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Research Article

MP-SeNPs; A Promising Cytokines Suppressor in Benzo[a]pyrene-Induced Mammal Tissue Injury in Rats

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Abstract

Background and Objective: *Moringa peregrina* (family Moringaceae) is a common flowering plant found in the Arabian Peninsula, Horn of Africa and Southern Sinai, Egypt. The purpose of this study was to investigate the protective activity of MP-SeNPs against BaP-induced mammal tissue injury in rats. **Materials and Methods:** MP-SeNPs were prepared and characterized in terms of particle size and zeta potential. Furthermore, the IC₅₀ of MP-SeNPs against the MCF7 breast carcinoma cell line and LD₅₀ was evaluated. Adult albino rats weighing approximately 187 ± 10 g was used to assess the lung protective activity of MP-SeNPs (28.7 and 71.75 mg kg⁻¹ b.wt.) against BaP-induced mammal tissue injury in rats. **Results:** The mean particle size of MP-SeNPs was 134.69 ± 8.24 nm with negative zeta potential of +26.04 with the observed shapes of nano particle was spherical. Also, IC₅₀ of MP-SeNPs against MCF7 breast carcinoma cell line = 89.57 µg mL⁻¹ and LD₅₀ equals and 1435 mg kg⁻¹ b.wt., respectively. The daily oral administration of MP-SeNPs at concentrations of 28.7 and 71.75 mg kg⁻¹ b.wt. for 30 days to rats treated with BaP (20 mg kg⁻¹ b.wt.) resulted in a significant improvement of IL-2, IL-6 and IL-10. Oral administration of MP-SeNPs, on the other hand, increased the levels of SOD, GPx, TNF-α, iNOs and GSH as well as decreased the level of MDA in mammal tissue of BaP-treated rats. Furthermore, MP-SeNPs almost normalized these effects in mammal tissue histoarchitecture and MRI examination. **Conclusion:** The biochemical, histological and MRI findings in current study demonstrated that MP-SeNPs have protective activity against BaP-induced mammal tissue injury in rats.

Key words: *Moringa peregrina*, MP-SeNPs, BaP, mammal tissue toxicity and cytokine storm

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Benzo[a]pyrene (BaP) is a long-lasting environmental contaminant that can be absorbed orally, inhaled, or dermally¹⁻³. BaP is distributed into the liver, kidney and bladder after being absorbed from food or contaminated aerosols⁴⁻⁸. BaP has also been shown to cross the placenta after oral, intravenous, or subcutaneous administration⁹. The liver contains the most enzymes required for the bioactivation of BaP⁵. Because BaP is lipophilic, it preferentially distributes and stores in fatty tissues such as mammary fat and bone marrow. BaP that enters the bloodstream is thought to be transported *in vivo* by chylomicrons and lipoproteins¹⁰.

Furthermore, Reactive Oxygen Species (ROS) are produced¹¹ during phases I and II via B[a]P biotransformation and can react with DNA, resulting in strand breaks¹².

By inducing oxidative stress, ROS can deplete endogenous antioxidants¹³, resulting in lipid peroxidation as well as nucleic acid and protein oxidation¹⁴. Even though cellular oxidative damage caused by B[a]P exposure and the link between oxidative stress and disease¹⁵.

Natural products have been shown to scavenge free radicals, modify the antioxidant defence system and aid in the detoxification of carcinogens. Natural products rich in flavonoids have been shown to be effective in the treatment of diabetes, neurodegenerative disease, cardiovascular disease and cancer in several epidemiological investigations^{16,17}. One of these plants, *Moringa peregrina* occurs in the countries bordering the Red Sea, from Somalia and Yemen to Jordan, Palestine and Syria¹⁸. It is considered as a very important medicinal and economic tree. Leaves of *M. peregrina* are also eaten as a vegetable and it is used traditionally in folk medicine and sold in local markets in Oman and other Arab Gulf countries as antioxidant and wound healer¹⁹. A literature survey indicated that the presence of quercetin flavonoids²⁰, sterols²¹, tocopherols (γ and α), β -carotene and other antioxidants²² have been reported from the plant. The different extracts of the plant were also screened for *in vitro* anti-inflammatory and antioxidant activities²³. As an extension of our interested research program in the extraction and therapeutic evaluation of rare medicinal plants²³⁻²⁶, we report here, a facile route to explain the lung protective and antioxidant effects of *M. peregrina* aqueous extract-Selenium nanoparticles against BaP-induced mammal tissue injury in rats, which may pave the way for possible therapeutic application.

MATERIALS AND METHODS

The current study was carried out at the Faculty of Applied Medical Sciences, October 6 University, Egypt during September, 2020.

Materials: The seeds of *M. peregrina* were purchased from the local market and identified by Prof. Heba A Elgizawy, Prof. of Phytochemistry, Faculty of Pharmacy, October 6th University, Giza, Egypt. Vitamin C (Vit. C), Benzo [a]pyrene and propylene glycol were obtained from Sigma Chemical Co. (St. Louis MO, USA). All other chemicals used in this study were of the analytical grade.

Preparation of aqueous extract: The aqueous extract of *M. peregrina* seeds was made by stirring 50 g of powdered *M. peregrina* seeds in 500 mL of warm distilled water at 60-70°C for 45 min with a magnetic stirrer. It was then filtered, evaporated under reduced pressure to dryness and the residue weighed (3.5 g). To facilitate handling, an aqueous suspension was prepared, which is the most common form of folk medicine.

