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# Evaluation of the analgesic, anti-inflammatory, anti-oxidant, phytochemical and toxicological properties of the methanolic leaf extract of commercially processed *Moringa oleifera* in some laboratory animals

## Abstract

**Background:** *Moringa oleifera* Lam (Moringaceae) is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value.
**Methods:** The commercially processed *M. oleifera* was extracted using methanol as its solvent. Phytochemical analysis as well as the anti-oxidant properties of this supplement were also investigated. Acute toxicity was carried out in fasted mice. Carrageenan and histamine tests were used to assess anti-inflammatory effects in rats, while analgesic activities were assessed using the acetic acid-induced writhing test and formalin-induced paw lick test in mice. In the anti-oxidant tests, 1,1-diphenyl-2-picrylhydrazyl, ferrous reducing activity power, $2,2^1$-azino-bis-(3-ethylbenthialozine)-6-sulphonic acid and total polyphenolic (TPP) assays were deployed at concentrations of 10 mg/mL and 20 mg/mL.
**Results:** The phytochemical analysis showed that the extract contained flavonoids, terpenoids, glycosides, tannins and saponins. In the acetic acid-induced writhing test, the extract significantly reduced the number of writhes at 100 and 200 mg/kg but not so much at 50 mg/kg. In the formalin-induced paw lick test, the effect was similar to that of the acetic writhing test. The analgesic effects were comparable to that of indomethacin used at 10 mg/kg. In the anti-inflammatory test, the extract reduced the formation of oedema especially at a dose of 200 mg/kg. In the anti-oxidant test, the extract was found to possess a free radical-scavenging property and is concentration related.
**Conclusions:** The use of this extract for medicinal and nutritional purposes may have thus been justified; however, caution must be exercised in its use to prevent the toxic effect.

## Introduction

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants [1]. It is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process [2]. The classical signs of acute inflammation are pain, heat, redness, swelling and loss of function [3]. Thus, any inflammatory studies must also consist of the analgesic effect. Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases. The conventional drugs used to ameliorate this phenomenon are either too expensive, toxic and not commonly available to the rural people who constitute the major populace of the world [4–6].

A chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent is called oxidation which can produce free radicals which in turn can start chain reactions. When the chain reaction occurs in

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a cell, it can cause damage or death to the cell. The role of anti-oxidants is to terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves; hence, anti-oxidants are often reducing agents, such as thiols, ascorbic acid or polyphenols [7, 8]. Plants and animals maintain complex systems of multiple types of anti-oxidants, such as glutathione, vitamin C, vitamin A and vitamin E, as well as enzymes such as catalase, superoxide dismutase and various peroxidases [8]. Insufficient levels of anti-oxidants, or inhibition of the anti-oxidant enzymes, cause oxidative stress and may damage or kill cells. Oxidative stress seems to play a significant role in many human diseases, including cancers [9], and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases [10], as well as inflammation and problems caused by cell and cutaneous ageing [11]. Accordingly, attention is focused on the protective biochemical functions of naturally occurring anti-oxidants in the cells of the organisms containing them [12]. The use of anti-oxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. For these reasons, oxidative stress can be considered to be both the cause and the consequence of some diseases. Anti-oxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness.

*Moringa oleifera* Lam (Moringaceae) is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics [13]. *Moringa oleifera* is the most widely cultivated species of the monogeneric family Moringaceae (order Brassicales), which includes 13 species of trees and shrubs distributed in sub-Himalayan ranges of India, Sri Lanka, North-eastern and Southwestern Africa, Madagascar and Arabia [14]. It is a bush of the African savannah that is used in folk medicine for the treatment of rheumatic and articular pain. The whole plant is believed to possess medicinal properties. It is used to treat ascites, rheumatism, venomous bites and pneumonia [15]. Flowers and young leaves are also consumed for nourishment. The methanolic extracts of the *M. oleifera* root can act as a central nervous system depressant [16], and the aqueous root extracts have been shown to possess some anti-infertility properties in rats [17]. This plant has been well documented for its medicinal importance for a long time. The stem bark, root bark,

fruit, flowers, leaves, seeds and gum are widely used in Indian folk medicine. The pods and seeds are tastier while they are young and before they turn brown. In Malaysia, the young tender pods are cut into small pieces and added to curries [18].

