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POTENTIAL THERAPEUTIC ROLE OF ROBINETIN AGAINST PARAQUAT-INSTIGATED HEPATIC TOXICITY VIA REGULATING *NRF-2*/*KEAP-1*, OXIDATIVE STRESS AND INFLAMMATION

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ABSTRACT

Paraquat (PQ) is an extensively used herbicide throughout the world, which is extremely harmful to living organisms. Robinetin (ROB) is a naturally occurring flavonoid with reported pharmacological potential. The current research was focused on identifying the hepato-protective potential of ROB against PQ-intoxicated liver damage in rats. The animals were randomly divided into four groups following a completely randomized design (CRD); control, PQ (5 $mgkg^{-1}$) group, PQ (5 $mgkg^{-1}$) + ROB (30 $mgkg^{-1}$) group and only ROB (30 $mgkg^{-1}$) supplemented group. The results of the current trial demonstrated that PQ exposure reduced the expressions of *NRF-2* and antioxidant enzymes while elevating the expressions of *KEAP-1*. Additionally, PQ exposure decreased the enzymatic activities of glutathione reductase (GSR), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) as well as glutathione (GSH) content while increasing malondialdehyde (MDA) and reactive oxygen species (ROS) levels, which indicated oxidative stress (OS) in the PQ administered group. Furthermore, PQ exposure increased the levels of liver serum enzyme i.e., AST, ALP and ALT. Moreover, PQ administration increased the levels of inflammatory cytokines, including NF-κB, TNF-α, COX-2, IL-1β, and IL-6. Besides, *CASPASE-3* and *BAX* (pro-apoptotic markers) expressions were augmented while *BCL-2* (anti-apoptotic marker) expressions were reduced due to PQ exposure. Furthermore, PQ treatment led to multiple histopathological damages in the hepatic tissues. However, ROB supplementation markedly restored the abovementioned alterations due to its anti-apoptotic, antioxidative and anti-inflammatory potentials.

Keywords: Paraquat, Robinetin, Oxidative stress, Hepatic damages, Antioxidant, Reactive Oxygen Species

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INTRODUCTION

PQ is a highly toxic herbicide that is extensively used in agriculture due to its effectiveness against weeds (Robinson *et al.,* 2016). Owing to its high-water solubility and low volatility, humans and animals are more susceptible to PQ-induced damage, upon accidental or intentional ingestion. PQ-instigated acute toxicity may cause organ dysfunction that consequently causes higher rates of morbidity as well as mortality (Villanueva-Paz *et al.,* 2021). PQ enters the human body via dermal contact, inhalation as well as ingestion (Badibostan *et al.,* 2024). After getting access to systemic circulation, it is disseminated into various tissues and organ systems. Previous literature indicates that the average annual mortality rate associated with PQ poisoning ranges from 60% to 70%, a consequence of multi-organ damage, particularly liver injury (Zhang *et al.,* 2020).

Reactive oxygen species (ROS) and oxidative stress are the key components of PQ toxicity. PQ undergoes oxidation and produces peroxynitrite, superoxide anion, and hydroxyl free radicals (Guerbette *et al.,* 2022). The liver is especially susceptible to oxidative injury because it is the primary site for xenobiotic metabolism and possesses a higher capacity for ROS generation. As a result, it has been proposed that PQ poisoning primarily targets the liver and causes hepatotoxicity in humans and rats (Mooli *et al.,* 2022). Since there is no precise remedy or efficient treatment against PQ-induced hepatic toxicity, developing novel therapeutic approaches is crucial to mitigate liver damage from PQ poisoning.

Flavonoids are polyphenolic agents that are present in multiple parts of plants. These phytochemicals are generally used in medications and dietary supplements due to their promising pharmacological

activities. Robinetin (ROB) is a naturally occurring flavonoid that demonstrates different biological and pharmacological properties (Tarahovsky *et al.,* 2008). ROB exhibits anti-oxidant (Menacer *et al.,* 2022), anti-viral (Mahmud *et al.,* 2021), and anti-cancer (Ogbodo *et al.,* 2023) properties. Moreover, ROB may be an active agent against Alzheimer's disease as it inhibits amyloid-β aggregations (Hanaki *et al.,* 2022). *In vivo* research has demonstrated that ROB exhibits significant protective efficacy against pulmonotoxicity in rats (Hayat *et al.,* 2024). As far as we are aware, this is the first research that explores the hepatoprotective effect of ROB against PQ induced toxicity. The current study demonstrates the protective efficacy of ROB against PQ-provoked liver damages in rats via assessing Nrf-2/Keap-1 signaling pathway, antioxidant, inflammatory and apoptotic markers as well as hepatic histology.

MATERIALS AND METHODS

Chemicals: ROB (Cas No. 490–31-3) and PQ (Cas No. 75365-73-0) were acquired from Merck (USA).

