Full Length Article

Pachypodol attenuates arsenic triggered cardiac damage via rectifying oxidative stress, inflammation, apoptosis and histopathological alterations

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ABSTRACT

Arsenic is a lethal toxicant found ubiquitously in the ecosystem which adversely affects the body organs including the heart. Pachypodol (PCHP) is a natural flavone which exhibits substantial pharmacotherapeutic potentials. The rats (n = 24) were distributed into 4 different groups i.e., control, arsenic-intoxicated group (50 mg/kg), arsenic + PCHP-supplemented group (50 mg/kg + 10 mg/kg) & PCHP-treated (10 mg/kg) group. It was assessed that arsenic administration subsided catalase (CAT), glutathione reductase (GSR), superoxide dismutase (SOD), glutathione peroxidase (GPx), & glutathione S-transferases (GST) activities while augmenting the levels of reactive oxygen species (ROS) and malondialdehyde (MDA). Furthermore, arsenic exposure increased the levels of cardiac injury markers such as creatine phosphokinase (CPK), creatine kinase-MB (CK-MB), troponin I & lactate dehydrogenase (LDH). Besides, the levels of inflammatory markers nuclear factor kappa B (NF-κB), interleukin-1β (IL-1β), Tumor necrosis factor α (TNF-α), Interleukin 6 (IL-6) levels and cyclooxygenase 2 (COX-2) activity were increased following the arsenic exposure. Similarly, Caspase-3, Bax and Caspase-9 levels were upsurged whereas Bcl-2 level was reduced after arsenic intoxication. In addition, the histopathological assessment revealed a substantial cardiac tissues impairment in the arsenic exposed group. Nonetheless, PCHP supplementation substantially (p < 0.05) recovered the abovementioned arsenic −induced impairments owing to its anti-inflammatory, antioxidative and anti-apoptotic abilities. Therefore, the current research revealed that PCHP might be a promising ameliorative agent to cure arsenic instigated cardiac damages.

1. Introduction

Arsenic is a well-known heavy metal which is ubiquitously present in our surroundings and considered as a major health concern across the globe. It is documented that arsenic leaches into drinking water through various sources such as bedrocks and earth crust (Vahter, 2008). Humans as well as other animals are exposed toarsenic via contaminated food and water, air as well as seafood (mussels, prawns, oyster, fish) (McCarty et al., 2011). It is reported that high concentrations of arsenic are present in urine, hoof, hair as well as nails (Roy et al., 2008). Furthermore, workers at chemical factories, mining sites and smelters are exposed to high concentration of arsenic. It is estimated that arsenic concentrations vary from one place to another such as the areas of low anthropogenic activities have 1–3 ng/m^3 while industrial zones have

20–30 ng/m^3 (ATSDR, 2000). Owing to high rate of absorption, slow elimination as well as small particle size, arsenic adversely affect various organs of body (Bergin et al., 2016).

It is revealed that arsenic exposure damages various body organs including skin, liver, lungs, heart, and urinary bladder (States et al., 2009; Moon et al., 2012). Various epidemiological investigations elucidated a strong relationship between arsenic contaminated water and cardiovascular disorders (Sanchez-Soria et al., 2012). Chowdhury and Daalen (2019) revealed that chronic exposure to arsenic instigates different cardiac impairments such as ischemia, arrhythmia as well as heart failure. It is reported that myocardial oxidative stress & apoptosis are primary factors underlying the arsenic instigated cardiac damages (Jiang et al., 2021). Furthermore, a long-term arsenic intoxication escalates the systolic blood pressure, total cholesterol, AST, bilirubin,

creatinine, urea as well as reduces the levels of high-density lipoproteins (HDL) which ultimately instigate hypertension (Balarastaghi et al., 2022).

However, the management of arsenic induced toxicities is still complicated owing to lack of efficient treatment (Flora, 2020). Natural compounds are widely used as adjuvant therapy to treat various disorders (Men et al., 2022; Aboubakr et al., 2023). It is reported that plant-based flavonoids have potential to bind with heavy metals owing to their antioxidative abilities (Tay et al., 2013). PCHP is a plant derived flavonoid which demonstrated various pharmacological potentials including anti-microbial, cytoprotective, anti-apoptotic as well as anti-oxidative (Kim et al., 2019; Krithika et al., 2021; Zhang et al., 2021; Kim et al., 2019). Therefore, this investigation was executed to investigate the attenuative potential of PHCP to rectify arsenic provoked cardiac toxicity in rats.

