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Cardioprotective potential of sakuranetin to counteract polyethylene microplastics induced cardiotoxicity

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ABSTRACT

Polyethylene microplastics (PEMPs) are toxic environmental contaminants which can impair multiple organs including heart. Sakuranetin (SKN) is a potential flavonoid with diverse pharmacological benefits. This research was undertaken to analyze the defensive impact of SKN to avert PEMPs-induced cardiotoxicity. 24 male albino rats were randomly allocated into 4 separate groups: control, PEMPs (1.5 mg kg^{-1}), PEMPs + SKN (1.5 mg kg^{-1} + 10 mg kg^{-1}) and only SKN (10 mg kg^{-1}) treated group. After 30 days of treatment, our results revealed that PEMPs exposure reduced the expressions of Nrf2 and antioxidant genes while increasing Keap1 expression. Besides, PEMPs intoxication escalated the levels of cardiac markers (CPK, LDH, Troponin I & CK-MB). Additionally, it lessened the activities of GSH, GST, SOD, HO-1, CAT, GSR, GPx whereas the levels of MDA and ROS were increased. Conversely, the levels of inflammatory markers i.e., COX-2 activity IL-1β, TNF-α, NF-kB & IL-6 were augmented. Moreover, the expressions of apoptotic markers i.e., Bax and caspase-3 were elevated while the Bcl-2 expression was decreased. However, SKN treatment significantly restored the PEMPs-induced biochemical dysregulations. Therefore, SKN could be used as a therapeutic compound to ameliorate PEMPs-induced cardiac impairments in rats, possibly due to its tremendous pharmacotherapeutic potential.

1. Introduction

The growing environmental pollution caused by plastics has attracted a significant global attention (Laskar and Kumar, 2019). Plastic materials are widely adopted as substitutes for various traditional materials, including paper, glass, wood and metals (Raheem, 2013). It is reported that global plastic production has reached up to 0.368 billion MT and is expected to upsurge twofold in upcoming few days (Yao et al., 2022). When plastics enter the environment, they undergo continuous fragmentation into small particles known as microplastics (MPs). Smaller MPs with pointed structure are more susceptible to pass through membrane barriers present in the body of animals (Sharma and Sharma, 2007). MPs can get access to human body via the consumption of various edible products i.e., sugar (7-32particles/kg), honey (40–60 particles/kg), table salt (7–681 particles/kg) & beer (12–109 particles/L) (Bouwmeester et al., 2015).

The most frequently recognized polymer among microplastics in terrestrial environment is polyethylene microplastics (PEMPs) (de Souza Machado et al., 2018). Recent studies have discovered that PEMPs can induce various adverse effects including cytotoxicity, developmental toxicity & hematological disturbances in the body (Ge et al., 2021). Recent evidences have revealed that, PEMPs administration leads to reproductive abnormalities and disrupts the process of gametogenesis (Mak et al. 2019). Beyond that, exposure to PEMPs has been reported to elicit oxidative stress (OS), which can have potential harmful effects on the normal function of the cells and disrupt the redox status (Silva et al., 2021). Moreover, PE-MP exposure may also alter signalling pathways leading to autophagy and apoptosis (Zhao et al., 2020). Furthermore, it has been reported that exposure to polyethylene can reduce the heart rate and instigate pericardial edema in the living organisms (Sun et al., 2021).

Flavonoids are recognized as valuable compounds in the field of traditional medicine owing to their remarkable pharmacological properties (Ullah et al., 2020). Sakuranetin (SKN) is a flavonoid with

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bioactive potential which is sourced from different plants as well as honey derived from several floral sources (Shen et al., 2019). SKN has long been utilized in traditional medicine for their remedial impacts, particularly for the mitigation of diabetes, inflammatory ailments and cancer (Stompor, 2020). However, no research has been performed to assess the cardioprotective effects of SKN. Therefore, this study was commenced to measure the amelioratory role of SKN to cure PEMPs-induced cardiac toxicity.

2. Materials & methods

2.1. Chemicals

PEMPs and SKN were procured from Sigma-Aldrich, Germany.

2.2. Animals

The research trial was conducted on male albino rats (n = 24) in the animal research center of the University of Agriculture, Faisalabad. The rats were kept in cages and maintained under uniform conditions (temperature, 24 ± 1 °C; 12 h day/night cycle) and treated with standard diet and water. Rats were acclimatized for 1 week before the initiation of the trial. The animals were treated and handled according to the guidelines of EU Directive 2010/63/EU for animal experiments.

