Original Research Article

Effect of Combined Ethanol Leaf Extracts of *Moringa oleifera* and *Gongronema latifolium* on Body weight and Blood glucose Concentration of Streptozotocin-Nicotinamide-Induced Diabetic Albino Rats

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ABSTRACT

Background: Diabetes is a major health challenge globally and it is on the increase as carbohydrate is the main food consumed by an average human. Up till now, no synthetic drug has been able to provide permanent cure for diabetes.

Aim: This study investigated the effect of the combined ethanol leaf extracts of *Moringa oleifera* and *Gongronema latifolium* on body weight and blood glucose levels of streptozotocin-nicotinamide-induced diabetic albino rats.

Methods: Experimental diabetes was induced intraperitoneally with 65mg/kg bodyweight of streptozotocin and 230mg/kg bodyweight of nicotinamide. A total of forty male albino rats were divided into eight groups of five rats each which were normal control, diabetic control and diabetics administered 500mg/kg *M. oleifera,* 500mg/kg *G.latifolium,* 250mg/kg *M.oleifera*+500mg/kg *G.latifolium,* 500mg/kg *M.oleifera*+250mg/kg *G. latifolium,* 500mg/kg *M.oleifera*+500mg/kg *G. latifolium* and 10mg/kg bodyweight glibenclamide respectively.

Results: There was a significant ($p \leq 0.05$) reduction in blood glucose levels of the treated groups when compared to the diabetic control. A significant body weight gain was observed across the treated groups compared to the diabetic untreated. Statistical analysis was done using One-Way Analysis of Variance (ANOVA) at a significance level of $p \leq 0.05$, the post hoc test was done using Turkey multiple comparison test.

Conclusion: Based on the results obtained, it is obvious that there is synergy in the anti-diabetic efficacies of *Moringa oleifera* and *Gongronema latifolium.*

Key words: *M. oleifera, G. latifolium,* streptozotocin, nicotinamide, diabetes

INTRODUCTION

According to the world health organization, diabetes mellitus (DM) commonly referred to as diabetes is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst and increased hunger. If left untreated, diabetes can cause many complications. Acute complications can include diabetic ketoacidiosis, hyperosmolar hyperglycaemic state or death. Serious long term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes [1]. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced [2]. Diabetes is one of the five leading causes of death worldwide, with type 2 diabetes occurring more frequently than type 1. Management of diabetes without side effect is still a challenge and therefore, new strategies need to be examined [3].

The lesions in the pathophysiology of diabetes are multiple and therefore would require more than a single drug agent to reverse all or a majority of the aspects of the disease. The effective therapeutic approach should be multimodal and in this light, several traditional medicinal herbs have been proffered, giving the plethora of active ingredients present in a single herb [4]. Polyherbal therapy which is the use of a combination of various agents from different plant sources for therapeutic purposes is a current pharmacological principle and has the advantage of producing maximum therapeutic efficacy with minimum side effects.

Moringa oleifera is the most widely cultivated species of the mono-generic family *Moringaceae*, which includes 13 species of trees and shrubs distributed in sub Himalayan ranges of India, Sri Lanka, North-eastern and Southwestern Africa, Madagascar and Arabia. *Moringa* is also native to parts of West Africa particularly Nigeria [5]. The whole *Moringa oleifera* plant is used in the treatment of psychosis, eye diseases, fever and as an aphrodisiac, the aqueous extracts of roots and barks were found to be effective in preventing implantation [6]. The *Moringa* tree is a multifunction plant. It has been cultivated in tropical regions all over the world for the following characteristics: high protein, vitamins, mineral and carbohydrate content of entire plants; high value of nutrition for both humans and livestock; high oil content (42%) of the seed which is edible, and with medicinal uses; the coagulant of seeds could be used for wastewater treatment [5].

Different parts of the *Moringa oleifera* (Mo) tree have been established as being good sources of unique glucosinolates, flavonoids and phenolic [7, 8], carotenoids [9], tocopherols [10], polyunsaturated fatty acids (PUFAs) [11], highly bioavailable minerals [12], and folate [13]. Among glucosinolates, 4-O-(a-Lrhamnopyranosyloxy)-benzylglucosinolate (glucomoringin) is the most predominant in the stem, leaves, flowers, pods and seeds of *M. oleifera* [7]. Although in the roots, benzyl glucosinolate (glucotropaeolin) is the most prominent. The highest content of glucosinolate is found in the leaves and seeds. The enzymatic catabolism of glucosinolates by the endogenous plant enzyme myrosinase produces isothiocyanates, nitriles, and thiocarbamates that are known for strong hypotensive (blood pressure lowering) and spasmolytic (muscle relaxant) effects [14]. In the leaves, the amount of quercetin and kaempferol was found to be in the range of 0.07–1.26 and 0.05–0.67 %, respectively. The potent antioxidant activity of *Moringa* is attributed to the high concentration of these polyphenols. Medicinally, the antioxidant, wound healing, hypotensive, and diuretic effects of this plant have been reported [15, 16]. Airaodion *et al*., [17] has reported its protective effect on haematological indices in crude oil treated diet.

