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Original Article

Dose-dependent hepatoprotective effects of ochnaflavone against paraquat-induced hepatic damage in albino rats

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Abstract

Paraquat (PQ) is a quaternary ammonium compound extremely toxic to humans and animals, causing multi-organ toxicity, particularly hepatic damage. Ochnaflavone (OCH), a biflavonoid isolated from *Ochna integerrima*, possesses potent anti-cancer, anti-inflammatory, anti-diabetic and antioxidative properties. This investigation was intended to estimate the dose-dependent remedial efficacy of OCH to combat PQ-mediated liver toxicity in male albino rats. The experimental period lasted for 8 weeks. 96 adult male rats (*Rattus norvegicus*; albino strain) were randomly allocated into 8 experimental groups: Control, PQ-intoxicated (5mg/kg), PQ+OCH1 (5mg/kg+25mg/kg), PQ+OCH2 (5mg/kg+50mg/kg), PQ+OCH3 (5mg/kg+100mg/kg), OCH1 (25mg/kg), OCH2 (50mg/kg), and OCH3 (100mg/kg). OCH was administered concurrently with PQ in the co-treated groups. Following oral administration, biochemical and liver function markers were determined along with histopathological observation. Results revealed that PQ intoxication downregulated the antioxidant enzymes activities such as glutathione reductase (GSR), heme oxygenase-1 (HO-1), catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathione (GSH) content, while concurrently upregulated the malondialdehyde (MDA) level and reactive oxygen species (ROS) concentration. Furthermore, PQ administration significantly provoked the concentrations of serum hepatic enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), concurrently elevating the concentrations of inflammatory indices: tumor necrosis factor-α (TNF-α), cyclooxygenase 2 (COX-2), nuclear factor kappa-B (NF-κB), interleukin-6 (IL-6) as well as interleukin-1β (IL-1β). Additionally, PQ-treatment instigated histopathological abnormalities in liver tissues. However, OCH co-treatment significantly mitigated these biochemical and histopathological changes instigated by PQ exposure. Therefore, OCH attenuates PQ-induced hepatotoxicity in a dose-dependent manner through its potent antioxidative and anti-inflammatory potential.

Keywords: Paraquat, Ochnaflavone, Antioxidant, Anti-inflammatory, Liver

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Introduction

Herbicides are agrochemicals effective for weed control, but they pose significant toxicological risks to humans, fishes, and other animals (Khan et al., 2023). Paraquat (PQ) is classified as quaternary ammonium herbicide that poses severe toxic risk to organisms, with several incidents of acute poisoning and fatalities documented globally (Garcia Carrillo et al., 2025). PQ is widely used for its easy availability, low cost, and potent herbicidal effects in crops such as tobacco, rice, tea, coffee, and sugarcane (Manju et al., 2022). Conversely, persistent application of PQ has led to increasing environmental contamination, with detectable residues in soil, water and air owing to its relatively slow degradation (Donaher and Van den Hurk, 2023).

The World Health Organization estimates that accidental pesticide poisoning affects ~385 million individuals and causes 11,000 deaths annually, particularly in Southeast Asia (Nihash et al., 2025). PQ toxicity constitutes a major public health threat in Asia-Pacific region, where intentional ingestion drives alarming rates of morbidity and mortality. In clinical toxicology, PQ ranks as one of the most lethal herbicides, with associated mortality rates spanning 33.0–91.7% (Tajai and Kornjirakasemsan, 2023). Human exposure occurs mainly through oral ingestion or dermal contact, and acute poisoning can lead to death within a few hours, depending on the dose (Chatterjee et al., 2024). Studies have reported that PQ-intoxication is associated with severe systemic multi-organ toxicity, affecting lungs, kidneys, testes, brain and liver (Liu et al., 2024).

Liver plays a vital role in maintaining metabolic homeostasis through enzymatic regulation and detoxification (Contreras-Zentella et al., 2022). In normal conditions, mitochondrial-derived ROS is usually neutralized by antioxidant enzymes to maintain physiological balance (Meng et al., 2025). PQ administration produces superoxide anions, hydroxyl radicals, and peroxynitrites, which substantially reduce antioxidant enzymes activities, culminating in oxidative stress (OS)-driven histopathological anomalies (Blanco-Ayala et al., 2014; Alizadeh et al., 2022). Moreover, PQ exposure triggers immune responses, elevating pro-inflammatory cytokines and aggravating oxidative damage, thereby impairing liver function (Chen et al., 2025). Evidence from human epidemiology and rodent models documented PQ induced hepatotoxicity (Yu et al., 2022). Furthermore, PQ poisoning is of serious concern in many developing countries, primarily due to absence of effective therapeutic options (Shahsavarinia et al., 2024).

