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Sakuranetin counteracts polyethylene microplastics induced nephrotoxic effects via modulation of Nrf2/Keap1 pathway

Ali Akbar [a,*], Fatima Amin [a], Moazama Batool [b], Aisha Khatoon [c], Zubair Ahmad [d], Usman Atique [e]

[a] Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan
[b] Department of Zoology, Govt. College Women University, Sialkot, Pakistan
[c] Department of Pathology, University of Agriculture, Faisalabad, Pakistan
[d] Department of Zoology, College of Science, King Saud University, P.O. Box: 2455, 11451 Riyadh, Saudi Arabia
[e] Department of Bioscience and Biotechnology, Chungnam National University, South Korea

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ABSTRACT

Polyethylene microplastics (PEMPs) are widely distributed in environment and exerts deleterious effects on animal as well as human health. Sakuranetin (SKN) is a natural flavonoid that manifests profound therapeutic potential. Albino rats (n = 24) were partitioned into 4 groups i.e., Control, PEMPs 1.5 mg/kg, PEMPs 1.5 mg/kg + SKN 10 mg/kg and SKN 10 mg/kg administered group. After 30 days of treatment, our results revealed that PEMPs exposure reduced nuclear factor erythroid 2–related factor 2 (Nrf-2) and antioxidant genes while enhancing the expression of kelch-like ECH-associated protein 1(Keap-1). Besides, PEMPs intoxication reduced the level of renal biomarkers i.e., creatinine clearance and increased the level of creatinine, urea, neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1). Additionally, it lessened the activities of glutathione S-transferase (GST), superoxide dismutase (SOD), glutathione reductase (GSR), catalase (CAT), glutathione peroxidase (GPx) and heme oxygenase-1 (HO-1) whereas the levels of malondialdehyde (MDA) and reactive oxygen species (ROS) were increased. Conversely, it increased the levels of nuclear factor-kappa B (NF-kB), tumor necrosis factor-alpha (TNF-a), interleukin-1beta (IL-1b) and IL-6 as well as escalated the activity of cyclooxygenase-2 (COX-2). Furthermore, the expression of bcl-2-associated X protein (Bax) and caspase-3 were elevated, while the expression of B-cell lymphoma 2 (Bcl-2) was lowered. However, SKN treatment significantly ($P < 0.05$) restored aforementioned renal impairments. Therefore, it is proposed that SKN may be applied as a nephroprotective agent against the PEMPs-prompted renal toxicity.

1. Introduction

The increasing environmental pollution caused by plastics has garnered substantial global attention (Hwang et al., 2020). Recent reports have revealed that plastic pollution has become ubiquitous in terrestrial as well as marine ecosystems (Borrelle et al., 2020). Microplastics (MPs) originate from the deterioration of plastic materials due to biological factors as well as the incineration of plastic materials (Wright and Kelly, 2017; Andrady, 2011). Humans are exposed to MPs through several ways, such as inhalation and oral administration, owing to their occurrence in air, water and food (Zhang et al. 2020). Once MPs invade the human body, they travel via the circulatory network to various body parts, affecting normal physiological functions by provoking cellular stress (Vethaak and Legler, 2021).

The most commonly recognized microplastics in the terrestrial and aquatic environments are polyethylene microplastics (PEMPs) (de Souza Machado et al., 2018). Recent studies have reported that PEMPs can induce various adverse effects including cytotoxicity, developmental toxicity and hematological disturbances in the body (Ge et al., 2021). It is evident that, PEMPs treatment leads to neurotoxicity and disrupts the normal physiological mechanism of germ cell differentiation (Mak et al. 2019). In addition to this, PEMPs treatment has been reported to elicit oxidative stress (OS), which can have potential detrimental influence on intracellular metabolism and disrupt the redox equilibrium (Silva et al., 2021). Recent investigations have reported that PEMPs may increases OS and lipid peroxidation (Ijaz et al., 2024).

* Corresponding author.
E-mail addresses: ali6265207@gmail.com, ali0703593@gmail.com (A. Akbar).

