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Research Article

THE POSSIBLE PROPHYLACTIC EFFECT OF EGYPTIAN *MORINGA OLEIFERA* SEED OIL AS ANTI-ULCER ACTIVITY AND ANTI-INFLAMMATORY IN RATS.

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ABSTRACT

Moringa oleifera Lam. is the multipurpose tree (therapeutic potential and nutritive value). In this work, authors focused on importance of *Moringa oleifera* seed oil (MSO) as antioxidant, anti-inflammatory and antiulcer. The search describes the lethal dose of 50 % (LD_{50}), some Physico-chemical, antioxidant, antiulcer and anti-inflammatory of MSO. The results illustrated that the LD_{50} of MSO is more than 62.5 g/kg. MSO treated animals (250 and 500 mg/kg) a significantly decreased in the gastric juice, free acidity(FA) and total acidity (TA), also occurs improvement in pH of gastric juice(PH). There was a significant anti-ulcer activity, in ethanol-induced gastric ulcer of the MSO treated groups compared to ranitidine (R)(197 mg/kg)as standard. Also, MSO (20 mg/kg, 72%) inhibited the carrageenan-induced inflammation in the hind paw of rats compared with ibuprofen (20 mg/kg, 33%) and mefenamic acid (100 mg/kg, 39%) as standard.

Keywords: lethal dose of 50 %, ibuprofen, *Moringa oleifera Lam.*, pylorus ligation, antiulcer activity, anti-inflammatory.

INTRODUCTION

Moringa species (Moringaceae) are one of the most useful trees in the tropics and subtropics of Asia and Africa. These species are the most widely cultivated and utilized by the ancient Egyptians, Romans and Greeks. Traditionally, *Moringa* flower, fruits and roots are edible and consumed as the vegetable. It used in folk Medicine for the treatment of many diseases such as rheumatic and anticular pain, abdominal tumors, hysteria, scurvy, paralytic attacks, helminthic bladder, prostate troubles, sores and skin infections **[1].** *Moringa oleifera* has been reported as analgesic and anti-inflammation **[2]** and hence the analgesic and anti-inflammatory effects may be attributed due to flavonoids, steroids and tannins by its extract. However, its pharmacological actions and mechanisms have not been precisely documented in spite of its increasing usage recently. *Moringa oleifera* seed can be used in a variety of ways including as medicinal and herbal remedies, nutritional supplements, and for industrial and agricultural purposes. Its seeds contain 19 to 47 % oil **[3],** similar as olive oil and are rich in palmitic, stearic, behenic and oleic acids **[4].** This oil contains about 75% oleic acid, a monounsaturated fatty acid that is less vulnerable to oxidative stress than polyunsaturated fats. The protective agents of oleic acid in reducing cardiovascular disease, breast and skin cancer levels may be attributed to its ability to reduce the inflammation **[5].** It has been used for the treatment of inflammation, infectious diseases and gastrointestinal **[6].** *Moringa* seed oil content and its properties show a wide variation depending mainly on the species and environmental conditions **[7].** Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. The body defense of inflammation reaction occurs by eliminating the spread of injurious agents as well as to remove the consequent necrosis cells and tissue and to manifest the body's response to tissue damage and infection. The inflammatory reaction may be beneficial as the body defense against agents deranging its homeostasis or harmful as damage the surrounding tissues **[8].** The inflammation associated chronic diseases can be possible ameliorate with the potent anti-inflammatory activity of *MO* bioactive compounds **[9].** The biphenols are abundant in MSO that have

