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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-κB), 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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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 alternantive 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-1β), 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 zylene, embedded in paraffin and sectioned at 5µm 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±0.22, density at 25 °C was 0.902±0.01 g/ml. There are some of important values of MOO were displayed including iodine (83.2±4.72 g of iodine/100 g of oil), and saponification value (189.5±7.28 mg of KOH/g of oil). It should be noted that MOO has low peroxide levels (8.11±0.42 meq. O2/kg oil), while acid value was (1.93±0.22 mg KOH/g oil). In addition to the content of Unsaponifiable matter was 1.13±0.03%.

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

Variable	
Acid value (mg KOH/g oil)	1.93±0.22
Peroxide value (meq.O2/kg oil)	8.11±0.42
Saponification value (mg KOH/g oil)	189.5±7.28
Iodine value (g I_2/100g oil)	83.2±4.72
Unsaponifiable matter (%)	1.13±0.03
Density at 25 °C (mg/mL)	0.902±0.01
Refractive index (40 °C)	1.4638±0.22

The values represent the means ±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 plamitic 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 querectin was the major flavonoid compound (25.39 µg/ml) followed by Hesperetin with much lesser value (5.28 µg/ml) from six flavonoid substances were detected. Whilet, the gallic acid (0.66 µg/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 (C18:1n9c)	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 (C18:2n6c)	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
Querectin	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-$_\gamma$, IL6, IL-1β, TLR4, and TNF-α levels (Table 5). When MOO and EMOO were given plus CsA, levels of INF-α, IL6, IL-1β, 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.

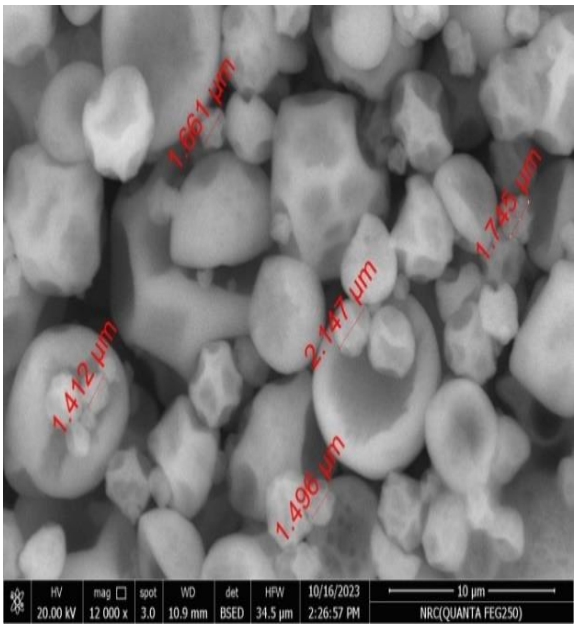

Figure 1: Scanning electron microscopy image of encapsulation of M. oleifera seed oil

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^{ac} ±0.17	404.67^{c} ±2.68

The values in every column with distinct letters differ dramatically ($p \leq 0.05$).
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-$_\gamma$, IL 6, IL-1β, TLR4 in serum and TNF-α in liver homogenates of all groups.

	CD4 (pg/ml)	CD8 (pg/ml)	INF-$_\gamma$ (pg/ml)	IL 6 (pg/ml)	IL-1β (pg/ml)	TLR 4 (pg/ml)	TNF-α (pg/g)
CN	702.67^{a} ±2.40	332.50^{a} ±3.77	29.64^{a} ±0.73	78.48^{a} ±0.72	10.89^{a} ±0.29	0.33^{a} ±0.01	15.29^{a} ±0.41
CsA	520.33^{b} ±2.97	497.50^{b} ±3.06	40.35^{b} ±0.85	164.65^{b} ±1.24	34.05^{b} ±0.35	0.90^{b} ±0.01	31.61^{b} ±0.49
MOO + CsA	694.50^{c} ±2.79	346.00^{c} ±1.77	30.83^{a} ±0.64	84.83^{c} ±0.95	15.94^{c} ±0.40	0.50^{c} ±0.01	20.55^{c} ±0.22
EMOO + CsA	691.17^{c} ±3.86	341.00^{c} ±1.75	30.26^{a} ±0.44	83.28^{c} ±0.88	13.42^{c} ±0.88	0.48^{c} ±0.02	19.72^{c} ±0.53