Phytochemical screening: *Moringa peregrina* seeds were phytochemically analyzed for alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oil, coumarins and cyanophoric glycosides²⁷.

Synthesis of *M. peregrina*-selenium nanoparticles (MP-SeNPs): To begin, a 20 mM ascorbic acid solution was freshly prepared by dissolving 35.2 g in 10 mL of deionized water. *Moringa peregrina* water extract was dissolved in deionized water and diluted in deionized water (90 mL) in a conical flask as follows: Dissolved selenious acid (H_2SeO_3 , 0.013 g, 0.01 mmol in 10 mL deionized water was added to the solution, with continuous stirring and heating at 60°C for 10 hrs. Forming in-situ after which 200 μ L of 40 mM ascorbic acid was added as a catalyst the ruby red SeNPs were suspended and characterized by TEM.

Characterization of MP-SeNPs: The crystal-line characteristics and grain dimensions of MP-SeNPs were determined by the X-ray diffraction pattern at 25-28°C with nickel (Ni) (D8 Advance X-ray diffractometer) filtered using $CuK\alpha$ ($\beta = 1.54184$ Å) radiation as X-rayed source. Scanning electron microscope and field transmission microscope at an accelerating voltage of 15 and 200 Kv have investigated the morphology and size of the MP-SeNPs.

Table 1: Description of treatment groups

Groups	Group name	Treatment description
I	Normal control A	3 mL of distilled water, orally for 30 days
II	BaP	was given benzo[a]pyrene orally (20 mg kg ⁻¹ b.wt. in propylene glycol) in a single daily dose for 30 days ²⁹
III	MP-SeNPs+BaP	Oral suspension of 1/50 LD ₅₀ (28.7 mg kg ⁻¹ b.wt. MP-SeNPs) in water+oral administration of 20 mg kg ⁻¹ b.wt. BaP in propylene glycol in a single daily dose for 30 days
IV	MP-SeNPs+BaP	Oral suspension of 1/20 LD ₅₀ (71.75 mg kg ⁻¹ b.wt. MP-SeNPs) in water+oral administration of 20 mg kg ⁻¹ b.wt. BaP in propylene glycol in a single daily dose for 30 days
V	Vitamin C+BaP	Was simultaneously given vitamin C (1.0 g kg ⁻¹ b.wt.) and benzo[b]pyrene (20 mg kg ⁻¹ b.wt.) for 30 days ²⁶

Determination of MP-SeNPs cytotoxicity on cells: Cells were plated into a 24-well plate at a density of 1.0×10^6 cells/well. The particle concentration range was selected based on the minimum concentration showing low toxicity to concentration showing maximum toxicity. The MCF7 cells were exposed to MP-SeNPs at concentration of 7.81, 15.62, 31.25, 62.50, 125 and 250 $\mu\text{g mL}^{-1}$ for 24 hrs. Cells free of particles were used as control cells throughout each assay.

MTT assay was used to determine the effect of MP-SeNPs on the viability of MCF7 cell lines. After exposure, the MCF7 cells were cultured for 4 hrs with MTT (20 μL /well of 5 mg mL⁻¹ stock). Mitochondrial dehydrogenases in living cells convert yellowish water-soluble MTT into water-insoluble formazan crystals that can be dissolved in DMSO. The medium was then withdrawn from each well and 200 μL of DMSO was added to dissolve the formazan crystals. The medium was removed from the suspension culture by centrifugation and then DMSO was added. A microplate reader was used to detect optical density at 570 nm after full mixing (Biotek, USA).

Animals: Female albino rats weighing approximately 187 ± 10 g (90 rats; 60 for LD₅₀ estimation and 30 rats for estimation of MP-SeNPs lung protective activity) were obtained from the animal house of Cairo University, Giza, Egypt. At the National Cancer Institute Animal House, they were housed in plastic cages with stainless steel covers. In a light-controlled room, the animals were kept at a temperature of $21 \pm 2^\circ\text{C}$ and a humidity of 55-60%. The animals were kept for one week to acclimate and were fed a standard diet and given unlimited water.

Determination of LD₅₀ of MP-SeNPs: Preliminary tests were performed on groups of four rats. MP-SeNPs were administered orally in various doses to determine the range of doses that cause zero to 100% mortality in animals. The LD₅₀ was determined in groups of ten animals by administering resveratrol nanoemulsion at different doses of 750, 1000, 1250, 1500, 1800 and 2000 mg kg⁻¹ orally. Animals were observed individually every hour for the first day and every day for the

next five days following administration of the tested MP-SeNPs. Throughout the experiment, animals' behaviour and clinical symptoms were recorded. Finney's²⁸ method was used to calculate the LD₅₀ using the following Eq.:

$$LD_{50} = D_m - \left[\frac{\sum (Z \cdot d)}{n} \right]$$

where, D_m is the largest that kill all animals, $\sum$ is the sum of (z × d), Z is the mean of dead animals between 2 successive groups, d is the constant factor between 2 successive doses and n is the number of animals in each group.

