



Full Length Article



Pinostrobin attenuated cadmium instigated cardiotoxicity in albino rats: A biochemical, inflammatory, apoptotic and histopathological examination

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ABSTRACT

Cadmium (Cd) is a noxious and non-biodegradable heavy metal which instigates various organ toxicities such as cardiac injuries. Pinostrobin (PSB) is a potent dietary bioflavonoid, which shows various pharmacological potentials. The current research was designed to evaluate the ameliorative effects of PSB against Cd elicited cardiac dysfunction in rats. Twenty-four albino rats were apportioned into four equal groups viz. control, Cd (5 mg/kg), Cd (5 mg/kg) + PSB (40 mg/kg) and PSB (40 mg/kg) only treated group. It was observed that Cd intoxication reduced catalase (CAT), glutathione reductase (GSR), superoxide dismutase (SOD), glutathione peroxidase (GPx), activities and glutathione S-transferase (GST) contents while escalating the levels of reactive oxygen species (ROS), hydrogen peroxide (H₂O₂) and malondialdehyde (MDA). Furthermore, Cd exposure escalated the levels of cardiac injury markers such as creatine phosphokinase (CPK), creatine kinase-myocardial band (CK-MB), troponin I and lactate dehydrogenase (LDH). Besides, the levels of inflammatory cytokines nuclear factor- κ B (NF- κ B), interleukin 1beta (IL-1 β), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) levels and cyclooxygenase-2 (COX-2) activity were upregulated in Cd intoxicated group. Similarly, Caspase-3, Bax and Caspase-9 levels were augmented, and Bcl-2 levels were reduced after Cd administration. In addition, the histopathological examination revealed a notable cardiac tissue impairment in the Cd exposed group. Nonetheless, PSB treatment significantly ($p < 0.05$) recovered the abovementioned Cd-induced impairments. Therefore, the current study revealed that PSB might be a promising ameliorative agent to ameliorate Cd instigated cardiac damages.

1. Introduction

Cd is considered as one of the most lethal heavy metals and designated a carcinogenic substance that instigates severe health risks to animals and humans (Al Olayan et al., 2020). Cd enters the body predominantly by ingestion, inhalation or dermal contact (Nduka et al., 2019). Moreover, Genchi et al. (2020) elucidated that Cd is extensively used in the manufacturing of various products including batteries, pigments, plastics, cigarette, metal coatings and polyvinyl chloride. People exposed to Cd during various operations such as mining, metal ores, production of nickel-cadmium (Ni-Cd) batteries and electronic devices. Owing to low rate of excretion, Cd accumulates in different organs &

instigates various organ damages such as hepatotoxicity, cardiotoxicity and nephrotoxicity (Pi et al., 2015; Oyinloye et al., 2016; Dong, 2014).

The heart is one of the vital organs of body that performs pivotal role in living organisms (Peate, 2021). Cd disrupts the cardiovascular system (CVS) by disrupting cytochrome P450 as well as antioxidant balance in the body. When Cd reaches the cardiac tissues, it prompts oxidative stress as well as DNA damage which ultimately disregulates the normal gene transcription (Guo et al., 2020). The exposure to Cd disrupts the antioxidant defense system that involves neutralization of free radicals by lowering ROS production and protecting the macromolecules (proteins, DNA & lipids) from damage (Jan et al., 2015). Furthermore, Cd intoxication instigates peripheral arterial disease, increased vascular

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intima media thickness, morphological lesions, disorders of cardiac muscles & myocardial infarction that ultimately leads to congenital heart disease & cardiovascular disease (CVD) (Ray et al., 2023).

Flavonoids are secondary metabolites of plants that are extensively used against various disorders (Juca et al., 2020). Pinostrobin (PSB) is a bioflavonoid which demonstrated various pharmacological abilities including anti-oxidative (Hidajati et al., 2018), antifungal (Kanchana-piboon et al., 2020), anticancer (Sun et al., 2020), antiapoptotic (Jadaun et al., 2019) antiparasitic (Vechi et al., 2021) & anti-inflammatory (Patel et al., 2014). However, the cardioprotective potential of PSB is yet to be reported. Consequently, the current trial was executed to ascertain the ameliorative effect of PSB against Cd-instigated heart damages in rats.