In Nigeria, it is very common to find the processed plant being sold freely in the open market as food supplements with acclaimed medicinal effects. It is for this reason one of such products sold as Bari-Frank Moringa is being assessed for its pharmacological properties so as to validate its claim in this wise.

# Materials and methods

### Extract preparation

A commercially prepared *M. oleifera* food supplement containing powdered leaves and marketed by Bari-Frank Moringa Nigeria Limited was used in this study. They were packaged in containers with each container weighing 153.91 g of leaves, which were dissolved in 1 L of methanol and shaken vigorously. The sample was then filtered after 3 days using a Buckner funnel and Wartman No. 1 filter paper. The filtrated sample was exposed to atmospheric air to evaporate the methanol. The extract was further concentrated using a water bath. The weight of the extract was 14.7 g. The graded solutions of the extract were prepared and used for the experiments.

### Experimental animals

Eighty healthy female white Wister strain albino rats (100–160 g) and seventy female mice (20–30 g) bred in the experimental animal house of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria, were used for the study. The animals were kept in cages within the animal house and allowed free access to water and standard livestock pellets. They were examined and found to be free of wounds, swellings and infections before the commencement of the experiment. All experimental procedures were in conformity with the University of Ibadan Ethics Committee on Research in Animals (ADE/FVM/2013A) as well as internationally accepted principles for laboratory animal upkeep and use.

### Chemicals and drugs

Chemicals used include carrageenan, acetic acid and formalin, all from Sigma-Aldrich Chemie Gmbh (Steinheim, Germany). Other chemicals were acetate buffer, hydrochloric acid, gallic acid, ascorbic acid, 2,2$^t$-azinobis-(3-ethylbenthialozine)-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri[2-pyridyl]-s-triazine (TPTZ) and ferric acid (Sigma-Aldrich, St. Louis, MO, USA). The standard drugs used were ibuprofen and histamine, which were also purchased from Sigma-Aldrich Chemie GmbH. All the chemicals and drugs used were of analytical grade.

## Phytochemical screening

The phytochemical analysis was performed on the food supplement for the identification of the constituents. The constituents tested for were alkaloids, tannins, saponins, anthraquinones, cardiac glycosides and flavonoids as described by Shale et al. [19], Moody et al. [20] and Sawadogo et al. [21].

## Acute toxicity test

The acute toxicity of the food supplement extract was determined in mice according to the method of Hilaly et al. [22] with slight modifications. Mice fasted for 16 h were randomly divided into six groups with five mice per group. Graded doses of the plant's extract (100, 200, 400, 800 and 1600 mg/kg per os) and control (3 mL/kg distilled water) were separately administered to the mice in each of the groups by means of a bulbed steel needle. All the mice in the groups were then allowed free access to food and water and observed over a period of 48 h for signs of acute toxicity. The number of deaths within this period of time was recorded.

## Anti-inflammatory activities

**Carrageenan-induced paw oedema in rats:** The rats were pretreated with intraperitoneal injection of normal saline 3 mL/kg body weight, indomethacin 10 mg/kg body weight and Moringa leaf extract at doses 50, 100 and 200 mg/kg body weight; after an hour, an injection of 0.1 mL of 1% carrageenan was administered into the right hind paw of the rats under the subplanter aponeurosis to induce pedal inflammation according to the method described by Moody et al. [20], Sawadogo et al. [21] and Gupta et al. [23]. Increases in the linear diameter of the paw were taken as an indication of oedema which signifies inflammation. The level of inflammation induced by the carrageenan injection was determined by measuring the diameter of the paw at the 0 h, 1 h, 2 h and 3 h after the administration of carrageenan using a micrometer screw gauge. The anti-inflammatory effect of the extract and reference drug was calculated from the formula

$$\% \text{ Inhibition} = D_0 - D_t / D_0 \times 100$$

where $D_0$ was the average inflammation, i.e., mean paw size, of the control group, and $D_t$ was the average inflammation, i.e., mean paw size, of the drug-treated groups (indomethacin and plant extract).