2.3. Experimental plan

24 rats were randomly distributed in 4 different groups (n = 6) i.e., Control, PEMPs (1.5 $mgkg^{-1}$), PEMPs (1.5 $mgkg^{-1}$ + SKN 10 $mgkg^{-1}$) and only SKN (10$mgkg^{-1}$) administrated group. Following the completion of experiment, the animals were anesthetized, decapitated and heart was excised. The heart was homogenized, centrifuged and the resulting supernatant was kept at −20 °C which was used for further analysis

2.4. Estimation of cardiac markers

The levels of LDH, CPK, CK-MB and Troponin-I were measured by following the protocols of Bais & Philcox (1994), Tietz et al. (1983) and Panteghini et al. (2004), respectively.

2.5. Evaluation of biochemical parameters

The CAT activity was measured through the technique of Aebi (1974). The SOD activity was calculated by following the technique documented by Kakkar et al. (1984). For the quantification of GPx Rotruck et al. (1973) technique was employed. Carlberg and Mannervik (1975) and Jollow et al. (1974) protocol was followed for the estimation of GSR and GSH. The Younis et al. (2018) protocol was followed for the measurement of GST. The levels of ROS and MDA were ascertained by using Hayashi et al. (2007) and Ohkawa et al. (1978) approaches, respectively.

2.6. RNA isolation & qRT-PCR

qRT-PCR was employed to estimate the expressions of Nrf2/Keap1, apoptotic markers (Bcl-2, caspase-3, and 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 & Schmittgen (2001). Table 1 demonstrates the primer sequence of β-actin as well as apoptotic markers and Nrf-2/Keap1 and its target genes, as previously reported by Ijaz 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	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
GST	F: TCGACATGTATGCAGAAGGAGT R: CTAGGTAAACATCAGCCCTGCT	NM_031509.2
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

2.7. Estimation of inflammatory markers

The analysis of inflammatory markers (TNF-α, Nf-kB, IL-6, IL-1β, and Cox-2) 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 ± SEM. One-way analysis of variance (ANOVA) & Tukey's test was carried out to compare different groups using Minitab (V17) Software. $P < 0.05$ was set as level of significance.

3. Results

3.1. Results of PEMPs & SKN on Nrf2/Keap1 pathway

Exposure to PEMPs substantially ($P < 0.05$) decreased the expressions of Nrf2 and antioxidative genes while escalating the Keap1 expression, contrary to the control. Combined treatment with SKN + PEMPssubstantially ($P < 0.05$) increased the expressions of Nrf2 and antioxidant genes while decreasing the expression of keep1 in comparison to PEMPs-group. No substantial alterations were detected in Nrf2/Keap1 expressions among the SKN-supplemented rats and the control (Fig. 1).

3.2. Impact of PEMPs & SKN on biochemical parameters

PEMPs exposure remarkably ($P < 0.05$) lowered the activities of GSH, GST, SOD, HO-1, CAT, GSR, GPx and escalated ROS and MDA levels, contrary to the control rats. Conversely, the concurrent treatment of PEMPs + SKN significantly ($P < 0.05$) recovered the PEMPs-induced dysregulations in contrast to the PEMPs- group. Only SKN administration showed insignificant differences contrary to the control rats as depicted in Table 2.

3.3. Impact of PEMPs & SKN on cardiac function markers

Analysis of cardiac function markers showed that PEMPs treatment instigated cardiac disturbances as confirmed by a notable ($P < 0.05$) elevation in CPK, LDH, CK-MB & troponin I levels, as compared to the

