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Palliative potential of robinetin to avert polystyrene microplastics instigated pulmonary toxicity in rats

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ABSTRACT

Polystyrene microplastics (PSMPs) are noxious environmental pollutants that pose a significant threat to vital body organs including the lungs. Robinetin (ROB) is a flavonoid which demonstrates various pharmacological potentials. This trial was designed to assess the protective ability of ROB to avert PSMPs provoked pulmonary toxicity in rats. Twenty-four rats were divided into four groups i.e., control, PSMPs (0.1 mg/kg), PSMPs (0.1 mg/kg) + ROB (30 mg/kg) and ROB (30 mg/kg) only supplemented group. PSMPs exposure led to a notable reduction in the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), glutathione reductase (GSR) as well as GSH contents while causing a pronounced elevation in the concentration of MDA and ROS. Furthermore, PSMPs significantly augmented the levels of myeloperoxidase (MPO), macrophages, neutrophils, and lymphocytes in BALF. Moreover, the levels of IL-1β, TNF-α, NF-κB, IL-6 and COX-2 activities were increased following the PSMPs exposure. Nonetheless PSMPs remarkably decreased Bcl-2 levels, coupled with an escalation in Caspase-9, Caspase-3 and Bax levels. Despite this, severe histological alterations were observed in lungs tissues after PSMPs provision. Nevertheless, ROB provision markedly protected the lungs via regulating aforementioned dysregulations. This investigation validated the shielding strength of ROB to counteract PSMPs caused pulmonary toxicity.

1. Introduction

Plastics pollution has emerged as a substantial environmental concern owing to its widespread use and inadequate disposal (Barnes et al., 2009). The use of plastics products is increasing day by day due to their low costs and high resistance to degradation (Andrady, 2017). These plastic materials undergo physical, chemical, and biological processes which convert them into smaller particles having a size less than 5 mm and are referred to as "Microplastics" (MPs) (Liu et al., 2022). Humans are subjected to MPs through different pathways such as food consumption, breathing as well as dermal contact (Kannan and Vimalkumar, 2021). MPs accumulates in various body organs and instigate renal-toxicity, hepatic impairments, neurotoxicity and cardiac damages (Yee et al., 2021). PSMPs are one of the most prevalent environmental MPs that resulted from the degradation of styrene polymers (Hartmann et al., 2019).

PSMPs exposure inflicts reproductive toxicity, hepatic impairments, and neurotoxicity in various models of rats (Yu et al., 2018; Lu et al., 2016). PSMPs accumulate in testicular tissues and dysregulate the process of spermatogenesis (Wei et al., 2022). Furthermore, PSMPs administration induces various histopathological damages in hepatic tissues (Mu et al., 2022). It is reported that the atmosphere contains a high number of PSMPs. These airborne particles are inevitably inhaled by living organs leading to the deposition in lungs (Xu et al., 2019). The amassment of PSMPs for a long period of time in lungs instigate chronic inflammation (Chen et al., 2020). Moreover, PSMPs exposure elevates oxidative burden levels, apoptosis, & inflammatory responses in lungs (Cao et al., 2023).

Since decades, plant-based compounds are employed in medical interventions to cure various health conditions (Sayadi et al., 2023). Flavonoids are a class of polyphenolic compounds which are extensively found throughout vascular plants. Owing to their remarkable efficacy as

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well as low systemic toxicity, these compounds are widely used as therapeutic agents against different diseases (Oteiza et al., 2005). Robinetin is a plant-based flavonoid which demonstrated numerus biological potentials such as antioxidant, anti-cancerous, anti-mutagenetic and anti-leishmanial (Tasdemir et al., 2006). The current investigation was carried out to assess the ameliorative potential of ROB against PSMPs instigated pulmonary toxicity in rats.

2. Materials and Methods

2.1. Chemicals

Both PSMPs (Cas No. 9003–53-6, Purity: HPLC<98 %) & ROB (Cas No. 490–31-3 Purity: HPLC<98 %) were procured from Sigma-Aldrich, (Germany).

2.2. Animals

Twenty-four *Rattus norvegicus* rats (200 ± 20 g) having age of 16–18 weeks, were kept in cages (rodent) in the animal station of University of Agriculture Faisalabad. Animals were given optimum laboratory environment (22–25 °C temperature, 55–60 % humidity & 12 h of light and dark cycle) during the experiment. A balanced rodent nutrition & tap water was provided during the entire trial. The standard guidelines of EU Directive 2010/63/EU were strictly observed to handle the rats.

