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Eugenol Attenuates Paraquat-Induced Hepatic and Renal Biomarker Dysregulation in Wistar Rats: A Dose-Dependent Protective Assessment

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Abstract

Paraquat is a toxic herbicide, posing a severe health risk at minimal exposure. This experiment evaluated the efficacy of eugenol on PQ-dysregulation of hepato-renal biomarkers in Wistar rats. 32 Wistar rats from the AE-FUNAI animal house were acclimatized for 14 days and divided into four groups of 8 rats per Group. Group A served as normal control, while Groups B, C, and D received 10 mg/kg of paraquat for 28 days at one-day intervals. Group C and D were later treated with 300 mg/kg and 600 mg/kg of eugenol, respectively, for 14 days. After sacrifice, the liver and kidneys were harvested. A lobe of the liver and one of the kidneys were removed and homogenized separately to estimate hepato-renal biomarkers such as creatinine, urea, cystatin, phosphatase, amino acid-leucine, β-trace protein, albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), Total protein (TP), lactate dehydrogenase (LDH), bicarbonate and prothrombin time (PrT). Paraquat significantly increased LDH, PrT, ALT, β-trace protein, cystatin, leucine and ALP while lowering AST, albumin, creatinine, urea, phosphatase, bicarbonate and TP ($p<0.05$). In the eugenol group, ALT decreased at 600 mg, ALP increased at 300 mg, AST increased at 600 mg relative to 300 mg, albumin and TP increased at 600 mg, LDH decreased at 600 mg relative to 300 mg, creatinine and urea increased at 600 mg relative to 300 mg, Cystatin and β-trace protein decreased at 600 mg relative to 300 mg, phosphate and HCO_3^- increased at 600 mg relative to 300 mg, and amino acid-leucine decreased at 300 mg ($p<0.05$). These findings show that eugenol is very efficacious against paraquat-induced hepato-renal toxicity that leads to the dysregulation of biomarkers.

Keywords: Biomarker; Dysregulation; Eugenol; Hepatorenal; Paraquat

INTRODUCTION

Some nations around the world prohibit the use of paraquat, a herbicide that is part of the class bipyridines (Jodeh *et al*., 2019; Sharma *et al*., 2019). Paraquat's rapid and non-selective action in destroying green plants gives it an advantage over other herbicides (Stuart *et al*.,

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2023). Nonetheless, PQ is among the most hazardous substances utilized by farmers and presents a serious risk to health, especially when exposure occurs accidentally or on purpose (Donaher & Van-Den, 2023). Reactive oxygen species (ROS), which cause oxidative stress and harm to essential organs, are the main way that PQ causes its toxic effects (Subbiah & Tiwari, 2021). Due to its accessibility and affordability, PQ is still widely used in many underdeveloped nations despite efforts to control its usage in some areas. This has resulted to high incidence of poisoning and organ damage incidents. The liver and kidneys, which are essential for detoxification and waste removal, are at serious risk due to PQ's extremely toxic nature (Flafel *et al*., 2024; Pathak *et al*., 2022; Alamri, 2018; Li *et al*., 2020; Gupta, 2020). Exposure of these organs to harmful compounds like PQ, their normal function is disrupted, leading to serious structural and metabolic damage (Chen *et al*., 2021). Lipid peroxidation, protein deterioration, and DNA damage are all consequences of paraquat poisoning that lead to tissue scarring, inflammation, and ultimately organ failure (Mateen *et al*., 2019). The primary source of paraquat toxicity's clinical symptoms is the generation of intracellular reactive oxygen species, which harm cells by activating nuclear factor kappa B, causing lipid peroxidation, damaging mitochondria, and causing apoptosis in numerous organs (Liu *et al*., 2022). The primary mechanisms affecting the liver are mitochondrial damage and endoplasmic reticulum degeneration. The severity of this damage is indicated by the rapid and severe rise in liver enzymes like aspartate transaminase (AST) and alanine transaminase (ALT) in the blood (Gheshlaghi *et al*., 2022; Zuñiga-Aguilar & Ramírez-Fernández, 2022). Paraquat intoxication also damages the kidneys, causing tubular necrosis and vacuolation of proximal tubular nephron cells, which raises creatinine and urea levels (Badroo *et al*., 2020; Gheshlaghi *et al*., 2022).