2. Materials and methods

2.1. Chemicals

Arsenic (CAS No. 7440–38-2, Purity HPLC $\geq$ 98.0 %) & PCHP (CAS No. 33708–72-4, Purity HPLC $\geq$ 98.0 %) were bought from Sigma-Aldrich (Germany).

2.2. Experimental animals

Twenty-four albino rats, aged 6–7 weeks and weighing between 180–200 g, were housed in adequately ventilated enclosures under standard environmental conditions at the Animal Research Station of the University of Agriculture, Faisalabad (UAF). Standard laboratory conditions included a 12-hour light–dark photoperiod, a humidity level maintained at 60 $\pm$ 5 % and a temperature range of 20–26 °C. The rats were provided with a commercial diet (composition: Fibers, oils, proteins, carbohydrates, flour, minerals, and vitamins) and ad libitum access to tap water throughout the study period. Rats were handled according to the ethical guidelines provided by European Union of Animals Care and Experimentation (EU Directive 2010/63/EU).

2.3. Experimental protocol

Twenty-four rats were randomly distributed into 4 different groups, each comprising of six rats. The treatment regimens were as follows: the control group, the arsenic (50 mg/kg), PCHP + arsenic (50 mg/kg + 10 mg/kg), and the group (10 mg/kg). The experimental duration spanned a period of 30 days. On the 31st day of the experiment, the rats were decapitated, and cardiac blood samples were collected to assess the serum profile. Subsequently, the heart was surgically excised from the rats. The one part of heart tissue was placed in the zipper bags and preserved at −20 °C to facilitate the assessment of the biochemical biomarkers. Conversely, the 2nd part was taken in a formalin solution (10 %) to facilitate the subsequent examination of histopathological changes in cardiac tissues.

2.4. Cardiac injury markers estimation

The levels of CPK (Catalog no. E4608-100), LDH (CSB-E11324r), troponin I (CSB-E08594r) & CK-MB (Catalog No. MBS2515061) were estimated by employing ELISA kits.

2.5. Biochemical analysis

The assessment of CAT activity was carried out using the technique developed by Aebi, (1974). The activity of SOD was quantified following the methodology described by Kakkar et al. (1984). The measurement of GPx activity was performed by using the method established by Rotruck et al. (1973). The concentration of GSR was estimated following the protocol outlined by Carlberg and Mannervik, (1975). Additionally, the content of GSH was evaluated using the methodology demonstrated by Jollow et al. (1974).

2.6. Estimation of oxidative stress indices

The concentration of ROS was determined using the method established by Hayashi et al. (2007). The quantification of MDA levels was performed following the procedure outlined by Ohkawa et al. (1979).

2.7. Inflammatory markers assessment

The quantification of inflammatory biomarkers was carried out using ELISA kits followed by manufacture's guidelines.

2.8. Apoptotic markers analysis

The ELISA kits were used to measure the level of apoptotic biomarkers according to the instruction of manufacturers.

2.9. Histopathological observation

A formaldehyde (10 %) solution was used to fix the cardiac tissues, after that dehydrated them with ascending grades of alcohol and trimmed into 4 μm pieces with the help of microtome (rotary). By using eosin as well as hematoxylin stains the staining was performed and the prepared slides were examined under light a microscope to study the histopathological disruption.

2.10. Statistical evaluation

All the data were illustrated as Mean $\pm$ 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. Impact of PCHP on biochemical profile

Arsenic intoxication substantially ($p < 0.05$) downregulated the GSH, GSR, GST, GPx, SOD as well as CAT activity. Furthermore, co–treatment of arsenic + PCHP considerably ($p < 0.05$) augmented these enzyme's activities in comparison to arsenic exposed rats. Moreover, insignificant differences were observed among PCHP alone and the control group (Table 1).