Traditional herbal remedies have been used for centuries to cure human and animal diseases (Izah et al., 2024). Flavonoids exhibit complex polyphenolic frameworks and functions as secondary metabolites in plants. They are abundantly present in vegetables, fruits, barks, roots, tea and wine (Bhatla and Lal, 2023). They display a diverse array of pharmacological properties (Mohi-Ud-Din et al., 2022). Ochnaflavone (OCH) is a naturally occurring and structurally complex biflavonoid mainly isolated from members of the Ochnaceae family, including *Ochna integerrima, Ochna squarrosa* and *Ochna pumila* (Patel and Patel, 2024). It is reported that OCH exhibits antidiabetic, anticancer, and anti-inflammatory activities (Lin et al., 2024). Despite this, the potential hepatoprotective effects of OCH against PQ-induced liver injury have not yet been investigated. Therefore, this investigation was purposed to examine the dose-dependent remedial potential of OCH against PQ-elicited liver toxicity.

Material and Methods

Chemicals

PQ (CAS No. 75365-73-0; Molecular Weight: 257.16; Purity: 98%) was obtained from Sigma-Aldrich (Germany) and OCH (CAS No. 50276-96-5; Molecular Weight: 538.46; Purity: 98.30%) was obtained from MedChemExpress (USA), respectively.

Animals

Ninety-six adult male *Rattus norvegicus* (albino strain), each weighing approximately 230-250g, were housed in ventilated stainless-steel enclosures at UAF Animal Research Facility in Faisalabad. The animals were allowed to acclimatize for one week before the study commenced. Standard laboratory environmental parameters such as relative humidity 45±5%, temperature 25±1°C and a 12 h light and 12 h dark cycle were maintained. The rats consumed a standard pellet-based diet and water without restriction. All animal handling and experimental procedures adhered to the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020) and EU Directive 2010/63/EU for animal research. Additionally, the UAF Institutional Bioethics Committee formally approved the

experimental protocol (No. BE-1130/ORIC/15-09-2025).

Experimental layout

96 adult male rats (albino strain) were allocated randomly into eight experimental groups, with *n*=12 rats per group as given below:

Group I: Served as a control group.

Group II: PQ-administered group was provided 5mg/kg of PQ through oral gavage.

Group III, IV and V: Concurrently treated groups labeled as PQ+OCH1, PQ+OCH2 and PQ+OCH3, received a constant dose of 5mg/kg of PQ along with varying doses of OCH at 25mg/kg, 50mg/kg and 100mg/kg, respectively, to assess the potential dose-related efficacy of OCH against PQ-mediated hepatic toxicity.

Group VI, VII and VIII: OCH was only administered to the groups designated as OCH1, OCH2, and OCH3, which received 25, 50 and 100mg/kg of OCH, respectively.

The study was conducted for 8 weeks. The specific OCH doses (25, 50 and 100mg/kg) were selected from the study performed by Wei et al. (2024), involving ginkgetin, a structurally similar biflavonoid with well-documented pharmacological and antioxidative properties. The 5mg/kg dose of PQ was selected from Edward et al. (2024). Following the experimental period, rats were anaesthetized with xylazine (6mg/kg) & ketamine (60mg/kg) via intraperitoneal injection, and subsequently decapitated. Blood samples were obtained from the trunk using syringes containing heparin as an anticoagulant. Plasma was isolated from blood via centrifugation at 2000 x g for 15 minutes and stored at -20°C. The liver was excised and divided into two halves. For subsequent biochemical evaluations one half was stored in resealable polyethylene bags at -80°C, and the remaining portion was fixed in a 10% neutral-buffered formalin solution for histopathological analysis.

Biochemical assay

Antioxidant enzymes GST, CAT, HO-1, GSR, GPx, and SOD activities, were measured by the techniques of Habig et al. (1974); Aebi (1984); Tenhunen et al. (1968); Carlberg and Mannervik (1975); Rotruck et al. (1973) and Kakkar et al. (1984) respectively. GSH content was estimated with the help of established protocol of Moron et al. (1979). ROS concentration as well as MDA level were measured according to the technique of Hayashi et al. (2007) and Ohkawa et al. (1979) respectively.

Estimation of hepatic function parameters

Hepatic function markers such as ALP, ALT and AST were measured through ELISA kits bought from AMP diagnostics, Abcam, MA (USA). The process was carried out in accordance with the manufacturer's instructions.

Estimation of inflammatory parameters

Key inflammatory mediators, including IL-6, TNF-α, COX-2, IL-1β, and NF-κB were quantified by using ELISA kits bought from Cusabio Technology LLC, Houston, Texas, USA. Every step strictly followed the manufacturer's guidelines.

Histopathological assessment

Hepatic tissue samples were fixed by immersion in 10% neutral-buffered formalin for 24 hours. Subsequently, tissues were dehydrated through an ascending ethanol gradient (80, 90, and 100%), encased in paraffin wax, and sectioned at a thickness of 4-5 µm using a microtome. Afterwards, hematoxylin and eosin staining was applied to the sections, which were then placed on glass slides using a permanent mounting medium. Histopathological assessment was performed by a pathologist blinded to the treatment groups to ensure an unbiased evaluation. Finally, a Leica microscope was employed to examine the prepared slides at a magnification of 200x.

Statistical analysis

Data were presented as Mean ± SE. For statistical comparisons, a one-way ANOVA was performed, and differences between groups were calculated using Tukey's post hoc test via Minitab 17 statistical software, with significance evaluated at $p < 0.05$ threshold.

