Original Research Article

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

ABSTRACT

Background: Diabetes mellitus (DM) is one of the leading metabolic disorder as well as among the major cause of death in developing countries. Several plants were investigated as a possible remedy for the management of DM, however, *Moringa oleifera* (MO) is one of the widely used plants. Thus, the high demand and scarcity of MO 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* (MI), MO and combinatorial formulation of ethanolic extract of *both plants (MOMI).*

Methods: Diabetes was induced by intraperitoneal injection of 100 mg alloxan per kg body weight. Diabetes was confirmed in experimental animals three days after the injection. MI, MO and MOMI (a mixture of both) were administered to groups of animals receiving MI, MO and MOMI respectively. Blood glucose level was estimated three weeks after treatment and one week after withdrawal of treatment.

Results: The blood glucose of animals of all groups reduced significantly ($P < 0.01$) compared to diabetic control (DC) group. A significant increase in blood glucose ($P < 0.01$) in animals of MI group was observed one week after withdrawal of treatment whereas, the increase in MO and MOMI groups were statistically insignificant. Furthermore, a significant increase in body weight ($P < 0.01$ and $P < 0.05$) was observed in treated groups (especially MOMI) compared to DC group.

Conclusion: The results of the study showed MO has a more antidiabetic effect compared to MI. Combination of both at 1:1 increases the antidiabetic effect of MI. Increase in body weight could not be a direct influence of the leaves. Hence mixing MO and MI may be a good alternative for managing DM.

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 been a natural source of therapeutic agents for several diseases including diabetes [1]. 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]. It 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 increasing metabolic disorders in the world [4]. Due to these reasons, there is a need for other 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. The two major types of DM are type I and type II DM. Other types of DM include gestational DM. Type I DM (T1DM) is characterized by negligible or complete lack of endogenous insulin due to the immunological destruction of β-cells of langerhans [7] while type II DM (T2DM) 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]. It is associated with many consequences such as coronary artery, heart, and peripheral vascular diseases, atherosclerosis, hyperlipidemia and obesity if left untreated [10]. Based on world

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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 antidiabetic (hypoglycemic) activities [11]. Some of the most documented include *Monringa oleifera,* [12] *Aloe vera and Aloe barbadensis,* [13] *Vernonia amygdalina,* [14] *Persea Americana,* [15] *Psidium guajava,* [16] and *Mangifera indica* [17]. MO and MI have many medicinal benefits including anti-inflammatory, [18,19] anti-malarial, [20] antiulcer, [21] antidiabetic, [22,23]. This study compared the effect of MI, MO and combinatorial formulation of ethanolic extract of MI and MO on alloxan induced diabetic rats.

MATERIALS AND METHODS

Materials/ Reagents

The albino rats were purchased from National Veterinary Research Institute Vom, Plateau State, Nigeria. Alloxan was purchased at Jos, form Zayo-Sigma chemical company, Nigeria. The MO leaves were purchased from Rimi Market (Kasuwar Rimi), Kano whereas fresh MI leaves were obtained from Bayero University Kano (BUK), old campus. Both plant leaves were authenticated by a Botanist at the Biological sciences Department BUK.

Experimental Design

Thirty (30) adult albino rats of same sex weighing 130-140g were used in the study. They were kept in the animal house of the department of Biological sciences, BUK, Nigeria under optimal conditions for 7 days to acclimatize and fed with a standard diet and have free access to drinking water ad libitum. They were randomly divided into 5 groups containing 6 rats each (Table 1).

Table 1. Rats grouping and type of treatment administered

Group	Title	Treatment
NDC	Non-Diabetic Control (Normal Control)	Standard feed + water ad libitum
DC	Diabetic Control	Standard feed + water ad libitum
MI	Diabetic treated with *MI*	Standard feed + 200 $mgkg^{-1}$ BW day^{-1} of MI + water libitum
MO	Diabetic treated with *MO*	Standard feed + 200 $mgkg^{-1}$ BW day^{-1} of MO + water libitum
MOMI	Diabetic treated with *MOMI*	Standard feed + 200 $mgkg^{-1}$ BW day^{-1} of MO and MI (1:1 of MO and MI) + water libitum

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

Plants Extracts Preparation

The MI and MO leaves were thoroughly cleaned with distilled water, air dried under a shade and grounded into powder using motor and pestle. Ethanolic extract of MI and MO were formed by soaking 400g of each in absolute ethanol and allowed to stay at 25^{o}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.