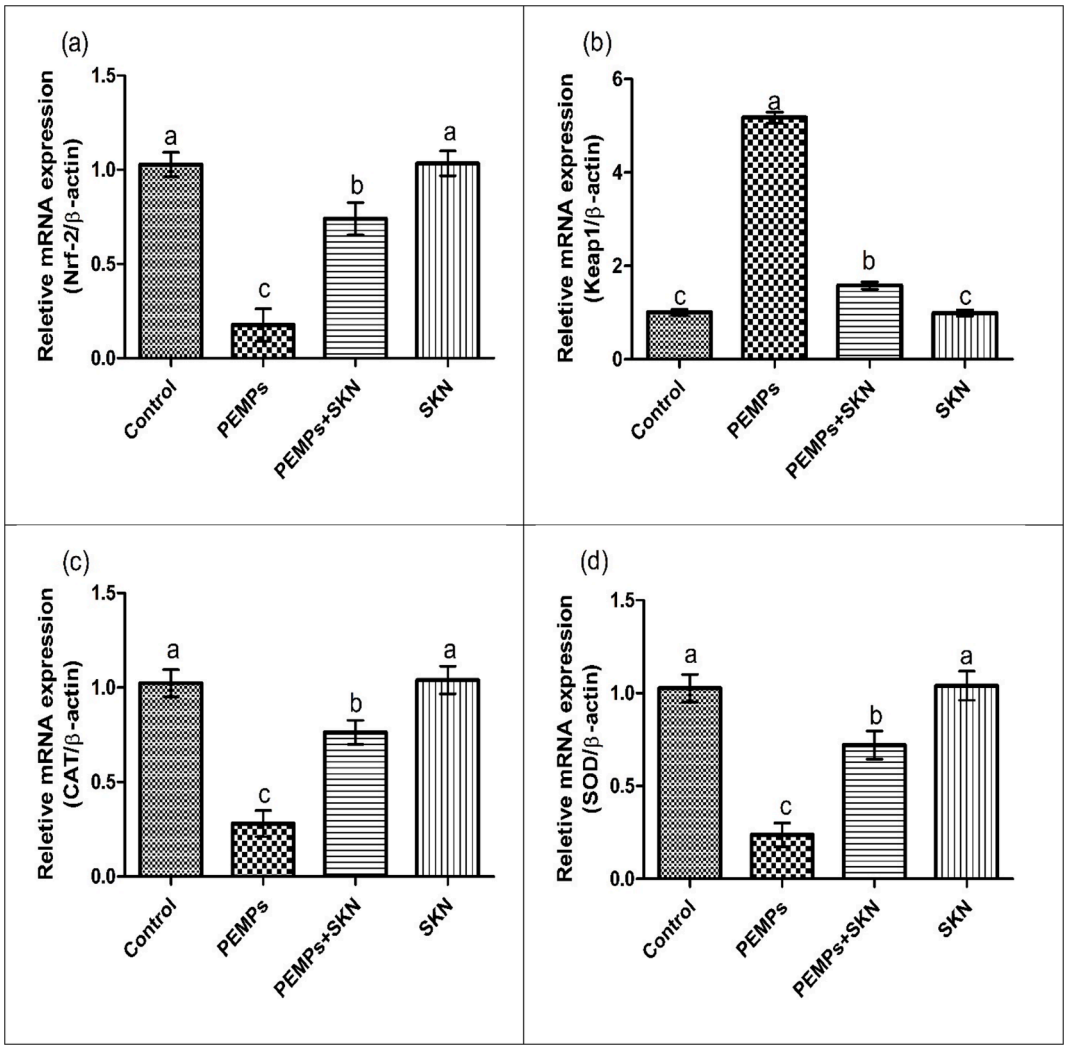

Fig. 1. Effects of PEMPs and SKN on the expression of (a) Nrf2, (b) Keap1, (c) CAT, (d) SOD, (e) GPx, (f) GSR and (g) HO-1, (h) GST. Dissimilar superscripts show significant difference among different groups ($p < 0.05$).

control. However, concurrent treatment of SKN & PEMPs markedly ($P < 0.05$) restored their levels, in comparison to the PEMPs-exposed animals. SKN (only) exposed group displayed mean values of cardiac markers approximately similar to the control as depicted in Table 3.

3.4. Results of PEMPs & SKN on inflammatory indices

PEMPs treatment considerably ($P < 0.05$) upregulated COX-2 activity and IL-1β, TNF-α, NF-kB & IL-6 levels, contrary to the control. The co-administration of SKN and PEMPs decreased the levels of inflammatory markers, in contrast PEMPs administrated rats. Only SKN supplementation displayed normal level of these markers almost near to the control as demonstrated in Table 4.

3.5. Impact of PEMPs & SKN on apoptotic markers

PEMPs treatment notably ($P < 0.05$) elevated the expressions of Bax and caspase-3 while lowering the expression of Bcl-2, in comparison to the control. SKN and PEMPs co-adminisration markedly ($P < 0.05$) restored the expressions of these markers, contrary to PEMPs animals. No considerable alterations were examined in SKN only treated and control group (Fig. 2).