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-$_\gamma$: interferon gamma, IL 6: Interleukin 6, IL-1β: Interleukin 1β, 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 histo-pathological 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	32.78^{a} ±0.76	21.55^{a} ±0.82	7.31^{a} ±0.14	4.28^{a} ±0.11	1.40^{a} ±0.05	27.55^{a} ±0.31
CsA	64.21^{b} ±1.10	38.37^{b} ±0.57	4.13^{b} ±0.06	2.34^{b} ±0.12	1.89^{b} ±0.05	32.41^{b} ±0.84
MOO + CsA	34.02^{a} ±0.31	26.11^{a} ±0.47	7.08^{a} ±0.16	4.03^{a} ±0.05	1.43^{a} ±0.06	28.35^{a} ±0.55
EMOO + CsA	33.34^{a} ±0.19	25.85^{a} ±0.48	7.02^{a} ±0.16	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.

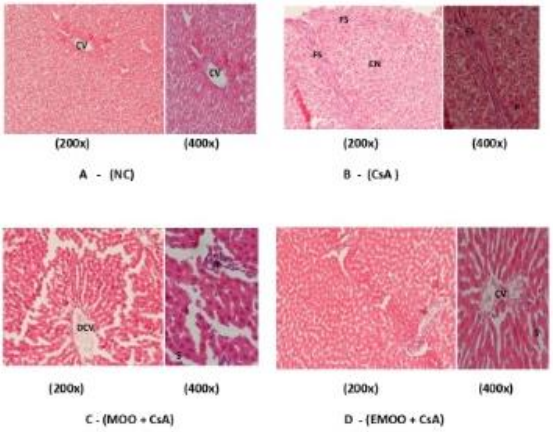

Figure 2: Photomicrograph of hepatic tissues show (A) Normal control (CN) shows normal liver architecture with average thickness of hepatic cords around central vein (CV). (B) cyclosporine A (CsA) shows destructed hepatic cells by fibrous strands (FS) , forming cirrhotic nodules (CN) , some hepatic cells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

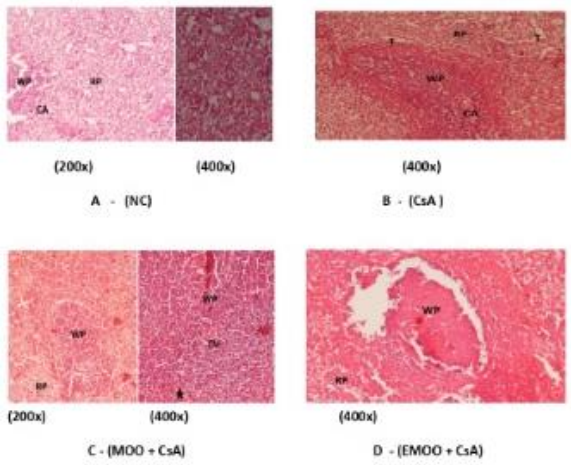

Figure 3: Photomicrography of splenic tissue. (A) Normal control (CN) show normal splenic tissue as normal white pulp (WP) formed of lymphoid aggregate, surrounded by red pulp (RP), average thickness of central artery (CA). (B) Cyclosporin A (CsA) show widing of white pulp (WP), average thickness of CA, surrounded by red pulp (RP) with widen trabecule (T). (C) The MOO+ CsA shows widing of (WP), in (400x) show average thickness of (CA), surrounded by RP, area of extramedullary haemopoesis (*). (D) The EMOO+ CsA, shows, normal splenic tissue with widing 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 consistence 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 immune-modulatory 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-1β., 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 consistence 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 finding 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.

