 (p<0.01) respectively.The co-administration of MOE (375 mg/kg)/Metformin and MOE (750 mg/kg)/Metformin significantly reduced FBS on day 7 (p<0.05), days 14 and 21 (p< 0.01) and day 28 (p< 0.001) respectively when compared to control MOE (1500 mg/kg). Metformin also produced significant (p< 0.001) reductions on days 7, 14, 21 and 28. Metformin alone showed significant (p<0.05), (p< 0.01) reduction in FBS on day 14 and days 21 and 28 respectively when compared to the diabetic control (Table 2).

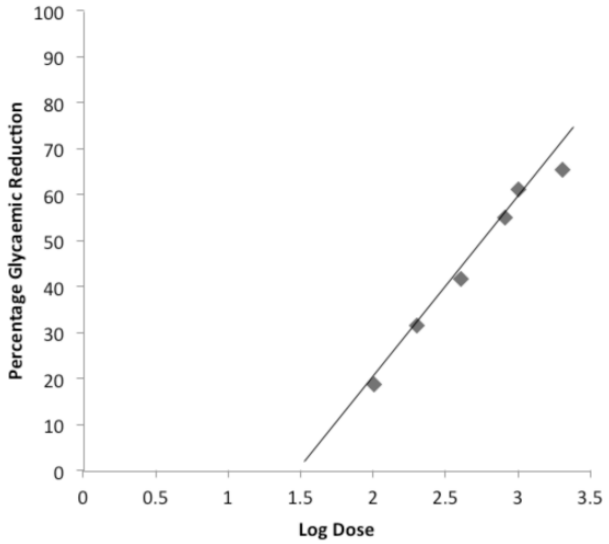

Figure 1: Log dose-Response Curve of *M. Oleifera* leave extract 4 hours post-treatment.

Effect of Ethanol Extract of M. oleifera Leaves (MOE), Metformin (Met) and MOE/Metformin co-administration on some Serum Total Protein and Albumin Concentrations of Rats.

Rats in groups that were treated alone with MOE (1500 mg/kg), 375 mg/kg MOE/Metformin, 750 mg/kg/Metformin, 1500 mg/kg MOE/Metformin and Metformin produced highly significant (p<0.001) increase in total protein and albumin compared to diabetic control (Figure 2).

Effect of Ethanol Extract of *M. oleifera* Leaves (MOE), Metformin (Met) and MOE/Metformin Co-administration on Serum Total Cholesterol Level of Diabetic Rats. MOE at the doses of 750 and 1500mg/kg significantly (p<0.01), (p<0.001) respectively decreased the serum level of total cholesterol when compared to the diabetic control. At all the doses of the extract co-administered with metformin, a highly significant (p<0.001) reduction in serum total cholesterol levels was observed compared to diabetic control. Similar observation was made with metformin alone group rats (Figure 3).

Effect of Ethanol Extract of *M. oleifera* Leaves (MOE), Metformin (Met) and MOE/Metformin Co-administration on Serum Triglyceride Level of Diabetic Rats.

There were statistically significant (p<0.05), (p<0.01) decreases in the level of serum triglyceride in the diabetic groups treated with MOE doses of 750 and 1500 mg/kg respectively. Metformin alone at 150 mg/kg showed highly significant (p<0.01) decrease in serum triglyceride level when compared to diabetic control group. Meanwhile the 375, 750 and 1500 mg/kg of MOE co-administered with metformin produced very highly significant reduction (p>0.001), when compared to the diabetic control (Figure 4).

Table 2: Effect of Ethanolic Extract of *M. oleifera* Leaves (MOE), Metformin (Met) and MOE /Metformin Co-administration on FBS of Hyperglycaemic Rats.

Treatment (mg/kg)	Post- Treatment Time (Day) 0	7	14	21	28
NC	84.80±5.60	72.40±4.42	76.40±3.41	79.20±2.67	73.80±2.44
DC	454.4±67.21	517.6±43.91	539.6±13.07	517.6±25.28	487.4±27.112
MOE 375	448.0±55.49^{c}	445.5±57.19^{c}	417.5±56.00^{c}	368.5±47.98^{c}	316.2±60.14*^{c}
MOE 750	415.0±56.17^{c}	356.5±49.78^{c}	360.0±56.00^{c}	281.2±50.23**^{c}	265.0±51.61**^{c}
MOE 1500	433.8±61.75^{c}	307.0±47.57*^{c}	288.8±51.25**^{c}	284.5±45.31**^{c}	244.3±50.94**^{c}
MOE 375 + Met	430.2±60.56^{c}	303.0±53.55*^{c}	288.7±59.35**^{c}	251.7±53.36***^{c}	132.3±19.01***^{b}
MOE 750 +Met	435.7±59.45^{c}	283.0±45.53*^{c}	268.8±47.21**^{c}	200.6±37.98***^{c}	112.8±13.74***^{b}
MOE 1500 +Met	423.2±65.87^{c}	198.0±14.59***^{c}	156.7±12.45***^{c}	119.5±8.84***^{c}	96.33±5.57***^{a}
Met150	416.3±57.81^{c}	373.5±53.17^{c}	308.7±54.79*^{c}	291.2±47.13**^{c}	257.0±47.81**^{c}

