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Prashantkumar Waghe
Department of Veterinary Pharmacology and Toxicology, Karnataka Veterinary, Animal and Fisheries Sciences University, Veterinary College, Nandinagar, Bidar, Karnataka, India

Dr. Prakash Nadoor
Department of Veterinary Pharmacology and Toxicology, Karnataka Veterinary, Animal and Fisheries Sciences University; Veterinary College, Vinobanagar, Shivamogga, Karnataka, India

Dr. Vijaykumar M
Department of Veterinary Pharmacology and Toxicology, Karnataka Veterinary, Animal and Fisheries Sciences University, Veterinary College, Nandinagar, Bidar, Karnataka, India

Dr. Shridhar NB
Department of Veterinary Pharmacology and Toxicology, Karnataka Veterinary, Animal and Fisheries Sciences University; Veterinary College, Vinobanagar, Shivamogga, Karnataka, India

Dr. Pavithra BH
Department of Veterinary Pharmacology and Toxicology, Karnataka Veterinary, Animal and Fisheries Sciences University, Veterinary College, Hebbal, Bengaluru, Karnataka, India

Dr. Vinay P Tikare
Department of Veterinary Pharmacology and Toxicology, Karnataka Veterinary, Animal and Fisheries Sciences University, Veterinary College, Gadag, Karnataka, India

Dr. Rashmi Rajashekaraiah
Department of Veterinary Pharmacology and Toxicology, Karnataka Veterinary, Animal and Fisheries Sciences University, Veterinary College, Hebbal, Bengaluru, Karnataka, India

Corresponding Author:
Prashantkumar Waghe
Department of Veterinary Pharmacology and Toxicology, Karnataka Veterinary, Animal and Fisheries Sciences University, Veterinary College, Nandinagar, Bidar, Karnataka, India

Experimental studies on potential utility of Pimobendan in arsenic induced cardiovascular dysfunction in wistar rats

Prashantkumar Waghe, Dr. Prakash Nadoor, Dr. Vijaykumar M, Dr. Shridhar NB, Dr. Pavithra BH, Dr. Vinay P Tikare and Dr. Rashmi Rajashekaraiah

Abstract
Today, contamination of drinking water with arsenic is a burning public health issue of global significance. Among the various deleterious effects of arsenic, cardiovascular disorders in the exposed population require urgent therapeutic intervention. The present study was aimed to assess whether pimobendan, a phosphodiesterase (PDE) - III inhibitor can attenuate the arsenic-induced cardiovascular dysfunction and to assess the involvement of mechanistic pathways related to reactive oxygen species (ROS) and nitric oxide (NO) signaling in rats. Wistar rats were exposed to arsenic through drinking water (100 ppm) for 90 days. These rats were treated with pimobendan (1 mg/kg) by oral gavage during the last 30 days of exposure to arsenic. At term, after overnight fasting, the experimental rats were sacrificed (91st day) and the aorta and heart tissues were dissected out to determine various parameters. Arsenic exposure favored the production of ROS such as $O_2^{\bullet}$ and decreased the activities of SOD, CAT, GPx, GR, and GSH content, thus leading to lipid peroxidation (LPO). Pimobendan administration restored the activities of enzymatic (SOD, CAT, GPx, and GR) and the level of GSH in the aorta and heart of arsenic exposed rats and reduced LPO. Experimental rats exposed to arsenic resulted in increased *i*NOS-derived nitrite production, while pimobendan significantly decreased its level. Similarly, arsenic-induced increase in IL-1β, IL-6, and TNF-α was significantly ($p< 0.05$) attenuated by pimobendan in the aorta and heart. In summary, the study revealed that sub-chronic exposure to arsenic can induce oxidative stress in rat aorta and heart leading to cardiovascular dysfunction, and pimobendan has the potential to ameliorate the arsenic-mediated alterations through the restoration of redox homeostasis and NO signaling.

Keywords: Arsenic, sub-chronic, aorta, redox homeostasis, pimobendan, wistar rats

Introduction
Arsenic is widely distributed in the environment and its exposure occurs primarily through groundwater contamination. Arsenic contamination of ground water much higher than the maximum permissible level is reported across several countries, particularly in the Indian sub-continent *viz*., Bangladesh, India, Taiwan, Chile, Argentina, and the USA [1, 2]. The presence of arsenic in foods of animal origin is a major problem in developed as well as developing countries, as its presence in food commodities attracts domestic and international trade restrictions by several countries around the world. In India what is worse is that arsenic contamination of groundwater is slowly spreading to several states like Bihar, Jharkhand, Uttar Pradesh, Assam, Chhattisgarh, and Chandigarh [3]. According to the World Health Organization guidelines and Bureau of Indian Standards (BIS) the maximum permissible limit of arsenic in drinking water is 0.01 ppm [4] and 0.05 ppm [5], respectively. In certain areas in the Indian subcontinent, the maximum arsenic concentration in groundwater was found to be around 3.7 ppm to 4.7 ppm [6]. In West Bengal (India), people were exposed to arsenic-contaminated water even in the range of 0.05-14.2 ppm [7]. Additionally, ground water levels of arsenic in Yadgir and Raichur districts of Karnataka State (India) are also known to contain more than the permissible limits (0.668 ppm) [8]. Arsenic exposure poses several health problems *viz*., vascular dysfunction including peripheral vascular disease [9], hypertension [10], ischemic heart disease [11] and carotid atherosclerosis [12, 13].
Evidence of high-level arsenic exposure (>0.2 ppm) on vascular disease is largely based on a series of epidemiologic studies in southwestern Taiwan, collectively suggesting that arsenic exposure induces atherosclerosis, the most common pathologic process underlying cardiovascular disease (CVD) that is often manifested clinically as coronary disease, stroke, or peripheral arterial disease [14].

In a cross-sectional analysis, a positive association was found between arsenic exposure and high pulse pressure [15], an indicator of arterial stiffness associated with an increased risk of atherosclerosis and CVD [16].

Pimobendan acts as a calcium sensitizer and phosphodiesterase (PDE) - III inhibitor and causes peripheral vasodilatation and also hasten the cardiac contractility both through itself or its metabolite [17]. The blended effect of those two moves ends in expanded cardiac output without a growth in myocardial oxygen demand [18]. This effect is crucial as different high quality inotropes have a destructive impact on survival times in humans with congestive heart failure [19].

Pimobendan is a singular agent which can be surprisingly applicable within the clinical management of congestive heart failure secondary to each dilated cardiomyopathy and chronic degenerative valvular disorder in dogs. A overview of to be had data indicates that pimobendan is secure, nicely-tolerated, and ends in better quality of existence in dogs with congestive heart failure secondary to dilated cardiomyopathy or persistent valvular disease when utilized in mixture with furosemide or different traditional treatment plans. In India, pimobendan is used in veterinary clinical practice in the treatment of acute and chronic heart failure in pet animals. It is also used as a marker of oxidative stress in dogs with heart failure [20]. Based on previous literature, we hypothesize that pimobendan could reduce the risk of development of cardiovascular dysfunctions in the arsenic exposed subjects.

Material and Methods

Male Wistar rats (N=24; 4-6 weeks old) obtained from the Laboratory Animal House of the Veterinary College, Shivamogga were employed for the study. The animals were maintained under standard practices as per the guidelines of the committee for the supervision and control of animal experiments (CPCSEA, New Delhi). The experimental study was imitated after necessary approvals from the Institutional Animal Ethics Committee (Ref. No.VCS/IAEC/13/2017-18 dated 10.06.2017) before initiating the experimental study.