**Histamine-induced pedal oedema in rats:** Paw oedema was induced by the administration of 0.1 mL of 0.1% freshly prepared histamine into the subplantar region of the right hind paw of the rats; the animals were previously treated with normal saline 3 mL/kg in the vehicle control group, indomethacin 10 mg/kg in the reference group and plant extract at doses 50, 100 and 200 mg/kg body weight. These drugs were all administered intraperitoneally an hour before the injection of histamine. Oedema was taken as a sign of inflammation and paw oedema was measured using a micrometer screw gauge at 0 h, 1 h, 2 h and 3 h after the administration of histamine. This is in accordance with the method described by Perianayagam et al. [24]. The percentage inhibition was calculated using the formula

$$\% \text{ Inhibition} = D_0 - D_t / D_0 \times 100$$

where $D_0$ was the average inflammation, i.e., mean paw size, of the control group and $D_t$ was the average inflammation, i.e., mean paw size, of the drug-treated groups (indomethacin and plant extract).

## Analgesic activities

**Acetic acid writhing test in mice:** To evaluate the analgesic effects of the plant extract, the method described by Dharmasiri et al. [5] was used with slight modifications. Five groups (A, B, C, D, E) of four mice each were treated orally with normal saline 3 mL/kg body weight, indomethacin 10 mg/kg body weight and plant extract at doses 50, 100 and 200 mg/kg body weight. Thirty minutes later, 0.6% acetic acid (10 mg/kg) solution was injected intraperitoneally to all the animals in the different groups. The number of writhes (abdominal constrictions) occurring between 5 and 20 min after acetic acid injection was counted. A significant reduction in the number of writhes in tested animals compared to those in the control group was considered as an antinociceptive response.

The percentage inhibition of the writhing response was calculated using the formula

$$\% \text{ Inhibition} = D_0 - D_t / D_0 \times 100$$

where $D_0$ was the average writhing response of the control group and $D_t$ was the average writhing response of the drug-treated groups (indomethacin and plant extract).

**Formalin paw lick test in mice:** The formalin paw lick test was conducted as described by Dharmasiri et al. [5]. The mice in groups (four per group) were treated with 3 mL/kg normal saline (control group), indomethacin 10 mg/kg (reference group) and 50, 100, 200 mg/kg body weight of the plant extract. These were administered orally. After 30 min, the mice were injected with 0.05 mL of 2.5% formalin into the right hind foot pad and immediately placed in a cage where they can be observed easily. The licking time and frequency of the injected paw were recorded for 30 min [25].

The percentage inhibition of the paw lick response was calculated using the formula

$$\% \text{ Inhibition} = D_0 - D_t / D_0 \times 100$$

where $D_0$ was the average paw lick response of the control group and $D_t$ was the average paw lick response of the drug-treated groups (indomethacin and plant extract). A significant reduction in the number of licks in the treated animals compared to the control group was taken into account as an anti-pain response.

## Anti-oxidant studies

**DPPH radical-scavenging assay:** DPPH (24 mg) was dissolved in 100 mL of methanol and stored at −20 °C as the stock solution. Twenty millilitres of stock was added to 90 mL of methanol and taken as the working solution. Then, 2850 μL of this working solution was mixed with 300 μL of extract (10 and 20 mg/mL), and left for 30 min at room temperature. The absorbance of the mixture was measured using a spectrophotometer at 515 nm. Ascorbic acid was

used as reference [26]. The ability to scavenge DPPH radical was calculated using the following equation based on the calibration curve: $y=1E-04x$, $R^2=0.987$, where $x$ was the absorbance and $y$ was the ascorbic acid equivalent.

**Determination of total polyphenolics:** Total phenol contents were determined by the modified Folin-Ciocalteu method [27]. Folin was diluted with distilled water in the ratio 1:10, and 7.5 g of sodium bicarbonate was dissolved in 100 mL of distilled water. One hundred microlitres of extract at 10 and 20 mg/kg was mixed with 0.5 mL of folin; after 5 min, 0.4 mL of $Na_2CO_3$ was added. The tubes were allowed to stand for 2 h at room temperature for colour development. Absorbance was then measured spectrophotometrically at 760 nm. Results were expressed in μmol/L and compared with gallic acid.

