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Full Length Article

Rhamnetin abrogates polystyrene microplastics prompted hepatic damage by regulating Nrf-2/Keap-1 pathway

Saba Yaqoob [a], Ali Hamza [a,*], Moazama Batool [b], Aisha Khatoon [c], Shaik Althaf Hussain [d], Mian Nadeem Riaz [e]

[a] Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan
[b] Department of Zoology, Govt. College Women University, Sialkot 51310, Pakistan
[c] Department of Pathology, University of Agriculture, Faisalabad, Pakistan
[d] Department of Zoology, College of Science, King Saud University, P.O. Box - 2454, Riyadh - 11451, Saudi Arabia
[e] Texas A & M University, College Station, TX 2476, USA

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ABSTRACT

Polystyrene microplastics (PS-MPs) are environmental toxicants that exert adverse effects on organisms. Rhamnetin (RHM) is a natural flavone that shows multiple therapeutic potentials. Therefore, the present study was planned to determine the mitigative effect of RHM against PS-MPs induced liver damage. 48 rats were divided into 4 groups. Control group, PS-MPs (0.01 mg/kg) administered group, PS-MPs (0.01 mg/kg)+ RHM (50 mg/kg) co-administered group and RHM alone (50 mg/kg) administered group. PS-MPs reduced the expressions of Nrf-2 and anti-oxidant gene expressions, while increasing Keap-1 expression. PS-MPs also decreased the activities of catalase (CAT), glutathione reductase (GSR), heme oxygenase-1 (HO-1), glutathione-S-transferase (GST), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH), besides elevating the levels of MDA and ROS. Additionally, PS-MPs augmented the levels of alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST). Furthermore, there was an upsurge in the levels of inflammatory indices in PS-MPs treated group. PS-MPs intoxication also increased Bax and Caspase-3 expressions, while lowering the Bcl-2 expression. Nevertheless, RHM mitigated all the damages due to its hepatoprotective, anti-inflammatory, anti-oxidant and anti-apoptotic potentials.

1. Introduction

Environmental pollution emerging from plastic is a prevailing issue due to its extensive manufacturing and application in industry and agricultural sector (Hwang et al., 2020). Plastic manufacture has accelerated exponentially reaching to 368 million metric tons in 2019, moreover within 20 years it is expected to be two folds (Al Mamun et al., 2023). Plastics production has surpassed the production of all other synthetic products worldwide. This shows that billions of kilograms of plastics are produced per day, of which only 9 % are recycled, 12 % are incinerated, whereas remaining 79 % are added in the natural environment (Geyer et al., 2017; Almadhi et al., 2023). The remaining plastics (79 %) undergo weathering and breaking down into minute particles that are known as microplastics (MPs) (<5 mm in diameter). Previous literature have described the presence of MPs in facial skin, hair, placenta, saliva, testis and liver. Additionally, MPs pollution contributes to global issues such as ocean acidification, global warming, and ozone depletion (Abbasi and Turner, 2021).

Polystyrene (PS) is one of the most renowned and frequently used type of plastics, which is valued for its persistent physical characteristics and inexpensive price (Cincinelli et al., 2017; Wang et al., 2022). Polystyrene microplastics (PS-MPs) is frequently used to manufacture disposable cups, paper clips, toys cosmetics, pharmaceuticals and trays. PS-MPs exposure to human occur directly through the drinking water, inhalation, skin and ingestion. PS-MPs can enter easily into the organisms owing to low density, smaller size and relatively large surface area; thus, they assemble in organic food chains (Campbell et al., 2017).

Mounting evidences have revealed that PS-MPs intoxication leads to gastrointestinal toxicity, nephrotoxicity, neurotoxicity, testicular toxicity and hepatotoxicity (Ge et al., 2023; Hamza et al., 2023a). PS-MPs treatment provokes ROS generation, which in turn enhances the oxidative and immunological processes. Excessive ROS production leads

* Corresponding author.
E-mail address: alihamzatabassum7@gmail.com (A. Hamza).

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Table 1
Primers sequences for the real-time quantitative reverse transcription-polymerase (RT-qPCR).

Gene	Primers 5′ –> 3′	Accession number
Nrf-2	F: ACCTTGAACACAGATTTCGGTG	NM_031789.1
	R: TGTGTTCAGTGAAATGCCGGA	
Keap-1	F: ACCGAACCTTCAGTTACACACT	NM_057152.1
	R: ACCACTTTGTGGGCCATGAA	
CAT	F: TGCAGATGTGAAGCGCTTCAA	NM_012520.2
	R: TGGGAGTTGTACTGGTCCAGAA	
SOD	F: AGGAGAAACTGACAGCTGTGTCT	NM_017051.2
	R: AAGATAGTAAGCGTGCTCCCAC	
GPx	F: TGCTCATTGAGAATGTCGCGTC	NM_030826.4
	R: ACCATTCACCTCGCACTTCTCA	
GSR	F: ACCAAGTCCCACATCGAAGTC	NM_053906.2
	R: ATCACTGGTTATCCCCAGGCT	
GST	F: TCGACATGTATGCAGAAGGAGT	NM_031509.2
	R: CTAGGTAAACATCAGCCCTGCT	
HO-1	F: AGGCTTTAAGCTGGTGATGGC	NM_012580.2
	R: ACGCTTTACGTAGTGCTGTGT	
Bax	F: GCACTAAAGTGCCCGAGCTG	NM_017059.2
	R: CCAGATGGTGAGTGAGGCAG	
Bcl-2	F: ACTGAGTACCTGAACCGGCA	NM_016993.1
	R: CCCAGGTATGCACCCAGAGT	
Caspase-3	F: GTACAGAGCTGGACTGCGGT	NM_012922.2
	R: TCAGCATGGCGCAAAGTGAC	
β-actin	F: AGGAGATTACTGCCCTGGCT	NM_031144
	R: CATTTGCGGTGCACGATGGA	

to DNA damage, impaired protein expression at biochemical as well as cellular level (Yu et al., 2018).

