



Exploring the Adverse Impacts of Cyclosporine A and the Preventative Abilities of *Moringa oleifera* Seed Oil: A Therapeutic Approaches Contrary to Negative Effects



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Abstract

Cyclosporine A (CsA) is a popular immunosuppressive medication for organ transplant recipients which its injection associated with oxidative stress. *Moringa oleifera* Seed oil (MOO) own high biological active ingredients, which makes it valuable medicinal plant-based dietary supplement that is used to address various nutrition-related wellness issues. The research is targeted to figure out the protective ability of MOO and encapsulated MOO (EMOO) against CsA toxicity, and assessing their antioxidant and anti-inflammation qualities. Rats (n=24) were separated into four groups: (1) received distilled water (control); (2) rats injected by CsA. (3) and (4) received MOO and EMOO coupled with CsA for 8 weeks. The oxidation, inflammation and minerals indices were measured in serum, liver and bone. Administration of MOO or EMOO significantly inhibited the development of bone loss in femur and tibia, improved hepatic and renal function, alleviate oxidative damage and inflammation compared to CsA rats. The histopathological examination of hepatic- and splenic tissue of MOO or EMOO showed improvement in tissue structure compared to CsA rats tissues. Encapsulating-MOO showed more effectiveness impact than MOO against CsA toxicity. Considering the potential antioxidant and anti-inflammatory characteristics of MOO, this research highlights its potential as a complementary therapeutic option when undergoing cyclosporine A.

Keywords: Moringa seed oil - Cyclosporine A – encapsulation - Antioxidant – Anti inflammation

Introduction

The body uses inflammation as a vital defensive reaction, which is marked by the formation and set free of pro-inflammatory cytokines like Interleukin 6 (IL 6), tumor necrosis- α (TNF- α), IL 17, and IL 4. Inducible nitric oxide synthase (iNOS) as well cyclooxygenase-2 (COX-2) enzymes are upregulated in this process. Additionally, intracellular signaling pathways are stimulated, especially nuclear factor kappa-B (NF- κ B), a transcription factor that influencing multiple tissues' inflammatory reactions[1,2]. Numerous chronic illnesses, including obesity, diabetes, colitis, pancreatitis, heart disease,

bone necrosis, and several types of cancer, have been linked to a persistent low-grade inflammatory response [3,4]. Many anti-inflammatory medications have been created to lower the production of inflammatory mediators, which helps regulate chronic inflammation. However, prolonged use of these medications can cause adverse effects such ulceration and bleeding in the stomach [5]. The CsA is a popular agent that suppresses immunity for autoimmune diseases and post-transplantation treatment. However, CsA-induced toxicity has been associated with oxidative stress, as suggested by numerous studies[6]. To mitigate the side effects of

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EJCHEM use only: Received date 11 September 2024; revised date 10 October 2024; accepted date 20 October 2024

DOI: 10.21608/ejchem.2024.320114.10402

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cyclosporine and maintain its clinical dosage, the co-administration of antioxidant or anti-inflammatory agents has been explored. Antioxidants and inflammation reduction action of medicinal plants have been verified. They can lower oxidative stress and regulate inflammation via modulation of signaling pathways, scavenging of oxidants directly, or generation of antioxidant enzymes, thereby helping to prevent chronic inflammation-related illnesses [7]. Thus, *Moringa oleifera* plant attracted a lot of interest lately due to its extensive medical potential. Science is beginning to recognize that *Moringa oleifera* is a valuable source of naturally occurring phytochemicals, which is putting the plant on the map for potentially successful future advances. Thus, *Moringa oleifera* plant attracted a lot of interest lately due to its extensive medical potential. Science is beginning to recognize that *Moringa oleifera* is a valuable source of naturally occurring phytochemicals, which is putting the plant on the map for potentially successful future advances[8]. According to the aforementioned discussion, oxidative damage, inflammatory processes, and apoptosis are thought to be important pathogenic mechanisms in the toxicity that CsA induces in different human organs. Consequently, targeting these pathways may prove to be a profitable strategy in combating CsA-associated toxicity. As a result, there is increasing interest in natural alternative therapies that can be included in the diet[9]. Research has been done on MOO as a potential remedy option for combating oxidative stress and inflammation resulting from CsA treatment[10]. Studies have indicated that the molecular architecture and biological function of Moringa polysaccharides' are substantially influenced by methods used for extraction. In order to incorporate bioactive substances into food, encapsulation has been mentioned as a way to minimize stress caused by oxidation to capture acting components enclosed in a carrier substance[11]. Because MOO's preventive benefits have not been fully explored to yet, extensive research is required to support previous findings in this area. Thus, assessing the prophylactic impact of MOO opposed to Cyclosporine A-induced in Wistar albino animals was the primary goal of this investigation. Also, this study aims to provide antioxidants and anti-inflammatory properties to cyclosporine while minimizing its adverse effects.