**Ferrous reducing activity power (FRAP) assay:** The Benzie and Strain [28] method was adopted with slight modifications for this assay. The stock solutions included 300 mM acetate buffer (prepared), pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3·6H_2O$ solution. The stock solution was prepared by mixing 225 mL acetate buffer, 0.625 g of TPTZ in 20 mL of (0.3 mL of HCl in 250 mL of distilled water), 0.108 g of $FeCl_3$ in 20 mL of (0.3 mL of HCl in 250 mL of distilled water). One hundred microlitres of plant extract (10 and 20 mg/kg) was allowed to react with 300 μL of the FRAP solution and incubated at 37 °C in an oven for 30 min. Readings were taken with the spectrophotometer at 593 nm. Results were calculated and expressed in μmol/L and compared with ascorbic acid.

**ABTS radical-scavenging assay:** The method described by Re et al. [29] was followed. The stock solution was prepared by mixing equal quantities of ABTS and potassium per sulphate ($K_2S_2O_8$) in 40 mL of distilled water. One hundred microlitres of this solution was added to 39 mL of methanol. Two hundred microlitres of plant extract (10 and 20 mg) was mixed with 2400 μL of ABTS, incubated for 15 min at room temperature and read with the spectrophotometer at 734 nm. Results were calculated and expressed in% and compared with gallic acid.

## Statistical analysis

The data generated were presented as mean±SD; statistical analysis was carried out by using GraphPad Prism 5. The results were compared in percentile.

# Results

In the acute toxicity test, no death or abnormal behaviour was observed in group A – the vehicle control group (normal saline 3 mL/kg). In groups B, C and D, i.e., 200, 400 and 800 mg/kg, the animals were slightly dull few minutes after administration with improvement after an hour; the animals were fully recovered and very active 48 h after administration. No death was recorded at these doses. In group E, i.e., 1600 mg/kg, the animals showed

signs of dullness and severe lethargy. Two out of the five animals died on administration. The surviving animals were slightly recovered after 48 h. In group F, i.e., 3200 mg/kg, the animals showed severe signs of lethargy, with rough hair coat. Four out of the five animals died within 24 h after administration. The surviving animal still did not recover after 48 h.

The results of the anti-inflammatory and analgesic activities of the methanol extract of *M. oleifera* food supplement are presented in Tables 1–4. Using the carrageenan method, the effect of the extract at 100 mg/kg and that of the reference drug were pronounced at 1 h after carrageenan injection, while that of 50 mg/kg was highest at 2 h after carrageenan injection and that of 200 mg/kg was pronounced at 3 h after carrageenan administration. The extract showed its most pronounced effect at 200 mg/kg, 3 h after the injection of carrageenan. The effect of the extract at 50 mg/kg and that of the reference drug were similar at 1 h after the administration of carrageenan (Table 1).

**Table 1:** Anti-inflammatory activities of the methanolic extract of *Moringa oleifera* and indomethacin on carrageenan-induced oedema in the right hind limb of rats.

| Time | Control | Extract, mg/kg | | | Indomethacin |
|---|---|---|---|---|---|
| | | 50 mg | 100 mg | 200 mg | |
| 1 | 27.7±1.5 (0) | 26.5±2.5 (4.2) | 24.8±1.3 (10.6) | 22.5±1.3 (18.6) | 26.5±2.5 (4.2) |
| 2 | 28.0±1.0 (0) | 25.5±1.7 (8.9) | 25.8±1.7 (8.0) | 22.5±3.0 (20.5) | 27.3±1.2 (2.68) |
| 3 | 26.3±2.5 (0) | 24.3±2.2 (7.8) | 24.8±1.5 (5.9) | 20±0.8 (23.9) | 25.3±2.5 (4.0) |

Data in mean±SD; n=4. Percentage inhibition of the carrageenan-induced inflammation produced by test extract and indomethacin are indicated in parentheses. Measurement was in mm.

**Table 2:** Anti-inflammatory activities of the methanolic extract of *Moringa oleifera* and indomethacin on histamine-induced oedema in the right hind limb of rats.

| Time | Control | Extract, mg/kg | | | Indomethacin |
|---|---|---|---|---|---|
| | | 50 mg | 100 mg | 200 mg | |
| 1 | 28±1.0 (0) | 27.8±3.3 (0.9) | 24.5±1.7 (12.5) | 23.3±3.2 (16.9) | 25.8±3.8 (9.1) |
| 2 | 31.3±1.5 (0) | 26±1.6 (17.1) | 23.0±2.0 (22.6) | 22.0±2.2 (29.8) | 24.3±1.7 (22.6) |
| 3 | 29.0±1.0 (0) | 23.3±2.2 (19.8) | 21.8±2.1 (25.0) | 20.5±1.0 (29.3) | 23.3±2.1 (19.8) |

Data in mean±SD; n=4. Percentage inhibition of the histamine-induced inflammation produced by test extract and indomethacin are indicated in parentheses. Measurement was in mm.

