



Rhoifolin protects cisplatin mediated pulmonary toxicity via attenuation of oxidative stress, inflammatory response, apoptosis and histopathological damages

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ABSTRACT

Cisplatin (CP) is a ubiquitous antineoplastic medicine that has been recognized to have severe toxic effects on different organs including lungs. Rhoifolin (RHO) is a therapeutic compound with significant pharmacological activities. The present study was designed to evaluate the protective effect of RHO against CP induced pulmonary toxicity. Twenty-four rats were randomly divided into 4 groups: control group, CP treated group (20 mgkg⁻¹), CP + RHO treated group (20 mgkg⁻¹ + 10 mgkg⁻¹) and RHO supplemented group (10 mgkg⁻¹). Following 30 days of administration, our results showed that CP treatment decreased the activity of antioxidant enzymes such as glutathione (GSH), glutathione reductase (GSR), glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) while elevated the level of malondialdehyde (MDA) along with reactive oxygen species (ROS). Furthermore, levels of inflammatory cytokines involving interleukin-6 (IL-6), nuclear factor-kappa B (NF-κB), interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α) and cyclooxygenase-2 (COX-2) activity were escalated. Besides, treatment with CP enhanced the activities of apoptotic proteins i.e., Bax, caspase-9 along with caspase-3 while reducing the activity of Bcl-2. Additionally, the histopathological examination revealed significant pulmonary tissue impairments in the CP exposed group. However, RHO treatment considerably (P < 0.05) recovered the abovementioned CP-induced toxic effects. Therefore, the current research demonstrated that RHO may be used as a promising pharmacological compound to cure CP-instigated pulmonary damages due to its antioxidant, anti-inflammatory, antiapoptotic and histo-protective properties.

1. Introduction

Cancer has emerged as an alarming health concern and a primary cause of mortalities worldwide (Aboubakr et al., 2023). Cisplatin (CP) is a platinum-derived antineoplastic drug that acts as a DNA alkylating compound and has been clinically applied in the management of a broad range of malignancies i.e., liver, kidney, ovarian, testicular, bladder as well as lung cancer (Elsherbiny et al., 2016). The chemotherapeutic action of CP is associated with its ability to provoke biochemical cross-linkage between DNA strands, obstructing DNA replication and impeding gene expression, ultimately leading to an apoptotic response in the body (Melnikov et al., 2016). Despite its remarkable clinical benefits, CP has been reported to have limited therapeutic index owing

to its severe toxic effects including pulmonary toxicity (Afsar et al., 2018).

CP has been documented to induce pulmonary oxidative stress (OS) by increasing oxidant levels, decreasing antioxidant concentration and accelerating the secretion of proinflammatory cytokines (Unver et al., 2019). It also provokes amplification in H₂O₂ and MDA levels along with a significant increase in the apoptotic response in the lung tissue (Han et al., 2021). Furthermore, CP treatment also triggers an acute inflammatory condition in the biological system by reducing the activity of anti-inflammatory protein along with a substantial amplification in pro-inflammatory biomarkers (Ijaz et al., 2023a). Recent studies have reported that CP exposure can instigate histological abnormalities including edema, alveolar septal fibrosis and infiltration of polymorpho-

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nuclear leukocytes in lung tissue (Unver et al., 2019).

Flavonoids have garnered remarkable attention in current times due to their effective pharmacotherapeutic actions against various pathologies (Ferreira et al., 2015; Men et al., 2022). Rhoifolin (RHO) is an important flavonoid glycoside that has gained a substantial attention in recent years due to its broad range of pharmacological activities i.e., antioxidant, anti-inflammatory and hepatoprotective effects (Refaat et al., 2015). Regardless of these potential curative actions, the ameliorative effect of RHO to antagonize pulmonary toxicity is unascertained. Therefore, this study was designed to assess the alleviative potential of RHO against CP-induced pulmonary toxicity.