Induction of Diabetes

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

Blood Glucose and Weight Determination

The blood glucose and weight of the animals were determined before induction of diabetes and weekly afterward. The blood glucose was determined using Accu-Chek Performa Apparatus (93 x 52 x 22 mm

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(LWH), Rocha Diagnostic GmbH, Germany) Abunasef *et al.* [25]. While the body weight was determined using digital animal weighing scale (Kent Scientific).

STATISTICAL ANALYSIS

Data were analysed using Excel 2016 and Statistical Package for Social Sciences (SPSS) 16.0 Students version for windows. Results were expressed as mean ± SD and statistically analysed using one-way ANOVA followed by Tukey's honest significant different (HSD) test as a post hoc test. Differences in means were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Blood Glucose Level During and After Withdrawal of Treatment

Prior to induction of diabetes, 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 ($P < 0.05$) compared to NDC. Administration of the extracts (MI, MO or MOMI) for three weeks lead to significant decrease in the blood glucose level ($P < 0.01$) compared to levels in animals of DC group (Figure 1). However, an increase was observed after one week of treatment withdrawal. Although the increase was statistically not significant in animals receiving MO and MOMI extract respectively (Table 3).

The blood glucose level for different periods within all the groups were compared using Tukey HSD post-test (Table 3). Surprisingly, the increase in blood glucose level in animals receiving MI was significant one week after withdrawal of treatment. Whereas, the increase in levels was non-significant in MO and MOMI groups. This may be an indicator that MO is more effective than MI in the management of DM, although the combination of extract of both (i.e MOMI) shows more activity than observed with extract of MI only. Therefore, using both extracts in the ration of 1:1 may be a good alternative in places where MO demand is very high.

Table 2: Fasting blood glucose (Mean ± SD) of rats before and after induction of diabetes, during treatment (with MI, MO or MOMI) and after withdrawal of treatment.

Group	Dose ($mgkg^{-1}$ BW day^{-1})	Fasting Blood Sugar (mmol/L)			
		BI	AI	3WKT	1WKWT
NDC	0	4.47 ± 0.63	4.43 ± 0.64	4.57 ± 0.60	4.28 ± 0.74
DC	0	5.05 ± 0.54	16.97 ± 1.47*	19.93 ± 5.53*	22.65 ± 3.27*
MI	200	4.97 ± 0.65	17.22 ± 0.90*	10.22 ± 0.80$^{\#}$	13.47 ± 1.99$^{\#}$
MO	200	4.47 ± 0.70	16.52 ± 2.24*	9.12 ± 0.88$^{\#}$	10.03 ± 1.67$^{\#}$
MOMI	100MO + 100MI	4.23 ± 0.80	15.48 ± 2.62*	11.17 ± 1.23$^{\#}$	12.18 ± 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 ($P < 0.05$) compared with NDC; $^{\#}$Statistically different ($P < 0.05$) compared with DC.

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Table 3. Comparison of fasting blood glucose levels in all animals within respective groups.

Comparison	Group							
	DC		MI		MO		MOMI	
	Mean Difference	P value	Mean Difference	P value	Mean Difference	P value	Mean Difference	P value
BI vs AI	-11.92	*P < 0.01*	-12.25	*P < 0.01*	-12.05	*P < 0.01*	-11.25	*P < 0.01*
BI vs 3WKT	-14.88	*P < 0.01*	-5.25	*P < 0.01*	-4.65	*P < 0.01*	-6.94	*P < 0.01*
BI vs 1WKAWT	-17.6	*P < 0.01*	-8.50	*P < 0.01*	-5.56	*P < 0.01*	-7.95	*P < 0.01*
AI vs 3WKT	-2.96	NS	-7.00	*P < 0.01*	7.40	*P < 0.01*	4.31	*P < 0.01*
AI vs 1WKAWT	-5.68	*P < 0.05*	3.75	*P < 0.01*	6.49	*P < 0.01*	3.30	*P < 0.05*
3WKT vs 1WKAWT	-2.72	NS	-3.25	*P < 0.01*	-0.91	NS	-0.01	NS

