Original Article

Protective Effects of Eugenol Against Iron Overload-induced Nephrotoxicity in Male Rats

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ABSTRACT

Background: Nephrotoxicity is a term used to describe when the renal system suffers from reduced renal function due to both direct and indirect toxin damage caused by exposure to certain drugs.

Objectives: This study aimed to investigate the protective effects of eugenol against iron overload (IOL)-induced nephrotoxicity in male rats.

Methods: Thirty rats were randomly divided into six equal groups: The first group, control negative C-, received intraperitoneal (IP) injection of distilled water. The second group, control positive C+, received iron dextran only at 100 mg/kg body weight (BW) IP. The third and fourth groups (iron+eugenol [IE]1 and IE2) received iron dextran 100 mg/kg BW IP and eugenol 50,100 mg/kg BW orally, respectively. The fifth and sixth groups (E3 and E4) received eugenol only at 50 100 mg/kg BW orally.

Results: The results revealed significant improvements in biomarkers and histological characteristics in rats treated with eugenol compared to those in the control group (C+). Rats treated with eugenol exhibited decreased levels of creatinine, blood urea nitrogen (BUN), malondialdehyde (MDA), erythropoietin (EPO) and kidney injury molecule-1 (KIM-1), along with increased concentrations of glutathione (GSH). Microscopic examination of kidney tissue from the control (C-) and eugenol-treated (E3 and E4) groups showed typical histological features, indicating preserved kidney architecture. In contrast, the control group (C+) showed epithelial cell necrosis in the renal tubules and inflammatory processes, particularly in the glomeruli and interstitial sections of the proximal renal tubules. The (IE1 and IE2) groups exhibited varying degrees of renal damage, with IE1 showing moderate epithelial cell necrosis and inflammation, while IE2 displayed relatively normal cortical architecture with mild inflammatory changes in the medulla.

Conclusion: Eugenol ameliorated IOL-induced nephrotoxicity in male rats.

Keywords: Eugenol, Histopathology, Iron overload (IOL), Kidney score, Nephrotoxicity

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Introduction

Nephrotoxicity occurs when kidney damage experiences diminished efficiency due to direct or indirect toxin damage caused by exposure to certain medications in the renal system, frequently precipitating toxic effects (Sales & Foresto, 2020; Polaka et al., 2023). Since the kidney is the main organ responsible for eliminating xenobiotics, it is especially vulnerable to the detrimental impacts of drugs and their metabolites during excretion. Additionally, several medications exhibit a preference for nephrotoxicity, which raises the risk of kidney injury (Sharma & Singh, 2023).

Iron overload (IOL) is a prevalent form of metal toxicity that is known to increase the risk of several acute and chronic diseases (Ige et al., 2019). IOL disorders include a range of illnesses characterized by elevated levels of iron throughout the body, which can cause damage to various organs, such as the liver, kidney, heart, and spleen (Hsu et al., 2022; Seyednejad et al., 2023). Kidney illnesses are also affected by the toxic effects of iron, and ferroptosis is recognized as a pathophysiological mechanism that can be targeted therapeutically to prevent kidney damage or disease development. Ferroptosis is a form of cell death linked to iron-induced oxidative stress (Ríos-Silva et al., 2023). Several studies have associated IOL with organ dysfunction and damage, such as cardiac, hepatic, renal, and diabetic diseases (Udani et al., 2021; Verna and Estuningtyas, 2022; Koohkan et al., 2023; Heriatmo et al., 2023).

Eugenol is a weakly acidic phenolic component of clove oil, comprising 83–95% of the oil. It is slightly water-soluble, easily soluble in organic solvents, and colorless or pale yellowish. The name is derived from the scientific names for clove, *Eugenia aromaticum* or *Eugenia caryophyllid* (Dable-Tupas et al., 2023). Eugenol is a phenylpropanoid formally derived from guaiacol with an allyl chain substituted para to the hydroxy group. It is a significant component of clove essential oil and is a natural compound used in traditional medicine. Several studies reported the healthy benefit effect of eugenol in vivo and in vitro, the antioxidant, anti-inflammatory, neuroprotective, analgesic, and antibacterial properties (Ikawati et al., 2022; Shahsavari et al., 2023).

Its protective effect makes it a therapeutic agent for various chronic diseases that involve metabolic syndrome due to abnormalities in chronic kidney disease (Gharaei et al., 2022), renal toxicity induced by vitamin D (Elkhadragy et al., 2022) and certain cancers (Melo et al., 2023). It is widely used in dentistry to treat toothache and pulpitis (Vilela et al., 2023). Thus, our study was designed to evaluate the effects of eugenol on IOL and nephrotoxicity.

Materials and Methods

Experimental animals

Thirty albino rats weighing 200-250 g were housed in the animal facility at the Faculty of Veterinary Medicine, University of Kufa. The rats were housed in sterile plastic enclosures, with each cage accommodating five rats. Wood shavings were used as bedding material. The animals were maintained at a regulated room temperature ranging from 23 ˚C to 25 ˚C, with a 12-hour alternating light and dark cycle and enough ventilation. The animals were acclimated for two weeks before the commencement of the trial. Ad libitum access to ordinary pellets and water was provided throughout the experiments.

Experimental design

The animals were randomly divided into six equal groups (n=5 per group) for treatment administrations over 30 experimental days. Control negative group (C-): Five healthy male rats were injected intraperitoneally (IP) with distal water. Control positive group (C+): Five male rats received iron dextran (LYFEXT®/USP/ India BN: ML22395) 100 mg/kg body weight (BW) IP every 72 h. Group iron+eugenol (IE)1: Five male rats received iron dextran 100 mg/kg BW IP injection every 72 h and were orally administered (Solarbio-China CN: 97-53-0) 50 mg/kg-BW of eugenol per day. Group IE2: Five male rats received an iron dextran 100 mg/kg BW IP every 72 h and were administered 100 mg/kg BW of eugenol daily. Group E3: Five male rats received oral eugenol 50 mg/kg-BW daily. Group E4: Five male rats received 100 mg/kg BW of eugenol orally per day. At the end of the experiment, the animals were anesthetized using an intramuscular injection of ketamine 80 mg/kg BW combined with xylazine 8 mg /kg BW (Jirkof & Lofgren, 2023).