After acclimatization for a period of one week, the rats were divided into four groups of six each. Rats belonging to Group I and II were received only arsenic free portable drinking water. Animals in Group III were received as sodium arsenite @ 100 ppm through drinking water. The animals in Group IV were subjected to evaluate the ameliorative potential of pimobendan in arsenic-induced cardiovascular dysfunction. The rats in Group IV were exposed to arsenic as in Group III. Animals in groups II and IV were administered pimobendan (1 mg/kg) through the *oro-gastric* route once daily during the last 30 days (*i.e*., day 61 to 90) of the experimental study.

On 91st day, experimental rats were sacrificed by bleeding from the posterior vena cava after intraperitoneal (*i.p*) administration of anesthetic cocktail (Ketamine @ 80 mg/kg b.wt and diazepam 2.2 mg/kg b.wt). Further, thoracic aorta and heart were collected and cleared of the perivascular adhering tissues, washed with ice-cold phosphate-buffered saline (PBS), and kept at -80˚C until further analysis.

Preparation of tissue homogenate

Each one hundred milligram of heart and aortic tissue was taken in 1 ml of ice-cold phosphate buffer saline (PBS) (Composition: NaCl 8 g; KCl 0.2 g; KH_2PO_4 0.24 g; Na_2HPO4 1.44 g in 1 liter of distilled water; pH 7.4). Another 20 mg of tissue was taken separately in 0.2 ml of 0.02 M EDTA for estimation of reduced glutathione (GSH). The homogenate (10%) was prepared with a homogenizer (IKA, Germany) under ice-cold condition and centrifuged for 10 min at 3000 rpm. The supernatant was stored at -20ºC until biochemical assays.

Protein estimation

The protein content in the supernatant of aortic and heart tissue homogenates was determined according to the method described by Lowry *et al*. [21] using bovine serum albumin as an internal standard.

Assessment of Peroxidative (LPO) damage

Aortic and heart peroxidative damage was assessed by evaluating lipid peroxidation (LPO) in terms of thiobarbituric acid-induced reducing substances (TBARS) called malondialdehyde (MDA) production as described by Paula *et al*. [22]. Results have been expressed as nmol MDA formed/g of tissue.

Determination of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity in the aorta and heart was determined by the procedure of Madesh and Balasubramanian [23]. The absorbance was read at 570 nm and the activity was expressed as Unit/mg protein.

Determination of Catalase (CAT) Activity

Catalase activity in the aortic and heart homogenate was assayed by the spectrophotometric method of Aebi [24]. The activity was expressed as mmol H_2O_2 utilized/min/mg protein.

Determination of Glutathione Reductase (GR) Activity

GR activity in the aorta and heart were measured following the method of Goldberg and Spooner [25]. The activity of GR was expressed as µmol NADPH oxidized to NADP/mg protein/min.

Determination of Glutathione Peroxidase (GPx) Activity

GPx activity was determined by the method of Paglia and Valentine [26]. The enzyme activity was expressed as µmol of NADPH oxidized to NADP/mg protein/min.

Determination of Reduced Glutathione (GSH) Content

GSH content was measured in the aortic and heart homogenate by the method of Sedlak and Lindsay [27]. The level of GSH was expressed as mmol of GSH/g of wet tissue.

Estimation of Superoxide Radical Anion ($O_2^{\cdot -}$) formation

Superoxide radical anion generation was estimated indirectly in terms of formazan (blue color) formed due to the reduction of nitroblue tetrazolium (NBT) as an index of superoxide anion generation and measured (formazan) by using a spectrophotometer at a wavelength of 540 nm [28].

Measurement of nitrite level

Nitric oxide (NO) can react with molecular oxygen and water to form the stable oxidized products, nitrite, and nitrate. So the measurement of nitrite level is used to assess cellular/tissue NO generation. The nitrite level was estimated on the day of the sacrifice of animals by the method described by Zhang *et al*. [29].

Enzyme-linked immunosorbent assay (ELISA)

The level of Interleukin -1β (IL-1β), Interleukin-6 (IL-6), Tumor Necrosis factor- α (TNF-α) in aortic and heart

homogenate were measured by using ELISA kits as per the protocol described by manufacturers (RayBiotech Inc., USA).

Statistical analysis

The values obtained from the various experiments were expressed as Mean + S.E with 'n' equal to the number of animals. Data obtained were statistically subjected to One-way analysis of variance (ANOVA) followed by Duncan's *post hoc* multiple comparison test using SPSS statistic software (IBM® SPSS® statistic software, Version 20.0.0, 2011, Armonk, NY, USA). The difference were considered significant at $p<0.05$ or lower. Graphical presentation of the data was carried out by using the Graph Pad Prism software program (GraphPad® software Inc., Version 7.0; San Digo, CA, USA).

Results and Discussion

Figure 1 summarizes the effects on lipid peroxidation (nmole MDA /g tissue). The level of LPO was 107.12±5.67 and 130.12±5.67 in the aorta and heart in the control group, respectively. Pimobendan alone did not alter LPO in the aorta (90.93±4.05) as well as in the heart (113.93±4.05). Arsenic exposure significantly increased MDA level to in the aorta (118.46±8.59) and heart (145.46±8.59), which was significantly ($p<0.05$) reduced by pimobendan (88.64±4.32; aorta) and (114.64±4.32; heart).

Figure 2 presents the effects of pimobendan on the activity of SOD (Units/mg protein). In the control rats, its activity was 9.15±0.35 in the aorta and 13.48±0.50 in the heart. It was not altered with pimobendan in the aorta (9.42±0.57) and heart (14.76±0.90). In the arsenic-exposed rats, it was significantly ($p<0.05$) decreased to 7.10±0.21; 9.95±0.99 in the aorta and heart, respectively.

Pimobendan brought the activity back (9.33±0.44; aorta) and (14.83±1.68; heart) to the control level.

Effects of pimobendan on the activity of catalase (mmol H2O2 utilized/min/mg protein) have been presented in figure 3. Its activity in the control is 123.30±6.08 (aorta) and 135.90±9.15 (heart). Pimobendan treated groups its activity significantly increased to 141.23±6.30 and 161.23±6.30 in the aorta and heart, respectively. Arsenic significantly ($p<0.05$) decreased its activity to 92.69±4.12 (aorta) and 107.69±2.33 (heart) and this effect were significantly attenuated with pimobendan (117.75±5.32; aorta and 139.42±5.65; heart) to the control level.

Effects on the activity of GR (µmol NADPH oxidized to NADP/ min/mg protein) have been presented in figure 4. The activity was 121.14±5.58 (aorta) and 141.14±4.30 (heart) in the control group and pimobendan treated 134.92±3.90 (aorta) and 173.22±3.48 (heart) were comparable with control. In the arsenic-treated group, GR activity was significantly ($p<0.05$) increased **(**89.10±5.09; aorta and 116.66±5.09; heart), which was significantly reduced to 117.97±7.46 (aorta) and 154.07±7.46 (heart) with pimobendan.

Figure 5 presents the effects of pimobendan on the activity of GPx (µmole of

NADPH oxidized to NADP/min/mg protein). In the control rats, its activity was 27.87±1.63 and 35.43±2.01 in the aorta and heart, respectively. It was not altered with pimobendan in the aorta (29.10±1.14) as well as in the heart (39.44±1.27). In the arsenic-exposed rats, it was significantly ($p<0.05$) decreased to 20.04±1.88 in the aorta and 29.27±1.69 in the heart. Pimobendan restored the activity (27.91±1.26; aorta and 41.12±1.26; heart) to the control level.