2.3. Experimental protocol

Four groups of twenty-four rats were formed and each group encompassed six rats (n = 6/group). All the groups were subjected to different regimens except the control group. The control group received only tap water & rodent nutrition. Group 2nd was exposed to PSMPs (0.01 mg/kg) while the group 3rd was subject to PSMPs (0.01 mg/kg) + ROB (30 mg/kg). The 4th group was administrated with ROB (30 mg/kg) alone through oral gavage. The dose of PSMPs was selected according to the previous investigation of Akbar and Ijaz (2024) while the dose of ROB was selected by following the previous study of Ijaz et al. (2024). After 4 weeks of experiment, rats were made unconscious by giving ketamine (60 mg/kg) & xylazine (6 mg/kg) through intraperitoneal route and slaughtered. Heparin syringes were employed to obtain blood samples. Lungs were excised from the abdominal cavity. The right lung was preserved in formalin (10 %) for histological assessment while the left lung was packed in zipper bag & stored at −20 °C for biochemical evaluation. The left lung was homogenized & centrifuged at 12000 rpm for a timeframe of 15 min. The resultant supernatant was used to assess biochemical parameters.

2.4. Biochemical assays

CAT activity was quantified following the principle outlined by Aebi (1984). SOD activity was calculated by adhering to the protocol of Kakkar et al. (1984). Rotruck et al. (1973) method was executed to ascertain GPx activity. GSR activity was quantified in accordance with the method expounded by Carlberg and Mannervik (1975). The strategies designed by Hayashi et al. (2007) and Ohkawa et al. (1979) were followed to evaluate ROS & MDA levels respectively.

2.5. Evaluation of lungs functional makers

The levels of pulmonary markers MPO (MBS704859), neutrophils (MBS438228), macrophages (MBS246615), lymphocytes (MBS140061) in BALF were measured via standard rat's kits (ELISA) in accordance with the guidelines provided in the literature book of product.

2.6. Inflammatory markers assessment

The determination of NF-κB (CSB-E13148r), IL-6 (CSB-E04640r), TNF-α (CSB-E07379r) and IL-1β (CSB-E08055r) as well as COX-2 (CSB-E13399r) activity were carried out by using standard ELISA kits (Cloud-clone Corp. USA). The protocol was performed by following the guidelines of Cloud-clone Corp. USA.

2.7. Evaluation of apoptotic markers

Caspase-9 (CSB-E08863r), Caspase-3 (CSB-E08857r), Bcl-2 (CSB-E08854r) and Bax (CSB-EL002573RA) levels were quantified by employing ELISA kits as instructed by the manufacturer.

2.8. Statistical analysis

The values were denoted as mean ± SEM. One-way ANOVA followed by Tukey's test were applied for comparison among the groups while the level of significance was adjusted at $p < 0.05$. The statistical evaluations were carried out by using Minitab V17 software and normality of data was checked by Shapiro-wilk test.

3. Results

3.1. Impacts of ROB & PSMPs on biochemical markers

Exposure to PSMPs markedly ($p < 0.05$) reduced CAT, SOD, GPx, GSH, GSR & GST activities while increasing ROS and MDA concentrations. However, the administration of PSMPs + ROB remarkably ($p < 0.05$) increased CAT, SOD, GSR, GPx and GST activities while downregulating MDA and ROS levels. Nevertheless, the ROB only treated group and control group showed insignificant disparities between the mean values (Table 1).

3.2. Impacts of ROB & PSMPs on lungs functional markers

Exposure to PSMPs notably ($p < 0.05$) escalated the levels of MPO, macrophages, neutrophils, lymphocytes, LDH, ALP, and total protein in BALF. However, the co-treatment of PSMPs + ROB noticeably ($p < 0.05$) reduced the levels of MPO, macrophages, neutrophils, lymphocytes, LDH, ALP, and total protein in BALF than the PSMPs treated group. Nevertheless, there was no considerable difference was noticed in the lung functional markers among the control group and ROB-only treated group (Table 2).

3.3. Impacts of ROB & PSMPs on inflammatory markers

Intoxication to PSMPs markedly ($p < 0.05$) increased IL-1β, NF-κB, TNF-α and IL-6 concentrations and COX-2 activity relative to untreated group. However, PSMPs + ROB concurrent intervention markedly ($p < 0.05$) reduced the concentrations of aforementioned inflammatory biomarkers. Nevertheless, the levels of these biomarkers in ROB and the control group were comparable to each other (Table 3).

3.4. Impacts of ROB & PSMPs on apoptotic markers

The treatment of PSMPs considerably ($p < 0.05$) lowered the levels of Bcl-2, while escalating the levels of Caspase-9, Caspase-3 and Bax. Furthermore, PSMPs + ROB treatment considerably ($p < 0.05$) escalated Bcl-2 levels while downregulating Caspase-9, Bax and Caspase-3 levels. Noticeably, control and ROB alone treated group showed negligible disparities among their mean values (Table 4).

4. Discussion

Plastics pollution becomes a global issue owing to its detrimental

Table 1
Variations in superscripts predicted the differences among different groups.