Results

Effect of treatments on biochemical assay

PQ administration noticeably ($p < 0.05$) reduced the antioxidant enzymes activities, whereas escalated MDA level and ROS concentration in PQ-exposed animals in comparison to the control group as presented in Table 1 and Table 2. Nonetheless, OCH+PQ co-administration improved antioxidant enzymes activities in a dose-related manner, while

substantially reducing the concentrations ROS & MDA as compared to PQ-intoxicated group. Additionally, OCH3 at a dose of 100mg/kg exhibited maximum elevation in antioxidant enzymes activity. Notably, the OCH only treated groups displayed no significant change in the aforementioned markers versus control rats.

Table-1. Effects of OCH and PQ treatments on hepatic antioxidant enzymes activities in albino rats.

Groups	Parameters						
	CAT (U/mg protein)	**SOD (U/mg protein)**	**GPx (U/mg protein)**	**GSR (nM NADPH oxidized/min /mg tissue)**	**GST (nM/min/mg protein)**	**GSH (µM/g tissue)**	**HO-1 (pmole bilirubin/min/mg protein)**
Control	17.83±0.77^{abc}	17.08±0.88^{a}	30.97±1.19^{a}	12.52±0.85^{a}	38.49±0.68^{a}	23.96±0.79^{a}	459.94±7.33^{a}
PQ	8.17±0.58^{d}	6.33±0.55^{c}	10.86±0.95^{d}	4.71±0.38^{c}	14.84±0.87^{e}	10.04±0.87^{c}	79.84±3.27^{e}
PQ+OCH1	14.75±0.58^{c}	10.64±0.40^{b}	19.15±0.87^{c}	8.80±0.60^{b}	26.67±0.65^{d}	15.34±0.40^{b}	263.19±5.95^{d}
PQ+OCH2	15.48±0.60^{bc}	15.01±0.79^{a}	23.43±0.53^{bc}	11.26±0.44^{ab}	30.71±0.91^{c}	17.14±0.22^{b}	318.19±7.04^{c}
PQ+OCH3	17.67±0.55^{abc}	17.41±0.53^{a}	25.54±0.66^{b}	11.57±0.26^{ab}	34.67±0.79^{b}	18.46±0.55^{b}	384.72±8.93^{b}
OCH1	18.13±0.74^{ab}	17.47±0.85^{a}	31.25±1.26^{a}	12.75±0.88^{a}	38.88±0.75^{a}	24.11±0.82^{a}	464.29±6.99^{a}
OCH2	18.91±0.69^{a}	17.82±0.88^{a}	31.51±1.35^{a}	12.96±0.92^{a}	39.49±0.64^{a}	24.21±0.79^{a}	468.86±7.41^{a}
OCH3	19.88±0.47^{a}	18.26±0.90^{a}	32.11±1.46^{a}	13.43±0.80^{a}	39.82±0.69^{a}	24.59±0.98^{a}	471.89±8.18^{a}

Values represent Mean±SE. Different superscripts (a-e) within a column show statistically significant variation between the groups.

Table-2. Quantitative assessment of oxidative stress indicators in response to treatments.

Groups	Parameters	
	MDA (nmol/mg protein)	**ROS (µmol H_2O_2 equivalents/mg protein)**
Control	0.59±0.20^{d}	1.23±0.29^{d}
PQ	4.21±0.25^{a}	8.14±0.51^{a}
PQ+OCH1	2.17±0.14^{b}	3.71±0.27^{b}
PQ+OCH2	1.66±0.18^{bc}	3.21±0.19^{bc}
PQ+OCH3	1.10±0.27^{cd}	2.04±0.16^{cd}
OCH1	0.54±0.21^{d}	1.14±0.28^{d}
OCH2	0.47±0.22^{d}	1.08±0.31^{d}
OCH3	0.32±0.17^{d}	1.01±0.34^{d}

Values represent Mean±SE. Different superscripts (a-e) within a column show statistically significant variation between the groups.

Effect of treatments on serum hepatic parameters

The impacts of OCH and PQ on serum hepatic enzymes are displayed in Table 3. Results highlight that, PQ intoxication remarkably ($p < 0.05$) raised liver markers (AST, ALT and ALP) levels in contrast to the control group. However, OCH+PQ co-treatment dose-dependently reduced the elevation of AST, ALT and ALP levels in 25, 50 and 100mg/kg treatment groups compared to PQ intoxicated group. Nevertheless, the most prominent reduction was seen in PQ+OCH3 group. In addition, OCH1, OCH2 and OCH3 only treated groups displayed no significant variations in liver function markers relative to the control group.

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Table-3. Displays the effects of treatments on serum hepatic markers.

Groups	Parameters		
	AST (U/I)	**ALT (U/I)**	**ALP (U/I)**
Control	82.97±1.02^{e}	39.53±1.34^{d}	97.45±1.23^{e}
PQ	224.61±3.62^{a}	85.64±1.10^{a}	379.28±5.43^{a}
PQ+OCH1	157.83±2.70^{b}	62.29±1.14^{b}	284.13±4.02^{b}
PQ+OCH2	129.34±1.74^{c}	56.22±1.21^{b}	223.12±2.75^{c}
PQ+OCH3	113.38±2.68^{d}	47.77±0.97^{c}	168.45±5.18^{d}
OCH1	81.87±1.21^{e}	39.13±1.47^{d}	96.73±0.99^{e}
OCH2	81.22±1.56^{e}	38.95±1.44^{d}	95.60±0.91^{e}
OCH3	80.49±1.49^{e}	38.70±1.51^{d}	96.41±1.14^{e}

Values represent Mean±SE. Different superscripts (a-e) within a column show statistically significant variation between the groups.