Materials and methods

Moringa oleifera seed samples

The *Moringa oleifera* seeds (MOO) were provided from the National Research Center, Giza, Egypt. The seeds were separated right away, cleaned to get rid of

any contaminants, and allowed to air dry beneath the shadow.

Moringa oleifera seed Oil extraction

The Moringa seeds were cold-pressed to extract the oil. A twin-screw extruder with a co-rotated conical design ground and crushed two kilograms of moringa seeds. The oil was then filtered in a centrifuge at 5000 rpm for 20 minutes to remove plant debris, with additional filtering done subsequently. The extracted seed oil was stored frozen at -20°C for further analysis [12].

The MOO physico-chemical evaluation

For Moringa seed oil, the following were determined using standard methods AOCS: specific gravity, unsaponifiable matter, refractive index, free fatty acid quantities, peroxide value, iodine value, and saponification value. The specific absorptivity values K232 and K270 were determined by measuring the absorbance of a 1% solution of seed oil in cyclohexane at 232 and 270 nm using an ultraviolet (UV) spectrophotometer [13].

The MOO emulsion formulation and encapsulation:

To form the MOO emulsion, Oil-in-water microemulsions were prepared by high-speed homogenization. The B-290 mini Spray Dryer B-290 (Büchi, Flawil, Switzerland) was utilized concurrently. Gum Arabic, maltodextrin (MD), oil emulsion, and spray drying were used to coat the extracted MOO[14].

The Experimental protocol

In this study, 24 male albino rats were assigned to different treatment groups. As the control normal group (CN), received distilled water with no treatment, whereas second group (CsA) injected by 10 mg/Kg bw of CsA intra-peritoneally (ip) each day[15]. While group 3 and 4 received orally MOO (CsA + MOO) and EMOO (CsA + EMOO) 5 ml/kg/d from each[16], coupled with CsA injection for a duration of 8 weeks. All experiments were performed in line with the ethical guidelines approved by the Medical Research Ethics Committee (MREC) at National Research Centre in Egypt that approved the experimental protocol (Code No. 19182).

Blood sampling

After a 12-hour fast, the animals were euthanized with diethyl ether. Blood was drawn from each rat's post-caval vein, serum collected before spun for 15 min at 3000 rpm, then frozen at -20°C for biochemical analyses. The spleens and livers were then removed, cleaned, dried, and weighed. Sections

of these tissues were preserved in a 10% phosphate-buffered formalin solution for histological analysis, and any remaining samples were stored at -20°C before analysis

Biochemical evaluation:

Serum biochemical assays

The alanine transaminase (ALT), and aspartate transaminase (AST), urea, creatinine, albumin and total protein were measured in serum[17-21]. Serum cluster of differentiation 4 (CD4), cluster of differentiation 8 (CD8), INF- γ , IL-6, interleukin 1-beta (IL-1 β), and TLR 4 were determined using rats enzyme-linked immunosorbent assay kits (ELISA) (Elabscience Biotechnology Co., Ltd., Wuhan, China).

Liver homogenates antioxidant enzymes assays

Glutathione S. transferase (GST), catalase (CAT), and superoxide dismutase (SOD) activities and MDA were measured in liver homogenate[22-25]. Liver TNF- α was evaluated using an ELISA kit (Elabscience Biotechnology Co., Ltd., Wuhan, China).

Measuring the bone (femur and tibia) minerals content

The dry ashing method was used to mineralize bone samples to determine calcium (Ca), phosphorus (P), and magnesium (Mg) levels in the left femur and tibia[26]. The bones were dried at 105°C, defatted, and finally ashed at 550°C. Analyzed using the vanadate-molybdate method for P[27], and atom-absorption spectroscopy for Ca and Mg content.