2. Materials & methods

2.1. Chemicals

Cd & PSB were bought from Sigma-Aldrich (Germany).

2.2. Animals

Rats (n = 24) having weight approximately (200–220 g) and age (16–18 weeks) were used as model animals during the current investigation. Steel cages were used to accommodate rats in the animal house of University of Agriculture, Faisalabad. All the experimental animals were acclimatized to the laboratory condition (12 h day/night period, standard temperature 22–25°C) for 7 days before the execution of trial. Rats were provided standard feed & tap water. European Union of Animal Care & Experimentation (CEE Council 86/609) protocols were followed to handle the experimental animals.

2.3. Experimental layout

Albino rats (n = 24) were apportioned into 4 groups (n = 6). The group 1st was considered as control group. The 2nd group was exposed to Cd (5 mg/kg). The 3rd group was exposed to PSB (40 mg/kg) + Cd (5 mg/kg) while the 4th group was exposed to PSB (40 mg/kg) only. After the completion of experiment (4 weeks), the rats were anesthetized by using ketamine (60 mg/kg) and xylazine (5 mg/kg), beheaded and the blood samples were collected in sterile syringes (heparin containing). Serum samples obtained from blood were homogenized and centrifuged (3000 rpm) for 15 min. Heart was removed and sliced into two equally divided parts. One part was stored in zipper bags & retained at –20 °C for further biochemical examination, while the other half was fixed in 10 % formalin for histopathology.

2.4. Biochemical analysis

Chance & Maehly (1995) approach was used to evaluate the CAT activity. The method described by Kakkar et al. (1984) was used to assess SOD levels. The method of Carlberg & Mannervik (1975) was employed to measure GSR activity. GST activity was assessed using the methods developed by Couri & Abdel-Rahman (1979). The technique of Jollow et al., 1974 was employed to evaluate GSH content. MDA level was quantified by using the strategy of ohkawa et al., (1979). The ROS level was assessed by following the method of Hayashi et al. (2007). The technique developed by Pick & Keisari (1981) was used to quantify H₂O₂ levels.

2.5. Evaluation of cardiac injury biomarkers

The cardiac injury markers such as CK-MB (Catalog No. MBS2515061), troponin I (CSB-E08594r), LDH (CSB-E11324r) and CPK (Catalog no. E4608-100) were determined via utilizing ELISA kits in consistent to the instructions of manufacturer.

2.6. Evaluation of inflammatory indices

For the assessment of inflammatory indices IL-1 β (CSB-E08055r), IL-6 (CSB-E04640r), NF- κ B (CSB-E13148r), TNF- α (CSB-E07379r) & COX-2 (CSB-E13399r) levels were carried out by using standardized ELISA kits (TX, Houston, Cusabio Technology Llc, USA).

2.7. Apoptotic markers assessment

For the evaluation of Caspase-9 (CSB-E08863r), Bax (CSB-EL002573RA), Caspase-3 (CSB-E08857r) & Bcl-2 (CSB-E08854r) standard ELISA kits were used according to the guidelines provided by the manufacturers (Cusabio Technology Llc, USA).

2.8. Histological examination

The cardiac samples were kept in formalin (10 %) solution and dehydrated by higher grades of ethanol. Then tissues were carefully kept in paraffin wax. Then thin slices (4–5 μ m) of paraffin coated tissues were sliced with rotary microtome & stained by using stain “Hematoxylin-Eosin”. Slides were carefully observed by using compound microscope (Nikon, Japan) and microphotographs were taken by MoticTM camera (5 megapixels).

2.9. Statistical evaluations

All the data were illustrated as Mean \pm SEM. The Tukey's test & ANOVA (one way interaction) were used to statistically analyze the data by using Graph pad Prism 5. The significance level was kept at p < 0.05.

3. Results

3.1. Effects of PSB on antioxidant enzymes activity

Antioxidant enzymes SOD, GSR, CAT, GSH, GST & GPx activities were markedly (p < 0.05) reduced in Cd intoxicated group in relation to control rats. Nonetheless, PSB supplementation substantially (p < 0.05) escalated antioxidant enzyme activities in Cd + PSB administered animals as compared to Cd intoxicated animals. Furthermore, no substantial variation was observed in these activities among the rats of control & PSB only supplemented group (Table 1).