Animals and treatments: Twenty-four male albino rats (*Rattus norvegicus*), aged 6 to 8 weeks and weight 180g-220g, were obtained and kept in rodent cages at the animal rearing facility of University of Agriculture, Faisalabad (UAF), Pakistan. The experiment was conducted in September 2023. They were given optimum laboratory conditions: 12-hour light and dark cycle, humidity: 55-60%, and temperature: 22-25°C. Throughout the research, a balanced diet for rodents consisting of refined groundnut oil, vitamins, casein and salt combination, and wheat flour was given, along with tap water. Animals were handled as per ARRIVE guidelines (Percie du Sert *et al.,* 2020). The rats were allowed to acclimate for seven days prior to the commencement of the experiment. Animals were divided into four groups having six rats each using CRD. Group I served as experimental control and received normal saline equivalent to treatment volume used in other groups while group II was administered with PQ (5 mg/kg). Group III was subjected to PQ+ROB (5 mg/kg + 30 mg/kg) co-treatment while group IV was given ROB (30 mg/kg) only. The ROB-only group was included to assess whether ROB itself exerts any toxic or physiological effects in the absence of PQ exposure.

Collection of blood and organ specimens: After a 28-days experimental period, rats were given xylazine and ketamine intraperitoneally to render them unconscious and then decapitated. Blood samples were taken using heparin syringes. All the liver samples were removed and preserved for histological and biochemical analysis The analyses were conducted at the Animal Physiology Lab, Department of Zoology, Wildlife & Fisheries, UAF.

Determination of gene expressions: qRT-PCR was used to determine the expression of *NRF-2*/*KEAP-1* and its cytoprotective genes, and apoptotic markers **(***CASPASE-3, BAX & BCL-2*). TRIzol reagent was used for the extraction of RNA from liver samples, and the RNA was then reversed transcribed to obtain cDNA. The methodology provided by Livak and Schmittgen (2001) was used to determine the expression changes of the aforementioned parameters via $2^{-\Delta\Delta CT}$. Moreover, *β-ACTIN* functioned as an internal control. Table 1 depicts the primer sequences as evidenced in the study of Ijaz *et al.* (2023).

Measurement of liver antioxidant and oxidative stress markers: MDA and ROS levels determined via the methodologies of Ohkawa *et al.* (2007) and Hayashi *et al.* (1979), respectively. The activities of CAT, SOD, HO-1 and GSR were assessed via the prescribed methodologies of Aebi (1974), Kakkar *et al.* (1984), Magee *et al.* (1999), and Carlberg and Mannervik (1975), respectively. Moreover, the activities of GST and GPx were determined via the techniques of Habig *et al.* (1974) and Rotruck *et al.* (1973), respectively. Meanwhile, GSH contents were determined as per the research of Jollow *et al.* (1974).

Measurement of hepatic serum indices: The levels of ALT (Cat Number: ELK2683), ALP (Cat Number: ELK5635) and AST (Cat Number: ELK5657) were assessed via ELISA kits from ELK Biotechnology CO., Ltd (USA). The guidelines given by the manufacturer were strictly followed during the assessment.

Measurement of hepatic inflammation assessing markers: COX-2 (Cat Number: ELK7718), IL-6 (Cat Number: ELK1158), NF-κB (Cat Number: ELK1693), IL-1β (Cat Number: ELK1272) and TNF-α (Cat Number: ELK1396) levels were determined with rat ELISA kits from ELK Biotechnology CO., Ltd. (USA). The standards given by the manufacturer were strictly followed during the assessment.

Histopathological staining: The liver was excised from the rats and put in 10% formalin for 24 hours. Then tissues were dehydrated in increasing ethanolic concentrations. Ultimately, liver was put in paraffin wax and sliced into 4μm parts via rotatory microtome and fixed on slides. Sample containing slides were then colored with H&E and changes in histology were seen at 400X using a compound microscope.

Statistical evaluation: The results are presented as Mean ± SE. All the data were continuous and were assessed using Minitab (v17) software. The differences among experimental groups were estimated through one-way ANOVA. For multiple comparisons among group means, Tukey's test was executed. $p < 0.05$ was considered

statistically significant. No categorical data was included in this study.

RESULTS

Influence of PQ and ROB on *NRF-2*/*KEAP-1*: PQ exposure significantly ($p < 0.05$) increased the expressions of *KEAP-1* (2.77-fold) and reduced *NRF-2* (0.65-fold), *HO-1* (0.52-fold), *CAT* (0.65-fold), *GSR* (1.14-fold), *SOD* (0.39-fold) and *GPX* (0.77-fold) when compared to the control group. However, the co-supplementation of PQ and ROB substantially ($p < 0.05$) upregulated *NRF-2* (0.44-fold), *HO-1* (0.85-fold), *CAT* (0.96-fold), *GSR* (1.27-fold), *SOD* (0.33-fold) and *GPX* (0.45-fold), while lowering *KEAP-1* (5.19-fold), as compared to PQ group. Nonetheless, the values of the above-mentioned parameters in the control and ROB-only treated groups were comparable (Figure 1).