3.2. Impact of PCHP on oxidative stress biomarkers

Arsenic treatment remarkably ($p < 0.05$) elevated MDA along with ROS concentration in arsenic group in contradiction to the control rats. PCHP + Arsenic group substantially ($p < 0.05$) reduced their levels when matched with arsenic rats. No substantial changes were detected in the mean values of these markers in the control & PCHP rats (Table 1).

3.3. Impact of PCHP on cardiac injury markers

Arsenic supplementation substantially ($p < 0.05$) elevated LDH, CPK & CK-MB in comparison to the control rats. C-administration of PCHP notably ($p < 0.05$) restored their levels to normal state arsenic as compared to arsenic group. Furthermore, there was no substantial changes noticed in the mean values of only PCHP and the control groups (Table 2).

3.4. Impact of PCHP on inflammatory biomarkers

Exposure to arsenic significantly ($p < 0.05$) augmented the TNF-α,

Table 1
Impacts of PCHP on biochemical profile.

Parameters	Groups			
	Control	**Arsenic**	**Arsenic + PCHP**	**PCHP**
CAT (U/mg protein)	13.99 ± 1.58[a]	6.74 ± 0.50[b]	11.53 ± 1.32[a]	14.13 ± 1.94[a]
SOD (U/mg protein)	9.37 ± 1.13[a]	3.81 ± 0.22[c]	6.79 ± 0.40[b]	9.42 ± 1.10[a]
GSR (nM NADPH oxidized/min/mg tissue)	7.28 ± 0.25[a]	2.29 ± 0.20[c]	5.54 ± 0.33[b]	7.32 ± 0.29[a]
GPx (U/mg protein)	25.12 ± 1.93[a]	9.09 ± 1.18[c]	16.93 ± 1.61[b]	25.83 ± 2.43[a]
GSH (U/mg protein)	15.34 ± 2.07[a]	4.01 ± 0.27[b]	13.62 ± 1.11[a]	15.79 ± 2.54[a]
GST (U/mg protein)	25.12 ± 1.93[a]	9.09 ± 1.18[c]	16.93 ± 1.61[b]	25.83 ± 2.43[a]
MDA (nmol/g)	0.75 ± 0.12[c]	7.22 ± 0.33[a]	3.19 ± 0.20[b]	0.71 ± 0.10[c]
ROS (nmol/g)	0.66 ± 0.12[c]	8.66 ± 0.50[a]	2.51 ± 0.28[b]	0.63 ± 0.11[c]

Distinct superscripts on various values demonstrated discrepancies among other groups.

Table 2
Impacts of PCHP on cardiac injury biomarkers.

Parameters	Groups			
	Control	**Arsenic**	**Arsenic + PCHP**	**PCHP**
LDH (mg/dl)	15.81 ± 1.51[c]	56.85 ± 2.52[a]	25.70 ± 2.03[b]	15.69 ± 1.51[c]
CPK (mcg/L)	146.16 ± 9.99[c]	429.7 ± 24.1[a]	237.38 ± 12.69[b]	141.99 ± 10.24[c]
CK-MB (ng/mL)	32.83 ± 1.50[a]	92.37 ± 2.11[a]	222 ± 286[a]	32.79 ± 1.51[a]
Troponin (pg/ml)	0.66 ± 0.20[c]	4.30 ± 0.23[a]	1.72 ± 0.16[b]	0.64 ± 0.20[c]

Distinct superscripts on various values demonstrated discrepancies among other groups.

IL- 6, IL-1β, NF-κB along with the COX-2 activity in the arsenic rats in contrast to control rats. PCHP administration along with arsenic markedly ($p < 0.05$) decreased the activity and levels of the aforesaid inflammatory biomarkers as compared to arsenic rats. Moreover, no substantial alterations were observed among the control groups and PCHP (Table 3).

Table 3
Impacts of PCHP on inflammatory indices.

