Hesperidin attenuates arsenic trioxide−induced cardiac toxicity in rats

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ABSTRACT

Objective: To explore the cardioprotective effect of hesperidin against arsenic trioxide-induced cardiac toxicity in rats.

Methods: Cardiac toxicity was induced by oral administration of 4 mg/kg arsenic trioxide for 30 days. Hematological, biochemical, electrocardiography, echocardiography, and histopathological examinations were performed.

Results: Hesperidin decreased the neutrophil-to-lymphocyte ratio, calcium, creatine kinase-myoglobin binding, lactate dehydrogenase, IL-6, and lipid peroxidation, as well as increased sodium and potassium concentration and superoxide dismutase and catalase activity in arsenic trioxide-intoxicated rats. Moreover, it reduced peak systolic velocity and end-diastolic velocity while increasing heart rate. Arsenic trioxide-induced histopathological damage to cardiac tissue was prominently alleviated by hesperidin treatment.

Conclusions: Hesperidin attenuates arsenic trioxide-induced cardiac toxicity in rats. Therefore, it can be further explored as a cardioprotective agent.

KEYWORDS: Arsenic trioxide; Hesperidin; Cardiotoxicity; ECG; CK-MB; LDH

1. Introduction

The clinical application of arsenic trioxide for the treatment of acute promyelocytic leukemia (APL) started in the 1970s at Harbin Medical University[1]. Arsenic trioxide is promptly used as an antileukemic medicine for the cure of newly diagnosed and relapsed APL[2]. Unfortunately, as it caused lethal renal, hepatic, and cardiac toxicity, its clinical utility is limited. Arsenic trioxide is responsible for inducing toxic cardiac effects such as prolongation of QT interval, torsades de pointes, and abrupt cardiac fatality[3]. Adverse impacts like arrhythmic cardiac changes, ventricular tachycardia, or sudden cardiac death were seen when it was used clinically[4].

Drugs that suppress the cardiotoxic potential of arsenic trioxide would permit us to take advantage of the complete chemotherapeutic performance of arsenic trioxide. Several mechanisms are associated with the myocardial toxicities of arsenic trioxide, which include the generation of highly reactive oxygen molecules (ROS) in cardiomyocytes, oxidative damage to DNA, disruptions in DNA repair mechanisms, deposition of arsenic, and alterations in ion channels of cardiac tissue & apoptosis[5,6]. The bioactively rich agents present in several natural sources have essentially acquired

Significance

Arsenic trioxide can cause cardiac toxicity. At present, there is no study on the effect of hesperidin on arsenic trioxide-induced cardiotoxicity *in vivo*. The present study shows that hesperidin has cardioprotective effects against arsenic trioxide-induced cardiotoxicity in rats. Thus, hesperidin can be further explored as a potential agent for the treatment of arsenic trioxide-induced cardiac toxicity.

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importance in modern systems of drugs, reducing the risks of cardiac unrest by eliminating free radical generation and contributing to the entire antioxidant protective system[7].

Hesperidin is a bioflavonoid glycoside that is obtained largely from citrus fruits and isolated from *Citrus aurantium* and various other species of the genus *Citrus* (family: Rutaceae). Various studies have indicated that hesperidin has various favorable therapeutic properties like immuno-modulator, anti-hypertensive, anti-allergic, anti-platelet, anti-carcinogenic, anti-apoptotic, radio-protective, and antioxidant characteristics[8]. The anti-inflammatory effects of hesperidin are said to be caused by inhibition of the p38 MAPK signaling pathway[9]. Various studies have suggested the antioxidant properties of hesperidin are associated with improved production of cellular antioxidative enzymes and scavenging of ROS. ERK/Nrf2 signaling is supposed to augment the antioxidant cellular defenses of hesperidin, owing to which it ideally relieves the stress associated with cancer chemotherapy and radiation therapy[10]. More crucially, hesperidin is capable of protecting the cell membrane of erythrocytes from oxidative damage induced by the oxidation of low-density lipoprotein[11]. It has been shown that hesperidin protects the cardiac tissues against the cardiotoxic effect of doxorubicin in rats as manifested by amelioration of histopathological changes and normalization of cardiac biochemical parameters[12]. Very recently, use of hesperidin was reported to have antioxidant, anti-inflammatory, and anti-apoptotic effects on sodium arsenite-induced toxicity in Sprague-Dawley rats[13]. Hesperidin has been shown in a rodent model to reduce inflammation through suppression of cytokine production, NF-κB activity, and oxidative stress[14]. Hesperidin is also reported to inhibit oxidative stress and inflammation by downregulation of TNF-α, IL-β, IL-6, and IL-10[15]. Hesperidin also possesses a cardioprotective effect against cardiac ischemia and reperfusion injury in diabetic rats *via* the PPAR-γ pathway[16]. Based on the abovementioned properties of hesperidin, we aimed to evaluate the effect of hesperidin against arsenic trioxide-induced cardiac toxicity.