Histological examination

All specimens were immediately fixed in 10% formalin at room temperature, treated with a conventional grade of alcohol, cleared in xylene, embedded in paraffin and sectioned at 5 μ m thickness. The sections were stained with haematoxylin and eosin (H&E) in order to study the histopathological changes using a light microscope

Statistics

The data are presented as mean values with their standard errors. Statistical analysis was conducted using one-way ANOVA and the differences among groups were determined using Bonferroni's multiple comparison test with GraphPad Prism version 7.01 for Windows (GraphPad Software Inc., San Diego, CA, USA).

Results

The MOO physico-chemical characteristics:

The physico-chemical characteristics of MOO obtained by cold pressing were displayed in Table (1). The findings reveal that the refractive index at 40 °C was 1.4638 \pm 0.22, density at 25 °C was 0.902 \pm 0.01 g/ml. There are some of important values of MOO were displayed including iodine (83.2 \pm 4.72 g of iodine/100 g of oil), and saponification value (189.5 \pm 7.28 mg of KOH/g of oil). It should be noted that MOO has low peroxide levels (8.11 \pm 0.42 meq. O₂/kg oil), while acid value was (1.93 \pm 0.22 mg KOH/g oil). In addition to the content of Unsaponifiable matter was 1.13 \pm 0.03%.

Table 1: The physical-chemical Characterization of *M. oleifera* seed oil (MOO)

Variable	
Acid value (mg KOH/g oil)	1.93 \pm 0.22
Peroxide value (meq.O ₂ /kg oil)	8.11 \pm 0.42
Saponification value (mg KOH/g oil)	189.5 \pm 7.28
Iodine value (g I ₂ /100g oil)	83.2 \pm 4.72
Unsaponifiable matter (%)	1.13 \pm 0.03
Density at 25 °C (mg/mL)	0.902 \pm 0.01
Refractive index (40 °C)	1.4638 \pm 0.22

The values represent the means \pm SD of three findings

The MOO Fatty acids (FAs) composition:

The *M. oleifera* oil's fatty acid composition were presented in table (2), which comprises oleic acid and palmitic acid, accounts for over 85% of the plant oil overall. The total content of saturated FAs (10.16%) was much lesser than total content of unsaturated FAs (80.85%). The most abundant saturated FA was palmitic acid (10.2%), followed by margaric- and stearic-acids (2.92% and 2.45%). The oil was found to contain a high amount of oleic acid (C18:1 n-9) up to 75.2%. A small quantity (2.44%) palmitoleic acid (C16:1 n-9) and (1.54%) of linoleic acid (C18:2 n-6) were also detected. The presented findings in Table (2) have demonstrated that the MOO fell in the category of high-oleic oils most of the characteristics and quality attributes of *M.oleifera* oil

The MOO Phenolic constituents:

Findings in the table (3) showed the flavonoid and phenolic acids of MOO determined by HPLC. The analysis showed that quercetin was the major flavonoid compound (25.39 μ g/ml) followed by Hesperetin with much lesser value (5.28 μ g/ml) from six flavonoid substances were detected. Whilet, the gallic acid (0.66 μ g/ml) was the highest quantity among four detected phenolic acids in MOO. These phenolic chemicals are mostly in charge of giving the oil its important sensory characteristics, such as astringency and pungency.

Table 2: Fatty acid constituents of *M. oleifera* seed oil (MOO)

Fatty acids (FAs)				
Saturated FAs	(%)	Unsaturated FAs	(%)	
Palmitic acid (C16:0)	10.18±0.62	Oleic acid (C18:1 n9c)	75.19±3.22	
Margaric acid (C17:0)	2.92±0.07	Palmitoleic acid (C16:1), n9	2.44±0.12	
Stearic acid (C18:0)	2.45±0.09	Linoleic acid (C18:2 n6c)	1.54±0.02	
Arachidic acid (C20:0)	1.42±0.021	11-eicosenoic acid	1.68±0.12	
Behenic acid (C22:0)	2.19±0.22			
Total			10.16	Total 80.85

The values represent the means ±SD of three findings

Table 3: Polyphenol components (µg/ml) of *M. oleifera* seeds oil (MOO)

