

Antiepileptic and antioxidant evaluation of methanol extract of *Moringa oleifera* Lam Seed

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Abstract

The present study was to investigate the antiepileptic and *in vivo* antioxidant effects of methanol seed extract of *Moringa oleifera* Lam (MSEMO). Mice of both sexes, weighing 25-35 g, were randomly divided into five groups of five each. The groups represented by control (distilled water), 0.5 mg/kg phenobarbitone, 100, 200 and 400 mg/kg MSEMO. Treatment was administered to the groups prior to inducement with epilepsy using graded doses of 100, 200 and 400 mg/kg of MSEMO as well as 0.5 mg/kg of phenobarbitone. Epilepsy was induced by passing a current of 50 Amps for 2 sec using an electro-convulsimeter with the aid of two electrodes clipped to the earlobes of the mice and careful observation was carried out to check for the inhibitory effect of the extract. At the end of the three weeks experimental period, the brains of the mice were taken, homogenized and the effects of the extract were tested on nitrite, malondialdehyde (MDA) and endogenous antioxidants. The extract significantly, ($P < 0.05$), showed the most potent protective effect against epilepsy at 200 and 400 mg/kg concentrations. Reduction in catalase level was complimented by 100 and 200 mg/kg of MSEMO. Similarly, that of glutathione peroxidase was complimented by 100, 200 and 400 mg/kg of MSEMO. Superoxide dismutase showed the most potency among the endogenous antioxidants used in the study, with no significant difference, $P > 0.05$, in relation to the control (2.39 ± 0.02). The study showed that MSEMO will be efficacious as an antiepileptic drug.

Keywords: Antiepileptic, antioxidant, methanol extract, *Moringa oleifera*

Introduction

Epilepsy is a chronic disease condition in humans involving seizures. Excessive discharge of neurotransmitters is the cause of this neuropsychological disorder (Muralidharan *et al.*, 2009).

Weakened gamma-amino butyric acid (GABA) inhibition, weakened activation of neurons, and an increase in excitatory neurotransmitters are implicated in the pathophysiology of epilepsy (Kong and Lin 2010).

It has been estimated that a major population of people worldwide live with epilepsy, and that low-income and lower-middle-income countries have more than eighty-five percent (85 %) of this disease (Diop *et al.*, 1996). Meanwhile, the prevalence of epilepsy in the sub-Saharan part of Africa was two or three times more than the rate in developed world (Ngoungou *et al.*, 2007; Ngugi, *et al.*, 2010; Newton and Garcia, 2012).

In the modern era, many synthetic drugs are used as anticonvulsants/antiepileptic drugs. However, there are associated side effects, chronic toxicity, serious drug interactions and adverse effects on cognition and behaviour (Muralidharan *et al.*, 2009). In addition, one patient out of three is resistant to antiepileptic drugs (Malvi *et al.*, 2011). The need for alternative drugs, with lower side effects minimum interaction and improved therapeutic effectiveness, is quite evident.

Possible mechanisms in the manner of development of epilepsy are oxidative and nitrosative stress (REF). Free radical productions play a role in regulating biological function and damage to structures of cell, including the manner of development of neurodegenerative diseases such as epilepsy.

Various studies have shown the role of oxidative stress in the pathogenesis of psychiatric, neurodegenerative disorders and atherosclerosis. Lipid peroxidation induced by free radical is considered as one of the major causes of damage to cell membrane resulting to different pathological conditions. The brain

is highly vulnerable to stress caused by oxidation because of the high content of lipid in it. A balanced redox state between oxidative and reactive conditions is generally experienced by the brain. However, reactive oxygen species (ROS) have deleterious effects when produced in excess and unable to be removed by endogenous antioxidants. The brain is particularly susceptible to stress caused by oxidation because its demand for oxygen is high. The oxygen is needed because oxidative phosphorylation in mitochondrial respiratory chains is the process whereby the brain gets energized (Lohr, 1991; Coyle, and Puttfarcken, 1993; Christen, 2000; Bashkatova, *et al.*, 2003; Erakovic *et al.*, 2003; Patsoukis *et al.*, 2004; Habbu *et al.*, 2008; Menon, *et al.*, 2012).

Moringa oleifera Lam.