Figure 6 shows the effects of pimobendan on the GSH content (mmol GSH/g tissue) in rat aorta and heart. In the control rats, its level was 2.48±0.09 in aorta and 4.48±0.09 in heart, which was not altered with pimobendan in aorta (2.69±0.08) as well as in heart (5.09±0.28). In the arsenic-exposed rats, GSH content was significantly ($p<0.05$) decreased to 1.59±0.11 and 3.32±0.13 in aorta and heart, respectively. However, pimobendan treatment (2.86±0.24; aorta and 5.18±0.35; heart) in the arsenic-exposed rats restored its level back to the control value.

The generation of superoxide anion radical (pmol/min/mg protein) has been presented in figure 7. The reduced NBT level was 15.62±1.94 (aorta) and 35.56±1.71 (heart) in the control group. It was not altered with pimobendan treatment in the aorta (14.52±1.83) and heart (30.62±1.94). Arsenic significantly increased ($p<0.05$) its generation (21.85±1.21; aorta and 43.35±2.30; heart), which was significantly reduced (17.40±1.01; aorta and 23.68±1.14; heart) to the control level with pimobendan.

The *i*NOS-mediated nitrite production (pmol/mg wet tissue) has been presented in figure 8. In the control rats, its concentration was 13.95±1.39 (aorta) and 11.45±0.54 (heart). It was not altered with pimobendan treatment (11.68±0.63; aorta and 10.35±0.67; heart). But it was significantly ($p<0.05$) increased by arsenic in the aorta (22.35±0.77) and heart (21.35±1.02). In the arsenic-exposed rats, pimobendan brought the level (13.23±0.94; aorta and 11.40±0.80; heart) statistically similar to its level in control group.

Figure 9 summarizes the effects of pimobendan on the IL-1β (pg/mg protein) level. It was decreased marginally with pimobendan treatment (74.52±4.29; aorta and 68.28±5.00; heart) compared to the control level (87.11±2.16; aorta and 79.70±4.69; heart), but arsenic significantly ($p<0.05$) increased its level in the aorta to 117.53±5.32 and 105.44±6.97 in heart. In the arsenic-exposed rats, pimobendan co-administration significantly reduced its concentration which was statistically (61.62±5.08; aorta and 64.52±4.88; heart) to the control level.

Figure 10 summarizes the effects on IL- 6 level (pg/mg protein). In the control rats, its concentration was 281.87±11.5 in the aorta and 546.68±37.42 in the heart. It was not altered with pimobendan treatment (234.52±09.37; aorta and 491.60±59.16; heart). Arsenic alone significantly ($p<0.05$) increased its level to 330.94±08.78 in the aorta and 637.35±55.63 in the heart.

Pimobendan brought the arsenic-mediated rise back to the control level in the aorta (230.28±11.77) as well as in the heart (437.85±25.30).

Figure 11 shows the effects on the level of TNF-α (pg/mg protein). TNF-α concentration was 52.85±3.08 and 79.28±1.78 in the aorta and heart respectively, in the control group. Pimobendan treatment marginally reduced it to 48.38±5.33 in the aorta but significantly ($p<0.05$) reduced it to 67.35±2.97 in the heart, while arsenic significantly increased to 68.02±4.68 (aorta) and 88.18±1.96 (heart). However, pimobendan reversed the effect of arsenic by bringing the level to 33.49±3.03 (aorta) and 68.71±1.40 (heart).

Cardiovascular dysfunctions in arsenic-epidemic geographical areas in humans, and experimental studies are a well known fact. The primary objective of the present study was to whether pimobendan could reduce the risk of development of cardiovascular dysfunctions in the arsenic exposed subjects. In the present study, subchronic exposure to arsenic through

drinking water with respect to induction of oxidative stress and modulation of antioxidant status in the cardiovascular tissue. We also sought to assess whether the outcome of the cardiovascular disturbance could be associated with the changes in inflammatory mediators, such as IL-1β, IL-6, and TNF-α, which are considered classical indicators of toxicity development. The major findings of the current study are: arsenic-induced oxidative stress, decreased antioxidant status, increased in *i*NOS-mediated nitrite production, and inflammatory mediators in the aorta and heart which was ameliorated by pimobendan.

Maintaining the redox balance in the cardiovascular system is important because impaired redox signaling leading to oxidative stress contributes to endothelial dysfunction and cardiovascular disease. LPO is regarded as one of the basic mechanisms of tissue damage caused by free radicals and extensively used as a marker of oxidative stress [30]. Several studies have shown that arsenic causes the generation of free radicals, including ROS such as $O_2^{\bullet}$ and hydrogen peroxide (H_2O_2), in vascular smooth muscle cells [31], vascular endothelial cells [32], rat thoracic aorta [33, 34] and in the heart [35]. These free radicals can react with biological molecules such as lipids, proteins, and DNA resulting in structural and functional abnormalities [36]. A fine balance between the presence of ROS and antioxidants is essential for the proper normal functioning of the cell [37]. ROS has been recognized as an important risk factor in cardiovascular dysfunction and the development of several vascular diseases such as hypertension [33], arteriosclerosis, myocardial infarction, and stroke [38]. In the present study, arsenic increased LPO and $O_2^{\bullet}$ suggesting that arsenic causes ROS-mediated induction of oxidative stress in rat aorta and heart. Our observations in the present study can be correlated with the fact that long-term exposure to arsenic through drinking water at high groundwater contamination level could lead to oxidative stress-mediated cardiovascular dysfunctions and consequent cardiovascular diseases. A similar observation in the aorta was also made in a previous study [33, 34] and the authors opined that pimobendan decreased the production of $O_2^{\bullet}$ via reducing NADPH-dependent oxidase activity.

Glutathione is an endogenous antioxidant and protects cells in opposition to ROS [39]. The enzymatic antioxidants are the primary line of defense against oxidative stress [30]. Sulfhydryl organization of cysteine moiety in GSH has a sturdy affinity for arsenic or GSH may be oxidized due to the interplay with the unfastened radicals prompted by means of arsenic [40]. Similarly, in another study the metabolites of arsenic formed within the cellular by means of reaction with GSH alter ratio of GSH: GSSG with the aid of inhibiting GR [41]. In our study, a growth in LPO changed into associated with depletion of GSH in arsenic exposed rats indicating dependency of LPO on GSH. It was additionally associated with a lower in the pastime of GR, which regenerates GSH from GSSG thru an NADPH Pathway to maintain GSH deliver to its established enzymes, along with GPx. In the present study, depletion of GSH within the aorta and heart may also relate to its interaction with arsenic and the ROS generated with the aid of arsenic, and additionally to the reduction of GR interest. SOD has the primary defence line towards oxygen derived catalyzes the dismutation of the $O_2^{\bullet}$ into H_2O_2, that's then converted into H_2O and O_2 with the aid of catalase [42], where as GPx removes excess H_2O_2 through a GSH-dependent mechanism [43]. Arsenic decreased the activities of SOD, catalase, and GPx, GR in the aorta and heart, indicating suppression of the antioxidative defense mechanisms. Pimobendan co-administration to arsenic exposed rats significantly improved the arsenic mediated inhibition in the levels of antioxidant enzymes and the GSH content in the aorta and heart. The reduced antioxidant enzyme levels could be attributable to increased utilization of enzymes for maintenance of redox homeostasis and direct binding of arsenicals to dithiol- targets [44].

Further, suggested that the ROS-mediated aortic and heart LPO was compounded by the accumulation of ROS due to a reduction in the neutralization of ROS by the antioxidants. Thus, the rise in arsenic-mediated ROS levels in the aorta and heart could relate to its generation as well as accumulation. Decreased GSH content and GR activity could be a reason for the reduced GPx activity. The decreased SOD activity suggests that accumulation of $O_2^{\bullet}$ due to reduction in dismutation of $O_2^{\bullet}$. On the other hand, the decreased catalase and GPx activities indicate a reduction in H_2O_2 neutralization, implying its accumulation. Furthermore, it is known that $O_2^{\bullet}$ can also be spontaneously converted to H_2O_2 [43]. In the present study, the decrease in the activities of SOD and catalase in cardiovascular tissue may be attributed to their mutual functioning for eliminating ROS.