**Table 3:** Analgesic effect of the methanolic extract of *Moringa oleifera* leaf and indomethacin using acetic acid induced writhing response.

| Parameters | Control | Extract, mg/kg | | | Indomethacin |
|---|---|---|---|---|---|
| | | **50 mg** | **100 mg** | **200 mg** | |
| No. of writhes/20 min | 46.3±21.8 | 9.8±7.0 | 7.5±4.7 | 4.5±4.4 | 8±5.9 |
| % Inhibition | 0 | 78.9 | 83.8 | 90.3 | 82.2 |

Data in mean±SD; n=4. Percentage inhibition of the writhing response induced by acetic acid produced by test extract and indomethacin are indicated.

**Table 4:** Analgesic effect of the methanolic extract of *Moringa oleifera* leaf and indomethacin using the formalin-induced paw lick test in mice.

| Parameters | Control | Extract, mg/kg | | | Indomethacin |
|---|---|---|---|---|---|
| | | **50 mg** | **100 mg** | **200 mg** | |
| No of licks | 23.8±9.8 | 22.3±3.6 | 14±2.4 | 12.5±3.5 | 16±4.1 |
| % Inhibition | 0 | 6.3 | 41.1 | 47.4 | 32.6 |

Data in mean±SD; n=4. Percentage inhibition of the paw lick response induced by formalin produced by test extract and indomethacin are indicated.

The effect of the extract at 200 mg/kg and reference drug on paw oedema induced by histamine was highest at 2 h after the administration of histamine, while the 50 and 100 mg/kg doses of the extract exhibited pronounced activity at 3 h after injection of histamine. The highest effect of the extract was seen at 200 mg/kg after 2 h of histamine injection. The effect of the extract at 100 mg and that of the reference drug were also similar at 2 h after histamine injection. The anti-histaminic effect of the extract increased with the increase in the dose of the extract, i.e., dose dependent (Table 2).

The methanolic extract of *M. oleifera* and indomethacin significantly reduced the number of writhes when compared to the control group. The extract at 50, 100 and 200 mg/kg and indomethacin at 10 mg/kg exhibited a high analgesic effect at 78.9%, 83.8%, 90.3% and 82.2%, respectively, indicating that the extract (100 and 200 g/kg) has a higher analgesic effect than indomethacin, the reference drug used in this study (Table 3).

The administration of the methanolic extract of *M. oleifera* at 50, 100 and 200 mg/kg and indomethacin at 10 mg/kg body weight caused a reduction in the licking time and frequency of the paw injected with formalin of the rats. The most pronounced effect was seen at 200 mg/kg of the extract (Table 4).

In Table 5, the anti-oxidant properties of the aqueous leaf extract of the plant are shown. The anti-oxidant ability of the extract was found to be dose dependent as the anti-oxidant effectiveness was higher at 20 mg than at 10 mg.

**Table 5:** Anti-oxidant properties of the methanolic extract of *Moringa oleifera* supplement using ABTS, FRAP, DPPH and TPP assays.

| Extracts | ABTS | DPPH | FRAP, AAE μmol/L | TPP, GAE μmol/L |
|---|---|---|---|---|
| 10 mg | 80% | 14.0% | 17.54 | 16.2 |
| 20 mg | 86.34% | 49% | 87.72 | 17.2 |

AAE, ascorbic acid equivalent; GAE, gallic acid equivalent.

In Table 6, the phytochemical screening of the extract shows that it contained tannin, saponin, flavonoids, terpenoids and cardiac glycosides.

## Discussion

The outcome of the acute toxicity test carried out revealed that 200, 400 and 800 mg/kg doses of the extract are harmless for oral therapeutic use, as there were no adverse signs seen at these doses. However at doses 1600 and 3200 mg/kg, the animals showed severe lethargic signs. This might suggest that at a dose of 1600 mg/kg and above, the food supplement may be toxic, signifying that care must be taken in its use for this purpose. In the acute toxicity test of the aqueous extract of this plant, death was recorded in 1600 and 2000 mg/kg dose groups [30]. This therefore agreed with the findings from this study that at doses 1600 mg/kg and above, a lot of caution should be exercised in the use of the plant for both medicinal and nutritional purposes.