Moringa oleifera is the most broadly grown species of a monogeneric family, Moringaceae, which is indigenous to the sub-Himalayan tracts of Bangladesh, India, Pakistan and Afghanistan. The species is commonly referred to horseradish tree, drumstick tree, or Ben oil tree (Fahey, 2005). It is an important food source. Many countries, especially Hawaii, Philippines, India, Pakistan and many parts of Africa use the immature pods, fruits, leaves and flowers of this tree as a highly nutritive vegetable (D'souza and Kulkarni, 1993; Anwar *et al.*, 2005).

In Indian's folklore, reports abound that the root of *M. oleifera* has been used to treat epilepsy (Kalita and Bora, 2008). The root extract of *Moringa oleifera* showed a significant reduction in the various phases of epileptic seizure when compared with the standard, Oxcarbazepine 20 mg/kg, p. o. and this reduction was dose-dependent. Dopamine, serotonin and noradrenaline levels significantly increased in the forebrain region for extract-treated animals. There was an observed significant reduction in the recovery time, a proof that extract of *M. oleifera* roots provides a beneficial effect in controlling MES (Maxima Electro Shock)-induced

seizures (Rajasree *et al.*, 2012). The study aimed to determine the antiepileptic and antioxidant properties of methanol seed extract of *M. oleifera*.

Materials and Methods

Collection and identification of plant material

Fresh samples of *Moringa oleifera* seeds were collected at Evbuotubu Quarters, Benin City, Edo State, Nigeria and were authenticated with the voucher number UBH-M340, in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria.

Extract preparation

M. oleifera seeds were washed and air-dried at room temperature, then further oven-dried at a temperature of 30°C before reducing to powder with the aid of a milling machine. The powder (1200 g) was extracted by cold maceration in 1.4 litres of methanol (analytical grade). After 24 hours, the mixture was filtered and the filtrate was concentrated over a water bath to obtain the paste of methanol seed extract of *M. oleifera* (MSEMO). The extract was preserved in a sample bottle until required for further use.

Preparation of doses

Various concentrations (100, 200 and 400 mg/kg) of MSEMO were prepared by dissolving a weighed portion of the extract in an appropriate volume of distilled water.

Experimental animals

Fifty-five mice (25-35 g) and sixty Wistar rats (150-200 g) were obtained and allowed two weeks acclimatization period in the Animal Unit, Department of Biochemistry, University of Benin, Benin City, Edo State, Nigeria. They were kept in plastic cages, with unrestricted access to food and water. All animals were subjected to experimental protocols in compliance with the ethical standards of the Faculty of Life Sciences.

Experimental design

The mice were randomly distributed into five groups, consisting of five mice each, while the Wistar rats were divided into five groups consisting of six rats respectively. The groups represented control (distilled water), reference standard (0.5 mg/kg phenobarbitone), MSEMO (100, 200 and 400 mg/kg) respectively. Treatment was administered to the groups prior to induction with epilepsy using graded doses of 100, 200 and 400 mg/kg of MSEMO as well as 0.5 mg/kg of phenobarbitone.

Maximal electro shock (MES) model

The procedure was according to Mittal (2009). The ear-lobes of the mice were clipped with an ear-clip electrode and connected to the electro-convulsimeter using an electrical wire. A knob on the electro-convulsimeter, which triggers electricity flow, was pressed for 2 seconds allowing a current of 50 amperes to flow into the brain of the mice; this elicited shock in the mice. The animals were monitored for signs of seizures. Electroshock was given two weeks after treatment administration.

Nitric oxide assay

Greiss reagent was used to indicate nitric oxide production (Green *et al.*, 1982). The following procedure was initiated by adding 500 μ L of brain homogenate to 500 μ L of Greiss reagent (1:1 solution

of 1% sulphanilamide in 5% phosphoric acid and 0.1% naphthalylamine diamine dihydrochloric acid in water). Incubation of the mixture followed for 10 minutes in a cupboard at room temperature in the dark. Measurement of absorbance was then taken at 546 nm. Finally, using a standard concentration for sodium nitrite, calculation of nitrite level was done and units were written as micromoles per milliliter.

Malondialdehyde (MDA) assay

Malondialdehyde was determined using the method specified by Madhava and Sresty (2000).