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Plants are recognized as extensive reservoirs of therapeutic compounds. Flavonoids are phenolic compounds that are abundantly found in grains, flowers, vegetables and fruits. Elsayed et al. (2022) reported that flavonoids can reduce the levels of OS via eliciting the activity of antioxidant enzymes. Sakuranetin (SKN) is a derivative of naringenin that is isolated from multiple plants as well as honey derived from. SKN has been extensively documented for its numerous pharmacotherapeutic characteristics i.e., anti-inflammatory, antitumor, antioxidant as well as neuroprotective (Stompor, 2020). Therefore, ongoing research was aimed to estimate the alleviative action of SKN to counteract PEMPs prompted renal impairments.

2. Materials and methods

2.1. Chemicals

PEMPs (CAS No:9002–88-4) and SKN (CAS No:520–29-6) were obtained from Sigma-Aldrich, Germany.

2.2. Animals

Trial was conducted on albino rats (n = 24) in the institutional research center of the University of Agriculture, Faisalabad. Rats were kept in enclosures and maintained under uniform conditions (temperature, 24 ± 1 °C) and treated with standard diet and water. Rats were acclimated for 1 week. Rats were treated in accordance with the approved protocol of the European Union for Animal Care and Experimentation (CEE Council 86/609).

2.3. Layout of experiment

24 albino rats were apportioned among 4 different groups (n = 6) i.e., Control, PEMPs 1.5 mg/kg, PEMPs 1.5 mg/kg + SKN 10 mg/kg and SKN 10 mg/kg administered group. After 30 days of administration. Animals were anesthetized and decapitated. Kidneys were dissected, and washed employing normal saline. One kidney was preserved in 10 % solution of formaldehyde for histological assessment while, second kidney was utilized for the biochemical assessment

2.4. Assessment of renal biomarkers

The estimation of renal biomarkers was carried out with the help of ELISA kits. The analysis was accomplished following the recommended protocol of the manufacturer.

2.5. Biochemical analysis

The CAT activity was measured through the Aebi (1984) technique. The SOD activity was calculated by following the technique documented by Kakkar et al. (1984) approach. For the quantification of GPx Rotruck et al. (1973) technique was employed. Carlberg and Mannervik (1975) along with Jollow et al. (1974) protocol was followed for the measurement of GSR along with GSH. The Younis et al. (2018) protocol was followed for the measurement of GST. The ROS and MDA level was ascertained using Hayashi et al. (2007) and Ohkawa et al. (1979) approaches.

2.6. Assessment of Nrf2/Keap1, Bcl-2, caspase-3, Bax and antioxidative genes

qRT-PCR was employed to estimate the expressions of Nrf2/Keap1, Bcl-2, caspase-3, Bax and antioxidative genes. Total RNA isolation was accomplished using the TRIzol reagent, followed by reverse transcription to produce cDNA. The evaluation of changes in the gene expression was carried out following $2^{-\Delta\Delta CT}$ method and β-actin served as an internal control, as outlined by Livak and Schmittgen (2001). Table 1 demonstrates the primer sequence of β-actin as well as target genes as previously stated by Ijaz et al. (2022) and Hamza et al. (2023).

Table 1
Primers sequences for the real-time quantitative reverse transcription polymerase (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: GGC CTT TTT GCT ACA GGG TT R: AGC TCC ATG TTG TTG TCC AG	NM_017059.2
Bcl-2	F: ACA ACA TCG CTC TGT GGA T R: TCA GAG ACA GCC AGG AGA A	NM_016993.1
Caspase-3	F: ATC CAT GGA AGC AAG TCG AT R: CCT TTT GCT GTG ATC TTC CT	NM_012922.2
β-actin	F: TACAGCTTCACCACCACAGC R: GGAACCGCTCATTGCCGATA	NM_031144

2.7. Inflammatory biomarkers

The analysis of inflammatory biomarkers was executed using ELISA kits. The analysis was accomplished following the recommended protocol of the manufacturer.

2.8. Statistical analysis

Data were shown as Mean ± SE. The normal distribution of the data was checked by Shapiro–Wilk test, whereas the homogeneity of variances was checked and confirmed by using Levene test. Using one-way ANOVA and Tukey's test, data were statistically examined through Minitab software. Significance level was set at $P < 0.05$.

3. Results

3.1. Impact of PEMPs and SKN on Nrf2/keap1 pathway

PEMPs exposure substantially ($P < 0.05$) decreased Nrf2 and antioxidative genes while escalating the Keap1 expression contrary to control. Co-treatment of SKN + PEMPs substantially increased Nrf2 and antioxidant gene's expression while decreasing the expression of keap1 in comparison to PEMPs-group. No substantial alterations were detected in Nrf2/keap1 expression among the SKN-supplemented rats and the control (Fig. 1).

