

Original Research Article

Comparison of antidiabetic effect of ethanolic extract of *Mangifera indica* and *Moringa oleifera* leaves on alloxan induced diabetic rats

ABSTRACT

Background: Diabetes mellitus (DM) is one of the fastest growing metabolic disorder as well as the important cause of death in developing countries. Several plants are used in the management of DM, however, *Moringa oleifera* is the widely used plant. Thus, the high demand and scarcity of *Moringa oleifera* in certain places necessitate an alternative plant for management of DM.

Aim: The aim of this study is to compare the antidiabetic effects of *Mangifera indica*, *Moringa oleifera* and combinatorial formulation of ethanolic extract of *Mangifera indica* and *Moringa oleifera*.

Methods: Diabetes was induced by intraperitoneal injection of 100 mg alloxan per kg body weight. The experimental animals were confirmed diabetic three days after the injection. *Mangifera indica*, *Moringa oleifera* and both were administered to MI, MO and MO + MI group respectively. Blood glucose level was estimated after three weeks treatment and one week after treatment withdrawal.

Results: The blood glucose of all groups reduce significantly ($P < .05$) compared to DC group. A significant increase in blood glucose ($P < .05$) in MI group was observed one week after treatment withdrawal while the increase in MO and MO + MI groups were insignificant. There was a significant increase in body weight ($P < .05$ and $P = .05$) in treated groups (especially MI and MO) compared to DC group.

Conclusion: The results of the study shows *Moringa oleifera* has more antidiabetic effect compare to *Mangifera indica*. Combination of both (1:1) increases the antidiabetic effect of MI. Increase in body weight could not be a direct influence of the leaves. Hence mixing MI and MO may be a good alternative to managing DM in places where MO is scarce.

Keyword: Diabetes Mellitus, *Mangifera indica*, *Moringa oleifera*, Alloxan

INTRODUCTION

The utilisation of different local herbs, vegetables and fruits by humans is believed to contribute notably to human health in preventing and or curing many diseases. Plants have being a natural source of therapeutic agents for several diseases including diabetes [1]. Diabetes mellitus (DM) is a group of the metabolic disorder generally characterized by increased blood glucose due to insufficient secretion, the action of endogenous insulin or both [2]. DM is still one of the major cause of death and disability in both developed and developing countries [3] and is probably one of the fastest growing metabolic disorders in the world [4]. Due to these reasons, there is a need for more alternative and appropriate therapies.

Many factors such as oxidative stress [5], genetic and environmental [6] are attributed to the pathogenesis of DM. Families with a history of DM, obesity, physical inactivity, poor dietary and exercise habits are at high risk of diabetes. DM can be divided into many types. The two major types of DM are DM type I and type II. Other types of DM include gestational DM. On the basis of aetiology type I DM is referred to Insulin Dependent DM (IDDM) or juvenile-onset diabetes whereas, type II DM is considered as Non-Insulin Dependent DM (NIDDM) or maturity-onset diabetes respectively. Type I DM is characterized by little or completely lack endogenous insulin due to the immunological destruction of β -cells, patients here solely rely on insulin therapy for survival [7] while type II is characterized by abnormal secretion and resistance to insulin [8]. DM symptoms include polyurea, polydipsia, polyphagia, weight

loss, fatigue, cramps, constipation, blurred vision, and candidiasis [9]. DM is associated with many consequences such as coronary artery, heart, and peripheral vascular diseases, atherosclerosis, hyperlipidaemia and obesity if left untreated [10]. DM is a major global health problem. Based on world health organization (WHO) prediction, the prevalence of the disease may probably increase by 35% by the year 2020.

Many plants (about 800 species) are known to have anti-diabetic (hypoglycemic) activities [11]. Some of the most documented plants include *M. oleifera*, [12] *Aloe vera* and *Aloe barbadensis*, [13] *V. amygdalina*, [14] *Persea Americana*, [15] *Psidium guajava*, [16] and *M. indica* [17]. *M. indica* and *M. oleifera* have many medicinal benefits including anti-inflammatory, [18], [19] anti-malarial, [20] antiulcer, [21] antidiabetic, [22] *M. indica* powder showed a significant reduction in blood glucose level in an experiment conducted by Patnaik [23]. This study compared the effect of *M. indica*, *M. oleifera* and combinatorial formulation of ethanolic extract of *M. indica* and *M. oleifera* on alloxan induced diabetic mice.

MATERIALS AND METHODS

Materials/ Reagents

The albino rats were purchased from National Veterinary Research Institute Vom, Plateau State, Nigeria. Alloxan was also purchased in Jos, from Zayo-Sigma chemical company. The *M. oleifera* leaves were purchased from Rimi Market (Kasuwar Rimi), Kano whereas fresh *M. indica* leaves were obtained from Bayero University Kano (BUK), old campus.