4. Discussion

Over the last few years, MPs have garnered great interest due to their ubiquitous prevalence and harmful effects on ecosystem as well as living organisms (Laskar and Kumar, 2019). Previous investigations have demonstrated that MPs intoxication causes nephrotoxicity (Ahmad et al., 2023; Ehsan et al., 2023), neurotoxicity (Yang et al., 2019), gastrointestinal toxicity (Dong et al., 2020), testicular toxicity (Alvi et al., 2024) and cardiotoxicity (Umamaheswari et al., 2021) in the living system. Moreover, bioactive compounds with effective antioxidative abilities may impede the formation of oxidative radicles (Erdemli et al., 2018). SKN is a natural compound with significant therapeutic properties. However, the existing research was intended to explore the impact of SKN to avert PEMPs prompted cardiac impairments in rats.

In our current investigation, PEMPs exposure resulted in a downregulation in Nrf2 expression and upregulation in Keap1 expression which decreased the expressions of cytoprotective genes including GSR, SOD, GPx, CAT & HO-1. It has been documented that regulation of cytoprotectant gene expression through the Nrf2/Keap1 pathway serves as an inducible defense mechanism to mitigate OS (Yamamoto et al., 2018). Nrf2 functions as the central element to coordinate the cellular antioxidative immune response that can effectively neutralize ROS.