Flavonoids, is a group of polyphenolic compounds that exhibit remarkable pharmacological potentials (Alvi et al., 2022; Surien et al., 2023). Rhamnetin ($C_{16}H_{12}O_7$) is a potent dietary bioflavonoid that is naturally extracted from diverse plant species i.e., halophytes (*Halimione portulacoides*, *Salicornia europaea* and *Arthrocnemum macrostachyum*) vegetables *(Thymus nummularius,* Ziziphus mistol) and fruits (clove, apple, sour cherries). RHM exhibits excellent therapeutic properties including neuro-protective, anti-tumor, anti-cancer and anti-bacterial (Mondal et al., 2013). Keeping these attributes under consideration, the present study was planned to determine the protective role of RHM against PS-MPs induced hepatic damage in rats.

2. Material and methods

2.1. Chemicals

PS-MPs (CAT No. 9003-53-6) and RHM (CAT 90-19-7) were purchased from Sigma-Aldrich, (Germany). The size of PS-MPs was 100 nm with monodispersed shape and 1±2 mV zeta potential. All the other chemicals used were of ultra-pure molecular biology grade. Formalin, sodium bicarbonate, ethanol, NADH, hydrogen peroxide, EDTA, ferric chloride and TRIzol reagent were purchased from Sigma-Aldrich, Germany.

2.2. Animals

The present research was conducted on 48 rats weighing 200–220 g. Rats were retained in the animal room at temperature (22–25 °C), 55–70 % humidity and 12 h day/night cycle at University of Agriculture, Faisalabad (UAF). All the rats were given free access to diet and water. The rats were treated by following the protocol of the European Union of Animal Care and Experimentation (CEE Council 86/609) that was further approved by the ethical committee of UAF.

2.3. Experimental design

48 rats were divided in 4 groups. Control group, PS-MPs administrated (0.01 $mgkg^{-1}$), PS-MPs (0.01 mg/kg)+ RHM (50 mg/kg) co-administered and only RHM treated (50 $mgkg^{-1}$). The doses were selected in compliance with the previous study (Hamza et al., 2023b). Furthermore, the oral toxicity analysis of RHM was performed by using 50, 100, 120, 150 and 200 $mgkg^{-1}$ doses. Health parameters such as diet, changes in weight, fluid intake, and psychomotor changes were measured. RHM did not show any toxicity at all the tested doses. So lowest dose (50 $mgkg^{-1}$) was selected. The experiment was performed for 30 days. Rats were anesthetized, beheaded, blood was collected in sterile tubes and centrifuged for 15 min (3000 rpm). Plasma was preserved at −20 °C for lateral observation. The hepatic samples were stored at −80 °C in a zipper bag for biochemical analysis.

2.4. Antioxidant enzymes activity

The technique demonstrated by Aebi (1984) was used to measure CAT activity. SOD activity was examined by following the protocol of Kakkar et al. (1984). GSH level was measured by using the methodology of Sedlak and Lindsay (1968). While, GPx activity was assessed by following the technique of Rotruck et al. (1973). GSR level was determined via the methodology of Carlberg and Mannervik (1985). The activity of GST was estimated by the method of Habig et al. (1974), while the activity of HO-1 was quantified by using the protocol of Magee et al. (1999). Moreover, MDA content was assessed by the protocol of Placer et al. (1966), while ROS content was analyzed by Hayashi et al. (2007) method.

2.5. Real-time polymerase chain reaction (qRT-PCR)

Anti-oxidant genes (CAT, SOD, GPx, GSR, GST and HO-1), Nrf-2/Keap-1 and apoptotic markers expressions were appraised by qRT-PCR. RNA was isolated with the help of TRIzol reagent that was converted to cDNA by employing Fast Quant RT kit. Alterations in the expressions were determined by $2^{-\Delta\Delta CT}$, taking β-actin as inner regulator. Table 1 shows the primer sequences of the target genes as reported earlier (Hamza et al., 2023a; Ijaz et al., 2022).

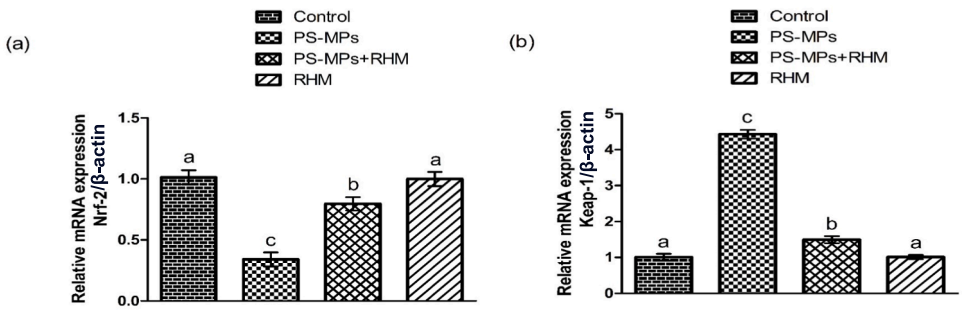

Fig. 1. Impact of RHM and PS-MPs on a) Nrf-2 and b) Keap-1 expressions. The data were interpreted with the help of One-way ANOVA and Tukey's test. Different superscripts on the graphs are presenting significant difference.

