

ACUTE HYPOGLYCAEMIC ACTIVITIES AND FATTY ACID PROFILE OF SEED OIL OF *MORINGA OLEIFERA* LAM

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Abstract

The adverse effect of oral hypoglycaemic drugs necessitated the search for efficient and safer antidiabetic drugs from herbal formulation. As such, the fatty acids profile and acute antidiabetic activities of *Moringa oleifera* seed oil extract of petroleum ether (PEEMO) and *Moringa oleifera* seed oil extract of dichloromethane (DCMMO) were investigated. The 2.0 mL/kg body weight (kg.bw) of both oils, 500 µg/kg.bw of glibenclimide and 2.0 mL/kg.bw of dimethyl sulphoxide (DMSO) were given orally to rats in their respective groups after induction with 2 g/kg.bw of glucose solution orally. Unsaturated fatty acids contents were in high proportion in both oils when compared to saturated fatty acids content. Administration of glucose solution significantly elevated the blood glucose level to 24.71, 47.83, 44.05, 44.78 and 30.86% for normoglycaemic, control, glibenclimide, DCMMO and PEEMO respectively at 30 minutes from their respective basal blood glucose level. However, the blood glucose level of the glibenclimide, PEEMO, DCMMO treated groups were significantly ($p < 0.05$) reduced at 60 (24.57, 15.61 and 10.69%), 90 (43.87, 30.08 and 15.45%) and 120 (57.98, 19.82 and 41.33%) minutes respectively when compared with that of 30 minutes' blood glucose levels. Therefore, *Moringa oleifera* seed oil extracts demonstrated acute hypoglycaemic effects in glucose fed rats.

Keywords: *Moringa oleifera*, glibenclimide, antidiabetic, diabetes, unsaturated fatty acids.

Introduction

Diabetes mellitus is a metabolic disorder, characterized by continuous increase in blood glucose with disturbances in metabolism of carbohydrate, protein and lipids caused by the abnormal secretion of insulin, insulin action, or both (Holt, 2004; Sabitha & Vinjay, 2017). Insulin, a hormone produced by beta cells of pancreas located in the Islet of Langerhans enables the cells of the body to absorb glucose

for metabolic process. However, when the body cells devoid of glucose via failure to absorb the glucose, accumulation occurs in the blood and results in many abnormalities (Wild *et al.*, 2004; Rother, 2007). Retinopathy, nephropathy, and / or neuropathy are among the long-term effects of diabetes mellitus that develop progressively during the illness (Nathan, 1993). Furthermore, cardiovascular diseases are also implicated in the patients with diabe-

tes mellitus (Collins *et al.*, 2016). Studies have shown that oxidative stress, caused mainly by hyperglycemia-induced free radical generation leads to the growth and progression of diabetes as well as its complications (Ceriello, 2003; Rahimi *et al.*, 2005). When the free radicals are abnormally high, it results in membrane damage due to protein glycation, membrane lipid peroxidation, followed by the simultaneous reduction of antioxidant defense mechanisms (Maritim, 2003). According to World Health Organization, the number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014 and categorized as seventh leading cause of death in 2016 (WHO, 2018). Diabetes prevalence has been rising more rapidly in middle- and low-income countries which is the major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation (WHO, 2018). In 2016, an estimated 1.6 million deaths were directly caused by diabetes and 2.2 million deaths were attributable to high blood glucose in 2012 which normally occurred before the age of 70 years (WHO, 2018).

About 90-99% of diabetes patients have type 2 diabetes (T2D). T2D contributes to combination of an adequate compensatory insulin secretory response and insulin resistance (Aditya *et al.*, 2012). Oral antidiabetic drug such as thiazolidinediones, abiguanides and sulfonylureas, are used for type 2 diabetes treatments, but these drugs have serious adverse effects and are not active against some complications of long-term diabetes (Aditya *et al.*, 2012). Herbs are alternative medicines for diabetes treatment due to their perceived acceptability, effectiveness, affordability and safety with lesser side effects in clinical experience coupled with low cost (Aditya *et al.*, 2012). As such the World Health Organization recommends the use of traditional and plant-

based medicines for the management of diabetes mellitus (WHO, 1994).