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References

[1] Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. 2012. Into the Eye of the Cytokine Storm. Microbiol Mol Biol Rev 2012;76(1):16–32. doi: 10.1128/MMBR.05015-11

[2] Schaper F, Rose-John S. Interleukin-6: Biology, signaling and strategies of blockade. Cytokine Growth Factor Rev 2015;26(5):475–487. doi. 10.1016/j.cytogfr.2015.07.004

[3] Geginat J, Larghi P, Paroni M, Nizzoli G, Penatti A, Pagani M. et al., The light and the dark sides of Interleukin-10 in immune-mediated diseases and cancer. Cytokine Growth Factor Rev 2016: 30:87–93. doi: 10.1016/j.cytogfr.2016.02.003.

[4] Ibrahim JN, Jéru I, Lecron JC, Medlej-Hashim M. Cytokine signatures in hereditary fever syndromes (HFS). 2017 Cytokine Growth Factor Rev 2017;33:19–34. doi: 10.1016/j.cytogfr.2016.11.001.

[5] Rose-John S. Interleukin-6 Family Cytokines. Cold Spring Harb Perspect Biol 2018; 10(2):a028415.doi: 10.1101/cshperspect.a028415

[6] El-Mancy EM, Elsherbini DMA, Al-Serwi RH, El-Sherbiny M, Shaker GA, Abdel-Moneim AH, et al., α-Lipoic acid protects against cyclosporine A-induced hepatic toxicity in rats: Effect on oxidative stress, inflammation, and apoptosis. Toxics 2022;10(8):442. doi. 10.3390/toxics10080442

[7] Mucha P, Skoczynska A, Małecka M, Hikisz P, Budzisz E. Overview of the antioxidant and anti-Inflammatory activities of selected plant compounds and their metal ions complexes. Molecules 2021; 26(16):4886. doi. 10.3390/molecules26164886

[8] Paliwal R, Sharma V, Pracheta SS. A review of Horse Radish tree (*Moringa oleifera*): A multipurpose tree with high economic and commercial importance. Asian J Biotech 2011;3(4):317-328. doi. 10.3923/ajbkr.2011.317.328

[9] Neha K, Haider MR, Pathak A, Yar MS. Medicinal prospects of antioxidants: A review. Eur J Med Chem 2019;178: 687-704. doi:10.1016/j.ejmech.2019.06.010

[10] Minaiyan M, Asghari G, Taheri D, Saeidi M, Nasr-Esfahani S. Anti-inflammatory effect of Moringa oleifera Lam. seeds on acetic acid-induced acute colitis in rats. Avincenna J Phytomed 2014; 4(2):127–136. doi: 10.22038/AJP.2014.1072.

[11] Pop OL, Kerezsi AD, Ciont C. Comprehensive Review of *Moringa oleifera* Bioactive Compounds—Cytotoxicity Evaluation and Their Encapsulation. Foods 2022;11(23): 3787. doi: 10.3390/foods11233787.

[12] Gharsallah K, Rezig L, Msaada K, Chalh A, Soltani T. Chemical composition and profile characterization of *Moringa oleifera* seed oil. South African J Botany 2021;137: 475-482. doi. 10.1016/j.sajb.2020.11.014.

[13] AOCS. Official Methods and Recommended Practices of the American Oil Chemist's Society, 5th ed. 1997; AOCS Press, Champaign, USA.

[14] Monica P, Sharma HK. Effect of different combinations of maltodextrin, gum arabic and whey protein concentrate on the encapsulation behavior and oxidative stability of spray dried drumstick (*Moringa oleifera*) oil. Intr J Biol Macromol 2017;105(1): 1232-1240. doi: 10.1016/j.ijbiomac.2017.07.160

[15] Matsunaga T, Shigetomi M, Hashimoto T, Suzuki H, Gondo T, Tanaka H, et al., Effects of bisphosphonate treatment on bone repair under immunosuppression using cyclosporine A in adult rats. Osteoporos Int 2007; 18(11):1531–1540. doi: 10.1007/s00198-007-0387-z.

