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Original article

The Effect of Addition of Extracts of *Vernonia amygdalina* and *Moringa oleifera* in the Nutrition of Alloxan-Induced Diabetic Wistar Rats

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ABSTRACT:
The antidiabetic effect of co-administration of extracts of *Vernonia amydalina* and *Moringa oleifera* on alloxan-induced diabetic Wistar rats was evaluated. The study design involved 64 adult Wistar rats. They were randomly divided into eight different experimental groups of 8 animals each. The experiment lasted for 21 days for which animals were treated with extracts of of *Vernonia amydalina* and *Moringa oleifera*. Their body weight and blood glucose level were determined using weighing scale and hand held glucometer. The results showed that the co-administration of different combination doses of *V. amygdalina* and *M. oleifera* significantly ($p<0.05$) resulted to an increase in the body weight of experimental rats and significantly ($p<0.05$) reduced in a dose dependent manner the blood glucos in experimental rats when compare to diabetic control rats. The antidiabetic potentials of the co-administration of the leaf extracts of *V. amygdalina* and *M. oleifera* can be attribute to secondary constituents present in the plants which stimulates insulin production and release from the beta-cells, resulting in a fall in blood glucose to normal levels.

Keywords: Diabetes, *Vernonia amydalina, Moringa oleifera,* alloxan-induced, co-administration, Nutrition

1. INTRODUCTION

Diabetes mellitus is an endocrine system metabolic condition that causes complications with glucose, lipid, and protein homoeostasis [1]. The disease is present in all and is spreading quickly across the globe. However, the Kitava study, which examined the non-Westernized population of Kitava, one of the Trobriand Islands of Papua New Guinea, between 1989 and 1993, concluded that Kitava residents reportedly did not have high blood pressure, diabetes, obesity, ischemic heart disease, or stroke [2].

Absolute or relative impairments in insulin secretion and action, along with persistent hyperglycemia and disruptions of the metabolism of carbohydrates, lipids, and proteins, are characteristics of diabetes mellitus (DM) [3-5]. It is caused by a defect with the pancreas' ability to secrete insulin or by the cells' failure to use the insulin made by the beta-cells of the pancreatic islet of Langerhans [6, 7].

DM is a global burden that can lead to serious side effects like heart attack, stroke, and kidney failure. A person with diabetes mellitus must live with continual awareness of their condition, one or two daily insulin shots, and regular finger pricks to check their blood sugar levels, a restricted diet, and worry about complications. Even after taking into account enhanced surveillance, the prevalence of diabetes has more than doubled globally over the past three decades, greatly outpacing model estimates as diabetes today affects almost one in ten persons worldwide [8-10].

Over 86 percent of people in underdeveloped countries, according to the World Health Organization (WHO), rely on traditional remedies like herbs for their everyday requirements, and about 855 traditional medications contain crude plant extracts [8]. Sokiprim and colleagues showed that patients with type 2 DM received unintentionally suboptimal treatment due to the high cost of oral hypoglycaemic drugs. Additionally, because people in lower socioeconomic classes commonly choose alternative solutions, their health-seeking behaviors aren't necessarily consistent with conventional orthodox care. This is supported by confirmation from the World Health Organization, which encourages research into alternative

diabetes treatments due to issues with insulin and the hazardous side effects of currently available synthetic oral glucose-lowering medications. Therefore, it is of utmost scientific interest to understand how food sources, natural antioxidants, and free radical scavengers contribute to the prevention and treatment of disease [11-14]. Over 150 million people worldwide have diabetes, making it one of the main causes of death [15]. Over 1.70 million Nigerians over the age of 15 have diabetes, and every year, about 70,000 children under the age of 15 are diagnosed with Type 1 diabetes. Obesity, population increase, and sedentary lifestyles are all contributing to the prevalence of diabetes, which is expected to reach over 360 million cases by 2030 [16].

In traditional medicine, more than 50% of plants are used to treat diseases that impact people, including diabetes, toothaches, diarrhea, dysentery, and skin infections. Numerous conventional medications have been developed from model compounds found in therapeutic plants. Over 400 traditional plant remedies for diabetes have been documented to date, but only a small number of these remedies have undergone scientific and medical testing to determine their usefulness [17]. The attributed hypoglycemic effects of these plants result from their capacity to restore pancreatic tissue function by increasing insulin secretion, inhibiting intestinal glucose absorption, or by facilitating metabolites in insulin-dependent processes. Therefore, using herbal medications for therapy has an impact on preserving beta-cells and reducing glucose fluctuation. The majority of these plants have been discovered to contain chemical elements, such as glycosides, alkaloids, phenols, terpenoids, and flavonoids that are frequently associated with having antidiabetic properties [18].