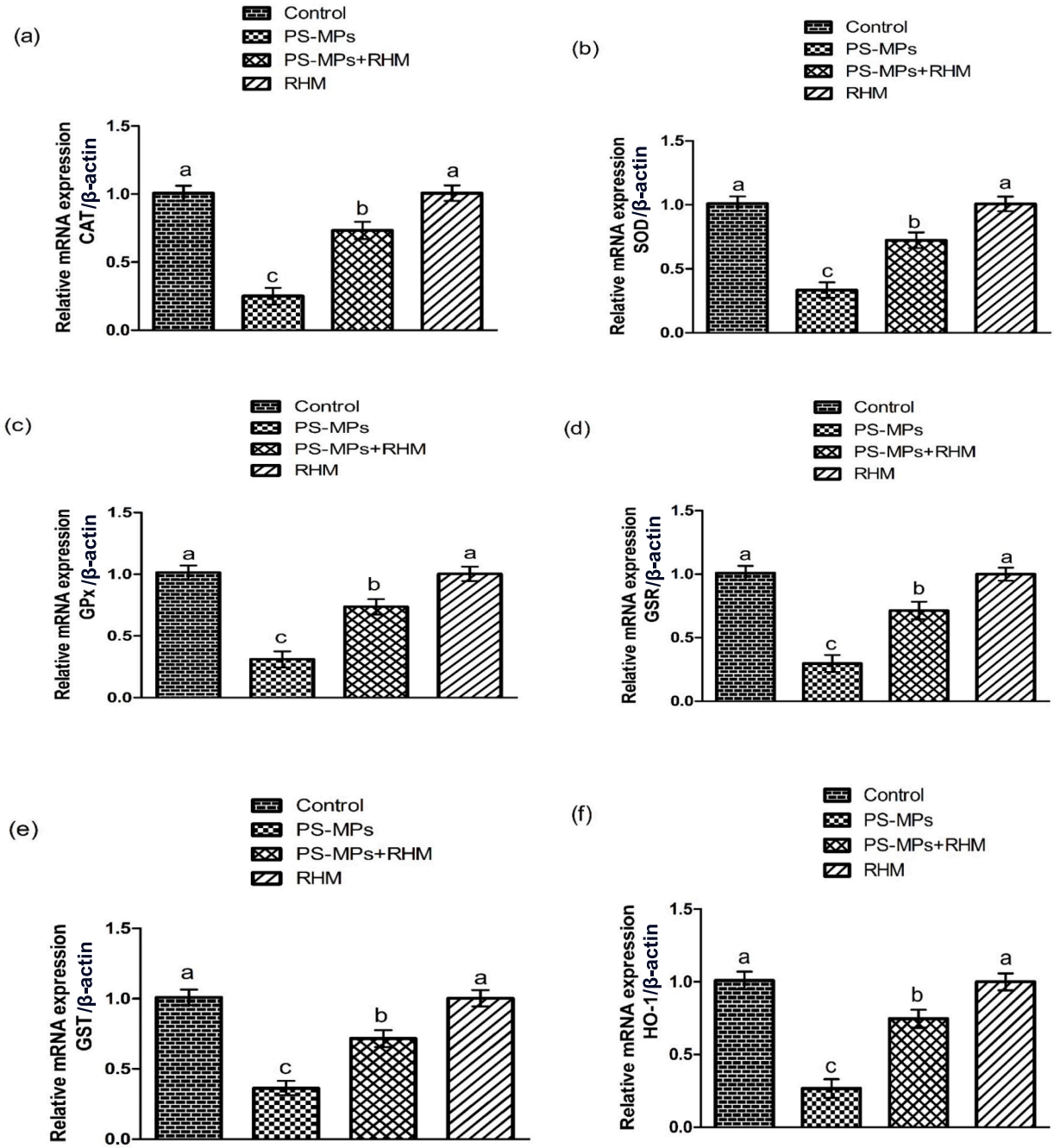

Fig. 2. Impact of RHM and PS-MPs on a) CAT, b) SOD, c) GPx, d) GSR, e) GST and f) HO-1 expressions. The data were interpreted with the help of One-way ANOVA and Tukey's test. Different superscripts on the graphs are presenting significant difference.

2.6. Assessment of liver serum enzymes

The level of ALP, AST and ALT were determined with the help of standardized ELISA kits purchased from Wiesbaden (Wiesbaden, Germany).

2.7. Inflammatory indices assessment

The levels of inflammatory markers were evaluated by using ELISA kits. The quantifications were accomplished employing ELISA plate reader in compliance with instructor's recommendations (BioTek, Winooski, USA).

2.8. Statistical analysis

Data were shown as Mean ± SEM. The whole data were interpreted with the help of One-way ANOVA & Tukey's test through Minitab software. The $P<0.05$ was taken as level of significance.

3. Results

3.1. Protective role of RHM on the expressions of Nrf-2/Keap-1

PS-MPs treatment induced a significant ($P<0.05$) reduction in the expressions of Nrf-2 and antioxidant genes, on the other hand elevated Keap-1 expression in PS-MPs administered rats in contrast to the control rats. Nevertheless, treatment of RHM increased the Nrf-2 and anti-

Table 2
Role of RHM and PS-MPs on biochemical markers.