Flavonoids	µg/ml	Phenolic acids	µg/ml
Rutin	0.12±0.004	Gallic acid	0.66±0.02
Naringenin	0.08±0.001	Chlorogenic acid	0.18±0.01
Daidzein	0.10±0.003	Syringic acid	0.13±0.002
Quercetin	25.39±1.42	Cinnamic acid	0.09±0.001
Kaempferol	0.48±0.02		
Hesperetin	5.28±0.22		
Total	31.45	Total	1.06

The values represent the means ±SD of three findings

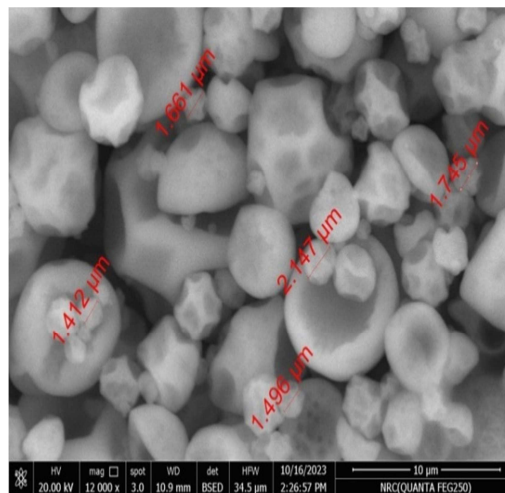
The MOO Encapsulation form

The combination of particle size and carrier agents had a considerable influence on the features of EMOO. When the viscosity and droplet sizes of EMOO emulsions were increased, stable emulsions and microcapsules were made with less oil on the surface and better encapsulation. Additionally, microcapsules made with EMOO showed a reduced span value (Fig. 1), which contributes to improved flowability since the microcapsule particle size has a smaller width distribution.

The MOO and EMOO capabilities as antioxidant and anti-inflammatory:

The in vivo results performed a notable decrease in liver antioxidant enzymes (CAT, SOD, and GST) activity, along with total antioxidants coupled with a notable increase of liver MDA in CsA group when contrasted with NC group (Table 4). These results may indicate the

activation of oxidative stress due to CsA treatment. Intriguingly, concomitant administration of MOO and EMOO with CsA notably improved both total antioxidants and antioxidant enzymes activity coupled with lower MDA toward normal values contrary to CsA group. These levels were comparable to those noticed in the normal control (Table 4). This confirmed the prophylactic function of MOO against toxicity caused by CsA. Comparing the CsA to NC groups revealed a significant reduction in CD4, coupled with notable increment in CD8, INF- γ , IL6, IL-1 β , TLR4, and TNF- α levels (Table 5). When MOO and EMOO were given plus CsA, levels of INF- α , IL6, IL-1 β , and TLR4 notably went down, while CD4 levels went up in contrast to CsA group (Table 5). These findings illustrated that the oxidation and pro-inflammatory indicators were markedly regulated following MOO and EMOO administration.

**Figure 1:** Scanning electron microscopy image of encapsulation of *M. oleifera* seed oil**Table 4:** Impact of Cyclosporine (CsA), *Moringa oleifera* oil (MOO) and encapsulating MOO on total antioxidant, malondialdehyde (MDA) and antioxidant enzymes in liver homogenates of all groups

	MDA (nmol/g)	Total AO (mM/l)	GST (U/g)	SOD (U/g)	CAT (µmol/g)
CN	16.80 ^a ±0.56	2.07 ^a ±0.07	28.18 ^a ±0.42	4.85 ^a ±0.12	443.67 ^a ±3.85
CsA	33.00 ^b ±0.86	1.04 ^b ±0.04	15.84 ^b ±0.50	2.64 ^b ±0.17	241.50 ^b ±2.95
MOO + CsA	24.20 ^{cb} ±1.00	1.82 ^{ab} ±0.15	22.44 ^c ±0.79	3.21 ^c ±0.15	400.83 ^c ±3.04
EMOO + CsA	20.92 ^{cb} ±0.71	1.95 ^{ab} ±0.09	24.97 ^c ±0.24	3.60 ^{bc} ±0.17	404.67 ^c ±2.68

The values in every column with distinct letters differ dramatically ($p < 0.05$).