Experimental setup: This experiment was carried out to examine the protective effect of MP-SeNPs against nicotine-induced lung toxicity. This experiment was conducted in accordance with guidelines established by the Animal Care and Use Committee of October 6th University. Adult albino rats were divided into six groups with six animals in each. The treatment groups were described in Table 1.

After 30 days of treatment, blood samples were drawn from each animal's retro-orbital vein and collected in heparin-containing tubes. The heparinized blood samples were centrifuged for 20 min at $1000 \times g$. The separated plasma was used to calculate IL-2 and IL-6 levels using ELISA kits from RayBiotech, Inc., USA, Quest Diagnostics Nichols Institute, San Juan Capistrano, California and R and D Systems Inc., Minneapolis, MN, USA, respectively.

Preparation of lung samples: Cervical dislocation was used to kill the animals and then the mammary tissues were quickly removed. To prepare a 25% w/v homogenate, a portion of each lung was weighed and homogenised in a glass homogenizer (Universal Lab. Aid MPW-309, mechanika precyzyjna, Poland) with ice-cold saline. Three aliquots of the homogenate were prepared. The first was deproteinized with ice-cold 12% trichloroacetic acid and the supernatant obtained after centrifugation at $1000 \times g$ was used to calculate GSH³⁰.

The second aliquot was centrifuged at $1000\times g$ and the supernatant was used to calculate the levels of malondialdehyde (MDA)³¹, nitric oxide (iNOs)³² and tumour necrosis factor- α (TNF- α)³³. The third aliquot of homogenate was used to prepare a cytosolic fraction of the mammary tissue by centrifuging it at $10500\times g$ for 15 min at 4°C in a cooling ultra-centrifuge (Sorvall comiplus T-880, Du Pont, USA) and the clear supernatant (cytosolic fraction) was used to determine the activities of superoxide dismutase (SOD)³⁴ and glutathione peroxidase (GPx)³⁵.

Histological assessment: The mammary tissue was cut into pieces and fixed in a 10% buffered formaldehyde solution for histological study. An automated tissue processing machine was used to process the fixed tissues. Tissues were embedded in paraffin wax using standard techniques. Sections of 5 mL thickness were prepared and stained with hematoxylin and eosin for light microscopy analysis using the Bancroft and Steven method³⁶. Following that, the sections were examined under the microscope for histopathological changes and photomicrographs were taken.

Magnetic Resonance Imaging (MRI) examination: All groups (including the control group) underwent an MRI scan (Closed MRI, PHILIPS 1.5 TESLA) at Smart Scan Radiology Center-Cairo, Egypt; all experimental Ethics procedures were achieved.

Once placed on the handling platform, each mouse was fixed in a supine recumbence position and then introduced into the RF coil inside the MRI gantry. Many images and sequences are taken for all rats to evaluate the effect of MP-SeNPs against BaP-induced mammary injury in rats, including CORONAL T1, T2, SAGITAL T1, T2 and STAIR.

Statistical analysis: For each of the eight separate determinations, the results were expressed as Mean $\pm$ SD. SPSS/18 Software was used to perform statistical analysis on all of the data³⁷. One-way analysis of variance was used to test hypotheses, followed by the least significant difference test. The p-values of 0.05 are considered statistically significant.

RESULTS

TEM analysis shows that MP-SeNPs had size of around 134.69 ± 8.24 nm with negative zeta potential of $+26.04$ (Fig. 1).

Figure 2a-b showed that the IC_{50} of MP-SeNPs against MCF7 breast carcinoma cell line = $89.57 \mu g mL^{-1}$.

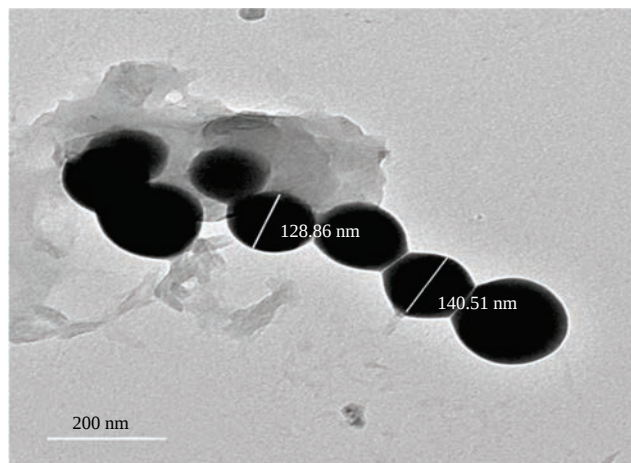


Fig. 1: TEM analysis of MP-SeNPs

The results were reported in Table 2 showed that oral administration of MP-SeNPs in doses of 750, 1000, 1250, 1500, 1800 and 2000 mg kg^{-1} b.wt., resulted in mortalities of 0, 1, 3, 6, 8 and 10 respectively. The dose of MP-SeNPs that killed half of the rats (LD_{50}) was 1435 mg kg^{-1} b.wt.

Table 3 show plasma IL-2, IL-6 and IL-10 levels. Oral administration of BaP led to significant increase of IL-2 and IL-6 while significantly decreasing of IL-10 plasma level as compared with the normal control group ($p < 0.05$), indicating acute mammary tissue injury. Treatment of animals with MP-SeNPs at 28.7 and 71.75 mg kg^{-1} b.wt., as well as vit. C (1 g kg^{-1} b.wt.) significantly reduced the level of IL-2 and IL-6 as well as significantly increased IL-10 ($p < 0.05$), as compared with the BaP treated group. The effect of MP-SeNPs at two different doses more pronounced than vit. C.