**Table 6:** Preliminary phytochemical screening of the methanol extract of *Moringa oleifera*.

| Test | Observation | Inference |
|---|---|---|
| Test for tannins:<br>  0.5 g of powdered sample+boil in 20 mL distilled water, filtered+few drops of 0.1% $FeCl_3$ | A blue black colouration was observed in the test tube | Tannin is present |
| Test for saponins:<br>  2 g of powdered sample+boil in 20 mL distilled water, filtered. 10 mL fitrate+5 mL distilled water+vigorous shaking+3 drops of olive oil | Formation of froth and the presence of emulsion after the addition of olive oil | Saponin is present |
| Test for flavonoids:<br>  Aqueous extract+few drops of 1% $NH_3$ | A yellow colouration is observed | Flavonoid is present |
| Test for terpenoids:<br>  5 mL of aqueous extract+2 mL $CHCl_3$+3 mL conc. $H_2SO_4$ | An interface of a reddish brown colouration is observed | Terpenoid is present |
| Test for glycosides:<br>  5 mL of aqueous extract+2 mL glacial acid $CH_3CO_2H$+1 drop $FeCl_3$+1 mL conc. $H_2SO_4$ | A brown ring appears | Cardiac glycoside is present |
| Test for alkaloids:<br>  1 g of methanolic extract+5 mL of 2 M HCl+few drops of Meyers and Wagner's reagent | Turbidity is not formed, precipitate not observed | Alkaloid is absent |

The processed leaf was found to contain tannin, saponin, flavonoids, terpenoids and cardiac glycosides which all have medicinal properties. Flavonoids and tannins are phenolic compounds and plant phenolics are also a major group of compounds that act as primary anti-oxidant or free radical scavengers [31–33]. Tannins and saponins are also found to be effective anti-oxidants, anti-microbial and anti-carcinogenic agents [34]. Flavonoids are known to target prostaglandins which are observed in the late phase of acute inflammation and pain perception [35]. Hence, flavonoids, tannins and saponins are contributory to the analgesic, anti-inflammatory and anti-oxidant activities of the methanolic leaf extract of the plant. Non-steroidal anti-inflammatory drugs such as indomethacin act by reduction of sensitization of pain receptors caused by prostaglandins at the inflammation site [36]. Inflammation in this study was induced with carrageenan and histamine. Carrageenan-induced oedema involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, especially the E series, histamine, bradykinins, leukotrienes and serotonin, all of which also cause pain and fever [37]. Inhibitions of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorate the inflammation and other symptoms. This study has shown that the methanolic extract of the processed *M. oleifera* leaf possesses a significant anti-oedematogenic effect on paw oedema induced by carrageenan and histamine. Development of oedema induced by carrageenan is commonly correlated with an early exudative stage of inflammation [38]. Carrageenan oedema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be biphasic. The first phase (1 h) involves the release of serotonin and histamine, while the second phase (over 1 h) is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins [24, 38]. Since the carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation [21], the results of this study are indications that *M. oleifera* are effective in treating acute inflammatory disorders. The extract also reduced the histamine-induced oedema. The results tend to suggest that the anti-inflammatory activity of the extract is possibly backed by its anti-histamine activity. Histamine is an important inflammation mediator, a potent vasodilator which increases vascular permeability [39]. Since the extract effectively suppressed the oedema produced by histamine, it exhibited anti-inflammatory actions by inhibiting the synthesis, release or action of inflammatory mediators such as histamine, serotonin and prostaglandins.