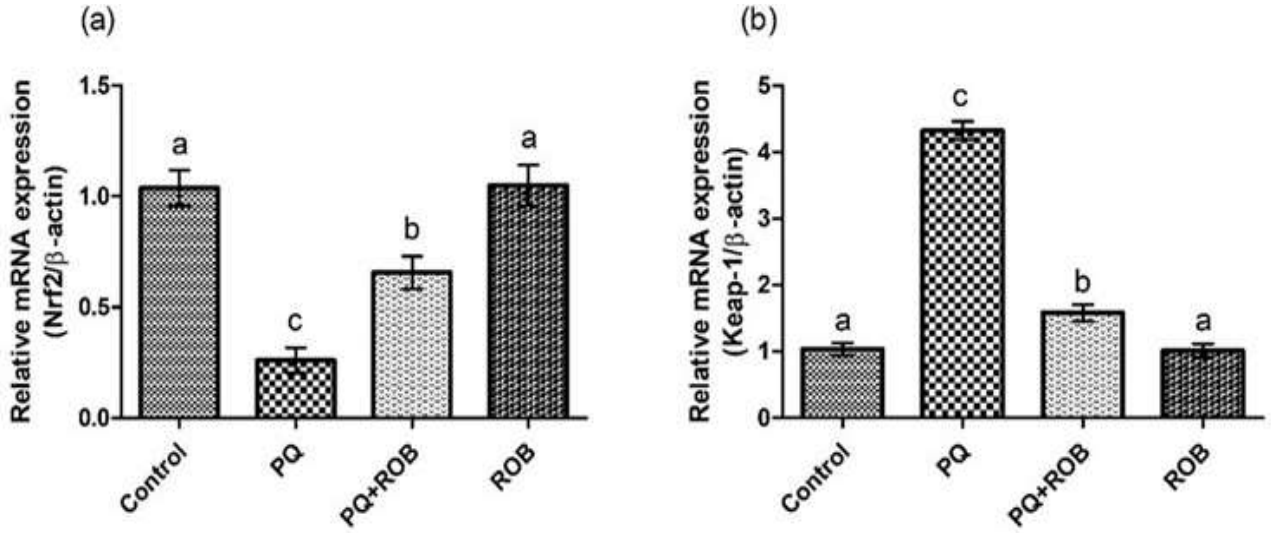
Influence of PQ and ROB on biochemical profile: PQ exposure significantly ($p < 0.05$) increased MDA and ROS concentrations while decreasing antioxidant enzyme activities, as compared to the control. However, PQ + ROB co-administration markedly ($p < 0.05$) increased the activities of these antioxidants while suppressing MDA and ROS concentrations, as matched to PQ group. Nonetheless, only ROB supplemented group presented negligible variations as compared to the control (Table 2).

Influence of PQ and ROB on hepatic function enzymes: PQ inebriation significantly ($p < 0.05$) augmented the levels of hepatic serum indices (ALT, AST, ALP), as compared to the control. This reveals the adverse effect of PQ on hepatic tissues. The co-supplementation of ROB & PQ markedly ($p < 0.05$) reduced the levels of hepatic markers, as compared to PQ group, and improved the hepatic profile. However, these levels were almost similar in the control and ROB groups (Table 3).

Influence of PQ and ROB on inflammatory markers: PQ-administration substantially ($p < 0.05$) elevated the levels of inflammation markers, as compared to the control. This outcome suggests that PQ disturbed the hepatic inflammatory indices and lead to inflammation. Nevertheless, ROB and PQ co-treatment noticeably ($p < 0.05$) lowered the levels of these markers, as compared to PQ group. Besides, ROB only treatment exhibited the levels of these markers approximately similar to the control animals (Table 4).

Influence of PQ and ROB on apoptotic markers: PQ exposure markedly ($p < 0.05$) elevated the expressions of *BAX* (2.77-fold) and *CASPASE-3* (3.15-fold) while lowering *BCL-2* (4.47-fold) expressions. Nonetheless, co-administration of PQ and ROB substantially ($p < 0.05$) lowered the expressions of *CASPASE-3* (1.65-fold) and *BAX* (1.97-fold), while *BCL-2*(1.91-fold) expressions were elevated, as compared to PQ group. However, no significant difference was found in the expressions of apoptotic markers in ROB and the control group (Figure 2).

Influence of PQ and ROB on hepatic histopathology: Histopathological analysis revealed that the histology of hepatic tissues remained normal in both the control and ROB-only treated group. Moreover, PQ exposure noticeably caused liver damage such as nuclei degeneration, fat accumulation, hepatocyte membrane disruptions as well as sinusoid dilation and various impairments in central venules of hepatic tissues. However, the co-treatment of PQ + ROB markedly recovered the aforementioned histological damages. (Figure 3).