N=6, *p <0.05, **p <0.01, ***p <0.001 and ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 compared with the diabetic control (DC) and normoglycaemic control (NC) respectively.

Effect of Ethanol Extract of *M. oleifera* Leaves (MOE), Metformin (Met) and MOE/Metformin co-administration on serum HDL-C Level of Diabetic Rats

Serum HDL-C level was significantly elevated (p<0.001) only in all the MOE/metformin co-administered groups when compared to the diabetic control (Figure 5).

Effect of Ethanol Extract of *M. oleifera* leaves (MOE), Metformin (Met) and MOE/Metformin Co-administration on Serum LDL-C level of Diabetic Rats

There were dose-dependent significant (p<0.05, p<0.01 and p<0.001 respectively) reductions in the serum level of LDL-C in the groups administered with MOE at the doses of 375, 750 and 1500 mg/kg when compared to the diabetic control. The extract at all doses co-administered with metformin showed highly significant decrease (p<0.001) in serum level of LDL-C while metformin alone group rats showed a significant (p<0.05) decrease in serum LDL-C when compared to the diabetic control (Figure 6).

DISCUSSION

The acute toxicity study of the extract revealed that the oral LD_{50} value is greater than 5000 mg/kg. This is in agreement with the study of Bakre *et al.* (2013) in which it was reported that the oral LD_{50} of the ethanol extract of *M. oleifera* leaves was greater than 6400 mg/kg in mice. The extract is therefore considered practically non-toxic. This implies that the extract can be consumed at higher doses without fear of toxicity. This study also estimated the median effective dose (ED_{50}) of the extract to be 750 mg extract/kg. This further confirms that the extract possesses a wide safety margin since the LD_{50} value is far greater than the ED_{50}.

Alloxan, used to induce diabetes acts by selectively destroying the insulin- producing beta cells of the Islet of Langerhans producing hypoinsulinaemia (Szkudelski, 2001). This resultant insulin deficiency, leads to various metabolic alterations in the animals such as, increase in blood glucose, dyslipidemia and alterations in serum protein profile (Vivek *et al.*, 2010).

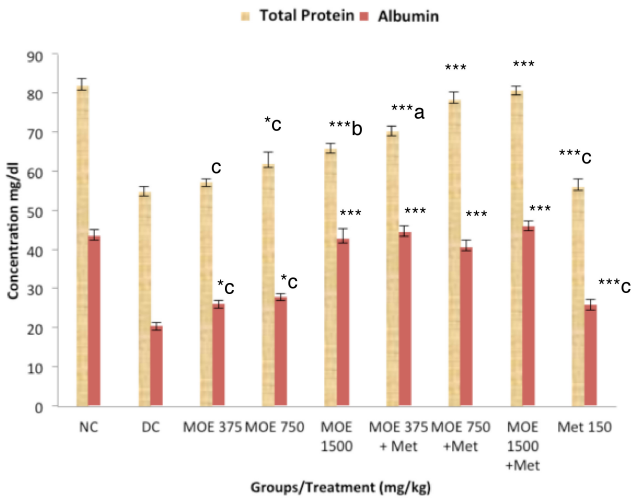

Figure 2: Effect of Ethanol Extract of *M. oleifera* Leaves (MOE), and MOE/Metformin co-administration on serum Total protein and Albumin levels of Alloxan-induced diabetic Wistar rats. n=6 , *p <0.05, **p <0.01, ***p <0.001 and [a]p<0.05, [b]p<0.01, [c]p<0.001 compared with the diabetic control (DC) and normoglycaemic control (NC) respectively.