Parameters	Groups			
	Control	PS-MPs	PS-MPs + RHM	RHM
CAT (U/mg protein)	9.11 ±0.21[a]	4.33 ±0.16[c]	8.11 ±0.14[b]	9.09 ±0.21[a]
SOD (U/mg protein)	8.08 ±0.16[a]	2.81 ±0.08[c]	6.53 ±0.12[b]	8.12 ±0.17[a]
GSR (nM NADPH oxidized/min/mg tissue)	6.86 ±0.13[a]	1.83 ±0.15[c]	5.23 ±0.12[b]	6.84 ±0.13[a]
GPx (U/mg protein)	25.63 ±1.08[a]	7.13 ±0.35[b]	21.55 ±0.77[a]	25.42 ±1.16[a]
GSH (μM/g tissue)	17.44 ±1.06[a]	4.52 ±0.28[c]	12.10 ±0.91[b]	17.94 ±1.15[a]
GST (nM/min/mg protein)	33.62 ±0.98[a]	12.73 ±0.59[c]	28.17 ±0.91[b]	34.48 ±1.37[a]
HO-1 (pmoles bilirubin/ mg protein/h)	287.09 ±3.88[a]	58.22 ±1.56[c]	162.12 ±3.65[b]	285.01 ±3.74[a]
MDA (nmol/mg protein)	0.54 ±0.08[a]	6.33 ±0.13[c]	1.63 ±0.15[b]	0.52 ±0.08[a]
ROS (Umg^{-1} tissue)	1.63 ±0.09[a]	7.81 ±0.33[c]	2.34 ±0.12[b]	1.61 ±0.09[a]

Values with different superscripts are significantly (P<0.05) different. The data were interpreted with the help of One-way ANOVA and Tukey's test.

Table 3
Role of RHM and PS-MPs on liver function enzymes.

Parameters	Groups			
	Control	PS-MPs	PS-MPs + RHM	RHM
ALT (U/I)	46.09±1.81[a]	87.93±1.74[c]	65.02±2.04[b]	45.29±1.74[a]
AST (U/I)	90.06±3.02[a]	188.05 ±4.03[c]	125.37±4.43[b]	89.30±3.21[a]
ALP (U/I)	125.87 ±4.84[a]	343.81 ±6.55[c]	187.31±3.75[b]	124.48 ±4.68[a]

Values with different superscripts are significantly (P<0.05) different. The data were interpreted with the help of One-way ANOVA and Tukey's test.

oxidant genes expression and down-regulated the expression of Keap-1. Additionally, RHM alone administered rats presented these expressions near to the control rats (Figs. 1, 2 presenting the findings).

3.2. Protective role of RHM on biochemical markers

PS-MPs treatment markedly (P<0.05) reduced the activities of GPx, GSR, HO-1, GST, CAT, GSH and SOD, while a remarkable elevation in ROS and MDA levels was observed in PS-MPs administered group as matched with the control group. Moreover, RHM + PS-MPs treatment noticeably increased the activities of anti-oxidants, while MDA and ROS contents were lowered relative to PS-MPs administered group. RHM only administered group exhibited these markers close to the control group (Table 2 presenting the findings).

3.3. Protective role of RHM on liver serum enzymes

PS-MPs significantly (P<0.05) augmented the levels of hepatic serum enzymes (ALP, AST & ALT) in PS-MPs treated rats as compared to the control rats. However, PS-MPs + RHM supplementation substantially lowered these levels in contrast to the rats treated with PS-MPs. RHM alone treated group showed the levels of these markers close to the control group (Table 3 presenting the findings).

3.4. Protective role of RHM on the expressions of apoptotic markers

PS-MPs intoxication markedly (P<0.05) increased Bax and Caspase-3 expressions. Although, Bcl-2 expression was lowered in rats treated with PS-MPs in contrast to the control rats. Nevertheless, RHM+PS-MPs supplementation considerably restored these expressions in contrast to the PS-MPs rats. RHM alone treated rats these expression were same as in the control rats (Fig. 3 presenting the findings).

3.5. Protective role of RHM on the levels of inflammatory markers

PS-MPs significantly (P<0.05) elevated the levels of inflammatory indices such as NFκB, TNFα-, IL6, IL1β, and COX-2 as matched with the control group. Nevertheless, RHM + PS-MPs treatment considerably lowered inflammatory indices in contrast to the PS-MPs group. Only RHM administered rats presented these markers near to the control rats (Table 4 presenting the findings).

4. Discussion

The pollution of plastic is one of the most prevalent issues, due to its massive production and detrimental effects on organisms (Zhang et al., 2020). PS-MPs are ubiquitous environmental contaminants that pose adverse effect on human health such as cancer, infertility, respiratory disease, brain damage, heart disease, gastrointestinal blockage, impairment in placental tissues and blood as well as remarkable dysfunctions in intestine, excretory systems and liver (Zolotova et al., 2022). PS-MPs have been reported in beer, salt and honey. According to the study of Hwang et al. (2019) humans are exposed to PS-MPs via skin, ingestion, drinking water and inhalation. PS-MPs exposure leads to OS and inflammatory responses (Kim et al., 2021). Moreover, PS-MPs administration disturb the apoptotic markers, anti-oxidants activities as well as liver serum marker enzymes in rats. RHM is a flavone that is abundantly reported in halophytes plants (*Halimione portulacoides, Arthrocnemum macrostachyum*), vegetables (*Moringa oleifera), Haplopappus multifolius* and fruits i.e. grapes, oranges, mangoes (Ahmad et al., 2023). RHM exhibits various protective activities i.e., anti-tumor, anti-arthritic, anti-tumor and anti-oxidant (Jia et al., 2016). Therefore, the present study was planned to assess the mitigative role of RHM against PS-MPs induced hepatotoxicity.