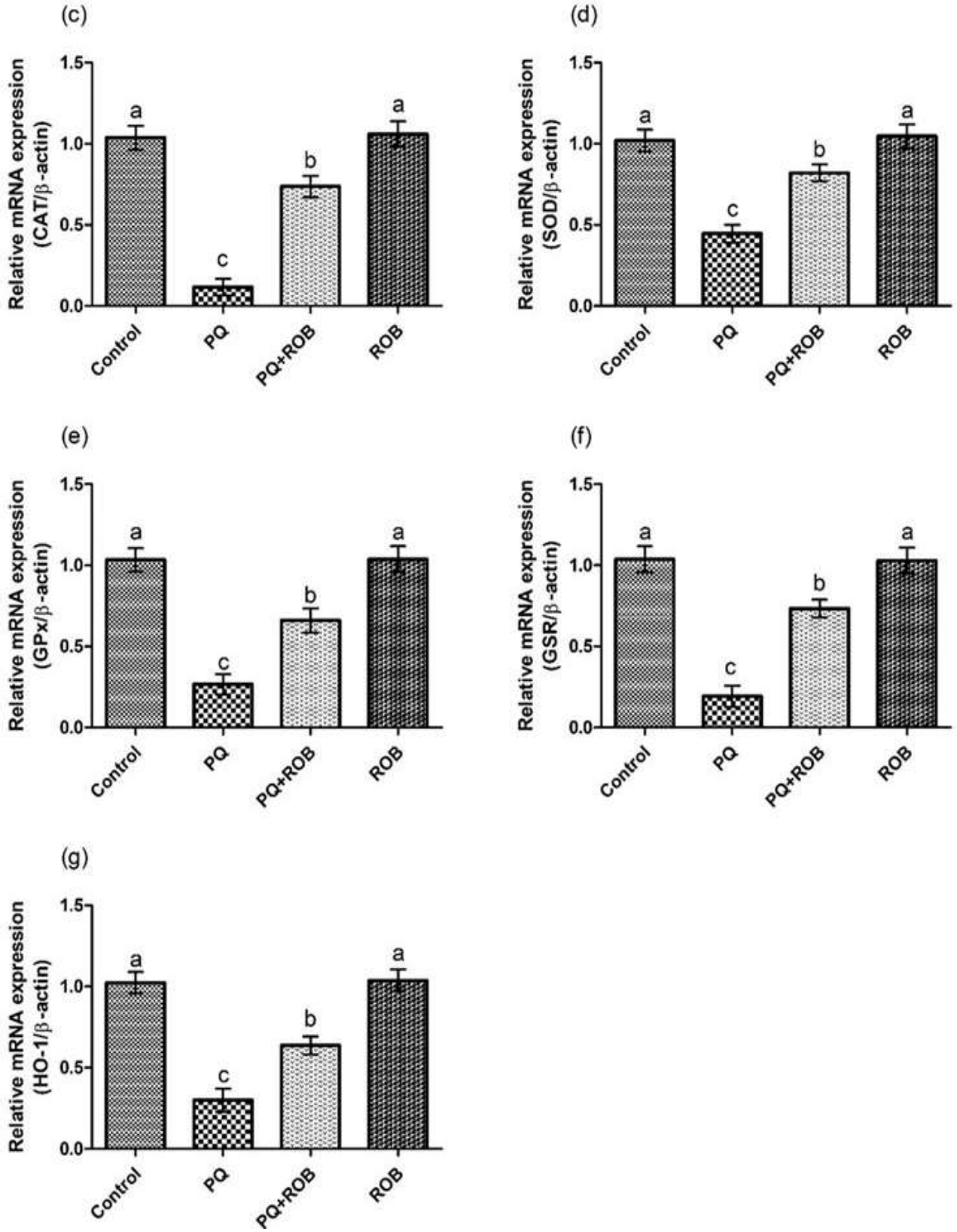


Fig. 1. Impact of PQ and ROB on expressions of (a) *NRF-2*, (b) *KEAP-1*, (c) *CAT*, (d) *SOD*, (e) *GPX*, (f) *GSR* & (g) *HO-1*. Bars are shown on the basis of Mean ± SE. [abc] Different superscripts are presenting substantial differences.