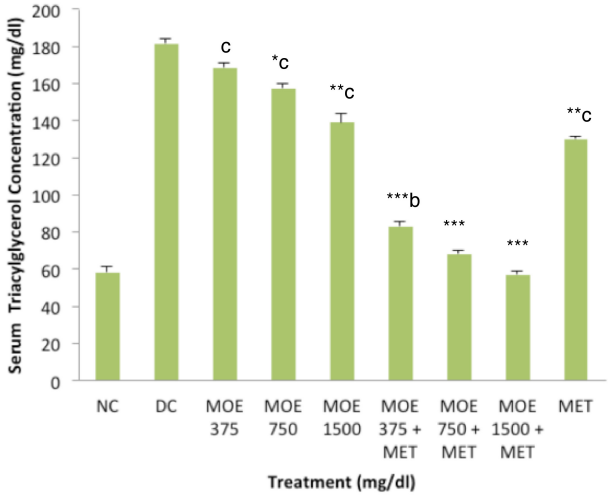

Figure 4: Effect of ethanol extract of *M. oleifera* leaves (MOE) and MOE/Metformin co-administration on serum triglyceride level of Alloxan-induced diabetic Wistar rats. n=6. *p <0.05, **p <0.01, ***p <0.001 and [a]p<0.05, [b]p<0.01, [c]p<0.001 compared with the diabetic control (DC) and normoglycaemic control (NC) respectively.

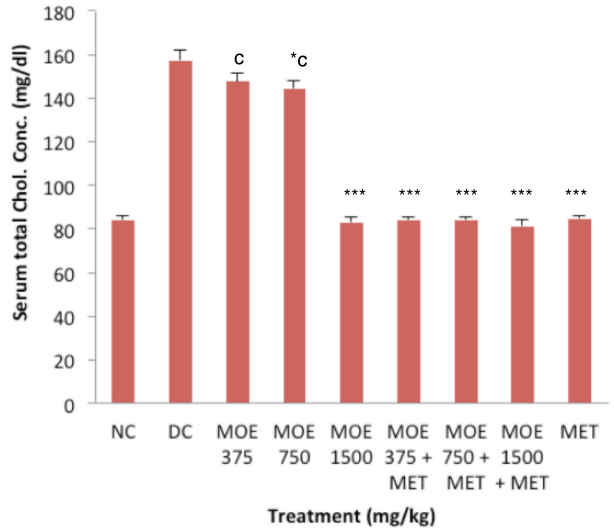

Figure 3: Effect of Ethanol Extract of *M. oleifera* (MOE) and MOE/Metformin Co-administration on Serum Total Cholesterol Level of Alloxan-induced Diabetic Wistar Rats. n = 6,*p <0.05, **p<0.01, ***p <0.001 and [a]p<0.05, [b]p<0.01, [c]p<0.001 statistically significant compared with the diabetic control (DC) and normoglycaemic control (NC) respectively.

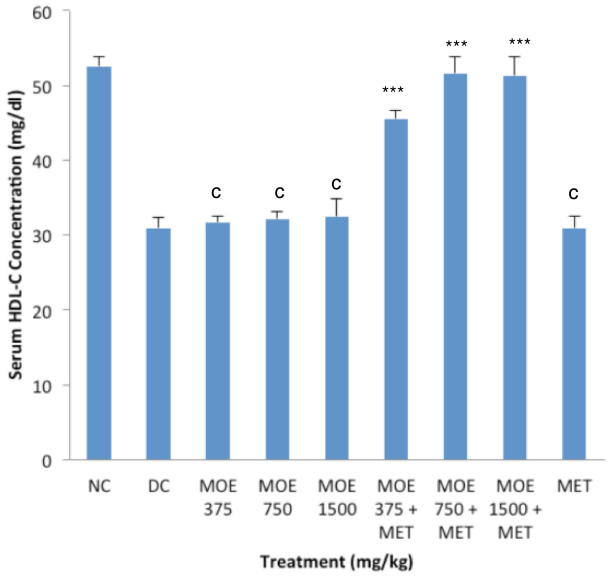

Figure 5: Effect of Ethanol Extract of *M. oleifera* leaves (MOE) and MOE/Metformin co-administration on serum HDL-C level of Alloxan-induced diabetic Wistar rats. n=6. *p <0.05, **p <0.01, ***p <0.001 and [a]p <0.05, [b]p<0.01, [c]p<0.001 compared with the diabetic control (DC) and normoglycaemic control (NC) respectively.