NS = Non-significant

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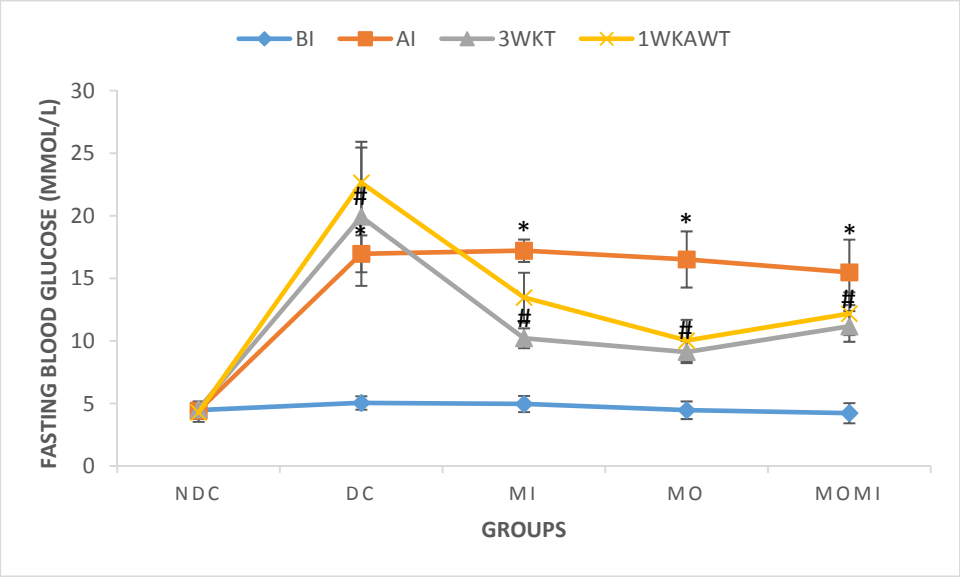

Figure 1. Fasting blood glucose expressed in mean ± SD before and after induction of diabetes, treatment with appropriate plant and doses as mentioned above. * Significant difference before and after induction ($P < 0.01$); # significant difference after 3 weeks treatment with appropriate leaves ($P < 0.01$).

Body weight during and After Withdrawal of Treatment

The body weight of rats was measured prior to and after induction of diabetes, after 3 weeks treatment and one week after withdrawal of treatment (Table 4). Significant weight loss was observed after induction of diabetes while three weeks of extract administration lead to significant weight gain. Surprisingly, there was a significant difference ($P < 0.01$) in the body of the weight of rats in MOMI group one week after withdrawal of treatment (3WKT vs 1WKAWT; $P < 0.01$). This finding indicated the extract may not have a direct effect on body weight because the difference in blood glucose at that period (3WKT vs 1WKAWT) was statistically insignificant. Thus, the gain in body weight could be the effect of the feed.

The body weight of all the rats was compared within the respective groups (Table 5). The body weight of all the rats reduced significantly after induction of diabetes. The body weight increased significantly in all the treated groups after the treatment and reduced drastically after withdrawal of the treatment in animals receiving MI and MOMI. Surprisingly, the difference was statistically insignificant in animals receiving MO. Thus, MO is more effective in regaining body weight. A non-significant difference was observed when AI was compared with 1WKAWT in animals receiving MI and MO while the difference was significant in animas receiving MOMI.

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Table 4: Body weight (Mean ± SD) of rats before and after induction of diabetes, during treatment (with MI, MO or MOMI) and after withdrawal of treatment.

Group	Dose ($mgkg^{-1}$ BW day^{-1})	Body Weight (g)			
		BI	AI	3WKT	1WKAWT
NDC	0	130.50 ± 1.87	133.33 ± 3.88	135.67 ± 3.39	137.5 ± 2.88
DC	0	132.67 ± 5.20	125.00 ± 5.55*	119.83 ± 1.94*	117.00 ± 4.38*
MI	200	130.17 ± 1.17	120.83 ± 2.04*	128.33 ± 5.24#	121.50 ± 2.88#
MO	200	131.67 ± 4.68	122.00 ± 3.85*	128.83 ± 2.14#	126.00 ± 3.85#
MOMI	100MO + 100MI	130.33 ± 1.86	122.00 ± 2.10*	131.33 ± 1.21#	127.5 ± 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 ($P < 0.05$) compared with NDC; #Statistically different ($P < 0.05$) 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 centuries, plants have been used in the treatment of many diseases including DM. However, certain plants are reported to lead to hypoglycaemia as a side effect [27]. Thus, there is need to identify herbal medications 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 MI or MO in the treatment of many diseases including diabetes [32]. The demand for MO is increasing due to its medicinal value, nutritional value, [33] and 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 of ethanolic leaves extract of MI, MO and combination of both in the management of DM.

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Table 5. Comparison of body weight in all animals within respective groups.