Table 1
Impacts of PSB & Cd on biochemical profile.

| Parameters | Groups | | | |
|--|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | Control | Cd | Cd + PSB | PSB |
| CAT (U/mg protein) | 12.88 \pm 1.47 ^a | 5.06 \pm 0.29 ^c | 8.06 \pm 0.62 ^b | 12.79 \pm 1.50 ^b |
| SOD (U/mg protein) | 10.39 \pm 0.85 ^a | 4.57 \pm 0.36 ^c | 7.82 \pm 0.36 ^b | 10.32 \pm 0.93 ^a |
| GSR (nM NADPH oxidized/min/mg tissue) | 9.34 \pm 0.22 ^a | 3.26 \pm 0.27 ^c | 6.40 \pm 0.46 ^b | 9.39 \pm 0.25 ^a |
| GPx (U/mg protein) | 31.28 \pm 2.28 ^a | 8.90 \pm 0.83 ^c | 18.72 \pm 1.43 ^b | 31.39 \pm 2.39 ^a |
| GSH (U/mg protein) | 19.59 \pm 1.72 ^a | 6.17 \pm 0.41 ^c | 13.67 \pm 1.00 ^b | 19.64 \pm 2.15 ^a |
| GST (U/mg protein) | 41.91 \pm 1.63 ^a | 14.61 \pm 1.49 ^c | 32.86 \pm 1.78 ^b | 42.03 \pm 2.01 ^a |
| MDA (nmol/g) | 0.74 \pm 0.07 ^c | 5.86 \pm 0.51 ^a | 2.34 \pm 0.20 ^b | 0.72 \pm 0.07 ^c |
| ROS (nmol/g) | 0.44 \pm 0.13 ^c | 7.40 \pm 0.44 ^a | 2.33 \pm 0.21 ^b | 0.41 \pm 0.14 ^c |
| H ₂ O ₂ (μ M/min/ mg protein) | 1.38 \pm 0.19 ^b | 6.54 \pm 0.66 ^a | 2.34 \pm 0.28 ^b | 1.36 \pm 0.19 ^b |

Values having different superscripts are significantly (p < 0.05) different from the other groups.

3.2. Effects of PSB on oxidant profile

The levels of ROS & MDA were markedly ($p < 0.05$) increased in Cd provided rats in compliance to control group. On the other hand, PSB administration remarkably ($p < 0.05$) decreased the ROS & MDA levels in Cd + PSB supplemented animals as compared with Cd exposed animals. Furthermore, ROS and MDA contents in only PSB supplemented rats were close to control group (Table 1).

3.3. Effects of PSB on cardiac function biomarkers

The levels of CPK, LDH, CK-MB & troponin I was markedly ($p < 0.05$) elevated in Cd administered animals in comparison to control group animals. Contrarily, Cd + PSB supplementation notably ($p < 0.05$) reduced the levels of abovementioned cardiac injury biomarkers as compared to Cd administered rats. Moreover, no discrepancies were noted among the control & only PSB supplemented rats (Table 2).

3.4. Effects of PSB on inflammatory biomarkers

The levels of IL-1 β , NF- κ B, COX-2, IL-6 & TNF- α were substantially ($p < 0.05$) augmented in Cd provided animals in relation to control group. Nonetheless, these levels were notably ($p < 0.05$) reduced in co-treated (Cd + PSB) animals as compared to Cd exposed animals. Furthermore, PSB (only) supplemented animals expressed these levels close to untreated group (Table 3).

3.5. Effects of PSB on apoptotic profile

Cd intoxication notably ($p < 0.05$) upsurged Caspase-9, Caspase-3 & Bax, while downregulating the levels of Bcl-2 in Cd exposed animals as compared to control animals. However, the exposure to PSB + Cd markedly ($p < 0.05$) decreased Caspase-9, Caspase-3 & Bax while escalating Bcl-2 levels as compared to Cd exposed animals. Furthermore, the rats of control as well as PSB only supplemented group showed no variation in their mean values (Table 4).

3.6. Effect of PSB on cardiac histopathology

Cd intoxication led to various histopathological disruptions in cardiac tissues such as myocardial damage, focal necrosis, vacuolization of cytoplasm, myofibril disarray, interstitial fibrosis, inflammation, and alterations in cellular morphology in comparison to control group. Nevertheless, PSB supplementation remarkably ($p < 0.05$) mitigated abovementioned histopathological disruptions prompted by Cd exposure. However, control and PSB only supplemented group showed (Fig. 1).