Blood sample collection

Blood samples were collected via cardiac puncture using an EDTA tube. Serum was separated from the gel tube by centrifugation at 3000 rpm for 15 minutes and stored at -20 ˚C (Abdul & Mlaghee, 2023) until use in the kidney function test, creatinine test (Biolabo- France REF: 80107) and blood urea nitrogen (BUN) (MTD Diagnostic-Italy REF: CC1450) measurements using spectrophotometric according to (Wu & Tietz, 2006; Rifai, 2022). Oxidative status parameter glutathione (GSH) (Solarbio-China BC: 1170) and malondialdehyde

(MDA) (Solarbio-China BC: 0020) were measured using spectrophotometric content according to (Alpert et al., 1985; Spitz et al., 1989). Serum erythropoietin (EPO) and kidney injury molecule-1 (KIM-1) levels were quantitatively measured using an enzyme-linked immunosorbent assay (ELISA) kit (BT LAB Kit- China CN: E0293Ra) and (BT LAB Kit- China CN: E0549Ra) using an ELISA reader (Stat Fax-USA).

Histopathological examination

The rats were allowed to sleep, and the abdominal area was opened. Kidney tissue samples were collected. After sample collection, tissue samples were removed and fixed in 10% formalin for 24 h. After the tissues were fixed, they were dehydrated by passing them through 70%, 80%, 90% and 100% ethyl alcohol twice each for 2 h, then cleaned with xylene for 1/2 hour. Samples were filled with paraffin wax at 58–60 ˚C and then covered with paraffin wax to prepare paraffin blocks. Using a rotary microtome, sliced to 0.5 cm thick sections, placed in plastic cassettes, stained using hematoxylin and eosin, and then examined under a microscope examination at 40x, 100x magnifications (Luna, 1968; Saleh mehdy Alzeiny & Abbas, 2017). The injured cortex and medulla areas were evaluated using a scoring system divided into four scores, as shown in the Table 1 (Jablonski, 1983).

Statistical analysis

The experimental data were statistically analyzed using GraphPad Prism software, version 7. Tukey's multiple comparisons and analysis of variance (ANOVA) were conducted to determine the significance of the variations among the groups. The data were presented as Mean±SEM, with a statistically significant P<0.05 (Al-Sharafi & Al-Sharafi, 2014; Ntumi, 2021).

Results

Effects of iron dextran and eugenol administration on serum parameters

Serum creatinine concentration (mg/dL)

The effect of iron and different concentrations of eugenol on serum creatinine concentration (mg/dL) after 30 days of treatment. The control positive (C+) group, which received an injection of iron dextran 100 mg/kg BW every 72 h, exhibited a significant increase in serum creatinine concentration compared to all other groups (P≥0.05). No significant differences (P≤0.05) were observed in serum creatinine concentrations among the eugenol-treated groups (Figure 1).

Different letters indicate significant differences among groups, while similar letters denote non-significant differences. The error bars represent the Mean±SEM for P≥0.05. ANOVA one-way statistical analysis was used to calculate the significance differences among groups.

Serum BUN concentration (mg/dL)

Mean values of serum BUN concentration (mg/dL) in the control group and the groups treated with iron and different concentrations of eugenol throughout the experimental period. The results demonstrated a significant increase (P≥0.05) in BUN concentration in the control positive (C+) group, which received iron dextran injections, compared to all experimental groups. Furthermore, the results revealed significant differences between the eugenol groups, since the IE1 group (iron dextran+eugenol 50 mg/kg BW) exhibited a significantly lower BUN concentration compared to all other eugenol groups (IE2, E3 and E4). However, BUN concentra-

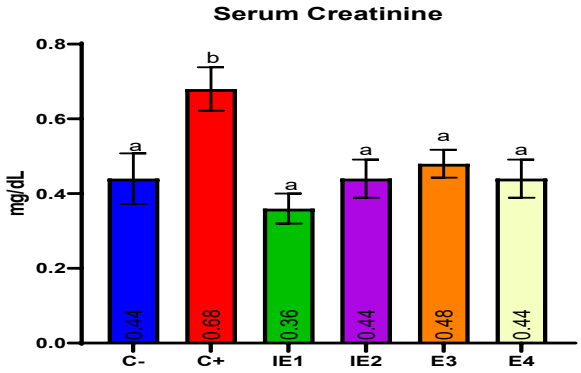

Figure 1. The effect of eugenol and iron dextran injection on serum creatinine concentration (mg/dL) in male rats

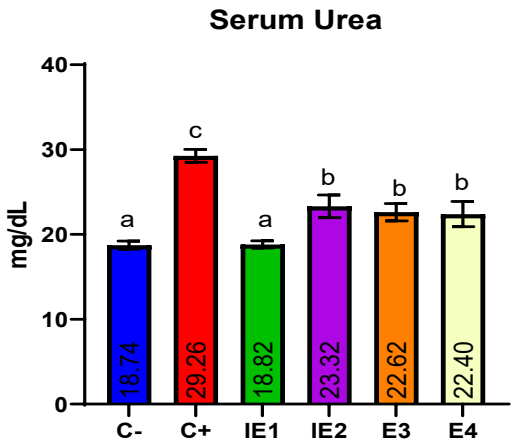

Figure 2. The effect of eugenol and iron dextran injection on serum BUN concentration (mg/dL) in male rats

tion in the IE1 group was not significantly different from that in the negative control group (Figure 2).

Different letters indicate the significant differences among groups, while similar letters denote non-significant differences among groups. The error bars represent the Mean±SEM for P≥0.05. ANOVA one-way statistical analysis was used to calculate the significance differences among groups.

Effects of iron dextran and eugenol administration on some oxidative status parameters

Detection of serum-reduced GSH µg/mL

Figure 3 shows the concentration of GSH (µg/mL) in male rats in the control group and the groups treated with iron and varying concentrations of eugenol throughout the experimental period. The results indicated a significant decrease (P≤0.05) in GSH concentration in the control positive (C+) group compared to all experimental groups. Further analysis of the eugenol groups revealed a significantly lower GSH concentration in the IE2 (iron dextran+eugenol 100 mg/kg BW) group compared to all other groups. Conversely, the GSH concentration in the E4 (eugenol 100 mg/kg BW) group was significantly higher than that in the other groups. GSH concentrations in the IE1 and E3 (eugenol 50 mg/kg BW) groups were not significantly different. The GSH concentration in the control negative (C-) group was not significantly different from that in IE1, IE2 and E3 groups. However, GSH concentration in the negative control group was considerably lower than in the E4 group.