Sub-chronic exposure to arsenic showed a significant increase in levels of

pro-inflammatory cytokines *viz*., IL-1β, IL-6, and TNF-α and their level brought back to control by pimobendan in the aorta and heart. Similar to our study, Kesavan *et al*. [45] also observed a similar trend of increase in the levels of cytokines in aortic tissue. The probable mechanism involved might be the activation of signaling pathways like ERK, JNK, p38-MAPK, and NF-κB, which in turn induces the expression of a variety of pro-inflammatory genes [46]. Pimobendan attenuates cardiac dysfunction by blocking proinflammatory cytokine production and nitric oxide synthesis [47, 48]. In the present study, pimobendan ameliorates the arsenic mediated cardiovascular damage is due to increased cAMP levels as a response to adenylyl cyclase agonists might block fibroblast proliferation and protein synthesis *viz*. type I collagen, cytokines, and smooth muscle actin [48] and PDE_3 inhibition suggested cardioprotective effects through cAMP [49].

In our previous studies have shown that vascular endothelial damage and reduced vasodilatation are two main events of cardiovascular endothelial dysfunction by arsenic exposure [33, 34]. The above results demonstrated that arsenic exposure causes endothelial cell damage. We also measured the iNOS mediated nitrite production to expansively determine whether cardiovascular endothelial dysfunction was significantly impaired?. NO is the most important vasodilator factor secreted by endothelial cells and catalyzed by nitric oxide synthase [32]. In the present study, we found that iNOS mediated nitrite production significantly increased, but pimobendan administration reduced it. Based on the changes an *in-vivo* model of arsenic-induced cardiovascular endothelial dysfunction and recovery from it was successfully established by pimobendan.

Based on our results, it may be concluded that sub-chronic exposure to arsenic through drinking water can cause cardiovascular dysfunction and oxidative damage in rat aorta and heart. Pimobendan has the potential to ameliorate the arsenic-mediated biochemical disorders by restoring pro-inflammatory cytokine production, NO synthesis by *i*NOS, and ROS-mediated redox signaling.

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Competing Interests
The authors have no conflict of interest to declare.

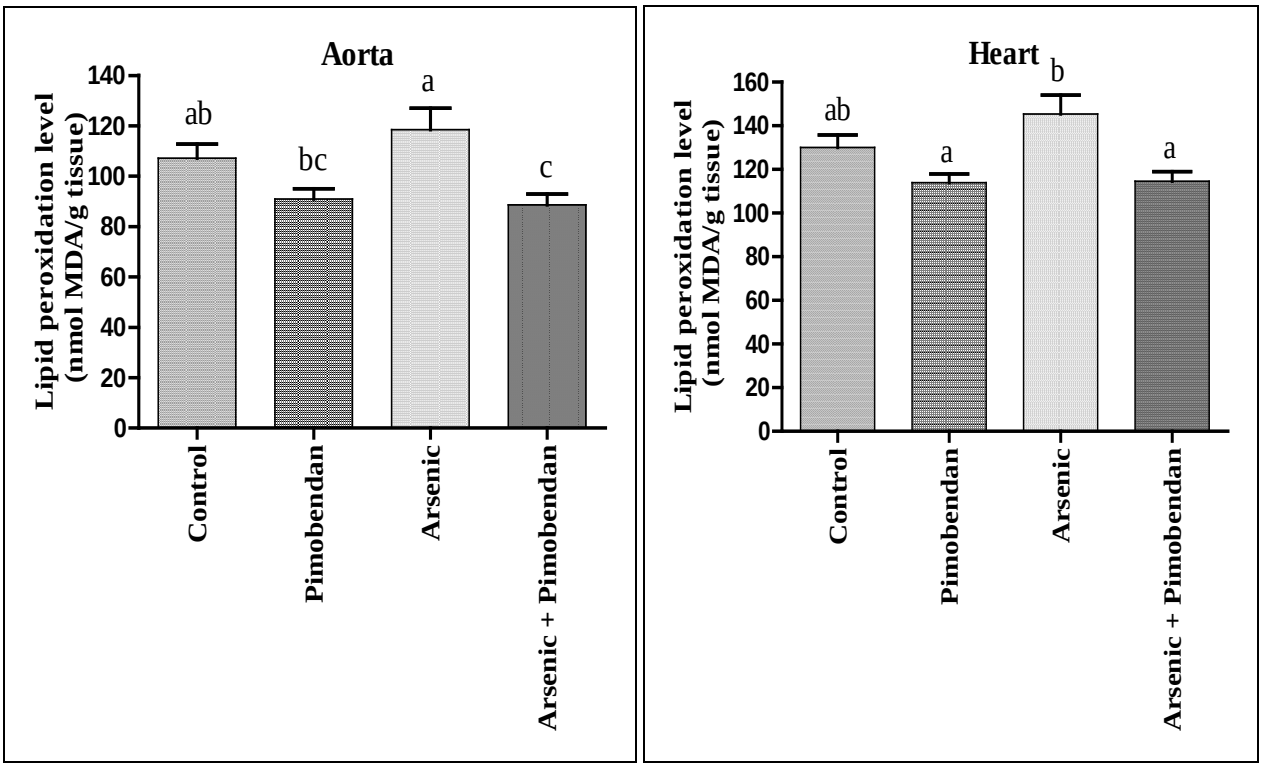

Fig 1: Effects of pimobendan on lipid peroxidation (LPO) level in the arsenic-exposed rat aorta and heart. Each bar represents mean ± SE (n=6). Bars bearing no superscripts common vary significantly ($p< 0.05$) in Duncan's multiple comparison *post-hoc* test.