4. Discussion

Cd is one of the most prevalent contaminants with a continuous

Table 2
Impacts of PSB & Cd on cardiac injury biomarkers.

| Parameters | Groups | | | |
|------------------|--------------------------------|---------------------------------|--------------------------------|--------------------------------|
| | Control | Cd | Cd + PSB | PSB |
| LDH (mg/dl) | 15.97 \pm 0.82 ^c | 57.72 \pm 1.31 ^a | 25.95 \pm 1.41 ^b | 15.91 \pm 0.67 ^c |
| CPK (mcg/L) | 126.34 \pm 4.67 ^c | 354.78 \pm 12.72 ^a | 227.41 \pm 7.68 ^b | 125.26 \pm 4.46 ^c |
| CK-MB (ng/mL) | 29.53 \pm 2.93 ^c | 91.13 \pm 2.65 ^a | 48.07 \pm 2.83 ^b | 28.70 \pm 3.55 ^c |
| Troponin (pg/ml) | 0.60 \pm 0.08 ^c | 3.84 \pm 0.09 ^a | 1.62 \pm 0.10 ^b | 0.59 \pm 0.08 ^c |

Values having different superscripts are significantly ($p < 0.05$) different from the other groups.

Table 3
Impacts of PSB & Cd on inflammatory indices.

| Parameters | Groups | | | |
|------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | Control | Cd | Cd + PSB | PSB |
| NF- κ B (ng/g tissue) | 18.96 \pm 1.68 ^c | 84.15 \pm 2.18 ^a | 35.77 \pm 1.55 ^b | 18.52 \pm 1.36 ^c |
| TNF α (ng/g tissue) | 8.23 \pm 0.28 ^c | 26.25 \pm 1.66 ^a | 15.74 \pm 1.74 ^b | 8.11 \pm 0.19 ^c |
| IL-1 β (ng/g tissue) | 27.55 \pm 2.03 ^c | 76.20 \pm 2.42 ^a | 42.24 \pm 2.36 ^b | 27.30 \pm 2.07 ^c |
| IL-6 (ng/g tissue) | 6.26 \pm 0.27 ^c | 51.71 \pm 1.84 ^a | 18.80 \pm 1.72 ^b | 6.21 \pm 0.28 ^c |
| COX-2 (ng/g tissue) | 16.85 \pm 1.51 ^c | 55.81 \pm 1.24 ^a | 28.90 \pm 2.11 ^b | 16.71 \pm 1.63 ^c |

Values having different superscripts are significantly ($p < 0.05$) different from the other groups.

Table 4
Impacts of PSB & Cd on apoptotic markers.

| Parameters | Groups | | | |
|-------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | Control | Cd | Cd + PSB | PSB |
| Bax (pg/mL) | 1.84 \pm 0.08 ^b | 6.74 \pm 0.53 ^a | 2.62 \pm 0.34 ^b | 1.82 \pm 0.09 ^b |
| Caspase-3 (ng/mL) | 1.58 \pm 0.21 ^c | 14.26 \pm 0.46 ^a | 3.18 \pm 0.39 ^b | 1.54 \pm 0.23 ^c |
| Caspase-9 (pg/mL) | 2.16 \pm 0.16 ^c | 22.85 \pm 1.61 ^a | 4.82 \pm 0.35 ^b | 2.13 \pm 0.17 ^c |
| Bcl-2 (pg/mL) | 17.25 \pm 1.33 ^a | 6.26 \pm 0.50 ^c | 11.60 \pm 1.28 ^b | 17.43 \pm 1.41 ^a |

Values having different superscripts are significantly ($p < 0.05$) different from the other groups.

increasing concentration because of agricultural and industrial activities, causing unavoidable hazards to humans (Wang et al., 2023). Setia et al. (2020) documented that Cd penetrates the body through skin, gastrointestinal & respiratory tract. It is reported that Cd exposure induces various damages such as nephrotoxicity, bone disease, infertility, liver toxicity as well as cardiac damages (Mitra et al., 2022). In cardiac tissues, Cd exposure generates oxidative stress, inflammation, cardiomyocyte apoptosis and histological damage (Alpsoy et al., 2014). Recent literature shows that antioxidants can prevent the body from adverse effects of Cd intoxication. PSB is a poly-phenolic compound that is effective in scavenging free radicals & well-known for its broad range of pharmacological properties (Athapaththu et al., 2023).