Moringa oleifera popularly called “horse-radish tree” is widely naturalized among the 13 species of *moringa* believed to have originated from sub-Himalayan tracts, part of north-western India. All parts of *moringa* are used in the folkloric systems of human medicine for the management and treatment of several diseases (Berushka & Himansu, 2012). The leaves, roots, and flowers are used to treat ailments such as venomous bites, ascites and rheumatism. It can also be used as circulatory stimulants in folk remedies (Anwar *et al.*, 2007). Recently, the use of oil for prevention or treatment of many chronic diseases such as diabetes has been reported by various researchers. Oils such as *Nigella sativa* (Mohashami *et al.*, 2011), walnut oil (Parivash *et al.*, 2011), coconut oil (Mahadevappa *et al.*, 2011), *Picralima nitida* (Calistus & Vincent, 2011) and garlic oil (Cheng-Tzu *et al.*, 2006) have all been reported to possess hypoglycaemic effects. The hypoglycaemic activities of *Moringa oleifera* seed oil in normoglycaemic rats and alloxan induced diabetic rats were also reported by Busari *et al.* (2014) and Abdullah *et al.* (2003). It is therefore necessary to evaluate the acute hypoglycaemic effect of the *Moringa oleifera* seed oil in glucose induced diabetic rats as this can form a basis to unravel possible mechanisms involved its hypoglycaemic activities.

Experimental

Plant materials

The dried pods of *M. oleifera* were collected from Bosso estate at Bosso Local Government Area, Niger State, Nigeria, in June 2013. The plant material was authenticated at the herbarium of Department of Biological Sciences, Federal University of Technology Minna, Ni-

ger State. The authentication was done using a taxonomic aid by Abdullah *et al.* (2003) and Schippers (2000). The seeds were threshed from the pods, air dried, pulverized into powdered form, using a rotary blender, and kept for further analysis.

Experimental animals

Wistar male rats weighing between 120–145 g used for the experiment were obtained from Biochemistry and Chemotherapy section of the National Institute for Trypanosomiasis and Onchocerciasis Research (NITR) Vom, Plateau State, Nigeria. The animals were allowed to acclimatize in the Department of Biochemistry laboratory, Federal University of Technology, Minna, for two (2) weeks. The Rats were fed on pelletized grower mash (vital feeds product, Kaduna) with access to distilled water *ad libitum*. All experiments involving the animals were conducted in compliance with the internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care guidelines on animal use protocol review (1997) and as also described by Adamu *et al.* (2010).

Extraction of Moringa oleifera oil

Fifty grams (50 g) of *M. Oleifera* seeds powder were placed into a cellulose paper cone and fed to a soxhlet extractor fitted with a 500 ml round-bottom flask and a condenser. The extraction was carried out for about 8 hours with 300 ml of petroleum ether. Same process was adopted for dichloromethane extraction. The extracts were condensed using rotary evaporator as reported by Adamu *et al.* (2010)

Determination of fatty acid

The fatty acid composition was determined using the Gas Chromatography Spectrophotometer (GC-QP2010, Shimadzu, Japan) and stand-

ard fatty acids after the oils were converted to fatty acid methylesters by using sodium hydroxide/methanol method which were already dissolved in hexane (Christie,1982). The identification of the peaks was obtained by comparing their retention time with standard fatty acids analysed under the same conditions. The relative percentage of fatty acid was calculated based on the peak area of a fatty acid species in the oil sample as related to that of the standard fatty acids.

Animal grouping

The rats used for the acute hypoglycaemic effect also known as oral glucose tolerance test (OGTT) of *Moringa oleifera lam.* oil consist of 20 animals that comprised 5 groups of 4 animals in each group.

NRML: Normoglycemic rats received 2.0 mL/kg dimethyl sulphoxide (DMSO) orally.

CNTRL: Diabetic rat received 2.0 mL/kg DMSO orally.

STND: Diabetic rats received 500 µg/kg of standard drug glibenclimide orally.

DCMMO: Glucose loaded rat received 2.0 mL/kg of *M. Oleifera* seed oil extract of Dichloromethane (dissolve in DMSO) orally.