Parameters	Groups			
	Control	**Arsenic**	**Arsenic + PCHP**	**PCHP**
NF-kB (ng/g tissue)	19.89 ± 1.74[c]	85.82 ± 1.34[a]	44.29 ± 2.50[b]	19.67 ± 1.52[c]
TNFα (ng/g tissue)	9.33 ± 1.44[c]	34.65 ± 3.00[a]	15.81 ± 1.33[b]	9.26 ± 1.33[c]
IL-1ß (ng/g tissue)	19.94 ± 2.95[c]	78.30 ± 2.96[a]	43.87 ± 1.57[b]	19.79 ± 2.78[c]
IL-6 (ng/g tissue)	9.24 ± 0.11[c]	54.91 ± 2.58[a]	24.24 ± 2.01[b]	9.21 ± 0.08[c]
COX-2 (ng/g tissue)	22.82 ± 1.70[c]	65.18 ± 2.23[a]	34.62 ± 2.10[b]	22.71 ± 1.75[c]

Distinct superscripts on various values demonstrated discrepancies among other groups.

3.5. Impact of PCHP on apoptotic biomarkers

Arsenic administration considerably ($p < 0.05$) esclated caspase-9, Bax and caspase-3, while reducing the concentration of Bcl-2 in arsenic rat in contrast to control. PCHP supplementation recovered the altered levels of these contrary to arsenic treated group. However, insignificant difference was observed in apoptotic markers level among PCHP and the control group (Table 4).

3.6. Impact of PCHP on histology

Arsenic intoxication instigated inflammation, degeneration of endocardium, cardiac edema, as well as abnormal shape of cardiomyocytes in contrast to control group. However, PCHP + arsenic treatment remarkably ($p < 0. 05$) restored aforementioned histopathological impairments. Nonetheless, control and PCHP alone treated group showed normal histology of cardiac tissues (Fig. 1).

4. Discussion

Our findings demonstrated that arsenic intoxication reduced the antioxidant enzyme's concentration (CAT, GPx, SOD, GSR & GSH) while downregulating the levels of ROS & MDA. Antioxidant enzymes are primary barriers against oxidative damage (Berroukeche et al., 2022). Various studies showed a strong association between ROS and arsenic instigated cellular damages (Liu and Jan, 2000). The overproduction of ROS retards the levels of these enzymes and disrupts the normal architecture of cells which ultimately leads to the progression of various pathologies (Mukherjee et al., 2015). Moreover, excessive production of free radicals decreases the activities of antioxidant enzymes which ultimately impairs the endogenous cellular defense system (Ahmad et al., 2023). It is reported that arsenic reduces the activities of SOD along with CAT via decreasing the availability of NADH while escalating the levels of superoxide anions (Das et al., 2010). Our results matched with the investigations of Ijaz et al. (2023) who elucidated that administration of arsenic reduced these enzyme's concentration while escalating the levels of lipid peroxidation. Plant-based flavonoid has potential to increase the activities of antioxidant enzymes (Ijaz et al., 2022). However, co-treatment of PCHP remarkably reduced antioxidant enzymes activities via regulating the levels of ROS owing to its ROS scavenging abilities.

Our investigation manifested that arsenic exposure augmented the levels of CK-MB, LHD, CPK as well as troponin-1 in cardiac tissues of rats. Abovementioned cardiac biomarkers are primary indicators of cardiac injury therefore, elevated levels of these biomarkers in the bloodstream indicate severe cardiac damage (Warings et al., 2000). Our findings are corroborated by the previous investigation which elucidated that arsenic administration triggers various alterations in cardiac tissues which are characterized by escalated levels of abovementioned cardiac injury markers (Jalaludeen et al., 2015). Nonetheless, the

Table 4
Impacts of PCHP on apoptotic markers.