Total AO: Total antioxidants, CN: normal control, GSH: reduced glutathione, SOD: superoxide dismutase, CAT: catalase

Table 5: Impact of Cyclosporine (CsA), Moringa oleifera seed oil (MOO) and encapsulating MOO on CD4, CD8, INF- γ , IL 6, IL-1 β , TLR4 in serum and TNF- α in liver homogenates of all groups.

	CD4 (pg/ml)	CD8 (pg/ml)	INF- γ (pg/ml)	IL 6 (pg/ml)	IL-1 β (pg/ml)	TLR 4 (pg/ml)	TNF - α (pg/g)
CN	702.6 7 ^a ± 2.4 0	332.5 0 ^b ± 3.7 7	29.6 4 ^a ± 0.7 3	78.48 a ± 0.7 2	10.8 9 ^a ± 0.2 9	0.33 ^a ± 0.0 1	15.2 9 ^a ± 0.4 1
CsA	520.3 3 ^b ± 2.9 7	497.5 0 ^b ± 3.0 6	40.3 5 ^b ± 0.8 5	164.6 5 ^b ± 1.2 4	34.0 5 ^b ± 0.3 5	0.90 ^b ± 0.0 1	31.6 1 ^b ± 0.4 9
MO O + CsA	694.5 0 ^c ± 2.7 9	346.0 0 ^c ± 1.7 7	30.8 3 ^a ± 0.6 4	84.83 c ± 0.9 5	15.9 4 ^c ± 0.4 0	0.50 c ± 0.0 1	20.5 5 ^c ± 0.2 2
EM OO + CsA	691.1 7 ^c ± 3.8 6	341.0 0 ^c ± 1.7 5	30.2 6 ^a ± 0.4 4	83.28 c ± 0.8 8	13.4 2 ^c ± 0.8 8	0.48 ^c ± 0.0 2	19.7 2 ^c ± 0.5 3

The values in every column with distinct letters differ dramatically ($p \leq 0.05$).

CD4: cluster of differentiation 4, CD8: cluster of differentiation 8, INF- γ : interferon gamma, IL 6: Interleukin 6, IL-1 β : Interleukin 1 β , TLR4: Toll-like receptor 4, TNF- α : tumor necrosis factor- α .

Effect of MOO and EMOO against CsA-Induced Hepatorenal malfunction

The ip injection of CsA resulted in a considerable disturbance in liver and kidney function indices; these were shown from the marked increment in serum AST, ALT, urea and creatinine, in addition to notable decline in serum total protein and albumin compared with NC group (Table 6). Administration of either MOO or EMOO plus CsA injected rats significantly recovered the deteriorated serum liver and kidney function markers, coupled with notable rise in serum total protein and albumin (Table 6).

Conformity with these findings, the histopathological examination demonstrated that the differences between CsA and NC rats were more pronounced in the hepatic than in the splenic tissues (Figure 2 and 3). The (MOO + CsA) group has been improved rather than CsA group, except for inflammatory cell infiltrates in hepatic tissue and a widening of white pulp in splenic tissue (Fig 2C & 3C). Whereas, EMOO + CsA rats tissues showed hepatic and spleen normal like morphological structure (Figures 2D and 3D).

Table 6: Impact of Cyclosporine (CsA), M. oleifera seed oil (MOO), and encapsulating MOO on hepatic and renal functions in serum of all groups

	AST (U/l)	ALT (U/l)	TP (g/dl)	Albumin (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)
CN	32.7 8 ^a ± 0.7 6	21.5 5 ^a ± 0.8 2	7.31 a ± 0.1 4	4.28 ^a ± 0.11	1.40 ^a ± 0.05	27.55 a ± 0.31
CsA	64.2 1 ^b ± 1.1 0	38.3 7 ^b ± 0.5 7	4.13 b ± 0.0 6	2.34 ^b ± 0.12	1.89 ^b ± 0.05	32.41 b ± 0.84
MOO + CsA	34.0 2 ^a ± 0.3 1	26.1 1 ^a ± 0.4 7	7.08 a ± 0.1 6	4.03 ^a ± 0.05	1.43 ^a ± 0.06	28.35 a ± 0.55
EMO O + CsA	33.3 4 ^a ± 0.1 9	25.8 5 ^a ± 0.4 8	7.02 a ± 0.1 6	4.03 ^a ± 0.08	1.47 ^a ± 0.03	28.03 a ± 0.56

The values in every column with distinct letters differ dramatically ($p \leq 0.05$).