2. Materials and methods

2.1. Experimental animal

Healthy adult male albino Wistar rats weighing 150-180 g were procured from CPCSEA registered organization along with their pellet feed. Initially, all the animals were kept as such for one week for acclimatization inside the lab animal house in separate clean polypropylene cages with stainless steel grills and soft bedding material. Animals were given free access to preformulated pellet feed and fresh drinking water and housed on a 12-h light and dark cycle at a constant temperature (22.0+1.0) ℃ and humidity (55.0±1.0)%.

2.2. Drugs and chemicals

Arsenic trioxide (CDH Chemicals, India), hesperidin (Sigma), ethanol (Bengal Chemicals), normal saline solution (freshly prepared), 1 mol/L NaOH, phosphate buffered saline (pH 7.4), buffered natural formalin, xylazine, and ketamine injection (NEON Laboratories Limited), TRIS-HCl (SRL, India), H_2O_2 (SRL, India), and trichloroacetic acid (CDH Chemicals, India) were used in the study.

2.3. Experimental design

Arsenic trioxide stock solution was prepared by dissolving 0.1 g of arsenic trioxide in 10 mL of 1 mol/L NaOH, making the concentration of the stock solution 0.01 g arsenic trioxide/mL of 1 mol/L NaOH. This stock solution was stored in a refrigerator at 4 ℃. Every time during dosing of animals, 1 mg/mL arsenic trioxide was prepared fresh with normal saline from stock solution by ten-fold dilution. Hesperidin was prepared by dissolving 30 mg in 200 μL of 1 mol/L NaOH and the volume was made to 1 mL by adding 800 μL of distilled water by five-fold dilution. Animals were administered hesperidin solution at 100 mg/kg body weight *p.o.*

Rats were randomly divided into four groups of six rats each. The first group received normal saline orally and served as the control. The second group received arsenic trioxide administration at 4 mg/kg[17] orally for 30 d. The third group was treated with hesperidin at 100 mg/kg[16] *p.o.* 1 h before oral administration of arsenic trioxide for 30 d. The fourth group was administered only hesperidin at 100 mg/kg *p.o.* for 30 d. Sodium hydroxide (1 mol/L) was used for dissolving the drugs and further diluted to 10 fold and 5 fold with normal saline, respectively, for oral gavage in rats as per Sibley *et al*[18].

2.4. Hematological analysis

Briefly, 0.5-1 mL of whole blood was collected from the retroorbital sinus of rats with the help of capillary tubes and placed in a 4 mL Becton Dickinson Vacutainer spray-coated with anticoagulant K2EDTA tubes for hematological analysis on day 0 and 24 h after the last treatment. Hematological parameters including hemoglobin (Hb) (g/dL), packed cell volume (PCV), total platelet count, total red blood cell (RBC) count, total white blood cell (WBC) count, neutrophils, eosinophils, lymphocytes, and monocytes were measured using an automated hematology analyzer (Model-H560, Transasia Erba).

2.5. Biochemical analysis

The serum samples were collected from freshly collected blood in a Becton Dickinson Vaccutainer SST tube containing spray-coated silica and a polymer gel for serum separation. The serum was

collected aseptically after separation and stored immediately at –20 ℃. Biochemical parameters including sodium, potassium, calcium, creatine kinase-myoglobin binding (CK-MB), and lactate dehydrogenase (LDH) were measured by the Microlab 300 Semi-automatic clinical chemistry analyzer (ELITech Group, France) using commercially available kits (CORAL Kits).

2.6. Antioxidant enzyme and proinflammatory cytokine assay

Cardiac tissue homogenate was prepared by rinsing the heart tissue in ice-cold isotonic saline and drying it with filter papers. Then, 10% tissue homogenate was prepared in Tris-HCL buffer (0.02 mol/L, pH=7.4) and centrifuged at 1 000 rpm for 2 min at 4 ℃. The supernatant was used for determination of lipid peroxidation and antioxidant enzymes [superoxide dismutase (SOD) and catalase]. Thiobarbituric acid reactive substance activity was measured as per Botsoglou *et al*.[19]. SOD and catalase were measured according to the methods of Madesh and Balasubramanium[20] and Aebi[21], respectively.

The serum samples were collected aseptically from freshly collected blood in Becton Dickinson Vaccutainer SST tubes containing spray-coated silica and a polymer gel. IL-6 was assayed by using an Invitrogen IL-6 rat ELISA kit (Thermo Fisher Scientific) following the manufacturer's instructions.

2.7. Electrocardiography (ECG)

On day 0 and 24 h after the last dose, all the rats were anesthetized with ketamine at 100 mg/kg body weight and xylazine at 5 mg/kg body weight *i.p.* 20 min before ECG recording. The dose of ketamine and xylazine was decided as per Hemmati *et al*[22]. The hypodermic needles attached to ECG electrodes were secured under the skin of the right forelimb (negative electrode), left hindlimb (positive electrode), and right hindlimb (earthing electrode). After fixing the electrodes, readings were taken for 1 h and 30 min after which animals were almost recovered from anesthesia. ECG recordings were taken in an 8/32 power lab (AD Instrument, Australia) with an animal Bio-Amp and analyzed by Lab Chart Pro-7 software. Each channel was amplified and sampled at a rate of 2 kHz and 5 mV range with a high pass filter setting of 1 Hz. The RR interval, PR interval, P duration, QRS interval, heart rate, QT interval, and corrected QT interval were measured and analyzed.