3.2. PEMPs and SKN impact on biochemical indices

PEMPs treatment remarkably lowered GSH, GST, GSR, SOD, HO-1, CAT, GPx and escalated the ROS and MDA level contrary to the control rats. Conversely, the concurrent treatment of PEMPs + SKN recovered the aforesaid dysregulations in contrast to the PEMPs-group. However, only SKN administration showed insignificant differences contrary to the animal in the control as displayed in Table 2.

3.3. PEMPs and SKN impact on renal parameters

Analysis of the renal function biomarkers showed that PEMPs

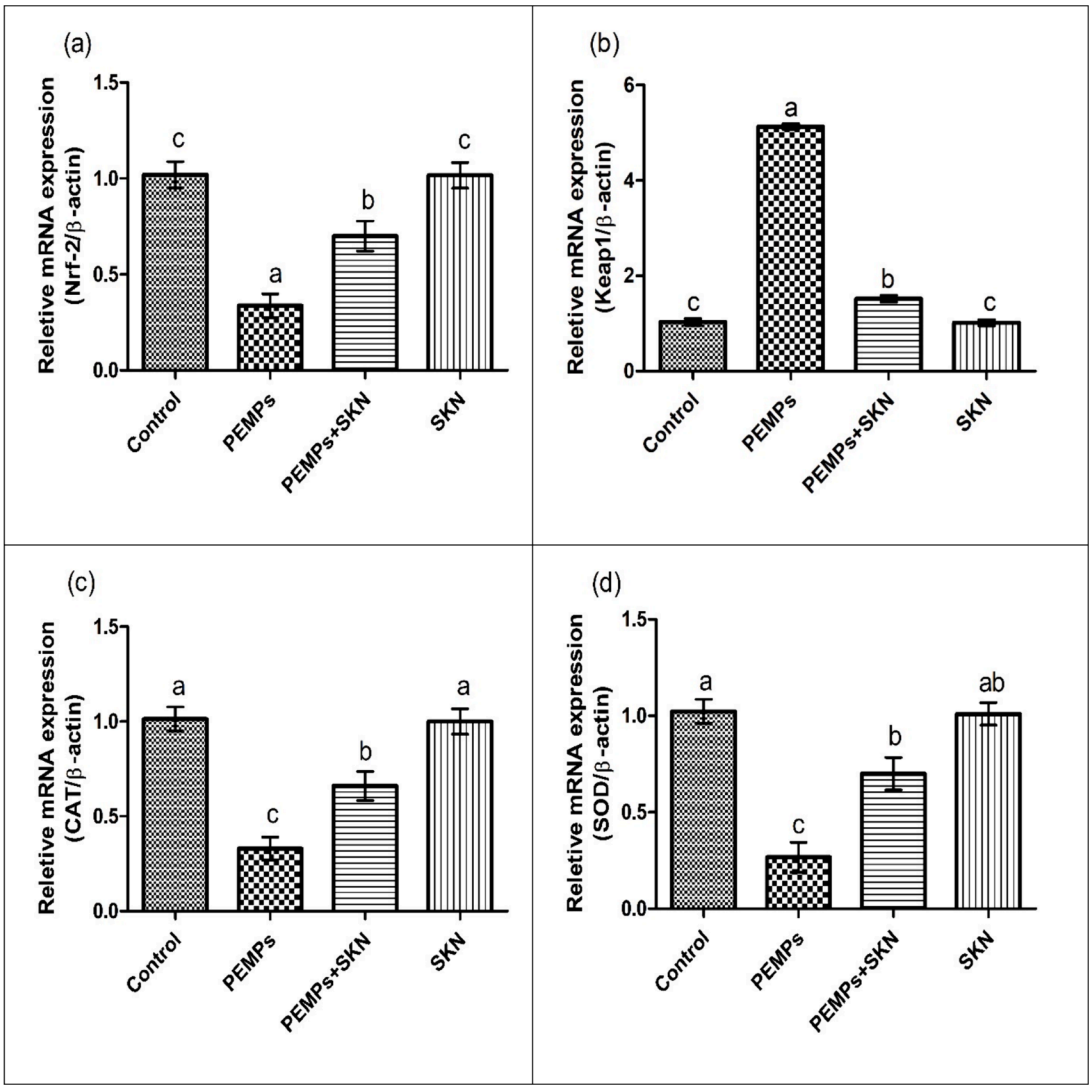

Fig. 1. The PEMPs and SKN effect on the expression of (a) Nrf2, (b) Keap1, (c) CAT, (d) SOD, (e) GPx, (f) GSR and (g) HO-1. Data were shown as Mean ± SEM. Dissimilar letters on graph bars denoting substantial distinctions at $P < 0.05$.

