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Prophylactic and Therapeutic Efficacy of *Rhus coriaria L.* (Sumac) Against Benzo[a]Pyrene-Induced Systemic and Reproductive Toxicity: A Comparative Study Against Vitamin E

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Abstract

Background: Benzo[a]pyrene is a dietary carcinogen in grilled and smoked foods that causes multiorgan toxicity via oxidative stress. Despite the traditional consumption of *Rhus coriaria L.* (Sumac) with BaP-rich foods, no study has addressed the protective efficacy of Sumac against BaP-induced toxicity and its superiority over vitamin E. Therefore, the present study aimed to investigate these aspects.

Methods: Seventy male C57BL/6 mice were randomly assigned to 14 groups: control, BaP (20 mg/kg), BaP + vitamin E (250 mg/kg), and BaP + hydroalcoholic extract of Sumac at three doses (50, 150, and 300 mg/kg), which were administered under two separate regimens, coadministration (therapeutic model) and pretreatment (prophylactic model). After 4 weeks, oxidative stress markers, hepatorenal function, lipid profiles, testosterone levels, sperm quality parameters (motility, concentration, DNA integrity, chromatin status, and oxidative indices), and histopathology of the liver and kidney were evaluated.

Results: BaP exposure significantly increased the levels of MDA, liver enzymes (ALT, AST, and ALP), total bilirubin, BUN, and creatinine; dyslipidemia; sperm DNA damage; sperm lipid peroxidation; protamine deficiency; and intracellular ROS while decreasing the TAC, albumin, testosterone, and total and progressive sperm motility. Vitamin E has shown limited efficacy, improving only the levels of albumin, cholesterol, LDL, and BUN; sperm lipid peroxidation; and protamine deficiency while failing to affect the levels of oxidative markers, liver enzymes, bilirubin, triglycerides, creatinine, and testosterone; and most sperm quality parameters.

In contrast, the Sumac extract, especially at higher doses (150 and 300 mg/kg), dose-dependently restored the antioxidant status, normalized liver and kidney function, corrected the lipid profile, and significantly improved chromatin integrity and sperm motility. These effects were markedly more pronounced with the prophylactic regimen than with the therapeutic regimen. Notably, while Sumac coadministration induced only mild hepatocellular degeneration, the pretreatment regimen unexpectedly led to severe hepatocellular degeneration, despite normal or decreased serum liver

enzyme levels. This apparent contradiction is explained by the key physiological principle that liver enzyme leakage occurs only in necrosis (membrane damage) and not in degeneration (membrane integrity preservation). Therefore, normal serum liver enzyme levels are by no means equivalent to those of healthy liver tissue. Furthermore, Sumac significantly reduced BaP-induced proliferative glomerulonephritis and tubular degeneration in the kidney.

Conclusion: The Sumac extract is superior to vitamin E in preventing BaP-induced systemic and reproductive toxicity because it reduces oxidative stress, preserves sperm chromatin, improves hepatorenal function, and corrects the lipid profile. These findings support Sumac as an effective, safe, and accessible dietary chemopreventive agent.

Keywords: Benzo[a]pyrene, oxidative stress, DNA damage, *Rhus coriaria L.*, Sumac, reproductive toxicity, *nephrohepatotoxicity, lipid profile*

1. Introduction

Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon (PAH) composed of five fused benzene rings. It is formed via the incomplete combustion of organic matter and is commonly found in coal tar, cigarette smoke, and charred foods [1]. Following metabolic activation by cytochrome P450 enzymes, particularly CYP1A1 and CYP1B1, BaP is ultimately converted to the carcinogenic metabolite BaP-r-7,t-8-dihydrodiol-t-9,10-epoxide, which forms DNA adducts and induces mutagenesis [2]. These genotoxic and oxidative stress–mediated mechanisms contribute to apoptosis, carcinogenesis, and tissue injury in multiple organs, including the respiratory, digestive, and reproductive systems [3]. Chronic exposure has also been associated with hepatic inflammation and fibrosis [4], as well as nephrotoxic and reproductive toxic effects in experimental models [5]. Given the well-established carcinogenic and systemic toxicity of BaP, increasing attention has been given to dietary-based preventive and therapeutic strategies to mitigate its adverse effects. *Rhus coriaria* L. (sumac) is a widely used spice in Mediterranean and Middle Eastern diets and is commonly consumed with grilled meats and kebabs [6]. This traditional dietary habit represents a potential link between nutrition and chemoprevention. Sumac is rich in bioactive phytochemicals, particularly flavonoids, tannins, and polyphenolic compounds, which are responsible for its potent antioxidant and anti-inflammatory properties [6, 7]. These constituents may counteract the oxidative damage induced by food-derived carcinogens.

Despite the widespread use of Sumac in traditional diets, limited scientific evidence exists regarding its comparative protective efficacy against BaP-induced multiorgan toxicity, particularly in the context of reproductive damage. Moreover, most previous studies have focused primarily on therapeutic interventions, while the potential role of early or prophylactic nutritional intervention remains underexplored. In addition, direct comparative studies between Sumac extract and established antioxidants such as vitamin E are scarce.

Therefore, the present study hypothesized that the hydroalcoholic extract of Sumac may exert protective effects against BaP-induced systemic and

reproductive toxicity through its antioxidant and anti-inflammatory properties. Furthermore, both prophylactic (pretreatment prior to BaP exposure) and coadministration (simultaneous administration of BaP) were evaluated to determine their efficacy at different stages of exposure. Finally, the protective potential of Sumac was compared with that of vitamin E as a reference antioxidant to determine whether Sumac has comparable or superior effects.

2. Materials and methods

2.1. Ethical Approval and Animal Housing

All these experiments in animals were approved by the Institutional Animal Ethics Committee of Islamic Azad University, Shahrekord Branch, Iran (IR.IAU.SHK.REC.2024.035), and conducted on the basis of ARRIVE guidelines. Seventy C57BL/6 male mice (6 weeks of age, 15–20 g) were obtained from the Pasteur Institute of Iran and allowed to acclimatize in individual ventilated cages for seven days under conditions of constant temperature (25±1°C) humidity (55±5%) and a 12 h light/dark cycle. The animals were given normal rodent food and sterile water ad libitum and were housed in standard cages enriched with various elements for behavioral enrichment, e.g., plastic tunnels, wooden blocks and nesting papers, with the aim of minimizing the stereotypy of the housing conditions and the stress of isolation over the duration of the experiment. [8]. The allocation of the mice to the different treatment groups is detailed in **Section 2.4**. All histopathology and biochemistry analyses were performed in a blinded manner; the researchers did not know which treatment group the samples belonged to until all analyses were completed. The mice were checked daily for humane endpoints (i.e., >20% weight loss or lethargy); none of the mice required euthanasia within a 4-week duration of the study.

2.2. Chemicals and reagents

All the chemicals and reagents were of analytical grade (≥99% purity, Merck, Darmstadt, Germany), and double-distilled water was used for dilution in

all the cases. BaP with a purity of ≥96% was obtained from Sigma–Aldrich. To maintain stability, the BaP chemical was diluted in pharmaceutical grade corn oil, which was then sealed in the dark by amber glass vials and incubated at 40°C. To avoid photodegradation, the working solution was made fresh weekly, and a uniform toxicological dosage was given for the entire 4-week study.

2.3. Preparation of hydroalcoholic extract of Sumac

The dried fruits of *Rhus coriaria* L. used in this study were collected from gardens margin (cultivated area) in Naghan, Mount Kallar, Chaharmahal and Bakhtiari province, Iran (31.9532°N, 50.7328°E; altitude: 2250 m a.s.l.) on 05 July 2025. A voucher specimen was prepared from the same collected material and was formally identified by Dr. Amin Zeraatkar. The voucher specimen was deposited at the Chaharmahal and Bakhtiari Agricultural and Natural Resources Research and Education Center Herbarium (Dimeh Herbarium) under the voucher number 7772. As *Rhus coriaria* L. is a widely cultivated species and the material was collected from a cultivated area (gardens margin), no specific collection permit from natural habitats was required. The study complies with all relevant institutional, national, and international guidelines and legislation, and *Rhus coriaria* L. is not listed as a species at risk of extinction according to the IUCN Red List.

The fruit of Sumac was ground into a fine powder. The hydroalcoholic extract was prepared via Soxhlet extraction via 96% ethanol at 78–80°C for 12 hours with a solid-to-solvent ratio of 1:10 (w/v). The final extract was dried and stored for subsequent use. For animal treatment, the dried extract was dissolved in distilled water to achieve three different doses of 50, 150, and 300 mg/kg body weight, corresponding to low, medium, and high doses, respectively.

2.4. Experimental Design

A total of 70 male mice were randomly assigned to 14 experimental groups (n=5 per group) via a computerized random number generator. The sample

size was determined via a power analysis performed via G*Power software (version 3.1.9.7). On the basis of one-way ANOVA with an expected large effect size (f=0.40), a significance level of 0.05, and a statistical power of 0.80, a minimum total sample size of 70 animals was calculated. This design ensured adequate statistical power to detect biologically relevant differences while adhering to the principles of the 3Rs (reduction), thereby minimizing the unnecessary use of animals.