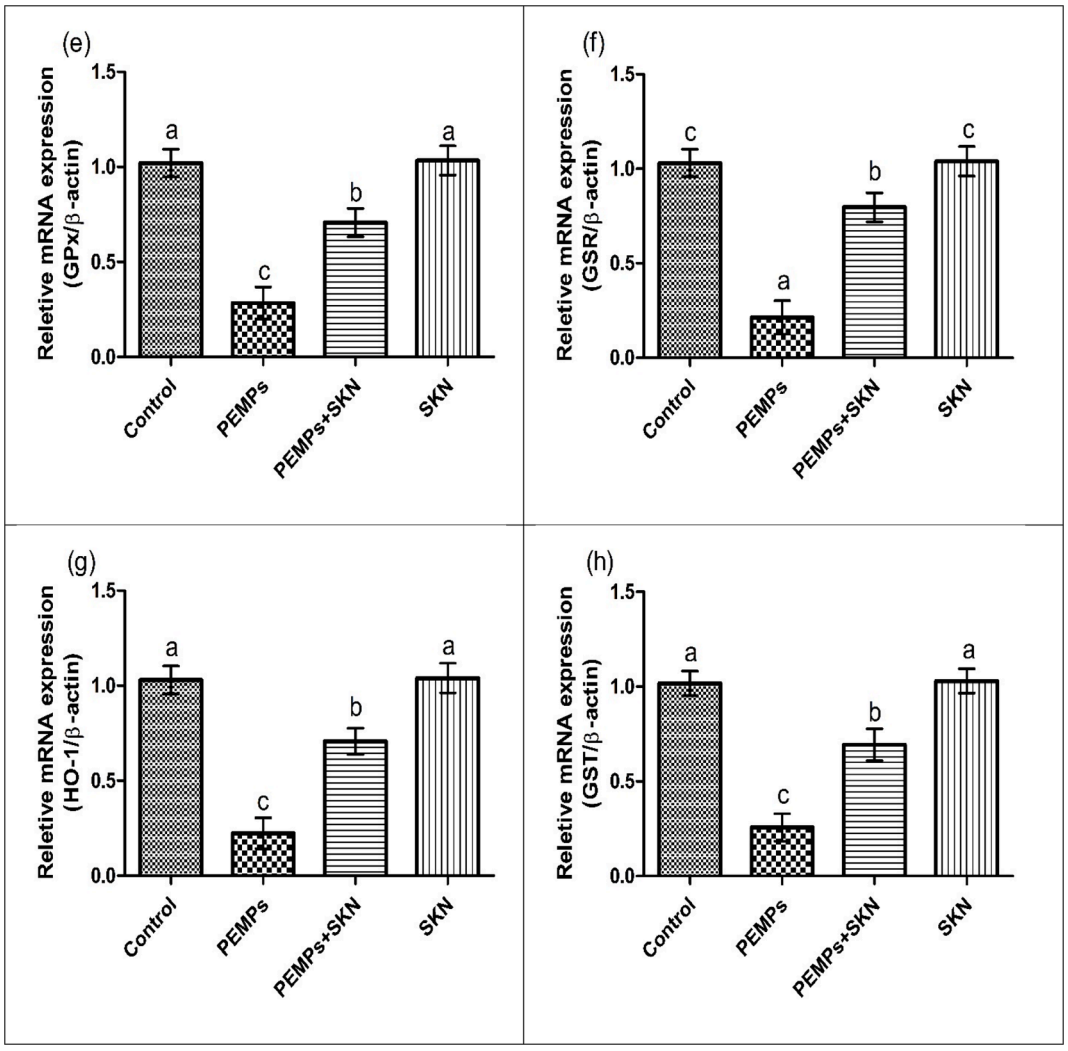

Fig. 1. (*continued*).

Table 2
Impacts of PEMPs & SKN impact on biochemical profile.

Parameters	Groups			
	Control	**PEMPs**	**PEMPs + SKN**	**SKN**
CAT (U/mg protein)	15.153 ± 1.49[a]	5.273 ± 0.23[c]	11.563 ± 1.13[b]	15.290 ± 1.46[a]
SOD (U/mg protein)	10.397 ± 1.01[a]	4.430 ± 0.45[b]	8.463 ± 0.59[a]	10.507 ± 1.13[a]
GSR (nM NADPH oxidized/min/mg tissue	7.590 ± 0.63[a]	3.703 ± 0.22[c]	5.513 ± 0.43[b]	7.543 ± 0.58[a]
GPx (U/mg protein)	19.943 ± 1.52[a]	7.960 ± 0.69[c]	15.607 ± 1.16[b]	20.87 ± 2.19[a]
GSH (U/mg protein)	26.35 ± 2.04[a]	9.810 ± 0.64[c]	21.940 ± 1.23[b]	27.06 ± 2.45[a]
GST (U/mg protein)	35.263 ± 1.73[a]	11.480 ± 0.97[c]	27.820 ± 1.35[b]	36.08 ± 2.21[a]
HO-1 (**pmoles bilirubin/ mg protein/h**)	263.5 ± 21.20[a]	66.03 ± 9.96[c]	188.79 ± 14.13[b]	269.5 ± 25.91[a]
MDA (nmol/g)	0.433 ± 0.21[c]	2.570 ± 0.28[a]	1.070 ± 0.36[b]	0.370 ± 0.25[c]
ROS (nmol/g)	1.270 ± 0.34[c]	6.420 ± 0.41[a]	2.297 ± 0.26[b]	1.217 ± 0.37[c]

Dissimilar superscripts show significant difference among different groups ($p < 0.05$).

Table 3
Impact of PEMPs & SKN on cardiac function markers.

Parameters	Groups			
	Control	**PEMPs**	**PEMPs + SKN**	**SKN**
CK-MB (ng/ mL)	17.60 ± 1.85[c]	84.70 ± 2.57[a]	31.30 ± 2.50[b]	16.79 ± 2.28[c]
CPK (mcg/L)	113.27 ± 11.18[c]	520.46 ± 16.51[a]	199.20 ± 14.73[b]	108.96 ± 10.50[c]
Troponin-I (pg/ ml)	0.540 ± 0.29[b]	12.227 ± 1.68[a]	2.407 ± 0.37[b]	0.483 ± 0.35[b]
LDH (mg/dl)	9.900 ± 1.62[c]	52.67 ± 2.80[a]	19.74 ± 2.52[b]	9.47 ± 1.80[c]

Dissimilar superscripts show significant difference among different groups ($p < 0.05$).

Conversely, Keap1 acts as an inhibitor of Nrf2 that facilitates Nrf2 degradation (Bellezza et al., 2018). Notably, supplementation of SKN significantly recovered the expression of the aforementioned cytoprotective genes by modulating the Nrf2/Keap1 pathway. Our results were in line with the study of Akbar et al. (2024) who reported the Nrf2/ Keap1 modulating property of SKN.

Cardiac muscle injury results in the release of various cardiac damage markers in the blood. CK-MB is particularly recognized as cardiac injury marker which is used to detect certain coronary disorders

Table 4
Impacts of PEMPs & SKN impact on cardiac inflammatory markers.