treatment instigated renal disturbances as confirmed by a notable amplification in the creatinine, urea, NGAL, KIM-1 and reduction in the creatinine clearance contrary to control. However, combined treatment of SKN and PEMPs restored aforementioned disturbances in comparison to the PEMPs-exposed animals. SKN (only) treatment group displayed mean values of aforesaid markers approximately similar to the animals in the control as displayed in Table 3.

3.4. Pemps and SKN impact on inflammatory indices

PEMPs treatment considerably upregulated inflammatory biomarkers level contrary to the control. The co-administration of SKN and PEMPs decreased the levels aforesaid biomarkers in contrast to the PEMPs administrated rats. However, only SKN supplementation displayed normal level of these biomarkers almost equal to the animals in the control as displayed in Table 4.

3.5. Pemps and SKN impact on apoptotic biomarkers

PEMPs treatment notably amplified the caspase-3 and Bax while lowering the expression of Bcl-2 in comparison to control. SKN supplementation along with PEMPs restored the expression of these biomarkers as compared to PEMPs group. However, non-significant alterations were examined among SKN and control groups (Fig. 2).

4. Discussion

In our investigation, PEMPs exposure resulted in a downregulation of Nrf2 and an upregulation of Keap1 expression which decreased the expression of antioxidative genes including GSR, SOD, GPx, CAT and HO-1. It has been documented that regulation of antioxidative gene through the Keap1-Nrf2 pathway serves as an inducible defense mechanism to mitigate OS (Yamamoto et al., 2018). Nrf2 coordinates the cellular antioxidative response that can effectively neutralize ROS. Conversely, Keap1 acts as an inhibitor of Nrf2 that facilitates Nrf2 degradation (Bellezza et al., 2018). However, supplementation of SKN substantially recovered the expression of the aforementioned cytoprotectant genes by modulating the Nrf2/Keap1 pathway. Our outcomes are corroborated by the observation of Hamza et al. (2023) who revealed that flavonoids exhibit the potential to regulate Nrf2/Keap1 pathway.

PEMPs intoxication prompted a substantial upsurge in the concentration of creatinine, urea, NGAL, KIM-1 and decreased creatinine clearance. OS is recognized as the major culprit underlying disturbed level of these biomarkers. Changes in the structure of the glomerulus,