A fixed dose of 20 mg/kg body weight BaP was used, as previous studies have demonstrated its ability to induce multiorgan toxicity without causing acute lethality. In addition, vitamin E was administered at a dose of 250 mg/kg as a reference antioxidant control on the basis of earlier experimental evidence. Three doses of Sumac (50, 150, and 300 mg/kg) were evaluated to establish a dose–response relationship.

The 14-group design compared these doses under two treatment regimens: prophylactic (pretreatment prior to BaP exposure) and coadministration (simultaneous administration with BaP). All treatments were administered once daily by oral gavage between 08:00 and 10:00 AM for 4 weeks, unless otherwise stated [9]. A summary of group allocation is presented in **Table 1.**

2.5. Serum Biochemical Analysis

The mice were fasted for 12 h and then deeply anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (12 mg/kg). Blood was then collected into plain tubes via cardiac puncture while the animals were still under deep anaesthesia. The mice were subsequently euthanized by cervical dislocation in accordance with the AVMA Guidelines for the Euthanasia of Animals. The blood samples were centrifuged at 3000 × g for 15 minutes, and the serum was stored at −80°C for analysis [10].

2.5.1. ***Oxidative stress markers***

- The total antioxidant capacity (TAC) of the serum was measured via the ferric reducing ability of plasma (FRAP) assay [11]. This method was adapted to assess oxidative stress parameters as previously described in similar reproductive and biochemical studies [12]. In

brief, the assay measures the change in absorbance at 593 nm in terms of the reduction of ferric tripyridyltriazine (Fe3+-TPTZ) to its ferrous form (Fe2+).

- Superoxide dismutase (SOD) activity was evaluated by pyrogallol autoxidation inhibition [13]. The enzymatic activity was expressed as U/mg protein (units of activity) throughout the experiment. The activity of SOD in the current work was characterized as the quantity of enzyme required for the 50% reduction in the autoxidation rate of pyrogallol. Glutathione peroxidase (GPx) activity was measured on the basis of the rate of NADPH oxidation at 340 nm via an established enzyme assay methodology, as previously described, and was adopted for reproductive research to test the level of oxidative stress. [13]. In brief, GPx catalyzes the oxidation of glutathione by cumene hydroperoxide, and in the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with the concomitant oxidation of NADPH to $NADP^+$.
- Malondialdehyde (MDA) levels were identified via the thiobarbituric acid reactive substances (TBARS) assay [14].

*2.5.2. **Liver injury tests***

The activities of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were determined spectrophotometrically via commercial kits [15–17]. The total bilirubin concentration was measured via the diazo method, and the albumin concentration was determined via the bromocresol green method [18]. Fasting blood sugar (FBS) was measured via the enzymatic glucose oxidase–peroxidase (GOD–POD) method via animal biochemistry diagnostic kits (Ziest Chem Diagnostics, Tehran, Iran).

2.5.3. Lipid Profile

Triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels were estimated via the precipitation method [18].

2.5.4. ***Renal function tests***

Serum creatinine was measured via the Jaffe method, and blood urea nitrogen (BUN) was evaluated via the urease-Berthelot method [18].

2.5.5. Serum Testosterone Measurement

The amount of testosterone in the serum was measured via a testosterone ELISA kit from commercial resources (Cat. No. KGE010, R&D Systems, Minneapolis, MN, USA) with a kit manual as instructed by the manufacturer. The absorbance was read via a spectrophotometer, and the testosterone content was extrapolated from a standard curve [13].

2.6. Sperm parameters

2.6.1. Measurement of Sperm ***Concentrations***

The sperm concentration was evaluated with a specially designed sperm count chamber (Sperm Meter; Sperm Processor, Aurangabad, India) on the basis of the standard procedure described in the WHO laboratory manual for human semen analysis [19]. In brief, 10 μL of a sperm sample collected from the epididymis was diluted with 90 L of saline. Then, 10 μL of this suspension was loaded onto the chamber very carefully. For each sample, sperm counts were conducted over 25 predesignated squares. Counting and calculation procedures were performed according to previously mentioned reproductive studies [20]. The final concentration of sperm was given in millions per millilitre ($\times 10^6$/mL).

2.6.2. Evaluation of Sperm Motility

Ten microliters of epididymal sperm were placed on a slide that was maintained at 37°C at room temperature. A total of 200 sperm were assessed via a light microscope (CX31 OLYMPUS, Japan) at 40×. The percentage motility was assessed by the percentage of motile spermatozoa, including progressive and nonprogressive spermatozoa, and was divided by the total number of sperm counted and multiplied by 100 [19].

2.7. Oxidative and Molecular Assessments of Sperm Damage

*2.7.1. Analysis of **the** Histone-to-Protamine Transition*

Aniline blue staining: Residual histone assessment was performed via aniline blue staining of the sperm samples as outlined by Dadoune et al. (1988) [21]. A 20-µL aliquot of epididymal sperm cells was smeared onto a slide, air-dried, and fixed with 3% glutaraldehyde prepared in 0.2 M phosphate buffer (pH 7.2) for 2 hours. After that, the slides were dried one more time and then incubated in a 5% aniline blue mixture prepared with 4% acetic acid (pH 3.5) for approximately 90 minutes. Afterwards, under a light microscope at 100x magnification, 200 spermatozoa per sample were selected at random and evaluated. Those spermatozoa that showed strong, dark blue staining were counted as having abnormal chromatin condensation, reported as AB+.

CMA3 Staining: As Bianchi et al. (1993) [22] described earlier, the adequacy of protamine incorporation was assessed through CMA3 staining. For this purpose, incubation with 100 l of CMA3 solution (0.25 mg/ml) was performed for 3 min in 1:20 ml of dilution buffer postdistillation, followed by air drying and coverslip mounting on the slides. Twenty microliters of epididymal sperm suspension was dispersed in phosphate-buffered saline into the smear and stored in Carnoy's fixative at 4°C for 5 min. The slides were then washed with 2 washes of PBS, air dried and coverslipped. A minimum of 200 sperm per slide were examined via a fluorescence microscope (Olympus BX51, Tokyo, Japan) fitted with filters at 460–470 nm. The sperm that emitted bright yellow fluorescence were classified as CMA3 positive, which is indicative of protamine deficiency.

290

291 *2.7.2. Assessment of sperm DNA damage*

292 **Acridine Orange Staining:** The degree of DNA damage to the sperm was
293 determined on the basis of Tejada et al. (1984) [23]. An aliquot of 20 µL of
294 epididymal sperm was laid on a slide and then festooned with Carnoy's
295 fixative at -4°C for 2 hours. The slides were then washed in PBS and stained
296 with fresh acridine orange solution in citrate phosphate buffer (80 ml of 0.1
297 M citric acid and 5 ml of 0.3 M NaH2PO4, pH 2.5) for 90 minutes. The stain
298 was washed off the slides, which were then washed again in PBS. A
299 minimum of 200 sperm on each slide were analysed under a fluorescence
300 microscope at 460–470 nm, and any fluorescent green was labelled as having
301 intact DNA and anything orange–red as having DNA damage.

302

303 *2.7.3. Measurement of Sperm Lipid Peroxidation*

304 Lipid peroxidation in sperm cells was assessed via the use of fluorescent
305 BODIPY C11 dye as described by Guthrie et al. (2012) [24]. Two million
306 sperm were incubated at 37°C with 5 M BODIPY C11 for 30 min. The cells
307 were then washed two times with PBS and centrifuged at 500 × g for 5 min
308 to form a cell pellet. Lipid peroxidation was then quantified via flow
309 cytometry via a FACSCalibur system (Becton Dickinson, San Jose, CA, USA).
310 The percentage of sperm that showed BODIPY fluorescence was quantified.
311 The positive control used was H_2O_2.

312

313 *2.7.4. Assessment of Cytosolic reactive oxygen species (ROS) in Sperm*

314 The DCFH-DA assay was previously described by Mahfouz et al. [25] to
315 measure the cytosolic levels of ROS in spermatozoa. DCFH-DA is an
316 intracellular penetrant dye that, once formed and de-esterified in the cell,
317 becomes retained within the cytosol and reacts with the ROS, chiefly H2O2
318 in this case, to yield the fluorescent compound 2,7-
319 dichlorodihydrofluorescein (DCF). The sperm suspension was incubated in
320 PBS for 30 min with 0.5 M DCFH-DA. Flow cytometric analysis was

performed using a FACSCalibur (Becton Dickinson, San Jose, CA, USA). At least 10000 events were obtained for each sample. The electronic noise and the cell debris were gated out by the forward scatter (FSC) and side scatter (SSC) profiles of the sperm population. The fluorescence was detected in the FL1 channel (530/30 nm). The data were analysed with FlowJo v10 and are presented as the percentage of sperm exhibiting cytosolic ROS fluorescence.

2.8. Histopathology

Following blood collection, the liver and kidneys were excised, rinsed with normal saline, and fixed in 10% neutral-buffered formalin for at least 48 hours. The tissues were then processed via routine histological techniques, dehydrated through graded ethanol solutions, cleared in xylene, and embedded in paraffin wax. The paraffin blocks were sectioned at a thickness of 5 μm via a rotary microtome and stained with hematoxylin and eosin (H&E). Histopathological examination of liver and kidney sections was performed under a light microscope (Olympus, Japan) by a blinded veterinary pathologist. The severity of tissue lesions, including hepatocellular degeneration, necrosis, inflammatory cell infiltration, glomerular alterations, and tubular degeneration, was evaluated qualitatively.