TP: Total protein, CN: normal control, AST: Aspartate transaminase, ALT: Alanine transaminase

Effect of MOO and EMOO against CsA-Induced bone mineral loss

According to Table (7), injected CsA rats showed a worthy decline of minerals (Ca, P, and Mg) in femur and tibia bones contrasted with NC group. Conversely, concomitant administration of MOO or EMOO with CsA substantially raised the minerals in the femur and tibia, comparable with the CsA group but not to NC (table 7). Notably, EMOO showed a more effective impact than MOO in preventing CsA-induced mineral loss in rat tibia and femur bones.

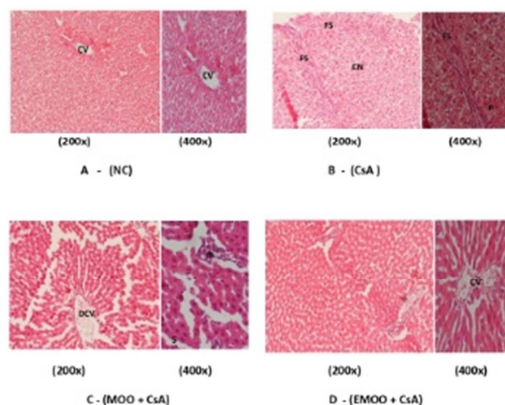


Figure 2: Photomicrograph of hepatic tissues show (A) Normal control (CN) shows normal liver architecture with average thickness of hepatic cords around central vein (CV). (B) cyclosporine A (CsA) shows destructed hepatic cells by fibrous strands (FS), forming cirrhotic nodules (CN), some hepatic cells display necrobiotic changes (n). (C) MOO+ CsA shows hepatic tissue with dilated central vein (DCV) surrounded by average thickness of hepatic cords, in (400x), widen sinusoids (S), inflammatory cells infiltrate (m) were displayed. (D) The EMOO+ CsA shows normal liver structure similar to NC group, with widen sinusoids (S) (400x).

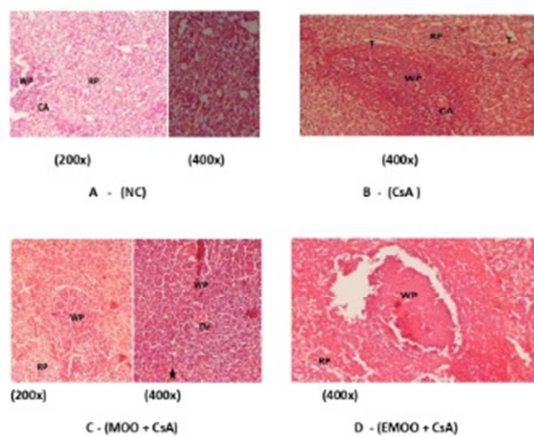


Figure 3: Photomicrography of splenic tissue. (A) Normal control (CN) show normal splenic tissue as normal white pulp (WP) formed of lymphoid aggregate, surrounded by red pulp (RP), average thickness of central artery (CA). (B) Cyclosporin A (CsA) show widening of white pulp (WP), average thickness of CA, surrounded by red pulp (RP) with widen trabecule (T). (C) The MOO+ CsA shows widening of (WP), in (400x) show average thickness of (CA), surrounded by RP, area of extramedullary haemopoiesis (*). (D) The EMOO+ CsA, shows, normal splenic tissue with widening of (WP) surrounded by (RP) (400x)

Discussion

Physical and chemical characteristics of oils play an important role in assessing these quality assurance palatability and consumer acceptability as well as they are related with the healthy safe quality criteria of these oils by using them. From Table (1), the specific gravitational value was similar to Indian MOO[28]. The MOO iodine value was 24% higher than Indian and Pakistani MOO[28,29], while it was corresponds to Nigerian MOO and olive oil[30,31]. This attests to the significant amount of unsaturated FAs in MOO. The MOO low peroxide value was far inferior to those set forth by the Codex Alimentarius[32], which allowed for highest possible acid values for vegetable oils not exceeding 10 mg KOH/mg oil. Nevertheless, the MOO peroxide value was consistency with Nigerian MOO but higher than Indian one[28,30,33]. The acid value of MOO was in compliance Pakistani one[29], which reflect the low content of free FAs and might have a long shelf life than other kinds of oil[34]. The MOO high saponification value was similar to cold-pressed Indian MOO[28], but higher than Tunisian and Malaysian MOO[12,33], which imply the existence of high molecular weight triacylglycerols[35]. The usaponifiable matter of our MOO was more than Indian and Pakistani MOO, but about the same as Tunisian MOO^[12,28,29].