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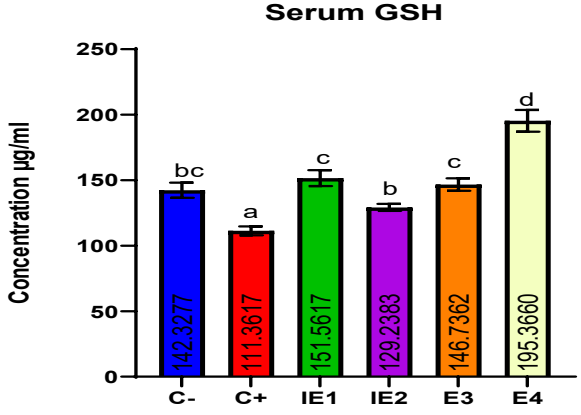

Figure 3. The effect of eugenol and iron dextran injection on kidney serum reduced GSH concentration µg/mL in male rats

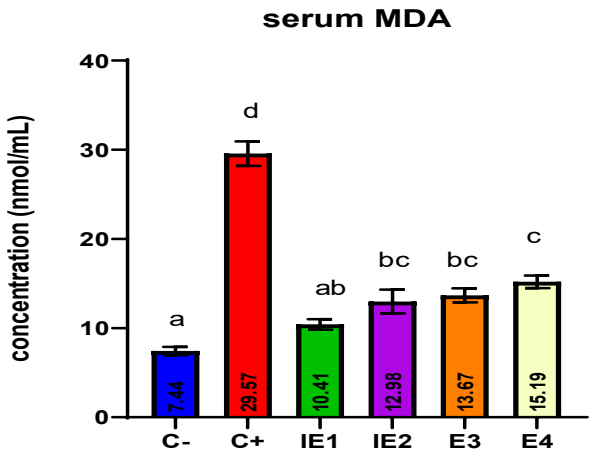

Figure 4. The effect of eugenol and iron dextran injection on kidney serum MDA concentration nmol/ml in male rats

the Mean±SEM for P≥0.05. ANOVA one-way statistical analysis was used to calculate the significance differences among groups.

Detection of serum MDA concentration nmol/mL

Figure 4 illustrates the concentration of MDA (mmol/mL) in male rats in the control group and the groups treated with iron and varying concentrations of eugenol throughout the experimental period. The results demonstrated a significant elevation (P≥0.05) in serum MDA concentration in the control positive (C+) group compared to all experimental groups. Further analysis of the iron+eugenol-treated groups (IE1 and IE2) showed no significant difference (P≤0.05) in MDA concentration between the two groups. However, MDA concentration in the IE1 group was significantly lower than in the E4 group. A non-significant difference was observed in the MDA concentration between the IE2, E3 and E4 groups. Additionally, the MDA concentration in the control negative (C-) group was significantly lower than in all other groups except IE1.

Different letters indicate the significant differences among groups, while similar letters denote non-significant differences among groups. The error bars represent the Mean±SEM for P≥0.05. ANOVA one-way statistical analysis was used to calculate the significance differences among groups.

Effects of iron dextran and eugenol administration on ELISA parameters

Serum EPO concentration pg/mL

The results in (Figure 5) elucidate the impact of iron dextran injection and daily oral eugenol administration on serum EPO concentration (pg/mL) in male albino rats. The findings showed a non-significant difference in the serum EPO levels between the C+ E3 and E4 groups. However, a significant reduction (P ≥ 0.05) was observed in the IE1, IE2 and C- groups compared to that in the C+ group. No significant difference in serum EPO levels was detected between the IE1 and IE2 groups. Nonetheless, the IE1 and IE2 groups exhibited significantly lower serum EPO levels than the E3 and E4 groups. The E4 group demonstrated the highest serum EPO levels. Additionally, a significant decrease in serum EPO levels was observed in the C- group relative to all eugenol-treated groups.

Different letters indicate significant differences among groups, while similar letters denote non-significant differences among groups. The error bars represent the Mean±SEM for P≥0.05. ANOVA one-way statistical analysis was used to calculate the significance differences among groups.

Serum KIM-1 concentration ng/mL

Figure 6 shows the impact of iron dextran injection and daily oral eugenol administration on KIM-1 levels in male albino rats. The results demonstrated a significant increase (P≥0.05) in KIM-1 concentration in the C+ group compared to all experimental groups. Additionally, the IE2 group exhibited a notable decrease in KIM-1

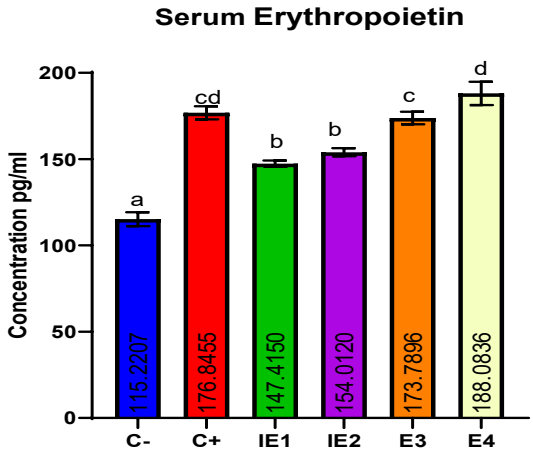

Figure 5. The effect of eugenol orally and iron dextran injection on serum EPO concentration pg/ml in male albino rats

levels compared to the IE1 and E4 groups while maintaining a non-significant difference from the E3 group. Furthermore, no significant differences were observed between the eugenol-treated groups (IE1, E3 and E4) or between the C- and IE2 groups.

Different letters indicate significant differences among groups, while similar letters denote non-significant differences. The error bars represent the Mean±SEM for the P≥0.05. The significant differences among groups were calculated using ANOVA one-way statistical analysis.

Microscopic examination

Microscopic examination of kidney rats in the negative control group (C-) did not show any histological changes in the kidney tissue (Figure 7) and eugenol 50 and eugenol 100 (E3, E4) treated groups showed typical histological architecture of the kidney (Figures 8 and 9). Rat kidneys of control positive groups (C+) showed necrosis of epithelial cells of the proximal and distal convoluted tubules, leading to spaces in the cortex area. Also, the infiltration of inflammatory cells in the affected cortex area filled the necrotic spaces (Figure 10). In the medulla, changes in necrosis of epithelial cells of the loop of Henle tubules led to space in the medulla area where inflammatory cells aggregated to form a cluster that occupied the necrotic cell spaces in the spaces of necrotic tissue (Figure 11).