Our investigation elucidated that Cd intoxication reduced the activities of antioxidant enzymes while augmenting the levels of oxidative stress markers in cardiac tissues of treated animals. Furthermore, excessive generation of ROS reduced the activities of antioxidant enzymes thereby impairing cellular defense system (Ahmad et al., 2023). Ighodaro and Akinloye (2018) elaborated that SOD transforms superoxide radicals into H₂O₂, whereas CAT utilizes the oxygen as a cofactor and stimulates the degradation of H₂O₂ into H₂O & O₂. GPx reduces oxidative stress by scavenging the hydrogen peroxide. GSH also protects the cells from oxidative stress (OS) by lowering the levels of H₂O₂ and other peroxides. GST participates in cellular detoxification of cytotoxic and genotoxic substances as well as in defending tissues against oxidative damage. (Hayes et al., 1999). Similarly, escalated levels of MDA indicate the onset of lipid peroxidation due to excessive generation of reactive oxygen species (Ijaz et al., 2022). Elsayed et al. (2022) reported that flavonoids can reduce the levels of OS by increasing the activity of antioxidant enzymes. In the current investigation PSB supplementation restored the balance between antioxidant and prooxidants which demonstrates its ROS neutralizing abilities.

Oral administration of Cd led to a remarkable upsurge in the level of cardiac function markers (CK-MB, CPK, LDH & troponin). Troponin, CPK, and CK-MB are the diagnostic biomarkers of cardiotoxicity due to