2.9. Statistical analysis

Statistical analysis was performed with SPSS software (version 24). To compare means for different groups, one-way ANOVA tests were conducted, and Tukey's post hoc test was applied to find specific group differences. All the results are presented as the means ± SEMs, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Oxidative stress

BaP exposure significantly impaired the antioxidant defense system ($p < 0.05$), as evidenced by a marked decrease in TAC and a significant increase in MDA levels. Specifically, the reference treatment (BaP + vitamin E) failed to significantly reduce MDA levels or improve TAC compared with the BaP group, indicating the limited efficacy of vitamin E in counteracting BaP-induced lipid peroxidation. Both pretreatment and coadministration of Sumac extract improved oxidative stress parameters in a dose-dependent manner. The 300 mg/kg dose had the strongest antioxidant effect, as evidenced by the highest TAC and the lowest MDA levels among all the treatment groups. This dose was also the only intervention that significantly increased GPx activity in both the pretreatment and coadministration regimens (**Table 2**).

3.2. Liver Enzyme Levels

BaP administration significantly increased the serum AST and ALP levels compared with those in the control group, whereas the ALT levels remained unchanged. Coadministration of vitamin E did not significantly improve the hepatic enzyme profile. In the groups that were coadministered and pretreated with Sumac, all three doses significantly suppressed AST and ALP levels. For the groups receiving the pretreatment regimen (i.e., Sumac administered before insult), 150 and 300 mg/kg significantly suppressed ALT activity (with a dose-dependent reduction), but in the coadministration regimen, only 300 mg/kg Sumac significantly suppressed the ALT level (**Table 3**).

3.3. Total bilirubin, albumin, and FBS levels

In the BaP-treated group, total bilirubin levels were significantly increased, while the albumin concentration was significantly decreased, and the level of FBS did not significantly change. Coadministration of Sumac or vitamin E did not exert a beneficial effect on bilirubin or FBS levels; however, it increased the albumin concentration.

In the Sumac-treated groups that were coadministered, only the 300 mg/kg dose significantly reduced bilirubin levels, whereas in the pretreatment groups, all the tested doses resulted in a significant reduction in bilirubin levels. With respect to albumin, none of the Sumac doses in the coadministration groups were able to significantly reverse the BaP-induced decrease; however, in the pretreatment groups, the 300 mg/kg dose significantly increased the albumin level. All doses of Sumac, both in the coadministration and pretreatment regimens, significantly reduced FBS levels (**Table 4**).

3.4. Lipid Profile

In the BaP-treated group, the levels of TG, TC, LDL, and HDL were significantly increased ($p < 0.05$). Coadministration of vitamin E with BaP significantly reduced TC and LDL levels, while no significant effect was observed on TG or HDL levels.

In the Sumac-treated groups with coadministration, no significant change in TG was observed; however, in the pretreatment groups, higher doses of Sumac (150 and 300 mg/kg) significantly reduced TG levels.

In both the coadministration and pretreatment regimens, all three doses of Sumac significantly decreased TC, LDL, and HDL levels, indicating its notable protective effects on the lipid profile (**Table 5**).

3.5. Renal Function Markers

Nephrotoxicity induced by BaP was confirmed by a significant increase in the serum creatinine and BUN levels. In the group receiving vitamin E along with BaP, creatinine levels were not significantly different from those in the BaP group, whereas BUN levels were significantly lower ($p < 0.05$). All three doses of Sumac, administered either concurrently with or prior to BaP exposure, resulted in a significant reduction in the serum creatinine and BUN levels compared with those in the BaP-treated group, indicating a consistent renoprotective effect across both treatment regimens (**Table 6**).

3.6. Testosterone levels and sperm concentration

The serum testosterone concentration significantly decreased following BaP treatment. Higher doses of Sumac, 300 mg/kg in the coadministration regimen and 150 and 300 mg/kg in the pretreatment groups, significantly increased serum testosterone levels compared with those in the BaP group, effectively restoring testosterone concentrations toward control values. Although BaP caused a reduction in sperm density compared with that in the control group, this decrease was not statistically significant. Nevertheless, compared with the BaP group, the 300 mg/kg coadministration group and all pretreatment groups presented an improvement in sperm density (**Table 7**).

3.7. Sperm motility parameters (progressive, nonprogressive, and immobile)

Compared with the control, BaP significantly decreased total sperm motility and the percentage of progressively motile sperm. Neither vitamin E nor Sumac (in any treatment regimen) improved sperm motility.

However, both the coadministration and pretreatment regimens at all the tested doses significantly improved the percentage of progressively motile sperm compared with that in the BaP-treated group, with the most prominent effect observed at the 300 mg/kg dose of Sumac extract (**Table 8**).

BaP induced a nonsignificant increase in the percentage of nonprogressively motile sperm as well as immotile sperm. Higher doses of Sumac (150 and 300 mg/kg) in both the coadministration and pretreatment regimens significantly reduced the proportion of nonprogressively motile sperm compared with that in the BaP group; however, no significant changes in sperm immobility relative to that in the BaP-treated group were observed (**Table 8**).

3.8. Sperm DNA damage and lipid peroxidation

Compared with the control, BaP significantly increased both sperm DNA damage and sperm lipid peroxidation. Compared with BaP alone, vitamin E did not exert any significant protective effect on sperm DNA integrity; however, it significantly reduced sperm lipid peroxidation.

Compared with BaP, Sumac at all the tested doses significantly reduced sperm DNA damage and lipid peroxidation in all the coadministration and pretreatment groups (**Table 9**).

3.9. Sperm Intracellular ROS, Protamine Deficiency, and Persistence Histone Levels

Compared with the control condition, BaP significantly increased the level of intracellular ROS and markedly elevated the degree of sperm protamine deficiency. Vitamin E had no significant effect on the intracellular ROS levels; however, it significantly reduced the degree of sperm protamine deficiency.

Compared with BaP alone, 300 mg/kg Sumac in the coadministration regimen, as well as all the pretreatment groups, significantly reduced the ROS levels. Compared with that in the BaP group, the sperm protamine content in all coadministration and prophylactic groups was also restored. BaP did not significantly increase sperm histone retention; however, only the 150 and 300 mg/kg pretreatment groups presented significantly lower levels of this parameter than did the BaP group (**Table 10**).

3.10. Liver histopathology

In the Control and Olive Oil groups, the hepatic tissues maintained a normal architecture with no visible lesions (**Figure 1**). Conversely, the BaP group exhibited marked hepatocellular necrosis (**Figure 2**). Liver sections from the BaP + Vit E group presented mild hepatocellular necrosis and degeneration (Figure 3). Mild hepatic inflammation with infiltration of inflammatory cells within the hepatic parenchyma was observed (Figure 4). In the coadministration groups, the administration of Sumac extract markedly

attenuated BaP-induced hepatic injury, with only mild hepatocellular degeneration observed in the liver sections. Thus, the extract was capable of reducing toxicity and seemed to protect the liver (**Figure 5**). Animals in the Sumac preventive groups presented severe hepatocellular degeneration, with varying severity depending on the administered dose (**Figure 6**).

3.11. Kidney Histopathology

Renal morphology appeared normal and devoid of histological lesions in both the olive oil and control groups (**Figure 7**). However, histological changes were significant in the BaP-treated animals. The most evident change observed was severe proliferative glomerulonephritis (**Figure 8**). Renal sections from the BaP+Vit E group indicated considerable renal tubule degeneration. (**Figure 9**). In the groups receiving Sumac extract at various concentrations, administered either as prophylactic or therapeutic treatment, a mild degree of tubular degeneration was observed, which was notably less severe than that observed in the BaP group (**Figures 10 and 11**).

4. Discussion

This study, for the first time, evaluated the protective effects of Sumac extract against BaP-induced toxicity in male rats. Two treatment regimens, pretreatment and cotreatment, were compared, and the efficacy of Sumac was compared with that of vitamin E as a reference antioxidant. In summary, particularly at higher doses (150 and 300 mg/kg) and in the pretreatment regimen, vitamin E demonstrated superior multiorgan protection compared with vitamin E. Vitamin E only had marginal effects on specific parameters, such as albumin, LDL, and BUN, and failed to inhibit major oxidative damage, hepatic dysfunction, and reproductive impairment. In contrast, Sumac dose-dependently restored the antioxidant status, preserved sperm chromatin integrity, ameliorated dyslipidemia, and protected hepatic and renal tissue integrity. Oxidative stress plays a critical role in BaP-induced toxicity, which is mediated by CYP450 metabolic activation and ROS generation [26, 27]. In the present study, elevated MDA and reduced TAC

confirmed oxidative stress in BaP-treated rats [28, 29]. Serum SOD and GPx, however, remained unaltered, suggesting that MDA and TAC are more sensitive biomarkers than serum SOD and GPx are in this model. The unchanged serum enzyme levels likely reflect that SOD and GPx are regulated primarily intracellularly and that the serum values do not accurately represent the tissue antioxidant status. This finding is consistent with previous findings reporting decreased SOD and GPx activity in injured tissues but not in serum [28, 29]. Thus, unaltered serum SOD and GPx should not be interpreted as evidence against oxidative stress or tissue damage.