The rat kidneys of iron dextran and eugenol at 50 mg/kg BW (IE1) showed moderate necrosis of epithelial cells of proximal and distal convoluted renal tubules from the space in the cortex area. Also, aggregation of infiltration of inflammatory cells in spaces of necrotic tissue (Figure 12) and in the medulla histological chang-

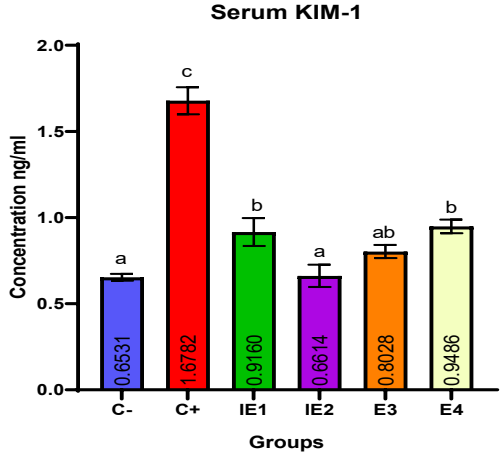

Figure 6. The effect of eugenol orally and iron dextran injection on serum KIM-1 concentration in male albino rats

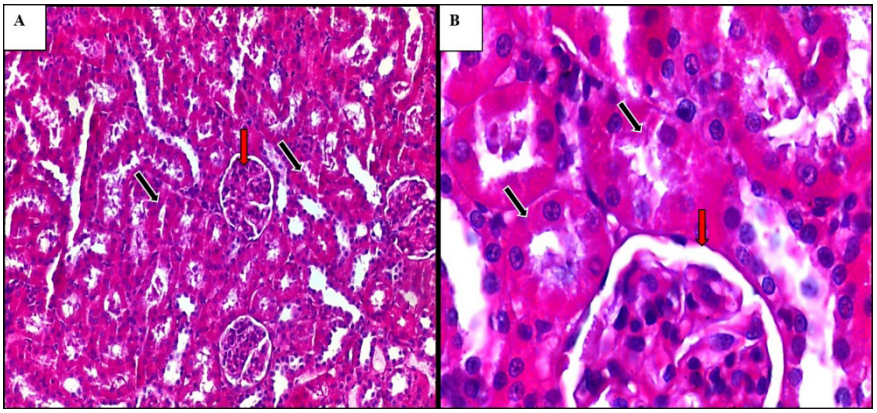

Figure 7. Photomicrograph of kidney of control negative group rat

A & B) Normal histological architecture of kidney; Note the glomerulus (red arrow) and the convoluted tubules (black arrow); H&E: A: ×100 and B: ×400.

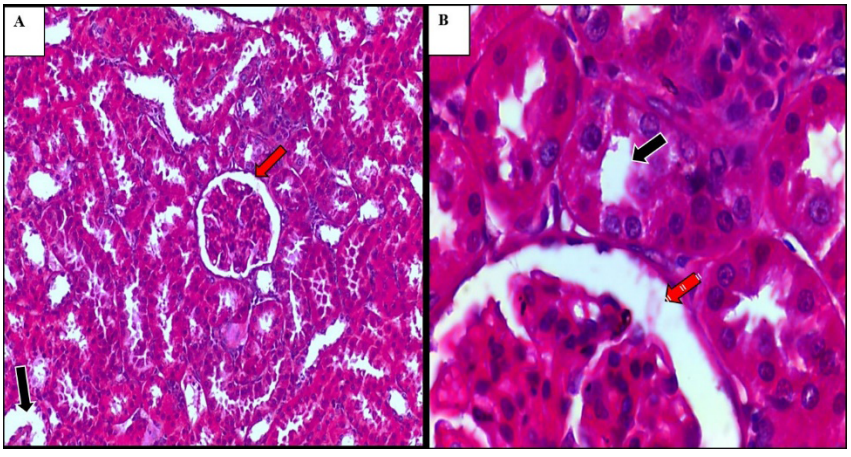

Figure 8. Photomicrograph of kidney of eugenol 50 (E3) group rat

A & B) Normal histological architecture of kidney; Note the glomerulus (red arrow) and convoluted tubules (black arrow); H&E: A: ×100 and B: ×400.

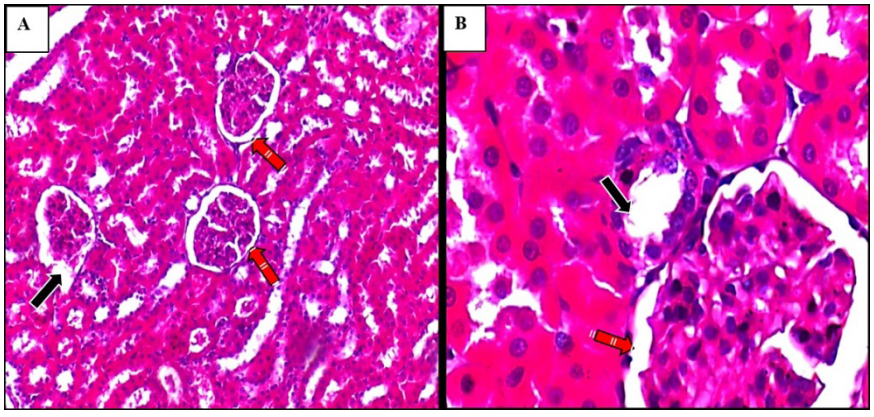

Figure 9. Photomicrograph of eugenol 100 (E4) group rat kidney

A & B) Normal histological architecture of kidney; Note the glomerulus (red arrow) and convoluted tubules (black arrow); H&E: A: ×100 and B: ×400.