2. Materials and methods

2.1. Chemicals

CP (CAT No: 15663-27-1) & RHO (CAT No: 17306-46-6) were purchased from Sigma-Aldrich (Germany).

2.2. Animals

Twenty-four albino rats (age, 8–12 weeks; weight, 250 ± 20 g) were used for this study. Rats were confined in steel cages at the experimental facility of University of Agriculture, Faisalabad (UAF). They were maintained at 12-h of dark & light cycles, at a constant temperature ($22\text{--}24$ °C). Furthermore, tap water & food chaw was provided. Experiment was conducted in accordance with protocol (CEE Council 86/609) from European Union of Animal Care and Experimentation.

2.3. Experimental design

The rats were randomly divided into 4 groups ($n = 6$). The treatment regimens were as follows: control group, CP treated group (10 mg kg^{-1}), CP + RHO administrated group ($10 \text{ mg kg}^{-1} + 20 \text{ mg kg}^{-1}$) and only RHO supplemented group (20 mg kg^{-1}) via oral gavage. The dosage of CP, 10 mg kg^{-1} and RHO, 20 mg kg^{-1} was administered in accordance with the previous study of Saher et al. (2023). On the 31st day of experiment, rats were anesthetized using ketamine (60 mg kg^{-1}) and xylazine (6 mg kg^{-1}), then decapitated & blood samples were taken for further analysis. The lungs were carefully removed, divided into two equal parts and cleaned with normal saline. One part was fixed in a formalin (10 %) solution for histological analysis. The second part was kept in plastic bag for biochemical evaluations.

2.4. Biochemical marker assessment

The activity of CAT was quantified in compliance with the protocol illustrated by Aebi (1984) while, SOD activity was measured by using the approach outlined Kakkar et al. (1984). GPx content was analyzed in accordance with the approach of Lawrence & Burk (1976). The activity of GSR was measured by employing the methodology explained by Carlberg & Mannervik (1975). The GSH activity was measured following the guidelines documented by Jollow et al. (1974). The quantification of GST activity was carried out as per the protocol illustrated by Younis et al. (2018). Hayashi et al. (2007) approach was followed to analyze ROS concentration. Ohkawa et al. (1979) procedure was considered for the assessment of the level of MDA.

2.5. Inflammatory markers assessment

For the evaluation of inflammatory markers in the lungs, commercially procured kits were employed. The quantification of NF- κ B (CSB-E13148r), IL-6 (CSB-E04640r), TNF- α (CSB-E07379r), IL-1 β (CSB-E08055r) along with COX-2 (CSB-E04640r) activity was performed with the help of ELISA kits specifically designed for rats, sourced from Shanghai, China. The evaluation was undertaken in compliance with the

guidelines provided by the manufacturer.

2.6. Assessment of apoptotic markers

The quantification of Caspase-3 (CSB-E08857r), Bax (CSB-EL002573RA), Bcl-2 (CSB-E08854r) and caspase-9 (CSB-E08863r) was carried out using ELISA kits specifically designed for rats, procured from Cusabio Technology Llc, Houston, TX, USA.

2.7. Histological examination

The lungs samples were kept in formalin (10 %) solution and passed through dehydration process using ascending grades of ethanol. Lung tissues were encased in paraffin wax and sliced into small pieces by using microtome machine. These slices were stained with eosin following hematoxylin & analyzed using light microscope, Olympus BX51 (Imagetec Pvt. Ltd., Chennai, India), for histo-pathological examination.

2.8. Statistical evaluation

Data were depicted as Mean \pm SEM. One-way analysis of variance (ANOVA) was performed, followed by the Tukey's test. $P < 0.05$ was set as level of significance.

3. Results

3.1. Impact of RHO on antioxidant profile

CP exposure significantly increased ($P < 0.05$) the activities of CAT, GSR, SOD, GPx, GSH & GST as compared to control group. RHO co-administration with CP substantially restored the activities of aforementioned antioxidant enzymes as compared to CP exposed rats. Supplementation of RHO (alone) displayed mean values of these biochemical marker close to the control rats (Table 1).