[16] Edeogu CO, Kalu ME, Famurewa AC, Asogwa NT, Onyeji GN, Ikpemo KO. Nephroprotective Effect of *Moringa Oleifera* Seed Oil on Gentamicin-Induced Nephrotoxicity in Rats: Biochemical Evaluation of Antioxidant, Anti-inflammatory, and Antiapoptotic Pathways. J Am Coll Nutr 2020; 39(4): 307-315. doi: 10.1080/07315724.2019.1649218

[17] Reitman S, Frankel S. Colorimetric methods for aspartate and alanine amino-transferase. Am J Clin Pathol 1957; 28(1): 55-63. doi: 10.1093/ajcp/28.1.56

[18] Fawcett J, Scott J. A rapid and precise method for the determination of urea. J Clin Pathol 1960;13(2):156-159. doi: 10.1136/jcp.13.2.156

[19] Larsen K. Creatinine assay by a reaction-kinetic principle. Clinica Chimica Acta 1972; 41:209-217. Doi. 10.1016/0009-8981(72)90513-x.

[20] Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta 1971; 31(1):87- 96. doi. 10.1016/s0009-8981(96)06447-9

[21] Rheinhold JG, Seligron D. Total protein, albumin and globulin. In Standard 664 methods of clinical chemistry, M. Reiner, (ed.). Academic press; Inc. I; New York 1953, pp. 88.

[22] Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249(22):7130-7139. doi:10.1016/S0021-9258(19)42083-8

[23] Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 1952; 195(1): 133-140.

[24] Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 1972; 46(2): 849-854. Doi. 10.1016/s0006-291x(72)80218-3

[25] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thio-barbituric acid reaction. Anal Biochem 1979; 95(2):351-358. Doi. 10.1016/0003-2697(79)90738-3

[26] Ščančar J, Milacic R, Benedik M, Bukovec P. Determination of trace elements and calcium in bone of the human iliac crest by atomic absorption spectrometry. Clin Chim Acta 2000: 293(1-2):187-97 Doi. 10.1016/s0009-8981(99)00239-9.

[27] Davies JL, Andrews GS, Miller R, Owen HG. Comparison of the stannous chloride and vanadate methods for estimation of serum inorganic phosphorus by use of the "SMA12-60". Clin Chem 1973; 19(4): 411-414. Doi. 10.1093/clinchem/19.4.411

[28] Ogunsina BS, Indira TN, Bhatnagar AS, Radha C, Debnath S, Gopala Krishna AG. Quality characteristics and stability of *Moringa oleifera* seed oil of Indian origin. J Food Sci Technol 2014; 51(3):503–510. doi: 10.1007/s13197-011-0519-5.

[29] Anwar F, Rashid U. Physico-chemical characteristics of *Moringa oleifera* seeds and seed oil from a wild provenance of Pakistan. Pak J Bot 2007; 39 (5):1443-1453.

[30] Ogbunugafor HA, Eneh FU, Ozumba AN, Igwo-Ezikpe MN, Okpuzor J, Igwilo IO. Physio-chemical and antioxidant peoperties of *Moringa oleifera* seed oil. Pak J Nutr 2011; 10(5):409-414. Doi. 10.3923/pjn.2011.409.414.

[31] Chouaibi M, Mahfoudhi N, Rezig L, Donsì F, Ferrari G, Hamdi S. A comparative study on physicochemical, rheological and surface tension properties of Tunisian Jujube (*Zizyphus lotus* L.) seed and vegetable oils. Int J Food Eng 2012; 8 (2):1-21. doi. 10.1515/1556-3758.2759

[32] Codex Alimentarius Commission, 1st ed. Recommended Internal Standards Edible Fats and Oils, 1982; Volume XI. FAO/WHO, Rome, Italy.