A significant elevation in mammary tissue TNF- α and iNOs levels ($p < 0.05$) in BaP treated rats when compared with control group was revealed in Table 4. The administration of MP-SeNPs 28.7 and 71.75 mg kg^{-1} b.wt., as well as vit. C (1 g kg^{-1} b.wt.) showed significantly decreased in TNF- α and iNOs levels relative to BaP treated the group of rats after 30 days ($p < 0.05$).

A significantly ($p < 0.05$) decreased of mammary tissue antioxidant parameters SOD, GPx and GSH while significantly increasing mammary tissue MDA, were observed in the BaP-treated rats as compared with the normal control group ($p < 0.05$), indicating acute mammary tissue damage (Table 5). MP-SeNPs 28.7 and 71.75 mg kg^{-1} b.wt., as well as vit. C (1 g kg^{-1} b.wt.) treatment significantly enhanced the lung enzymes activities SOD, GPx and GSH in rats and decrease MDA level, as compared to the BaP-treated group ($p < 0.05$).

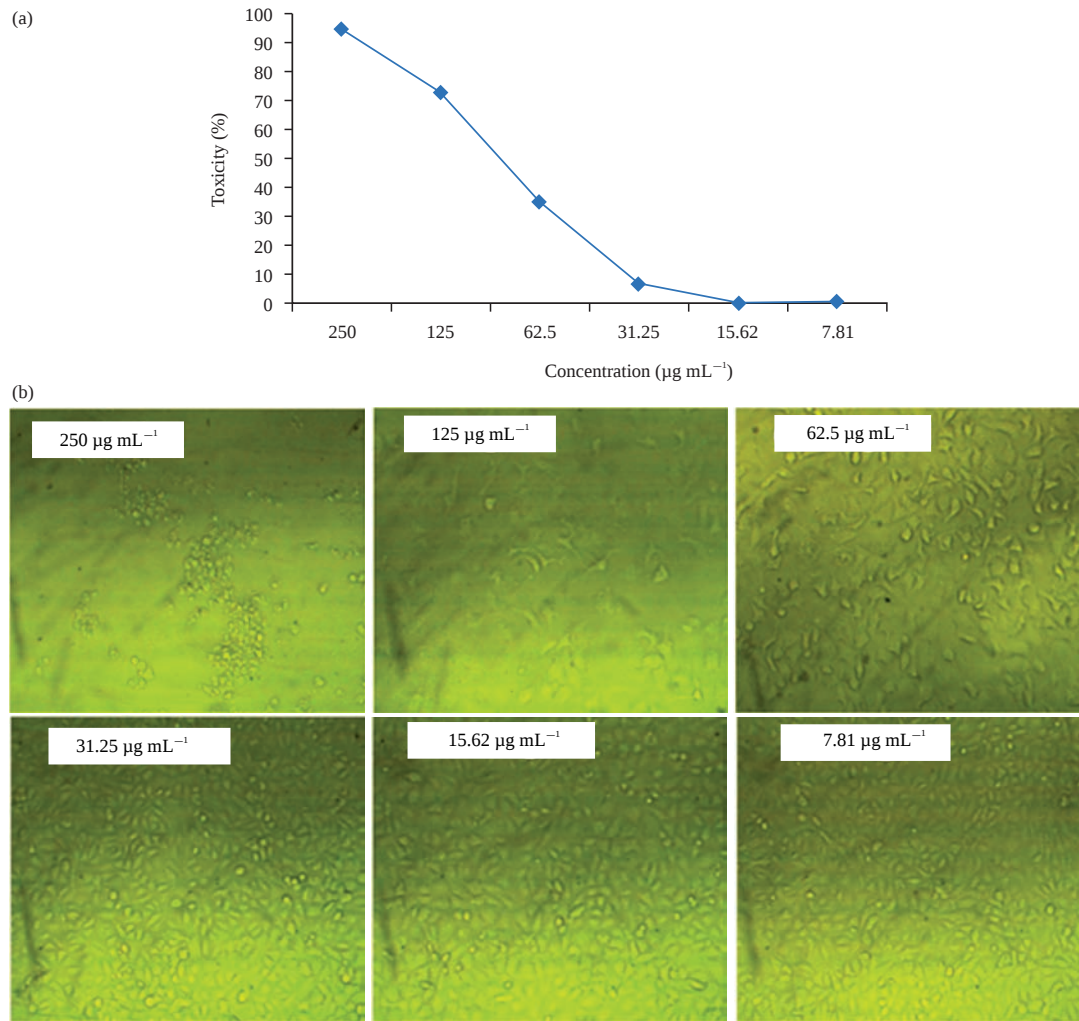


Fig. 2(a-b): Determination of MP-SeNPs cytotoxicity on cells (MTT protocol)

(a) Viability (%) of breast cancer cells (MCF7) after treatment with of MP-SeNPs was assessed by the MTT assay after 48 hrs of treatment with DMSO and
(b) Effect of MP-SeNPs on MCF7 breast carcinoma cell line at different concentrations