Effects of treatments on inflammatory biomarkers

PQ administration evidently ($p < 0.05$) upregulated inflammatory parameters (COX-2, IL-6, NF-κB, IL-1β, and TNF-α) concentrations versus control rats. Nonetheless, PQ+OCH co-treatment, in a dose-related manner, substantially lowered the concentrations of all above-mentioned inflammatory indices in contrast to only PQ exposed group. However, the most pronounced reduction was observed in PQ+OCH3 co-treated group. Furthermore, no substantial differences were observed in OCH1, OCH2 and OCH3 supplemented groups versus control (Table 4).

Table-4. Shows the effects of treatments on key inflammatory parameters.

Groups	Parameters				
	NF-κB (ng/g tissue)	**TNF-α (ng/g tissue)**	**IL-1β (ng/g tissue)**	**IL-6 (ng/g tissue)**	**COX-2 (ng/g tissue)**
Control	22.75±1.35^{d}	22.51±0.59^{e}	19.08±0.93^{de}	9.55±0.69^{d}	14.63±0.53^{d}
PQ	86.84±1.51^{a}	93.19±1.27^{a}	78.99±0.87^{a}	66.91±0.73^{a}	53.70±1.41^{a}
PQ+OCH1	38.42±1.19^{b}	43.91±0.90^{b}	35.86±0.71^{b}	22.51±1.23^{b}	27.94±0.86^{b}
PQ+OCH2	33.36±1.05^{bc}	36.95±0.85^{c}	29.42±1.06^{c}	16.51±0.43^{c}	23.71±0.60^{c}
PQ+OCH3	27.88±0.95^{cd}	32.06±1.22^{d}	22.70±0.78^{d}	12.65±0.88^{cd}	17.42±0.63^{d}
OCH1	22.50±1.41^{d}	22.19±0.72^{e}	18.61±0.97^{de}	9.32±0.72^{d}	14.44±0.48^{d}
OCH2	21.98±1.48^{d}	22.05±0.47^{e}	18.16±0.86^{e}	9.14±0.68^{d}	14.14±0.60^{d}
OCH3	21.70±1.53^{d}	21.54±0.65^{e}	17.86±0.91^{e}	8.79±0.84^{d}	13.97±0.55^{d}

Values represent Mean±SE. Different superscripts (a-e) within a column show statistically significant variation between the groups.

Effect of treatments on liver histology

Figure 1 illustrates the impacts of PQ and OCH on histology of liver tissues. PQ intoxication considerably ($p < 0.05$) elicited histopathological alterations, resulting in sinusoid dilation, damage to central venules, necrosis as well as obscured hepatocyte boundaries among PQ exposed rats as compared to the control rats. Conversely, concurrent treatment with OCH and PQ in a dose-related manner mitigated the severity of aforementioned histopathological changes and normalized the structural organization of hepatocytes as compared to PQ administered group. Nonetheless, the most prominent attenuation of histological damage was observed in PQ+OCH3 group. Moreover, OCH1, OCH2 and OCH3 only treated groups exhibited normal histology similar to control rats.