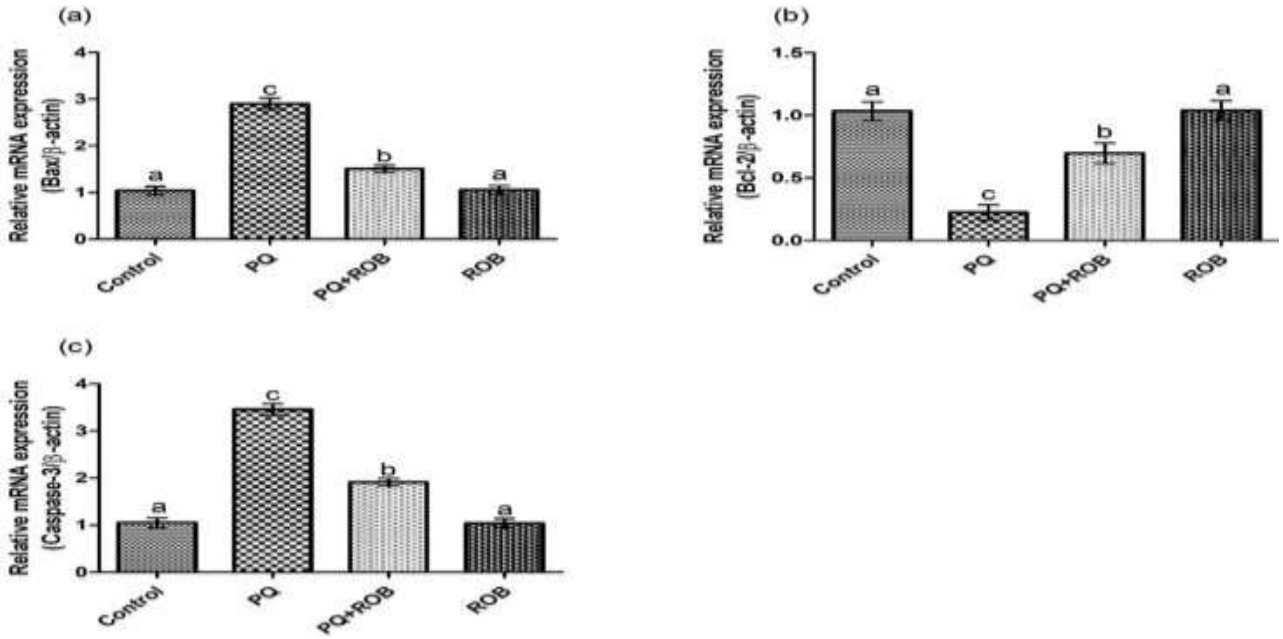


Fig. 2. Impact of PQ and ROB on apoptotic markers expression (a) *BAX*, (b) *BCL-2*, & (c) *CASPASE-3*. Bars are shown on the basis of Mean ± SE. [abc] Different superscripts are presenting substantial differences.