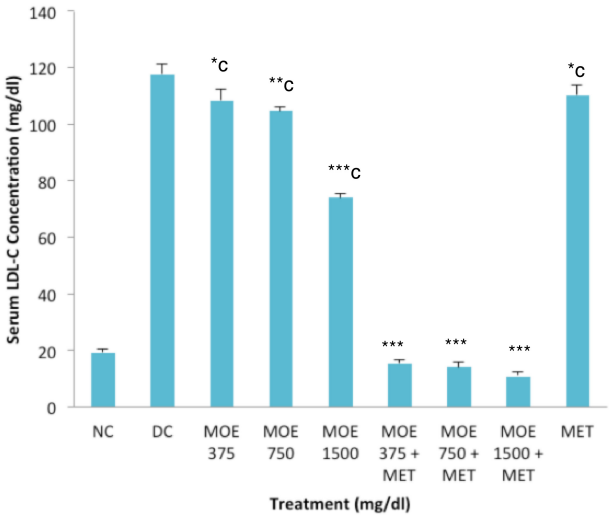

Figure 6: Effect of Ethanol Extract of *M. oleifera* leaves (MOE) and MOE/Metformin Co-administration on Serum LDL-C Level of Alloxan-induced Diabetic Wistar Rats. n=6, *p <0.05, **p <0.01, ***p <0.001 and [a]p<0.05, [b]p<0.01, [c]p<0. compared with the diabetic control (DC) and normoglycaemic control (NC) respectively.

The significant, dose and time- dependent reduction in blood glucose level produced in alloxan-induced hyperglycaemic rats by ethanol extract of *Moringa oleifera* leaves is in agreement with those of other researchers, who systematically demonstrated that extracts of various parts of the plant (leaves, flowers and seed) possess antidiabetic properties (Tende *et al.*, 2011; Jaiswal *et al.*, 2009). In a controlled study with untreated type 2 diabetes mellitus patients, (William *et al.*, 1993) reported the anti-hyperglycaemic activity of the *Moringa oleifera* leaves. Kumari (2010) also reported significant hypoglycaemic effect of *M. oleifera* leaf dietary consumption in Type 2 diabetes mellitus (T2DM) patients, and reported that it reduced glucose level significantly after a 40-day period.

The therapeutic actions of *Moringa oleifera* medication have been attributed to the relatively high antioxidant activity of its leaves, flowers, and seeds (Atawodi *et al.*, 2010). Quercetin and kaemferol found in *Moringa oleifera* leaves were shown to be antioxidant in nature (Fuglie, 1999). Hypoglycaemic activity of kaemferol derivatives from many medicinal plants has been reported by Desoky & Yousef (1997). These antioxidants might have played a role in scavenging the free radicals generated by alloxan leading to the regeneration of the beta-cells destroyed by alloxan, hence an increase in release of insulin and reduction in glycaemia. The extract might have also produced anti-hyperglycaemic activity through direct release of insulin by inhibiting the ATP-sensitive potassium channels in the membrane of the residual beta-cells just like sulfonylureas and meglitinides. It is also possible that the extract might have potentiated the action of insulin to stimulate glucose uptake and utilization by tissues, especially by the liver, skeletal muscle, and adipose tissue (Gerich, 2000).

Dyslipidemia which includes not only quantitative but also qualitative abnormalities of lipoprotein plays a significant role in the proatherogenesis of vascular complications in diabetes mellitus (Rotimi *et al.*, 2011). Lowering of serum lipid levels through herbal or drug therapy seems to be associated with a decrease in the risk of vascular disease in diabetes (Claudia *et al.*, 2006). In this study, following alloxan treatment, there was an elevation in serum concentration of total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) and a decrease in HDL-C in rats. Daisy *et al.* (2009) and Eze *et al.* (2012) also reported increased plasma cholesterol, triglycerides, LDLC and decreased HDL-C in streptozocin-induced hyperglycemic rats. According to Mathe (1995), the observed increase in serum cholesterol level results from increased intestinal absorption and synthesis of cholesterol. Nimenibo-Uadia (2003) suggested that diabetes-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose due to under utilization of glucose. Insulin deficiency and elevations of the counter-regulatory hormones lead to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissues which are mobilized for energy purpose (Rotimi *et al.*, 2011). The excess fatty acids are afterwards accumulated in the liver and converted to triglyceride (Suryawanshi *et al.* 2006). The unregulated action of lipolytic hormones on the fat depots is therefore responsible for the hyperlipidemia that characterizes diabetes (Claudia *et al.*, 2006).

The significant decrease in serum lipid profile levels observed with *Moringa oleifera* and its co-administration may presumably be mediated by a control of lipid metabolism by some of the reported phytochemicals present in the plant. *Moringa oleifera* leaves are a rich source of glycosides, phenols, saponins and sterols. Beta-sitosterol, one of such sterols in the leaves lowers cholesterol level by inhibiting its re-absorption from endogenous sources with a simultaneous increase in its excretion into faeces in the form of neutral steroids (Kumarappen *et al.*, 2007). Saponins also lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption and/or by binding with bile acids, causing a reduction in the entero-hepatic circulation of bile acids and increase in its fecal excretion (Gerich, 2000). Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol [29]. Polyphenolic compounds possess hypolipidemic properties (Kumarappen *et al.*, 2007). Thus, the observed anti-hypercholesterolemic effect of *Moringa oleifera* could be linked to the high polyphenolic content of its leaves.