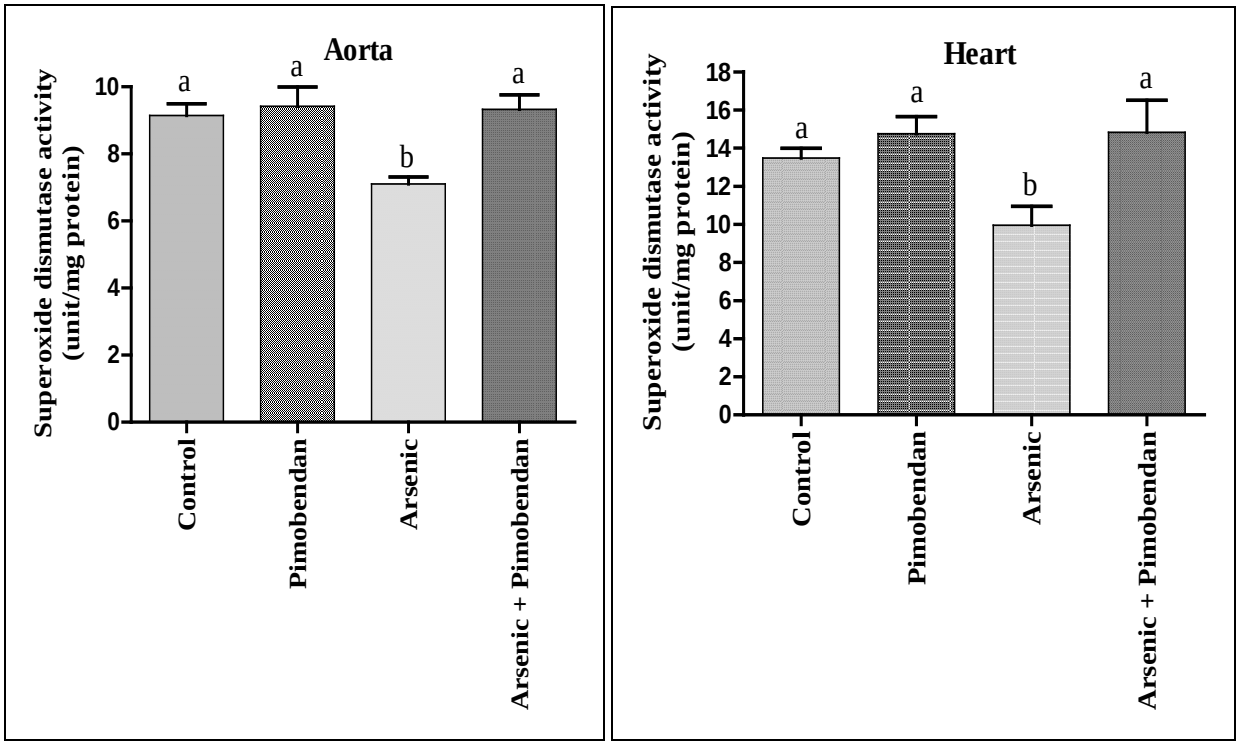

Fig 2: Effects of pimobendan on superoxide dismutase (SOD) activity in sodium arsenite-exposed on rat aorta and heart. Each bar represents mean ± SE (n=6). Bars bearing different superscripts vary significantly ($p< 0.05$) in Duncan's multiple comparison *post-hoc* test.

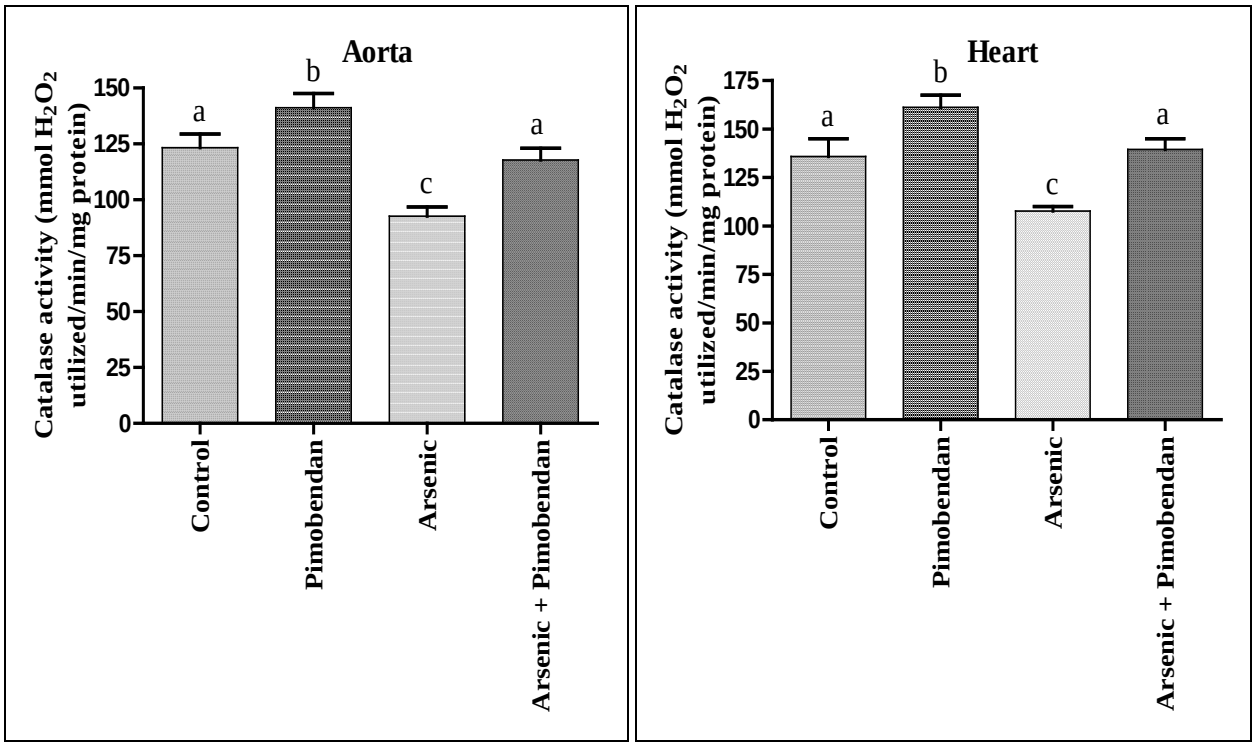

Fig 3: Effects of pimobendan on catalase (CAT) activity in sodium arsenite-exposed on rat aorta and heart. Each bar represents mean ± SE (n=6). Bars bearing different superscripts vary significantly ($p< 0.05$) in Duncan's multiple comparison *post-hoc* test.

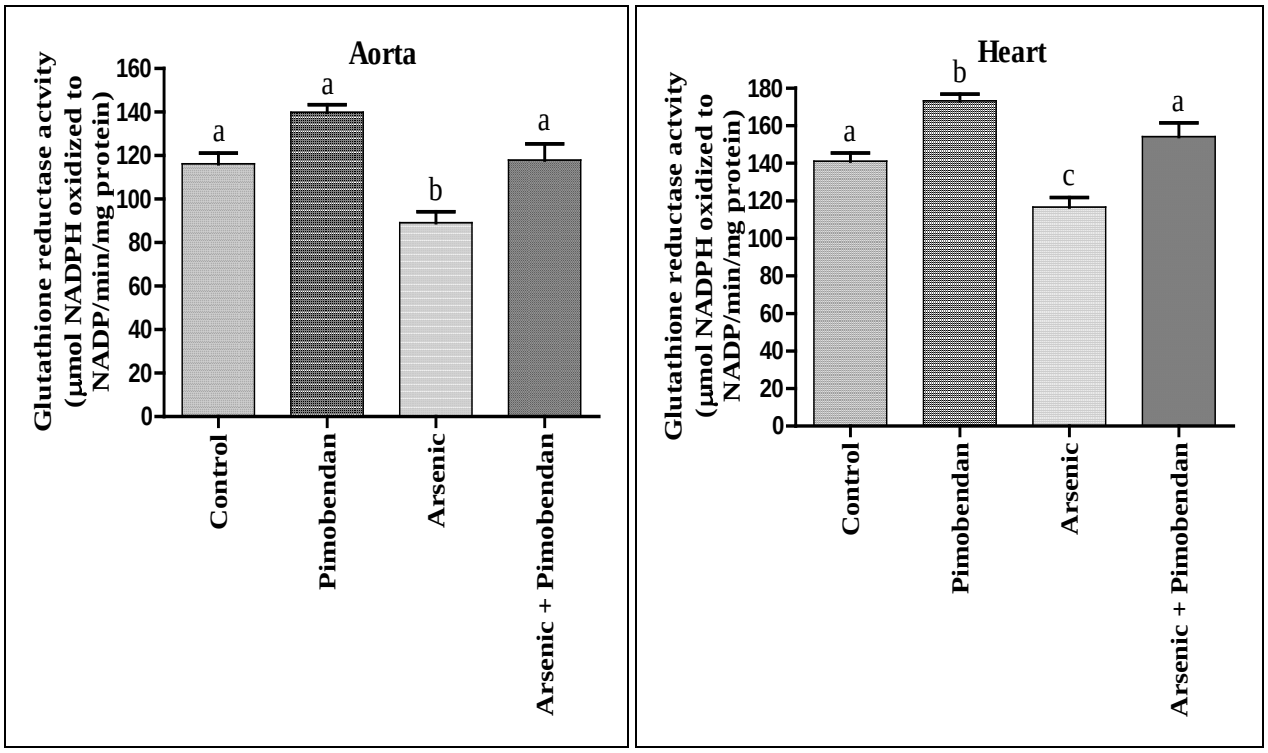

Fig 4: Effects of pimobendan on glutathione reductase (GR) activity in sodium arsenite-exposed on rat aorta and heart. Each bar represents mean ± SE (n=6). Bars bearing different superscripts vary significantly ($p< 0.05$) in Duncan's multiple comparison *post-hoc* test.