Experimental Design

Thirty (30) adult albino rats of same sex weighing 130-140g were used in the study. The rats were kept in the animal house of the department of Biological sciences, BUK, Nigeria under optimal conditions for 7 days to acclimatize. The animals were fed a standard diet with free access to drinking tap water ad libitum. They were randomly grouped into five groups of 6 mice in each group as shown below.

Table 1. Mice grouping and type of treatment administered

Group	Title	Treatment
NDC	Non-Diabetic Control (Normal)	Standard feed + water ad libitum
DC	Diabetic Control	Standard feed + water ad libitum
MI	Diabetic treated with <i>MI</i>	Standard feed + 200 mg kg ⁻¹ BW day ⁻¹ of <i>MI</i> + water libitum
MO	Diabetic treated with <i>MO</i>	Standard feed + 200 mg kg ⁻¹ BW day ⁻¹ of <i>MO</i> + water libitum
MO+ MI	Diabetic treated with both <i>MO</i> and <i>MI</i>	Standard feed + 200 mg kg ⁻¹ BW day ⁻¹ of <i>MO</i> and <i>MI</i> (1:1 of <i>MO</i> and <i>MI</i>) + water libitum

MI = *Mangifera indica*, *MO* = *Moringa oleifera*, *MO* + *MI* = Combination of *Manifera indica* and *Moringa oleifera*, BW = Body weight.

Diabetes Induction

Alloxan monohydrate was used to induce diabetes in the rats. Ajibola et al. [24] recommendation for diabetes induction was adopted. Diabetes was induced in all mice (except NDC) by a single (while twice in few mice) intraperitoneal (IP) of 100 mg alloxan per kg body weight. Animals were confirmed diabetic after 3 days and mice with a glucose level of 13.00 mmol/L and above were used in this study.

Plants and Extracts Preparation

The plant leaves were authenticated by Dr. Zainab T. of Department of Biological Sciences, BUK. The leaves were thoroughly cleaned with distilled water, air dried under a shade and grounded into powder using motor and pestle. Ethanolic extract of *M. indica* and *M. oleifera* were formed by soaking 400g of each in absolute ethanol and allowed to stay at 25°C for 3 days. The extracts were filtered and

evaporated in a cylindrical water bath for removal of the solvent. The extracts were obtained and stored in the refrigerator until used.

Weight and Blood Glucose Determination

The blood glucose and weight of the animals were determined before induction of diabetes and weekly after induction. The blood glucose was determined using Accu-Chek Performa Apparatus according to Abunasef *et al.* [25]. While the body weight was determined using digital animal weighing scale (Kent Scientific).

STATISTICAL ANALYSES

Data were analysed using Excel 2016 and Statistical Package for Social Sciences (SPSS) 16.0 Students version for windows. Results were expressed as mean $\pm$ SD and statistically analysed using one-way ANOVA followed by Tukey's as a post hoc test. Differences in means were considered statistically significant at $P = .05$.

RESULTS AND DISCUSSION

Blood Glucose Level During and After Treatment Withdrawal

Prior to diabetes induction, the difference in blood glucose level of the animals was statistically insignificant (Table 2). There was a significant increase in the blood glucose level after administration of alloxan i.e. diabetes induction ($P = .05$) compared to NDC. Administration of the extracts (MI, MO or MO + MI) for three weeks lead to significant decrease in the blood glucose level ($P < .05$) compared to DC group (Figure 1). However, an increase was observed after one week of treatment withdrawal. Although the increase was statistically insignificant in MO and MO + MI group (Table 3).

The blood glucose level for a different period in all the groups was compared using Tukey HSD post-test (Table 3). Surprisingly, the increase in blood glucose level in MI group was significant one week after the treatment withdrawal. Whereas, the increase was non-significant in MO and MO + MI groups. This may be an indicator that *M. oleifera* is more effective than *M. indica* in the management of DM, although the combination of *M. indica* and *M. oleifera* (1:1) shows more effect than *M. indica* only. Therefore, using both (1:1) may be a good alternative in places where *M. oleifera* demand is very high.

Table 2: Fasting blood glucose (Mean $\pm$ SD) of mice before and after induction of diabetes, during treatment with either *Mangifera indica* or *Moringa oleifera* or both, and after treatment withdrawal.