A well-known and extensively researched substance is eugenol (4-allyl-1-hydroxy-2-methoxybenzene) (Lone & Ahmad, 2020). Commercial manufacturing started in the USA in 1940 after it was initially identified as a volatile chemical from Eugenia caryophyllata in 1929. Because of its antiseptic, antibacterial, anti-inflammatory, antiviral, antioxidant, analgesic, antispasmodic, and apoptosis-promoting properties, this natural compound—which is extracted from the essential oils of plants like clove (*Syzygium aromaticum*), cinnamon, and basil—has been used for therapeutic purposes in traditional and medical applications (Haro-González *et al*., 2021; Karaman & Kaplan, 2022; Tavvabi-Kashani *et al*., 2024). Eugenol, a phenolic molecule, prevents oxidative stress, neutralizes free radicals, and shields cells from harm. Heavy metals, herbicides, and industrial chemicals are among the poisons that eugenol has been demonstrated to protect against (Silva *et al*., 2024; Tavvabi-Kashani *et al*., 2024). Nevertheless, little study has been done expressly on how it affects organ dysfunction brought on by PQ. For the treatment of toxin-induced organ damage, natural bioactive substances like eugenol are becoming more and more recognized as viable substitutes for traditional medications (Aladejana, 2023). Originating from plants like clove and cinnamon, eugenol is well-known for its capacity to treat several pathological processes at once (Liñán-Atero *et al*., 2024; Tavvabi-Kashani *et al*., 2024). It is especially useful in situations involving inflammation and oxidative stress, including paraquat (PQ) poisoning, because of its broad-spectrum activity (Flafel *et al*., 2024). Eugenol works through a number of interrelated pathways, in contrast to synthesized medications that frequently concentrate on a single biochemical pathway (Jaramillo Jimenez *et al*., 2024). By lowering the buildup of reactive oxygen species (ROS), which cause oxidative damage, it strengthens the body's antioxidant defense mechanisms (Mateen *et al*., 2019). By regulating the synthesis of pro-inflammatory chemicals and shielding cellular structures from harm, eugenol also reduces inflammation (Mateen *et al*., 2019; Schilrreff & Alexiev, 2022). Additionally, it maintains organ function by regulating cellular integrity, which inhibits programmed cell death (apoptosis) (Tavvabi-Kashani *et al*.,

2024; Yousef *et al*., 2022). In addition to making it more accessible, its natural origin reduces the possibility of serious adverse consequences that are frequently connected to synthetic substances (Tavvabi-Kashani *et al*., 2024). Eugenol is a strong remedy for PQ-induced liver and kidney failure because of these characteristics, which offer a comprehensive strategy for minimizing tissue damage and enhancing recovery results.

Poisoning instances have alarmingly increased as a result of the extensive use of paraquat (PQ) as a herbicide, especially in areas with lax regulation and control (Donaher & Van Den Hurk, 2023). Eugenol has the potential to reduce oxidative stress, maintain cellular integrity, and safeguard organ function by focusing on the pathways that underlie PQ toxicity. Investigating how well it protects renal and hepatic health may offer important clues about its potential for treatment. Furthermore, comprehending the ways in which eugenol works to protect will advance our understanding of its pharmacological advantages and aid in the creation of novel therapeutic approaches to address the serious health effects of PQ which lies in the evaluation of eugenol as a protective agent against PQ-induced hepatorenal damage. This study is the first to examine the dual protective effects of eugenol on both liver and kidney tissues within a single experimental model. In order to provide a basis for incorporating eugenol into the treatment of PQ-induced toxicity, this study aims to assess these potentialities.

MATERIALS AND METHODS

Ethical Clearance

This research strictly adhered to the National Institutes of Health for the use and care of animals and to the Faculty of Basic Medical Sciences Ethics and Animal Handling Committee of Alex Ekwueme Federal University Ndufu Alike (AEFUNAI) guideline with the reference number: AE-FUNAI/FBMS/EAHC/24/004.