Table 2: Determination of LD₅₀ of resveratrol nano emulsion given orally in adult rats

Group number	Dose (mg kg ⁻¹)	No. of animals/group	No. of dead animals	(Z)	(d)	(Z.d)
1	750	10	0	0.5	250	125
2	1000	10	1	2.0	250	500
3	1250	10	3	4.5	250	1125
4	1500	10	6	7.0	300	2100
5	1800	10	8	9.0	200	1800
6	2000	10	10	0	00	5650

$$LD_{50} = Dm - \left[\frac{\sum(Z.d)}{n} \right] \text{ and } LD_{50} = 2000 - \left[\frac{5650}{10} \right] = 1435 \text{ mg kg}^{-1} \text{ b.wt.}$$

Table 3: Effect of MP-SeNPs on plasma IL-2, IL-6 and IL-10 of BaP-treated rats

Groups	Treatment description	IL-2 (pg mL ⁻¹)	IL-6 (pg mL ⁻¹)	IL-10 (pg mL ⁻¹)
I	Normal control A	15.76±2.09 ^a	8.31±0.74 ^a	29.63±3.80 ^d
II	BaP (20 mg kg ⁻¹ b.wt.)	52.60±4.51 ^e	31.25±3.18 ^d	10.48±1.07 ^a
III	MP-SeNPs (28.7 mg kg ⁻¹ b.wt.)+BaP(20 mg kg ⁻¹ b.wt.)	34.25±3.74 ^c	16.50±1.62 ^b	18.94±1.86 ^b
IV	MP-SeNPs (71.75 mg kg ⁻¹ b.wt.)+BaP (20 mg kg ⁻¹ b.wt.)	22.18±3.10 ^b	12.84±2.20 ^a	24.25±2.61 ^c
V	Vit. C (1 g kg ⁻¹ b.wt.)+BaP (20 mg kg ⁻¹ b.wt.)	40.66±3.26 ^d	23.37±4.33 ^c	15.90±1.58 ^b

Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at p ≤ 0.05

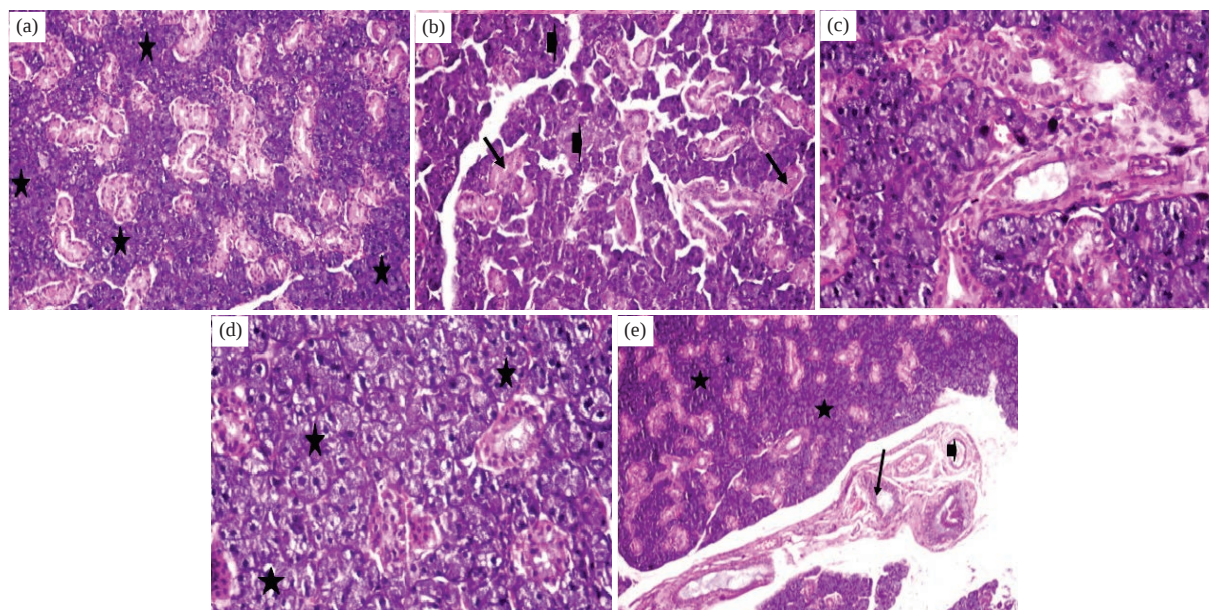


Fig. 3(a-e): Sections stained with hematoxylin and eosin (H and E; 400 $\times$) histological examination of rats' mammary tissue of different groups compared to control group

(a) Group I: Normal control, (b) Group II: BaP (20 mg kg⁻¹ b.wt.), (c) Group III: Was administrate MP-SeNPs (28.7 mg kg⁻¹ b.wt.)+BaP (20 mg kg⁻¹ b.wt.), (d) Group IV: Was administrate MP-SeNPs (71.75 mg kg⁻¹ b.wt.)+BaP (20 mg kg⁻¹ b.wt.) and (e) Group V: Was administrate Vit. C (1 g kg⁻¹ b.wt.)+BaP (20 mg kg⁻¹ b.wt.)