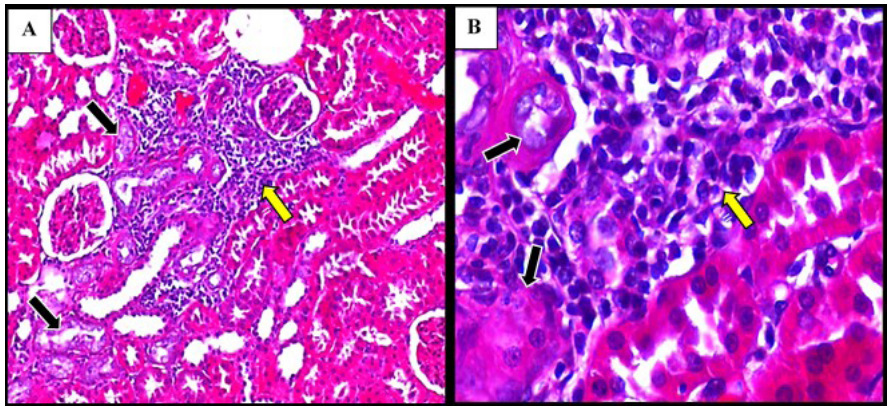

Figure 10. Photomicrograph cortex area of kidney of control positive group rat

A & B) Necrosis of epithelial cells of the proximal and distal convoluted tubules (black arrow) led to spaces in the cortex area. Also, infiltration of inflammatory cells (yellow arrow) in the affected cortex area fills the necrotic spaces; H &E: A: ×100 and B: ×400.

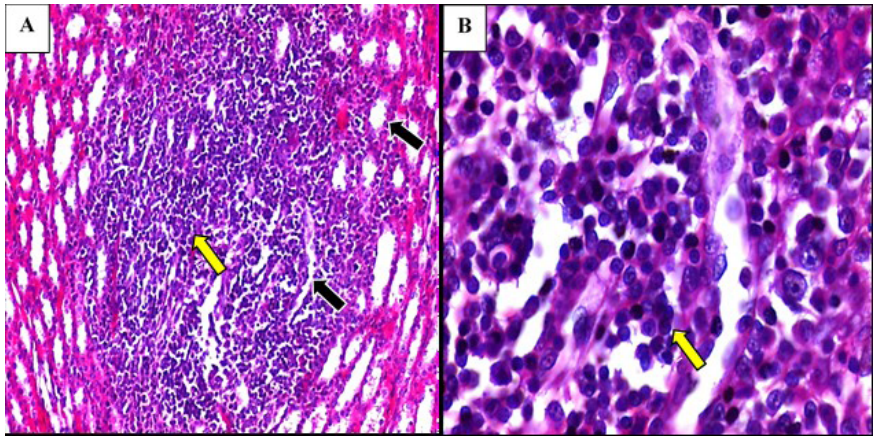

Figure 11. Photomicrograph medulla area of kidney of control positive group rat

A & B) Necrosis of epithelial cells of renal tubules (black arrow) led to space in the medulla area where inflammatory cells aggregated (yellow arrow) to form a cluster that occupied the necrotic cells' spaces in the spaces of necrotic tissue; H&E: A: ×100 and B: ×400.

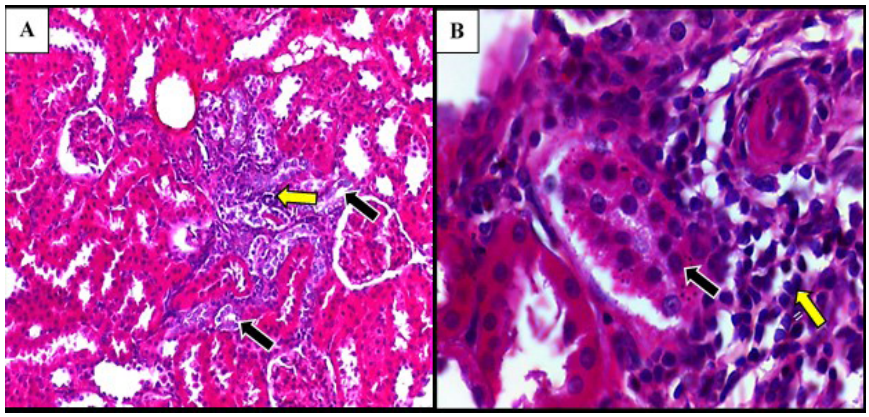

Figure 12. Photomicrograph cortex area of kidney of iron+eugenol (IE)1 group rat

A & B) Moderate necrosis of epithelial cells of the proximal and distal convoluted renal tubules (black arrow) led to space in the cortex area; Aggregation of inflammatory cell infiltration (yellow arrow) in necrotic tissue spaces; H&E: A: ×100 and B: ×400.

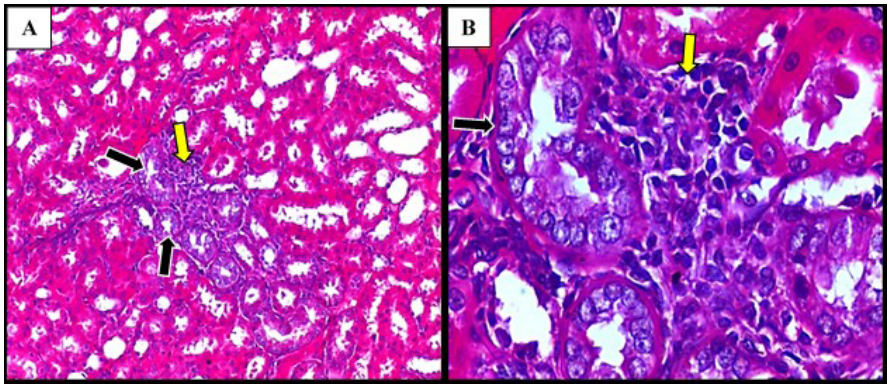

Figure 13. Photomicrograph medulla area of kidney of iron+eugenol (IE)1 group rat

A & B: Moderate infiltration of inflammatory cells (yellow arrow) with necrosis in epithelial cells of the renal tubules (black arrow) of the loop of Henle in the medulla area; H&E: A: ×100 and B: ×400.

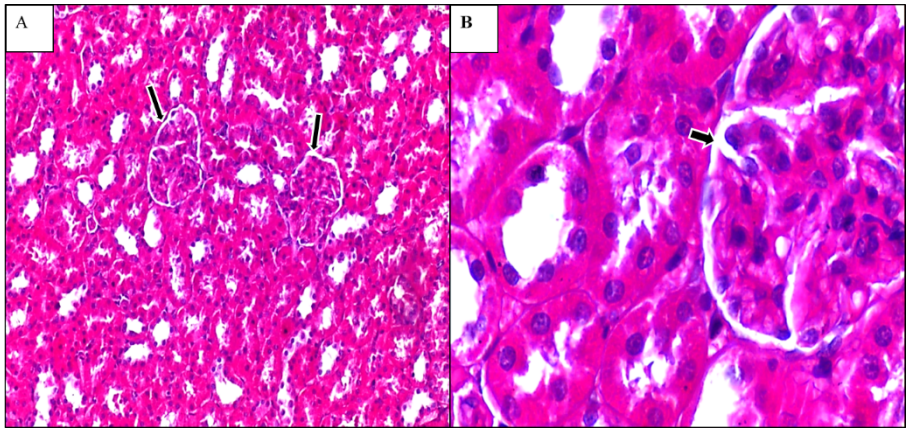

Figure 14. Photomicrograph cortex area of kidney of iron+eugenol (IE)2 group rat

A & B) Normal histological architecture of proximal & distal of convoluted tubules (black arrow) of cortex area of kidney; H&E: A: ×100 and B: ×400.