Previous studies have reported the antioxidant [18], anti-inflammatory [19] and pharmacological [7] properties of *M. oleifera*. *Moringa oleifera* leaf

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has been reported to be potent in the prevention of peptic ulcer [20]. However, Airaodion *et al*., [21] reported that the combination of *M. oleifera* leaves and turmeric root is more potent in the prevention of peptic ulcer. Further studies revealed that *M. oleifera* leaves possess hepatoprotective efficiency against hydrocarbon exposure [22].

Gongronema latifolium is a climber, characterized by greenish-yellow flowers [23]. It is wide spread in tropical Africa and can be found in Senegal, east to Chad and south to Democratic Republic of Congo [24]. The leafy vegetable can be propagated by seed or soft wood, semi-hard wood and hard wood cuttings.

The common name for *G. latifolium* is amaranth globe. The Efik/Ibibio people in south-southern Nigeria call the leaves "Utasi", the Igbos in South-Eastern Nigeria call it "Utazi", and the Yorubas in South-Western Nigeria call it "arokeke" or "madumaro" [25].

The leaves are sharp-bitter-sweet and are widely used as leafy vegetable and a spice for sauces, soups and salads. The plant has also been widely used in folk medicine for maintaining healthy blood glucose levels.

Management of diabetes without any side effect is still a challenge, hence, this study was intended to evaluate the antidiabetic efficacy of combined ethanol leaf extracts of *M. oleifera* and *G. latifolium* on Streptozotocin-nicotinamide-induced diabetic rats.

MATERIALS AND METHODS

Collection and preparation of plant extracts

Fresh and healthy leaves of *M. oleifera* and *G. latifolium* were harvested from a farm in Ihiagwa in Owerri West Local Government Area of Imo state, Nigeria and were identified by Dr. S. N. Mbagwu, a plant taxonomist at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. They were dried under shade for about two weeks and then ground into powder. Ethanol extracts were prepared using a modified maceration method reported by Etim *et al*. [29]. The leaf powders were soaked in 90 % ethanol for 72 hours, properly stoppered and labeled in a conical flask. The mixtures were filtered using Whatman No. 1 filter paper. The filtrates were concentrated to one-fifth of their original volumes in a rotary evaporator under reduced pressure at 40^{o}C, after which they were evaporated to the dried residues in a water-bath, and stored in a refrigerator.

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Experimental Animals

A total of seventy-six albino rats aged between 4-6 weeks and weighing about 100-150 g were used for this study. Thirty-six of the rats were used for acute toxicity studies and the remaining forty for the experiment. They were purchased from the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka and allowed to acclimatize for two weeks, prior to the commencement of the experiment. The rats were kept in well ventilated and clean cages at room temperature and their beddings changed every two days. They were fed with standard feed and water and water was given *ad libitum.*

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Acute Toxicity Studies (LD_{50})

Acute toxicity studies (LD_{50}) was carried out using the method of Lorke [26]. The method comprises two phases:

In the first phase, for each of the plant extracts, nine rats were subjected to a 12 hours fast, after which they were randomly divided into three groups of three rats each. The extracts were administered orally as follows:

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Group I: 10 mg/kg body weight of extract

Group II: 100 mg/kg body weight of extract

Group III: 1000 mg/kg body weight of extract

At the end of 24 hours observation time, all the animals were alive and active

In the second phase, for each extract, another nine rats fasted overnight and randomly divided into three groups of three rats each. Extracts were given through oral route as follows:

Group I: 1600 mg/kg body weight

Group II: 2700 mg/kg body weight

Group III: 5000 mg/kg body weight

At the end of the 24 hours observation time, all the rats were still alive and moving about actively.

Induction of Diabetes

After the acclimatization period, the rats were subjected to a 12 hours fast and diabetes was induced by intraperitoneal injection of nicotinamide (NA) at a dose of 230 mg/kg body weight, 15 minutes before intraperitoneal administration of streptozotocin (STZ) at a dose of 65 mg/kg body weight reconstituted in citrate buffer. Confirmation of diabetes after two weeks was done using accu-chek glucometer active, with blood obtained from tail puncture of the rats, and rats with fasting blood glucose level (FBGL) of 200 mg/dl and above were considered diabetic and used for the study.

Animal Grouping and Treatment

The forty rats were divided into eight groups of five rats each and were treated as follows:

Group I: 500 mg/kg *M. oleifera* extract only (MO).