3.2. Impact of RHO on oxidant profile

The levels of ROS & MDA were markedly ($P < 0.05$) augmented in CP exposed animals as compared to control rats. Co-administration of RHO with CP remarkably lowered the ROS & MDA levels as compared to CP exposed rats. However, only RHO supplemented rats resulted in ROS & MDA levels close to control rats (Table 2).

3.3. Impact of RHO on inflammatory biomarkers

CP intoxication remarkably ($P < 0.05$) upsurged the NF- κ B, IL-1 β , IL-

Table 1
Impact of RHO on antioxidant profile.

Parameters	Groups			
	Control	CP	CP + RHO	RHO
CAT (U/mg protein)	12.423 \pm 1.26 ^a	7.120 \pm 0.43 ^b	9.697 \pm 0.55 ^b	12.610 \pm 1.36 ^a
SOD (U/mg protein)	8.830 \pm 1.17 ^a	4.230 \pm 0.39 ^b	7.033 \pm 0.29 ^a	8.790 \pm 1.16 ^a
GSR (nM NADPH oxidized/min/mg tissue)	6.587 \pm 0.38 ^a	2.013 \pm 0.40 ^c	5.100 \pm 0.28 ^b	6.663 \pm 0.43 ^a
GPx (U/mg protein)	19.043 \pm 0.66 ^a	5.600 \pm 0.30 ^c	14.677 \pm 1.06 ^b	19.323 \pm 0.60 ^a
GSH (U/mg protein)	32.38 \pm 2.06 ^a	11.480 \pm 1.04 ^c	25.187 \pm 1.52 ^b	32.46 \pm 2.08 ^a
GST (U/mg protein)	35.480 \pm 1.72 ^a	14.56 \pm 1.88 ^c	25.910 \pm 1.52 ^b	35.92 \pm 2.22 ^a

Distinct superscripts on various values demonstrated discrepancies among other groups.

Table 2
Impact of RHO on oxidant profile.

Parameters	Groups			
	Control	CP	CP + RHO	RHO
MDA (nmol/g)	0.86 ± 0.09 ^c	6.52 ± 0.37 ^a	2.56 ± 0.20 ^b	0.83 ± 0.08 ^c
ROS (nmol/g)	0.49 ± 0.22 ^c	8.43 ± 0.44 ^a	2.33 ± 0.27 ^b	0.46 ± 0.23 ^c

Distinct superscripts on various values demonstrated discrepancies among other groups.

6, TNF- α levels along with activity of COX-2 as compared to control rats. CP + RHO treatment led to a substantial restoration in the aforesaid cytokines in contrast to CP exposed rats. No significant difference was analyzed in these cytokines level between the RHO treated rats & the control (Table 3).

3.4. Impact of RHO on apoptotic biomarkers

CP treatment significantly ($P < 0.05$) escalated the activities of Caspase-3, Bax, Caspase-9 while reduced the Bcl-2 activity as compared to control rats. However, the co-administration of RHO with CP culminated in a substantial restoration in the disturbed apoptotic biomarkers status as compared to rats treated with CP. Furthermore, supplementation of RHO resulted in mean values of these biomarkers close to the control animals (Fig. 1).

3.5. Impact of RHO on pulmonary histology

CP intoxication led to various histopathological disruptions in pulmonary tissues such as elevated interstitial cellularity, dilated inter-alveolar spaces, alveolar fibrosis, congested alveolar capillaries along with thickening of bronchiolar walls as compared to control group. Nevertheless, RHO supplementation remarkably ($P < 0.05$) mitigated abovementioned histopathological disruptions prompted by CP exposure. However, RHO only supplemented group showed normal histology as compared to control group.