With respect to the acetic acid-induced abdominal writhing, the result showed that all the doses produced a significant analgesic effect. This could be partly attributed to its anti-inflammatory effect as, in the visceral pain model, the processor releases arachidonic acid via the cyclooxygenase and prostaglandin biosynthesis which plays a role in the nociceptive mechanism [21]. The abdominal constriction induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors [35]. Therefore, an anti-inflammatory substance may also be involved in the peripheral analgesic activity,

because the inhibition of the acute inflammation by this extract leads to its inhibitory effect on pain development. In this study, the reference drug gave an 82.2% inhibition of writhing in the animals, while the extract gave a 78.9% inhibition of writhing at 50 mg/kg, an 83.8% inhibition of writhing at 100 mg/kg and a 90.3% inhibition of writhing at 200 mg/kg. These show that its effect is comparable to that of the reference drug with the extract exhibiting a higher analgesic effect than the reference drug at 100 and 200 mg/kg.

The result of the formalin test in this study showed that the 100 and 200 mg/kg doses of the extract produced 41.1% and 47.4% inhibition compared to the reference drug (indomethacin) at 32.6% inhibition (Table 4). It thus showed that the extract at these two doses is more effective than the reference drug which in turn had a greater inhibitory effect than the 50 mg/kg dose (6.3% inhibition). In the formalin test, the pain in the early phase was due to the direct stimulation of the sensory nerve fibres by formalin, while the pain in the late phase was due to inflammatory mediators, such as histamine, prostaglandins, serotonin and bradykinins [5]. The formalin test has been described as a convenient method for producing and quantifying pain in rats [40]. In the test, adequate painful stimulus is employed with the animals showing a spontaneous response. It is sensitive to commonly used analgesics. Intraplantar injection of 2.5% formalin evoked a characteristic licking response in the Wistar rats. The pain stimulus is rather a continuous transient one, having a resemblance to some kind of clinical pain, and observations are made on animals which are restrained only lightly or not at all [25, 41].

With respect to the anti-oxidant properties of this food supplement, it was found that the ABTS showed higher free radical-scavenging activities than DPPH at the two concentrations used. The ABTS and DPPH assays measure the ability of anti-oxidants to quench a radical cation, while the FRAP assay evaluates the reducing potential of the samples [42]. The principle of DPPH assay is based on the reduction of the purple coloured methanolic DPPH solution in the presence of hydrogen donating anti-oxidants by the formation of yellow coloured diphenyl-picryl hydrazine. In the case of ABTS, the principle is based on the ability of the assay to inhibit the absorbance of radical cation, ABTS$^+$, by anti-oxidants at a characteristic wavelength of 734 nm. The principle behind the technique involves the reaction between the ABTS and potassium per sulfate to produce the radical which is a blue green chromogen [43]. In the study, the food supplements exhibited anti-oxidant properties by inhibiting and scavenging for free radicals as shown by the 80% and 86.3%

performance by the 10 and 20 mg concentrations on ABTS. This effect however is not as pronounced for DPPH. The extract also exhibited anti-oxidant properties as shown by the FRAP and TPP tests.

The anti-oxidant properties exhibited by this extract may be attributed to the presence of polyphenolics contained therein. Phytochemical analysis showed that the extract contained tannin, saponin, flavonoids, terpenoids and cardiac glycosides which all have medicinal properties. Flavonoids and tannins are phenolic compounds and plant phenolics are also a major group of compounds that act as primary anti-oxidant or free radical scavengers [31–33]. Tannins and saponins are also found to be effective anti-oxidants, antimicrobial and anti-carcinogenic agents [34]. Phenolics constitute one of the major groups of compounds which act as primary antioxidants [42, 44]. In fact, phenolic compounds are very important plant constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals [45]. This activity is believed to be mainly due to their redox properties [46], which play an important role in adsorbing and neutralizing free radicals, quenching singlet or triplet oxygen, or decomposing peroxides. The level of phenolics in this study was found to be concentration dependent. They inhibit autoxidation of unsaturated lipids, thus preventing the formation of oxidized low-density lipoprotein, which is considered to induce cardiovascular diseases.

Sreelatha and Padma [47] reported that the aqueous extract of *M. oleifera* exhibited a strong scavenging effect on DPPH free radical, superoxide, nitric oxide radical and inhibition of lipid peroxidation, and this effect was comparable with that of the reference antioxidants. This therefore supports the observation from this study that even the extract of *M. oleifera* that is used as food supplements has antioxidant properties.

From this study, it can be concluded that the use of Bari-Frank Moringa as a food supplement is justified; however, a lot of caution should be exercised not to consume this product in large quantities at a time because the toxicity study implies that the extract may be toxic at a dose of 1600 mg/kg and above.

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