Tropical Africa is home to the plant *Vernonia amygdalina* [19]. It belongs to the Asteraceae family and is the source of a number of traditional medicines in the West African region [20-22]. It is frequently referred to as bitter leaf. The leaves are the plant parts that are most frequently used. The plant's young, succulent, and fresh leaves are typically recommended for laxative and treating conditions like diabetes, kidney function, malaria, fever, constipation, and high blood pressure [23].

The drumstick tree, *Moringa oleifera* (*M. oleifera*), is a member of the Moringaceae family. It is indigenous to the sub-Himalayan regions, although it is now widely grown and has naturalized in many tropical areas [24]. It is a fast-growing tree that is used in African folk medicine. Its production and administration are made simple by the ease with which it spreads by sexual and asexual mechanisms as well as the little requirement it has after being planted for soil nutrients and water [25]. It is an edible plant with significant medical and nutritional benefits that is used to heal a variety of illnesses, hence the term "wonder tree" [26]. Comprehensive research has been done on *M. oleifera* plant parts for the treatment of a variety of illnesses, including typhoid fever, arthritis, malaria, swellings, skin conditions, parasitic diseases, hypertension, diabetes, liver disorders, and for boosting the immune system in patients with immunosuppressed individuals [27, 28]. Previous published scientific investigations on laboratory animals support the traditional usage of *M. oleifera* in treating diabetes [29]. *Moringa oleifera* extract has been linked to a wide range of pharmacological effects, including anticancer, anti-inflammatory, hypocholesterolemic, anti-atherosclerotic, antioxidant, neuro-protective, kidney-protective, and hepato-protective effects [27-33].

This study aimed to evaluate the effectiveness of combined extracts of *Vernonia amygdalina* and *Moringa oleifera*, two proven antidiabetic herbs, in the management of lipid problems often linked to chronic diabetes. According to Ugochukwu *et al*., some minor food ingredients and secondary plant metabolites alter biological processes, which lower the risk of chronic diseases in people like diabetes mellitus. Understanding the manner of action of such agents may also require this [34]. Exploring and implementing newer approaches to attenuate DM is essential to reducing the burden from DM due to the high predictable rise in its burden, particularly in limited resource situations [35].

2. METHODOLOGY

2.1. Preparation of Plant materials

Fresh leaves of *Vernonia amygdalina* Del. and *Moringa oleifera* Lam. were collected from a botanical garden at Aluu Community in the Obio/Akpor Local Government Area of Rivers State, Nigeria, and identified in the herbarium of the Department of Plant Science and Biotechnology in the University of Port Harcourt. To get rid of dust and dirt, the leaves were rinsed multiple times with clean tap water and given time to thoroughly drain. A knife was used to separate and chop the plant components, and then one kilogram (1kg) of each of *V. amygdalina* and *M. oleifera* was homogenized in 1.95 and 2.25 liters of ethanol that was 80% (v/v) by volume. The mixtures were placed in the refrigerator at 4°C for 48 hours to fully extract the active ingredients from the plants. These were first filtered using cheesecloth and then Whatman No. 1 filter paper, and the filtrates were then concentrated using a rotary evaporator in a vacuum at a low temperature (37–40^{o}C) to about one tenth the original volume. For *V. amygdalina* and *M. oleifera*, the concentrates produced 41.05g (4.105 percent) and 35.21g (3.521 percent) of greenish/greenish-brown oily substances when left uncovered in a water bath at 40°C for complete drying. The extracts were then kept in a refrigerator at a temperature of 2 to 8^{o}C until usage.

2.2. Toxicity studies

Acute toxicity employed for *Vernonia amygdalina* was the method by Lorke [36] as described by Okoli *et al.* [9] while the acute toxicity and LD_{50} of *Moringa oleifera* ethanolic leaves extract in rats was estimated using the method as described by Osman *et al.* [37].

2.3. Phytochemical Screening of *Vernonia amygdalina and Moringa oleifera* leaves

Alkaloids, tannins, saponnins, flavonoids, phenol, Terpenes, steroid and anthraquinones were quantitatively determined using standard methods [38-41].