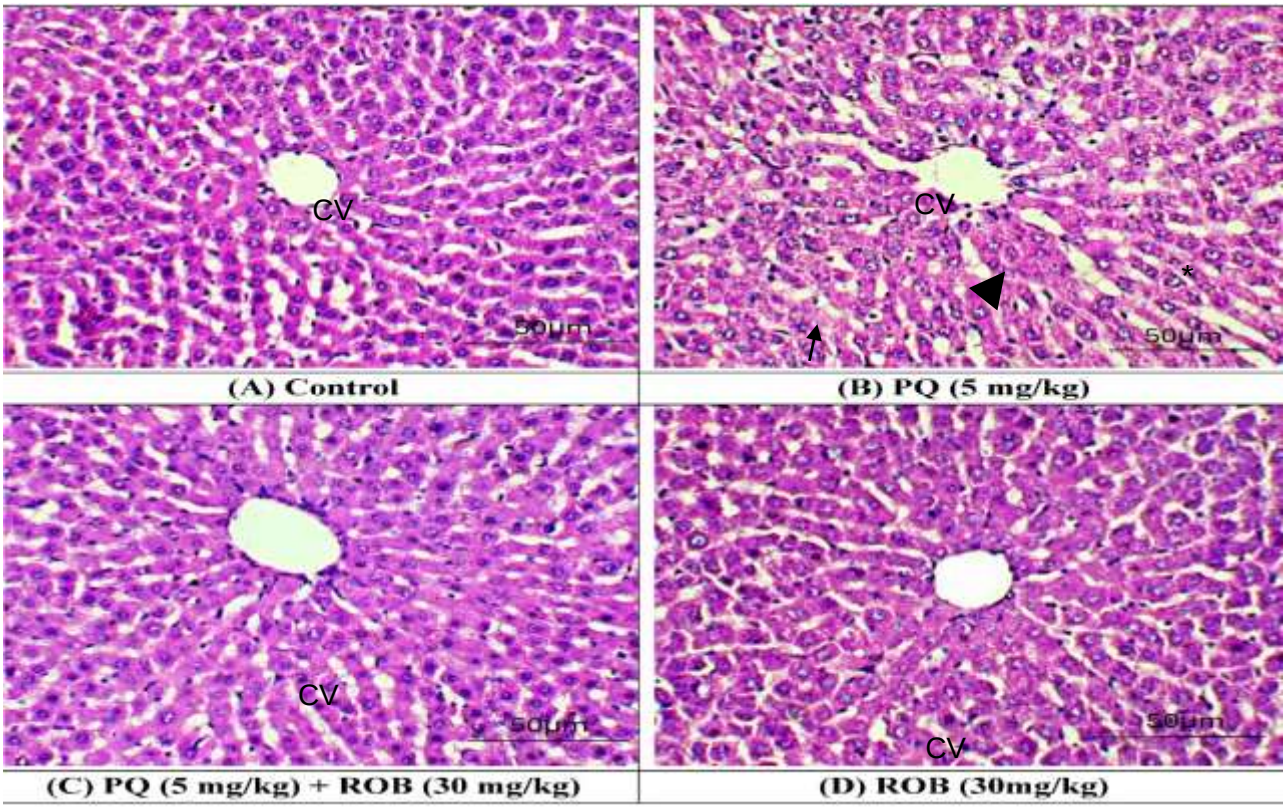


Fig. 3. Microphotographs of liver tissues of *Rattus norvegicus* (H&E, 400X): (A) Control group demonstrates normal architecture of hepatocytes with organized hepatic lobules and central veins. (B) PQ exposed group shows degenerated hepatocytes (arrow), vacuolization (asterisk), disrupted central vein (CV) as well as degenerated sinusoids (arrowhead). (C) Co-treated group exhibits improved morphology of liver tissues with recovered abovementioned tissue damages. (D) Only ROB supplemented group depicts normal as histology as in control group.

Table 1: Primers sequences for targeted genes (RT-qPCR)

Gene	Primers 5' -> 3'	Accession number
NRF-2	F: ACCTTGAACACAGATTTCGGTG R: TGTGTTCAGTGAAATGCCGGA	NM_031789.1
KEAP-1	F: ACCGAACCTTCAGTTACACACT R: ACCACTTTGTGGGCCATGAA	NM_057152.1
CAT	F: TGCAGATGTGAAGCGCTTCAA R: TGGGAGTTGTACTGGTCCAGAA	NM_012520.2
SOD	F: AGGAGAAACTGACAGCTGTGTCT R: AAGATAGTAAGCGTGCTCCCAC	NM_017051.2
GPX	F: TGCTCATTGAGAATGTCGCGTC R: ACCATTCACCTCGCACTTCTCA	NM_030826.4
GSR	F: ACCAAGTCCCACATCGAAGTC R: ATCACTGGTTATCCCCAGGCT	NM_053906.2
HO-1	F: AGGCTTTAAGCTGGTGATGGC R: ACGCTTTACGTAGTGCTGTGT	NM_012580.2
BAX	F: GGCCTTTTTGCTACAGGGTT R: AGCTCCATGTTGTTGTCCAG	NM_017059.2
BCL-2	F: ACAACATCGCTCTGTGGAT R: TCAGAGACAGCCAGGAGAA	NM_016993.1
CASPASE-3	F: ATCCATGGAAGCAAGTCGAT R: CCTTTTGCTGTGATCTTCCT	NM_012922.2
β-ACTIN	F: TACAGCTTCACCACCACAGC R: GGAACCGCTCATTGCCGATA	NM_031144

Table 2: Protective role of ROB on antioxidant and oxidative stress profile

PARAMETERS	GROUPS			
	Control	PQ	PQ + ROB	ROB
CAT (Umg^{-1} protein)	15.53 ± 1.02^{a}	4.65 ± 0.39^{c}	11.71 ± 0.65^{b}	15.67 ± 1.09^{a}
SOD (Umg^{-1} protein)	12.21 ± 1.15^{a}	3.29 ± 0.35^{c}	8.80 ± 0.62^{b}	13.14 ± 1.41^{a}
GPx (Umg^{-1} protein)	26.08 ± 1.25^{a}	7.57± 0.79^{c}	15.39±1.06^{b}	27.52 ± 1.03^{a}
GSR (nM NADPH oxidized/min/mg tissue)	7.42 ± 0.65^{a}	2.23 ± 0.26^{c}	5.26 ± 0.32^{b}	7.78 ± 0.92^{a}
GST (nM/min/mg protein)	38.76 ± 1.37^{a}	12.25±1.12^{c}	31.14 ± 0.83^{b}	39.62 ± 1.59^{a}
GSH (µM/g tissue)	20.70 ± 1.32^{a}	8.39 ±0.64^{c}	18.57 ± 0.78^{b}	21.61± 1.55^{a}
HO-1(pmoles bilirubin/mg protein/h)	469.81± 9.27^{a}	97.16 ±3.77^{c}	358.91±7.51^{b}	482.43 ±8.79^{a}
ROS (Umg^{-1} tissue)	1.52 ± 0.13^{c}	9.37 ± 0.65^{a}	2.82 ± 0.23^{b}	1.48 ± 0.14^{c}
MDA (nmol/mg protein)	0.66 ± 0.17^{c}	4.39 ± 0.23^{a}	1.8 ± 0.09^{b}	0.62 ± 0.18^{c}

abc Distinct superscripts on various values (Mean±SE) demonstrate significant difference among other groups.