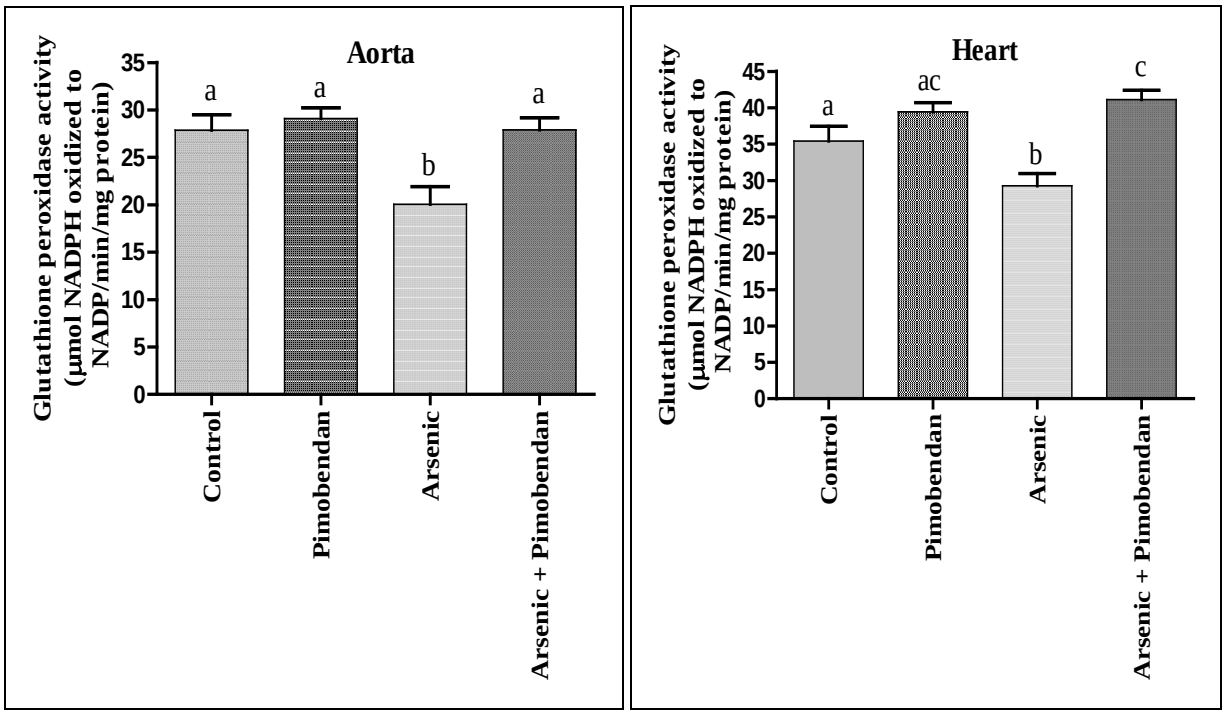

Fig 5: Effects of pimobendan on glutathione peroxidase (GPx) activity in sodium arsenite-exposed on rat aorta and heart. Each bar represents mean ± SE (n=6). Bars bearing no superscripts common vary significantly (p<0.05) in Duncan's multiple comparison *post-hoc* test.

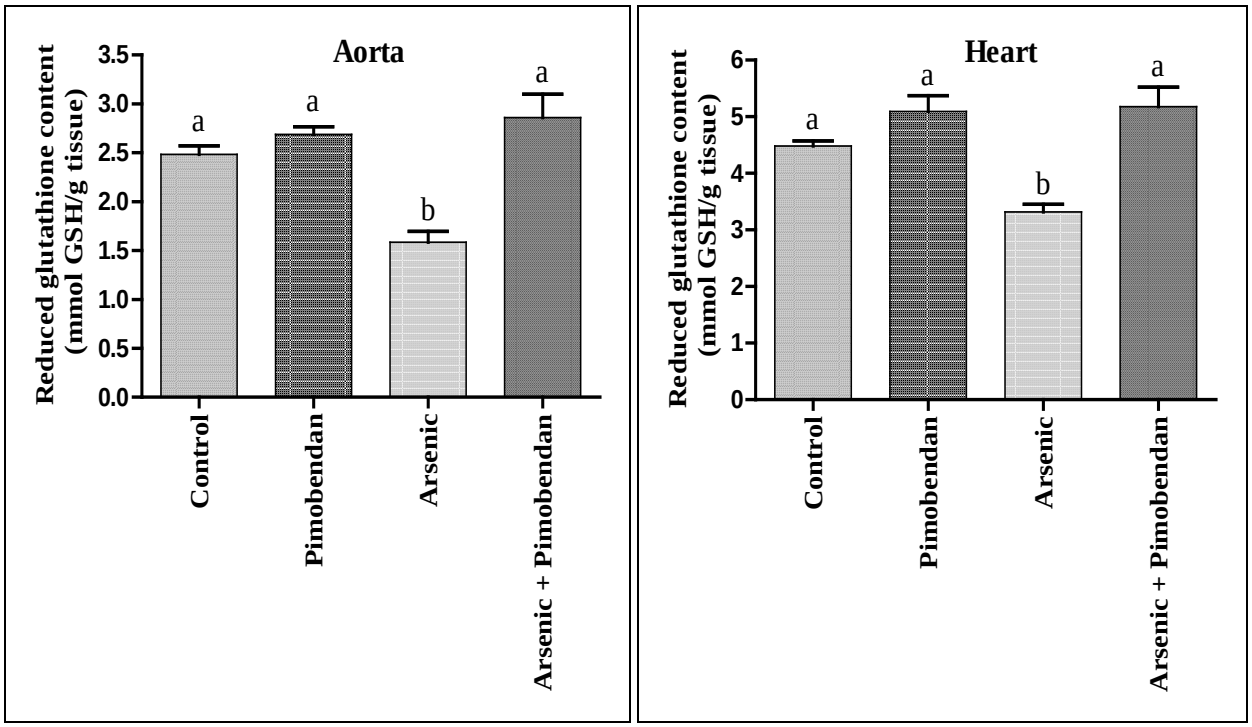

Fig 6: Effects of pimobendan on glutathione (GSH) content in sodium arsenite-exposed on rat aorta and heart. Each bar represents mean ± SE (n=6). Bars bearing different superscripts vary significantly (p<0.05) in Duncan's multiple comparison *post-hoc* test.