Notably, despite comparable serum SOD and GPx levels between the Sumac and vitamin E groups, Sumac exhibited greater efficacy in reducing MDA and restoring TAC. These findings suggest that the protective effects of Sumac are not solely dependent on serum antioxidant enzymes but may involve direct ROS scavenging, enhancement of nonenzymatic antioxidant systems, or activation of intracellular pathways such as Nrf2. The antioxidant effects of Sumac extract were dose dependent, with the 300 mg/kg dose exerting the greatest reduction in MDA levels and the most pronounced improvement in TAC. Furthermore, a significant increase in GPx activity was observed exclusively at this dosage compared with that in the BaP-treated group, whereas no such effect was detected in the vitamin E-treated group. Given the pivotal role of GPx in detoxifying hydrogen peroxide and lipid peroxides, the elevated GPx activity at the highest Sumac dose suggests that the protective mechanisms of this plant may extend beyond direct radical scavenging and may involve the upregulation of endogenous antioxidant defense systems. One of the possible mechanisms underlying these effects is the activation of the Nrf2/ARE signalling pathway by Sumac polyphenolic compounds, particularly gallotannins and quercetin. Activation of this pathway can lead to increased expression of phase II antioxidant enzymes, including GPx and glutathione-S-transferase (GST) [26, 30]. The observed significant increase in GPx activity, exclusively at the highest Sumac dose, is partially consistent with this mechanism. However, further studies are needed to directly assess the expression of Nrf2-related genes and proteins in target tissues to confirm this hypothesis.

Analysis of hepatic enzyme levels revealed that, compared with those in the control group, BaP exposure significantly increased ALT, AST, and ALP, although these increases, despite being statistically significant, remained within the upper limits of the physiological range. However, the significant increase in total bilirubin in the same group indicated impaired hepatic function in terms of bilirubin uptake and excretion, which may be independent of extensive cellular necrosis and suggestive of functional hepatic impairment. One notable finding of the present study was the partial discrepancy between biochemical indicators and histopathological findings. In the Sumac-pretreated groups, despite severe hepatocellular degeneration, no significant increase in serum enzyme levels was detected, whereas the coadministration groups exhibited milder degeneration accompanied by limited increases in enzyme levels. The protective effects of Sumac in this study were primarily evident at the systemic and biochemical levels, whereas the hepatic tissue response, particularly under pretreatment conditions, displayed a more complex pattern that warrants further mechanistic investigations.

The observed discrepancy between the biochemical and histopathological findings can be interpreted on the basis of the fundamental distinction between degeneration and necrosis. Elevations in hepatic enzymes occur when cellular membrane integrity is disrupted and when intracellular enzymes leak into the bloodstream—a phenomenon characteristic of necrosis. In contrast, in cases of cellular degeneration, even in severe cases, the membrane structure remains largely intact; therefore, considerable tissue damage may be observed histopathologically without a proportional increase in serum enzyme levels [18, 31]. This finding suggests that the relative normality of liver enzyme levels does not necessarily indicate hepatic tissue health, and accurate interpretation of liver injury requires concurrent evaluation of both biochemical and histopathological findings.

The severe degeneration observed in the pretreatment group may be explained by several possible mechanisms. First, long-term pretreatment with Sumac may have induced phase I enzymes, particularly CYP1A1—an induction that has been reported for certain polyphenolic compounds.

Under such conditions, BaP metabolism could shift toward increased production of reactive and toxic metabolites, such as BaP-7,8-dihydrodiol-9,10-epoxide (BPDE), thereby exacerbating intracellular damage to hepatocytes [32, 33]. Although the pro-oxidant effects of polyphenols at high concentrations and the phenomenon of hormesis are theoretically conceivable, the pattern of the present findings is more consistent with altered BaP metabolism through phase I pathways.

The absence of a marked elevation in liver enzymes alongside hepatocellular degeneration further indicates that the damage occurred primarily at the intracellular level and was not accompanied by extensive necrosis. Thus, the possible induction of CYP1A1 and enhanced production of reactive BaP metabolites represent one plausible explanation for the observed damage in the pretreatment group; however, confirmation of this mechanism requires direct assessment of relevant enzyme activities and metabolites. In contrast, coadministration of Sumac and BaP likely exerted greater protective effects through neutralization of reactive oxygen species and reduced formation of toxic metabolites.

The vitamin E-treated group presented milder histopathological alterations, including limited necrosis, mild hepatocellular degeneration, and inflammatory cell infiltration. These findings are consistent with the well-established role of vitamin E as a chain-breaking antioxidant in inhibiting lipid peroxidation and mitigating oxidative damage to cellular membranes [34].

Reproductive toxicity was one of the most prominent consequences of BaP exposure in the present study. Decreased testosterone levels, reduced progressive sperm motility, increased DNA fragmentation, and increased protamine deficiency demonstrated that even in the absence of a significant decline in sperm count, the functional quality and genetic integrity of sperm were considerably impaired. These findings are consistent with studies reporting that molecular and genetic damage to sperm becomes evident prior to the manifestation of quantitative changes. In contrast, treatment with Sumac significantly reduced DNA fragmentation, improved protamine deficiency, and largely restored testosterone levels. These results suggest

that the protective effects of Sumac likely extend beyond direct neutralization of reactive oxygen species. Since protamine deficiency often arises from disruption of the histone-to-protamine replacement process and alterations in the expression of nuclear transition proteins (TNP1 and TNP2) during spermiogenesis, the Sumac-induced reduction in oxidative stress may have safeguarded these sensitive processes [35, 36]. Furthermore, sumac polyphenolic compounds, including gallic acid and myricetin, may contribute to improved sperm quality through preservation of mitochondrial function, reduction of germ apoptosis, and modulation of regulatory pathways governing cell survival and metabolism [37, 38]. However, confirmation of these mechanisms requires dedicated molecular studies.

The restoration of testosterone levels, particularly at the higher Sumac dose, further indicates partial protection of Leydig cell function. This effect may be associated with reduced oxidative stress and inflammation induced by BaP, thereby preserving steroidogenic capacity—a process likely facilitated by the inhibition of inflammatory pathways, including nuclear factor kappa-B (NF-κB).

BaP-induced nephrotoxicity in the present study was associated with elevated BUN and creatinine levels, as well as evidence of proliferative glomerulonephritis, indicating both functional and structural renal impairment. In both pretreatment and coadministration regimens, the combination of these methods largely ameliorated these alterations and reduced the severity of renal tissue lesions. In contrast, vitamin E improved only the BUN level and did not significantly affect the creatinine level. These findings suggest that the protective effects of Sumac are not limited to the liver or reproductive system but also protect the kidneys against BaP-induced damage. The relative superiority of Sumac over vitamin E is likely attributable to its concurrent antioxidant and anti-inflammatory properties, which may contribute to reducing glomerular injury and preserving renal function.

BaP exposure in the present study was associated with significant alterations in the lipid profile, manifested as increased TC, LDL, HDL, and triglyceride

levels. These findings indicate that BaP toxicity extends beyond oxidative and tissue damage and affects lipid metabolism. In contrast, Sumac, particularly at higher doses, was able to substantially correct these alterations and restore the lipid profile to values closer to normal. These effects are likely related to the presence of active polyphenolic compounds in Sumac, which may act through the modulation of lipid metabolism, reduction of lipoprotein oxidation, and improvement of cholesterol homeostasis [39]. Therefore, in addition to the protective role of Sumac against BaP-induced damage, the observed improvement in lipid parameters suggests that this plant may also exert beneficial effects from a metabolic health perspective.

From a nutritional perspective, Sumac is not merely a traditional food season but rather a rich source of bioactive compounds, including gallotannins, gallic acid, quercetin, myricetin, and other polyphenols. Unlike single antioxidants such as vitamin E, this complex array of compounds may exert synergistic effects on oxidative, inflammatory, and metabolic pathways. The observed superiority of Sumac over vitamin E across many of the parameters examined in the present study is likely attributable to this multicomponent, multitarget nature.

The present findings are also important from a food safety perspective. BaP is one of the most significant contaminants resulting from the thermal processing of foods and is found in grilled, smoked, and roasted products. Although the most effective strategy for risk reduction is to prevent the formation of PAHs during food processing and preparation, the results of this study suggest that naturally occurring polyphenolic compounds in Sumac may partially mitigate the biological damage associated with exposure to these contaminants. Furthermore, the addition of Sumac to meat products can reduce the formation of certain heat-induced carcinogenic compounds [6]. Therefore, Sumac may play a role both in reducing contaminant formation and in mitigating their biological consequences. Since Sumac is both consumed as a common food season and has demonstrated the ability to reduce damage caused by thermal process contaminants, this plant may serve as an example of the "reduction of food

toxicity by dietary compounds" approach—a strategy that has gained attention among food safety researchers in recent years.

Notably, the consumption of Sumac alongside grilled foods has a long-standing history in many Middle Eastern cultures, particularly in Iran. Although the results of the present study partially support the potential benefits of this dietary pattern, extrapolation of these findings to humans requires further clinical and epidemiological investigations.

