

REVIEW

Review of the Safety and Efficacy of *Moringa oleifera*

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Moringa oleifera leaves, seeds, bark, roots, sap, and flowers are widely used in traditional medicine, and the leaves and immature seed pods are used as food products in human nutrition. Leaf extracts exhibit the greatest antioxidant activity, and various safety studies in animals involving aqueous leaf extracts indicate a high degree of safety. No adverse effects were reported in association with human studies. Five human studies using powdered whole leaf preparations of *M. oleifera* have been published, which have demonstrated anti-hyperglycemic (antidiabetic) and anti-dyslipidemic activities. These activities have been confirmed using extracts as well as leaf powders in animal studies. A rapidly growing number of published studies have shown that aqueous, hydroalcohol, or alcohol extracts of *M. oleifera* leaves possess a wide range of additional biological activities including antioxidant, tissue protective (liver, kidneys, heart, testes, and lungs), analgesic, antiulcer, antihypertensive, radioprotective, and immunomodulatory actions. A wide variety of polyphenols and phenolic acids as well as flavonoids, glucosinolates, and possibly alkaloids is believed to be responsible for the observed effects. Standardization of products is an issue. However, the results of published studies to date involving *M. oleifera* are very promising. Additional human studies using standardized extracts are highly desirable. © 2015 The Authors Phytotherapy Research Published by John Wiley & Sons Ltd.

Keywords: *Moringa oleifera*; leaf extract; anti-hyperglycemic; anti-dyslipidemic; antioxidant; chemoprotectant.

INTRODUCTION

Moringa oleifera Lam. is a tree that grows widely in many tropical and subtropical countries. It is grown commercially in India, Africa, South and Central America, Mexico, Hawaii, and throughout Asia and Southeast Asia. It is known as the drumstick tree based on the appearance of its immature seed pods, the horse-radish tree based on the taste of ground root preparations, and the ben oil tree from seed-derived oils. In some areas, immature seed pods are eaten, while the leaves are widely used as a basic food because of their high nutrition content (Thurber and Fahey, 2009; Mbikay, 2012; Razis *et al.*, 2014). No human clinical trials have been conducted looking at the efficacy of *M. oleifera* for treating undernutrition.

Seeds, leaves, oil, sap, bark, roots, and flowers are widely used in traditional medicine. *Moringa* leaves have been characterized to contain a desirable nutritional balance, containing vitamins, minerals, amino acids, and fatty acids (Moyo *et al.*, 2011; Teixeira *et al.*, 2014; Razis *et al.*, 2014). Additionally, the leaves are reported to contain various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids (Alhakmani *et al.*, 2013; Vongsak *et al.*, 2014). According to several commentaries (Anwar *et al.*, 2007; Mbikay, 2012; Razis *et al.*, 2014), various preparations of *M. oleifera* are used for their antiinflammatory, antihypertensive, diuretic, antimicrobial, antioxidant, antidiabetic,

antihyperlipidemic, antineoplastic, antipyretic, antiulcer, cardioprotectant, and hepatoprotectant activities. The therapeutic potential of *M. oleifera* leaves in treating hyperglycemia and dyslipidemia was reviewed by Mbikay (2012). Razis *et al.* (2014) summarized potential health benefits of *M. oleifera*, focusing on their nutritional content as well as antioxidant and antimicrobial characteristics.

SAFETY STUDIES

No adverse effects were reported in any of the human studies that have been conducted to date, and these studies will be described in more detail later in the text. Furthermore, various preparations have been and continued to be used around the world as foods and as medicinals without the report of ill effects. Several animal studies have specifically assessed the potential toxicity of various preparations on *M. oleifera*.

The safety of an aqueous leaf extract given orally to rats at doses of 400, 800, 1600, and 2000 mg/kg body weight was examined (Adedapo *et al.*, 2009). The treatment was either an acute single dose or given daily for 21 days except the highest dose. Various parameters were assessed including blood cell counts and serum enzyme levels. The authors concluded that consumption of *M. oleifera* leaves at doses of up to 2000 mg/kg were safe. A dose-dependent decrease in body weights of the rats occurred over the 21 days of the study.

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Asare *et al.* (2012) examined the potential toxicity of an aqueous leaf extract of *M. oleifera* in several different experimental systems. In one set of experiments, human peripheral blood mononuclear cells were exposed *in vitro* to graded doses of the extract and cytotoxicity was assessed. Cytotoxicity occurred at 20 mg/kg, a concentration not achievable by oral ingestion. In another set of experiments, rats were given 1000 and 3000 mg/kg of the extract, and the animals were assessed for up to 14 days. The *M. oleifera* leaf extract was shown to be genotoxic based on blood cell analysis at the 3000 mg/kg dose, a dose that greatly exceeds commonly used doses. A dose of 1000 mg/kg was deemed safe and did not produce genotoxicity when given to rats, a dose still in excess of commonly used doses.

Ambi *et al.* (2011) divided 24 rats into four groups and fed varying amounts of *M. oleifera* powdered leaves mixed with standard livestock feed (25%, 50%, 75%, and control) for 93 days. Total amount of *M. oleifera* leaves consumed was not quantified. Following the experimental period, some organs of the treated animals had observable microscopic lesions with the 75% group developed necrosis of hepatic cells, splenic blood vessels, and neuronal glial cells. The control animals had no observable microscopic lesions in all organs examined. No photomicrographs of any tissues were provided. The amounts of leaves consumed, although not quantified by the authors, greatly exceeded doses that would be typically used in either rats or humans. For example, if the rats consumed an average of 15–20 g of chow per day, even at the low dose of 25% of the chow, the daily dose would be approximately 15–20 g of leaves per kilogram for an adult rat, which would equate to 195–260 g for an 80-kg human.