4. Discussion

The antioxidant enzymes (GPx, GSR, SOD, GSH, GST & CAT) activities were remarkably decreased in CP supplemented rats, while MDA & ROS levels was notably increased. Antioxidant enzymes are the primary protective barrier that safeguards the biomolecules (lipids, DNA and proteins) from oxidative injury by decreasing ROS generation (Ighodaro and Akinloye, 2018). H_2O_2 , OH, O^{-2} & NO are main reactive molecules that are engaged in cellular injury (Mijatović et al., 2020). SOD enzymatically mitigates the O^{-2} via catalyzing the conversion of O^{-2} into O_2 & H_2O_2 (Xu et al., 2015), whereas H_2O_2 is transformed into H_2O via the biocatalytic function of GPx & CAT (Weydert and Cullen, 2010). GSR maintains the level of GSH, which retains the uninterrupted GPx levels

Table 3
Impact of RHO on inflammatory biomarkers.

Parameters	Groups			
	Control	CP	CP + RHO	RHO
NF- κ B (ng/g tissue)	23.99 ± 1.28 ^c	88.60 ± 2.40 ^a	47.60 ± 2.62 ^b	23.81 ± 1.36 ^c
TNF α (ng/g tissue)	9.84 ± 1.380 ^c	37.49 ± 1.75 ^a	19.56 ± 1.40 ^b	9.72 ± 1.35 ^c
IL-1 β (ng/g tissue)	25.02 ± 1.82 ^c	83.97 ± 1.48 ^a	52.92 ± 3.07 ^b	24.38 ± 1.16 ^c
IL-6 (ng/g tissue)	9.823 ± 1.29 ^c	62.75 ± 2.84 ^a	27.38 ± 3.65 ^b	9.78 ± 1.29 ^c
COX-2 (ng/g tissue)	24.99 ± 1.56 ^c	73.52 ± 2.02 ^a	39.10 ± 1.99 ^b	24.86 ± 1.61 ^c

Distinct superscripts on various values demonstrated discrepancies among other groups.

(Rojo et al., 2014). CP stimulates OS in the pulmonary tissues (Ali et al., 2015). OS is a state in which the elevated level of ROS detriments the cells, tissue & organ (Hajam et al., 2022). When the ROS contents in the tissues are upsurged, they attack PUFA in the cellular plasma membrane & brought on cascade of bio-catalytic process "lipid peroxidation (LP)" (Unver et al., 2019). The concentration of LP is positively correlated with the generation of superoxide radicals, as evidenced by the levels of MDA, the ultimate product of LP (Kaynar et al., 2005; Ehsan et al., 2023). Our findings are further supported by the study of Unver et al. (2019) who reported that CP treatment increased the levels of ROS & LP as well as decreased the levels of antioxidative enzymes in the lungs. However, CP induced OS was notably averted by RHO supplementation as evidenced by the escalated antioxidant enzymes levels, declined MDA & ROS level owing to its ROS neutralizing nature.

CP treatment culminated in a considerable augmentation in inflammatory biomarkers (IL-6, NF- κ B, TNF- α , IL-1 β levels & activity of COX-2). NF- κ B is considered as an integral factor underlying initiation of inflammatory events in the cells (Dorrington and Fraser, 2019). NF- κ B is an oxidation-reduction modulated transcriptional factor that has been documented as the mediator of OS (Sequeira, 2021). Augmented cellular ROS concentration subsequently amplify NF- κ B initiation (Ijaz et al., 2020a). IL-6 is recognized as a pro-inflammatory biomarker that serves as an indispensable cytokine in the acceleration of inflammatory reaction (Hou et al., 2008). Besides, COX-2 is a principal cytokine that governs the systemic inflammatory condition (Wu et al., 2012). OS instigated on account of CP intoxication elicits NF- κ B activation that accelerates the biosynthesis of aforesaid biomarkers (Ijaz et al., 2020b; Ijaz et al., 2023a). However, RHO supplementation notably restored the state of these inflammatory cytokines. Our outcomes confirm the hypothesis that RHO could suppress NF- κ B initiation & declined levels of proinflammatory cytokines on account of its anti-inflammatory action & ROS neutralizing attributes. The fact that the anti-oxidants supplementation mitigates the initiation of NF- κ B evident that ROS are engaged in NF- κ B initiation, which trigger the inflammatory cascade (Elliott and Chithan, 2017).