The protective effects of Sumac are not limited to the BaP animal model. Published studies have also demonstrated that plant bioactive compounds can act through similar molecular mechanisms. For example, an enzymatic extract derived from grape pomace has been shown to reduce the expression of the TNF-α and IL-1β genes through the inhibition of the NF-κB inflammatory pathway [40]. Likewise, phenolic acids present in blueberries, particularly chlorogenic acid, exert anti-inflammatory effects not only through strong antioxidant properties but also via modulation of the NF-κB pathway [41]. This consistency substantiates our view that Sumac polyphenols confer protective effects against BaP toxicity.

5. Conclusion

In conclusion, the hydroalcoholic extract of Sumac in the present study was able to significantly attenuate a considerable portion of the systemic and reproductive toxicity induced by BaP and exhibited effects comparable to or even superior to those of vitamin E across many parameters. The protective effects of Sumac are likely exerted through a combination of mechanisms, including modulating oxidative stress, enhancing endogenous antioxidant defense, preserving sperm chromatin integrity, and improving metabolic disturbances. The findings suggest that coadministration of Sumac with BaP may represent an effective approach to mitigate the damage caused by exposure to this contaminant, whereas long-term pretreatment, despite improving certain systemic parameters, is associated with hepatic tissue alterations, underscoring the importance of concurrent evaluation of biochemical and histopathological indices.

From a nutritional and food safety perspective, the present findings support the potential of Sumac as a protective dietary agent against the biological effects of thermal processing contaminants, particularly PAHs. However, further mechanistic studies, chronic toxicological evaluations, and clinical trials are needed to elucidate the precise molecular mechanisms, long-term safety, and efficacy of these methods in humans.

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Competing interests

The authors declare that they have no competing interests.

Ethics approval

Ethical approval for this study was obtained from the Research Ethics Committee of the Department of Veterinary Medicine, Islamic Azad University, Shahrekord, Iran (IR.IAU.SHK.REC.1402.130). All procedures in this study were conducted according to the ethical standards of the Research Ethics Committee.

Availability of data and materials

Data available on request from the authors.

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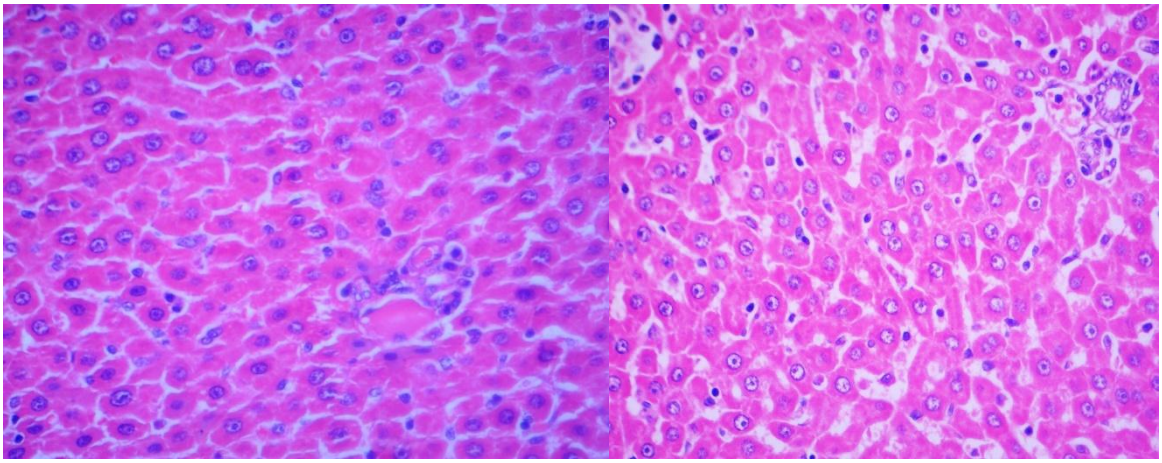

Figure 1. Normal liver parenchyma with no observable lesions in the Control and Olive Oil groups (400×).

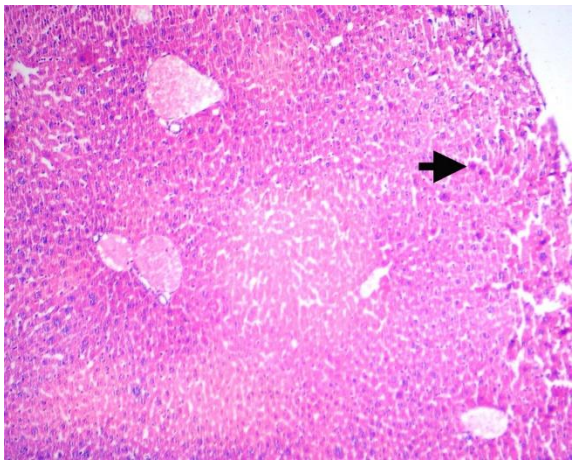

Figure 2. Marked hepatocellular necrosis (arrow) in the B[a]P group (100×)

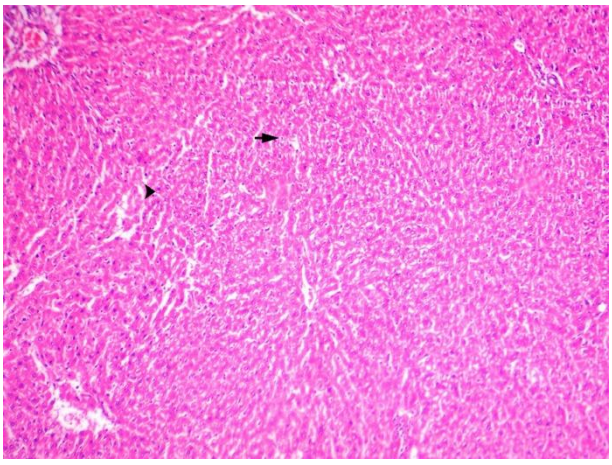

Figure 3. Mild hepatocellular necrosis (arrow) and mild degeneration (arrowhead) in the B[a]P + Vit E group (100×).

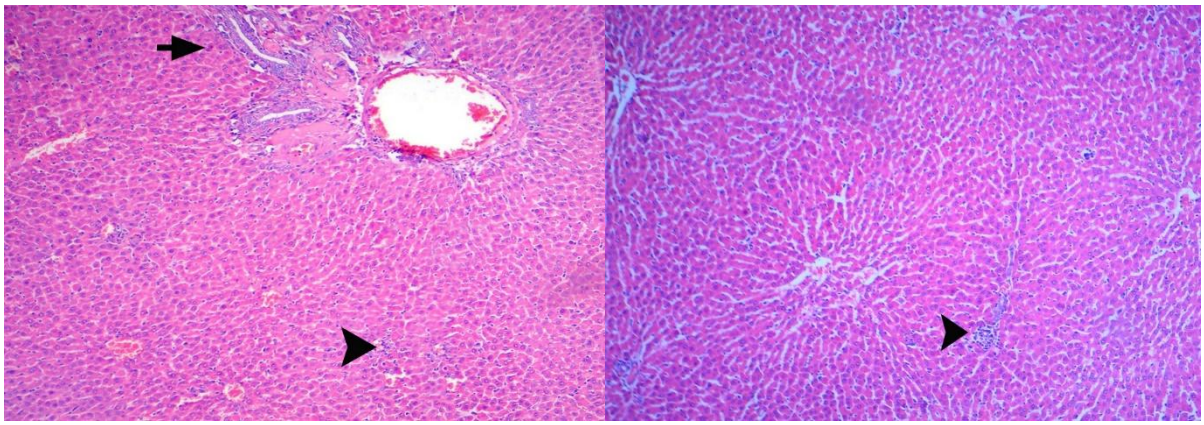

Figure 4. Mild hepatocellular necrosis (arrow) and mild inflammatory infiltration (arrowhead) in the B[a]P + Vit E group (100×).

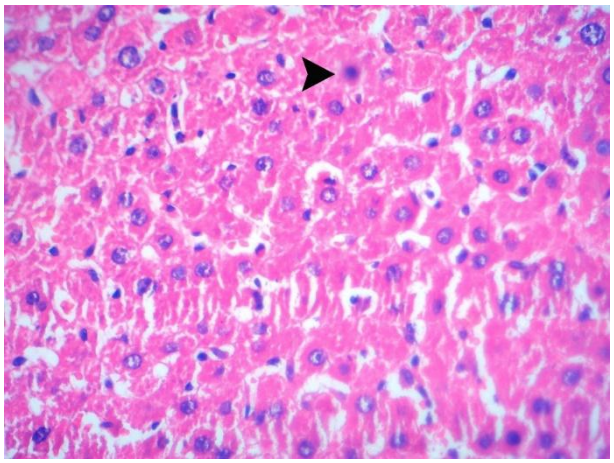

Figure 5. Mild hepatocellular degeneration (arrowhead) in the B[a]P + sumac (Co-administration groups) (400×).