Animal Management and Research Protocol

A total of 32 adult Wistar rats were purchased from the animal house of Alex Ekwueme Federal University Ndufu Alike, Ikwo (AE-FUNAI) and housed in netted cages under standard conditions of 25-28^{o}C and relative humidity of 35% and 70%, respectively, in the same institution. The rats were allowed to be fed and water *ad libitum*. The procedures for animal care and handling, according to the AE-FUNAI guidelines, were strictly adhered to. After 14 days of acclimatization, the animals were rando mLy assigned to four groups (eight rats in each group). Group A (the control group) received normal water and fed. Group B received 10 mg/kg of paraquat + feed + water. Group C received 10 mg/kg paraquat + 300 mg/kg eugenol + feed + water. Group D received 10 mg/kg paraquat + 600 mg/kg eugenol + feed + water. All administrations were done orally using oral gavage. Paraquat administration was at one-day intervals and lasted for 28 days. Eugenol was administered to Groups C and D as a post-treatment (sequential treatment). No eugenol-only group was included in this study, as the experimental design was focused on evaluating the ameliorative effects of eugenol on paraquat-induced toxicity.

Animal Sacrifice and Sample Collection

The animals were sacrificed and the liver and kidney were harvested and immediately homogenized separately with the aid of homogenizer. Phosphate buffer was added to it and well mixed before transferring it to a vial. These vials were passed through the centrifuge at 4000 rpm for 10 minutes and thereafter decanted to separate the supernatant from the residue. The supernatants were then used to estimate the hepato-renal biomarkers.

DETERMINATION OF UREA AND CREATININE

The urea concentration was estimated spectrophotometrically as described by (Searcy *et al*., 1967). Briefly, 10 µL of the sample was added to 0.1 mL of sodium nitrous side-urease reagent after which the mixture was incubated for 10 min at 37 °C. 2.5 mL of 120mM diluted phenol and 2.5 mL of 27 mM sodium hypochlorite solution containing 0.14 N sodium hydroxide was then added to the reaction mixture. The mixture was hereafter incubated for 15 min at 37 °C and the absorbance at 546 nm was taken against reagent blank within 8 hours. Urea concentration was calculated relative to the standard. The level of creatinine was determined spectrophotometrically using the alkaline Jaffe picrate method as described by (Spierto *et al*., 1979). Briefly, 50 µL of distilled water was added to 2 mL of the working reagent before 50 µL of the sample was added. The mixture was allowed to remain for 30 s before the absorbance was measured. The absorbance at 492 nm was measured twice, after 30 s, and then 2 min later. The creatinine concentration was calculated against the standard using the change in sample absorbance.

DETERMINATION OF ASPARTATE AMINOTRANSFERASE (AST), ALANINE AMINOTRANSFERASE (ALT), ALKALINE PHOSPHATASE (ALP), AND ALBUMIN LEVELS

The method described by (Reitman & Frankel, 1957) was used to determine AST levels. Briefly, 100 µL of the test sample was mixed with 500 µL of buffer (containing 100mM phosphate buffer pH 7.4, 100mM l-aspartate for AST, 200mM l-alanine for ALT, and 2mM α-oxoglutarate) and the mixture was incubated for 30 min at 37 °C. Thereafter, 500 µL of 2 mM 2,4-dinitrophenylhydrazine was added to the reaction mixture and allowed to stand for 20 min at 25 °C. Then, 500 µL of 0.4mM NaOH was added and thoroughly mixed; the absorbance was read after 5 min at 546nm against a reagent blank and the AST and ALT activity were determined. ALP concentration was assayed spectrophotometrically by the technique of (Adefegha *et al*., 2015). This was done by adding 20 µL of the test sample and 1 ml of the reaction. The absorbance was read at 1 min intervals for 3 min at 405 nm, and ALP activity was subsequently determined (DGKC 1972).