Group	Dose (mgkg ⁻¹ BW day ⁻¹)	Fasting Blood Sugar (mmol/L)			
		BI	AI	3WKT	1WKWT
NDC	0	4.47 $\pm$ 0.63	4.43 $\pm$ 0.64	4.57 $\pm$ 0.60	4.28 $\pm$ 0.74
DC	0	5.05 $\pm$ 0.54	16.97 $\pm$ 1.47*	19.93 $\pm$ 5.53*	22.65 $\pm$ 3.27*
MI	200	4.97 $\pm$ 0.65	17.22 $\pm$ 0.90*	10.22 $\pm$ 0.80 [#]	13.47 $\pm$ 1.99 [#]
MO	200	4.47 $\pm$ 0.70	16.52 $\pm$ 2.24*	9.12 $\pm$ 0.88 [#]	10.03 $\pm$ 1.67 [#]
MO + MI	100MO + 100MI	4.23 $\pm$ 0.80	15.48 $\pm$ 2.62*	11.17 $\pm$ 1.23 [#]	12.18 $\pm$ 1.71 [#]

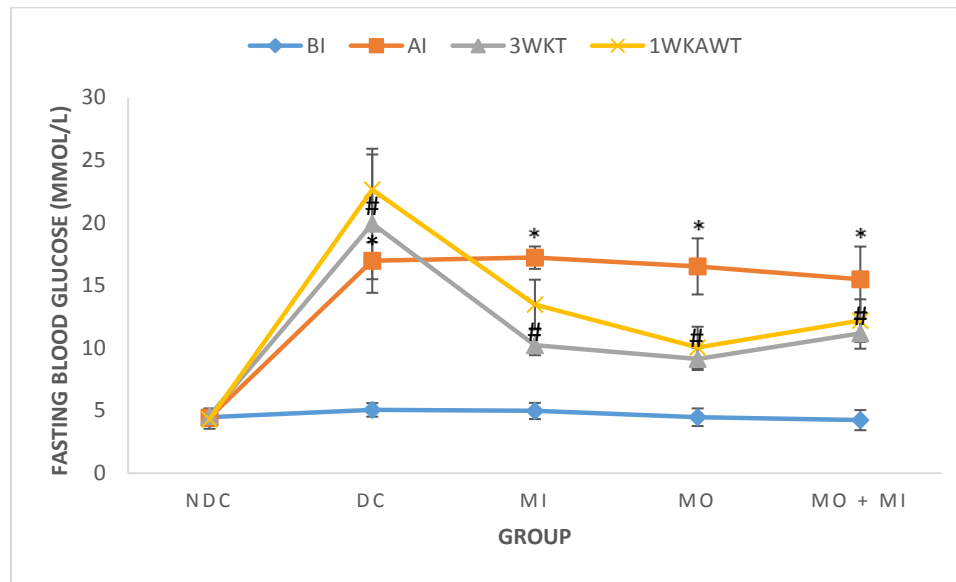
BI = Before Induction of diabetes, AI = After Induction of diabetes, 3WKT = 3 Weeks of Treatment with either MI or MO or both, 1WKAWT = 1 Week After Withdrawal of Treatment. *Statistically different ($P = .05$) compared with NDC; [#]Statistically different ($P = .05$) compared with DC.

1 Table 3. Blood glucose levels for different period (BI, AI, 3WKT and 1WKATW) in respective groups were compared using Tukey HSD test

Comparison	Group							
	DC		MI		MO		MO + MI	
	Mean Difference	P value	Mean Difference	P value	Mean Difference	P value	Mean Difference	P value
BI vs AI	-11.92	$P < .05$	-12.25	$P < .05$	-12.05	$P < .05$	-11.25	$P < .05$
BI vs 3WKT	-14.88	$P < .05$	-5.25	$P < .05$	-4.65	$P < .05$	-6.94	$P < .05$
BI vs 1WKAWT	-17.6	$P < .05$	-8.50	$P < .05$	-5.56	$P < .05$	-7.95	$P < .05$
AI vs 3WKT	-2.96	NS	-7.00	$P < .05$	7.40	$P < .05$	4.31	$P < .05$
AI vs 1WKAWT	-5.68	$P = .05$	3.75	$P < .05$	6.49	$P < .05$	3.30	$P = .05$
3WKT vs 1WKAWT	-2.72	NS	-3.25	$P < .05$	-0.91	NS	-0.01	NS

2 NS = Non-significant

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3 Figure 1. Fasting blood glucose expressed in mean $\pm$ SD before and after induction of diabetes, treatment
 4 with appropriate plant and doses mentioned as above. * Significant difference before and after induction
 5 ($P < .05$); # significant difference after 3 weeks treatment with appropriate leaves ($P < .05$).

6 Body weight during and After Treatment Withdrawal

7 The body weights of mice were measure prior to and after diabetes induction, after 3 weeks treatment
 8 and one week after treatment withdrawal (Table 4). Significant weight loss was observed after diabetes
 9 induction while three weeks of extract administration results in significant weight gain. Surprisingly, there
 10 was a significant difference ($P < .05$) in the body of the weight of mice in MO + MI group one week after
 11 treatment withdrawal (3WKT vs 1WKAWT; $P < .05$). This finding indicates that extract may not have a
 12 direct effect on body weight because the difference in blood glucose at that period (3WKT vs 1WKAWT)
 13 was statistically insignificant. Thus, the gain in body weight could be the effect of the feed.