Nine FA were detected among which four were unsaturated (Table 2). The oleic acid accounts for 75.2% of MOO, which were relatively close to Indian, Pakistani, Malaysian and Tunisian MOO[12,28,29,33]. In Indian MOO, saturated FA of

cold-pressed oil was somewhat more than the amount in oil extracted by solvent[28]. The MOO falls into the high-oleic oil category with a considerable proportion of omega-6 and 9, which makes it MOO a favorable choice for nutrition and substantially contributor for certain disease prevention[36-39]. Additionally, MOO has monounsaturated FAs content similar to olive oil, that made it a suitable as primary source of fat in areas that cultivate *M. oleifera* trees [11,40]. According to its content of FAs, the MOO is highly suitable for both edible and non-edible applications [11,41]. Phenolic compounds are part of unsaponifiable matter, which are minor oil constituents. It play a decisive role to avoid the oxidation damage owing to their antioxidant quality[42]. Four phenolic and six flavonoid substances were detected in MOO (table 3). Recent study reported that MOO has an antioxidant efficacy comparable to commercial tea oil and peanut oil[43]. Nevertheless, there is little information available for comparison on the phenolic compounds in MOO[44]. Ten phenolic components were detected including quercetin and gallic acid in moringa seed flour [45]. According to its content of phenolics, the MOO is extremely resistant to autoxidation which can be used as an antioxidant for the long term stabilization of commercial edible oils [11,41]. The encapsulation technique is proposed to minimize oxidative stability and improve accessibility to the MOO active biomolecules[11]. This method preserves the compounds and serves as a good physical barrier against a variety of environmental factors, including light, pH, oxygen, temperature, and water activity. Encapsulation enhances stability and the surface area of the active ingredient to allows for controlled release at a desired rate[46,47]. Extracting the bioactive components is necessary before encapsulation. A variety of recent techniques are used including spray drying and extraction by supercritical fluid and by microwave[48]. In future, should test the new material for encapsulation on Moringa extract and develop new techniques that consider both encapsulation cost and efficiency.

This study designed to evaluate the safety and effectiveness of MOO and EMOO against CsA as a novel immunosuppressive therapy in a model based on rat. Injection with CsA was accompanied by different adverse effect on redox balance, lipid peroxidation, pro-inflammatory markers, hepato- and renal malfunction (toxicity and tissue damage) (Table 4-6). Cyclosporine has a narrow therapeutic index, that possibly initiate oxidative stress, which ultimately caused toxicity[49-52]. Previous studies reported the potential therapeutic capability of MOO against oxidative stress and inflammation[12,45].

Table 7: Impact of Cyclosporine (CsA), Moringa oleifera seed oil (MOO) and encapsulating MOO (EMOO) on femur and tibia minerals in all groups

	Dry bone (g)		Tibia minerals (mg/tibia wt)			Femur minerals (mg/femur wt)		
	Tibia	Femur	Ca	P	Mg	Ca	P	Mg
CN	0.202 ^a ±0.01	0.234 ^a ±0.011	76.00 ^a ±3.61	43.750 ^a ±3.626	4.931 ±0.72	84.6 ^a ±1.112	37.368 ^a ±2.231	5.690 ^a ±0.045
CsA	0.153 ^b ±0.008	0.154 ^b ±0.0024	47.40 ^b ±3.69	27.776 ^b ±3.379	3.189 ±0.461	62.9 ^b ±1.312	26.758 ^b ±1.507	3.958 ^b ±0.395
MOO + CsA	0.244 ^a ±0.016	0.244 ^a ±0.0046	69.51 ^a ±4.8	47.718 ^a ±4.890	5.029 ±0.382	67.3 ^a ±1.941	41.361 ^a ±4.406	5.796 ^a ±0.509
EMOO + CsA	0.250 ^a ±0.023	0.244 ^a ±0.0044	74.3 ^a ±3.7	48.085 ^a ±4.515	5.033 ±0.346	75.2 ^a ±1.181	44.950 ^a ±1.721	5.683 ^a ±0.223

The values in every column with distinct letters differ dramatically ($p \leq 0.05$).