Table 3: Protective role of ROB on liver function markers

PARAMETERS	GROUPS			
	Control	PQ	PQ + ROB	ROB
ALT (U/L)	34.54 ± 1.72^{c}	95.81 ± 2.02^{a}	44.94 ± 1.47^{b}	33.68 ± 1.81^{c}
AST (U/L)	85.78 ± 2.32^{c}	325.54 ± 6.62^{a}	174.96 ± 3.70^{b}	84.43 ± 2.63^{c}
ALP (U/L)	110.18 ± 2.81^{c}	438.33 ± 7.81^{a}	194.73. ± 5.43^{b}	105.88 ± 1.99^{c}

^{abc} Distinct superscripts on various values (Mean±SE) demonstrate significant difference among other groups.

Table 4: Protective role of ROB on inflammatory indices

PARAMETERS	GROUPS			
	Control	PQ	PQ + ROB	ROB
NF-κB (ngg^{-1} tissue)	25.95 ± 1.36^{c}	68.35 ± 2.01^{a}	34.09 ± 1.23^{b}	24.89 ± 1.47^{c}
TNF-α (ngg^{-1} tissue)	20.82 ± 2.28^{c}	77.68 ± 1.81^{a}	35.91 ± 0.87^{b}	19.91 ± 2.55^{c}
IL-1β (ngg^{-1} tissue)	17.34 ± 1.58^{c}	57.74 ± 1.43^{a}	29.15 ± 1.09^{b}	16.48 ± 1.44^{c}
IL-6 (ngg^{-1} tissue)	14.19 ± 1.07^{c}	87.08 ± 1.83^{a}	25.33 ± 0.90^{b}	13.49 ± 1.27^{c}
COX-2 (ngg^{-1} tissue)	8.77 ± 0.93^{c}	52.87 ± 0.84^{a}	14.6 ± 1.06^{b}	8.45 ± 0.81^{c}

^{abc} Distinct superscripts on various values (Mean±SE) demonstrate significant difference among other groups.

DISCUSSION

PQ is an extensively used herbicide known for its effectiveness in controlling weeds and grass in agricultural settings. It has been revealed that PQ induces hepatic toxicity via stimulating OS, inflammation and degenerative changes in hepatic tissues (Ijaz *et al.,* 2024). Elevated generation of free radicals along with a compromised antioxidant defense system drive oxidative stress, leading to the manifestation of PQ-induced pathological conditions (Kheiripour *et al.,* 2021). ROB belongs to flavonoids group that exhibits tremendous pharmacological and biological potential (Erdemli *et al.,* 2018). To address the hepatotoxicity associated with PQ treatment, this research was performed to observe the potential role of ROB.

PQ intoxication lowered the expressions of *NRF-2* and cytoprotective genes while increasing *KEAP-1* expressions. *NRF-2* protein plays a crucial role in regulating electrophilic and oxidative stresses. *KEAP-1* is a negative regulator of *NRF-2* and regulates the activation of *NRF-2* during oxidative stress conditions (Hussein *et al.,* 2021). The separation of *NRF-2* from *KEAP-1*, in response to cellular injury, leads to its activation and translocation into the nucleus, where it stimulates the expressions of antioxidant genes. However, extreme oxidative stress leads to the overexpression of *KEAP-1* and reduced expressions of *NRF-2* (Yang *et al.,* 2022). Nevertheless, our research exhibited that co-administration of ROB escalated *NRF-2* while downregulating the *KEAP-1* expressions. It is reported that quercetin and silymarin, some of the established flavonoids, exhibit *NRF-2/KEAP-1* modulating properties (Hussein *et al.,* 2021). Similarly, ROB also exhibits *NRF-2*/*KEAP-1*-modulating properties.

This research showed that PQ exposure decreased the activities of antioxidants and increased the levels of ROS and MDA. PQ treatment regulates ROS generation via disrupting the balance among the generation and removal of ROS. Unnecessary ROS production results in oxidative stress, which causes adverse damage to the membranes and internal biomolecules (Selamoglu Talas *et al.,* 2009). CAT is a significant anti-oxidant enzyme that cleaves hydrogen peroxide (H_2O_2) to produce oxygen and water. Additionally, SOD is responsible for the conversion of superoxide to H_2O_2 and oxygen (Fois *et al.,* 2018). Moreover, GPx lowers H_2O_2 and lipid peroxidation to reduce oxidative stress. GSR controls GSH levels, which influences the activity of GPx (Ali *et al.,* 2020). Additionally, MDA levels show lipid peroxidation and oxidative stress as it is a by-product of lipid peroxidation (Agarwal *et al.,* 2020). In the current research, ROS supplementation elevated the activities of antioxidant enzymes and lowered MDA and ROS levels. Hence, ROB exhibits antioxidant properties comparable to those reported for key flavonoids, such as silymarin (Okiljević *et al.,* 2024), and other plant extracts (Ahmed *et al.,*2025).

