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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 & non-biodegradable heavy metal which instigates various organ toxicities such as cardiac injuries. Pinostrobin (PSB) is a potent dietary bioflavonoid, which shows various pharmacological capabilities. The existing research was executed to explicate the ameliorative abilities of PSB against Cd elicited cardiac dysfunction in rats. Albino rats (n=24) 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 lowered CAT, GSR, SOD, GPx, & GST activities while escalating the levels of ROS, H_2O_2 & MDA. Furthermore, Cd exposure escalated the levels of cardiac damage biomarkers such as CPK, CK-MB, Troponin 1 & LDH. Besides, the levels of inflammatory cytokines (NF-κB, IL-1ß, TNF-α, IL-6 levels & COX-2 activity) were upregulated in Cd intoxicated group. Similarly, Caspase-3, Bax & Caspase-9 levels were augmented, & Bcl-2 levels were declined after Cd administration. In addition, the histopathological examination revealed a notable cardiac tissue impairment in the Cd exposed group. Nonetheless, PSB treatment considerably ($p<0.05$) recovered the abovementioned Cd-induced impairments. Therefore, current research revealed that PSB might be a promising ameliorative agent to cure Cd instigated cardiac damages.

Keyword: Pinostrobin, Cadmium, Cardiotoxicity, Oxidative stress, Inflammation

1. Introduction

Cd is considered as the 7th most lethal heavy metal and designated a carcinogenic substance that instigates severe health risks to different life forms (Al Olayan et al., 2020). Cd primarily enters the body predominantly by ingestion, inhalation or by the dermal contact (Nduka et al., 2019). Moreover, Genchi et al. (2020) elucidated that Cd is extensively utilized 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 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 & nephrotoxicity (Pi et al., 2015; Oyinloye et al., 2016; Dong, 2015).

Heart is one of the vital organs of body that performs significant function (pumping blood) in living organisms (Peate, 2020). Cd disrupts the cardiovascular system (CVS) by disrupting the normal structure of "cytochrome P450" as well as antioxidant balance in the body. When Cd reaches the cardiac tissues by cell membranes it prompts oxidative stress and prompted DNA damage which ultimately disrupts the normal gene transcription (Guo, et al., 2020). The exposure to Cd disrupted the antioxidant defense system that involve in 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 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 with polyphenolic and therefore 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 (Kanchanapiboon et al., 2020), anticancer (Sun et al., 2020), antiapoptotic (Jadaun et al., 2019) antiparasitic (Vechi et al., 2020) & anti-inflammatory (Patel et al., 2014). However, the cardioprotective potential of PSB has yet to be reported. Consequently, 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) were used as model animals during 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 (12h day/night period, standard temperature 22-25ºC) for 7 days before the execution of trial. Rats were provided standard feed & tap water. European Union of Animal Care & Experimentation 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 assigned as control group. The second group was exposed to Cd (5 mg/kg). The third 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 execution of experiment period (4 weeks), the rats were anesthetized by using xylazine and ketamine, 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 preserved in zipper bags & retained at -20 ºC for further chemical examination, while the other half was fixed in 10% formalin for histopathology.

2.4.Biochemical analysis

Chance & Maehly (1995) approach was utilized to evaluate the CAT activity. The approach ascribed 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 calculate GSH content. MDA level was quantified by utilizing the strategy of ohkawa et al., (1979). The ROS level was quantify by utilizing the

strategy described by Hayashi et al. (2007). The technique developed by Pick & Keisari (1981) was used to quantify H_2O_2 levels.

2.5.Evaluation of cardiac injury biomarkers

The cardiac damage biomarkers such as CK-MB, troponin-1, LDH & CPK 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-1β, IL-6, NF-κB, COX-2 & TNF-α) levels was carried out by using standardized ELISA kits from (TX, Houston, Cusabio Technology Llc, USA).

2.7.Apoptotic markers assessment

For the evaluation of Caspase-9, Bax, Caspase-3 & Bcl-2 standard ELISA kits used according to the guidelines stipulated 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 (4-5μm) 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 ±SEM. The Tukey's test & ANOVA (one way interaction) were used to statistically analyze the data 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 ($p < 0.05$) declined 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 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).

3.2.Effects of PSB on oxidant profile

The levels of ROS & MDA were markedly ($p < 0.05$) enhanced in Cd provided rats in compliance to control group. On the other hand, PSB administration remarkably ($p < 0.05$) declined 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 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-1β, NF-κB, 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 ($p < 0.05$) lowered 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 (figure 1).