Table 4: Effect of MP-SeNPs on levels of mammary tissue TNF- α and iNOs of BaP-treated rats

Groups	Treatment description	TNF- α (pg mL ⁻¹)	iNOs (pg mL ⁻¹)
I	Normal control A	9.65 $\pm$ 0.93 ^a	21.09 $\pm$ 2.76 ^a
II	BaP (20 mg kg ⁻¹ b.wt.)	44.87 $\pm$ 3.22 ^e	62.27 $\pm$ 6.08 ^e
III	MP-SeNPs (28.7 mg kg ⁻¹ b.wt.)+BaP (20 mg kg ⁻¹ b.wt.)	23.91 $\pm$ 1.27 ^c	38.64 $\pm$ 4.18 ^c
IV	MP-SeNPs (71.75 mg kg ⁻¹ b.wt.)+BaP (20 mg kg ⁻¹ b.wt.)	18.70 $\pm$ 1.60 ^b	31.40 $\pm$ 4.81 ^b
V	Vit. C (1 g kg ⁻¹ b.wt.)+BaP (20 mg kg ⁻¹ b.wt.)	32.16 $\pm$ 1.64 ^d	44.10 $\pm$ 6.90 ^d

Data shown are Mean $\pm$ standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at $p \leq 0.05$

Table 5: Effect of MP-SeNPs on levels of mammary tissue superoxide dismutase (SOD) and glutathione peroxidase (GPx), malondialdehyde (MDA) and reduced glutathione (GSH) of BaP-treated rats

Groups	Treatment description	SOD	GPx	MDA (nmol mg ⁻¹ protein)	GSH (mg % ⁻¹)
I	Normal control A	19.54 $\pm$ 0.66 ^d	17.65 $\pm$ 1.08 ^d	0.98 $\pm$ 0.08 ^a	12.25 $\pm$ 1.11 ^d
II	BaP (20 mg kg ⁻¹ b.wt.)	4.37 $\pm$ 0.52 ^a	6.49 $\pm$ 0.60 ^a	2.84 $\pm$ 0.30 ^c	3.17 $\pm$ 0.40 ^a
III	MP-SeNPs (28.7 mg kg ⁻¹ b.wt.)+BaP (20 mg kg ⁻¹ b.wt.)	10.55 $\pm$ 1.06 ^b	11.69 $\pm$ 0.67 ^b	1.08 $\pm$ 0.07 ^b	8.25 $\pm$ 0.61 ^c
IV	MP-SeNPs (71.75 mg kg ⁻¹ b.wt.)+BaP (20 mg kg ⁻¹ b.wt.)	15.65 $\pm$ 1.25 ^c	13.40 $\pm$ 0.91 ^c	0.99 $\pm$ 0.08 ^a	10.89 $\pm$ 0.85 ^c
V	Vit. C (1 g kg ⁻¹ b.wt.)+BaP (20 mg kg ⁻¹ b.wt.)	11.08 $\pm$ 0.74 ^b	8.70 $\pm$ 0.48 ^a	1.25 $\pm$ 0.70 ^b	8.53 $\pm$ 0.54 ^b

Values are given as Mean $\pm$ SD for groups of six animals each. Values data followed by the same letter are not significantly different at $p \leq 0.05$. SOD: One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1 min mg⁻¹ protein, GPx: GSH (μ g) consumed/min mg protein

Histopathological examination of mammary tissue sections of the normal group (I) showed normal mammary tissue; note the normal round mammary acini (*) lined with cuboidal epithelium, (H and E X200) (Fig. 3a). On the other hand, in the mammary tissue of BaP-treated control group (II), histological examination showing massive necrosis in the mammary acini (arrow head) and ducts (arrows), (H and E X200) (Fig. 3b).

Histopathological examination also showed showing multi-focal areas of regenerated mammary acini (arrows) with absence of the interlobular blood vessels congestion and lactiferous duct hyperplasia (H and E X400) BaP-induced mammary tissue toxicity by MP-SeNPs 28.7 and 71.75 mg kg⁻¹ b.wt., as compared with the BaP-treated group and showed almost the same records as Groups III and IV (Fig. 3c-d).