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anti-inflammatory, antioxidant **[10],** and immunomodulatory **[11]** properties suggest that they may have beneficial effects on inflammatory bowel diseases (IBD) which is characterized by chronic intestinal inflammation. Although its etiology is still unknown, immune dysfunction, inflammatory mediators, reactive oxygen species (ROS) and cytokines play crucial roles in its development **[12-13].** There are two main approaches for treating peptic ulcer; first reducing the production of gastric acid and reinforcing gastric mucosal protection **[14].** Treat of gastric ulcer by commercially available anti-ulcer drugs is usually accompanied by various side effects **[15]** such as H_2-receptor antagonists (e.g. cimetidine) may cause gynaecomastia in men and galactorrhoea in women **[16],** while proton pump inhibitors (e.g. omeprazole and lansoprazole) can cause nausea, abdominal pain, constipation and diarrhea **[17].** As a result of these problems, there is a need to find new antiulcer agents that are highly effective with potentially less or no side effects. Medicinal plants have always been the main source of new drugs for the handle of gastric ulcers **[18].**The clout of research is to investigate some physico-chemical, antioxidant properties, anti-inflammatory and anti-ulcer of Egyptian *Maringa oleifera* seed oil.

MATERIALS and METHODS

Plant material

Moringa oleifera seeds (MS) were collected from National Research Center, Dokki, Cairo, Egypt. The seeds coat and wings were removed manually and it has crushed into powder using a laboratory blender.

Extraction of oil using n- hexane:

500 g of MS powder was added to 1 L of n-hexane and continuously shaken for 4 h and then allowed to stay overnight and the oil was decanted. The filtrate was concentrated using a rotary evaporator at 40˚C. The recovered oil was used for physicochemical and biochemical analysis **[19].**

Physicochemical properties

The physico-chemical properties of the oil as (density, refractive index, iodine value, peroxide value, acidity, saponification value, and unsaponifiable matter were carried out using the method described by A.O.A.C. **[20].**

Biochemical Analysis:

Median lethal dose (LD_{50}):

For determination of acute toxicity, each animal was carefully given single orally dose of MSO and death was observed after 24h of treatment **[21].**

Antioxidant Activity:

Determination of Total phenolic content

Total phenolic content of MSO was spectrophotometrically determined using Folin-Ciocalteu reagent **[22].**

Determination of total flavonoid content

The total flavonoid content was assayed according to Wong, *et al*., **[23].**

Determination of radical DPPH scavenging activity

Free radical scavenging capacity of MSO was determined using the stable 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) according to Hwang, *et al*., **[24].**

Experimental Animals

Anti-inflammatory activity

Carrageenan-induced paw oedema test

Carrageenin-induced rat hind paw oedema model The method adopted resembles essentially **[25, 26].** The animals were studied for toxicity of DMSO up to 10% v/v in saline, and 5% DMSO was selected as a vehicle to suspend the standard drugs and the test compounds. Albino rats weighing between 150 and 250 g of either sex were starved for 18 h prior to the experiment. The animals were weighed, marked for identification and divided into groups of six. The standard drugs, ibuprofen (20 mg/kg body weight), mefenamic acid (100 mg/kg body weight) and three graded doses (10, 20 and 40 mg/kg body weight) of the test compounds were given orally as a suspension using 5% DMSO as a vehicle. One hour later foot paw oedema was induced by injecting 0.1 ml of 1% carrageenin subcutaneously into the planter portion of the right hind paw of each rat. Initial foot paw volume was measured immediately by mercury plethysmometer. Oedema was measured 3 h after carrageen in administration. The swelling in test group animals was used to calculate the % inhibition M ± SEM of oedema achieved by the compound at the test dose compared with the vehicle control group. The % protection of oedema

was calculated according to the formula, % anti-inflammatory activity = 100 X (1 - Vt/Vc) where Vt and Vc are the volume of oedema in test compounds and control groups, respectively.

Antiulcer activity

Animal

rats were fasted for 18 hours before pylorus ligation with water *ad libitum* and it has divided into four groups (each: n=6): Group I: Ulcer induced control; Group II: MSO extract (250 mg/kg body wt p.o.) in ulcer rats; Group III: MSO extract (500 mg/kg body wt p.o.) in ulcer rats; Group IV-Ranitidine (179 mg/kg body wt p.o.) in ulcer rats.