Following 24 hours incubation, treated blood culture was centrifuged at 3000 g speed for 20 min, and consequently, the supernatants were isolated. A volume of 1300 μ L of R1 was withdrawn from the microcentrifuge tube. Furthermore, a volume of 1 mL of the supernatant was diluted ten times in Tris HCl and 200 μ L. The supernatant was further diluted and an addition of 200 μ L of distilled water and vortexes was made. Furthermore, all the test tubes received an addition of 300 μ L of R2 and vortexes and were incubated at 45°C for 40 mins. Following the incubation process, each tube was chilled in ice and centrifugation was done at 15,000 g speed for 10 mins at a temperature of 4°C. Measurements of all the samples were done in a spectrophotometer at 586 nm.

Determination of The Effects of Endogenous Antioxidants (SOD, Catalase and Glutathione peroxidase)

Superoxide dismutase (SOD) assay

Superoxide dismutase determination was done according to Misra and Fridovich (1972) method. Aliquant mixture of plasma, 0.20 mL of the diluted microsome, was enclosed using 2.5 mL solution of 0.05 M carbonate buffer. The reactions started on addition of 0.3 mL of 0.3 mM adrenaline. While the reference combination was 2.5 mL of 0.05 M carbonate buffer, 0.3 mL of 0.3 mM Adrenaline and 0.20 mL of distilled water. Absorbance was measured after 30 seconds to 150 seconds using wavelength of 480 nm.

Catalase

This was assayed following the protocol by Cohen *et al.* (1970). Tissue homogenate (10 μ L) (100-150 μ g of protein) was added to 2.8 mL solution of potassium phosphate buffer (pH 7.0) of 50 mM concentration in a 3 mL cuvette. Chemical reaction was started by the addition of a solution of 30 mM H_2O_2 having a volume of 0.1 millilitre, prepared freshly, to decomposed rate of hydrogen peroxide that was measured at 240 nm for 300 seconds in spectrophotometer. Molar loss coefficient of $0.041 \text{ M}^{-1}\text{cm}^{-1}$ was utilized by calculating catalase effect in H_2O_2 mole reduced/min/mg/protein.

Glutathione Peroxidase (GPx)

Glutathione peroxidase activity was measured in line with Fatemeh *et al.* (2016) protocol. Volumes of 940 μ L, 890 μ L and 890 μ L of Glutathione peroxidase assay buffer were pipetted into 1 mL cuvette of blank, positive control and sample respectively. The assay buffer temperature in the spectrophotometer was kept at 25 °C with the aid of a cell holder having a thermostat. The reaction initialized by adding a solution of 30 mM *tert*-butyl hydroperoxide with a volume of 10 μ L to the cuvettes and mixed by inversion. Glutathione Peroxidase activity in the sample was then computed with the following formula:

$$\text{Activity per extract (mmol/min/mL} = \text{Units/mL)} \\ = \frac{\Delta A_{340} \times DF}{\text{Volume of sample}}$$

$$6.22 \times V$$

$$\Delta A_{340} = A_{340}/\text{blank}_{\min} - A_{340}/\text{sample}_{\min}$$

6.22 = NADPH constant

DF = sample dilution factor before adding to reaction

V = volume of sample in mL

Results

Phytochemical Screening

Cardiac glycosides, saponin and alkaloids were found in MSEMO, with cardiac glycosides and saponin showing the same degree of abundance (+) while alkaloids, which were relatively more abundant (++) , exceeded both (Table 1).

Table 1: Phytochemical screening of methanol seed extract of *M. oleifera* (MSEMO)

Parameters
Flavonoids
Tannins
Cardiac glycosides
Terpenoids
Phenols
Steroids
Saponins
Alkaloids

Key: + = present; ++ = highly present; - = not detected

Maximal Electro Shock-Induced Convulsion

The effects of methanol seed extract of *M. oleifera* on Maximal Electro Shock (MES)-induced seizure in mice are shown in Table 2. The time for the onset of jerks, seizure, percentage inhibition against the onset of jerks and percentage inhibition against seizure for the control group, were 0.1 ± 0.00 s, 1.05 ± 0.05 s, 0 and 0 % respectively. Also, the group treated with 100 mg/kg methanol seed extract of *M. oleifera* (MSEMO) had 0.17 ± 0.17 s and 0.75 ± 0.00 s as their onset of jerks and seizure, respectively; percentage inhibition against onset of jerks and seizure were -70 and 28.6 % respectively. There was no occurrence of seizure in the 200 and 400 mg/kg MSEMO; a 100 % inhibition against seizure.