In the present research, CP intoxication upsurged Caspase-9, Bax & Caspase-3, while it subsided Bcl-2 concentration. Bax & Bcl-2 are main cytokines of the Bcl-2 protein family. Bcl-2 (anti-apoptotic cytokine), restrains the process of cellular apoptosis, while Bax facilitate cellular apoptotic events (Santana, 2018). In addition to this, Caspase-3 & 9 are engaged in cleavage of intracellular enzymes, consequently provoking structural modifications in cells that elicit the mechanism of apoptotic (Uğuz et al., 2009). Any imbalance in these biomarkers changes permeability of mitochondrial membranous channels, which promotes cytoplasmic secretion of heme protein "cytochrome c" (Akao et al., 2001). The elevated concentration of this heme protein ultimately activates Caspase-3 which culminated in cellular apoptosis (Porter and Jänicke, 1999). Therefore, RHO treatment declined Bax along with Caspase-3, while upregulated Bcl-2 concentration due to its anti-apoptotic effects. Present research results are in accordance with the findings of Saher et al. (2023), who also documented that RHO treatment suppresses apoptosis in neuronal cells by reducing Caspase-3 & Bax as well as enhancing Bcl-2 expression.

CP intoxication culminated in histopathological disruptions in pulmonary tissues i.e., elevated interstitial cellularity, dilated inter-alveolar spaces, alveolar fibrosis, congested alveolar capillaries along with thickening of bronchiolar walls. OS recognized as the key event underlying the structural irregularities in the pulmonary tissues. ROS instigate an inflammatory response, which culminates in enhanced interstitial cellularity (Ijaz et al., 2023b). Moreover, it prompts oxidative damage leading to tissue remodeling i.e., dilated inter-alveolar spaces and alveolar fibrosis, disrupts vascular function (congested inter-alveolar capillaries), and induces airway inflammation as well as thickening of bronchiolar walls (Fekri et al., 2018; Unver et al., 2019). Nevertheless, RHO effectively mitigated these adverse histological damages provoked by CP owing to its antioxidative attributes and its capability to