[33] Abdulkarim SM, Long K, Lai OM, Muhammad SKS, Ghazali HM. Frying quality and stability of high-oleic *Moringa oleifera* seed oil in comparison with other vegetable oils. Food Chem 2007; 105 (4):1382-1389. doi. 10.1016/j.foodchem.2007.05.013

[34] Nyam KL, Tan CP, Lai OM, Long K, Che Man YB. Physicochemical properties and bioactive compounds of selected seed oils. Food Sci Technol 2009; 42:1396-1403. doi.10.1016/j.lwt.2009.03.006

[35] Vidal NP, Adigun OA, Pham TH, Mumtaz A, Manful C, Callahan G. et al., The effects of cold saponification on the unsaponified fatty acid composition and sensory perception of commercial natural herbal soaps. Molecules 2018;23(9):2356. DOi: 10.3390/molecules23092356.

[36] Abdulkarim SM, Long K, Lai OM, Muhammad SKS, Ghazali HM. Some physicochemical properties of *Moringa oleifera* seed oil extracted using solvent and aqueous enzymatic methods. Food Chem 2005; 93 (2):253-263. doi. 10.1016/j.foodchem.2004.09.023

[37] Athikomkulchari S, Tunit P, Tadtong S, Jantrawut P, Sommano SR, Chittasupho C. *Moring Oleifera* seed oil formulation physical stability and chemical constituents for enhancing skin hydration and antioxidant activity. Cosmatics 2020;8(1):2. Doi. 10.33390/cosmatics8010002.

[38] Fernández J, de la Fuente VG, García MTF, Sánchez JG, Redondo BI, Villar CJ. Et al., A diet based on cured acorn-fed ham with oleic acid content promotes anti-inflammatory gut microbiota and prevents ulcerative colitis in an animal model. Lipids Health Dis 2020; 19:28. doi: 10.1186/s12944-020-01205-x

[39] Fratianni F, d`Acierno A, Ombra MN, Amato G, De Feo V, Ayala-Zavala JF. Et al., Fatty acid composition, antioxidant, and *in vitro* anti-inflammatory activity of five cold-pressed *Prunus* seed Oils, and their anti-biofilm effect against pathogenic bacteria. Front Nutr 2021; 8:775751. doi: 10.3389/fnut.2021.775751.

[40] Boskou D. Olive oil. *In* Vegetable oils in Food Technology: Composition, Properties and Uses, F.D. Gunstone (ed.). Wiley-Blackwell: Hoboken NJ, USA. 2011, pp. 243–271.

[41] Nadeem M, Imran M. Promising features of *Moringa oleifera* oil: recent updates and perspectives. Lipids in Health and Disease 2016; 15:212. Doi. 10.1186/s12944-016-0379-0.

[42] Meddeb W, Rezig L, Abderrabba M, Lizard G, Mejri M. Tunisian milk thistle: an investigation of the chemical composition and the characterization of its cold-pressed seed oils. Int J Mol Sci 2017; 18:2582. Doi. 10.3390/ijms18122582.

[43][43] Chen X, Li Z, Smith SA, Chen M, Liu H, Zhang J. et al., Optimization of supercritical CO2 extraction of Moringa oleifera seed oil using response surface methodological approach and its antioxidant activity. Front Nutr 2022;8:829146.

[43] Chen X, Li Z, Smith SA, Chen M, Liu H, Zhang J. et al., Optimization of supercritical CO2 extraction of Moringa oleifera seed oil using response surface methodological approach and its antioxidant activity. Front Nutr 2022;8:829146. Doi. 10.3389/fnut.2021.829146.