Table 2: Effect of methanol seed extract of *M. oleifera* (MSEMO) on maximal electro shock (MES)-induced convulsions in mice

Treatment	Onset of jerks (sec)	% Inhibition Against Onset of jerks	Seizure duration (sec)	% Inhibition Against Seizure
Control	0.10 ± 0.00	0	1.05 ± 0.05	0
Phenobarbitone	-	100	-	100
100 mg/kg MSEMO	0.17 ± 0.17	-70	0.75 ± 0.00	28.57
200 mg/kg MSEMO	-	100	-	100
400 mg/kg MSEMO	-	100	-	100

MSEMO = Methanol Seed Extract of *Moringa oleifera*; Result for onset of jerks and seizure were given as Mean \pm SEM, (n = 5); - = nil.

Comparative analyses of the effects of 100, 200 and 400 mg/kg methanol seed extract of *M. oleifera* (MSEMO) on nitrite, malondialdehyde and endogenous antioxidants in experimental mice

Table 3 shows the comparative analysis of 100, 200, and 400 mg/kg MSEMO on nitrite, malondialdehyde (MDA) and endogenous antioxidants in experimental mice.

Nitrite

The administration of 100, 200, and 400 mg/kg MSEMO significantly, $P < 0.05$, increased nitrite levels with values of 4.93 ± 0.16 , 4.59 ± 0.14 and 3.91 ± 0.53 compared to the control group, which had a value of 2.26 ± 0.01 .

Malondialdehyde (MDA)

Upon treatment with 100 and 200 mg/kg MSEMO, there was a non-significant, ($P > 0.05$) increase in MDA level and a non-significant, ($P > 0.05$) decrease in the same parameter was observed upon treatment with 400 mg/kg of the same extract compared with the control. The values were 1.84 ± 0.04 , 1.83 ± 0.01 and 1.62 ± 0.08 for 100, 200 and 400 mg/kg MSEMO respectively, while that of the control was 1.71 ± 0.00 .

Catalase +

Upon treatment of the mice with 100 mg/kg of MSEMO, there was a significant, $P < 0.05$, decrease in catalase level, with 40.16 ± 0.03 as the value for MSEMO against the value for the control group which was 45.61 ± 0.00 . Also, there was a significant, $P < 0.05$, decrease in catalase level upon treatment with 200 mg/kg MSEMO against the control group and the value was 39.92 ± 0.02 . However, 400 mg/kg MSEMO had a non-significant, $P > 0.05$, decrease with the value: 43.57 ± 1.69 against the control (Table 3).

Glutathione Peroxidase (GPx)

Upon administration of 100, 200 and 400 mg/kg of MSEMO, there was a significant reduction ($P < 0.05$) in GPx level compared to the control and the values were 36.94 ± 0.02 , 36.75 ± 0.02 and 42.24 ± 2.81 for 100, 200 and 400 mg/kg of MSEMO respectively while that of the control was 53.56 ± 0.03 (Table 3).

Superoxide Dismutase (SOD)

There was no significant difference, $P > 0.05$ in the Superoxide Dismutase (SOD) of the treatment groups of mice upon treatment with 100, 200 and 400 mg/kg of MSEMO.

Table 3: Comparative analysis of the activities of 100, 200 and 400 mg/kg of methanol seed extract of *M. oleifera* (MSEMO) on nitrite, malondialdehyde and endogenous antioxidants of mice

Par	Ctrl	Phen	100 mg/kg MSEMO	200 mg/kg MSEMO	400 mg/kg MSEMO
NIT (mg/L)	2.26±0.01 ^a	2.25±0.08 ^a	4.93±0.16 ^c	4.59±0.14 ^c	3.91±0.53 ^c
MDA (×10 ⁻³ µmole/L)	1.71±0.00 ^a	1.69±0.04 ^a	1.84±0.04 ^a	1.83±0.01 ^a	1.62±0.08 ^a
CAT (U/mL)	45.61±0.00 ^a	45.43±0.16 ^a	40.16±0.03 ^b	39.92±0.02 ^b	43.57±1.69 ^a
GPx (U/mL)	53.56±0.03 ^a	53.57±0.02 ^a	36.94±0.02 ^d	36.75±0.02 ^d	42.24±2.81 ^d
SOD (U/mL)	2.39±0.02 ^a	2.44±0.04 ^a	2.17±0.07 ^a	2.23±0.06 ^a	2.38±0.11 ^a

Values are mean ± SEM, (n = 5); Par = Parameter; Ctrl = Control; Phen = Phenobarbitone; NIT = Nitrite; MDA= Malondialdehyde; CAT= Catalase; GPx= Glutathione Peroxidase; SOD= Superoxide dismutase. Data with same alphabet (s) are insignificantly (P> 0.05) different from each other in accordance with Dunnett's multiple comparison test.