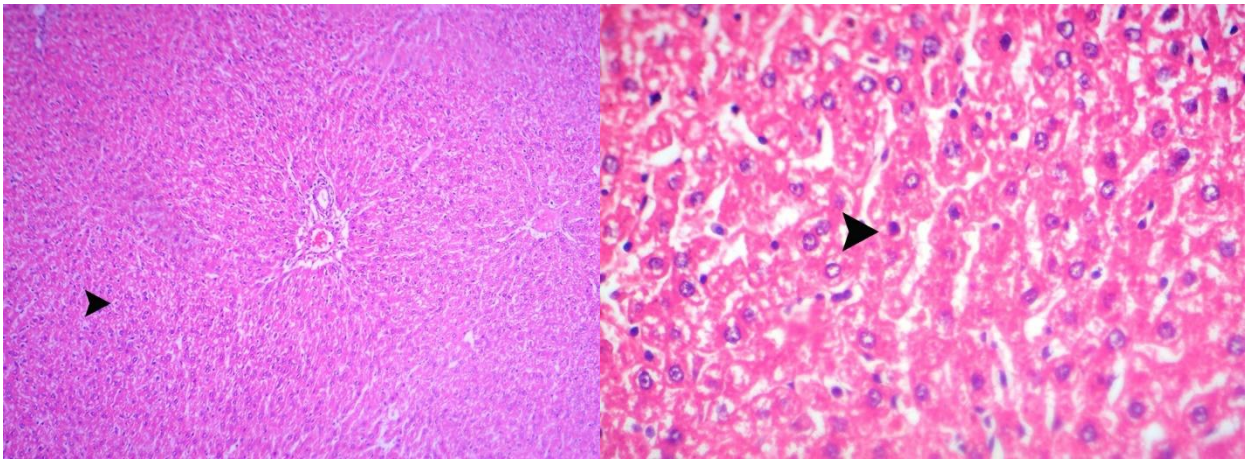

Figure 6. Severe hepatocellular degeneration (arrowhead) in the B[a]P + sumac (Pre-treaatment groups). (100× and 400×)

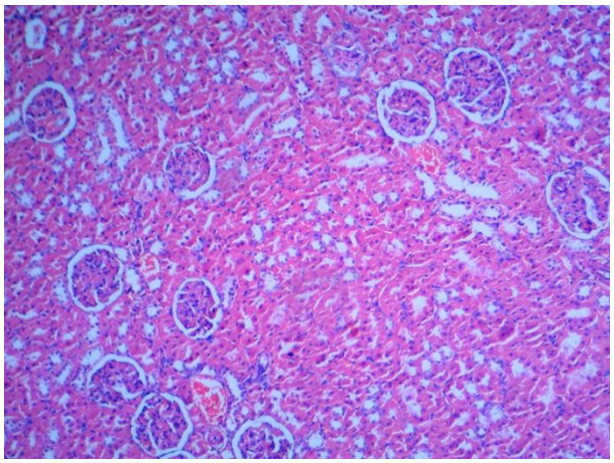

Figure 7. No detectable lesions in the Olive Oil and Control groups (100×).

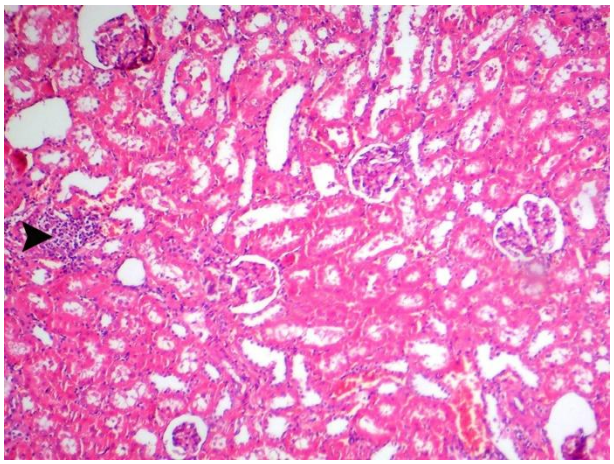

Figure 8. Proliferative glomerulonephritis (arrowhead) in the B[a]P group (100×).

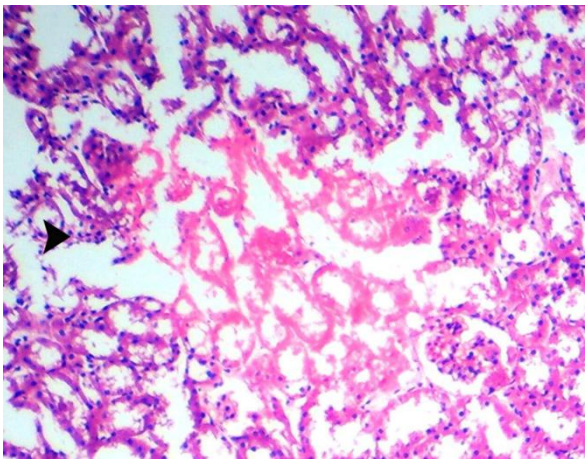

Figure 9. Marked degeneration of renal tubules (arrowhead) in the B[a]P + Vit E group (100×).

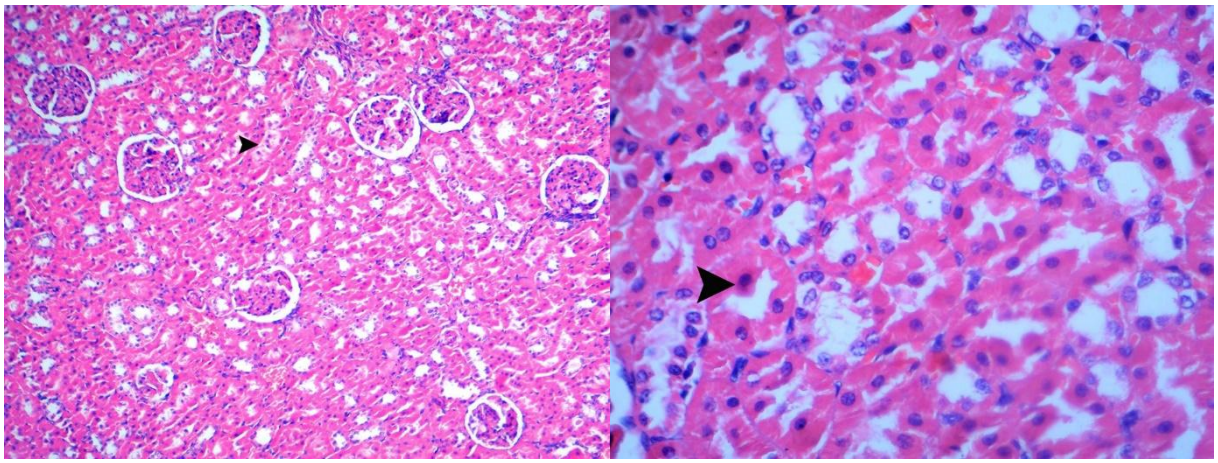

Figure 10. Mild degeneration of renal tubules (arrowhead) in the B[a]P + Sumac (Co-administration groups) (100× and 400×).

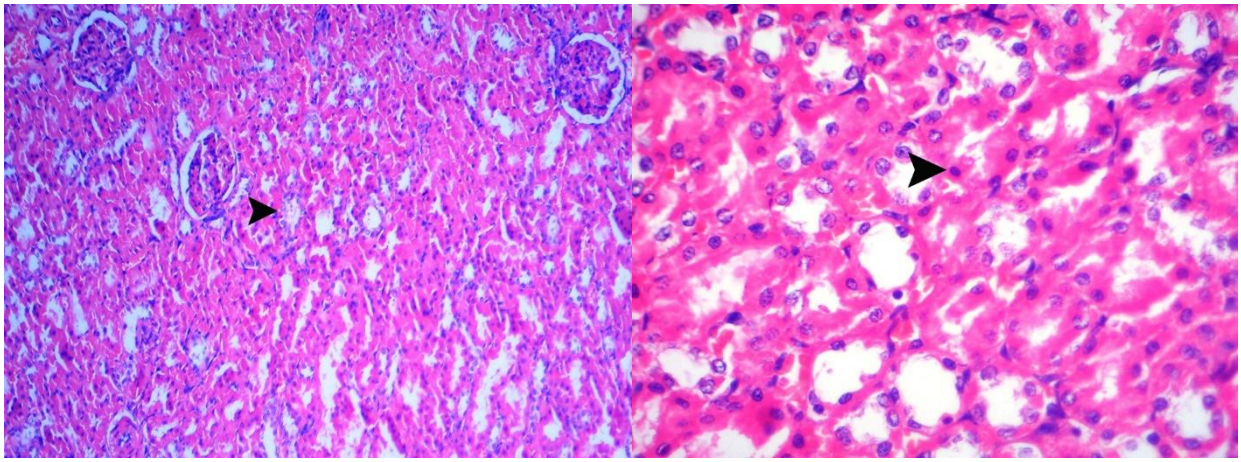

Figure 11. Mild degeneration of renal tubules (arrowhead) in the B[a]P + Sumac (Pre-treatment groups) (100× and 400×).