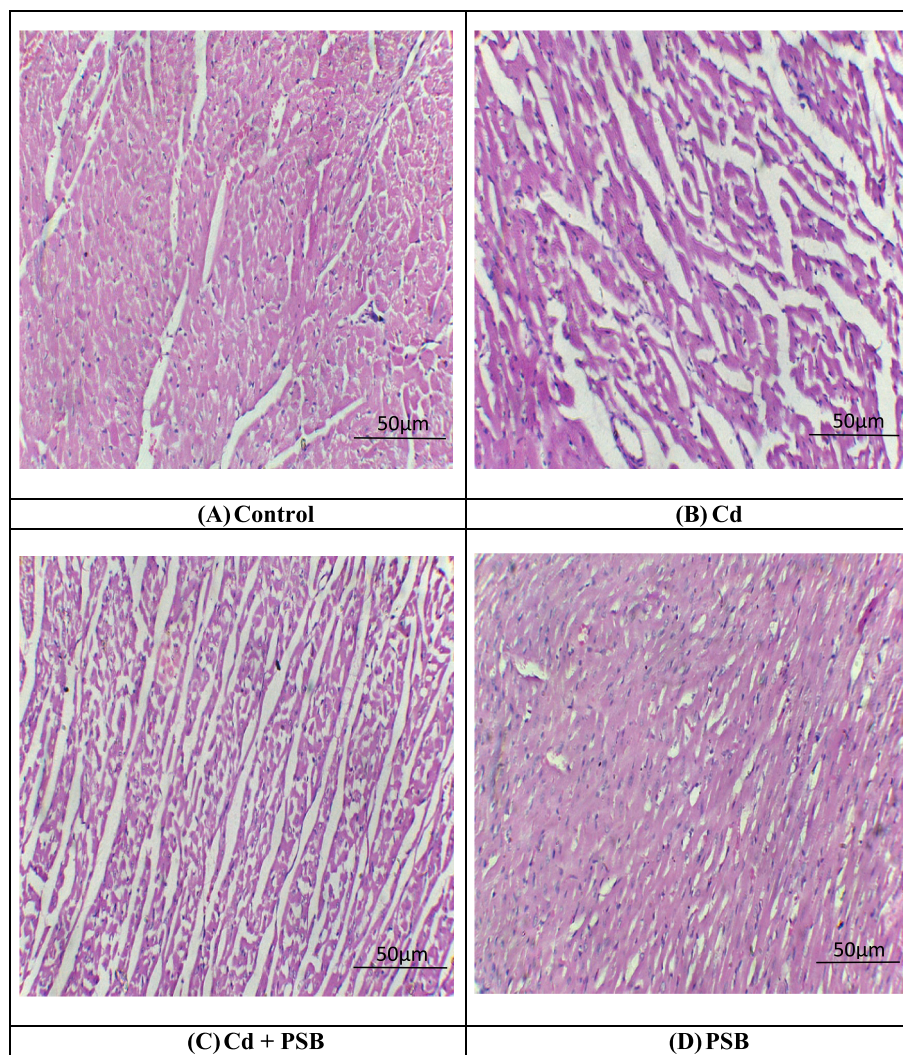


Fig. 1. Histopathological analysis of heart tissues. H&E stain; 40X (A) Control group exhibited normal architecture of heart tissues (B) Cd exposed group demonstrated fibrosis, inflammation as well as large interstitial spaces (edema) (C) Cd + PSB group showed a remarkable recovery in contrast to Cd exposed group (D) Only PSB supplemented group showed normal morphology of cardiac tissues as in control group.

their serum catalytic activity and tissue specificity (Zhang et al., 2015). A previous investigation elucidated that Cd is a cardio-toxic compound having the capability to destroy directly cardiomyocytes & release these cardiac damage biomarkers into the blood stream. Various types of heart injury, including myocarditis, heart failure & myocardial infarction have been associated with elevated levels of these enzymes (Saleh et al., 2017). However, PSB administration notably lowered the level of these enzymes that might be attributed to its cardio-protective ability.

In the current investigation, exposure to Cd instigated an augmentation in the levels of IL-6, TNF- α , NF- κ B, IL-1 β & COX-2 activity. NF- κ B, a cytoplasmic protein complex which is associated with elevated levels of ROS during various disorders (Ali et al., 2022) and its activation ultimately trigger the production of aforementioned inflammatory markers (Somade et al., 2019). Furthermore, COX-2 is an adaptive enzyme that stimulates the complete inflammation state (Kumar et al., 2022). Therefore, inflammatory responses in the cell can be blocked by preventing the activation of NF- κ B. However, PSB supplementation reduced the levels of inflammatory biomarkers which is attributed to anti-inflammatory property of PSB.

Cd administration induced a substantial upsurge in the levels of pro-apoptotic markers while downregulating the anti-apoptotic markers. Danial and Korsmeyer (2004) elaborated that Bax works as pro-apoptotic protein that mediates various events of apoptosis while Bcl-

2 functions antagonistic to Bax. An escalation in Bax levels & decline in Bcl-2 alters the mitochondrial membrane permeability & triggers the eviction of Cytochrome c into cytoplasmic matrix that in turn activates Caspase-3 to mediate apoptotic pathways (Santana, 2018). Caspase-3 belongs to the cysteine protease family which splits the cellular proteins & making structural variations that led to apoptosis (Hofmann, 2020). However, administration of PSB lowered the levels of pro-apoptotic proteins while elevating the levels of anti-apoptotic markers owing to its anti-apoptotic properties.

The present research demonstrated that Cd intoxication induced adverse histopathological damages in the architecture of cardiac tissues such as focal necrosis, perivascular and interstitial fibrosis, myofibril disarray, alterations in cellular morphology and increased levels of matrix metalloproteinases (MMPs). Our outcomes matched with the investigations of Chou et al. (2023) who stated that Cd exposure instigated various histopathological damages in heart including focal necrosis, myofibril disarray, disorganized sarcomere structures and interstitial & perivascular fibrosis. However, the supplementation of PSB significantly restored all the histopathological damages in the cardiac tissues of rats that might be ascribed to its anti-inflammatory, anti-oxidative & anti-apoptotic nature.

5. Conclusion

Taken together, the current research exposed that Cd intoxication prompted OS in cardiac tissues and disturbed the heart functional enzymes, inflammatory, apoptotic, and biochemical markers along with histopathological profile. However, PSB supplementation significantly restored all the cardiac damages provoked by Cd. As a result, it can be assumed that PSB might be used as a potential therapeutic drug in future to cure cardiac damage. However, further clinical investigations are indispensable to evaluate the efficacy of PSB to cure cardiac damages in humans.

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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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jksus.2023.103074>.

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Further reading

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