CN: Normal control, Ca: calcium, P: phosphorous, Mg: Magnesium

Our findings were harmonious with these studies, the combined administration of MOO or EMOO with CsA substantially recovered undesired changes of oxidation and inflammation indices close to normal state (Tables 4 and 5). The oil produced from Moringa seeds distinguished by the presence of many powerful phytochemicals, in particular polyphenols, which possess an ability to scavenge reactive oxygen and nitrogen species, in addition to its immunomodulatory effects on different immune cells [43,53-56]. Inflammation, and oxidative stress are regarded as key pathogenic pathways in toxicity induced by CsA. Previous studies have proved that MOO can inhibit stress cytokines (IL-1 β , IL6, TNF- α) that cause inflammation [16,55]. Moringa seeds, when combined with fruits, like avocado, may be a potential dietary supplement for treating inflammatory illnesses [57,58].

The CsA injection was accompanied by an ascent of serum hepatic function enzymes and renal function markers, associated with decline in albumin and total protein levels (Table 6). This was in line with numerous studies that triggered renal and hepatic damage in animal models using cyclosporine [59-61]. In this work, administration of MOO and EMOO may shield CsA damaging effect on hepatorenal function (Table 6). Our observation is consistent with a recent study proved that MOO can restore liver enzymes activity, reduce oxidative stress and increasing of liver protein content [62]. Previous studies had recommended the utilize of MOO to mitigate the harmful effects of different medicines on kidney tissue [16,55,36]. Our data aligned with their findings, as we observed a significant prevention of creatinine and urea up-regulation in rats taken MOO plus CsA.

The histo-pathological examination of hepatic and splenic tissues in CsA rats were more pronounced

than NC rats (Figure 2 and 3). Our observed hepatic lesions were in line with previous studies [6,64]. Histological assessment of MOO and EMOO rats tissue indicated their ability to lessen the alteration caused by CsA injection and bring the histo-architectural integrity back to a level similar to NC tissues (Figures 2 and 3). These findings align with recent studies [65,66].

The CsA adverse effect on femur and tibia bones minerals loss was exhibited in (Table 7). Earlier studies reached to same findings when administered rats with CsA at dose 10 mg/kg/day [15,67]. The authors describe two pathways by which CsA promotes turnover of bone. One is indirect by raising levels of parathyroid hormone in serum and the other is reduction in body weight gain. The administration of MOO or EMOO concomitantly with CsA can successfully improve the femur and tibia minerals loss (Table 7). Former research found that daily oral intake of MOO (0.25 ml/100 b.w) for 6 weeks, reduced Ca and P urinary levels and restored their serum levels in ovariectomized rats [68]. Recently, even moringa leaves perfectly affects on bone density, mineral content, strength, and integrity in rats [69]. When CsA is used to suppress the immune system, MOO, in particular encapsulated form may be effective in repairing mineral bone loss and could be hopeful options for halting bone resorption and osteoporosis.

Conclusions

In light of our findings, targeting oxidative stress, and inflammation pathways could be an efficient approach to prevail CsA-induced toxicity. The strong antioxidative and anti-inflammatory impacts of MOO, in particular encapsulated form, suggest their potential usefulness in the curing of autoimmune diseases, either by itself as monotherapy or coupled with conventional drugs. Further studies are required

to elucidate the potential underlying mechanisms of EMOO remedies.

Conflicts of interest

The authors have no conflicts to declare.

Funding sources

This study founded in partial fulfillment of the requirement of internal project submitted for Chemistry of Flavour and Aroma Department in title “Production of functional foods with natural flavors to enhance immune system and reduce non-communicable diseases associated with climate change”, project No. 13050213, National Research Centre, Egypt

Acknowledgments

The Authors would like to thank the National Research Centre, Egypt for ongoing co-operation to support research and that provided facilities necessary to achieve the desired goals of research.

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