Parameters	Groups			
	Control	**PEMPs**	**PEMPs + SKN**	**SKN**
NF-kB (ng/g tissue)	27.41 ± 2.22^{c}	83.54 ± 1.98^{a}	38.92 ± 2.13^{b}	27.02 ± 2.38^{c}
TNFα (ng/g tissue)	13.147 ± 1.66^{c}	63.26 ± 1.91^{a}	21.63 ± 2.22^{b}	12.773 ± 1.59^{c}
IL-1ß (ng/g tissue)	28.07 ± 2.20^{c}	83.80 ± 2.32^{a}	37.10 ± 2.70^{b}	26.98 ± 2.26^{c}
IL-6 (ng/g tissue)	5.743 ± 1.58^{b}	37.58 ± 2.64^{a}	9.853 ± 1.48^{b}	5.663 ± 1.64^{b}
COX-2 (ng/g tissue)	11.263 ± 1.16^{c}	78.99 ± 2.05^{a}	19.48 ± 2.11^{b}	10.900 ± 1.14^{c}

Dissimilar superscripts show significant difference among different groups ($p < 0.05$).

including critical myocardial damage (Christenson et al., 1997). As per the investigation of Ibrahim and Abdel-Daim, (2015), the extent of the cardiac injury markers in the blood stream indicates the myocardial injury, suggesting the leakage of these enzymes from damaged heart cells. Our investigation explored that the level cardiac damage markers were augmented owing to PEMPs dosage. Nonetheless, SKN supplementation exhibited tremendous restoration in the levels of these markers due to its cardioprotective abilities.

PEMPs administration reduced the activities SOD, GPx, CAT, HO-1 and escalated the levels of ROS and MDA. The aforementioned endogenous enzymes play a crucial role in regulating the levels of ROS and OS, ultimately preventing cellular damage in the body (Adejuwon et al., 2015). The incidence of lipid peroxidases is parallel to the creation of free radicals and can be diagnosed by the level of its end product i.e., level of MDA (Adejuwon et al., 2015). In addition to the body's endogenous defensive antioxidant system, these antioxidants from various plant sources can be supplemented in order to suppress OS (Nahid et al., 2017). Therefore, SKN dosage regulated PEMPs induced imbalance in pro-oxidants and antioxidants by reducing OS and elevating antioxidants in the cardiac tissues. The antioxidant characteristics of these bioactive compounds mainly exist on account of their multiple OH configuration, that enable them to avert OS (Teixeira et al. 2005).

PEMPS administration elevated the levels of inflammatory markers. NF-κB serves as a major element that activates the levels of pro-inflammatory cytokines which induce acute inflammation and ROS-associated damage in the body (Khan et al., 2020). Moreover, COX-2 is fundamental inflammatory mediator that serves a key element in inducing cardiac inflammation (Agarwal et al., 2009). However, SKN treatment not only suppressed the activation of NF-κB, a major culprit underlying cardiac inflammation but also regulated the other inflammatory markers concentration. These outcomes are in line with the research of Ali et al. (2024) who reported that SKN protects from gastric