Parameters	Groups			
	Control	**Arsenic**	**Arsenic + PCHP**	**PCHP**
Bax (pg/mL)	1.38 ± 0.15[c]	9.50 ± 1.04[a]	3.32 ± 0.20[b]	1.36 ± 0.16[c]
Caspase-3 (ng/mL)	3.32 ± 0.20[b]	19.33 ± 2.05[a]	5.23 ± 0.11[b]	3.29 ± 0.19[b]
Caspase-9 (pg/mL)	3.32 ± 0.20[b]	19.33 ± 2.05[a]	5.23 ± 0.11[b]	3.29 ± 0.19[b]
Bcl-2 (pg/mL)	14.52 ± 1.04[a]	3.85 ± 0.25[c]	11.03 ± 0.62[b]	14.76 ± 1.12[a]

Distinct superscripts on various values demonstrated discrepancies among other groups.
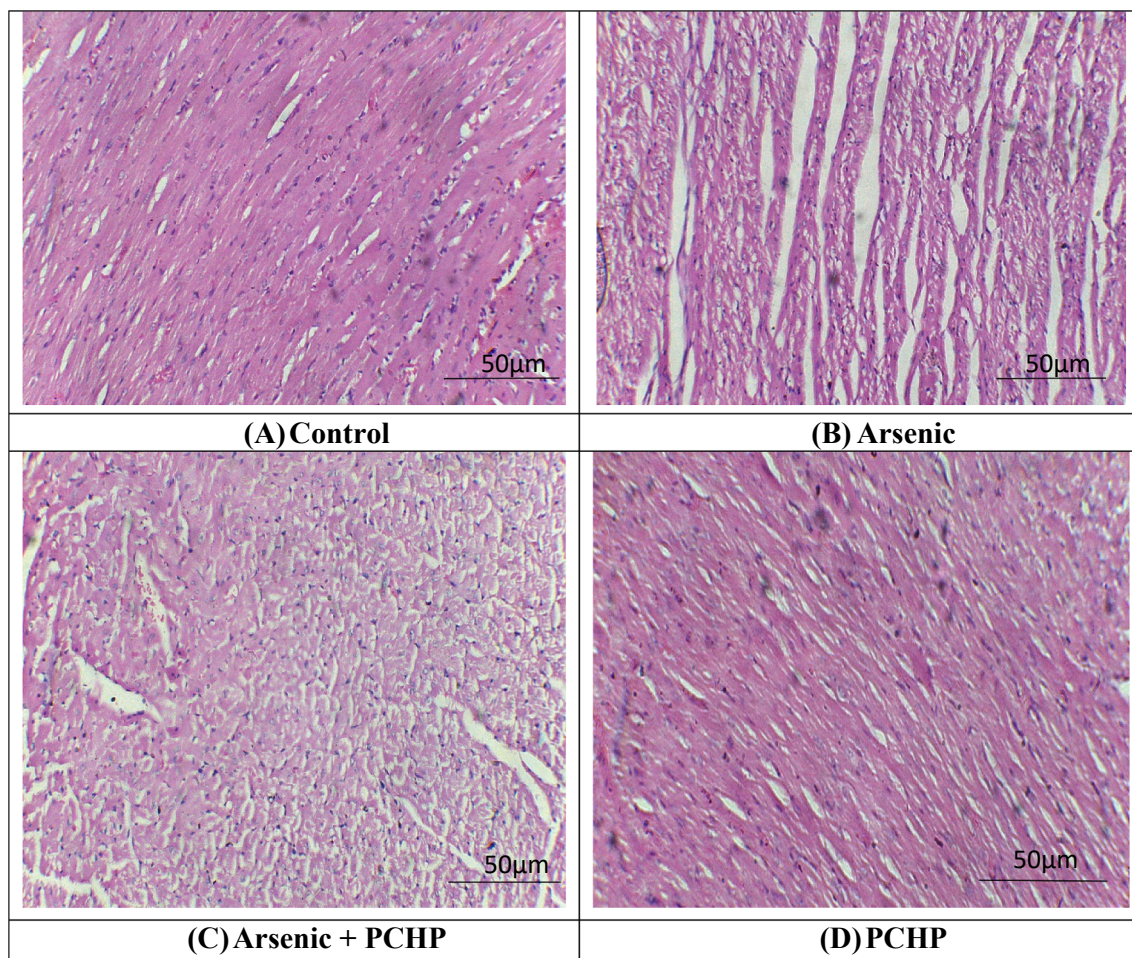
Fig. 1. Histopathological analysis of heart tissues. H&E stain; 40X **(A)** Control group exhibited normal architecture of heart tissues **(B)** Arsenic exposed group demonstrated fibrosis, inflammation as well as large interstitial spaces (edema) **(C)** Arsenic + PCHP group showed a remarkable recovery in contrast to Arsenic exposed group **(D)** Only PCHP supplemented group showed normal morphology of cardiac tissues as in control group.

supplementation of PCHP reduced the levels of these biomarkers which demonstrates its cardioprotective potential.