Parameters	Groups			
	Control	**PSMPs**	**PSMPs + ROB**	**ROB**
CAT (U/mg protein)	15.29 ± 2.04^{a}	6.88 ± 0.77^{b}	11.55 ± 1.24^{a}	15.69 ± 2.12^{a}
SOD (U/mg protein)	11.22 ± 1.02^{a}	5.86 ± 0.59^{b}	8.24 ± 0.72^{b}	11.42 ± 1.19^{a}
GSR (nM NADPH oxidized/min/mg tissue	9.81 ± 0.84^{a}	4.81 ± 0.33^{b}	8.17 ± 0.61^{a}	9.97 ± 0.95^{a}
GPx (U/mg protein)	26.25 ± 1.16^{a}	13.09 ± 1.79^{c}	21.18 ± 1.52^{b}	26.89 ± 1.57^{a}
GSH (nM/min/mg protein)	25.28 ± 0.62^{a}	10.63 ± 1.54^{c}	17.81 ± 1.34^{b}	25.48 ± 0.67^{a}
GST (nM/min/mg protein)	43.47 ± 1.28^{a}	14.11 ± 1.57^{c}	36.36 ± 1.77^{b}	44.36 ± 1.03^{a}
MDA (nmol/mg protein)	0.64 ± 0.16^{c}	3.34 ± 0.26^{a}	1.39 ± 0.34^{b}	0.61 ± 0.15^{c}
ROS (nmol/g)	1.19 ± 0.21^{c}	7.02 ± 0.50^{a}	2.15 ± 0.29^{b}	1.06 ± 0.33^{c}

Table 2
Variations in superscripts predicted the differences among different groups.

Parameters	Groups			
	Control	**PSMPs**	**PSMPs + ROB**	**ROB**
Neutrophils (10^6 cells/mL)	0.44 ± 0.38^{b}	15.35 ± 2.88^{a}	2.14 ± 0.56^{b}	0.41 ± 0.36^{b}
Macrophages (10^6 cells/mL)	1.41 ± 0.33^{c}	8.20 ± 0.80^{a}	2.98 ± 0.38^{b}	1.38 ± 0.30^{c}
Lymphocytes (10^6 cells/mL)	0.81 ± 0.22^{b}	9.88 ± 1.85^{a}	2.29 ± 0.39^{b}	0.71 ± 0.29^{b}
MPO (Units/min/mg protein)	2.12 ± 0.23^{c}	10.14 ± 0.73^{a}	3.79 ± 0.51^{b}	1.99 ± 0.37^{c}

effects on living organisms (Li et al., 2020a; Li et al., 2020b). It is estimated that industrial and household plastics account for 300 million tons of annual production (Ogunola et al., 2018). Various investigations have reported the adverse impacts of MPs health of human such as reproductive toxicity, hepato-renal damages, immune-depressant, and gastro-intestinal damages (Prata et al., 2020). Furthermore, MPs act as carriers for other environmental toxicants including heavy metals (Zhao et al., 2023). Since many years, flavonoids are widely employed as therapeutic agents due to their anti-bacterial, hepatoprotective, anti-inflammatory and antioxidative abilities (Santos et al., 2022). Therefore, this research trial was sought to explore the mitigative abilities of ROB to avert PSMPs instigated pulmonary toxicity via modulating biochemical, inflammatory, and apoptotic assessment.

Our research demonstrated that PSMPs intoxication reduced CAT, SOD, GPx, GSR and GST activities while escalating the MDA & ROS concentrations. Antioxidant enzymes act as a protective shield to avert oxidative stress (OS) (Papas et al., 2019). The disparities between the balance of antioxidant enzymes and ROS induce OS (Ijaz et al., 2023). Furthermore, excessive generation of ROS reduced the activities of antioxidant enzymes thereby impairing cellular defense system (Ahmad et al., 2023). SOD converts O_2^- into hydrogen peroxide and molecular oxygen (Bromfield, 2016) whereas CAT transforms hydrogen peroxide into water (Jonakova et al., 2010). It is reported that Nrf-2 is the primary factor that enters the nucleus and triggers the transcription of various antioxidant genes (Lu et al., 2022). Elsayed et al. (2022) reported that flavonoids can reduce the levels of OS via increasing the activity of antioxidant enzymes. Nonetheless, ROB+PSMPs treatment remarkably escalated antioxidant enzymes activities while reducing the levels of OS markers. Teixeira and da Costa (2005) elucidated that flavonoid exhibits antioxidative abilities due to the existence of hydroxyl groups in their structural configuration. ROS is also a plant flavonoid therefore demonstrate ROS scavenging properties.