Discussion

From the present study, alkaloids and saponins present in MSEMO, especially at 400 mg/kg of the extract, may be responsible for the 100 % inhibition against jerk and seizure due to the extract's antioxidant activities in the mice's brain. This corroborates an earlier study by Yang and Stöckigt (2010) that alkaloids provide pharmacological activities for human therapeutic drugs including antioxidant compounds, antitumoral drugs, analgesics, anti-inflammatories and stimulants. Furthermore, it also aligns with the report given by Sayyah *et al.* (2004) and Tatiya *et al.* (2007), that the significant ameliorating properties of saponins are partly due to the antioxidant activity which inhibits the formation of reactive oxygen species (ROS). More so, the synergistic effect of alkaloids and saponins undoubtedly increased the antioxidant capability of the extract in line with an earlier report by Liu (2004) and Dahlen *et al.* (2014) which stated that the additive and synergistic effects of phytochemicals in fruits and vegetables could be responsible for their potent antioxidant activities.

The present study showed that 400 mg/kg MSEMO-treated mice subjected to MES, had a significant, (P< 0.05) and a non-significant decrease, (P> 0.05) in GPx and MDA levels respectively. A significant increase, P< 0.05, was also seen in nitrite level. These account for the reason why 400 mg/kg of MSEMO-treated mice showed 100 % inhibition against seizure. These findings agree with the study carried out by Rajasree *et al.* (2012) which proved that extract of *Moringa oleifera* roots has a beneficial effect in controlling MES-induced seizures. They also align with the study carried out by Menon *et al.* (2012) on humans, who demonstrated that MDA and PC (Protein Carbonylation) levels were significantly, (P< 0.001) higher in patients with epilepsy than in the control group.

The most commonly used marker of oxidative damage is malondialdehyde (MDA) (Mateos *et al.*, 2005; Valko *et al.*, 2007; Chang and Yu, 2010; Kong and Lin 2010). MDA, which is the end product of lipid peroxidation and the main secondary oxidation product of polyunsaturated fatty acids, is mutagenic, carcinogenic and genotoxic. In several diseases related to free-radical damage, the levels of this compound rise (Mateos *et al.*, 2005; Valko *et al.*, 2007). Brain tissue is particularly vulnerable to free-radical damage for several reasons and the extent of damage corresponds to the amount of free radicals formed (Nilüfer *et al.*, 2016).

Nitrite

Nitric oxide (NO) is a diatomic free radical whose lifespan is very short in biological systems (less than 1 second in circulating blood). Nitrite is a major intravascular store for nitric oxide. The endogenous production of NO by NOS has been established as playing an important role in vascular homeostasis, neurotransmission, and host defense mechanisms. The stepwise oxidation to nitrite and nitrate is the major pathway for NO metabolism (Moncada *et al.*, 1991; Kelm, 1999; Bryan and Grisham, 2007). The presence of nitrite in living tissues shows the result of the metabolism of oxidized nitrogen species in organisms. The molecular weight of nitrite anions is low and its diffusive transport in water is rapid but is unable to cross lipid cell membranes. Hence, nitrite is presently considered to act as a hypoxic buffer and is helpful in the maintaining vasodilation and mitochondrial respiration under hypoxic conditions. Its importance emanates from the rich supply of nitrite in tissues and blood circulation. A fast growing body of clinical data affirms that nitrite is protective against ischemic damage to vascular and other tissues. Here, nitrite's beneficial effect appears to be mediated by the release of free-NO radicals or NO-like species under hypoxic condition (Faassen *et al.*, 2007).

Conclusion

Epilepsy has been an issue of health concern affecting people of all age categories. Already available synthetic drugs have not completely proffered lasting solutions for this ailment, and their side effects further aggravate the condition; thus, more research is needed into alternative sources of medicine. The study showed that the seed extract of *M. oleifera* is a potential source of efficacious antiepileptic drugs. The activities are dose-dependent, with the highest dose (400 mg/kg) giving the most performance. However, further studies are encouraged to determine the safety and mechanism of action of the extract.

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