The toxicity of an aqueous extract of *M. oleifera* leaves has also been evaluated in mice (Awodele *et al.*, 2012). In an acute study, mice were administered the extract at up to 6400 mg/kg orally and 1500 mg/kg intraperitoneally. In a subchronic study, mice received 250, 500, and 1500 mg/kg orally for 60 days. The lethal dose of 50% LD₅₀ was estimated to be 1585 mg/kg. No significant effects were observed with respect to hematological or biochemical parameters or sperm quality. A high degree of safety was observed on oral administration.

The toxicological effects associated with consumption of 50, 100, 200, or 400 mg/kg of methanol extract of *M. oleifera* for 8 weeks was performed in 30 rats (Oyagbemi *et al.*, 2013). The extract was a 30:1 concentration. All experimental animals that received *M. oleifera* had a significant increase in body weight in a dose-dependent manner, contrary to what is observed with an aqueous extract (Adedapo *et al.*, 2009). Rats that received *M. oleifera* at 200 and 400 mg/kg showed a significant increase in serum alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, and creatinine. It should be noted that the extract was prepared with methanol and not water. The 30:1 concentration of the methanol extract at a dose of 400 mg/kg would be equivalent to 12 g of leaves per kilogram, a very unrealistic dose. The composition of the extract was not reported, and it is not clear how the composition of the methanol extract relates to the composition of aqueous extracts, which are commonly used.

Bakre *et al.* (2013) determined that the lethal dose of 50% of an orally administered ethanol extract of *M. oleifera* leaves in mice was greater than 6.4 g/kg.

The dietary effects of *M. oleifera* leaves as a dietary supplement for liver function were performed by Zvinorova *et al.* (2014). Thirty-two weanling rats were randomly assigned to diets of normal rat feed fed at 20% and 14% of body mass, or *Moringa*-supplemented feeds fed at 20% and 14% of body mass for 5 weeks. *Moringa* supplementation did not affect blood metabolite concentrations, liver glycogen, or lipid storage.

The potential toxicological effects of a single oral dose of 5000 mg/kg of an aqueous *M. oleifera* extract as well as oral doses of up to 1000 mg/kg of the same extract for 14 days on rats were examined (Asiedu-Gyekye *et al.*, 2014). The authors noted that no overt adverse reactions were observed at these doses, and no histopathological findings were found. Small but statistically significant dose-dependent increases in several liver enzymes were observed. A dose of 1000 mg/kg in a rat is equivalent to over 30 times a typical 400 mg dose of an aqueous extract in an 80-kg human.

The genotoxicity of an aqueous *M. oleifera* seed extract was assessed using three separate assay systems including the Ames assay (Rolim *et al.*, 2011). The seed extract was not genotoxic without metabolic activation, and did not pose a risk to human health. The effect of a hexane extract of *M. oleifera* leaves on reproductive organs of male rats was examined (Cajuday and Pocsidio, 2010). The extract was given orally at doses of 17, 170, and 1700 mg/kg body weight for 21 days. A dose-dependent increase in testis and epididymis weights, in seminiferous tubule diameter, and epididymal epithelium thickness without change in plasma gonadotropin levels was observed. The authors concluded that the changes were associated with an increase in spermatogenesis.

For the sake of completeness, several studies involving *M. oleifera* seeds and roots will be described, although the results cannot be directly compared or equated with studies involving leaves. Cytotoxicity of an aqueous extract of *M. oleifera* seeds was evaluated by Araújo *et al.* (2013). Following 14 days of the extract administration (500 and 2000 mg/kg) in mice, no signs of systemic toxicity were observed, and all the animals survived. There were no changes in organ indices between treatment and control groups. Small but insignificant changes were observed in erythrocytes, platelets, hemoglobin, and hematocrit. All values remained within the normal range.

A methanol extract of seeds of *M. oleifera* were screened phytochemically for chemical components and used for acute and subacute toxicity studies in rats (Ajibade *et al.*, 2013). The phytochemical screening revealed the presence of saponins, tannins, terpenes, alkaloids, flavonoids, carbohydrates, and cardiac glycosides but the absence of anthraquinones. Although signs of acute toxicity were observed at an extract dose of 4000 mg/kg, mortality was recorded at 5000 mg/kg. No adverse effects were observed at concentrations lower than 3000 mg/kg. The authors concluded that methanol extracts of seeds of *M. oleifera* are safe for nutritional use.

Paul and Didia (2012) investigated the effect(s) of methanol extract of *M. oleifera* root on the histoarchitecture of the liver and kidney of 24 guinea-pigs. Experimental conditions included daily intraperitoneal injections of the root extract at doses of 3.6, 4.6, and 7.0 mg/kg, and control for 3 weeks. Histological sections

of all treated groups had ballooning degeneration of the liver, suggesting time-dependent hepatotoxicity rather than a dose-dependent response. Examination of the kidneys, demonstrated mild tubular damage and interstitial inflammation in the 4.6 mg/kg group, while the 7.0 mg/kg group had infiltration of the interstitium by inflammatory cells and amorphous eosinophilic materials. No information was provided regarding extract composition or degree of concentration. The results of this study cannot be compared or equated with studies involving aqueous extracts of leaves. This study involved a methanol extract of roots, which was given intraperitoneally and not orally.