Pylorus Ligated (PL) - Induced Ulcers

Rats weighing 150-200 g were used for Pylorus Ligated (PL) - Induced Ulcers using the method described by to Deshpande, *et al.,* **[27] .**
Scoring of ulcer will be made as follows **[28]**.
Normal colored stomach..... (0)
Red coloration..... (0.5)
Spot ulcer..... (1)
Hemorrhagic streak... (1.5)
Deep Ulcers..... (2)
Perforation..... (3)
Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:
Ulcer index (UI) was measured by using following formula: **[29].**

$UI = UN + US + UP \times 10^{-1}$

Where,
UI= Ulcer Index; UN = Average number of ulcers per animal; US = Average number of severity score; UP = Percentage of animals with ulcers
Percentage inhibition of ulceration was calculated as below:
% Inhibition of ulceration = (ulcer index control-ulcer index Test) × 100 /ulcer index control.

Ethanol-induced gastric ulcer in rats

Rats were deprived from food but had ad *libitum* access to tap water for 24 h before ulcer induction. Gastric mucosal damage was induced in conscious rats by gavage of 5.0ml/kg b.wt. of absolute ethanol (99.5%). The standard drug (Ranitidine 179 mg/kg) or MSO (250 & 500 mg/kg) were taken one hour before ethanol administration. The animals were anaesthetized with ether. Stomachs were isolated and ulcer index was determined **[30].**

Parameters measured: Ulcer index and % protection was measured. pH of the gastric juice was also measured.

STATISTICAL ANALYSIS

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet comparison test. The values are expressed as mean ± S.E. **[31].**

RESULTS

The results illustrated that the LD_{50} of MSO is more than 62.5g/kg b.w., as all doses below this incurred zero percentage of death among all groups of rats. Physicochemical property of MSO is illustrated in Table 1. As shown in Table 2, MSO exhibited higher phenolic content and good scavenging abilities against DPPH radicals. The total phenolic, total flavonoid and DPPH of MSO were 35.05 mg gallic acid / g extract, 2.29 mg quercetin /g extract and 75.75 % respectively. Carrageenan-induced edema in rat hind paw is the most widely used primary test for screening of anti-inflammatory agents. The percentage inhibition of edema at 1h in ibuprofen (20 mg/kg) and mefenamic acid (100 mg/kg) was 30.0% and 8.0% respectively, since the percentage inhibition of edema was 72.2% in hexane extract of MSO treated group at a dose 20 mg/kg were investigated in table 3. The percentage inhibition for hexane extracts of MSO was highest at the doses of 20 mg/kg (72%) ($p<0.05$) which was comparable to that of ibuprofen and mefenamic acid (30% and 8% respectively). Table 4 and 5 illustrate that MSO was effective in the all of tested models of gastric ulcers.

Table 1: Physicochemical property of *Moringa oleifera* seed oil

Properties	*Moringaoleifera* oil
Oil yelid %	38.23
Acidity (as oleic acid) %	1.50
Peroxide value (meq kg-1 of oil)	24.70 ±0 .24
Iodine value (g /100 g of oil)	66.11± 0.30
Density (g/cm^3) 25^{o}C	0.928±0 .02
Refractive index (*n*D 25 °C)	1.454± 0.04
Saponification value (mg of KOH/g of oil)	183.4± 0.27
Unsaponifiable matter (%)	0.85±0.09

Table 2: The total phenolic, total flavonoid contents and DPPH antioxidant test of Moringa oleifera seed oil extract.

Parameters	Total phenols (mg/g)	Total flavonoids (mg/g)	DPPH (%inhibition)
Oil extract	35.05±0.03	2.29±.04	75.75±0.1

Table 3: Anti-inflammatory activity carrageenin-induced rat hind paw oedema % protection.