The impact of toxicants on liver function can be quantified by estimating the levels of key functional markers (AST, ALT and ALP) (Carobene *et al.,* 2013). Due to their association with hepatocytes, these biomarkers are released into blood circulation by hepatic cells in response to any cellular injury. Oxidative stress causes hepatic toxicity by elevating the mitochondrial permeability, which allows the leakage of hepatic markers into the bloodstream. The elevation in the levels of hepatic markers in the blood shows liver injury, which might result in hepatic dysfunctions (Faheem *et al.,* 2019). In the current study, the levels of AST, ALT and ALP were increased following PQ administration. However, the administration of ROB decreased the harmful impacts of PQ on the liver, as shown by a decrease in hepatic function. The results indicate that ROB produced outcomes comparable to other flavonoids, such as silymarin (Okiljević *et al.,* 2024) and rhamnazin (Anjum *et al.,* 2025), by reducing hepatic enzyme levels, thereby demonstrating its hepatoprotective potential.

The research outcomes showed that PQ intoxication increased the levels of inflammatory markers. NF-κB activation due to excess OS markedly upregulated the production of inflammation mediators. The escalated levels of these markers lead to subsequent acute inflammation as well as ROS-provoked abnormalities. In addition to this, COX-2 is an inflammation mediator that initiates inflammation of the liver (Badr *et al.,* 2019). However, administration of ROB reduced the levels of inflammatory markers and prevented hepatic tissues due to its anti-inflammatory nature. Hence, ROB exhibited anti-inflammatory effects comparable to those of other flavonoids, such as quercetin (Liu *et al.,* 2015), astragalin (Hamza *et al.,*2023) and vitexin (Ijaz *et al.,*2022), that are reported to reduce inflammatory cytokines and protect the hepatocytes from inflammatory damage.

PQ exposure notably increased the expressions of *CASPASE-3* and *BAX* while downregulating *BCL-2* expressions. ROS-activated programmed cell death and oxidative stress are the ultimate reasons for liver toxicity (Maione *et al.,* 2015). *BCL-2* prevents apoptosis by neutralizing pro-apoptotic signals and maintaining mitochondrial integrity. Cytochrome (cyt)-c comes in cytoplasm from the mitochondrial membrane due to disturbance in *BAX* and *BCL-2* ratio. The elevated concentration of cyt-c in cytoplasm results in the activation of *CASPASE-3*. *CASPASE-3* cleaves the proteins in cells, change their structure and ultimately cause apoptosis, that results in cellular death (Somade *et al.,* 2019). However, ROB supplementation increased *BCL-2* expressions besides lowering *CASPASE-3* and *BAX* expressions, owing to its tremendous potential

against apoptotic pathways. These antiapoptotic effects of ROB are similar to various other reported flavonoids including resveratrol (Hajighasem *et al.,* 2019) silymarin (Gür and Bilgiç, 2023), and isorhoifolin (Ijaz *et al.,* 2025).

PQ intoxication adversely affected the normal architecture of the hepatic tissues including degenerated hepatocytes, vacuolization, disrupted central vein as well as degenerated sinusoids. Previous literature has shown that PQ exposure noticeably disrupted the normal histology of hepatic tissues of rats (Kheiripour *et al.,* 2021). Free radical production targets the lipids present in the plasma membrane, causing oxidative stress that eventually results in hepatotoxicity (Kheiripour *et al.,* 2021). Nonetheless, ROB treatment remarkably revoked the histopathological alterations in the hepatic tissues of rats.

Conclusions: Taken together, PQ exposure resulted in significant hepatic toxicity, characterized by disrupted *NRF-2/KEAP-1* signaling, oxidative stress, inflammation, apoptosis and altered hepatic enzyme levels. However, ROB co-administration mitigated the impact of PQ by restoring antioxidant defense, modulating apoptotic and inflammatory response and improving histopathological architecture. Therefore, ROB exhibits hepatoprotective effects against PQ induced hepatic damage. We recommend further preclinical and clinical investigations to validate the protective effects of ROB against hepatotoxicity, explore its safety profile, and assess its therapeutic potential in human liver disorders.

***Ethics Declaration*:** Rats were treated as per ARRIVE guidelines, which were approved by the institutional ethics committee, UAF (DGS No. 7725-28).

Conflict of Interest: None

Authors' Contribution Statements: NG designed and conducted the experiments and wrote the manuscript. MZS assisted in experimental design, execution, and manuscript writing. AA and MFH performed the experiments and contributed to the manuscript. HH and MB carried out statistical analyses. AA supervised the study. All authors approved the final manuscript.

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