In summary, based on human, animal, and *in vitro* studies, and the extrapolation of results from animal studies to humans, various preparations of *M. oleifera* leaves including aqueous extracts appear to be exceedingly safe at the doses and in the amounts commonly utilized.

HUMAN STUDIES

Moringa oleifera has long been used in traditional medicine. While data collected from human subjects are limited, several trials demonstrating potential benefits for treating hyperglycemia and dyslipidemia primarily in people with type 2 diabetes have been published.

In a single dose study with six type 2 diabetic subjects, the feeding of 50 g of a *M. oleifera* leaf powder with a standard meal on a one-time basis decreased blood glucose levels by 21% (William *et al.*, 1993). The authors concluded that the reduced blood glucose response to *M. oleifera* was not due to alterations in insulin secretion.

Kumari (2010) treated type 2 diabetic subjects with 8 g of powdered *M. oleifera* leaf in a tablet form per day for 40 days. A total of 46 subjects were involved in the study. At the end of the study, fasting blood glucose and postprandial blood glucose were 28% and 26% lower, respectively, in the treated subjects. Furthermore, total cholesterol, triglycerides, Low density lipoprotein (LDL)-cholesterol, and very low density lipoprotein-cholesterol were 14%, 14%, 29%, and 15% lower relative to the control group.

Nambiar *et al.* (2010) examined the anti-dyslipidemic effects of *M. oleifera* in 35 type 2 diabetic subjects. The treated group received 4.6 g of a leaf powder in a tablet form daily for 50 days. Compared with the control group, the treated subjects experienced a 1.6% decrease in total plasma cholesterol and a 6.3% increase in HDL. Comparing this study with the previous studies suggests that higher doses may be more effective.

Ghiridhari *et al.* (2011) conducted a study in which 60 type 2 diabetic subjects were given two *M. oleifera* leaf powder tablets per day or placebo for up to 3 months. Unfortunately, the weight of the tablets and therefore the actual dose of the leaf powder were not given. After 3 months, postprandial blood glucose had decreased by 29% relative to the control group, while hemoglobin A1C, an index of glycosylation related to blood glucose levels, decreased by 0.4%.

In another human study, Kushwaha *et al.* (2012) studied 30 postmenopausal women who were supplemented daily with 7 g of *M. oleifera* leaf powder for a period of 3 months. A control group also consisted of

30 postmenopausal women. The data revealed significant increases in serum glutathione peroxidase (18.0%), superoxide dismutase (10.4%), and ascorbic acid (44.4%), with decreases in malondialdehyde (16.3%; lipid peroxidation), markers of antioxidant properties. In addition, a significant decrease in fasting blood glucose levels (13.5%) as well as an increase in hemoglobin (17.5%) was observed. No adverse effects were reported.

In summary, the previous human studies indicate that whole leaf powders of *M. oleifera* given orally exhibit significant anti-hyperglycemic, anti-dyslipidemic, and antioxidant effects in human subjects without production of adverse effects. None of these studies involved the use of leaf extracts.

ANIMAL AND IN VITRO STUDIES

An ever-expanding number of animal studies have been conducted involving *M. oleifera* leaf powder, and aqueous and aqueous alcohol extracts. These studies have exhibited the following properties: anti-hyperglycemic, anti-dyslipidemic, antioxidant, tissue chemoprotectant, immunomodulatory, radioprotective, antihypertensive, and neuroprotective effects. These studies will be briefly summarized later in the text.

Several reports exist concerning the anti-hyperglycemic effects of *M. oleifera* leaf products in rats. Ndong *et al.* (2007a) administered 2 g of a leaf powder per kilogram to rats and demonstrated that the leaf powder decreased blood glucose levels by 23% relative to controls. Jaiswal *et al.* (2009) demonstrated that an aqueous extract of *M. oleifera* leaves decreased blood glucose levels in a dose-dependent manner when using doses of 100–300 mg/kg. A single oral dose of 200 mg/kg of an aqueous extract of *M. oleifera* leaves decreased blood glucose levels in mildly streptozotocin-induced diabetic rats following an oral glucose tolerance test by 33.8% and by 51.2% in severely diabetic animals.

Tende *et al.* (2011) examined the effects of an ethanol extract of *M. oleifera* leaves on blood glucose levels of streptozotocin-induced diabetic rats. Doses of the extract at 250 and 500 mg/kg were given intraperitoneally. Significant reductions in blood glucose levels were observed in fasted streptozotocin-induced diabetic animals but not when the extract was administered to control, normotensive animals. The authors postulated that the effect was due to the terpenoid content of the extract, but provided no direct evidence to support this contention.

Yassa and Tohamy (2014) have also assessed the antidiabetic and antioxidant potential of an aqueous extract of *M. oleifera* leaves in streptozotocin-induced diabetic rats. *M. oleifera* treatment significantly decreased fasting plasma glucose (380% to 145%), increased reduced glutathione (22% to 73%), and decreased malondialdehyde (385% to 186%) compared with control levels. Damage of islet cells was also reversed following *M. oleifera* leaf extract administration. The anti-hyperglycemic effects of an aqueous leaf extract may be due in part to the presence of an intestinal sucrose inhibitor (Adisakwattana and Chanathong, 2011), but this action cannot explain the effect of the leaf extract in response to a glucose tolerance test.