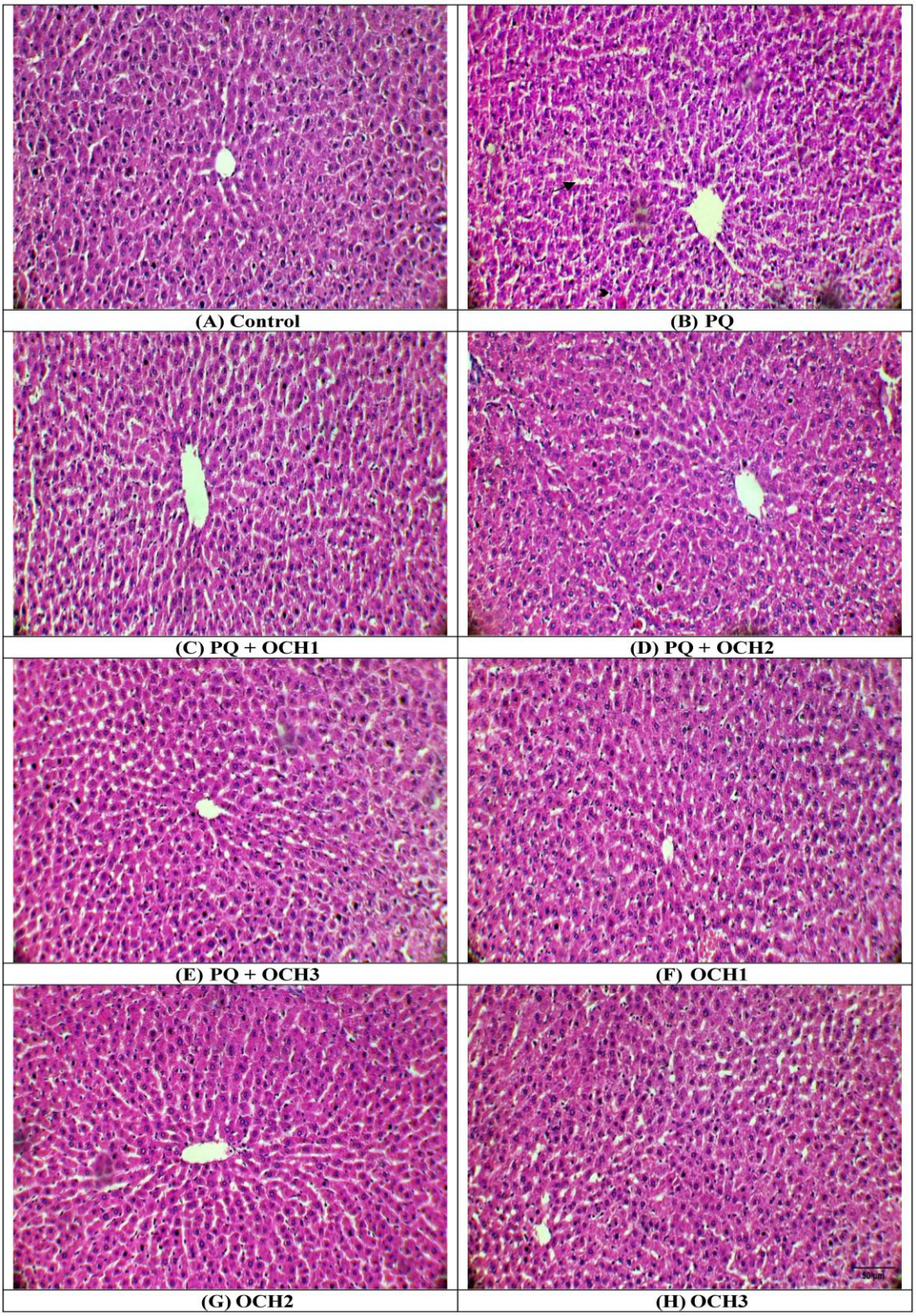


Figure-1. Illustrates dose-dependent protective efficacy of OCH to counteract PQ prompted histological damages in different groups such as: A) Control, B) PQ (5mg/kg), C) PQ+OCH1 (5mg/kg+25mg/kg), D) PQ+OCH2 (5mg/kg+50mg/kg), E) PQ+OCH3 (5mg/kg+100mg/kg), F) OCH1 (25mg/kg), G) OCH2 (50mg/kg), H) OCH3 (100mg/kg); Sinusoidal dilation (arrow), Central Venule (CV) and pyknotic nuclei (chevron).

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Discussion

Paraquat is a widely used herbicide that exerts severe toxic effects on living organisms (Ghafoor et al., 2026). Exposure to PQ causes severe hepatic injury by generating excessive free radicals and compromising the antioxidant defense system, which triggers OS inflammation, and degenerative histopathological alterations in the liver (Ijaz et al., 2024). In contrast, OCH is a biflavonoid known to exhibit a wide range of therapeutic properties (Lin et al., 2024). Therefore, this investigation was intended to analyze the dose-dependent effects of OCH in combating PQ-elicited hepatotoxicity.

In this work, biochemical analysis demonstrated that PQ intoxication markedly downregulated the antioxidative parameters, while escalating MDA and ROS levels. Antioxidant enzymes constitute 1st line of defense against free radicals, providing protection to the essential biomolecules (Roy et al., 2023). A reduction in the activities of antioxidant enzymes results in elevated ROS concentrations, thereby triggering OS (Jomova et al., 2024). Furthermore, OS induces lipid peroxidation that leads to membrane disruption and dysregulation of cellular functions (Wang et al., 2023). These findings indicate that PQ triggers severe oxidative imbalance and lipid peroxidation in hepatic tissues, aligning with earlier observations by Zhang et al. (2021). Contrarily, co-treatment with OCH and PQ substantially attenuated the decline in antioxidant enzymes activities and attenuated ROS and MDA levels in a dose-dependent manner. These protective effects may be associated with the phenolic hydroxyl groups in biflavonoids, which enable them to scavenge free radicals and stabilize reactive species (Devireddy and Lakshmi, 2026). As OCH is a biflavonoid, it is hypothesized that its phenolic hydroxyl groups contribute to its antioxidant potential.

PQ administration considerably increased the concentrations of hepatic serum markers (AST, ALP, and ALT), indicating membrane damage and hepatocellular injury. Jain et al. (2023) described that elevated concentrations of these markers in blood normally indicate liver dysfunction as well as OS. The present findings corroborate with earlier observations by Chikere et al. (2025), who validated that PQ exposure augmented the level of ALT, AST, and ALP in rats. However, our data suggested that co-treatment with OCH and PQ in a dose-related manner markedly attenuated these alterations. The hepatoprotective efficacy of OCH may be attributed to its antioxidative potential and its capacity to preserve membrane integrity against PQ-induced cellular damage.