Comparison	Group									
	NDC		DC		MI		MO		MOMI	
	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 < 0.01*	9.34	*P < 0.01*	9.67	*P < 0.01*	8.33	*P < 0.01*
BI vs 3WKT	-5.17	*P < 0.05*	12.84	*P < 0.01*	1.84	NS	2.84	NS	-1.00	NS
BI vs 1WKAWT	-7.00	*P < 0.01*	15.67	*P < 0.01*	8.67	*P < 0.01*	5.67	NS	2.83	NS
AI vs 3WKT	-2.34	NS	5.17	NS	-7.50	*P < 0.01*	-6.83	*P < 0.05*	-9.33	*P < 0.01*
AI vs 1WKAWT	-4.17	NS	8.00	*P < 0.05*	-0.67	NS	-4.00	NS	-5.50	*P < 0.01*
3WKT vs 1WKAWT	-1.94	NS	2.83	NS	6.83	*P < 0.01*	2.83	NS	3.83	*P < 0.01*

NS = Non-significant

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CONCLUSION

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

RECOMMENDATION

Since the combination of MI and MO has an effective antidiabetic effect. Its mechanism of action should be explored.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was conducted in accordance with the standard set for the Care and Use of Laboratory Animals. The protocol was approved by the Ethics Committee on Animal Use of the Bayero University, Kano, Nigeria.

CONFLICTS OF INTEREST

All authors declare no conflict of interest.

REFERENCES

[1] Robbers JE, Tyler VE. Tyler's herbs of choice. The therapeutic use of phytomedicinals. Tyler's herbs choice Ther use phytomedicinals. 1999.

[2] Joseph B, Jini D. Insight into the Hypoglycaemic Effect of Traditional Indian Herbs used in the Treatment of Diabetes. *Res J Med Plant*. 2011;5(4):352–76.

[3] Buowari OY. Diabetes Mellitus in Developing Countries and Case Series. In: *Diabetes Mellitus - Insights and Perspectives*. InTech; 2013: 131.

[4] Piyush M, Natvarlal M, Ramesh K. Holistic classification of herbal antidiabetics: A review. *Pharma Times*. 2006;38(5):19-25

[5] Gwarzo MY, Nwachuku VA, Lateef AO. Prevention of Alloxan Induced Diabetes Mellitus in Rats by Vitamin a Dietary Supplementation. *Asian J Anim Sci*. 2010;4(4):190–6.

[6] Fletcher B, Gulanick M, Lamendola C. Risk Factors for Type 2 Diabetes Mellitus. *J Cardiovasc Nurs*. 2002;16(2):17–23.

[7] Zimmet P, Cowie C, Ekoe J-M, Shaw J, Zimmet P, Cowie C, et al. Classification of Diabetes Mellitus and Other Categories of Glucose Intolerance. In: International Textbook of Diabetes Mellitus. Chichester, UK: John Wiley & Sons, Ltd; 2003

[8] DeFronzo RA, Ferrannini E, Zimmet P, Alberti G, editors. *International Textbook of Diabetes Mellitus*, 2 Volume Set. John Wiley & Sons; 2015 May 18.

[9] Bastaki S. Pharmacotherapy of nonnutritive sweeteners in diabetes mellitus. *Int J Diabetes Metab*. 2015;(23):11–22.

[10] Svensson M, Eriksson JW, Dahlquist G. Early glycemic control, age at onset, and development of microvascular complications in childhood-onset type 1 diabetes: a population-based study in northern Sweden. *Diabetes Care*. 2004;27(4):955–62.

[11] Maton A. Human biology and health. 1st ed. Englewood Cliffs N.J.: Prentice Hall; 1993;256.

[12] Ibrahim El-Desouki N, Aboulfotouh Basyony M, Abdelmonaim Hegazi MM, Samir El –Aama MI.

Email: harisbilkis@yahoo.com

Moringa oleifera Leaf Extract Ameliorates Glucose, Insulin and Pancreatic Beta Cells Disorder in Alloxan-Induced Diabetic Rats. *Research Journal of Pharmaceutical Biological and Chemical Sciences*. 2015;6(3):975–8585.

[13] Ajabnoor MA. Effect of aloes on blood glucose levels in normal and alloxan diabetic mice. *J Ethnopharmacol*. 1990;28(2):215–20.

[14] Efiong EE, Igile GO, Mgbeje BI, Otu EA, Ebong PE. Hepatoprotective and anti-diabetic effect of combined extracts of Moringa oleifera and Vernonia amygdalina in streptozotocin-induced diabetic albino Wistar rats. *Journal of Diabetes and Endocrinology*. 2013;4(4):45-50.

[15] Alhassan AJ, Sule MS, Atiku MK, Wudil AM, Abubakar H. Effects of aqueous avocado pear (Persea americana) seed extract on alloxan induced diabetes rats. *Greener J Med Sci*. 2012;2:5–11.