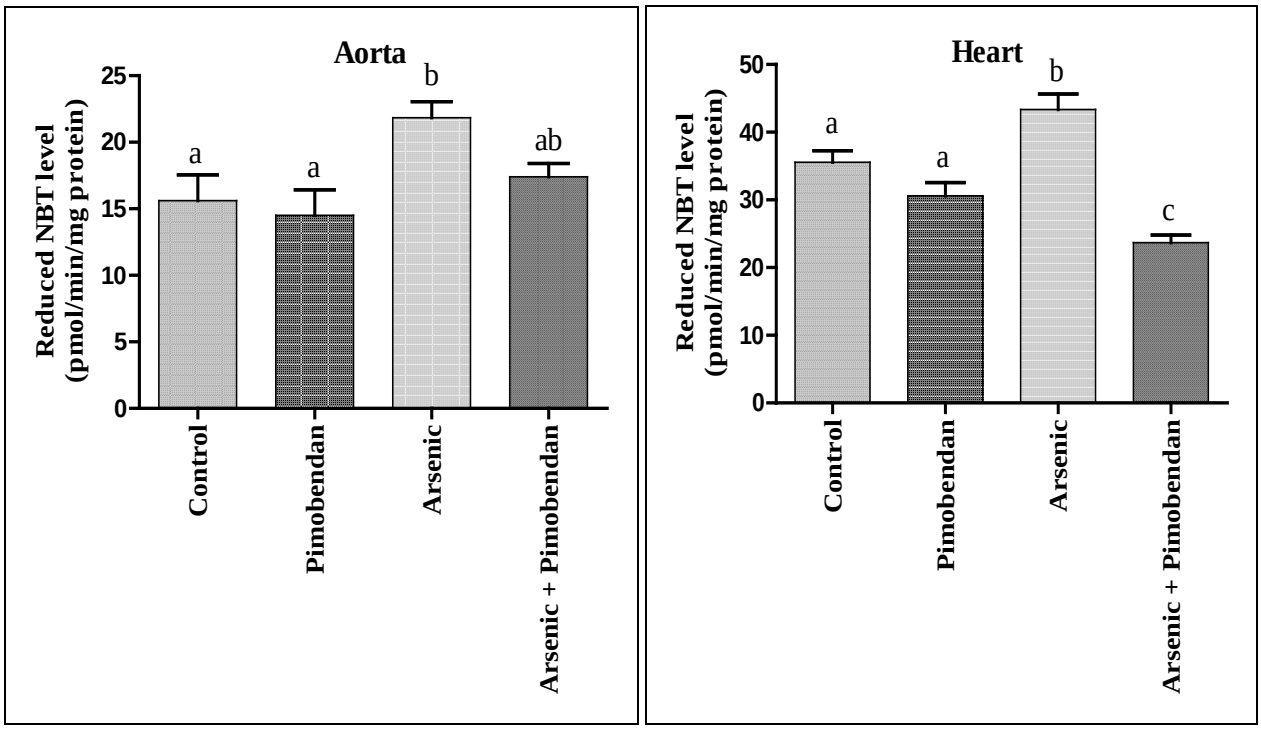

Fig 7: Effects of pimobendan on superoxide anion radical ($O_2^{\bullet-}$) generation in the arsenic-exposed rat aorta and heart. Each bar represents mean ± SE (n=6). Bars bearing no superscripts common vary significantly ($p<0.05$) in Duncan's multiple comparison post-hoc test.

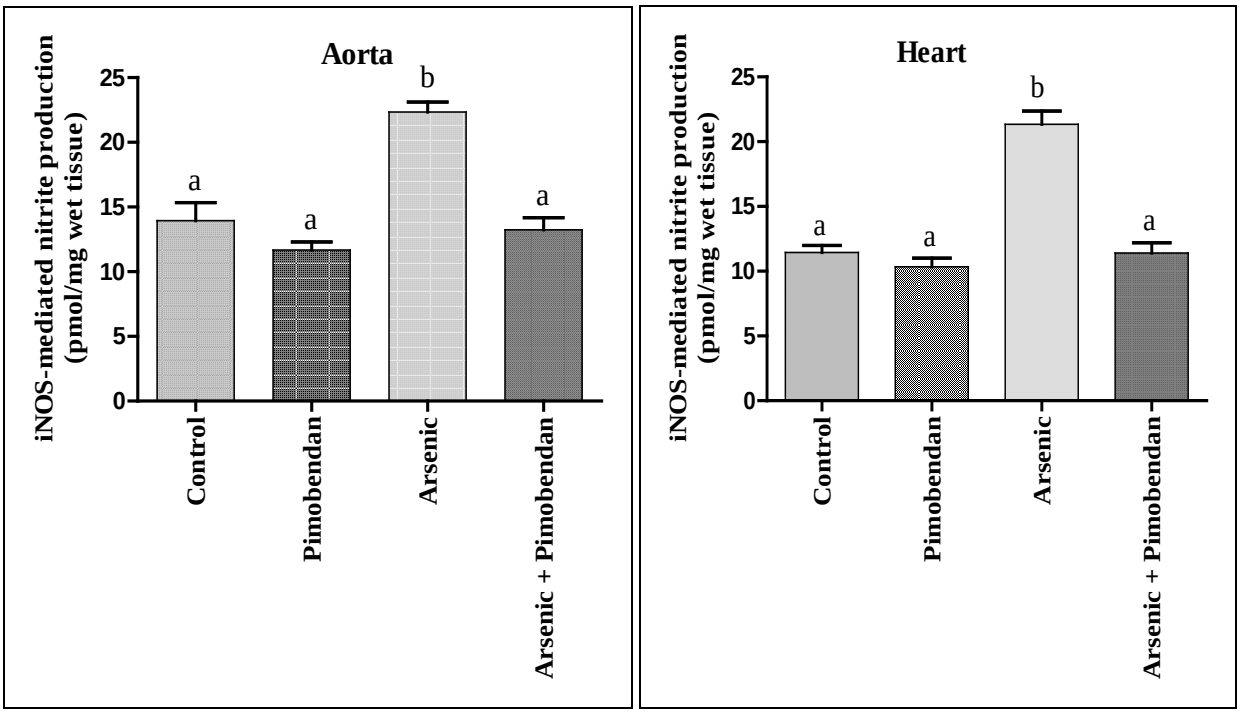

Fig 8: Effects of pimobendan on levels of inducible nitric oxide synthase (*i*NOS)-mediated nitrite production in the arsenic-exposed rat aorta and heart. Each bar represent mean ± SE (n=6). Bars bearing different superscripts vary significantly ($p<0.05$) in Duncan's multiple comparison *post-hoc* test.