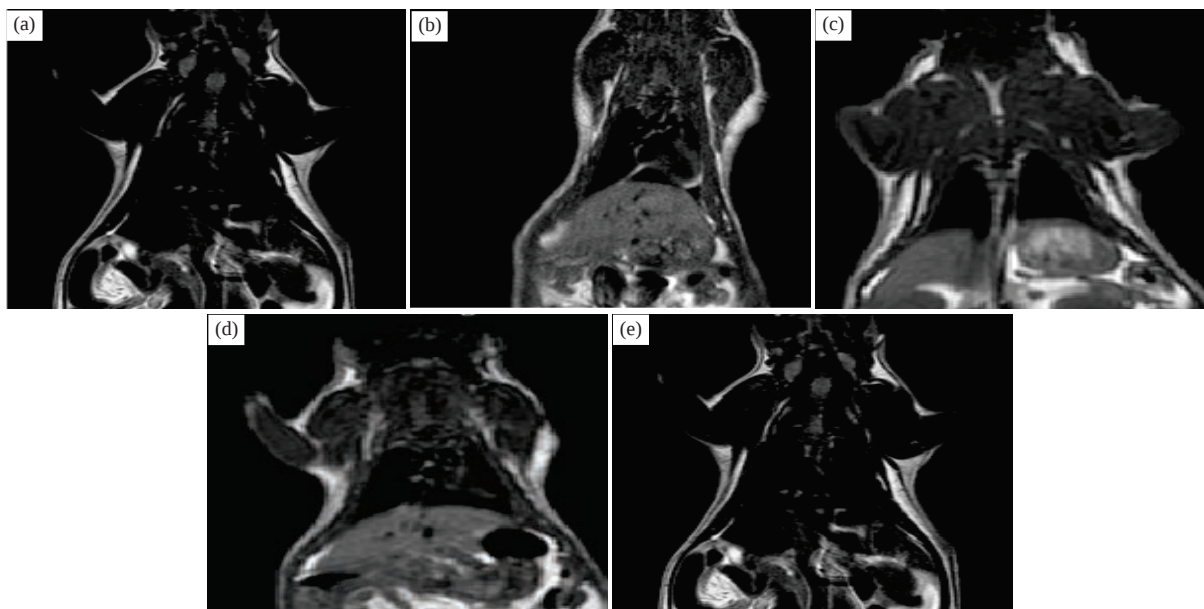


Fig. 4 (a-e): Magnetic resonance imaging (MRI) examination of rats mammary tissue of different groups compared to control group

(a) Group I: Normal control, (b) Group II: BaP (20 mg kg⁻¹ b.wt.), (c) Group III: Was administrate MP-SeNPs (28.7 mg kg⁻¹ b.wt.)+BaP (20 mg kg⁻¹ b.wt.), (d) Group IV: Was administrate MP-SeNPs (71.75 mg kg⁻¹ b.wt.)+BaP (20 mg kg⁻¹ b.wt.) and (e) Group V: Was administrate Vit. C (1 g kg⁻¹ b.wt.)+ BaP (20 mg kg⁻¹ b.wt.)

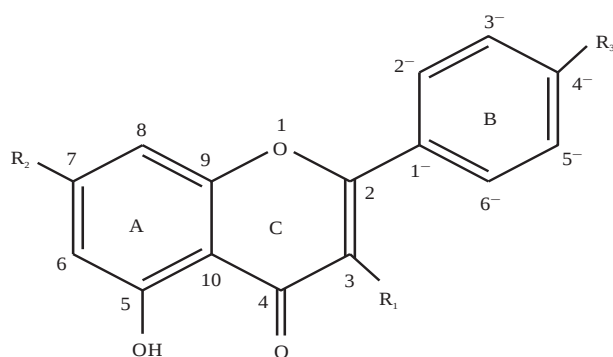


Fig. 5: Structure of flavonoid derivatives from *Moringa*⁴⁵

Group (V) all samples of BaP treated rats showing massive regeneration of the mammary acini (*) but the interlobular tissue still showing congestion in the interlobular blood vessels (arrow head) and hyperplasia in the lactiferous duct (arrow), (H and E X200) by treatment with vit. C (Fig. 3e).

Magnetic Resonance Imaging (MRI) examination of rats mammary tissue of the normal group (I) showed mammary parenchyma was free from any masses, with no abnormal vascularization and with normal intensity (Fig. 4a).

Also, the mammary tissue of BaP-treated control group (II), MRI showed a mammary mass that appear as irregular, heterogenous, with alternative intensity (Fig. 4b).

MRI also showed coarse texture and the abnormal focal lesion was regressed at different levels of BaP-bearing rats treated with MP-SeNPs 28.7 mg kg⁻¹ b.wt., as compared with the BaP-treated rats (group III) (Fig. 4c). In addition, mammary tissue examination by MRI from BaP-treated rats with MP-SeNPs 71.75 mg kg⁻¹ b.wt., group (IV) showed marked improvement with no injury no inflammatory cells was evaluated (Fig. 4d).

In addition, all samples of BaP-treated rats showed moderate lesion was regressed by treatment with Vit. C 1 g kg⁻¹ b.wt. group (V) (Fig. 4e).

DISCUSSION

The present study investigated the protective role of MP-SeNPs against BaP-mammal tissue damage in the target organs of rats. Although active metabolites BaP cause DNA damage in various tissues. Hence, we investigated whether MP-SeNPs suppressed BaP-induced ROS and cytokines formation in the target organs. Taken together, this study provides new insights into the novel mechanisms of MP-SeNPs acting on the regulation of BaP metabolism, which may be important for understanding BaP-induced carcinogenesis. Our data indicated that MP-SeNPs administration significantly inhibited BaP-induced mammal tissue by inhibiting BaP

metabolism. Therefore, we suggest that dietary MP-SeNPs may inhibit BaP-induced mammal tissue tumorigenesis by reducing the formation of active BaP metabolites, as well as increasing the detoxification of BaP metabolites.

Several chemo preventive agents that appeared to be highly promising after preclinical safety and efficacy studies have failed in clinical trials over the last decade³⁸. Because of these failures, there is a renewed focus on understanding the mechanistic aspects of a potential chemo preventive agent before it is tested in clinical trials. *M. peregrina's* chemo preventive efficacy has been demonstrated in a variety of experimental models^{23,39}.