Paraquat administration substantially instigated inflammatory cascade in hepatic tissues as demonstrated by augmented level of inflammatory indices; such as IL-1β, IL-6, TNF-α, NF-κB, and COX-2 in rats. During inflammatory process, NF-κB serves as a critical transcription factor that mediates the production of of IL-6, IL-1β, and TNF-α (Guo et al., 2024). Our results are in line with the study of Elkholy et al. (2023) who reported that PQ exposure increased the level of TNF-α and NF-κB in rats. Nonetheless, co-treatment with OCH along with PQ in a dose-related manner, lowered the concentrations of inflammatory indices that may be attributed to the anti-inflammatory properties of OCH.

Histopathological examination of liver tissues further supported the biochemical observations. PQ exposure caused severe histological alterations such as necrosis, dilations of sinusoids, pyknotic nuclei and damaged central venules. These pathological changes are likely to be associated with excessive production of free radicals and LP in hepatocytes. Similar histological abnormalities following PQ exposure have also been previously reported in the studies of Kheiripour et al. (2021) and Edward et al. (2024). Conversely, OCH treatment substantially ameliorated these histopathological alterations and restored hepatic architecture that further supports its protective efficacy against PQ-induced hepatic injury. These ameliorative effects of OCH may be attributed to the free radicals neutralizing as well as anti-inflammatory potentials of OCH.

Conclusion

PQ intoxication caused hepatotoxicity via triggering OS, as evidenced by elevated inflammatory markers, hepatic serum enzymes, and oxidative stress biomarkers, along with downregulated antioxidant enzymes activities and GSH content. Additionally, PQ administration resulted in notable histopathological anomalies in rat liver tissue. Conversely, OCH supplementation potentially mitigated the aforementioned PQ-elicited injuries in a dose-related manner owing to its anti-inflammatory and ROS-scavenging properties. Therefore, these findings suggest that OCH supplementation may exert protective effects against PQ-mediated hepatotoxicity by mitigating OS and inflammation.

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Limitations of the study

A limitation of the current investigation is the use of an animal model, which may restrict direct extrapolation to human physiology. However, further clinical trials are required to translate these findings to humans.

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Disclaimer: None.

Conflict of Interest: None.

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Ethical Approval Statement

The experimental protocol received authorization from the Institutional Bioethics Committee of the University of Agriculture of Faisalabad, under approval number BE-1130/ORIC (Dated: 15/09/2025).

Contribution of Authors

Khalil M: Conceptualized, planned and conducted the experiment and wrote original draft.

Ijaz MU: Designed and supervised the study and edited the manuscript.

Khalil M & Ijaz MU: Reviewed literature, edited the manuscript and interpreted the results.

Ehsan N & Imran M: Provided the statistical evaluations and helped in the manuscript preparation and write-up.

All authors read and approved the final draft of the manuscript.

References

Aebi H, 1984. Catalase *in vitro*. Methods Enzymol. 105:121-126. DOI: https://doi.org/10.1016/s0076-6879(84)05016-3

Alizadeh S, Anani-Sarab G, Amiri H and Hashemi M, 2022. Paraquat induced oxidative stress, DNA damage, and cytotoxicity in lymphocytes. Heliyon. 8(7): e09895. DOI: https://doi.org/10.1016/j.heliyon.2022.e09895

Bhatla SC and Lal MA, 2023. Secondary metabolites, pp. 765-808. In Plant physiology, development and metabolism. Springer Nature, Singapore. DOI: https://doi.org/10.1007/978-981-99-5736-1

Blanco-Ayala T, Andérica-Romero AC, Pedraza-Chaverri J. 2014. New insights into antioxidant strategies against paraquat toxicity. Free Radic. Res. 48(6):623-40. https://doi.org/10.3109/10715762.2014.899694

Carlberg I and Mannervik B, 1975. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J. Biol. Chem. 250(14):5475-5480. DOI: https://doi.org/10.1016/S0021-9258(19)41206-4

Chatterjee A, Ansar M, Chandra A, Chakraborty U and Dasgupta S, 2024. An overview of paraquat poisoning: a review. Bengal Physician J. 11(1):24-29. DOI: https://doi.org/10.5005/jp-journals-10070-8040

Chen C, Qi M, Zhang W, Chen F, Sun Z, Sun W, Tang W, Yang Z, Zhao X and Tang Z, 2025. Taurine alleviated paraquat-induced oxidative stress and gut-liver axis damage in weaned piglets by regulating the Nrf2/Keap1 and TLR4/NF-κB signaling pathways. J. Anim. Sci. Biotechnol. 16(1):117. DOI: https://doi.org/10.1186/s40104-025-01244-3

Chikere OU, Favour NC, Nzube OB, Uchechi EE, Ndubuisi OA and Kenechukwu ED, 2025. Hepatoprotective effects of Solanum macrocarpon leaf extract against paraquat-induced liver toxicity in Wistar rats. J. Adv. Med. Med. Res. 37(3):268-277. DOI: https://doi.org/10.9734/jammr/2025/v37i35763