PS-MPs treatment reduced anti-oxidant genes and Nrf-2 expressions, while increasing the expression of Keap-1. Vomund et al. (2017), stated that Nrf-2 performs crucial function in the regulation of OS. While Keap-1 acts as the inhibitor of Nrf-2 and controls its stability. Keap-1 detaches from Nrf-2 during ROS production via some physical modifications, then travels into the nucleus, where it attaches to small MAF proteins (Pintard et al. 2004). Nrf-2 along with MAF proteins attach to the antioxidant responsive elements and induces anti-oxidants genes expressions. Therefore, Nrf-2 has a critical part in monitoring the stimulation of anti-oxidant genes (Hawkes et al. 2014). Nevertheless, during extreme OS Nrf-2 expression is decreased, whereas increasing the Keap-1. Consequently, reduced Nrf-2 lowers the genes expressions of anti-oxidants, as Nrf-2 has a crucial role in regulating the genes expressions anti-oxidants (Yang et al., 2022). Nevertheless, RHM administration elevated anti-oxidant genes and Nrf-2 expressions, while lowering Keap-1 expression. Therefore, it is assumed that RHM has the ability to regulate Nrf-2 and Keap-1 expressions.

PS-MPs administration reduced the anti-oxidants activities such as GPx, GSH, GST, SOD, GSR, HO-1, CAT enzymes, besides increased ROS and MDA level. These anti-oxidants are the first wall of protection that shield DNA, lipids and proteins from oxidative damage (Ighodaro et al., 2018). CAT is referred as primary enzyme, which has important role in the catabolism of hydrogen peroxide (H_2O_2) (Selamoglu Talas et al., 2008). SOD splits superoxide radicals into oxygen (O_2) and H_2O_2. According to the study of Ulhaq and Tse (2023), GSR maintains reduced GSH levels in cells, via reducing ROS levels. HO-1 is an essential enzyme, which helps in the breakdown of heme and performs a crucial function in homeostasis. MDA is a marker of LP and directly indicates the level of LP and ROS (Samad et al., 2019). The body's anti-oxidant defense mechanism is overwhelmed when excessive level of ROS are created that