[16] Mazumdar S, Akter R, Talukder D. Antidiabetic and antidiarrhoeal effects on ethanolic extract of Psidium guajava (L.) Bat. leaves in Wister rats. *Asian Pac J Trop Biomed*. 2015;5(1):10–4.

[17] Gondi M, Basha SA, Bhaskar JJ, Salimath P V, Prasada Rao UJS. Anti-diabetic effect of dietary mango (Mangifera indica L.) peel in streptozotocin-induced diabetic rats. *J Sci Food Agric*. 2015;95(5):991–9.

[18] Jangir RN, Jain GC. Antidiabetic and antioxidant potential of hydroalcoholic extract of Moringa oleifera leaves in streptozotocin-induced diabetic rats. *European Journal of Pharmaceutical and Medical Research*. 2016;3:438-50.

[19] Kim H, Banerjee N, Ivanov I, Pfent CM, Prudhomme KR, Bisson WH, Dashwood RH, Talcott ST, Mertens-Talcott SU. Comparison of anti-inflammatory mechanisms of mango (Mangifera Indica L.) and pomegranate (Punica Granatum L.) in a preclinical model of colitis. *Molecular nutrition & food research*. 2016;60(9):1912-23.

[20] Venancio VP, Abrão LC, Kim H, Talcott ST, Mertens-Talcott SU. In vitro antimalarial activity of microbial metabolites from mango tannins (Mangifera indica L.). *The FASEB Journal*. 2016;30(9):916-6.

[21] Prabhu K, Rajan S. Assessment of antiulcer activity of ethanolic extract of Mangifera indica seed kernel using acid ethanol induced ulcer model. *Int J Curr Microbiol App Sci*. 2015;4(4):854-860.

[22] Irondi EA, Oboh G, Akindahunsi AA. Antidiabetic effects of Mangifera indica Kernel Flour-supplemented diet in streptozotocin-induced type 2 diabetes in rats. *Food Sci Nutr*. 2016;4(6):828–39.

[23] Patnaik R. Mango Leaves in Treating Diabetes: A Strategic Study. International Journal of Innovative Research and Development. 2014;3(12);432-441.

[24] Ajibola M, Eunice O, Nnnedinma Stephanie I. Effects of Aqueous Extract of Moringa oleifera Seeds on Alloxan Induced Hyperglycemia. *Basic Sci Med*. 2014;3(3):37–42.

[25] Abunasef SK, Amin HA, Abdel-Hamid GA. A histological and immunohistochemical study of beta cells in streptozotocin diabetic rats treated with caffeine. *Folia Histochem Cytobiol*. 2014;52(1):42–50.

[26] Packer M. Have We Really Demonstrated the Cardiovascular Safety of Antihyperglycemic Drugs? Rethinking the Concepts of Macrovascular and Microvascular Disease in Type 2 Diabetes. *Diabetes, Obes Metab.* 2018(In press);

[27] Gushiken LF, Beserra FP, Rozza AL, Bérgamo PL, Bérgamo DA, Pellizzon CH. Chemical and Biological Aspects of Extracts from Medicinal Plants with Antidiabetic Effects. *Rev Diabet Stud*.

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2016;13(2):96–112.

[28] El-Tantawy WH, Temraz A. Management of diabetes using herbal extracts: review. *Arch Physiol Biochem*. 2017;1–7.

[29] Bagherniya M, Nobili V, Blesso CN, Sahebkar A. Medicinal plants and bioactive natural compounds in the treatment of non-alcoholic fatty liver disease: A clinical review. *Pharmacol Res*. 2017

[30] Governa P, Baini G, Borgonetti V, Cettolin G, Giachetti D, Magnano A, et al. Phytotherapy in the Management of Diabetes: *A Review. Molecules*. 2018 Jan 4;23(1):105.

[31] Ojiako OA, Chikezie PC, Ogbuji AC. Blood glucose level and lipid profile of alloxan-induced hyperglycemic rats treated with single and combinatorial herbal formulations. *J Tradit Complement Med*. 2016;6(2):184–92.

[32] Dwivedi C, Daspaul S. Antidiabetic Herbal Drugs and Polyherbal Formulation Used For Diabetes: A Review. *J Phytopharm Jphyto*. 2013;2(23):44–51.

[33] Abdull Razis AF, Ibrahim MD, Kntayya SB. Health benefits of Moringa oleifera. *Asian Pac J Cancer Prev*. 2014;15(20):8571–6.

[34] Sánchez-Martín J, Beltrán-Heredia J, Peres JA. Improvement of the flocculation process in water treatment by using moringa oleifera seeds extract. *Brazilian J Chem Eng*. 2012;29(3):495–502.

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