Contreras-Zentella ML, Villalobos-Garcia D and Hernandez-Munoz R, 2022. Ethanol metabolism in the liver, the induction of oxidant stress, and the antioxidant defense system. Antioxidants. 11(7):1258. DOI: https://doi.org/10.3390/antiox11071258

Devireddy AR and Lakshmi AV, 2026. Isolation, Characterization, and β-Cyclodextrin Nanosponge Formulation of Ochnaflavone from Ochna Obtusata for Enhanced Antioxidant Activity and Improved Drug

Delivery. Nat. Resour. Hum. Health. 6(5):39-49. DOI: https://doi.org/10.53365/nrfhh.395

Donaher SE and Van den Hurk P, 2023. Ecotoxicology of the herbicide paraquat: effects on wildlife and knowledge gaps. Ecotoxicology. 32(9):1187-1199. DOI: https://doi.org/10.1007/s10646-023-02714-y

Edward TA, Ajibade AJ, Edward SS and Umeaku U, 2024. Histological and biochemical effects of Lutein on the liver of adult Wistar rats following paraquat-induced toxicity. J. Exper. Clin. Anat. 21(2):317-323. DOI: https://doi.org/10.4314/jeca.v21i2.24

Elkholy AR, El-Sheakh AR and Suddek GM, 2023. Nilotinib alleviates paraquat-induced hepatic and pulmonary injury in rats via the Nrf2/Nf-kB axis. Int. Immunopharmacol. 124:110886. DOI: https://doi.org/10.1016/j.intimp.2023.110886

Garcia Carrillo M, Madanes D and Blaustein M, 2025. Carcinogenic effects of paraquat-based herbicides: a comprehensive review. Environ. Sci. Pollut. Res. 32(26):15473-15492. DOI: https://doi.org/10.1007/s11356-025-36593-z

Ghafoor N, Salar MZ, Ashraf A, Hayat MF, Batool M and Hamdi H, 2026. Potential therapeutic role of robinetin against paraquat-instigated hepatic toxicity via regulating nrf-2/keap-1, oxidative stress and inflammation. J. Anim. Plant Sci. 36(3):669-679. DOI: https://doi.org/10.36899/JAPS.2026.3.0055

Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, Zeng C, Zhou T and Zhang J, 2024. NF-κB in biology and targeted therapy: new insights and translational implications. Sig. Transduct. Target Ther. 9(1):53. DOI: https://doi.org/10.1038/s41392-024-01757-9

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

Hayashi I, Morishita Y, Imai M, Nakamura M, Nakachi K and Hayashi T, 2007. High-throughput spectrophotometric assay of reactive oxygen species in serum. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 631(1):55-61. DOI: https://doi.org/10.1016/j.mrgentox.2007.04.006

Ijaz MU, Ghafoor N, Hayat MF, Almutairi BO and Atique U, 2024. Amentoflavone mediated hepatoprotection to counteract paraquat instigated hepatotoxicity via modulating Nrf2/keap1 pathway: A biochemical, inflammatory, apoptotic and histopathological study. Pestic. Biochem. Physiol. 198:105715. https://doi.org/10.1016/j.pestbp.2023.105715

Izah SC, Odubo TC, Ngun CT and Ogwu MC, 2024. Research needs of medicinal plants used in the management and treatment of some diseases caused by microorganisms. pp. 1797-1823. Herbal medicine phytochemistry: Applications and trends. Springer International Publishing, Cham, Switzerland. DOI: https://doi.org/10.1007/978-3-031-21973-3_11-1

Jain P, Batta AK and Singh P, 2023. Comparative study of serum levels of gamma-glutamyl transferase, Aspartate aminotransferase (AST), Alanine Transaminase (ALT), AST: ALT, and bilirubin in patients with chronic hepatitis. Ind. J. Med. Biochem. 26(3):73-76. DOI: https://doi.org/10.5005/jp-journals-10054-0208

Jomova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K and Valko M, 2024. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. Arch. Toxicol. 98(5):1323-67. DOI: https://doi.org/10.1007/s00204-024-03696-4

Kakkar P, Das B and Viswanathan PN, 1984. A modified spectrophotometric assay of superoxide dismutase. Ind. J. Biochem. Biophys. 21(2):130-132.

Khan BA, Nadeem MA, Nawaz H, Amin MM, Abbasi GH, Nadeem M, Ali M, Ameen M, Javaid MM, Maqbool R and Ikram M, 2023. Pesticides: impacts on agriculture productivity, environment, and management strategies, pp. 109-134. In: Emerging contaminants and plants: Interactions, adaptations and remediation technologies. Springer International Publishing, Cham, Switzerland. DOI: https://doi.org/10.1007/978-3-031-22269-6_5

Kheiripour N, Plarak A, Heshmati A, Asl SS, Mehri FF, Ebadollahi-Natanzi A and Hosseini A, 2021. Evaluation of the hepatoprotective

effects of curcumin and nanocurcumin against paraquat-induced liver injury in rats: Modulation of oxidative stress and Nrf2 pathway. J. Biochem. Mol. Toxicol. 35(5): e22739. DOI: https://doi.org/10.1002/jbt.22739