DETERMINATION OF CYSTATIN C

A particle-enhanced immunonephelometric assay was used to measure the quantity of cystatin C according to (Tanaka *et al*., 2004). Blood samples were treated with latex particles coated with monoclonal antibodies against human cystatin C, and nephelometry was used to assess the development of antigen–antibody complexes as enhanced light scattering. A standard calibration curve was used to determine concentrations. A particle-enhanced immunonephelometric assay was used to measure cystatin C levels according to (Tanaka *et al*., 2004). Briefly, serum samples were incubated with latex particles coated with monoclonal antibodies against human cystatin C. Antigen–antibody complex formation was quantified by nephelometry based on increased light scattering, and concentrations were derived from a standard calibration curve. The assay was performed using a commercial cystatin C reagent kit (BN II/BN ProSpec system, Siemens Healthineers, Germany) designed and calibrated for human samples. Although cystatin C is evolutionarily conserved across mammalian species, the antibodies used are human-specific, and no formal validation for Wistar rat samples was conducted in this study. Therefore, potential cross-reactivity with rat cystatin C was assumed based on molecular conservation and previously reported use of similar immunoassays in non-human species, but was not experimentally confirmed. This limitation may affect the accuracy of absolute concentration values; however, since all experimental groups were processed under identical conditions, the assay remains suitable for comparative analysis of relative changes in cystatin C levels.

DETERMINATION OF B-TRACE PROTEIN

The level of β-trace protein was measured using an enzyme-linked immunosorbent assay (ELISA) as described by (Oda *et al*., 1996). Serum samples were added to wells pre-coated with monoclonal antibodies specific for β-trace protein. After washing, an enzyme-conjugated secondary antibody was addeds, followed by incubation with substrate solution. The intensity of the developed color was measured at 450 nm and the concentration was calculated using a standard curve.

DETERMINATION OF LEUCINE (AMINO ACID)

Leucine concentration was determined using high-performance liquid chromatography (HPLC) with pre-column derivatization, following the protocol of (Pencharz *et al*., 2012).

DETERMINATION OF TOTAL PROTEIN (TP)

The total protein concentration was measured using the Biuret method, as described by (Buzanovskii, 2017). Briefly, 20 µL of serum was mixed with 1 mL of Biuret reagent containing copper sulfate, sodium potassium tartrate, and sodium hydroxide. The mixture was incubated for 10 min at room temperature and the absorbance was measured at 546 nm. The protein concentration was calculated using a standard calibration curve.

DETERMINATION OF LACTATE DEHYDROGENASE (LDH) ACTIVITY

The LDH activity was determined using the method described by (Akagawa *et al*., 2016). The assay measures the oxidation of lactate to pyruvate, with a concomitant reduction of NAD^+ to NADH. Briefly, 100 µL of the test sample was mixed with 1 mL phosphate buffer containing NAD + and sodium lactate. The increase in absorbance at 340 nm was monitored for 3 min and LDH activity was calculated using the molar absorptivity of NADH.

DETERMINATION OF PROTHROMBIN TIME (PrT)

Prothrombin time was assessed using a standard coagulometric method as described by (Casella *et al*., 1988). Briefly, citrated plasma (100 µL) was incubated at 37 °C for 2 min, after which 200 µL of a pre-warmed thromboplastin reagent containing calcium chloride was added. The time required for clot formation was measured using a coagulometer. Prothrombin time was expressed in seconds and compared against normal control plasma.

ANALYSIS OF DATA

The data generated were analyzed using GraphPad Prism 8.0.1 (La Jolla, California, USA) and are presented in bar charts. Statistically significant differences were determined using two-way analysis of variance (ANOVA), and the means were compared using post hoc tests with multiple comparisons (Tukey's post-hoc test). Differences were considered statistically significant at $P < 0.05$.

RESULTS

EFFECTS ON ALT, AST, ALBUMIN AND ALP

As shown in Figure 1a, the level of ALT significantly increased in the paraquat group ($p < 0.05$) compared to the control group. In contrast, ALT levels decreased significantly in the high-dose eugenol group compared to those in the paraquat group ($p < 0.05$). In Figures 1c and 1d, there was a significant decrease in the levels of AST and albumin in the paraquat-treated group compared with the control group ($p < 0.05$). A significant decrease in AST level was observed in the low-dose group, whereas the high-dose group showed a significant increase compared with the paraquat-only group. Additionally, a significant increase in albumin level was observed in both the low- and high-dose groups ($p < 0.05$). The ALP level

was significantly increased in the paraquat group compared to the control group at $p<0.05$, and it was further increased significantly in the low-dose group ($p < 0.05$) compared to the paraquat group, as shown in Figure 1b.