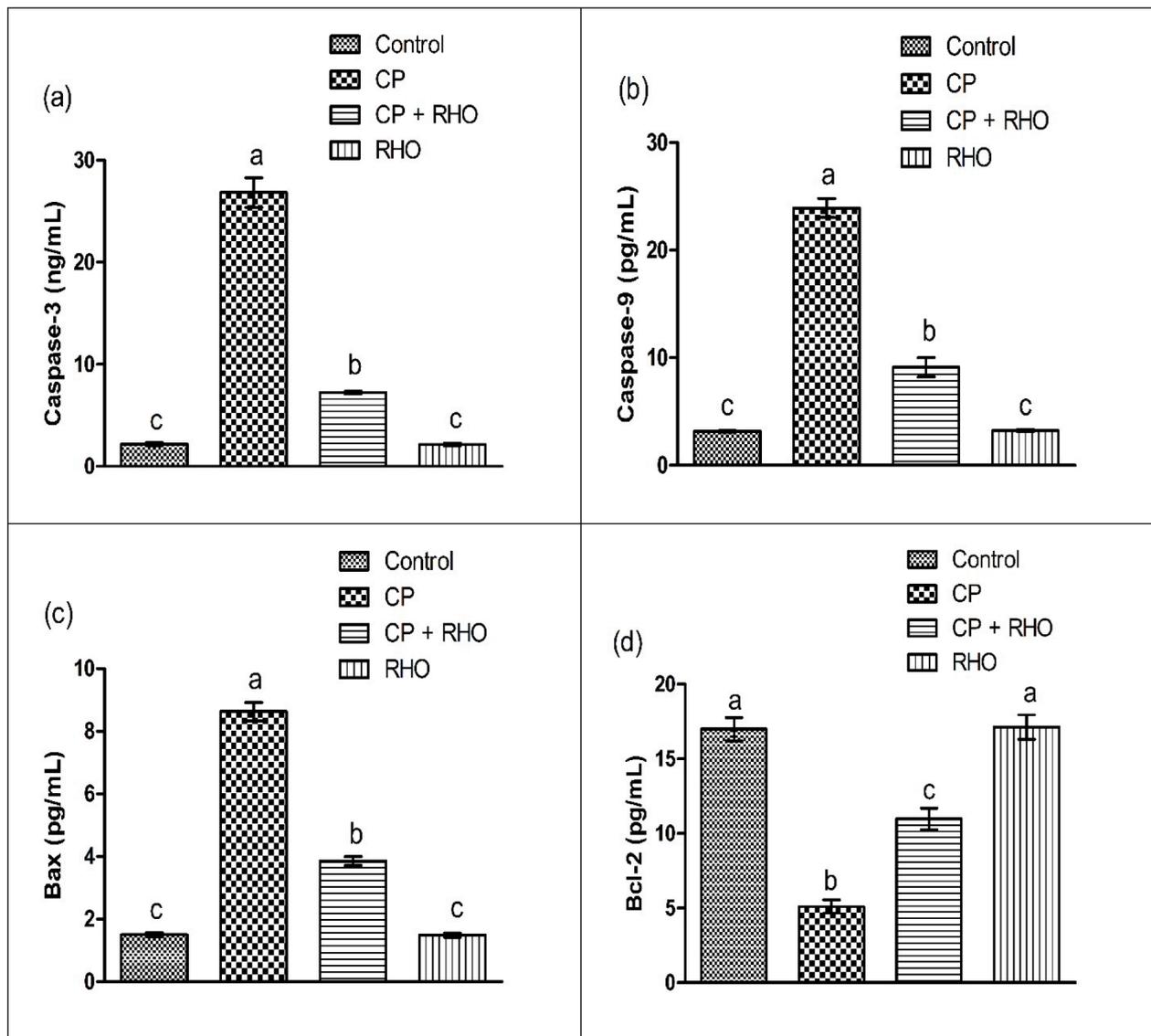


Fig. 1. Effect of CP & RHO on activity of (a) Caspase-3 (b) Caspase-9 (c) Bax (d) Bcl-2. Values are depicted as Mean \pm SEM. Significant differences displayed as ($P < 0.05$).

neutralize augmented ROS concentration. Our results are corroborated by Unver et al., (2019) who revealed that flavonoids exert effective therapeutic action to antagonize OS provoked pulmonary histological impairments.

5. Conclusion

CP intoxication provoked pulmonary toxicity via augmenting concentration of oxidants "MDA & ROS" following a substantial decline in the antioxidant biomarkers levels. Besides, a profound upsurge was observed in proinflammatory cytokines concentration and apoptotic biomarkers levels were also scaled up. Additionally, histoarchitectural modifications were noticed in the pulmonary tissues on account of CP intoxication. Nevertheless, RHO effectively counteract the impairments in the pulmonary tissues of rats indicating that the RHO may be utilized as an important pharmacotherapeutic agent to rectify CP provoked pulmonary toxicity.

CRediT authorship contribution statement

Ali Akbar: Conceptualization, Writing – original draft, Investigation,

Formal analysis. Rabia Azmat: Data curation, Writing – review & editing, Investigation. Moazama Batool: Methodology, Visualization, Formal analysis. Bader O. Almutairi: Funding acquisition, Project administration, Resources, Data curation. Mian Nadeem Riaz: Formal analysis and Software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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