[44] Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera leaves: An overview. Int J Mol Sci 2015:16(6):12791–12835. doi: 10.3390/ijms160612791

[45] Govardhan Singh RS, Negi PS, Radha C. Phenolic composition, antioxidant and

antimicrobial activities of free and bound phenolic extracts of Moringa oleifera seed flour. J Function Food 2013;5:1883–1891. doi. 10.1016/j.jff.2013.09.009

[46] Sun-Waterhouse D, Wadhwa SS, Waterhouse GIN. Spray-drying microencapsulation of polyphenol bioactives: A comparative study using different natural fibre polymers as encapsulants. Food Bioprocess Techol 2012; 6(9):2376-2388. Doi. 10.1007/s11947-012-0946-y.

[47] Kullawong O, Chaiyasat A, Chaiyasat P. Microencapsulation of moringa oil in bio-polymer by simple solvent evaporation technique. J Appl Res Sci Tech 2023;22(3):253714.

[48] Dzuvor CKO, Pan S, Amanze C, Amuzu P, Asakiya C, Kubi F. Bioactive components from Moringa oleifera seeds:Production, functionalities and applications—A critical review. Crit Rev Biotechnol 2022;42:271–293. DOI: 10.1080/07388551.2021.1931804

[49] Patocka J, Nepovimova E, Kuca K, Wu W. Cyclosporine A: Chemistry and Toxicity-A Review. Curr Med Chem 2021; 28(20): 3925-3934.doi: 10.2174/0929867327666201006153202

[50] Ershad A, Taziki S, Ebrahimian M, Abadi SSD. Acute cyclosporine overdose: A systematic review. Medicina Clinica Practica 2022; 6(2): 100358.

[51] Ibrahim SRM, Abdallah HM, El-Halawany AM, Mohamed GA, Alhaddad AA, Samman WA . et al., Natural Reno-Protective Agents against Cyclosporine A-Induced Nephrotoxicity: An Overview. Molecules 2022; 27(22):7771. doi: 10.3390/molecules27227771

[52] Cui S, Li L, Liu W, Zhao B, Zhong X. A real-world disproportionality analysis of cyclosporine from the FDA Adverse Event Reporting System (FAERS) database. Braz J Med Res 2024;57:e13392. doi: 10.1590/1414-431X2024e13392.

[53] Olatosin TM, Akinduko DS, Uche CZ, Bardi J. Effects of *Moringa oleifera* seed oil on acetaminophen-induced oxidative stress and liver damage in Wistar albino. J Pharm Biol Sci 2014;9:53-59. Doi. 10.9790/3008-09215359.

[54] Abarikwu SO, Benjamin S, Ebah SG, Obilor G, Agbam G. Protective effect of *Moringa oleifera* oil against HgCl2-induced hepato and nephrotoxicity in rats. J Basic Clin Physiol Pharmacol 2017;28(4):337–345. doi. 10.1515/jbcpp-2016- 0033.

[55] Famurewa A, Asogwa N, Aja PM, Akunna G, Awoke J, Ekeleme-Egedigwe CA. et al., *Moringa oleifera* seed oil modulates redox imbalance and iNOS/NF-kB/ caspase-3 signaling pathway to exert antioxidant, anti-inflammatory and antiapoptotic mechanisms against anticancer drug 5-fluorouracilinduced nephrotoxicity in rats. South African J Botany 2019; 127: 96-103. DOI: 10.1016/j.sajb.2019.08.038

[56]Oba EA, Opeyemi SS, Augustine WJ. Phytochemical screening of aqueous and ethanolic extracts of *Moringa oleifera* seed and physicochemical analysis on the seed oil cultivated in Lere local government area, Kaduna state. Int J Chem Sci 2019; 3(3): 22-24. doi: 10.1016/j.sjbs.2021.07.075

[57] Alkhalaf MI, Alansari WS, Ibrahim EA, EL halwagy MEA. Anti-oxidant, anti-inflammatory and anti-cancer activities of avocado (*Persea americana*) fruit and seed extract. J King Saud Univ Sci 2019; 31:1358–62. doi: 10.1016/j.jksus.2018.10.010