Tested Compounds	Ibuprofen (20 mg/kg)	Mefenamic acid (100 mg/kg)	MSO (20mg/kg)
% Protection	33	8	72

Table 4: demonstrated descriptive statistic of *Moringa oleifera seed oil* on Pylorus Ligation-Induced Gastric Ulcers

Group	Treatment	Gastric juice (GJ) (ml)	pH of gastric juice(PH)	Free Acidity(FA) mEq/litre	Total Acidity(TA)
I	**Control (C) (pyloric ligation)**	8.5 ± 0.21	3.21±0.04	120±0.25	95.1±1.5
II	**MSO (L) (250mg/kg)**	4.6 ± 0.10***	4.8±0.18**	70±0.30*	66±0.12*
III	**MSO (H) (500mg/kg)**	4.2 ± 0.10***	3.8±0.05*	65±0.28*	58 ±0.14**
IV	**Ranitidine (R) (197mg/kg)**	4.1 ± 0.10***	5.35±0.15***	3 ± 0.17***	37±0.24***

All values are mean ± *S.E.* ,n = 6 , ****p*<0.00 1, ***p*<0.01, **p*<.0.05,when compared with control group.

Table 5: demonstrated descriptive statistic of *Moringa oleifera* seed oil on ethanol-Induced Gastric Ulcers.

Group	Treatment	Doses(mg/kg)	Ulcer index (mm^2/rat)	% ulcer inhibition
I	**Ethanol**	-	3.6± 0.45	-------
II	***Morniga Oleifera* oil extract**	250	2.72± 0.15	55.13
III	***Morniga Oleifera* oil extract**	500	2.13± 0.18*	58.22
IV	**Ranitidine**	197	1.50 ± 0.20*	72.49

All values are mean ± *S.E.* , n = 6 , ****p*<0.001, ***p*<0.01, **p*<.0.05,when compared with control group.

DISSCUSION

Median lethal dose (LD_{50})

The results illustrated that the LD_{50} of MSO is more than 62.5g/kg b.w. This suggests that MSO possesses a degree of relative safety from acute intoxication. However, LD_{50} has not been regarded as a biological constant because many variables such as animal's species and strain, age, gender, diet, bedding, ambient temperature, aging conditions and time of the day can all affect the LD_{50} value obtained; hence there are considerable uncertainties in extrapolating LD_{50} value obtained for species to other species. Consequently, recognizing LD_{50} test as providing, at best, only a ballpark estimate of human lethality has been advocated **[32].**

Characteristics of *Moringa Oleifera* seed oil

Physicochemical property of MSO is illustrated in Table (1). The present data was consistent with other reported in the literature of others *Moringa* species **[33-34].**The percentage of Acidity (A% , 1.50), peroxides value (PV, 24.7 meq peroxide/kg) and iodine value (IV, 66.11 g /100 g of oil), density (D, 0.928 g/cm3), Refractive index (RI, 1.4545), saponifiable matter (SM, 183.4 mg KOH g-1 of oil) and unsaponifiable (USM, 0.85%) matters of MSO were comparable with others reported in the similar MSO **[35]** On other hand, some physicochemical constants of the oil was varied from those reported for different Moringa species. For example, the PV of MSO found in the present analysis was in agreement with those of MSO (69 mg I/100 g oil) from Saudi Arabia **[36].** Also, the values of AV, IV, PV, USM and SM in the present analysis for Egyptian of MSO was higher than those reported for MSO (0.04 mg KOH/g, 69.5 mg I/100g, 2.3 meq/kg, 0.3 % and 182.9 mg KOH/g) native to Saudi Arabia **[37]** Also, saponifiable value was comparable to those of MSO (185 mg KOH g-1 of oil) from Pakistan **[38].** However, variation in oil yield and physicochemical value between Egyptian MSO and same MSO grown in other countries might be attributable to the differences in variety of plant, environmental and geological conditions of the regions, ripening stage, the harvesting time of seeds and extraction methods used **[33]**.