948 **Table 1**: Group allocation summary

Row	Group Name	Dose and Treatment (4 week)	Protocol Type
1	Control	Standard Diet	Control
2	Olive Oil	0.04 mL Olive Oil	Control
3	B[a]P	20 mg/kg	Toxicity
4	Vitamin E	250 mg/kg	Reference (Monotherapy)
5	B[a]P + Vitamin E	B[a]P (20) + Vitamin E (250 mg/kg)	Reference (Co-administration)
6	Sumac 1 (Low)	50 mg/kg	Monotherapy
7	Sumac 2 (Medium)	150 mg/kg	Monotherapy
8	Sumac 3 (High)	300 mg/kg	Monotherapy
9	Therapeutic (Low)	B[a]P (20) + Sumac (50)	Co-administration (4 Weeks)
10	Therapeutic (Medium)	B[a]P (20) + Sumac (150)	Co-administration (4 Weeks)
11	Therapeutic (High)	B[a]P (20) + Sumac (300)	Co-administration (4 Weeks)
12	Prophylactic (Low)	Sumac (50) (First 2 Weeks) → B[a]P + Sumac (50) (Next 4 Weeks)	Pre-treatment
13	Prophylactic (Medium)	Sumac (150) (First 2 Weeks) → B[a]P + Sumac (150) (Next 4 Weeks)	Pre-treatment
14	Prophylactic (High)	Sumac (300) (First 2 Weeks) → B[a]P + Sumac (300) (Next 4 Weeks)	Pre-treatment

Table 2. Oxidative Stress Markers Across Study Groups

Groups	TAC (µmol/L)	SOD (U/mL)	GPx (U/mL)	MDA (µmol/L)
Control	6.38±0.45^{de}	63.10±1.19^{ab}	54.98±0.92^{abc}	5.60±0.36^{bc}
Olive oil	6.40±0.43^{de}	62.20±0.90^{ab}	54.52±1.04^{abc}	5.72±0.25^{c}
B[a]P	4.62±0.60^{a}	53.20±3.70^{ab}	50.08±0.72^{a}	6.76±0.19^{d}
Vitamin E	7.26±0.50^{e}	65.46±0.45 ^{b}	58.20±1.30^{abc}	5.04±0.23^{abc}
B[a]P + Vitamin E	5.22±0.19^{abc}	54.04±3.69^{ab}	51.66±0.65^{ab}	6.80±0.15^{d}
Sumac 1 (Low)	5.46±0.51^{abcd}	62.20±1.92^{ab}	52.60±3.50^{abc}	5.18±0.34^{abc}
Sumac 2 (Medium)	5.78±0.52^{bcd}	64.00±3.24^{ab}	54.60±3.64^{abc}	4.60±0.83^{ab}
Sumac 3 (High)	6.52±0.31^{de}	66.00±3.87^{b}	58.00± 2.54^{abc}	4.24±0.55^{a}
Therapeutic (Low)	5.12±0.54^{ab}	48.00±23.59^{a}	48.00±11.18^{a}	5.64±0.68^{c}
Therapeutic (Medium)	5.84±0.61^{bcd}	57.60±7.53^{ab}	56.20±8.67^{abc}	5.18±0.41^{abc}
Therapeutic (High)	6.22±0.41^{bcde}	62.20±2.28^{ab}	62.20±5.89^{bc}	4.86±0.25^{abc}
Prophylactic (Low)	6.16±0.77^{bcde}	61.80±5.54^{ab}	54.00±2.00^{abc}	4.84±0.48 ^{abc}
Prophylactic (Medium)	6.36±0.27^{cde}	60.40±3.78^{ab}	58.40±6.91^{abc}	4.52±0.57^{a}
Prophylactic (High)	6.44±0.75^{de}	64.80±3.34^{b}	63.20±2.16^{c}	4.18±0.58^{a}

*Based on Tukey's HSD, if two groups share at least one letter in common, it means that there is no significant difference between those groups.

TAC: Total antioxidant capacity; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; MDA: Malondialdehyde

Table 3. Liver Enzyme Levels Across Study Groups

Groups	**ALT (IU/L)**	**AST (IU/L)**	**ALP (IU/L)**
Control	44.60±1.51^{abc}	44.40±1.67^{cd}	51.00±0.70^{de}
Olive oil	45.40±1.67^{abc}	44.00±2.54^{cd}	53.60±0.89^{ef}
B[a]P	54.20±3.70^{c}	62.20±4.32^{f}	59.00±3.39^{f}
Vitamin E	45.94±1.52^{abc}	48.94±1.47^{de}	52.52±1.24^{ef}
B[a]P + Vitamin E	47.00±2.91^{abc}	54.78±1.15^{ef}	55.38±5.55^{ef}
Sumac 1 (Low)	40.80±4.96^{ab}	41.20±5.58^{bcd}	53.60±1.67^{ef}
Sumac 2 (Medium)	38.60±4.15^{a}	41.00±2.54^{bcd}	50.60±3.36^{de}
Sumac 3 (High)	37.00±11.51^{a}	34.40±3.71^{abc}	44.00±2.44^{cd}
Therapeutic (Low)	49.60±3.64^{bc}	42.00±3.3^{cd}	47.80±6.64^{cde}
Therapeutic (Medium)	44.00±6.89^{abc}	40.80±30.96^{abcd}	49.40±4.61^{cde}
Therapeutic (High)	42.00±2.23^{ab}	35.00±3.31 ^{abc}	34.20±3.42^{ab}
Prophylactic (Low)	44.80±5.06^{abc}	37.00±8.27 ^{abc}	41.60±3.20^{bc}
Prophylactic (Medium)	37.00±4.30^{a}	30.60±7.05 ^{a}	35.60±4.03^{ab}
Prophylactic (High)	37.40±5.12^{a}	31.60±4.66 ^{ab}	33.40±3.36^{a}

*Based on Tukey's HSD, if two groups share at least one letter in common, it means that there is no significant difference between those groups.

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase

Table 4. Total Bilirubin, Albumin, and FBS Levels in Study Groups

Groups	Total Bilirubin (mg/dL)	Albumin (mg/dL)	FBS (mg/dL)
Control	0.82±0.28^{a}	4.30±0.58^{d}	92.20±4.49^{cde}
Olive oil	0.90±0.10^{a}	4.02±0.59^{cd}	93.00±4.12^{cde}
B[a]P	1.59±0.22^{b}	2.78±0.27^{a}	107.80±25.10^{e}
Vitamin E	1.00±0.21^{a}	4.12±0.19^{cd}	89.80±4.38^{bcde}
B[a]P + Vitamin E	1.34±0.24^{ab}	3.76±0.41^{bcd}	99.80±12.93^{de}
Sumac 1 (Low)	0.92±0.13^{a}	3.70±0.32 ^{bcd}	79.20±5.89^{abcd}
Sumac 2 (Medium)	1.00±0.24^{a}	3.82±0.25 ^{bcd}	76.40±3.43^{abc}
Sumac 3 (High)	1.02±0.19^{a}	3.64±0.30 ^{bcd}	65.40±6.98^{a}
Therapeutic (Low)	1.06±0.30^{ab}	3.60±0.54^{abcd}	78.80±4.38^{abc}
Therapeutic (Medium)	1.04±0.24^{ab}	3.36±0.32^{abc}	75.00±5.43^{abc}
Therapeutic (High)	0.88±0.43^{a}	3.40±0.17^{abc}	75.40±8.53^{abc}
Prophylactic (Low)	1.02±0.27^{a}	3.12±0.21^{ab}	78.40±3.97^{abc}
Prophylactic (Medium)	0.90±0.25^{a}	3.40±0.22^{abc}	73.40±10.40^{abc}
Prophylactic (High)	0.82±0.27^{a}	3.70±0.48^{bcd}	70.00±6.00^{ab}

*Based on Tukey's HSD, if two groups share at least one letter in common, it means that there is no significant difference between those groups.

FBS: Fasting blood sugar

Table 5. Lipid Profile Parameters Across Study Groups

Groups	TG (mM)	TC (mM)	LDL (mg/dL)	HDL (mg/dL)
Control	1.10±0.15^{ab}	2.46±1.31^{a}	82.60±20.01^{cd}	65.00±7.21^{a}
Olive oil	1.06±0.23^{ab}	2.86±0.55^{a}	80.80±17.54^{bcd}	65.80±7.21^{a}
B[a]P	2.44±0.25^{c}	4.60±1.23^{b}	162.80±44.20^{e}	93.80±15.38^{b}
Vitamin E	0.72±0.17^{a}	1.72±0.85^{a}	71.00±14.61^{abcd}	64.80±6.30^{a}
B[a]P + Vitamin E	1.76±0.50^{abc}	2.02±0.94^{a}	99.20±10.56^{d}	78.80±6.61^{ab}
Sumac 1 (Low)	1.98±0.67^{abc}	2.82±0.46^{a}	78.60±9.60^{abcd}	64.80±8.43^{a}
Sumac 2 (Medium)	1.78±0.86^{abc}	2.56±0.62^{a}	61.40±9.55^{abc}	67.80±8.22^{a}
Sumac 3 (High)	1.14±0.55 ^{ab}	1.56±0.46^{a}	45.20±4.76^{ab}	63.60±9.28^{a}
Therapeutic (Low)	1.28±0.42^{abc}	2.78±0.39^{a}	67.60±11.08^{abcd}	71.20±9.06^{a}
Therapeutic (Medium)	2.04±0.79^{bc}	2.52±0.49^{a}	60.00±8.57^{abc}	69.80±2.86^{a}
Therapeutic (High)	2.00±0.71^{abc}	1.94±0.52^{a}	56.60±14.53^{abc}	67.00±6.20^{a}
Prophylactic (Low)	1.66±0.96^{abc}	1.78±0.75^{a}	47.60±4.82^{abc}	67.20±7.19^{a}
Prophylactic (Medium)	1.04±0.50^{ab}	1.74±0.67^{a}	46.00±2.44^{ab}	71.80±5.97^{a}
Prophylactic (High)	1.10±0.51^{ab}	1.50±0.58^{a}	44.80±3.83^{a}	67.00±6.85^{a}

*Based on Tukey's HSD, if two groups share at least one letter in common, it means that there is no significant difference between those groups.