Group II: 500 mg/kg *G. latifolium* extract only (GF)

Group III: 250 mg/kg *M. oleifera* and 500 mg/kg *G. latifolium* extracts (MO+GF1)

Group IV: 500 mg/kg *M. oleifera* and 250 mg/kg *G. latifolium* extracts (MO+GF2).

Group V: 500 mg/kg *M. oleifera* and 500 mg/kg *G. latifolium* extracts (MO+GF3).

Group V: 10 mg/kg glibenclamide (GB).

Group VII: Uninduced with diabetes and untreated (Normal Control)

Group VIII: Group VII: Induced with diabetes but untreated (Diabetic Control)

N.B: Glibenclamide is a standard drug used for diabetes management

Administration was done 12 hourly via orogastric intubation for 28 days.

Determination of Blood Glucose Concentration

Blood for determination of glucose concentration was collected by cutting the tails of the rats after sterilizing with 10% alcohol. A drop of blood was allowed to fall on a test strip which was then inserted into a glucometer (Accu-chek active). This gave direct reading in mg/dl after 5 seconds.

Statistical Analysis

Results were presented as mean ± standard deviation and data was analyzed for statistical significance by one-way analysis of variance (ANOVA). Post hoc analysis was done using Turkey's multiple comparison test at a significance level of $p \leq 0.05$.

RESULTS

Acute Toxicity Studies

Administration of ethanol extracts of *M. oleifera* and *G. latifolium* up to concentrations of 5000 mg/kg did not cause any mortality or any major acute toxicity. Thus, the LD_{50} of the both extracts was found to be greater than 5000 mg/kg.

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Comment [S9]: Mention the PH of the citrate buffer.

Table 1: Effect of Extracts on Body Weight (g) of The Animals Before And After 28days of Treatment.

The result for the effect of ethanol extracts of *M. oleifera* and *G. latifolium* on the body weight of the rats is presented on table 1 below.

TREATMENT	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	BODY WEIGHT CHANGE	%BODY WEIGHT CHANGE
NC	112.17±9.77[a]	161.77±5.11[b]	0.44	44.22
DC	122.07±17.28[a]	92.93±2.15[a]	-0.24	-23.87
MO	107.43±3.29[a]	144.23±5.32[b]	0.34	34.25
GF	102.47±1.38[a]	151.63±2.08[b]	0.48	47.98
MO+GF1	101.00±14.48[a]	139.37±3.91[b]	0.38	37.99
MO+GF2	112.03±9.22[a]	164.40±16.30[b]	0.47	46.75
MO+GF3	127.63±14.84[a]	159.87±19.12[b]	0.25	25.26
GB	116.17±6.65[a]	154.77±18.18[b]	0.33	33.23

Results are presented as mean ± standard deviation. Values with different superscripts along the same row are statistically different at $p<0.05$.

Legend: MO = group administered 500 mg/kg *M. oleifera*, GF = group administered 500 mg/kg *G. latifolium*, MO+GF1 = group administered 250 mg/kg *M. oleifera*, MO+GF2 = group administered 500 mg/kg *M. oleifera* +250 mg/kg *G. latifolium*, MO+GF3 = group administered 500m g/kg M. oleifera+500 mg/kg *G. latifolium*, GB = group administered 10 mg/kg glibenclamide, NC= normal control group, DC = diabetic control group.

Table 2: Effect of Extracts on Blood glucose concentrations (mg/dl) of The Rats Before And After 28days of Treatment.

The result for the effect of ethanol extracts of *M. oleifera* and *G. latifolium* on the blood glucose levels of the rats is presented on table 2 below.

TREATMENT	INITIAL GLUCOSE CONCENTRATION	FINAL GLUCOSE CONCENTRATION	CHANGE IN GLUCOSE CONCENTRATION	% CHANGE IN GLUCOSE CONCENTRATION
NC	90.33±4.62[a]	82.67±4.16[a]	-0.085	-8.48%
DC	261.70±32.87[a]	340.70±24.99[a]	0.30	30.19%
MO	296.30±88.68[a]	102.30±16.92[a]	-0.65	-65.47%
GF	295.30±71.45[a]	85.33±11.59[b]	-0.71	-71.10%
MO+GF1	322.30±36.69[a]	100.00±3.00[b]	-0.69	-68.97%
MO+GF2	256.70±77.11[a]	93.67±14.74[b]	-0.64	-63.51%
MO+GF3	318.70±70.50[a]	81.33±9.29[b]	-0.74	-74.48%
GB	271.00±75.90[a]	107.30±5.51[a]	-0.60	-60.41%

Results are presented as mean ± standard deviation. Values with different superscripts along the same row are statistically different at p<0.05.