Our findings showed that PSMPs notably escalated the levels of neutrophils, MPO, lymphocytes, macrophages, ALP, LDH & total proteins contents in BALF. Various inflammatory conditions are characterized by elevated levels of neutrophils and macrophages (Medan et al., 2002). During chronic inflammation, neutrophils escalate the production of MPO which ultimately damage pneumonocytes (Haegens et al., 2008). Lung cells damage is evident by increased levels of ALP, LDH and total protein contents in BALF (Li et al., 2020a; Li et al., 2020b). However, PSMPs + ROB reduced the levels of abovementioned lung function markers.

Our findings revealed exposure to PSMPs increased the concentrations of NF-κB and its associated cytokines in lungs tissues of rats. NF-κB is an essential regulator of inflammatory pathway which stimulates the initiation of other inflammatory cytokines (Fan et al., 2008). Kim et al. (2019) elucidated that COX-2 is widely used as diagnostic marker of inflammation during different disorders. Jung et al. (2008) revealed that ROS directly attacks the IκB that resulted in its dissociation which provoke the activation of NF-κB. Nonetheless, concurrent treatment of ROB considerably lessened earlier mentioned inflammatory cytokines concentrations.

PSMPs exposure escalated Caspase-9, Caspase-3, and Bax while reducing Bcl-2 levels. Bcl-2 is a sensitive biomarker which regulates the process of apoptosis (Eno et al., 2012). Similarly, Bax serves as antagonistic to Bcl-2 which accelerates apoptotic pathway. Elevated levels of Bax trigger the liberation of Cytochrome *c* into cytoplasm. Cytochrome *c* stimulates the activation of various caspases including caspase-9 & caspase-3 (Siddiqui et al., 2015). Nevertheless, administration of ROB remarkably reduced caspase-9, caspase-3 and Bax while enhancing Bcl-2 concentrations.

Table 3
Variations in superscripts predicted the differences among different groups.

Parameters	Groups			
	Control	**PSMPs**	**PSMPs + ROB**	**ROB**
NF-kB (ng/g tissue)	25.72 ± 1.68^{c}	77.21 ± 2.01^{a}	32.91 ± 1.62^{b}	25.51 ± 1.47^{c}
TNFα (ng/g tissue)	11.52 ± 1.38^{c}	55.31 ± 2.03^{a}	20.19 ± 2.32^{b}	11.26 ± 1.14^{c}
IL-1ß (ng/g tissue)	23.53 ± 0.90^{c}	85.61 ± 1.43^{a}	35.69 ± 2.06^{b}	22.71 ± 1.08^{c}
IL-6 (ng/g tissue)	5.91 ± 1.52^{c}	35.67 ± 2.81^{a}	14.01 ± 1.63^{b}	5.85 ± 1.51^{c}
COX-2 (ng/g tissue)	13.46 ± 2.23^{c}	84.93 ± 1.55^{a}	21.91 ± 1.82^{b}	13.20 ± 2.07^{c}

Table 4
Variations in superscripts predicted the differences among different groups.

Parameters	Groups			
	Control	PSMPs	PSMPs + ROB	ROB
Bax (pg/mL)	2.50 ± 0.43^{b}	9.73 ± 0.96^{a}	3.60 ± 0.64^{b}	2.36 ± 0.32^{b}
Caspase-3 (ng/mL)	1.64 ± 0.36^{b}	13.57 ± 1.25^{a}	2.57 ± 0.27^{b}	1.52 ± 0.48^{b}
Caspase-9 (pg/mL)	3.37 ± 0.28^{c}	24.59 ± 1.32^{a}	5.38 ± 0.35^{b}	3.14 ± 0.31^{c}
Bcl-2 (pg/mL)	18.37 ± 1.12^{a}	4.05 ± 0.23^{c}	11.40 ± 0.99^{b}	19.47 ± 1.43^{a}

5. Conclusion

Taken together, PSMPs disrupted the lungs tissues via inducing OS. Moreover, exposure to PSMPs subsidized antioxidant enzymes activities while escalating lung's function markers concentrations. Furthermore, exposure to PSMPs increased the markers of inflammation and apoptosis while lessening anti-apoptotic markers. However, ROB treatment markedly protected the lungs tissues via regulating abovementioned dysregulations. However, it is indispensable to conduct clinical trials on human to assess the palliative role of ROB against PSMPs induced lungs damage.

CRediT authorship contribution statement

Muhammad Faisal Hayat: Writing – original draft, Methodology, Investigation, Conceptualization. **Anees Ur Rahman:** Writing – original draft, Methodology, Investigation, Conceptualization. **Amara Tahir:** Software, Methodology, Investigation. **Moazama Batool:** Visualization, Validation, Software, Formal analysis, Data curation. **Zubair Ahmed:** Writing – original draft, Visualization, Resources, Funding acquisition. **Usman Atique:** Writing – review & editing, Software, Formal analysis.

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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