2.4. Procurement of Animal

For this investigation, adult Wistar rats of either sex weighing 140–210g were used. They were acquired from the Animal House of the Department of Pharmacology at the University of Port Harcourt in River State, Nigeria, and were acclimatized for two weeks. They were kept in a conventional laboratory environment with 28°C temperature (28±2^{o}C), relative humidity (46±6%), a 12-hour light/dark cycle, and adequate ventilation. The animals were given access to water and a commercial feed (Vital Feed Nig. Ltd.) *ad libitum*. Twelve hours prior to the experiments, food was withheld, although water was always available for free.

2.5. Ethical Clearance

According to the recommendations made by the University of Port Harcourt's Research Ethics Committee, all methods used in this study were carried out in compliance with the fundamental principles of animal-based research.

2.6. Drug Purchase and Preparation

Glibenclamide (GBC) was obtained from E-Blend Pharmacy, a licensed pharmacy located inside the University of Port Harcourt. In order to prepare the powder for administration to the test animals, the tablets were crushed into a fine powder and the proper concentrations produced in distilled water. Alloxan monohydrate, another substance utilized, was also bought from the same pharmacy to cause diabetes in rats.

2.7. Induction of Diabetes

Alloxan monohydrate, freshly made with distilled water as the vehicle, was diluted to a concentration of 150mg/kg body weight and administered intraperitoneally to rats to cause diabetes. Three days later, diabetes was identified in alloxan-induced rats with Random Blood Glucose (RBG) levels ≥200mg/dL. Glucose levels were monitored using a hand held glucometer (Accu-CHEK) to test blood samples taken from the tail vein.

2.8. Experimental Design

Sixty-four (64) rats were divided into eight different experimental groups of 8 animals each. Group 1 (normal control group) received 0.5ml dimethylsulfoxide (DMSO), group 2 (diabetic control group) received placebo, 0.5ml dimethylsulfoxide (DMSO), group 3 (glibenclamide group) received 0.2mg/kg body weight glibenclamide (GBC, standard drug) orally, group 4 (*V. amygdalina* group) received 200mg/kg body weight of *V. amygdalina* extract, group 5 (*M. oleifera* group)received 200mg/kg body weight of *M. oleifera* extract, group 6 (low dose combination of *V. amygdalina* and *M. oleifera* extracts) received 100mg/kg each of *V. amygdalina* and *M. oleifera* extracts, group 7 (medium dose combination of *V. amygdalina* and *M. oleifera* extracts) received 200mg/kg each of *V. amygdalina* and *M. oleifera* extracts and group 8 (high dose combination of *V. amygdalina* and *M. oleifera* extracts) received 300mg/kg each of *V. amygdalina* and *M. oleifera* extracts. Upon administration, experimental rats were allowed access to feed and water.

Table 1: Experimental design

Group	No. of Rats	Treatment
Group 1	8	Normal Control
Group 2	8	Alloxan Control
Group 3	8	0.2mg/kg Glibenclamide (GBC)
Group 4	8	200mg/kg *V. amygdalina*Extract
Group 5	8	200mg/kg *M. oleifera*Extract
Group 6	8	100mg/kg *V. amygdalina* + 100mg/kg *M. oleifera*Extracts
Group 7	8	200mg/kg *V. amygdalina* + 200mg/kg *M. oleifera*Extracts
Group 8	8	300mg/kg *V. amygdalina* + 300mg/kg *M. oleifera*Extracts

The plant extracts were administered orally. The dosage of the extract was determined from preliminary studies in the laboratory. The duration of treatment was 21 days after induction of diabetes.

2.9. Body weight and Blood glucose determination

Body weight and blood glucose levels were monitored in order to assess the combined effects of *V. amygdalina* and *M. oleifera* extracts in treated diabetic rats. The blood glucose level was monitored using a glucometer (Accu-CHEK) to measure the blood glucose level from the tail vein using a tail clip. Body weight of the animals was evaluated using a laboratory weighing scale. Experimental animals had their body weight and blood glucose levels checked before starting therapy, on days 7, 14, and 21, and also before and after inducing diabetes.

2.10. Method of Data Analysis

Using the statistical program SPSS for Windows version 23.0, descriptive statistics were used to examine the data from this investigation and provided as mean standard error of mean of three determinations (mean ± SEM) (SPSS Inc. Chicago IL). Multiple comparison tests and analysis of variance (ANOVA) were used to differentiate differences between means. P values less than 0.05 were considered significant. The mean ± SEM body weight and blood glucose values were given in g and mg/dL respectively.