HDL: High-density lipoprotein; LDL: low-density lipoprotein; TC: Total cholesterol; and TG: Triglycerides.

Table 6. Renal Function Markers in Study Groups

Groups	Creatinine (mg/dL)	BUN (mg/dL)
Control	0.64±0.31^{abc}	20.40±1.14^{ab}
Olive oil	0.76±0.08^{bc}	20.40±1.14^{ab}
B[a]P	1.78±0.22^{e}	32.60±7.50^{c}
Vitamin E	0.76±0.09^{bc}	20.60±1.67^{ab}
B[a]P + Vitamin E	1.34±0.39^{de}	25.54±3.32^{b}
Sumac 1 (Low)	0.60±0.22^{abc}	22.60±4.33^{ab}
Sumac 2 (Medium)	0.30±0.12^{ab}	24.00±3.80^{b}
Sumac 3 (High)	0.22±0.16^{a}	21.80±3.11^{ab}
Therapeutic (Low)	0.94±0.11^{cd}	21.00±1.22^{ab}
Therapeutic (Medium)	0.52±0.27^{abc}	20.00±1.22^{ab}
Therapeutic (High)	0.46±0.26^{abc}	16.20±1.78^{a}
Prophylactic (Low)	0.50±0.18^{abc}	19.80±2.58^{ab}
Prophylactic (Medium)	0.48±0.27^{abc}	20.60±1.94^{ab}
Prophylactic (High)	0.46±0.16^{abc}	18.80±1.30^{ab}

*Based on Tukey's HSD, if two groups share at least one letter in common, it means that there is no significant difference between those groups.

BUN: Blood urea nitrogen; Cr: Creatinine.

Table 7. Testosterone Levels and Sperm Concentration in Study Groups

Groups	**Testosterone (ng/dl)**	**Sperm Concentration (×10^6 million)**
Control	3.38±0.13[a]	49.4±9.94 [ac]
Olive oil	3.32±0.18[a]	51±10.1 [abc]
B[a]P	2.56±0.57[b]	41.4±7.4[c]
Vitamin E	3.62±0.28[a]	62±6.59[ab]
B[a]P + Vitamin E	2.98±0.38[ab]	56±3.81[abc]
Sumac 1 (Low)	3.02±0.54[ab]	56±7.05[abc]
Sumac 2 (Medium)	3.22±0.27[ab]	67.4±2.41[ab]
Sumac 3 (High)	3.36±0.29[a]	65.2±11.05[ab]
Therapeutic (Low)	3.2±0.25[ab]	52.4±2.61[abc]
Therapeutic (Medium)	3.2±0.32[ab]	59.6±11.72[abc]
Therapeutic (High)	3.44±0.39[a]	69.6±9.66[b]
Prophylactic (Low)	3.26±0.25[ab]	64.4±13.75[ab]
Prophylactic (Medium)	3.32±0.19[a]	65±6.74[ab]
Prophylactic (High)	3.32±0.19[a]	69.6±7.23[b]

*Based on Tukey's HSD, if two groups share at least one letter in common, it means that there is no significant difference between those groups.

Table 8. Sperm Motility Parameters (%) Across Study Groups

Groups	Sperm Motility	Sperm Progressive Motility	Sperm Nonprogressive motility	Immotile Sperm
Control	83.60±4.39 ^{abd}	45.2±5.07^{b}	38.4±1.52^{ab}	16.4±4.39^{ab}
Olive oil	87.2±2.17 ^{bd}	45±3.74^{b}	42.2±5.72^{b}	12.8±2.17^{ab}
B[a]P	62.6±2.07^{c}	30.6±2.4^{a}	42.4±6.43^{bc}	27±6.44^{a}
Vitamin E	88.2±9.18^{d}	55.2±5.54^{c}	36.6±7.3^{abd}	8.2±2.86^{b}
B[a]P + Vitamin E	72.4±13.43^{abc}	40.2±6.14^{ab}	37.6±6.07^{ab}	22.2±5.93^{ab}
Sumac 1 (Low)	65±10.56^{c}	51.6±5.50^{bc}	24.6±5.46^{ad}	23.8±5.76^{ac}
Sumac 2 (Medium)	64.6±3.91^{c}	54.6±10.71^{bc}	22.8±11.17^{ad}	15.2±5.26^{ab}
Sumac 3 (High)	64.6±7.47^{c}	61.4±5.18^{cd}	27±3.74^{abd}	11.6±5.94^{bc}
Therapeutic (Low)	61±6.08^{c}	50±3.67^{bc}	33.4±8.2^{abd}	16.6±8.11^{ab}
Therapeutic (Medium)	66.2±7.92^{c}	57.6±5.27^{cd}	25±4.95^{ad}	17.4±4.77^{ab}
Therapeutic (High)	65.4±8.2^{c}	64.8±3.96^{d}	22±7.38^{ad}	13.2±6.46^{ab}
Prophylactic (Low)	69±3.81^{ac}	58.2±5.97^{cd}	27.6±13.7^{abd}	14.2±12.73^{ab}
Prophylactic (Medium)	66.2±3.03^{c}	53.6±2.88^{bc}	22.8±7.26^{ad}	23.6±6.84^{ac}
Prophylactic (High)	65.8±2.77^{c}	64.4±7.5^{d}	20.4±7.44^{d}	17.2±9.44^{ab}

*Based on Tukey's HSD, if two groups share at least one letter in common, it means that there is no significant difference between those groups.

Table 9. Sperm DNA Damage, and Lipid Peroxidation in Study Groups

Groups	Sperm DNA Damage	Sperm Lipid Peroxidation
Control	17.8±3.77^{abd}	10±4.74^{ac}
Olive oil	21.8±9.55^{bcd}	11.6±5.55^{ac}
B[a]P	37±10.2^{c}	30±9.82^{b}
Vitamin E	11.6±5.98^{abd}	6.2±4.09^{a}
B[a]P + Vitamin E	24.8±16.11^{cd}	18.4±4.98^{c}
Sumac 1 (Low)	16.2±4.44^{abd}	8.6±2.61^{ac}
Sumac 2 (Medium)	14.8±9.68^{abd}	8.4±2.07^{a}
Sumac 3 (High)	7.4±4.04^{ab}	6.6±3.51^{a}
Therapeutic (Low)	16.4±4.04^{abd}	11.4±2.70^{ac}
Therapeutic (Medium)	11.2±3.27^{abd}	8.8±3.56^{ac}
Therapeutic (High)	10±6.06^{abd}	8.2±4.92^{a}
Prophylactic (Low)	7±4.41^{ab}	7.4±2.97^{a}
Prophylactic (Medium)	8.4±2.30^{ab}	4.6±2.07^{a}
Prophylactic (High)	4.8±2.77^{a}	5.2±2.77^{a}

*Based on Tukey's HSD, if two groups share at least one letter in common, it means that there is no significant difference between those groups.

Table 10. Sperm Intracellular ROS, Protamine Deficiency, and Persistence Histone Levels in Study Groups

Groups	Sperm intracellular ROS	Sperm Protamine Deficiency	Sperm Persistence Histone
Control	6.6±4.34^{ac}	10.4±3.78^{a}	9.8±1.79^{ab}
Olive oil	5.6±3.85^{ac}	10.6±1.14^{a}	10.2±1.92^{ab}
B[a]P	16.4±5.18^{b}	30±6.12^{b}	13.8±4.87^{b}
Vitamin E	4.2±3.56^{ac}	6.6±3.36^{a}	7±3.74^{a}
B[a]P + Vitamin E	9±5.34^{abc}	8.4±3.65^{a}	10.2±5.35^{ab}
Sumac 1 (Low)	9.8±3.11^{abc}	12.6±3.78^{a}	10.6±0.89^{ab}
Sumac 2 (Medium)	6±2.24^{ac}	10.4±5.41^{a}	8.4±2.07^{ab}
Sumac 3 (High)	2.2±0.83^{c}	10.2±2.49^{a}	8±1.58^{ab}
Therapeutic (Low)	10.4±2.88^{ab}	6.8±4.09^{a}	7.2±3.27^{ab}
Therapeutic (Medium)	8.8±5.17^{abc}	9.8±2.59^{a}	7.8±3.11^{ab}
Therapeutic (High)	6.4±1.14^{ac}	9.6±4.04^{a}	7.8±1.92^{ab}
Prophylactic (Low)	5.8±3.11^{ac}	8.2±3.03^{a}	9.4±2.19^{ab}
Prophylactic (Medium)	3.6±1.95^{ac}	6.4±2.30^{a}	6±3.39^{a}
Prophylactic (High)	5.4±1.14^{ac}	4.8±2.59^{a}	4.8±1.79^{a}

*Based on Tukey's HSD, if two groups share at least one letter in common, it means that there is no significant difference between those groups.