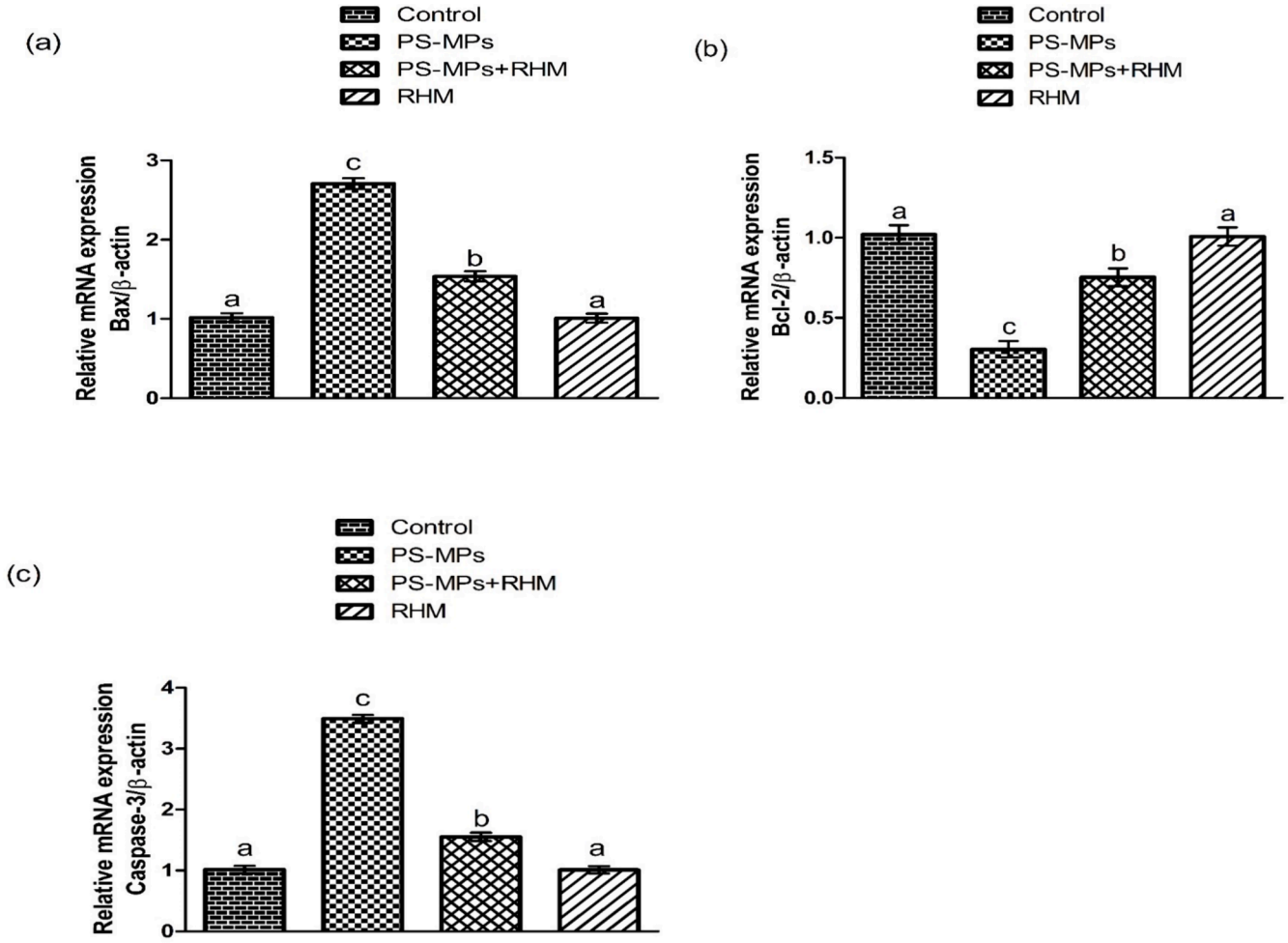

Fig. 3. Impact of RHM and PS-MPs on a) Bax, b) Bcl-2 and c) Caspase-3 expressions. The data were interpreted with the help of One-way ANOVA and Tukey's test. Different superscripts on the graphs are presenting significant difference.

Table 4
Role of RHM and PS-MPs on inflammatory markers.

Parameters	Groups			
	Control	PS-MPs	PS-MPs + RHM	RHM
NF-kB (ng/g tissue)	17.22 ±0.73^{a}	79.04 ±1.27^{c}	27.48±1.31^{b}	17.04 ±0.67^{a}
TNF-α (ng/g tissue)	7.62±0.46^{a}	57.50 ±1.29^{c}	16.88±0.74^{b}	7.57±0.44^{a}
1L-1β (ng/g tissue)	23.15 ±0.93^{a}	91.60 ±1.44^{c}	36.07±0.91^{b}	22.82 ±0.93^{a}
IL-6 (ng/g tissue)	8.51±0.64^{a}	65.55 ±1.45^{c}	18.07±0.94^{b}	8.44±0.66^{a}
COX-2 (ng/g tissue)	12.64 ±1.07^{a}	83.69 ±1.42^{c}	26.64±1.67^{b}	12.43 ±0.94^{a}

Values with different superscripts are significantly ($P<0.05$) different. The data were interpreted with the help of One-way ANOVA and Tukey's test.

results in OS. Besides natural anti-oxidants, these anti-oxidants from different plants may be used to treat OS (Nahid et al., 2017). Nevertheless, RHM+PS-MPs supplementation attenuated the PS-MPs damages by escalating anti-oxidants activity and lowering ROS and MDA contents owing to its ROS salvaging property. Moreover, according to previous study flavonoids have the anti-oxidant potential due to the presence of phenolic rings and OH groups in their structural formula (Rodríguez De Luna et al., 2020).

The intoxication of PS-MPs substantially escalated the levels of hepatic function enzymes. Hepatic function enzymes are suitable indicators to evaluate the hepatic function. When the plasma membrane of hepatocytes is disrupted, these enzymes are released from the cytosol and enter into the blood, resulting in hepatic dysfunction. Our outcomes are also evinced by the study of Cheng et al. (2022) who reported that PS-MPs intoxication increased the concentration of liver function enzymes, indicating the adverse condition of liver tissues that consequently leads to liver dysfunction (Kandemir et al., 2020). However, RHM administration dramatically reduced the levels of these enzymes due to hepato-protective and ROS scavenging properties.

PS-MPs-intoxication increased the expressions of Bax and Caspase-3, while decreasing the expression of Bcl-2. Caspase and Bcl-2 family play a major role in apoptosis (Hou et al., 2021). Bcl-2 protects the cells from apoptotic cell death. Contrarily, pro-apoptotic marker (Bax) triggers the cell death (Ehsan et al., 2023). A decrease in in Bcl-2 and escalation in Bax prompts cytochrome-C eviction in cytosol that instigates Caspase-3 (Yen et al., 2012). Caspase-3 break down the proteins and alters their structural makeup, leading to cell death. However, RHM+PS-MPs administration led to substantial reduction in Caspase-3 and Bax expression, besides a notable escalation in Bcl-2 expression was observed. This protective role might be ascribed to the anti-apoptotic property of RHM. Our results are further endorsed by the study conducted by Hamza et al. (2023b), who reported that RHM has to potential to inhibit apoptosis in the testicular tissues of rats.

The intoxication of PS-MPs increased the levels of inflammatory markers including NFκB, TNFα-, IL6, IL1β, and COX-2. The instigation of

NF-κB performs a pivotal function in the expressions of other inflammatory indices (TNFα-, IL6, IL1β, and COX-2) that shows severe inflammation. The stimulation of NF-κB boosts inflammatory cytokines (TNFα-, IL6, IL1β, and COX-2) production that results in inflammation. COX-2 is an additional biomarker that also contributes significantly to inflammation (Kim et al., 2019). However, RHM supplementation significantly decreased the levels of inflammatory markers due to its anti-inflammatory nature.

5. Conclusion

Our research revealed that RHM shows potential mitigative role against PS-MPs-instigated hepatic damage. The administration of RHM regulated the anti-oxidants activities, MDA and ROS levels and hepatic function enzymes disturbed by PS-MPs intoxication. Moreover, it also restored apoptotic markers expression as well as inflammatory markers level, owing to its hepato-protective, anti-inflammatory, anti-oxidant and anti-apoptotic activities. Under the light of these findings, it can be deduced that RHM could be used to treat PS-MPs induced liver toxicity.

6. Limitations and future directions

The study was performed by using rats as an animal model and clinical trials are recommend in future to check the relevancy of these findings in human. The testing of rhamnetin with other natural flavonoids or compounds is also recommended in future to check its efficacy. The histopathological examination was not performed in this study.