4. Discussion

Cd is one of the most prevalent contaminants with a constant 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 investigations elucidated 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. Ighodaro and Akinloye (2018) elaborated that SOD transforms superoxide radicals during OS into H_2O_2, whereas CAT utilizes the oxygen as a cofactor and stimulates the degradation of H_2O_2 into H_2O & O_2. GPx reduces oxidative stress by scavenging the hydrogen peroxide. GSH also protects the cells from OS by lowering the levels of H_2O_2 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). However, in our

investigation PSB supplementation restored the balance between antioxidant and oxidant biomarkers which is demonstrating 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 their serum catalytic activity and tissue specificity (Zheng et al., 2015). A previous investigation elucidated that Cd is a cardio-toxic compound having the capability to destroy directly cardiocytes & release these diagnostic biomarkers into the blood stream. Various sorts 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 ascribed to its cardio-protective ability.

In current investigation, exposure to Cd instigated an augmentation in the levels of IL-6, TNF-α, NF-κB, IL-1β & COX-2 activity. NF-kB, a cytoplasmic protein complex which is associated 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-κB. 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 biomarkers while downregulating the anti-apoptotic indicators. Danial and Korsmeyer (2004) elaborated that Bax works as pro-apoptotic protein that mediates various events of apoptosis while Bcl-2 functions function antagonistic to Bax. An escalation in Bax levels & decline in Bcl-2 alters the mitochondrial membrane permeability & trigger the eviction of Cytochrome C into cytoplasmic matrix that in turn activates Caspase-3 to mediate apoptotic pathways (Santana et al., 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, 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 an auspicious therapeutic drug in future to cure cardiac damages. However, further clinical investigations are indispensable to evaluate the efficacy of PSB against human cardiac toxicity.

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Table 1: Impacts of PSB & Cd on biochemical profile

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

Table 3: Impacts of PSB & Cd on inflammatory indices

Table 4: Impacts of PSB & Cd on apoptotic markers

Table 1

Parameters	Groups			
	Control	**Cd**	**Cd+ PSB**	**PSB**
CAT (U/mg protein)	12.88 ± 1.47^{a}	5.06 ± 0.29^{c}	8.06 ± 0.62^{b}	12.79 ± 1.50^{b}
SOD (U/mg protein)	10.39 ± 0.85^{a}	4.57 ± 0.36^{c}	7.82 ± 0.36^{b}	10.32 ± 0.93^{a}
GSR (nM NADPH oxidized/min/mg tissue	9.34 ± 0.22^{a}	3.26 ± 0.27^{c}	6.40 ± 0.46^{b}	9.39 ± 0.25^{a}
GPx (U/mg protein)	31.28 ± 2.28^{a}	8.90 ± 0.83^{c}	18.72 ± 1.43^{b}	31.39 ± 2.39^{a}
GSH (U/mg protein)	19.59 ± 1.72^{a}	6.17 ± 0.41^{c}	13.67 ± 1.00^{b}	19.64 ± 2.15^{a}
GST (U/mg protein)	41.91 ± 1.63^{a}	14.61 ± 1.49^{c}	32.86 ± 1.78^{b}	42.03 ± 2.01^{a}
MDA (nmol/g)	0.74 ± 0.07^{c}	5.86 ± 0.51^{a}	2.34 ± 0.20^{b}	0.72 ± 0.07^{c}
ROS (nmol/g)	0.44 ± 0.13^{c}	7.40 ± 0.44^{a}	2.33 ± 0.21^{b}	0.41 ± 0.14^{c}
H_2O_2 (μM/min/ mg protein)	1.38 ± 0.19^{b}	6.54 ± 0.66^{a}	2.34 ± 0.28^{b}	1.36 ± 0.19^{b}

Distinct superscripts on various values demonstrated discrepancies among other groups

Table 2

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

Distinct superscripts on various values demonstrated discrepancies among other groups