14 In MI group, the body weight reduced drastically one week after the treatment withdrawal (Table 5). A
 15 significant difference was observed when 1WKAWT was compared with 3WKT ($P < .05$) while the
 16 difference was non-significant compared to AI.

17 Table 4: Fasting blood glucose (Mean $\pm$ SD) of mice before and after induction of diabetes, during
 18 treatment with either *Mangifera indica* or *Moringa oleifera* or both, and after treatment withdrawal.

Group	Dose (mgkg ⁻¹ BW day ⁻¹)	Body Weight (g)			
		BI	AI	3WKT	1WKAWT
NDC	0	130.50 $\pm$ 1.87	133.33 $\pm$ 3.88	135.67 $\pm$ 3.39	137.5 $\pm$ 2.88
DC	0	132.67 $\pm$ 5.20	125.00 $\pm$ 5.55*	119.83 $\pm$ 1.94*	117.00 $\pm$ 4.38*
MI	200	130.17 $\pm$ 1.17	120.83 $\pm$ 2.04*	128.33 $\pm$ 5.24#	121.50 $\pm$ 2.88#
MO	200	131.67 $\pm$ 4.68	122.00 $\pm$ 3.85*	128.83 $\pm$ 2.14#	126.00 $\pm$ 3.85#
MO + M1	100MO + 100MI	130.33 $\pm$ 1.86	122.00 $\pm$ 2.10*	131.33 $\pm$ 1.21#	127.5 $\pm$ 1.87#

BI = Before Induction of diabetes, AI = After Induction of diabetes, 3WKT = 3 Weeks of Treatment with either MI or MO or both, 1WKAWT = 1 Week After Withdrawal of Treatment. *Statistically different ($P = .05$) compared with NDC; #Statistically different ($P = .05$) compared with DC.

DM is a serious metabolic disorder with several consequences, which may lead to death if not treated. Also, some diabetic medications may compromise the function of kidneys, peripheral nerves and retina [26]. For many centuries, plants are used in the treatment of many diseases including DM. However, certain plants are reported to have a side effect in the management of DM because they lead to hypoglycaemia [27]. Thus, there is need to identify more medications for the DM with less or no side effect. Several studies reported that plants are used in the management of DM [28–30]. The combinatorial herbal formulation has been reported as a good alternative for diabetes management [31] while many studies used either *M. indica* or *M. oleifera* in the treatment of many diseases including diabetes [32]. The demand for *M. oleifera* is increasing due to its medicinal value in the treatment of hundreds of diseases, nutritional value, [33] water treatment capacity [34]. Hence, there is need to discover other alternatives for treatment of diabetes due to its increasing prevalence. Thus, this study compared the antidiabetic effect ethanolic extract of *M. indica*, *M. oleifera* and combinatorial formulation of ethanolic extract of *M. indica* and *M. oleifera* in the management of DM.

1 Table 5. Body weight for different period (BI, AI, 3WKT and 1WKATW) in respective groups were compared using Tukey HSD test

Comparison	Group									
	NDC		DC		MI		MO		MO + M1	
	Mean Difference	P value	Mean Difference	P value	Mean Difference	P value	Mean Difference	P value	Mean Difference	P value
BI vs AI	-2.83	NS	7.67	$P < .05$	9.34	$P < .05$	9.67	$P < .05$	8.33	$P < .05$
BI vs 3WKT	-5.17	$P = .05$	12.84	$P < .05$	1.84	NS	2.84	NS	-1.00	NS
BI vs 1WKAWT	-7.00	$P < .05$	15.67	$P < .05$	8.67	$P < .05$	5.67	NS	2.83	NS
AI vs 3WKT	-2.34	NS	5.17	NS	-7.50	$P < .05$	-6.83	$P = .05$	-9.33	$P < .05$
AI vs 1WKAWT	-4.17	NS	8.00	$P = .05$	-0.67	NS	-4.00	NS	-5.50	$P < .05$
3WKT vs 1WKAWT	-1.94	NS	2.83	NS	6.83	$P < .05$	2.83	NS	3.83	$P < .05$

2 NS = Non-significant

CONCLUSIONS

The results of this study indicate that *M. oleifera* is more effective than *M. indica* in the management of DM. However, a combination of *M. indica* and *M. oleifer* is also effective in diabetes management. Therefore, a combination of both leaves (1:1) is an alternative for *M. oleifera* in a place where it is scarce or expensive.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki

CONFLICTS OF INTEREST

All authors declare no conflict of interest.

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