3. RESULTS

V. amygdalina and *Moringa oleifera* leaves both have oral LD50 values greater than 3000 mg/kg and 6500mg/kg body weight, respectively, according to an acute toxicity test [37,42]. *V. amygdalina* and *Moringa oleifera* leaves were examined for their phytochemical composition, which included alkaloids, tannins, saponins, flavonoids, phenol, terpenes, and steroids (Table 3). Table 2 displays the percentage yield of the ethanolic leaf extracts of *V. amygdalina* and *M. oleifera*.

Table 2: Percentage yield of Plant leaves extracts

Plant	Yield (%)	Yield (g)
V. amygdalina	4.105	41.05
M. oleifera	3.521	35.21

Table 3: Phytochemical (quantitative) analysis of the plants

Metabolites	Test	*V. amygdalina*	*M. oleifera*
Alkaloids	a. Mayer's test	+	+
	b. Dragendorf's test	+	+
	c. Wagners reagent	+	+
Tannins	a. Lead Sub-acetate	+	+
	b. Ferric chloride test	+	+
Saponins	a. Frothing test	+	+
Flavonoids	a. Ferric chloride test	+	+
	b. NaOH test	+	+
	c. Shinoda test	+	+
Phenols	a. Ferric chloride test	+	+
Terpenes	a. Lieberman-Buchard	+	+
	b. Salkowski test	+	+
Sterols	a. Lieberman-Buchard	+	+
	b. Salkowski test	+	+
Anthraquinones	a. Borntrager's test	-	-

Key: + presence of constituent, - absence of constituent

Table 4: Effect of *Vernonia amygdalina* and *Moringa oleifera* extract combination on the body weight of diabetic rats

	Body Weight (g)			
Group	**Pre-Treatment**	**Day 7**	**Day 14**	**Day 21**
Normal Control	165.86±4.20	170.57±2.66	174.66±4.83	181.23±5.24
Alloxan Control	151.96±13.0	142.20±8.71*	136.54±7.63*	118.62±9.12*
0.2mg/kg Glibenclamide (GBC)	152.56±0.61	168.61±9.31*#	174.24±7.48*#	171.40±10.22
200mg/kg VAE	153.52±5.74	170.45±8.70*#	177.10±6.12*#	175.47±12.21*#
200mg/kg MOE	154.15±6.05	168.65±4.95*#	176.88±7.65*#	173.15±11.62*#
100mg/kg VAE + 100mg/kg MOE	151.30±6.17	171.25±6.85*#	175.78±9.68*#	172.61±11.25*#
200mg/kg VAE + 200mg/kg MOE	153.32±11.18	174.40±10.05*#	178.85±6.10*#	171.43±7.44#
300mg/kg VAE + 300mg/kg MOE	154.30±10.13	175.40±10.15*#	180.28±7.23*#	172.54±6.57*#

Values are represented in mean±SEM, values marked with (*) differ significantly from normal control value (*p≤0.05) while those marked with (#) differ significantly from Alloxan control group (#p≤0.05). VAE = *Vernonia amygdalina* Extract, MOE = *Moringa oleifera* Extract

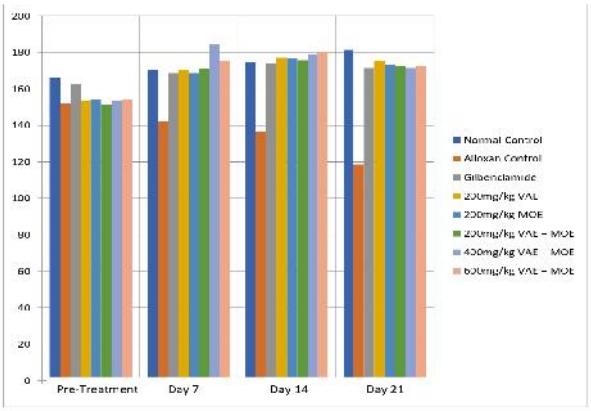

Fig 1: Bar Chart showing the effect of *Vernonia amygdalina* and *Moringa oleifera* extract combination on the body weight of diabetic rats

Table 5: Effect of *Vernonia amygdalina* and *Moringa oleifera* extract combination on blood glucose level of diabetic rats