[58] Sayed WAE, Omar FA, Emam MMAA, Farag MA. UPLC-MS/MS and GC-MS based metabolites profiling of *Moringa oleifera* seed with its anti-helicobacter pylori and anti-inflammatory activities. Natural Product Res 2022;36(24):6433-6438.doi: 10.1080/14786419.2022.2037088

[59] Faheem SA, El-Sayed NM, Moustafa YM, Saeed NM, Hazem RM. Mechanisms of cyclosporin A induced hepatotoxicity. Rec Pharm Biomed Sci 2022; 6(1):57-68. doi. 10.21608/RPBS.2022.129863.1133.

[60] Nouri A, Ghatreh-Samani K, Amini-Khoei H, Mohammadi A, Heidarian E, Najafi M. Ferulic acid prevents cyclosporine-induced nephrotoxicity in rats through exerting anti-oxidant and anti-inflammatory effects via activation of Nrf2/HO-1 signaling and suppression of NF-κB/TNF-α axis. Naunyn Schmiedebergs Arch Pharmacol 2022; 395(4):387–395. doi: 10.1007/s00210-022-02212-8.

[61] Abd-Eldayem AM, Makram SM, Messiha CBAS, Abd-Elhafeez HH, Abdel-Rehaim MA. Cyclosporine-induced kidney damage was halted by sitagliptin and hesperidin via increasing Nrf2 and suppressing TNF-α, NF-κB, and Bax. Sci. Reports 2024;14:7434.

[62] Fathil NM. The histological and physiological study of *Moringa oleifera*. Intr J Appl Sci Tech 2022; 4(3): 276-298.

[63] Mahran HA, Okdah YA, Zaky AA, Arisha SM. Ultrastructural and biochemical studies on the potential effects of *Moringa oleifera* seed oil against nephrotoxicity induced by sofosbuvir in albino rats. Egypt J Exp Biol (Zool.) 2022;18(2), 171-185. doi: 10.5455/egysebz.20221105123510

[64] Korolczuk A, Caban K, Amarowicz M, Czechowska G, Irla-Miduch J. Oxidative Stress and Liver Morphology in Experimental Cyclosporine A-Induced hepatotoxicity. Biomed Res Int 2016; 2016(1):5823271. doi: 10.1155/2016/5823271

[65] Abdalla OAM, Hashem MA, Abdelnaeim NS, Elazouney DMS. Hepatoprotective effects of *Moringa oleifera* seed oil and pumpkin seed oil on CCL4 induced hepatotoxicity in rats. SCVMJ 2022; XXVII (2): 315-331.

[66] Ali AH. The protective effect of *Moringa oleifera* seeds extract on liver damage in mice. Egy Acad J Biol Sci B Zoology 2022; 14(1):239-247. DOI: 10.21608/eajbsz.2022.233847

[67] Kanda J, Izumo N, Furukawa M, Shimakura T, Yamamoto N, Takahashi HE, Asakura T, Wakabayashi H. Effects of the calcineurin inhibitors cyclosporine and tacrolimus on bone

metabolism in rats. Biomed Res 2018; 39(3): 131-139. doi: 10.2220/biomedres.39.131

[68] Kusolrat P, Kupittayanant S. Effects of *Moringa oleifera* Lam. seed oil on lipid profiles and bone biomarker in ovariectomized rat. Sci Tech RMUTT J 2018; 8(1), 173-178.

[69] Sakr DM, Abd El-Shafea YM, Eissa MI, El oghazy GM. Composition, nutritive value of *Moringa oleifera* leaves and its clinico - nutritional role in conserving bone integrity of albino rats fed on purified diet. Int J Adv Res Biol Sci 2020.7(3): 100-113. DOI:10.22192/ijarbs.2020.07.03.012