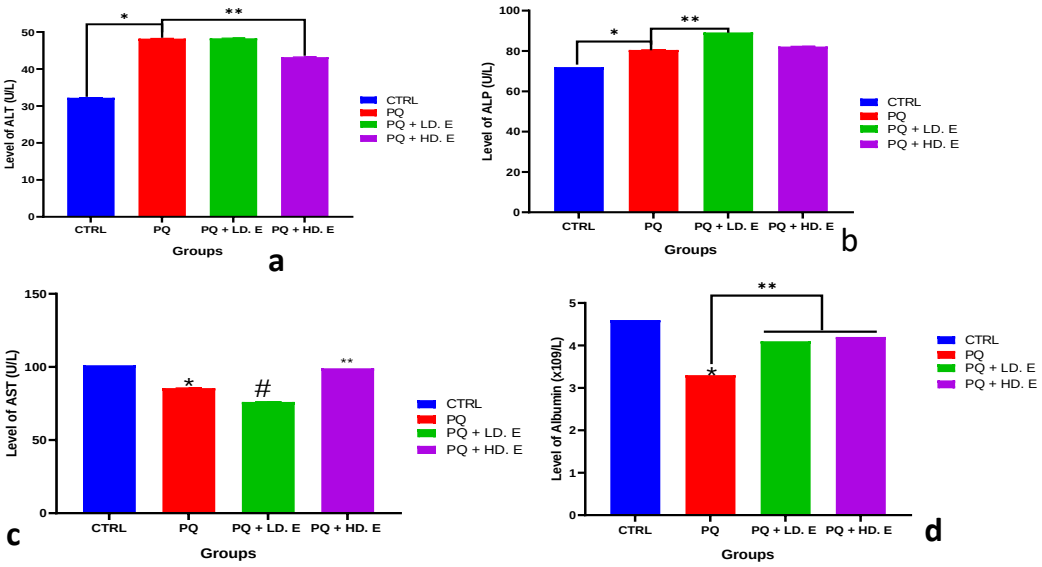


Fig 1: The effect of paraquat and eugenol on the level of (a) ALT, (b) ALP, (c) AST, and (d) albumin in experimental rats. *Significant difference at $p<0.05$ compared to the control; **Significant difference at $p<0.05$ compared to the untreated; #Significant difference at $p<0.05$ compared to the untreated.

EFFECTS ON PrT, LDH AND TP

Figures 2a-c represent the effects of paraquat and eugenol on the levels of prothrombin time (PrT), lactate dehydrogenase (LDH), and total protein, as assayed using liver homogenate. In the paraquat groups, the levels of PrT and LDH significantly increased ($p<0.05$), and the level of TP decreased significantly ($p<0.05$), compared to the control group, as shown in Figures 2a, b, and c. The levels of PrT and LDH were significantly reduced in the groups that received both low and high doses of eugenol ($p<0.05$), while the TP level significantly increased in the high-dose eugenol group alone ($p<0.05$) compared to the paraquat group (Figures 2a, b, and c).