PEEMO: Glucose loaded rat received 2.0 mL/kg of *M. oleifera* seed oil extract of Petroleum ether (dissolve in DMSO) orally.

Hypoglycaemic effects of Moringa oleifera seed oil extracts of petroleum ether (peemo) and dichloromethane (dcmmo) on glucose loaded rats

Oral glucose tolerance test was determined as described by Edith & Romuald (2016) with little modification. After 30 minutes of extracts administration and the standard drug, 2 g/kg bodyweight of glucose was given orally to each group of rats. The blood glucose concentration was determined before and af-

ter administration with the aid of glucometer (Accu-check; Roche Germany) via the blood taken from the cut at the tail end of the animals at 0 minute (Basal blood glucose level) and 30, 90 and 120 minutes after glucose loading. The blood glucose concentration values obtained at the end of the experiment were used to compare glucose tolerance in the various groups.

Results

Fatty acid profile of Moringa oleifera seed oil
The fatty acids detected from *Moringa oleifera* seed oil extracted with petroleum ether

and dichloromethane are presented in Table 1. Eight fatty acids were detected in PEEMO with highest proportion of oleic acid (54.45%) followed by Palmitic acid (9.74%), behenic acid (9.29%) and stearic acid (8.38%). Same numbers of fatty acids were detected from DCMMO with highest proportion of oleic acid (54.86%), followed by erucic acid (10.44%), palmitic acid (9.40%) and behenic acid (8.95%). Four saturated fatty acid and four unsaturated fatty acids were detected in both cases.

TABLE 1
Fatty Acid Profile of Moringa oleifera Seed Oil

Fatty Acid	Percentage Proportion	
	PEEMO	DCMMO
Hexadecanoic acid (Palmitic)	9.74	9.40
9-Hexadecenoic acid (Palmitoleic)	2.39	2.22
Octadecanoic acid (Stearic)	8.38	7.89
Docosanoic acid (Behenic)	9.29	8.95
Eicosanoic acid (Arachidic)	5.40	5.00
9-Octadecenoic acid (Oleic)	54.45	54.86
11-Eicosenoic acid (Gondoic acid)	3.79	1.24
13-Docosenoic acid (Erucic acid)	6.56	10.44
Total saturated fatty acid	32.81	31.24
Total unsaturated fatty acid	67.18	68.76
_Total	100	100

Key:

PEEMO: *Moringa oleifera* seed oil extract of petroleum ether

DCMMO: *Moringa oleifera* seed oil extract of dichloromethane

Effect of Moringa oleifera Seed Oil on Glucose Loaded Rats (Oral Glucose Tolerance Test; OGTT)

Fig.1 showed the elevation of blood glucose level in the treated rats after 30 minutes of oral glucose feeding when compared with their respective basal blood glucose levels. Increase in blood glucose levels was observed in all treated rats from their respective basal blood glucose levels by 24.71, 47.83, 44.05, 44.78 and 30.86% for normoglycaemic, control, glibenclimide, DCMMO and PEEMO respectively. Nevertheless, reduction of blood glucose levels was also observed among rats

in normoglycaemic, control, glibenclimide, DCMMO and PEEMO groups respectively by 8.18, 8.51, 24.57, 15.61 and 10.69% at 60 minutes when compared with their respective maximum blood glucose levels. Same observation was repeated at 90 minutes with the blood glucose reduction of 8.18, 21.47, 43.87, 30.08 and 15.45% among the aforementioned respective groups. However, at 120 minutes only PEEMO showed comparable ($p < 0.05$) hypoglycaemic activities with the standard drug at 41.33% and 57.98% respectively when compared with other groups.