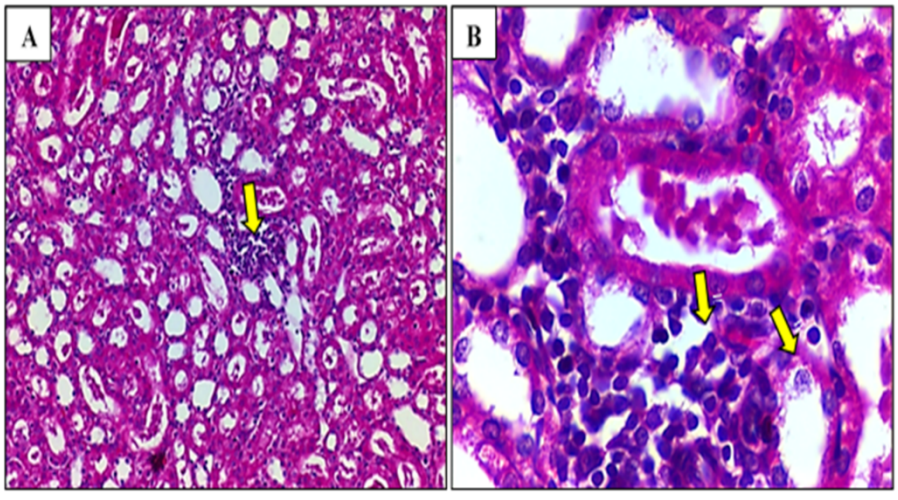

Figure 15. Photomicrograph medulla area of kidney of iron+eugenol (IE)2 group rat

A & B) Mild infiltration of inflammatory cells (yellow arrow) between the medullary renal tubules of the loop of Henle; H&E: A: ×100 and B: ×400.

Table 1. Renal tissue damage scoring system criteria

Score System Criteria		
Grade	**Necrosis (%)**	**Score**
Minimal	0-10 of the cortex or medulla area	1
Mild	10-20 of the cortex or medulla area	2
Moderate	20-30 of the cortex or medulla area	3
Severe	>30 of cortex or medulla area	4

es showed moderate infiltration of inflammatory cells with the presence of necrosis in epithelial cells of renal tubules of the loop of Henle in the medulla area (Figure 13). Finally, the rat kidneys treated with iron dextran and eugenol at a dosage of 100 mg/kg BW (IE2) showed normal histological architecture of the proximal and distal convoluted tubules of the cortex area of the kidney (Figure 14) and medulla, mild infiltration of inflammatory cells between the medullary renal tubules of the loop of Henle (Figure 15).

Kidney injury score

According to the Jablonski renal damage score system, the results showed different grades of injury in the renal tissues of rats. A significant increase was observed in the renal damage score (P≥0.05) for the cortex and medulla in the C+ group compared with the other experimental groups. However, non-significant changes were observed between the IE1 and IE2 groups; both IE1 and IE2 showed a significant increase in the damage score for the cortex of renal tissue compared with (C-, E3 and E4). In contrast, a substantial increase (P≥0.05) in the damage score was observed in the medulla of IE1 compared to that in IE2. The tissue damage scores were divided into four categories (minimal 0-10%, mild 10-20%, moderate 20-30% and severe 30≥) observed in the materials and methods in the Table 1.

In the cortex, severe lesions were observed in (60%) of the rats in the C+ group but not in the other IE1- and IE2-treated groups. Moderate cortical injury was observed in (40%) of C+ groups and (20%) of IE1 and was not observed in IE2 groups, respectively. Mild cortex injury was observed in 60% of IE1 group rats and in (20%) of IE2 group rats. However, mild grades of cortex injury were not observed in rats in the C+ group. Minimal grades of cortex injury were observed in (20%) of the IE1 group rats, and in (80%) of the IE2 group rats, mild grades of cortex injury were not observed in the C+ group rats (Figure 16A).

In the medulla, severe lesions were observed in (60%) of the C+ group rats but not in other groups IE1 and IE2-treated groups rats. Moderate grades of medulla injury were observed in (40%) of the rats in C+ group, 20% of IE1 was not observed in other group IE2 treated groups rats, and mild grades of medulla injury were observed in (80%) of IE1 and were observed in (40%) of IE2 rats. While not observed in C+ group rats. Minimal grades of medullary injury were observed in (60%) of IE2 group rats and did not appear in C+ and IE1 treated rats (Figure16B).

Discussion

This study investigated the protective effects of eugenol against iron-induced nephrotoxicity in albino rats by exploring biochemical, oxidative, and histopathological parameters to assess eugenol's impact on renal function. The anticipated benefits include a better understanding of eugenol's therapeutic potential and insights into potential preventive or complementary strategies for managing iron-induced renal disease.

The investigation of the role of eugenol against nephrotoxicity induced by IOL in albino rats revealed noteworthy outcomes after a 30-day treatment period. The positive control group, subjected to iron dextran injections, exhibited a significant elevation in serum creatinine concentration, indicating iron-induced renal dysfunction. In contrast, groups receiving eugenol and iron injections demonstrated no significant differences in serum creatinine levels. This suggested a potential protective effect of eugenol against iron-induced nephrotoxicity. These results are consistent with previous studies on eugenol's antioxidant and anti-inflammatory properties, highlighting its potential to mitigate renal impairment caused by oxidative stress (Said, 2011; Fathy et al., 2022).