	Blood Glucose Level(mg/dL)			
Group	**Pre-Treatment**	**Day 7**	**Day 14**	**Day 21**
Normal Control	60.5±2.14	62.2±2.21	62.8±1.84	61.1±2.18
Alloxan Control	255.6±6.31	268.4±7.16*	282.2±6.08*	310.5±12.23*
0.2mg/kg Glibenclamide (GBC)	248.4±7.28	196.5±6.82*#	200.4±8.12*#	178.0±7.26*#
200mg/kg VAE	238.6±6.18	241.7±7.30*#	220.2±8.24*#	198.5±5.84*#
200mg/kg MOE	235.9±6.08	238.5±6.71*#	217.8±5.12*#	196.2±4.92*#
100mg/kg VAE + 100mg/kg MOE	237.8±6.33	217.5±6.81*#	195.8±7.10*#	188.1±7.42*#
200mg/kg VAE + 200mg/kg MOE	248.9±7.22	202.4±8.47*#	190.5±5.11*#	181.7±6.73*#
300mg/kg VAE + 300mg/kg MOE	236.8±6.88	200.5±7.21*#	189.2±8.05*#	180.4±6.46*#

Values are represented in mean±SEM, values marked with (*) differ significantly from normal control value (*p≤0.05) while those marked with (#) differ significantly from Alloxan control group (#p≤0.05). VAE = *Vernonia amygdalina* Extract, MOE = *Moringa oleifera* Extract

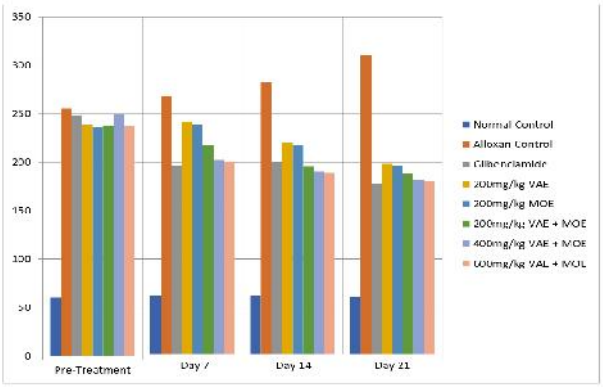

Fig 2: Bar Chart showing the effect of *Vernonia amygdalina* and *Moringa oleifera* extract combination on blood glucose level of diabetic rats

4. DISCUSSION

Numerous plant treatments have been utilized in traditional medicine for a number of ailments, including the treatment of diabetes mellitus [43]. Scientific research has proven that several of these herbal remedies have biological effects that counter diabetes mellitus and its consequences [44]. The biochemicals found in the plant components have been credited for some of these plants' therapeutic qualities. Nwaoguikpe [45], Adikwu *et al*. [46] and Asante *et al*. [47] investigated the effects of *V. amygdalina* on diabetic rat models and came to the conclusion that it has an anti-diabetic impact. In a similar vein, a study by Onyagbodor and Aprioku [48] confirmed the anti-diabetic activity of *Moringa oleifera*, while a review by Fatoumata *et al*. [49] provided comprehensive diabetes research employing *Moringa oleifera* that demonstrated the impact of the Moringa plant in diabetic rat models.

The goal of the current investigation is to determine whether co-administering *Vernonia amygdalina* and *Moringa oleifera* extracts in the nutrition of Wistar rats will have an anti-diabetic impact. Both plants contained alkaloids, tannins, saponins, flavonoids, phenol, terpenes, and steroids,

according to the phytochemical analysis. These phytochemicals help to explain some of the effects of using these plants as herbal remedies. Saponins [50,51], flavonoids [52-54], alkaloids [55,56], steroids and terpenes [57,58] have been reported to have good antidiabetic, hypolipidemic and antihyperglycemic activities [59-62].

Alloxan, a well-known diabetogenic chemical and cytotoxic glucose analogue utilized in diabetes research, was used to cause diabetes in Wistar rats. Alloxan has two distinct pathological effects. First, by specifically inhibiting glucokinase, the beta cell's glucose sensor, it inhibits glucose-induced insulin secretion. Second, by causing the formation of reactive oxygen species (ROS), which causes the selective necrosis of beta cells, it results in an insulin-dependent diabetes state. These two actions can be attributed to the particular chemical characteristics of alloxan, with the beta cell's selective uptake and accumulation of alloxan serving as the common denominator. This led to pancreatic malfunction and the bulk of the islets of Langerhans' -cells being destroyed. Untreated diabetic animals lost weight overall and their blood glucose levels rose as a result of pancreatic dysfunction brought on by the disease. The action of the alloxan results in the necrosis-induced death of beta-cells [24].