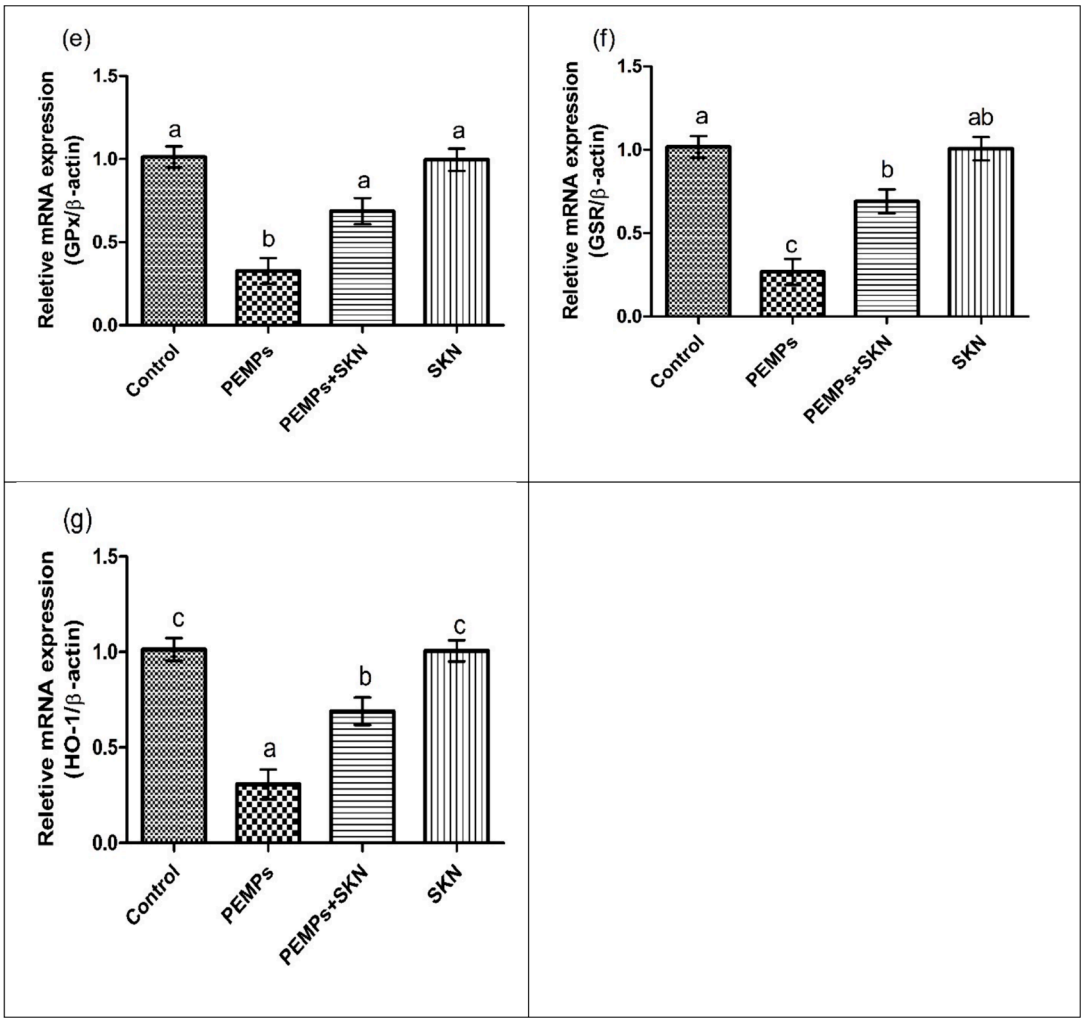

Fig. 1. (*continued*).

Table 2
PEMPs and SKN impact on biochemical markers.

Parameters	Groups			
	Control	PEMPs	PEMPs + SKN	SKN
CAT (U/mg protein)	14.21 ± 1.59[a]	5.99 ± 0.96[b]	11.28 ± 0.74[a]	14.75 ± 1.90[a]
SOD (U/mg protein)	11.48 ± 1.27[a]	4.56 ± 1.18[c]	8.18 ± 0.75[b]	11.77 ± 1.43[a]
GSR (nM NADPH oxidized/min/mg tissue	8.59 ± 0.88[a]	3.68 ± 0.47[c]	6.21 ± 0.62[b]	8.86 ± 0.96[a]
GPx (U/mg protein)	25.11 ± 1.43[a]	12.15 ± 1.84[b]	21.07 ± 1.52[a]	25.37 ± 1.92[a]
GSH (U/mg protein)	17.82 ± 1.28[a]	8.05 ± 0.44[c]	14.72 ± 1.17[b]	18.15 ± 1.50[a]
GST (U/mg protein)	37.88 ± 2.18[a]	16.390 ± 0.796[c]	31.080 ± 1.336[b]	38.52 ± 2.82[a]
HO-1 (pmoles bilirubin/ mg protein/h)	287.06 ± 8.35[a]	64.99 ± 9.48[c]	20.93 ± 11.38[b]	299.42 ± 14.54[a]
MDA (nmol/g)	0.27 ± 0.14[c]	2.48 ± 0.34[a]	1.58 ± 0.13[b]	0.25 ± 0.14[c]
ROS (nmol/g)	1.28 ± 0.22[b]	6.86 ± 0.81[a]	2.05 ± 0.14[b]	1.25 ± 0.22[b]

Dissimilar letters accompanying certain values serve to highlight disparity among dissimilar groups.

Table 3
Impact of PEMPs and SKN on serum biomarkers.