When rats fed with a high-fat diet were given an aqueous *M. oleifera* leaf extract at an oral dose of 1 g/kg body weight per day for 30 days, significant reductions in total cholesterol in serum, liver, and kidneys of 14.4%, 6.4%, and 11.1%, respectively, were observed (Ghasi *et al.*, 2000). Chumark *et al.* (2008) fed rabbits a high-cholesterol diet for 12 weeks. When these animals were concomitantly given an oral daily dose of 100 mg/kg of an aqueous *M. oleifera* leaf extract, total serum cholesterol and lipoprotein cholesterol were reduced by 50% and 75%, respectively, while carotid plaque was decreased by 97%. Jain *et al.* (2010) fed the rats with a high-fat diet for 30 days with and without a methanol extract of *M. oleifera* at daily doses of 150, 300, and 600 mg/kg body weight. A dose-dependent reduction in serum lipids was observed. At the highest dose, total cholesterol, LDL-cholesterol, VLDL-cholesterol, and total triglycerides were decreased by 37.5%, 61.4%, 23.5%, and 18.7%, respectively. A 50% reduction in plaque formation was also observed.

Numerous studies have examined the antioxidant properties of *M. oleifera*. Chumark *et al.* (2008) demonstrated the free radical scavenging ability of an aqueous extract of *M. oleifera* leaves in several *in vitro* systems, and also showed that the extract inhibited lipid peroxidation in both *in vitro* and *ex vivo* systems. Aqueous extracts of *M. oleifera* leaves, fruits, and seeds were assessed for their ability to inhibit oxidative damage to DNA (Singh *et al.*, 2009). The leaf extract was shown to exhibit the greatest antioxidant activity and to have the highest total phenolic content (105 mg gallic acid equivalents/100 g), the highest total flavonoid content (31 mg quercetin equivalents/100 g), and ascorbic acid content (107 mg/100 g).

The antioxidant properties of different fractions of *M. oleifera* leaves were examined both *in vitro* and *in vivo* by Verma *et al.* (2009). The polyphenolic fraction was shown to exhibit the greatest free radical scavenging activity *in vitro*. This fraction when administered to rats inhibited carbon tetrachloride-induced toxicity and hepatic lipid peroxidation while increasing hepatic glutathione content. Glutathione is the primary antioxidant in liver cells. This fraction also increased the antioxidant enzymes catalase and superoxide dismutase while decreasing lipid peroxidases, thus providing a biochemical rationale for the antioxidant and chemoprotectant effects.

The ability of an aqueous extract of *M. oleifera* leaves to scavenge free radicals associated with 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, superoxide, and nitric oxide as well as to inhibit lipid peroxidation was demonstrated by Sreelatha and Padma (2009). In an *in vitro* system involving rat liver slices, an extract of *M. oleifera* leaves was shown to attenuate the toxicity of carbon tetrachloride as demonstrated by decreases in lipid peroxidation and increases in the antioxidant enzymes glutathione peroxidase, glutathione reductase, catalase, superoxide dismutase, and glutathione S-transferase (Sreelatha and Padma, 2010).

These same investigators (Sreelatha and Padma, 2011) subsequently showed that an aqueous extract of *M. oleifera* leaves inhibited hydrogen peroxide-induced DNA damage and lipid peroxidation in KB (human tumor) cells. The aqueous extract also enhanced the antioxidant activity of the enzymes superoxide dismutase and catalase. In yet another study, Sreelatha *et al.* (2011) reported that an aqueous extract of *M. oleifera* leaves exhibited an

antiproliferative effect in conjunction with apoptosis (programmed cell death) and prevented DNA fragmentation in KB cells in culture. Santos *et al.* (2012) examined the antioxidant activity of *M. oleifera* ethanol and saline extracts from leaves, flowers, seeds, and stems. The greatest radical scavenging and antioxidant activity was associated with ethanol leaf extracts.

Jung (2014) has shown that an aqueous extract of *M. oleifera* leaves exhibited significant antineoplastic activity against a lung cancer cell line and several other types of cancer cells. The extract induced apoptosis, inhibited tumor cell growth, and lowered the internal level of reactive oxygen species in human lung cancer cells. Tiloke *et al.* (2013) have also shown that an aqueous extract of *M. oleifera* leaves exhibited antiproliferative activity against cancerous human alveolar epithelial cells. In neither of these studies were attempts made to relate specific ingredients of the extracts to the observed effects.

Jaiswal *et al.* (2013) have investigated the antioxidant activity of an aqueous extract of *M. oleifera* leaves in normal and diabetic rats. Oxidative free radical scavenging enzymes were measured in response to 200 mg/kg of lyophilized powder. A significant increase in activities of superoxide dismutase, catalase, and glutathione S-transferase and a decrease in lipid peroxidation were observed. It was suggested that the high phenolic and flavonoid contents in the extract can protect against oxidative damage in normal and diabetic subjects.

A number of studies have examined the tissue protectant activity of *M. oleifera* extracts. As noted earlier, several studies have demonstrated that an aqueous extract protects against carbon tetrachloride hepatotoxicity (Verma *et al.*, 2009; Sreelatha and Padma, 2010). Das *et al.* (2012) have shown that in mice fed with a high-fat diet, an aqueous extract of *M. oleifera* leaves protects against liver damage as demonstrated by reductions in tissue histopathology and serum activities of marker enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) as well as reduced lipid peroxidation and increases in reduced glutathione.

Pari and Kumar (2002) showed that an ethanol extract of *M. oleifera* leaves protected rats against the hepatotoxicity of various antitubercular drugs including isoniazid, rifampicin, and pyrazinamide. The extract decreased drug-induced levels of AST, ALT, ALP, and bilirubin, and inhibited drug-induced lipid peroxidation in the liver.