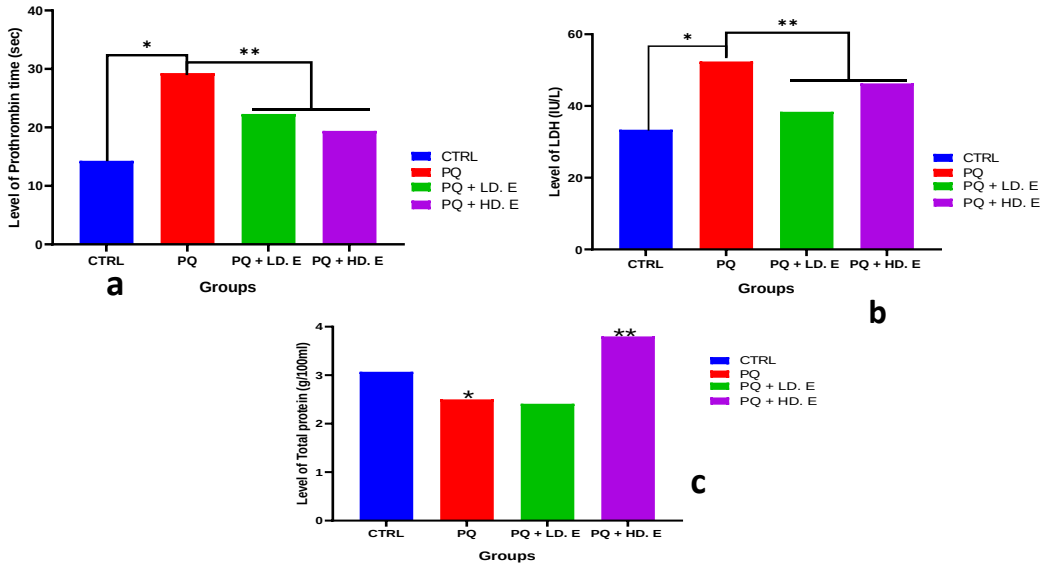


Fig 2: Effect of paraquat and eugenol on the levels of (a) prothrombin, (b) Lactate Dehydrogenase, and (c) total protein during the experiment. *Significant difference at $p<0.05$ compared to the control; **Significant difference at $p<0.05$ compared to the untreated

EFFECTS ON CREATININE, UREA, CYSTATIN AND B-TRACE ELEMENTS

Figures 3a-d are–charts show the effects of paraquat and eugenol on creatinine, urea, cystatin, and β-trace element levels. The levels of creatinine and urea were significantly reduced in the paraquat group compared to those in the control group ($p<0.05$). The low and high dose eugenol showed significant increase in the the level of creatinine and urea compared to the paraquat group ($p < 0.05$). In contrast to the above statements, the cystatin and β-trace element levels were all significantly increased in the PQ groups at $p<0.05$, compared to the control, while eugenol administered to the rats significantly lowered the levels of cystatin and β-trace elements at $p<0.05$, compared to the PQ group, as seen in Figures 3c and d below.