Table 3

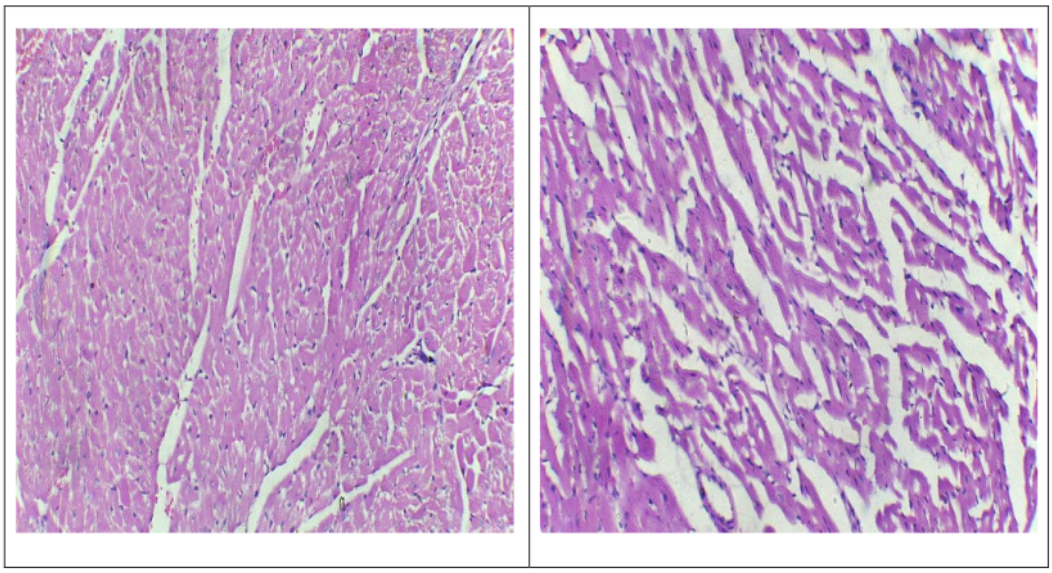
Parameters	Groups			
	Control	**Cd**	**Cd + PSB**	**PSB**
NF-kB (ng/g tissue)	18.96±1.68^{c}	84.15±2.18^{a}	35.77±1.55^{b}	18.52±1.36^{c}
TNFα (ng/g tissue)	8.23±0.28^{c}	26.25±1.66^{a}	15.74±1.74^{b}	8.11±0.19^{c}
IL-1ß (ng/g tissue)	27.55±2.03^{c}	76.20±2.42^{a}	42.24±2.36^{b}	27.30±2.07^{c}
IL-6 (ng/g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

Distinct superscripts on various values demonstrated discrepancies among other groups

Table 4

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

Distinct superscripts on various values demonstrated discrepancies among other groups



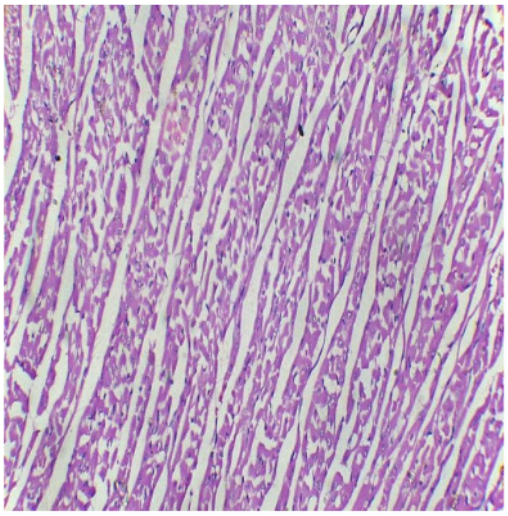
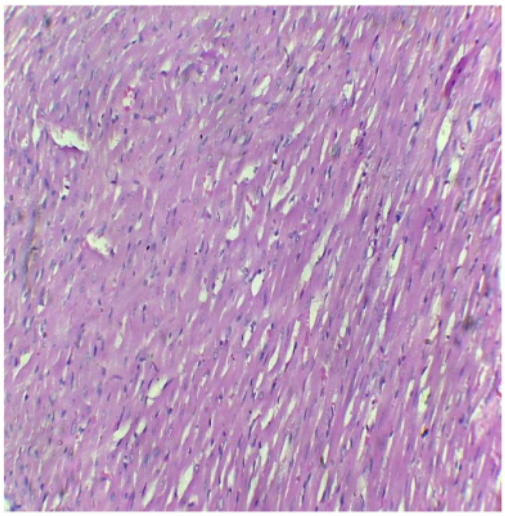
(A) Control	(B) Cd
	
(C) Cd + PSB	(D) PSB

Figure 1

Figure 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 + PSD 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.

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