Various studies have demonstrated that extracts of leaves can prevent liver toxicity because of acetaminophen (paracetamol). Fakurazi *et al.* (2008) showed that the administration of 200 and 800 mg/kg of aqueous ethanol extracts of *M. oleifera* leaves prevented acetaminophen-induced liver damage as determined by decreases in AST, ALT, and ALP as well as increases in hepatic glutathione. Fakurazi *et al.* (2012) expanded on these studies and demonstrated that intraperitoneal administration of 200 and 400 mg/kg body weight of hydroethanol extracts of *M. oleifera* leaf and flower protected against acetaminophen-induced liver damage. The extracts decreased hepatic lipid peroxidation, increased glutathione levels, and increased the levels of the antioxidant enzymes superoxide dismutase and catalase.

Using a hydroethanol extract (80%) of *M. oleifera* leaves at oral doses of 200 and 800 mg/kg (Uma *et al.*, 2010), the protective effect against the hepatotoxicity

of acetaminophen (paracetamol) was demonstrated in rats. The extract reduced hepatic lipid peroxidation while restoring levels of the enzymes glutathione S-transferase, glutathione reductase, and glutathione peroxidase to normal. Sharifudin *et al.* (2013) also reported on the ability of hydroethanol extracts of *M. oleifera* leaves and flowers at doses of 200 and 400 mg/kg given intraperitoneally to inhibit acetaminophen-induced hepatotoxicity. No changes were observed with respect to markers of kidney function.

An ethanol extract of *M. oleifera* leaf was shown to protect against chromium-induced testicular toxicity in rats (Sadek, 2013). When the extract was given orally on a daily basis (500 mg/kg) for 60 days to rats that received 8-mg potassium chromate intraperitoneally daily, the extract significantly ameliorated the testicular chromium effects on sperm parameters, local immunity, inflammatory markers, and antioxidant enzyme activities.

The ability of an extract of *M. oleifera* leaves to prevent selenite-induced cataractogenesis was demonstrated in rat pups weighing 10–12 g (Sasikala *et al.*, 2010). Pups were given sodium selenite (4 µg/g) subcutaneously on day 10 to induce cataracts. Some animals in addition received 2.5 µg/g of the extract from days 8 through 15. Cataracts were visualized on day 16. The extract effectively prevented morphological changes (cataracts) and oxidative damage to the lens. Furthermore, treatment with the *M. oleifera* extract prevented selenite-induced lipid peroxidation, and maintained glutathione levels in the lens as well as the activities of antioxidant enzymes.

The retinoprotective effects of *M. oleifera* in streptozotocin-induced diabetic rats were investigated by Kumar Gupta *et al.* (2013). Treatment with *M. oleifera* was shown to prevent diabetes-induced dilation of retinal vessels and the increase in inflammatory factors tumor necrosis factor- α and interleukin-1 β . In addition, *M. oleifera* decreased the diabetes-induced angiogenic factors vascular endothelial growth factor and protein kinase C- β . Therefore, the results indicated that an extract of *M. oleifera* may be useful in preventing diabetes-induced retinal dysfunction.

A hydroalcohol extract of *M. oleifera* leaves has been shown to exhibit cardioprotective, antioxidant, and anti-peroxidative activity in response to isoproterenol in rats (Nandave *et al.*, 2009). Rats were treated daily with saline, isoproterenol, or isoproterenol plus the leaf extract (200 mg/kg) orally for 1 month. The *M. oleifera* leaf extract prevented biochemical, histopathological, and ultrasound changes in the heart induced by isoproterenol. The extract prevented isoproterenol-induced hemodynamic changes in the heart including changes in heart rate, left ventricular end-diastolic pressure, left ventricular peak positive pressure, and left ventricular negative pressure.

In a more recent study, the cardioprotective effect of *N*, α -L-rhamnopyranosyl vincosamide, an indole alkaloid isolated from the leaves of *M. oleifera*, was demonstrated (Panda *et al.*, 2012). This alkaloid when administered at an oral dose of 40 mg/kg per day for 7 days markedly reduced isoproterenol-induced cardiotoxicity in rats. The cardioprotective effects were demonstrated by decreases in serum cardiac biomarkers, increases in cellular antioxidants and antioxidant enzymes, a reduction in cardiac necrosis, a decrease in cardiac lipid peroxidation, and a reduction in cardiac histopathology and electrocardiographic (ECG) changes.

An aqueous ethanol (hydroalcohol) extract of *M. oleifera* leaves was reported to prevent gentamicin-induced (80 mg/kg) nephrotoxicity in rabbits at doses of 150 and 300 mg/kg body weight (Ouedraogo *et al.*, 2013). The leaf extract significantly decreased markers of gentamicin-induced kidney toxicity including histological changes, lipid peroxidation, and serum urea and creatinine levels. The feeding of an iron-deficient diet to rats results in hepatic ultrastructural changes (Ndong *et al.*, 2007b). The addition of a leaf extract of *M. oleifera* to the diet normalized the mitochondria, and prevented hyperlipidemia and ultrastructural changes in the hepatocytes due to iron deficiency. This beneficial effect was due to the high iron content of the *M. oleifera* leaves (Teixeira *et al.*, 2014).