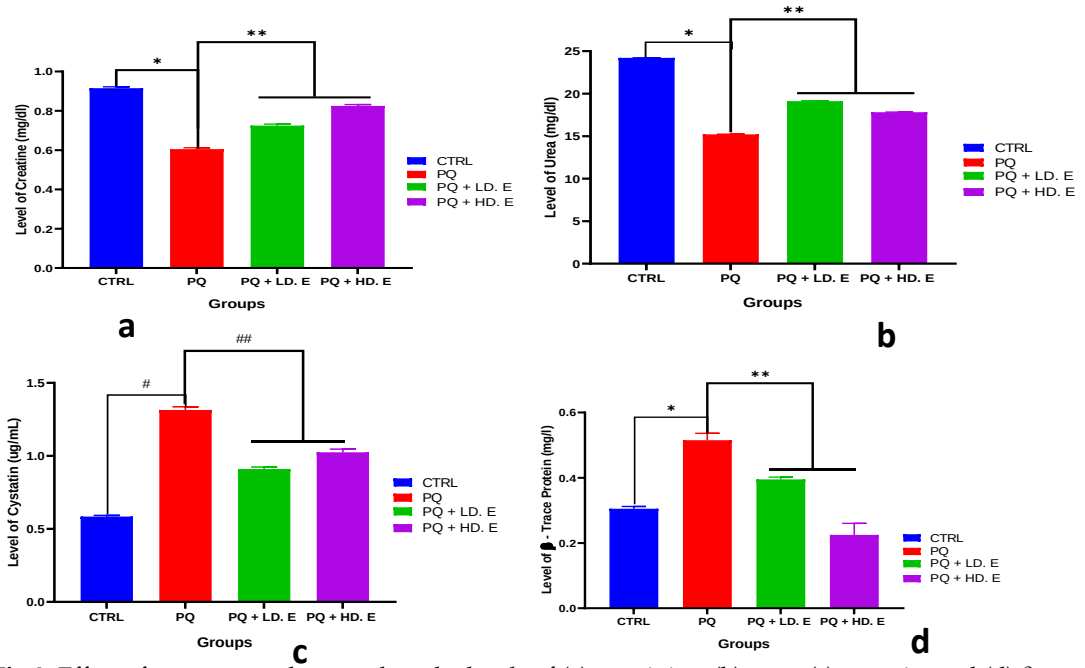
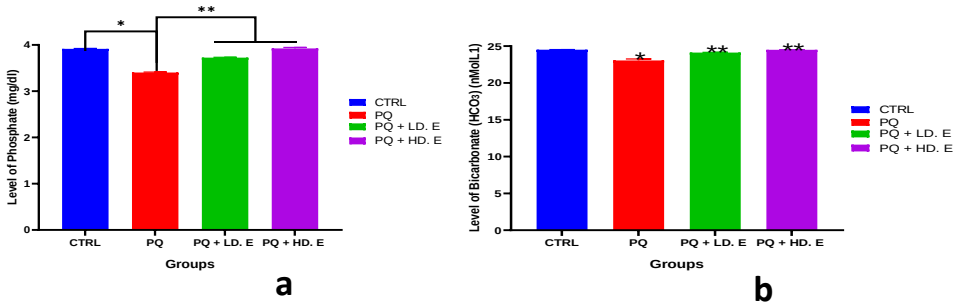
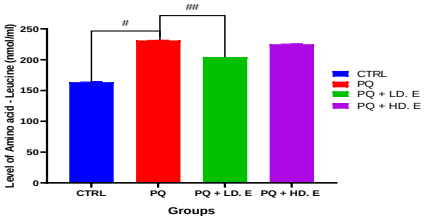


Fig 3: Effect of paraquat and eugenol on the levels of (a) creatinine, (b) urea, (c) cystatin, and (d) β-trace elements in experimental rats. *Significant difference at $p<0.05$ compared to the control; **Significant difference at $p<0.05$ compared to the untreated.

EFFECTS ON PHOSPHATASE, BICARBONATE, AND AMINO-ACID LEUCINE

Changes in the levels of phosphatase, bicarbonate, and amino acids are shown in Figures 4a-b below. The levels of phosphatase and bicarbonate significantly decreased in the PQ group ($p < 0.05$) compared to the control, while eugenol significantly increased the levels of phosphatase and bicarbonate ($p < 0.05$) compared to the PQ group (Figures 4a and b). It was observed that PQ significantly increased the level of the amino acid leucine in the body at $p<0.05$, compared to the control (Figure 4c). In contrast, the level of the amino acid leucine decreased significantly at $p<0.05$, compared to the PQ group (Figure 4c).





c

Fig 4: Effect of paraquat and eugenol on the levels of (a) phosphatase, (b) bicarbonate, and (c) leucine. *Significant difference at p<0.05 compared to the control; **Significant difference at p<0.05 compared to the untreated; #Significant difference at p<0.05 compared to the control; ##Significant difference at p<0.05 compared to the untreated.