Parameters	Groups			
	Control	PEMPs	PEMPs + SKN	SKN
Urea (mg/dl)	15.96 ± 2.15[a]	29.7 ± 22.2[a]	21.66 ± 2.28[a]	15.88 ± 2.16[a]
Creatinine (mg/dl)	1.47 ± 0.20[bc]	5.40 ± 0.39[a]	2.19 ± 0.29[b]	1.39 ± 0.21[c]
Creatinine Clearance (ml/min)	2.15 ± 0.16[a]	0.77 ± 0.22[c]	1.61 ± 0.135[b]	2.21 ± 0.14[a]
KIM-1 (mg/dl)	0.48 ± 0.12[c]	4.48 ± 0.26[a]	1.45 ± 0.21[b]	0.43 ± 0.15[c]
NGAL (ng/day)	0.83 ± 0.15[c]	6.30 ± 0.25[a]	1.56 ± 0.20[b]	0.79 ± 0.17[c]

Dissimilar letters accompanying certain values serve to highlight disparity among dissimilar groups.

impaired filtration rate and subsequent dysfunction of nephron are all linked to an increase in the concentration of nitrogenous end product i. e., creatinine and urea (Ijaz et al., 2023). Moreover, increased levels of KIM-1 and NGAL are positively correlated with the vascular damage and dysfunctions of the proximal tubules (Dobrek et al., 2017). However, SKN supplementation significantly restored the disrupted levels of the aforementioned parameters, primarily through its antioxidative and nephroprotective properties.

Table 4
PEMPs and SKN impact on renal inflammatory indices.

Parameters	Groups			
	Control	PEMPs	PEMPs + SKN	SKN
NF-kB (ng/g tissue)	21.47 ± 0.85^{c}	84.40 ± 2.37^{a}	26.26 ± 1.27^{b}	21.39 ± 0.89^{c}
TNFα (ng/g tissue)	9.63 ± 0.54^{c}	57.72 ± 1.54^{a}	17.20 ± 1.63^{b}	9.53 ± 0.61^{c}
IL-1ß (ng/g tissue)	15.10 ± 2.06^{c}	72.92 ± 2.16^{a}	23.77 ± 2.60^{b}	14.94 ± 2.01^{c}
IL-6 (ng/g tissue)	10.38 ± 0.99^{c}	52.48 ± 3.49^{a}	19.81 ± 1.78^{b}	10.12 ± 1.11^{c}
COX-2 (ng/g tissue)	11.99 ± 2.22^{c}	63.54 ± 2.11^{a}	19.64 ± 1.581^{b}	11.37 ± 1.94^{c}

Dissimilar letters accompanying certain values serve to highlight disparity among dissimilar groups.

PEMPs administration lowered the activities GSR, SOD, GST, GPx, CAT HO-1 and escalated the ROS and MDA level. Disturbed equilibrium between pro- and anti-oxidant results in formation of OS. The aforementioned endogenous antioxidants serve as a crucial factor in regulating the levels of ROS and OS, ultimately preventing cellular damage in the body (Sinha et al., 2013). It is reported that increased levels of OS disrupt the normal architecture of plasma membrane (Ishtiaq et al., 2022). Furthermore, excessive generation of ROS reduced the activities of antioxidant enzymes thereby impairing cellular defense system (Ahmad et al., 2023). The occurrence of LP and free radicals formation are correlated to each other and can be diagnosed by the level of its end product i.e., level of MDA (Adejuwon et al., 2015). However, SKN supplementation regulated PEMPs induced imbalance in pro-oxidants and antioxidants by reducing OS and elevating the antioxidant enzymes levels in the kidney. The antioxidative nature of flavonoids is mainly ascribed to their numerous OH groups, which facilitate their ability to alleviate OS (Teixeira et al. 2005).

PEMPS administration raised the inflammatory indices concentration. NF-κB serves as a major element that activates TNF-α, IL-1β along with IL-6 which induce acute inflammation and ROS-associated damage in the body (Khan et al., 2020). Moreover, COX-2 is a notable inflammatory mediator that plays a key part in inducing renal inflammatory response (Agarwal et al., 2009). However, SKN treatment not only suppressed the activation of NF-κB, a major culprit underlying renal inflammation but also regulated the levels of other inflammatory biomarkers. Outcomes of current investigation are in line with the research of Kim and Kang (2016) who reported that SKN constrains the inflammatory response in the macrophages.