Two reports have looked at the immunomodulatory effects of extracts of *M. oleifera* leaves. In a study in mice, it was shown that a methanol extract of *M. oleifera* leaves given orally at doses of 250 and 750 mg/kg stimulated both cellular and humoral immune responses (Sudha *et al.*, 2010). The low dose was found to be more effective than the high dose of the extract. Various assays were used to assess cellular and humoral immunity. In another study, the immunomodulatory effect of an ethanol extract of *M. oleifera* leaves was examined in mice treated with cyclophosphamide (Gupta *et al.*, 2010). The extract was given orally at doses of 125, 250, and 500 mg/kg per day for 15 days. The results demonstrated that the extract reduced the cyclophosphamide-induced immunosuppression by stimulating both cellular and humoral immunity.

The analgesic effect of methanol extracts of leaves and roots of *M. oleifera* was demonstrated in rats (Manaheji *et al.*, 2011). Extracts of both leaves and roots (200, 300, and 400 mg/kg) as well as a combination of the two extracts (200 mg/kg) were given intraperitoneally to rats after administration of complete Freund's adjuvant to induce arthritis in the animals. Both extracts at the two highest doses as well as the combination dose were effective in reducing pain induced by complete Freund's adjuvant on days 3 and 6.

In a wound healing study in rats, the administration of 300 mg/kg per day of an aqueous extract of *M. oleifera* leaves significantly increased wound closure rate, skin-breaking strength, and granuloma dry weight, and decreased scar area (Rathi *et al.*, 2006). In a study involving the use of human dermal fibroblasts, Muhammad *et al.* (2013) showed that an aqueous extract of *M. oleifera* leaves significantly increased cell proliferation and viability as compared with untreated controls. It was determined that the bioactive aqueous fraction contained vicenin-2 as well as quercetin and kaempferol, and this fraction may enhance faster wound healing.

The ability of an aqueous extract of *M. oleifera* to inhibit the ulcerogenic effects of aspirin (500 mg/kg) in rats was demonstrated (Debnath *et al.*, 2011). Maximum protection was afforded with an oral dose of 300 mg/kg of the extract. The authors provided evidence that the prevention of aspirin-induced ulcers by the extract involved the modulation of 5-hydroxytryptamine (5-HT) secretion. Choudhary *et al.* (2013) examined the anti-ulcer potential of an *M. oleifera* ethanol root bark extract with ethanol-induced and pylorus ligation-induced gastric ulceration in rats. *M. oleifera* at doses of 350 and 500 mg/kg for 15 consecutive days decreased the ulcer index significantly as compared with the control group

($p < 0.01$) and significantly reduced the free acidity, total acidity, and ulcer index ($p < 0.01$), and increased the pH of gastric content compared with the control group. The composition of this root extract was not determined, nor is it clear how this root extract relates to leaf extracts in terms of composition.

Two studies have shown that extracts of *M. oleifera* can provide radioprotection in mice. In the initial study, a methanol extract of *M. oleifera* leaves was given as a single dose of 150 mg/kg or divided into five doses of 30 mg/kg and given over the course of the day. The animals were exposed to lethal whole-body gamma radiation 1 h later (Rao *et al.*, 2001). Administration of the extract conferred significant radioprotection to bone marrow chromosomes. The fractionated administration of the extract afforded higher protection than the single dose. In the second study, mice were given 300 mg/kg of a *M. oleifera* leaf extract daily for 15 days followed by exposure to gamma radiation (Sinha *et al.*, 2012). Administration of the extract restored hepatic glutathione levels and prevented radiation-induced hepatic lipid peroxidation.

Two studies have examined the hypotensive effects of *M. oleifera* extracts in rats. In the initial study (Faizi *et al.*, 1998), ethanol extracts of seeds and pods exhibited equivalent hypotensive activity at 30 mg/kg. Bioassay-directed fractionation resulted in the isolation of thiocarbamate and isothiocyanate glycosides as well as hydroxybenzoate as the active principles, confirming the earlier but unpublished studies of Saleem (1995). In the second study, ethanol extracts of *M. oleifera* leaves were used (Chen *et al.*, 2012). Pulmonary hypertension was induced in the rats by injection of monocrotaline, which resulted in increased arterial blood pressure and thickening of the pulmonary arterial medial layer. Three weeks after induction, daily intraperitoneal injections of a freeze-dried extract resulted in a dose-dependent decrease in pulmonary arterial blood pressure that reached statistical significance at 4.5 mg/kg. Chronic administration of the extract reversed the monocrotaline-induced changes.

The neuroprotective effects of *M. oleifera* are an emerging area of study. Sutralangka *et al.* (2013) have determined that an aqueous *M. oleifera* leaf extract is a potential cognitive enhancer and neuroprotectant in an animal model of dementia-induced rats (intracerebroventricular bilateral administration of a cholinotoxin). Doses of 100–400 mg/kg of the extract were given for 7 days. Brain levels of lipid peroxidation and increases in the levels of the antioxidant enzymes superoxide dismutase and catalase were observed in response to the extract. The active constituents in the extract were not determined.

Kirisattayakul *et al.* (2013) have demonstrated that a hydroalcohol extract of *M. oleifera* leaves at oral doses of 100–400 mg/kg for 3 weeks attenuated brain dysfunction and brain damage induced by cerebral ischemia. The extract represented 17.5% of the starting material. The protective effects were believed to be due to decreased oxidative stress based on assessment of various antioxidant enzymes and decreases in brain lipid peroxidation.