Our findings demonstrated that the administration of arsenic culminated in an increase in the concentration of inflammatory biomarkers (IL-1β, TNF-α, IL-6, NF-κB & COX-2 activity). NF-κB is a principal biomarker contributing substantially to the augmentation of the aforementioned proinflammatory biomarkers, ultimately leading to the onset of acute inflammation and the subsequent damages associated with ROS (Zha et al., 2014). Furthermore, COX-2 serves as a substantial mediator that prompts the incipience of inflammatory cascade in the cardiac tissues (Gilroy and Colville-Nash, 2000). The outcomes of our study revealed that the arsenic caused a substantial escalation in the concentration of these inflammatory biomarkers owing to increased OS levels which reflects the state of inflammation in the heart tissues. Supplementation of PCHP effectively reduced the stimulation of NF-κB that serves as the primary catalyst for tissue inflammation. Therefore, it can be inferred that PCHP may have substantial therapeutic action in mitigating the raised proinflammatory biomarkers, owing to its noticeable anti-inflammatory nature.

Apoptosis can be instigated by dysregulation in the homeostasis between apoptotic and antiapoptotic enzymes which are mediated through mitochondrial-independent or dependent cascades (Selimovic et al., 2011). Downregulation of Bcl-2 and concurrent upregulation of Bax markedly disrupt the integrity of the mitochondrial membrane (Reed, 2006). Bcl-2 & Bax mediates the secretion of cytochrome *c* from the mitochondrial system which ultimately triggers the process of apoptosis (Shou et al., 2002). Caspase-3 is recognized as a pivotal

mediator of apoptosis which activates other pro-apoptotic markers (D'Amelio et al., 2012). Treatment with arsenic resulted in a substantial upsurge in the Bax & Caspase-3 following a remarkable decline in the Bcl-2 concentration, However, PCHP supplementation alleviated arsenic caused cardiac apoptosis by reducing the Caspase-3 & Bax levels while upregulating the levels of Bcl-2.

A normal histo-architectural integrity of any organs is essential for its physiological functions (Oyeyipo et al., 2010). In the current study arsenic exposure remarkably induced histopathological disruptions in cardiac tissues i.e., myocardial damage, fibrosis, inflammatory responses, modification in cellular morphology as well as cellular necrosis. OS is recognized as central element underlying these morphological disturbances (Goyal et al., 2016). ROS disrupts normal activity of mitochondria, resulting in decreased ATP formation which leads towards the cell death in the cardiac tissues. Besides, OS triggered cascade of inflammatory events, instigating myocardial inflammation (Fabiani et al., 2011). Furthermore, these detrimental alterations influence contractility and cardiac perfusion, thus triggering the proliferation of wide range of cardiovascular pathologies (Fabiani et al., 2011). Our findings demonstrated that PCHP is an effective curative compound which recovered aforesaid cardiac histopathological damages provoked by arsenic.

5. Conclusion

Taken together, arsenic reduced the activities of antioxidant enzymes while upregulating the levels of oxidative stress. Furthermore,

arsenic promoted the levels of cardiac injury as well as inflammatory markers. The levels of pro-apoptotic markers were escalated while the levels of anti-apoptotic markers were reduced following the arsenic treatment. Moreover, arsenic instigated various histopathological impairments. However, PCHP treatment protected the cardiac tissues via regulating abovementioned dysregulations.

CRediT authorship contribution statement

Syeda Sania Zahara: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Mehrab Khalil:** Writing – original draft, Validation, Methodology, Investigation. **Moazama Batool:** Visualization, Validation, Software, Formal analysis, Data curation. **Muhammad Faisal Hayat:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Bader O. Almutairi:** Writing – original draft, Resources, Funding acquisition. **Mian Nadeem Riaz:** Writing – original draft, Visualization, Validation.

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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