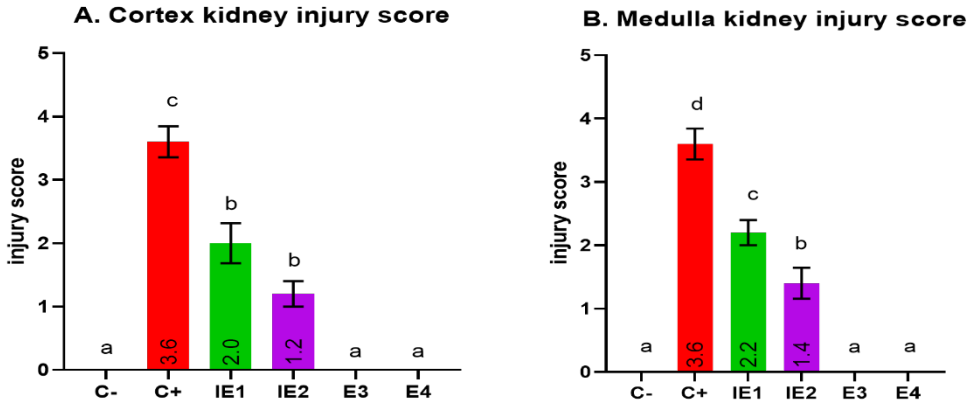

Figure 16. The tissue damage score values for renal tissue of rats

The graphs show the effect of eugenol and iron dextran on the cortex and medulla of the renal tissue. Different letters indicate the significant differences among groups, while similar letters denote non-significant differences among groups. The error bars represent the Mean±SEM for P≥0.05. ANOVA one-way statistical analysis was used to calculate the significance differences among groups.

The mechanism influencing the role of eugenol on the level of creatinine involves its multifaceted antioxidant and anti-inflammatory properties on creatinine can explain by which as explain eugenol (Said, 2011; Asker et al., 2021; Fathy et al., 2022), known for its free radical scavenging abilities effectively mitigate oxidative stress and lipid peroxidation in the renal tissue that by eliminating electrons from free radicals and preventing Fe^{2+} oxidation by H_2O_2, eugenol inhibit radical (OH^-) production, ultimately suppressing lipid peroxidation (Aboelwafa et al., 2022). Additionally, eugenol's anti-inflammatory actions include the modulation of cytokine levels by suppressing cyclooxygenase II and inhibiting cell proliferation. These combined effects contribute to reducing renal MDA levels, a marker of lipid peroxidation, and positively impact antioxidant defense mechanisms. Although the exact pathways by which eugenol influences creatinine levels may involve a complex interplay of its antioxidant and anti-inflammatory actions, the overall outcome has a significant protective effect on renal function in the context of IOL syndrome-induced renal injury (Bachiega et al., 2012; Aboelwafa et al., 2022; Gharaei et al., 2022).

The BUN observed increase in the concentration in the control positive (C+) group is consistent with previous studies indicating iron's adverse effects on renal function (Ige et al., 2019). However, the significant decrease in BUN concentration in the IE1 group (iron dextran+eugenol 50 mg/kg) compared to the other eugenol groups implies a potential dose-dependent protective effect of eugenol against iron-induced renal damage. This result aligns with previous studies suggesting that eugenol can ameliorate renal injury through its antioxidant and anti-inflammatory mechanisms (Said, 2011; Arase et al., 2020). Furthermore, the non-significant difference in BUN concentration between the IE1 group and the negative control group suggests that eugenol at a dose of 50 mg/kg may effectively counteract the iron-induced rise in BUN levels, bringing them closer to baseline levels (Gharaei et al., 2022).

Investigating the effects of iron injection and oral eugenol on biochemical serum concentration parameters in male rats revealed significant alterations in serum reduced GSH and increased serum MDA. GSH levels exhibited a notable decrease in the C+ group, indicative of oxidative stress induced by iron dextran injections, which showed dose-dependent effects, with the IE2 group displaying significantly lower GSH compared to the other groups due to the antioxidant effect of eugenol (Barhoma, 2019; Sharma et al., 2019; Abdel-Magied et al., 2020).

In terms of MDA concentration, the C+ group demonstrated elevated levels, suggesting oxidative damage, while eugenol-treated groups displayed varied responses since IE1 exhibited lower MDA than E3, E4 and IE2 showed comparable levels to E3 and E4; however, they had significantly lower MDA levels than both IE2 and E3. The negative control group displayed substantially lower MDA levels than all other groups. This study is consistent with previous research indicating eugenol's potential antioxidant effects, as evidenced by GSH modulation, and its ability to mitigate lipid peroxidation, as reflected in MDA levels (Gharaei et al., 2022). Additionally, the dose-dependent responses emphasize the nuanced impact of eugenol on oxidative stress mark-

ers (Sharma et al., 2019) and its therapeutic potential in iron-induced renal injury, consistent with earlier anti-inflammatory and antioxidant properties (Mateen et al., 2019ab; Kumar et al., 2021; Fathy et al., 2022).

EPO is a crucial hormone in the erythropoiesis regulatory cycle, which is produced by peritubular cells in the kidney. It responds to decreased oxygen delivery as hypoxia or elevated and acts on erythroid precursors; EPO prevents apoptosis and enhances transferrin receptor expression, promoting increased red blood cell production. Augmented RBC count alleviates hypoxia, initiating a negative feedback loop downregulating hypoxia-inducible factors. Consequently, this increases hemoglobin levels, contributing to the intricate balance of the EPO system (Hemani et al., 2021).

Examining serum EPO concentrations in male albino rats subjected to iron dextran injection and daily oral eugenol administration revealed significant variations in hematological parameters. As shown in the results of the C- group, EPO was within the normal range in the everyday physiological context. However, in the context of the IOL C+ group, where there is an excess of iron in the body, the relationship between EPO levels and iron is more complex since IOL can lead to oxidative stress agent damage in various tissues, including the kidneys, which can involve free superoxide radicals, hydrogen peroxide, singlet oxygen, nitric oxide, and peroxynitrite (Maiese et al., 2008; Yun et al., 2020; Mohammad et al., 2021).

The Kidneys are central to EPO production, and in the presence of IOL-induced kidney damage, there can be an increase in EPO production as a compensatory mechanism since the elevated EPO levels in IOL may be an attempt by the body to counteract the negative impact of iron-induced tissue damage, particularly in the kidneys, and to support the continued production of red blood cells (RBCs) (Dang et al., 2010; Sun et al., 2018). The increased EPO, in turn, stimulates the bone marrow to produce more RBCs. This contributes to an overall rise in RBC levels (Sun et al., 2018). Furthermore, it is noteworthy that EPO is primarily produced in the kidneys, with additional production and secretion occurring in the liver, brain, and uterus (Badi et al., 2022). EPO secretion in the brain seems to exhibit a more sustained pattern than that in peripheral organs, such as the kidney, suggesting a potential origin of EPO production in the brain (Maiese et al., 2008).