Bakre *et al.* (2013) have shown an ethanol extract of *M. oleifera* leaves possesses Central Nervous System (CNS) depressant and anticonvulsant activities in mice through the enhancement of central inhibitory mechanism involving release of γ -amino butyric acid. Significant

dose-dependent (250–2000 mg/kg) decreases in grooming, rearing, head dips, and locomotion were observed. Hannan *et al.* (2014) demonstrated neuroprotective properties of an ethanol extract of *M. oleifera* leaves when incubated with a primary culture of hippocampal neurons. The extract promoted neurite outgrowth in a concentration-dependent manner, with significant increases in the number and lengths of dendrites and axonal branches. These findings suggest that *M. oleifera* may provide a neuroprotective benefit through reductions in oxidative stress. However, further research regarding the active ingredient(s) is still required.

In summary, animal studies have demonstrated antihyperlipidemic, antidiabetic, antioxidant, tissue protectant (liver, kidneys, heart, and eyes), immunomodulatory, radioprotective, antihypertensive, cardioprotective, and neuroprotective effects.

CHEMISTRY

One of the earliest and most extensive studies on the chemical constituents of an ethanol extract of *M. oleifera* leaves was conducted by Saleem (1995). The study was published only as a thesis and never in a peer-reviewed journal. The author isolated and provided structure elucidation of 23 compounds using a variety of separation and spectroscopic techniques such as infrared and ultraviolet spectroscopy, mass spectroscopy, gas chromatography, gas chromatography–mass spectroscopy, and nuclear magnetic resonance spectroscopy. In addition to the rhamnosyloxy benzyl isothiocyanate niaziminins, niazinins, and niacinins, various methylated, ethylated, and acetylated rhamnosyloxy benzyl carbamates and rhamnosyloxy benzyl thiocarbamates were isolated and characterized. An additional 63 compounds were identified in an ethanol extract of *M. oleifera* pods.

As noted earlier, Faizi *et al.* (1998) used bioassay-directed fractionation of ethanol extracts of seeds and pods in the isolation of thiocarbamate and isothiocyanate glycosides as well as a hydroxybenzoate. They demonstrated that these fractions possessed hypotensive activity. The isolation of these chemical constituents confirmed the earlier but unpublished studies of Saleem (1995).

Guevara *et al.* (1999) isolated niazimicin and niazirin as well as a rhamnosyloxy benzyl carbamate, rhamnosyloxy benzyl isothiocyanate, and various derivatives of β -sitosterol from the seeds of *M. oleifera*. No determination of these compounds in leaves was made. Niacimicin was shown to have chemoprotective activity, serving as a potent antitumor-promoting agent *in vivo* in a two-stage carcinogenesis assay in mouse skin.

Niaziridin and niazarin were also isolated from leaves and pods of *M. oleifera* by Shankar *et al.* (2007). They noted that niazarin was present in higher concentrations in leaves, while niaziridin was present in approximately three times greater amounts in pods as compared with leaves. These investigators demonstrated that niaziridin enhanced the bioactivity of a number of antibiotics and also facilitated the gastrointestinal absorption of vitamins and other nutrients.

Bennett *et al.* (2003) analyzed the glucosinolates and phenolics in *M. oleifera* leaves, seeds, and roots. Present in leaves were rhamnosyloxy benzyl glucosinolates and

acetylated isomers thereof, quercetin 3-*O*-glucosides, kaempferol 3-*O*-glucosides, and caffeoylquinic acids. No proanthocyanidins or anthocyanidins were detected. Again, this study supports the earlier work of Saleem (1995).

Mbikay (2012) reviewed the bioactive phytochemicals that have been isolated from *M. oleifera* leaves, seeds, flowers, pods, and stems. Major classes of compounds that have been isolated include phenolic acids, glucosinolates, and flavonoids. The flavonol quercetin is present in concentrations as high as 100 mg/100 g of the leaves, which exists primarily as the glucoside. Other prominent ingredients include chlorogenic acid, rutin, kaempferol rhamnoglucoside, myricetin, benzylamine (moringanine), and the glycosides niaziminin and niazinin (Mbikay, 2012).

Various derivatives of salicylic acid, gallic acid, coumarin acid, and caffeic acid also exist in extracts of *M. oleifera*. In addition, indole alkaloid *N*, α -*L*-rhamnopyranosyl vincosamide has been isolated from *M. oleifera* leaves and shown to exert a cardioprotective effect on rats (Panda *et al.*, 2012). Waterman *et al.* (2014) isolated and characterized four isothiocyanates from *M. oleifera* leaves. An aqueous extract contained 1.66% isothiocyanates and 3.82% total polyphenols. The isothiocyanates were shown to exhibit antiinflammatory activity in an *in vitro* macrophage cell system. These studies support the initial findings of Saleem (1995).

A nutritional characterization of dried *M. oleifera* leaves was made by Moyo *et al.* (2011) who reported the content of calcium, phosphorus, magnesium, potassium, sodium, sulphur, zinc, copper, manganese, iron, and selenium. They noted that the dried leaves contained 77 mg/100 g of vitamin E and 18.5 mg/100 g of ascorbic acid. The dried leaves also contained 30.3% protein, 19.89% fiber, 1.8% lignin, 4.0% cellulose, 3.2% tannins, and 2.0% polyphenols.

The microelemental and macroelemental composition of an aqueous extract of *M. oleifera* leaves was determined by energy-dispersive X-ray technology with triaxial geometry (Asiedu-Gyekye *et al.*, 2014). The authors determined the amounts of 35 elements (14 macroelements and 21 microelements) in the aqueous extract. Elements present in the greatest abundance in decreasing order were sulphur, calcium, potassium, magnesium, sodium, phosphorus, silicon, and aluminum, in general agreement with the report of Moyo *et al.* (2011).