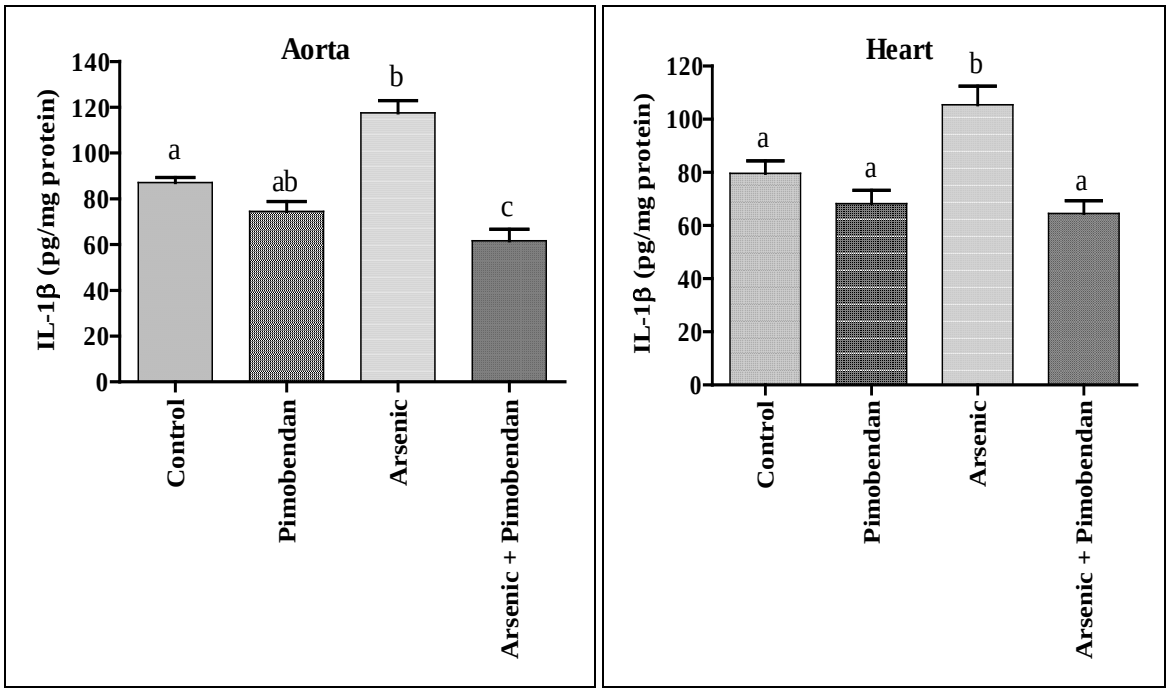

Fig 9: Effects of pimobendan on interleukin - 1β (IL-1β) in the arsenic-exposed rat aorta and heart. Each bar represent mean ± SE (n=6). Bars bearing no superscripts common vary significantly (p<0.05) in Duncan's multiple comparison *post-hoc* test.

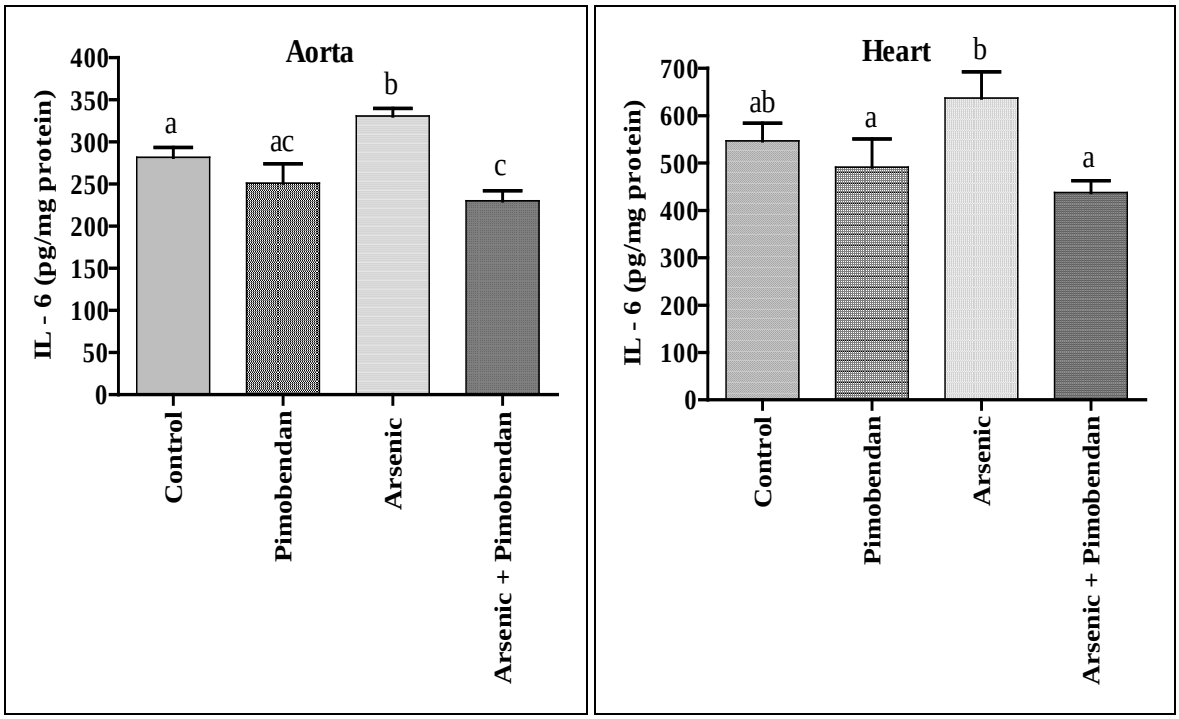

Fig 10: Effects of pimobendan on interleukin - 6 (IL-6) in the arsenic-exposed rat aorta and heart. Each bar represent mean ± SE (n=6). Bars bearing no superscripts common vary significantly (p<0.05) in Duncan's multiple comparison *post-hoc* test.

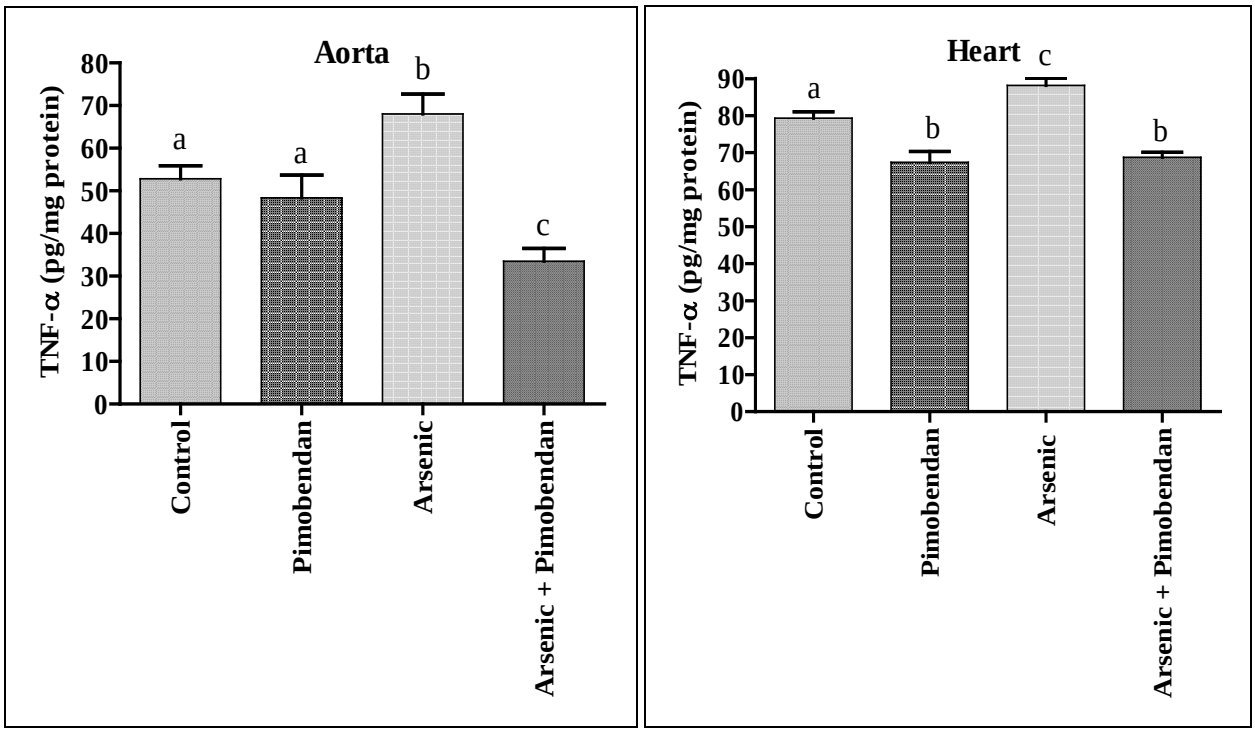

Fig 11: Effects of pimobendan on tumor necrosis factor - α (TNF- α) in the arsenic-exposed rat aorta and heart. Each bar represents mean ± SE (n=6). Bars bearing different superscripts vary significantly ($p< 0.05$) in Duncan's multiple comparison *post-hoc* test.

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