CRediT authorship contribution statement

Saba Yaqoob: Conceptualization, Investigation, Methodology, Writing – original draft. **Ali Hamza:** Conceptualization, Investigation, Methodology, Writing – original draft. **Moazama Batool:** Data curation, Formal analysis, Software, Validation. **Aisha Khatoon:** Validation, Visualization, Writing – review & editing. **Shaik Althaf Hussain:** Funding acquisition, Resources, Validation, Writing – review & editing. **Mian Nadeem Riaz:** Software, Validation, Visualization, Writing – review & editing.

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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References

Abbasi, S., Turner, A., 2021. Human exposure to microplastics: A study in Iran. J. Hazard. Mater. 403, 123799.
Aebi, H., 1984. Catalase in vitro. In Methods Enzymol. Academic Press. 105, 121–126.
Ahmad, M., Ehsan, N., Khan, H.A., Hamza, A., Azmat, R., Ijaz, M.U., 2023. Ameliorative effects of rhamnetin against polystyrene microplastics-induced nephrotoxicity in rats. Pak Vet J 43 (3), 623–627. https://doi.org/10.29261/pakvetj/2023.058.
Al Mamun, A., Prasetya, T.A.E., Dewi, I.R., Ahmad, M., 2023. Microplastics in human food chains: Food becoming a threat to health safety. Sci. Total Environ. 858, 159834.
Almadhi, A., Abdelhadi, A., Alyamani, R., 2023. Moving from linear to circular economy in saudi arabia: life-cycle assessment on plastic waste management. Sustainability 15, 10450.
Alvi, K., Ijaz, M.U., Hamza, A., Ashraf, A., Arooj, J., Ehsan, N., 2022. Nephroprotective Effects of Delphinidin against Bisphenol A Induced Kidney Damage in Rats. Pak. Vet. J. 43 (1), 189–193. https://doi.org/10.29261/pakvetj/2022.085.
Campbell, S.H., Williamson, P.R., Hall, B.D., 2017. Microplastics in the gastrointestinal tracts of fish and the water from an urban prairie creek. Facets 2, 395–409.
Carlberg, I., Mannervik, B., 1985. Glutathione reductase. In Methods Enzymol. Academic Press 113, 484–490.
Cheng, W., Li, X., Zhou, Y., Yu, H., Xie, Y., Guo, H., Wang, Y., 2022. Polystyrene microplastics induce hepatotoxicity and disrupt lipid metabolism in the liver organoids. Sci. Total Environ. 806, 150328.
Cincinelli, A., Scopetani, C., Chelazzi, D., Lombardini, E., Martellini, T., Katsoyiannis, A., Corsolini, S., 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): occurrence, distribution and characterization by FTIR. Chemosphere 175, 391–400.
Ehsan, N., Ali, S., Hamza, A., Batool, M., Al-Ghanim, K.A., Atique, U., Ijaz, M.U., 2023. Attenuative effects of ginkgetin against polystyrene microplastics-induced renal toxicity in rats. Pak Vet J 43 (4), 819–823. https://doi.org/10.29261/pakvetj/2023.104.
Ge, Y., Yang, S., Zhang, T., Wan, X., Zhu, Y., Yang, F., Liang, G., 2023. The hepatotoxicity assessment of micro/nanoplastics: A preliminary study to apply the adverse outcome pathways. Sci. Total Environ. 902, 165659.
Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, 1700782. https://doi.org/10.1126/sciadv.1700782.
Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.
Hamza, A., Ijaz, M.U., Ehsan, N., Khan, H.A., lkahtani, S., Atique, U., 2023a. Hepatoprotective effects of astragalin against polystyrene microplastics induced hepatic damage in male albino rats by modulating Nrf-2/ Keap-1 pathway. J. Funct. Foods 108, 105771. https://doi.org/10.1016/j.jff.2023.105771.
Hamza, A., Ijaz, M.U., Anwar, H., 2023b. Rhamnetin alleviates polystyrene microplastics-induced testicular damage by restoring biochemical, steroidogenic, hormonal, apoptotic, inflammatory, spermatogenic and histological profile in male albino rats. Hum. Exp. Toxicol. 42, 0960327123117378.
Hawkes, H.J.K., Karlenius, T.C., Tonissen, K.F., 2014. Regulation of the human thioredoxin gene promoter and its key substrates: a study of functional and putative regulatory elements. Biophys. Acta Gen. Subj. 1840, 303–314.
Hayashi, I., Morishita, Y., Imai, K., Nakamura, M., Nakachi, K., Hayashi, T., 2007. High-throughput spectrophotometric assay of reactive oxygen species in serum. Mutat. Res. Genet. 631, 55–61.
Hou, B., Wang, F., Liu, T., Wang, Z., 2021. Reproductive toxicity of polystyrene microplastics: In vivo experimental study on testicular toxicity in mice. J. Hazard. Mater. 405, 124028.
Hwang, J., Choi, D., Han, S., Choi, J., Hong, J., 2019. An assessment of the toxicity of polypropylene microplastics in human derived cells. Sci. Total Environ. 684, 657–669.
Hwang, J., Choi, D., Han, S., Jung, S.Y., Choi, J., Hong, J., 2020. Potential toxicity of polystyrene microplastic particles. Sci. Rep. 10, 7391.
Ighodaro, O.M., Akinloye, O.A., 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J. Med. 54, 287–293.
Ijaz, M.U., Tahir, A., Ahmed, H., Ashraf, A., Ahmedah, H.T., Muntean, L., Moga, M., Irimie, M., 2022. Chemoprotective effect of vitexin against cisplatin-induced biochemical, spermatological, steroidogenic, hormonal, apoptotic and histopathological damages in the testes of Sprague-Dawley rats. Saudi Pharm. J. 30 (5), 519–526.
Kakkar, P., Das, B., Viswanathan, P.N., 1984. A modified spectrophotometric assay of superoxide dismutase. 21, 130–132.
Kim, T.H., Kang, M.S., Mandakhbayar, N., El-Fiqi, A., Kim, H.W., 2019. Anti-inflammatory actions of folate-functionalized bioactive ion-releasing nanoparticles imply drug-free nanotherapy of inflamed tissues. Biomaterials 207, 23–38.
Kim, J.H., Yu, Y.B., Choi, J.H., 2021. Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and neurotoxicity in fish exposed to microplastics: A review. J. Hazard. Mater. 413, 125423.
Magee, C.C., Azuma, H., Knoflach, A., Denton, M.D., Chandraker, A., Iyer, S., Sayegh, M., 1999. In Vitroandin VivoImmunomodulatory Effects of RDP1258, a Novel Synthetic Peptide. J. Am. Soc. Nephrol. 10, 1997–2005.
Mondal, A., Rajalingam, D., Maity, T.K., 2013. Anti-inflammatory effect of O-methylated flavonol 2-(3,4-dihydroxy-phenyl)-3,5-dihydroxy-7-methoxy-chromen-4-one obtained from Cassia sophera Linn in rats. J. Ethnopharmacol. 147, 525–529.
Nahid, A., Neelabh, C., Navneet, K., 2017. Antioxidant and antimicrobial potentials of Artemisia Indica collected from the Nepal region. J. Pharm. Sci. Res. 9, 1822–1826.
Pintard, L., Willems, A., Peter, M., 2004. Cullin-based ubiquitin ligases: Cul3-BTB complexes join the family. EMBO J. 23, 1681–1687.
Placer, Z.A., Cushman, L.L., Johnson, B.C., 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal. Biochem. 16, 359–364.
Rodríguez De Luna, S.L., Ramírez-Garza, R.E., Serna Saldívar, S.O., 2020. Environmentally friendly methods for flavonoid extraction from plant material: Impact of their operating conditions on yield and antioxidant properties. Sci. World J. 679–2069 https://doi.org/10.1155/2020/6792069.
Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., Hoekstra, W.G., 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science 179, 588–590.
Samad, N., Jabeen, S., Imran, I., Zulfiqar, I., Bilal, K., 2019. Protective effect of gallic acid against arsenic-induced anxiety-/depression- like behaviors and memory impairment in male rats. Metab. Brain Dis. 34, 1091–1102.
Sedlak, J., Lindsay, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25, 192–205.