This production may involve crossing the blood-brain barrier to reach the bloodstream and peripheral organs. Hypoxia-inducible factor-la (HIF-1a) plays a pivotal role in the cellular response to hypoxia; hypoxia-induced factor1 controls the expression of EPO and EPOR during periods of reduced oxygen content. Previous studies have suggested that HlF-1a reduces intracellular reactive oxygen species (ROS) levels (Uchewa et al., 2023). Conversely, decreased levels of HlF-1a have been linked to elevated ROS levels, contributing to cell apoptosis in certain tumors. Furthermore, the activity of HlF-1a is influenced by the concentration of iron, a cofactor of the prolyl hydroxylase domain 2 (PHD2). PHD2 is an essential hydroxylase involved in oxygen sensing in HlF-1a cells. Excess iron impacts oxygen-sensing machinery, particularly HIF-la. HIF-1a is a crucial sensing regulator that responds to low oxygen conditions, and iron availability modulates its activity (Abed Al-Kareem et al., 2022). Iron serves as a cofactor for PHD2, an essential enzyme responsible for the degradation of HIF-la. When iron is abundant, PHD2 is activated, leading to the degradation of HIF-la and, consequently, a decrease in EPO production. Conversely, under conditions of iron deficiency or low iron availability, reduced PHD2 activity allows HIF-la to accumulate, stimulating EPO production as a compensatory response to low oxygen levels (Zheng et al., 2017; Hu et al., 2020).

In contrast, serum EPO levels were significantly reduced in the iron-treated groups (IE1 and IE2) compared to the control positive (C1) group, indicating a potential impact of IOL on erythropoiesis. The E4 group, receiving high-dose eugenol, demonstrated the highest serum EPO levels among all groups. Considering the modulatory effects of eugenol on EPO regulation, studies investigating the hematopoietic and antioxidant effects of eugenol are essential to understand further the potential therapeutic implications of eugenol in iron-induced hematological alterations (Arab et al., 2018; Mateen et al., 2019ab; Fathy et al., 2022).

KIM-1 is a transmembrane glycoprotein expressed by proximal tubular cells and is recognized as an early, sensitive, and specific urinary biomarker of kidney injury. It is associated with the severity of acute and chronic kidney damage. The ĶIM-1 acts as a phosphatidylserine phagocytosis and scavenger receptor, which binds to lipids on the surface of apoptotic and necrotic cells and oxidases low-density lipoprotein (LDL). Furthermore, it acts as a phagocytic receptor, helping engulf dead and necrotic debris in injured epithelial tubules, thus transforming epithelial cells into semi-professional phagocytes. KIM-1 plays a critical role in the progression of

kidney disease and is used as a reliable biomarker for kidney injury. It has been found to correlate with the amount of acute tubular necrosis and interstitial fibrosis/tubular atrophy on kidney biopsy, and elevated KIM-1 levels predicted the initial estimated glomerular filtration rate (Fazel et al., 2020).

KIM-1 also mediates fatty acid uptake by renal tubule cells to promote progressive diabetic kidney disease (Bonventre, 2009; Peng et al., 2020). The results of the study revealed significant findings concerning the impact of iron dextran injection and daily oral eugenol administration On KIM-I levels in male albino rats, which demonstrates a noteworthy increase in KIM-1 concentration in the C+ group compared to all experimental groups, as shown in previous studies (van Raaij et al., 2019) were illustrated that IOL increased the KIM-1. The 1E2 group exhibited a notable decrease in KIM-1 levels compared to the IE1 and E4 groups while maintaining a non-significant difference from the E3 group. Moreover, no significant differences were observed between the eugenol-treated groups (IE1, E3 and E4) or between the C- and IE2 groups. These results suggest a potential mitigating effect of eugenol, particularly at higher doses, on the iron-induced elevation of KIM-1 levels (Aboelwafa et al., 2022; Kuang et al., 2023).

The present study showed that pathological examination that controls negative and eugenol-treated groups (E3 and E4) maintained an unaltered kidney histology. Compared with the control positive (C+) group, iron dextran injection causes renal damage characterized by necrosis in proximal and distal convoluted tubules, accompanied by inflammatory cell infiltration (Ganz, 2013; Teschke, 2022). Eugenol has potential antioxidant properties and has been used to counteract IOL-induced oxidative stress. The iron dextran- and eugenol-treated groups (IE1 and IE2) exhibited standard histological architecture, reduced necrosis, and inflammatory cell infiltration compared to the positive control group. This suggests that eugenol protects against iron-induced renal damage (Jaganathan and Supriyanto, 2012; Barhoma, 2019; Gharaei et al., 2022).

Eugenol administration at varying dosages resulted in nuanced effects, with the IE1 group showing moderate necrosis and inflammatory cell aggregation. In contrast, the IE2 group restored normal architecture with mild inflammatory cell infiltration. These observations implicate IOL-induced oxidative stress due to excessive iron in the kidney, leading to elevated iron levels contributing to ROS production. ROS induces damage to cellular components, including lipids, proteins and DNA, leading to oxidative injury and subsequent inflammatory responses (Refaat et al., 2018; Baruah et al., 2023). Eugenol is known for its antioxidative properties, which are believed to mitigate oxidative stress, attenuate inflammatory reactions, and preserve renal tissue integrity (Adli et al., 2022). The dose-dependent impact of eugenol underscores the need for further investigation into the specific molecular pathways modulated by eugenol in iron-induced histopathological alterations in the kidney (Toprak et al., 2022).

Conclusion

In conclusion, our results suggest that eugenol exhibits nephroprotective effects against IOL-induced nephrotoxicity, as evidenced by a reduction in KIM-1 concentration and mitigated renal tissue damage. Administration of eugenol at a dose of 50 mg/kg BW demonstrated superior efficacy in attenuating nephrotoxicity compared to the higher dose of 100 mg/kg BW. These results highlight the dose-dependent eugenol's protective effects and its potential as a therapeutic agent for managing IOL-induced renal injury. Further mechanistic studies are required to elucidate the precise molecular pathways involved in eugenol-mediated nephroprotection.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the University of Kufa, Iraq (Code: UK.VET.2023/11/7.27151).

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interpretation of the results, and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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