PEMPs treatment enhanced Bax and Caspase-3 while lowering the Bcl-2 expression. Apoptosis occurs due to a disruption in the equilibrium between pro- and anti-apoptotic biomarkers. It has been revealed that impairment in the inner and outer mitochondrial membrane movement occurs as a result of disturbed ratio between Bax and Bcl-2 (Gu et al., 2017). Besides, Bax and Bcl-2 play an indispensable role in amplifying the liberation of cytochrome *c* from mitochondria, thereby triggering the apoptotic response (Caglayan et al., 2019; Kuzu et al., 2019). An upsurge in Bax and a drop in Bcl-2 serves as the mediator for the stimulation of discharge of caspase-3 which ultimately initiates the apoptotic damages (Eldutar et al., 2017). However, SKN treatment restored the expression of these biomarkers due to its anti-apoptotic properties.

5. Conclusion

Present study suggests that SKN possesses the mitigative potential to counteract PEMPs prompted detrimental nephrotoxic effects via

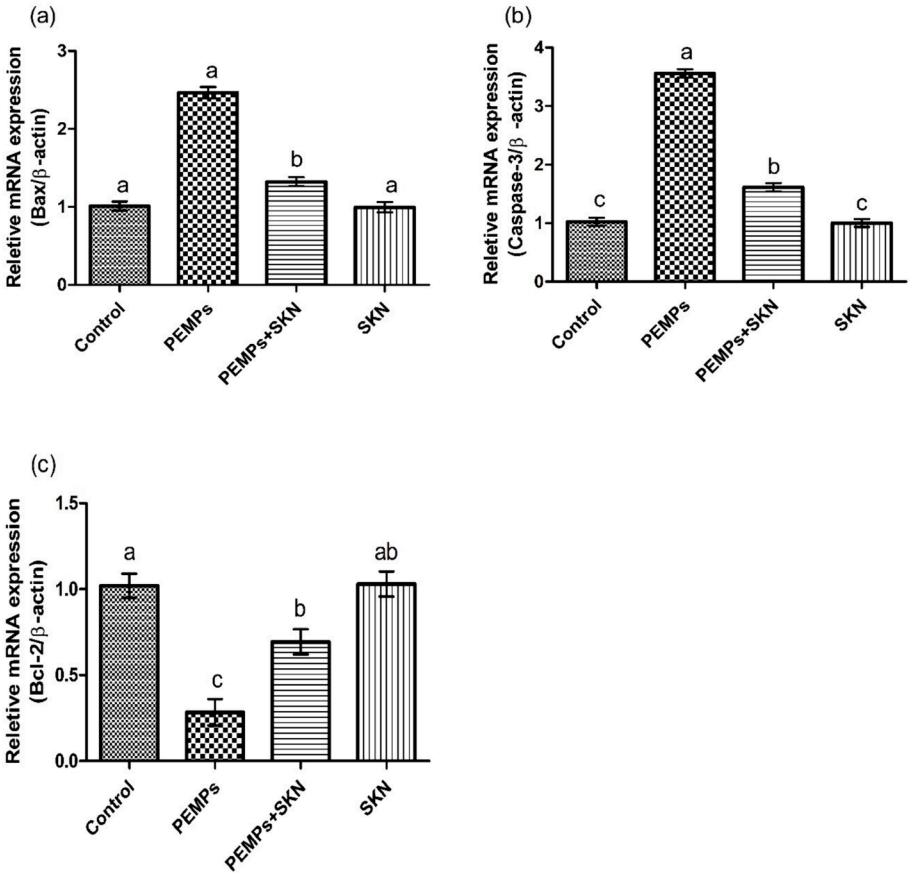

Fig. 2. The PEMPs and SKN effect on (a) Bax, (b) caspase-3 and (c) Bcl-2. Dissimilar letters on graph bars denoting substantial distinctions at $P < 0.05$.

modulating the Nrf2/keap1, antioxidative and apoptotic genes expression. Additionally, SKN supplementation restored the disturbed level of renal OS, LP, inflammatory and renal injury biomarkers. These findings provide evidence that SKN demonstrates defensive properties to counteract PEMPs provoked renal impairments. However, clinical trials are recommended in future to evaluate the efficacy and therapeutic potential of SKN against PEMPs-instigated renal impairments.

CRediT authorship contribution statement

Ali Akbar: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Fatima Amin:** Writing – original draft, Methodology, Investigation, Conceptualization. **Moazama Batool:** Visualization, Validation, Formal analysis, Data curation. **Aisha Khatoon:** Visualization, Software, Investigation. **Zubair Ahmad:** Writing – original draft, Resources, Funding acquisition. **Usman Atique:** Writing – review & editing, Validation, 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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