The findings of this study revealed that untreated diabetic rats (alloxan control) significantly lost weight when compared to non-diabetic rats (normal control), whereas experimental rats' body weights significantly increased after receiving doses of 200mg/kg of *V. amygdalina* and *M. oleifera*, respectively. The co-administration of different combination doses of *V. amygdalina* and *M. oleifera* also significantly resulted to an increase in the body weight of experimental rats when compared with non-diabetic (normal control) rats and untreated diabetic rats (alloxan control) as shown in Table 4 and Figure 1. Additionally, when compared to normal and alloxan control rats, experimental rats treated with gilbenclamide had a rise in body weight. Therefore, experimental rats gained weight when *V. amygdalina* and *M. oleifera* were administered together. This supports the conclusions reached by Minari [63] and Efiong *et al*. [64].

Fasting animals' blood glucose levels provide information on the amount of insulin in the bloodstream and the sensitivity of peripheral tissues to insulin's effects. Wistar rats' blood glucose levels are known to range from 50 to 135mg/dL, and readings above 200 mg/dL are typically indicative of severe hyperglycemia and the development of diabetes [65].

In this investigation, the control group consisted of water and dimethyl sulfoxide (DMSO). According to the findings, alloxan significantly raised blood glucose levels as compared to control rats, although blood glucose levels in control rats were within the previously mentioned normal range. Throughout the course of the experiment, the rats given alloxan showed a continuous rise in blood glucose levels (hyperglycemia). This is in line with other research that found that alloxan causes permanent diabetic mellitus 24 hours after administration [24,48,65].

Additionally, it was found that the elevated blood glucose levels of diabetic rats were decreased by individual doses of *V. amygdalina* and *M. oleifera* as well as by co-administration of *V. amygdalina* and *M. oleifera* extracts in a dose-dependent manner as shown in Table 5 and Figure 2. These effects were seen in comparison to non-diabetic rats (normal control) over time. Glibenclamide, a common medication, dramatically lowered the blood glucose level in diabetic rats. Gilbenclamide is a sulfonylurea that lowers blood sugar by encouraging pancreatic beta-cells to produce more insulin and by boosting the accumulation of glycogen in the liver. However, it may not be effective in alloxan-induced diabetic animals because alloxan treatment permanently destroys the beta cells [66]. As with the co-administered extracts, it also reduced the hyperglycemia brought on by alloxan. Co-administration of the extracts outperformed individual dose administration of *V. amygdalina* and *M. oleifera* extracts in terms of effectiveness. Therefore, experimental rats may develop normoglycemia if *V. amygdalina* and *M. oleifera* are administered together. The findings by Efiong *et al*. [64], Asante *et al*. [47], Ebong *et al.* [67] and Onyagbodor and Aprioku [48] are in agreement with this. The co-administered extract's antihyperglycemic efficacy may have been influenced by auxiliary components. This antihyperglycemic effect may be brought on by the terpenes and other bitter components found in these plants, which may also stimulate insulin production and release from beta cells. The findings imply that *V. amygdalina* and *M. oleifera* may play a dose-dependent protective role on pancreatic beta-cells and/or may have an insulin-like impact on peripheral tissues by either boosting glucose uptake/utilization or by blocking hepatic gluconeogenesis [68].

There is a chance of amplifying the insulin-like phytochemicals in these two extracts by combining them since the impact is similar to that of gilbenclamide, according to the evaluation of the antidiabetic effects demonstrated by the co-administration of *V. amygdalina* and *M. oleifera*. Some phytochemicals in anti-diabetic plants are known to have characteristics that mimic and function somewhat like insulin [69]. Therefore, it is possible that co-administration of these plants will stimulate insulin receptors, aid in the uptake and metabolism of glucose, and improve insulin sensitivity. Therefore, a combination of *V. amygdalina* and *M. oleifera* leaf extracts may be more advantageous and useful in managing diabetes as an antioxidant defense system, providing a comprehensive repertoire of free radical quenching components, and preventing atherosclerosis in diabetes mellitus.

5. CONCLUSION

Due to a potential synergy between the bioactive secondary chemicals from these two plants, the administration of mixed extracts from both plants' leaves demonstrated a potential impact. Because of this, it seems as though the leaves of these plants have a complement of bioactive substances, which could explain why they have a hypoglycemic effect. The utility of the combination herbal remedy as an anti-diabetic drug has been confirmed after Wistar rats that had been given alloxan-induced diabetes showed that it had an anti-diabetic effect. The current research backs up the traditional use of *M. oleifera* and *V. amygdalina* in diabetes control with scientific data.

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