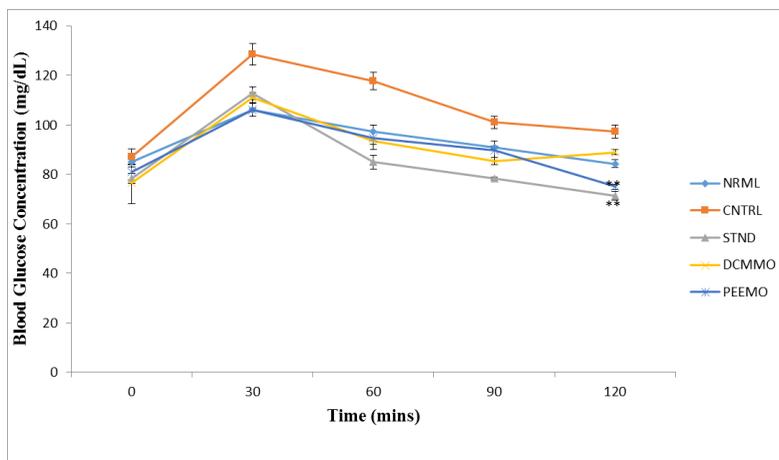


Fig.1: Effect of *Moringa oleifera* Seed Oil on Glucose Loaded Rats.

Values with hysteric symbol show comparable hypoglycaemic activity at the end of the experiment with the standard drug (glibenclimide) at ($p < 0.05$) level of significance while those values without hysteric symbol are significant difference with the standard drug.

Key:

NRML: Normoglycemic rats received 2.0 mL/kg dimethyl sulphoxide (DMSO) orally.

CNTRL: Diabetic rat received 2.0 mL/kg DMSO orally.

STND: Diabetic rats received 500 μ g/kg of standard drug glibenclimide orally.

DCMMO: Glucose loaded rat received 2.0 mL/kg of *M. oleifera* seed oil extract of Dichlorom ethane (dissolve in DMSO) orally.

PEEMO: Glucose loaded rat received 2.0 mL/kg of *M. oleifera* seed oil extract of Petroleum ether (dissolve in DMSO) orally.

Discussion

Variation in fatty acids with predomination of oleic acid and total unsaturated fatty acids in moringa oil makes it as good as olive oil and this prompted nutritionist to suggest its possibility of serving as edible oil (Alessandro *et al.*, 2016; Al-Ghamdi, 2018).

The reduction of blood glucose level by PEEMO and DCCMO from 106.00 and 110.00 mg/dL at 30 minutes to 75.00 and 89.00 mg/dL at 120 minutes respectively is not surprising as it has been reported by Busari *et al.* (2015) that the oral administration of *Moringa oleifera* seed oil extracts demonstrated antidiabetic properties in alloxan induced diabetic Wistar rats. Similarly, *Moringa oleifera* seed extract which is the source of the oil was also found to possess hypoglycaemic effects on streptozotocin induced diabetes and diabetic nephropathy in male rats.

The mechanisms by which *Moringa oleifera* oil elicit its hypoglycaemic effect in glucose loaded rats were not yet fully established, however, among the proposed mechanisms through which oils elicited such activities include but not limited to sensitization of peripheral tissues which enhances glucose absorption and assistance of insulin secretion from pancreatic β -cell (Sabu & Kuttan, 2002; Latha & Pari, 2004; Zereba *et al.*, 2005; Park *et al.*, 2008). The hypoglycaemic activity of the *Moringa oleifera* oil as this study revealed could be due to the presence of fatty acids in the oil. Monounsaturated fatty acid was reported to have the tendency of improving β -cell secretory function by preventing β -cell apoptosis, reducing impairment of β -cell activities and β -cell proliferation (Maedler *et al.*, 2003). It was also reported from Horváth *et al.* (2001), that vitamin E regulate the immunoresponse of cells and restores damaged liver function via their antioxidant activities

as *Moringa* oil contain reasonable quantity of vitamin E (S´anchez-Machado *et al.*, 2006). Antioxidant compounds either naturally or synthetic provide protective effect on various oxidative stress related diseases including diabetes mellitus (Batubara *et al.*, 2010; Nadeem & Imran, 2016).

Conclusion

Oral administration of *Moringa oleifera* seed oil extracts have demonstrated acute hypoglycaemic effects in glucose fed rats possibly but not limited to sensitization of peripheral tissues which enhances glucose absorption or via assistance of insulin secretion from pancreatic β -cell. Therefore, *Moringa oleifera* seed oil extract can serve as a source of potential antidiabetic agents oral or adjuvant for the management of diabetes mellitus.

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