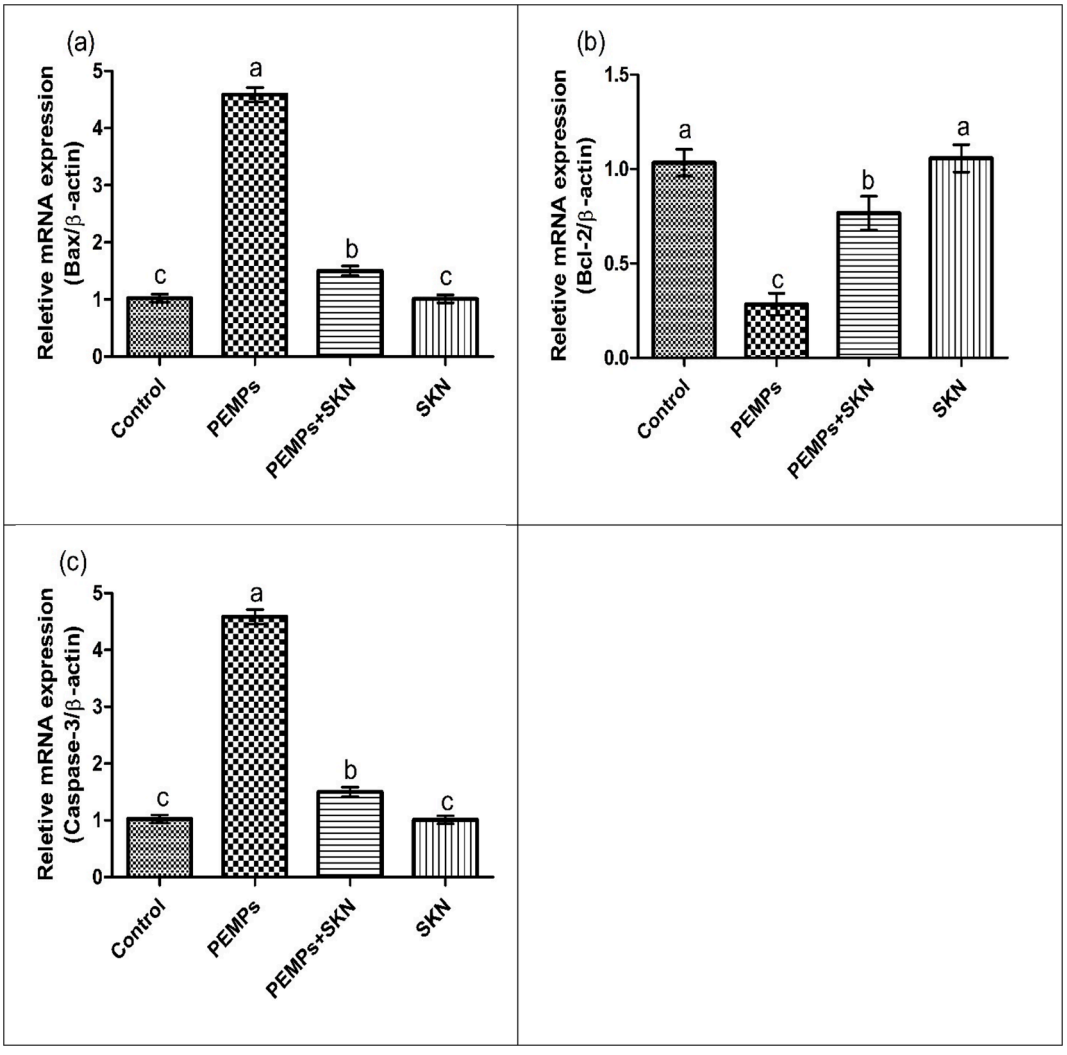

Fig. 2. Effects of PEMPs and SKN on (a) Bax, (b) Bcl-2 and (c) Caspase-3. Dissimilar superscripts show significant difference among different groups ($p < 0.05$).

ulcers due to its anti-inflammatory and antioxidant properties.

PEMPs treatment increased the expressions of Bax and Caspase-3 and lowered the expressions of Bcl-2. Apoptosis occurs on account of an imbalance in pro- and anti-apoptotic proteins. The downregulation of Bcl-2 and upregulation in Bax impairs the translocation between mitochondrial inner and outer layer (Gu et al., 2017). Their imbalance also mediates the discharge of cytochrome-C from mitochondria, initiating the death response in cells (Caglayan et al., 2019). Caspases particularly caspase-3 is reported as a fundamental mediator of apoptotic response as it triggers the death mechanism of cell by accelerating other enzymes (Eldutar et al., 2017). However, SKN treatment restored the expressions of these markers due to its anti-apoptotic properties.

5. Conclusion

In conclusion, PEMPs exposure induced cardiac impairments in rats by increasing the expressions of Keap1 while downregulating Nrf2 and its antioxidant genes. Moreover, PEMPs inebriation also elevated cardiac injury markers, inflammatory and pro-apoptotic mediators as well as OS. Additionally, PEMPs lessened the levels of cardiac anti-apoptotic markers and antioxidative genes Nevertheless, SKN supplementation restored all the impairments that were induced by PEMPs intoxication due to its antioxidant, anti-inflammatory and anti-apoptotic properties. However, the current study was performed on model animals, therefore, we recommend clinical trials of this compound to check its effectiveness on human beings.

CRediT authorship contribution statement

Nazia Ehsan: Writing – original draft, Methodology, Investigation, Conceptualization. **Muhammad Gulfam:** Writing – original draft, Methodology, Investigation, Conceptualization. **Ali Akbar:** Writing – review & editing, Writing – original draft, Methodology. **Moazama Batool:** Visualization, Validation, Software, Data curation. **Mohammad Z. Ahmed:** Writing – review & editing, Resources, Funding acquisition. **Mian Nadeem Riaz:** Writing – review & editing, Visualization, Data curation.

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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