DISCUSSION

Eugenol attenuated the biochemical changes induced by paraquat's systemic toxicity, which affected renal biomarkers, protein metabolism, and liver function. The pattern shows tissue dysfunction caused by oxidative stress as opposed to discrete organ damage. Paraquat exposure, ALT increased, signifying hepatocellular injury (Ujowundu *et al*., 2018; Zeinvand-Lorestani *et al*., 2018), while high dose of eugenol (600 mg/kg) reversed the effect by decreasing ALT, indicating better membrane stability and less liver damage. Paraquat exposure decreased albumin and total protein, although in albumin, both low and high doses (300 mg/kg and 600 mg/kg) of eugenol resulted in an increase, while TP only the high dose of eugenol (600mg/kg) showed increase, indicating impaired hepatic protein synthesis; their recovery with eugenol suggests restored synthetic function (Tavvabi-Kashani *et al*., 2024; Kumar *et al*., 2021; Abbas *et al*., 2019; Zhang *et al*., 2020; Ujowundu *et al*., 2018). For liver damage, it is abnormal; AST decreased with paraquat. Rather than less damage, this could be the result of changed systemic metabolism or enzyme inhibition (Ujowundu *et al*., 2018; Zeinvand-Lorestani *et al*., 2018). The opposing reactions to eugenol (increase at 600 mg/kg and reduction at 300 mg/kg) show that enzyme activity is dose-related. ALP increased with paraquat and much more with low-dose eugenol, indicating that 300 mg/kg was not enough to correct biliary or membrane-related disruption (Kumar *et al*., 2021; Abbas *et al*., 2019; Zhang *et al*., 2020). Better protection at the higher dose is shown by the lack of this pattern at 600 mg/kg. Paraquat exposure led to decrease in total protein while LDH and prothrombin time increased, which is consistent with cellular damage and lower hepatic synthetic ability (Hashemi Domeneh *et al*., 2025; Zhang, 2025; Zhang *et al*., 2024).

Eugenol, particularly at 600 mg/kg, improved these alterations. Following paraquat, creatinine and urea decreased, which is not indicative of kidney damage. Rather than better renal function, this probably indicates disturbed metabolic production. Cystatin and β-trace protein, on the other hand, increased, indicating renal involvement (Fathy Al-Mshaly *et al*., 2021; Abdel-Moaty *et al*., 2021; Dehkhoda *et al*., 2024). Both indicators were lowered by eugenol, suggesting renal protection. Following paraquat, phosphate and bicarbonate levels dropped, indicating a metabolic imbalance and potential acidosis (Fathy *et al*., 2022; Kumar *et al*., 2021). Treatment with eugenol benefited both. Leucine reduced with eugenol, indicating metabolic correction, and increased with paraquat, indicating disrupted amino acid metabolism. However, AST and ALP did not follow a linear dose pattern, indicating that the response is not strictly dose-dependent. The 600 mg/kg dose consistently generated better improvement than 300 mg/kg across most measures. One limitation of this study is the lack of a eugenol-only group, which makes it difficult to distinguish between its protective function against paraquat and its inherent effects. Wistar rat metabolic reactions, toxicity progression, and drug management are different from clinical paraquat poisoning, which entails more complicated and quickly progressing multi-organ failure, translation to humans

is restricted. Although some enzyme alterations point to non-linear biological reactions, eugenol lessened paraquat-induced biochemical disruption, with stronger effects at higher doses.

CONCLUSION

Paraquat exposure resulted in significant biochemical disturbances in hepatic and renal function, as evidenced by altered renal biomarkers, reduced protein synthesis, and altered enzyme activity. These changes demonstrate systemic toxicity consistent with oxidative stress-induced tissue damage. Eugenol treatment reduced most of these alterations, indicating a protective effect on liver and kidney function. The higher dose (600 mg/kg) often produced more biochemical improvement than the lower dose (300 mg/kg), despite certain non-linear patterns in the enzyme responses. This suggests that the protective effect of eugenol is dose-responsive but not always commensurate. However, the absence of a eugenol-only group limits the interpretation of its independent physiological effects. Furthermore, direct clinical extrapolation is limited due to variations between human paraquat toxicity and rodent odels. Eugenol showed quantifiable protective effect against biochemical toxicity caused by paraquat, indicating its promise as a therapeutic candidate that needs additional clinical and mechanistic confirmation.

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Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors are free of any personal or business association(s) that could represent any conflict of interest, and we have respected the ethical principles underpinning the research.

Authors Contribution

Conceptualization, IOA, OOU, and EJA; methodology, OOU and EJA; software, UOO, and EJA; validation, IOA and OOU; formal analysis, OOU and EJA; investigation, OOU and EJA; resources, IOA and OOU; data curation, OOU; writing—original draft preparation, OOU and EJA; writing—review and editing, IOA and UOO; visualization, UOO and IOA; supervision, IOA and OOU; project administration, OOU, and EJA; funding, all authors. All authors have read and agreed to the published version of the manuscript.

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