Additive cholesterol-lowering activity was observed with *Moringa oleifera*/ Metformin co-administeration as it produced highly significant reduction compared to either of the extract

or metformin alone. Metformin produces its anti-hypercholesterolemic effect mainly by correcting abnormal glucose metabolism (Defronzo & Goodman,1995).

The extract/metformin co-administration also produced a synergistic decrease in serum triacylglycerol level, which was more than that of the extract at all, the doses administered. Metformin is known to produce reduction in serum triglyceride levels as a result of decreased hepatic synthesis of very low-density lipoprotein (Chehade, 2000) and the extract might have acted through an alteration in the level of interleukin-6 (IL-6) which mediates energy mobilization in the muscles and fat tissues (Oyewo & Akanji, 2010). In addition, reduction in the serum cholesterol level in this study does not seem to be accompanied by lipolysis, as seen in the reduction in the serum triacylglycerol levels. From this study, it could be inferred that the extract will only produce synergistic serum triacylglyceride lowering activity with metformin at a high dose.

The dose-dependent and additive increase in the serum HDL-C level by the extract and extract/metformin co-administration respectively could possibly be due to an increase in the biosynthesis of HDL-C in the liver by the presence of flavonoids in the extract (Renaud *et al.*, 1999). With increase in HDL-C serum level, there was possibly an enhanced cholesterol excretion as more of it might have been transported from the peripheral tissues to the liver for excretion by HDL-C. The observed dose dependent decrease in the serum LDL-C levels may explain the serum cholesterol-lowering capability of *M. oleifera* extract. This effect could possibly be due to enhanced reverse cholesterol transport and bile acid excretion through inhibition of apo B production, needed for LDL-C production, transport and binding (Libby, 2011). The extract co-administered with metformin reduced serum level of LDL-C more than either metformin or the extract administered alone, this implies a synergistic interaction. The observed synergistic increase and decrease in the serum HDL-C and LDL-C respectively, is beneficial in diabetes as it reduces the risk of developing atherosclerosis (Libby, 2011).

Serum albumin and total protein concentration was significantly decreased in alloxan-treated rats when compared with the non-diabetic rats. Proteins are important building blocks of all cells and tissues. They form the structural part of most organs and make up enzymes and hormones that regulate body functions (Pagana & Pagana, 2006). The low protein level observed in this study may be as a result of overproduction of globulins, such as seen in multiple myeloma or autoimmune diseases or underproduction of albumin (Burtis et al, 2006). Albumin plays an important physiological role by maintaining osmotic pressure, transport of both endogenous and exogenous substance and serving as protein reserves (Saidu *et al.*, 2007). The liver's ability to synthesize albumin is reduced if the synthetic function of the liver is tampered with (Whitby *et al.*, 1989) and is an indication of hepatitis and liver cirrhosis. However, the results of this study showed that, the treatment of diabetic rats with *M. oleifera* and *M. oleifera* extract/metformin co-administeration brought about marked increase in serum total protein contents. The extract/metformin co-administeration showed synergistic effect as the total protein and albumin in these groups increased more than the groups administered the extract or metformin alone. This increment could be as a result of the reversal of hepatic damage. Hence, increase in hepatic uptake of amino acids, stimulation of amino acid incorporation into protein and decreased proteolysis by activating the enzyme that catalyzes amino acids transamination (Nahla *et al.*, 2006).

In this study, the anti-diabetic activity of the extract/metformin co-administeration was significantly higher than the anti-diabetic activity of the extract at all the doses administered and metformin. This suggests that the extract and metformin showed additive anti-hyperglycaemic activities possibly due to the additive beta-cell regeneration shown by the co-administeration which must have led to more insulin secretion. The wide safety margin of the extract and its positive effect on lipid and protein profile in the combination makes it useful in preventing arteriosclerosis and cardiovascular diseases associated with diabetes mellitus.

Conclusion

The anti-diabetic activity observed with co-administration of metformin and *Moringa oleifera* was significantly more than either of the drug or extract administered alone. It was concluded that *Moringa oleifera* shows synergistic effect with metformin on blood glucose levels, protein and lipid profiles. This could be important in reducing the dose of metformin to achieve enhanced therapeutic effect with minimum adverse effect.

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