Antioxidant activity

As shown in Table (2), MSO exhibited higher phenolic content and good scavenging abilities against DPPH radicals. Phenolic compounds are a class of antioxidant agents which act as free radical terminators and also involved in retardation of oxidative degradation of lipids **[39].** Thus, in MSO the presence of phenolic compounds gives additional value to its nutritional and health potential. Moreover, the occurrence of flavonoids that are also phenolic compounds in MSO similarly promotes the health potential of the oil. This finding is in accordance with other which reported that the flavonoids carry out antioxidant action through scavenging or chelating process and are play a preventive role in the ulcer, inflammation and heart disease **[40,41].** In the DPPH assay, the antioxidant effect was likely to be due to the hydrogen donating ability, **[42].** Therefore, the importance of the antioxidant constituents of *Moringa* oil in the maintenance of health is strengthened as the trend of the future toward using foods as medicine in the management of various chronic diseases. The suppression of DPPH free radicals by the antioxidant potential of *Moringa* oil was further highlighted in this study, which measured the extent of radical scavenging potential of the oils supported the findings of reducing power capacity of some compounds may serve as a significant indicator of its potential antioxidant activity which confirmed in the present study **[43-45].**

Effect of hexane Extract of *Moringa oleifera* seed oil on Carrageen an induced edema in rat hind paw.

The data depicted in Table (3) revealed that the percentage inhibition for hexane extracts of MSO was highest at the doses of 20mg/kg (72%) ($p<0.05$) which was comparable to that of ibuprofen and mefenamic acid 30% and 8% respectively. The development of edema in the paw of the rats development after injection of carrageenan is due to a release of histamine and serotonin and is known to be sensitive to cyclooxygenase which lead to a release of prostaglandin like substances. The beneficial effect of hexane extract of MSO could be due to inhibition of their release possibly due to inhibition of the enzyme cyclooxygenase leading to inhibition

of prostaglandin synthesis **[46].** This anti-inflammatory activity can be attributed to various phytochemicals - like alkaloids, flavonoids, sterols, glycosides, tannins and terpenoids reported in MSO **[47,48].** The prostaglandin syntheses which are involved in acute inflammation are attributed to the sterols and flavonoids present in *MSO* **[47-49].** Also**,** the anti-inflammatory action may also be due to antioxidants such as flavonoids, tocopherols and vitamin c present in *Moringa oleifera* seeds which decrease oxidative stress generated during inflammation **[49].**

Effect of hexane Extract of *Moringa oleifera* seed oil on Pylorus Ligation-Induced Gastric Ulcers

The results, in Table (4) make a clear dose-dependent anti-ulcerogenic activity of MSO. The effect of MSO on Pylorus-ligated-induced ulcer may due autodigestion of gastric juice and decrease mucosal blood flow and breakdown of mucosal barrier **[50].**

Effect of Hexane Extract of *Moringa oleifera* Seed oil On Ethanol-Induced Gastric Ulcers

Table (5) indicated the effect of MSO on induce gastric ulceration is mainly caused by the alteration of the antioxidant enzymes of the gastric mucosa with concomitant loss of cytoprotection due to decreased of the prostaglandins (PGs) synthesis **[51].** The flavonoids present in the seed is well known antiulcer agent. This explains more potent ulcer healing effect of ethanol extracts of the seed compared to control. The ulcer healing effect obtained with ethanolic extracts may be due to both anti-secretary and gastric cytoprotective constituents present in these extracts, as evident by a decrease in acidity in pylorus ligation and decrease in ulcer index in ethanol induced gastric ulcers **[52].**

CONCULSION

The study has highlighted the unique characteristics of Egyptian *Moringa oleifera* seed oil and indicated good quality oil. These results suggest that the MSO has medicine importance as an anti-inflammatory principle that may useful in the handle of both the acute and chronic inflammatory conditions. Also, MSO has ulcer protective effect as dose-dependent against pylorus-ligation, ethanol induced gastric ulcer in rats and this due to the strong antioxidant capacity of this the medicinal plant. Must increase the cultivation of these seed and consume oil to improve the public health.

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