Legend: MO = group administered 500 mg/kg *M. oleifera*, GF = group administered 500 mg/kg *G. latifolium*, MO+GF1 = group administered 250 mg/kg *M. oleifera*, MO+GF2 = group administered 500 mg/kg *M. oleifera* +250 mg/kg *G. latifolium*, MO+GF3 = group administered 500m g/kg *M. oleifera*+500 mg/kg *G. latifolium*, GB = group administered 10 mg/kg glibenclamide, NC= normal control group, DC = diabetic control group.

DISCUSSION

This study investigated the effect of ethanol extract of *M. oleifera* and *G. latifolium* on streptozotocin-nicotinamide induced diabetic albino rats in combined as well as single doses. Diabetes mellitus is a disease that is characterized by a derangement in carbohydrate, lipid and protein metabolism.

Management of this disease is considered a global challenge because an effective measure is yet to be discovered. Most of the modern antidiabetic drugs only control blood sugar levels as long as they are regularly administered and are associated with a myriad of undesired side effects [27].

The results from this study revealed significant loss of weight of the untreated diabetic rats by 23.87 % compared to the non-diabetic animals. This might be attributed to the loss in muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty acids [28]. It corroborates the findings of Mohamed *et al*.,[29] who reported a significant body weight loss in diabetic rats induced with streptozotocin.

There was 34.26 %, 47.98 %, 37.99 %, 46.75 %, 25.26 %, and 33.23 % increase in body weight in the groups treated with 500 mg/kg *M. oleifera* (MO), 500 mg/kg *G. latifolium* (GF), 250 mg/kg *M. oleifera* + 500 mg/kg *G. latifolium* (MO+GF1), 500 mg/kg *M. oleifera* + 250 mg/kg *G. latifolium* (MO+GF2), 500 mg/kg *M. oleifera* + 500 mg/kg *G. latifolium* (MO+GF3) and 10 mg/kg glibenclamide (GB) respectively. The body weight gain observed across the treated groups gave an insight into the ameliorative effects of the extracts used for treatment as well as the anti-diabetic drug, glibenclamide.

In type 2 Diabetes mellitus, the loss of a direct effect of insulin to suppress hepatic glucose production and glycogenolysis in the liver causes an increase in hepatic glucose production [30]. This corroborates with the hyperglycaemia observed in the rats after streptozotocin-nicotinamide administration. Oral administration of *M. oleifera* and *G. latifolium* and their co-administration had a hypoglycaemic effect on the treated rats. Results revealed a reduction of blood glucose concentration by 65.47 %, 71.11 %, 68.98 %, 63.51 %, 74.48 %,and 60.40 % in the groups administered 500 mg/kg *M. oleifera,* 500 mg/kg *G. latifolium*, 250 mg/kg *M.oleifera* + 500 mg/kg *G. latifolium*, 500 mg/kg *M. oleifera* + 250 mg/kg *G. latifolium,* 500 mg/kg *M. oleifera* + 500 mg/kg *G. latifolium* and 10 mg/kg glibenclamide respectively. Sylvester et al. [31] reported that experimental

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71.10%

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rats subjected to streptozotocin- induced diabetes mellitus and treated with G. latifolium extracts had a significant (p≤0.05) reduction of blood glucose by 66.43 %. The result of this study corresponds favourably with the blood glucose lowering effects of *Corchorus olitorius* [32], *Vernonia amygdalina* [33], *Talinum triangulare* [34], *Telfairia occidentalis* [35], and *Carica papaya* [36] among others.

The hypoglycaemic effects of *M. oleifera* leaves have been postulated to be associated with decreased intestinal glucose uptake and slowing gastric emptying time by fibre in the leaves whose fibre content is 12 % (w/w) [37]. The presence of three important bioactive phytochemicals; quercetin, chlorogenic acid and moringinine [38]. Chlorogenic acid has been

shown to inhibit glucose – 6 –phosphate translocase in rats" liver which resulted in a reduction of hepatic gluconeogenesis and glycogenolysis [39]. In a human study, chlorogenic acid caused a decrease in glycemic response during oral glucose tolerance test [40]. Moringinine demonstrated an improvement of glucose tolerance in rats models [41].

The group that was administered standard antidiabetic drug, glibenclamide, showed a reduction in blood glucose level due to its insulin–stimulating actions on the beta cells of the pancreas [42]. The significant (p≤0.05) reduction in blood glucose by the combined action of *G. latifolium* and *M. oleifera* extracts evident in the groups administered 250 mg/kg *M. oleifera* + 500 mg/kg *G. latifolium*, 500 mg/kg *M. oleifera* + 250 mg/kg *G. latifolium* and 500m g/kg *M. oleifera* + 500 mg/kg *G. latifolium* attests to a synergism between both extracts.

CONCLUSION

The present study demonstrates that there is synergy in the anti diabetic efficacies of Moringa oleifera and Gongronema latifolium.

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The extracts were also found to be safe up to 5000mg/kg body weight.

CONSENT

It is not applicable.

ETHICAL DISCLAIMER

Animal ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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