In the present study, the concentration of MP-SeNPs was found to increase with increasing extract concentration, i.e., increasing concentration of reducing agent⁴⁰. Also, attributed increased MP-SeNPs concentration to the availability of more reducing biomolecules for the reduction of Se⁻². The *in vitro* determination of anticancer activity of various concentrations of MP-SeNPs showed IC₅₀ against Mcf7 breast carcinoma cell line is 89.57 µg mL⁻¹. *Moringa peregrina* extracts may produce its anti-cancer effect due to the presence of tannins⁴¹, flavonoids⁴², saponins⁴³, unsaturated sterols and/or triterpenes⁴⁴. *In vivo* and *in vitro* anti-cancer effects have been reported for several flavonoids. Thus, some flavones have been found to be active in different experimental models. B-ring substituted flavones of this type are capable of inhibiting inflammation⁴⁶ (Fig. 5). Nevertheless, the influence of *Moringa peregrina* extracts on the inhibition of inflammation was not yet completely understood:

- **Compound 1:** Kaempferol 3-O-(2'',3''-diacetylglucoside), R₁ = O-2'',3''-Diacetylglucoside, R₂ = OH, R₃ = OCH₃
- **Compound 2:** Kaempferol 3-O-(2''-O-galloylrhamnoside), R₁ = O-[Glucosyl-(1'''-2'')]-[rhamnosyl(1'''-6'')]-glucoside, R₂ = O-rhamnose, R₃ = OH
- **Compound 3:** Kaempferol 3-O-(2''-O-galloyl rutinoside)-7-O-α-rhamnoside, R₁ = O-(2''-Galloylrhamnoside), R₂ = OH, R₃ = OCH₃

In the current study, BaP-treated rats had significantly higher levels of IL-2, IL-6, TNF-α, iNOs and MDA during the experimental period than normal control rats. BaP, on the other hand, lowers plasma levels of IL-10 as well as mammary tissue SOD, GPx and GSH which is a powerful protective factor against the development of endogenous antioxidant effect²³.

Evidence suggested that free radicals, oxidative stress and lipid peroxidation are present in organ damage⁴⁵. It has been demonstrated that in chronic lung toxicity, increased lung concentrations of TNF-, IL-6, iNOS and MDA, as well as decreased activity of IL-10,

Furthermore, oral administration of MP-SeNPs at 28.7 and 71.75 mg kg⁻¹ b.wt., as well as Vit.C at 1 g kg⁻¹ b.wt., provided significant protection against BaP-induced inflammation and free radicals production via normalize mammary tissue levels of TNF-α, IL-2, IL-6, IL-10 and iNOS in the BaP-treated group and induce mitochondrial toxicity and free radical generation⁴⁶.

The most extensively studied mitogenic and fibrogenic factors are TNF-, IL-6, IL-10 and iNOS. MP-SeNPs can also inhibit the expression of proinflammatory cytokines⁴⁷. Taken together, these findings suggested that MP-SeNPs' antifibrotic effect is linked to the inhibition of mitogenic and/or fibrogenic signalling. TNF-α has been shown to stimulate the formation of NO⁴⁸.

MP-SeNPs are a powerful reactive oxygen species (ROS) scavenger⁴⁹ and normalized the oxidative stress biomarkers SOD, GPx, GSH and MDA, resulting in decreased oxidative stress, which contributes to nicotine's suppression of lung inflammation. After nicotine administration, there was a significant decrease in lung SOD, GPx and GSH activity in the current study.

In the present study, the observed depletion of cytokines level and elevation in the activities of these antioxidant enzymes in mammal tissues of MP-SeNPs treated rats compared to the untreated ones reflects the antioxidant and anti-inflammatory potential of MP-SeNPs. A number of investigators have shown those MP-SeNPs containing flavonoids, tannins and other polyphenolic compounds (e.g., coumarins), triterpenoids and a host of other secondary plant metabolites possess analgesic, antioxidant and anti-inflammatory properties in various experimental animal models^{23,40}.

MP-SeNPs have a mammal tissue-protective effect, according to histological studies and MRI examinations. Because mammal tissue proliferation is an early event in toxicity-related changes, the attenuation of mammal tissue injury and fibrosis in rats by MP-SeNPs could be associated with a reduction in inflammatory response and induction of endogenous antioxidant enzymes. To the best of my knowledge, the prophylactic effect of MP-SeNPs against BaP-induced mammal tissue toxicity has never been reported and this study may be the first of its kind.

CONCLUSION

The current study found that MP-SeNPs have potent anticancer activity against Mcf7 breast carcinoma cell line and produce protective activity against BaP-induced mammal injury by normalizing the levels of oxidative stress biomarkers and inflammatory mediator gene expression.

Antioxidant and anti-inflammatory effects of MP-SeNPs due to the presence of flavonoids, tannins and other polyphenolic compounds (e.g., coumarins), triterpenoids and a variety of other secondary plant metabolites.

SIGNIFICANCE STATEMENT

This study discovers the protective activity of MP-SeNPs that can be beneficial for the treatment of mammal tissue toxicity. This study will help the researcher to uncover the critical areas that focus on evaluate of MP-SeNPs as a promising new agent in the treatment of certain type of cancers that many researchers were not able to explore. Thus, a new theory to explain the correlation between protective activity of MP-SeNPs and the degree cytokines suppression in mammal tissue may be arrived at.

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