Lin S, Ganesan AR, Vargas-De-La-Cruz C, Ortiz-Viedma J and Xiao J, 2024. Advances in resources, biosynthesis pathway, bioavailability, bioactivity, and pharmacology of ochnaflavone. Future Postharvest Food. 1(1):47-60. DOI: https://doi.org/10.1002/fpf2.12011

Liu T, Zheng F, Liu L, Zhou H, Shen T, Li Y and Zhang W, 2024. Paraquat disrupts the blood–brain barrier by increasing IL-6 expression and oxidative stress through the activation of PI3K/AKT signaling pathway. Open Med. 19(1):10-20. DOI: https://doi.org/10.1515/med-2024-1020

Manju B, Jamal S and Lokesh NK, 2022. Paraquat poisoning, what we should know: a review article. Int. J. Health Sci. 6(3):2274-2284. DOI: https://doi.org/10.53730/ijhs.v6nS3.5999

Meng L, Liu S, Luo J, Tu Y, Li T, Li P, Yu J and Shi L, 2025. Oxidative stress and reactive oxygen species in otorhinolaryngological diseases: insights from pathophysiology to targeted antioxidant therapies. Redox Rep. 30(1):2458942. DOI: https://doi.org/10.1080/13510002.2025.2458942

Mohi-Ud-Din R, Mir RH, Sabreen S, Jan R, Pottoo FH and Singh IP, 2022. Recent insights into therapeutic potential of plant-derived flavonoids against cancer. Anti-Cancer Agents Med. Chem. 22(20):3343-3369. DOI: https://doi.org/10.2174/1871520622666220421094055

Moron MS, Depierre JW and Mannervik B, 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim. Biophys. Acta –Gen. Subj. 582(1):67-78. DOI: https://doi.org/10.1016/0304-4165(79)90289-7

Nihash VL, Jayashree GC, Spandana BM, Chilur AY, Krishnamma PN and Dharshan MB, 2025. Unintentional pesticide poisoning and safety of the farmers-a review. Int. J. Agric. Food Sci. 7(9):362-268. DOI: https://doi.org/10.33545/2664844X.2025.v7.i9e.765

Ohkawa H, Ohishi N and Yagi K, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95(2):351-358. DOI: http://doi.org/10.1016/0003-2697(79)90738-3.

Patel K and Patel DK, 2024. Therapeutic Potential and Pharmacological Activities of Bioflavonoid 'Ochnaflavone' in Medicine: Diverse Scaffolds and Promise Leads for Drug Discovery. Anti-Infect. Agents. 22(4):32-37. DOI: https://doi.org/10.2174/0122113525284912240221115753

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U and Emerson M, 2020. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS biology. 18(7): e3000410. DOI: https://doi.org/10.1371/journal.pbio.3000410

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG and Hoekstra W, 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science. 179(4073):588-590. DOI: https://doi.org/10.1126/science.179.4073.588

Roy Z, Bansal R, Siddiqui L and Chaudhary N, 2023. Understanding the role of free radicals and antioxidant enzymes in human diseases. Curr. Pharm. Biotechnol. 24(10):1265-76. DOI: https://doi.org/10.2174/1389201024666221121160822

Shahsavarinia K, Balafar M, Tahmasbi F, Gharekhani A, Milanchian N, Kashgsaray NH, Maham M and Salehi-Pourmehr H, 2024. Evidence of efficacy the various management methods in paraquat poisoning: an umbrella review. Toxin Rev. 43(3):437-451. DOI: https://doi.org/10.1080/15569543.2024.2355457

Tajai P and Kornjirakasemsan A, 2023. Predicting mortality in paraquat poisoning through clinical findings, with a focus on pulmonary and cardiovascular system disorders. J. Pharm. Policy Pract. 16(1):123. DOI: https://doi.org/10.1186/s40545-023-00635-z

Tenhunen R, Marver HS and Schmid R, 1968. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. Proc. Natl. Acad. Sci. 61(2):748-755. DOI: https://doi.org/10.1073/pnas.61.2.748

Wang B, Wang Y, Zhang J, Hu C, Jiang J, Li Y and Peng Z, 2023. ROS-induced lipid peroxidation modulates cell death outcome: mechanisms behind apoptosis, autophagy, and ferroptosis. Arch. Toxicol. 97(6):1439-51. DOI: https://doi.org/10.1007/s00204-023-03476-6

Wei G, Liang X, Wu F, Cheng C, Huang S and Zeng Y, 2024. Ginkgetin attenuates bone loss in OVX mice by inhibiting the NF-κB/IκBα signaling pathway. PeerJ. 12: e17722. DOI: https://doi.org/10.7717/peerj.17722

Yu J, Zhang L, Li X, Lv K, Sun S, Wu W and Yang N, 2022. Comparison of biochemical parameters between mouse model and human after Paraquat Poisoning. Biomed Res. Int. 2022:1254824. DOI: https://doi.org/10.1155/2022/1254824

Zhang S, Song S, Luo X, Liu J, Liu M, Li W and Cai H, 2021. Prognostic value of liver and kidney function parameters and their correlation with the ratio of urine-to-plasma paraquat in patients with paraquat poisoning. Basic Clin. Pharmacol. Toxicol. 128(6):822-830. DOI: https://doi.org/10.1111/bcpt.13555