Teixeira *et al.* (2014) also characterized various chemical constituents in the dried, powdered leaves of *M. oleifera*. The leaf powder sample examined contained 28.7% crude protein, 7.1% fat, 10.9% ash, 44.4% carbohydrates, 3.0 mg calcium and 103.1 mg of iron per 100 g, 20.7 mg tannins/g, 17 mg nitrate/g, 10.5 mg oxalate/g, 161 μ g β -carotene/g, and 47 μ g lutein/g. The analysis of an aqueous (10:1) extract of *M. oleifera* leaves by the authors of this review was conducted. The dried extract contained 22.6% fiber, 2.73% ash, 3.78% protein, 9.53% total sugars, 0.00746% calcium, 0.0549% iron, and 0.0468% total catechins/flavonoids (0.0323% epicatechin). No carotenoids, vitamin C, or phyosterols were present in the extract (Stohs and Hartman, unpublished).

Vongsak *et al.* (2014) have conducted a quantitative analysis of an ethanol extract of *M. oleifera* leaves by HPLC, and have shown that the average values for cryptochlorogenic acid, isoquercetin, and astragaloside in

the dried extract were 0.081%, 0.120%, and 0.153%, respectively. They have suggested that these compounds and this analysis may serve as a guideline for the standardization of *M. oleifera* extracts. However, these standards could only be applied to ethanol extracts and not to aqueous extracts.

Several procyanidin compounds have been shown to be present in root and stem barks (Atawodi *et al.*, 2010). Additionally, the flowers of *M. oleifera* are reported to contain various types of antioxidant compounds such as ascorbic acid and carotenoids, as well as tannins, flavonoids, alkaloids, and cardiac glycosides (Alhakmani *et al.*, 2013).

The chemical composition of an ethanol extract of *M. oleifera* leaves, which represents the essential oil component, was determined by gas chromatography–mass spectroscopy (Chuang *et al.*, 2007). The extract represented 5.6% of the total dry weight of the leaves from which the extract was prepared. The authors identified a total of 44 compounds. Pentacosane, hexacosane, (E)-phytol, and 1-[2,3,6-trimethylphenyl]-2-butanone represented 17.4%, 11.2%, 7.7%, and 3.4%, respectively, of the extract. Thus, these four components represented approximately 40% of the total neutral oil constituents. It should be noted that while all of the 44 constituents are present in low concentrations in whole leaves and leaf powders, little if any of these compounds will be present in an aqueous extract. A number of these ingredients had been previously identified in the pods of *M. oleifera* by Saleem (1995).

Phytochemical variations of 13 *M. oleifera* cultivars collected from around the globe were compared (Ndhlala *et al.*, 2014). Aqueous methanol extracts were compared for total phenol content, total flavonoid content, free radical scavenging and antioxidant activity using three different assay systems, and antimicrobial activity. As might be expected, the results demonstrated variations between the cultivars from different locations. The variations could be due to many factors including genetic variations, soil, climate, time of harvest, and storage conditions.

In summary, a large number of potentially bioactive compounds are present in *M. oleifera*. As a consequence, extracts are generally unstandardized. However, extracts have been evaluated on the basis of their relative polyphenol, flavonoid, and glucosinolate contents, with aqueous leaf extracts exhibiting the greatest activities of these indicators (Singh *et al.*, 2009; Mbikay, 2012; Waterman *et al.*, 2014; Vongsak *et al.*, 2014).

SUMMARY AND DISCUSSION

The human and animal as well as *in vitro* studies described in the preceding text indicate that various preparations of *M. oleifera* leaves and other plant parts possess a wide range of physiological and pharmacological activities. All published studies in human subjects have used powdered leaf preparations, while the majority of animal studies have used aqueous, hydroalcohol, or alcohol (methanol or ethanol) extracts of *M. oleifera* leaves or other plant parts. The most research support exists for the antioxidant, antidiabetic (anti-hyperglycemic), anti-dyslipidemic, and chemoprotective effects of *M. oleifera* whole leaf powder and extracts thereof.

A rapidly growing number of research studies involving *M. oleifera* have been reported in recent years, primarily in rodents. Little effort to standardize extracts and to employ standardized extracts appears to have been made, and as a consequence, it is difficult to relate, compare, and contrast the results of one study with another. In addition, few bioactivity-based extraction procedures have been employed to determine the relationships between extraction procedures and solvents, chemical constituents, and pharmacological activities. It is not clear as to what extent the various constituents present in *M. oleifera* preparations interrelate through additive, synergistic, and/or inhibitory effects.

Various animal studies have assessed the general safety of extracts, and have demonstrated a very high degree of safety. No adverse effects were reported in a human study conducted with whole leaf powder at up to a single dose of 50 g or in a study using 8 g per day dose for 40 days. A typical dose of an aqueous extract

in rats is approximately 300 mg/kg, which would be equivalent to a dose of about 3.9 g in an 80-kg (176 lb) human. No human studies involving aqueous extracts have been reported, and little information is available regarding the percentage of the powdered whole leaf material that is typically solubilized by extraction with water or alcohol. If 10% of the whole leaf powder is solubilized by aqueous extraction, a 4 g dose of the whole leaf powder would equate to 400 mg of an extract.

The results from published research studies to date with *M. oleifera* are very promising. However, as is usually the case, additional studies are required to address various points raised in the earlier discussion.

Conflict of Interest

The authors have no conflicts of interest to report.

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