Selamoglu Talas, Z., Ozdemir, I., Yilmaz, I., Gok, Y., Orun, I., 2008. The investigation of the antioxidative properties of the novel synthetic organoselenium compounds in some rat tissues. Exp. Biol. Med. 233, 575–579.
Surien, O., Masre, S.F., Basri, D.F., Ghazali, A.R., 2023. Potential chemopreventive role of pterostilbene in its modulation of the apoptosis pathway. Int. J. Mol. Sci. 24, 9707.
Ulhaq, Z.S., Tse, W.K.F., 2023. Perfluorohexanesulfonic acid (PFHxS) induces oxidative stress and causes developmental toxicities in zebrafish embryos. J. Hazard. Mater. 457, 131722.
Vomund, S., Schäfer, A., Parnham, M.J., Brüne, B., von Knethen, A., 2017. Nrf2, the master regulator of anti-oxidative responses. Int. J. Mol. Sci. 18, 2772.
Wang, S., Han, Q., Wei, Z., Wang, Y., Xie, J., Chen, M., 2022. Polystyrene microplastics affect learning and memory in mice by inducing oxidative stress and decreasing the level of acetylcholine. Food Chem. Toxicol. 162, 112904.
Yang, S., Li, F., Lu, S., Ren, L., Bian, S., Liu, M., Zhao, D., Wang, S., Wang, J., 2022. Ginseng root extract attenuates inflammation by inhibiting the MAPK/NF-κB signaling pathway and activating autophagy and p62-Nrf2-Keap1 signaling in vitro and in vivo. J. Ethnopharmacol. 283, 114739.
Yen, Y.P., Tsai, K.S., Chen, Y.W., Huang, C.F., Yang, R.S., Liu, S.H., 2012. Arsenic induces apoptosis in myoblasts through a reactive oxygen species-induced endoplasmic reticulum stress and mitochondrial dysfunction pathway. Arch. Toxicol. 86, 923–933.
Yu, P., Liu, Z., Wu, D., Chen, M., Lv, W., Zhao, Y., 2018. Accumulation of polystyrene microplastics in juvenile Eriocheir sinensis and oxidative stress effects in the liver. Aquat. Toxicol. 200, 28–36.
Zhang, D., Liu, X., Huang, W., Li, J., Wang, C., Zhang, D., Zhang, C., 2020. Microplastic pollution in deep-sea sediments and organisms of the Western Pacific Ocean. Environ. Pollut. 259, 113948.
Zolotova, N., Kosyreva, A., Dzhalilova, D., Fokichev, N., Makarova, O., 2022. Harmful effects of the microplastic pollution on animal health: a literature review. PeerJ. 10, 13503.