2.8. Echocardiography

Before histopathological analysis, rats from different groups were subjected to Doppler ultrasonography of the heart to observe various ultrasonographic parameters in B-mode (Brightness or 2-D mode) and spectral Doppler modes like CW-mode (continuous wave) and PW-mode (Pulse wave) in a normal awake condition. Triplex

Doppler ultrasonography was performed with a Make-Wipro GE, Model - Logiq F8 Expert ultrasound machine.

2.9. Histological analysis

On the 30th day of post-treatment, rats were sacrificed by cervical dislocation. The thorax and abdomen were cut open, and heart tissue was removed and fixed in 10% buffered neutral formalin for 72 h after a gentle rinse with normal saline to remove the blood and debris adhering to them. Then the tissues were subjected to overnight washing under slow-running tap water, subsequently dehydrated through ascending grades of alcohol, cleared in xylene, and embedded in molten (temperature 60 ℃) paraffin wax to obtain paraffin blocks[23]. The tissue paraffin blocks were sectioned through a rotary microtome to obtain 5-6 μm thick serial paraffin sections, and stained with hematoxylin and eosin (H & E)[24]. The histopathological changes of each tissue slide were examined using a trinocular research microscope (Leica, DM 2500, Digital camera system DFC 290, Germany).

2.10. Statistical analysis

All data were presented as mean ± SD. Statistical analysis was performed using SPSS ver 1.0. One-way ANOVA followed by the Dunnett *post hoc* test was used to determine statistical significance among the groups, and $P<0.05$ was considered significant.

2.11. Ethical statement

The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Regd No. 433CPCSEA/CVS/2007).

3. Results

3.1. Effect of hesperidin on hematological parameters

Arsenic trioxide administration had no significant effect on Hb. PCV, total RBC count, and total platelet count were significantly decreased ($P<0.05$) while total WBC count was significantly increased ($P<0.05$) compared to the control group. In addition, significant lymphocytopenia and an increase in neutrophils and monocytes were observed with no significant change in eosinophils. Administration of hesperidin in arsenic trioxide-intoxicated rats significantly improved ($P<0.05$) the hematological parameters (Table 1).

3.2. Effect of hesperidin on the electrolyte balance

Arsenic trioxide administration significantly altered serum electrolytes concentration, as it caused a significant decrease (*P*<0.05) in sodium and potassium concentrations (Figure 1A & 1B) and a significant increase (*P*<0.05) in calcium concentration (Figure 1C) when compared to the control group. Treatment with hesperidin produced remarkable improvements in serum sodium, potassium, and calcium concentrations. Hesperidin administration alone did not affect the electrolyte balance significantly compared to the control group.

3.3. Effect of hesperidin on cardiac biomarkers

CK-MB and LDH levels were significantly higher (*P*<0.05) in the arsenic trioxide-intoxicated group compared to the control group, indicating a significant cardiac injury (Figure 2A & 2B). Treatment with hesperidin significantly decreased CK-MB and LDH levels (*P*<0.05) compared to the arsenic trioxide-intoxicated group (Figure 2A & 2B).

3.4. Effect of hesperidin on antioxidant status and proinflammatory cytokine

Table 2 shows the lipid peroxidation activity (TBARS activity)

Table 1. Effect of hesperidin (100 mg/kg) on hematological parameters in ATO-intoxicated rats.

Parameter	Control	ATO	ATO+Hesperidin	Hesperidin
Hb (g/dL)	15.67±0.21	14.40±0.76	14.35±0.48	15.30±0.37
PCV (%)	50.00±1.12	43.67±1.28[a]	47.65±0.99[b]	52.10±0.96
Total platelet count (×10^3/μL)	787.00±189.13	281.17±30.33[a]	722.50±30.74[b]	1015.67±32.61
Total RBC count (×10^6/μL)	8.93±0.40	5.67±0.20[a]	9.60±0.22[b]	10.07±0.06[a]
Total WBC count (cells/μL)	9433.33±589.16	12248.33±267.84[a]	12072±344.80[a]	10162.33±953.03
Neutrophils (%)	32.33±1.43	40.67±1.26[a]	13.00±2.29[ab]	24.33±1.58[a]
Eosinophils (%)	4.67±0.42	4.00±0.26	3.60±0.74	4.16±0.70
Lymphocytes (%)	71.67±3.13	54.00±2.28[a]	82.83±3.05[b]	73.17±2.89
Monocytes (%)	0.83±0.31	2.83±0.17[a]	2.00±0.26[a]	1.50±0.42

Data are presented as mean ± SD (*n*=6) and analyzed by one way ANOVA analysis followed by the Dunnett *post hoc* test. [a]*P*<0.05 compared with the control group, [b]*P*<0.05 compared with the ATO group. PCV: packed cell volume; Hb: hemoglobin; RBC: red blood cell; WBC: white blood cell.
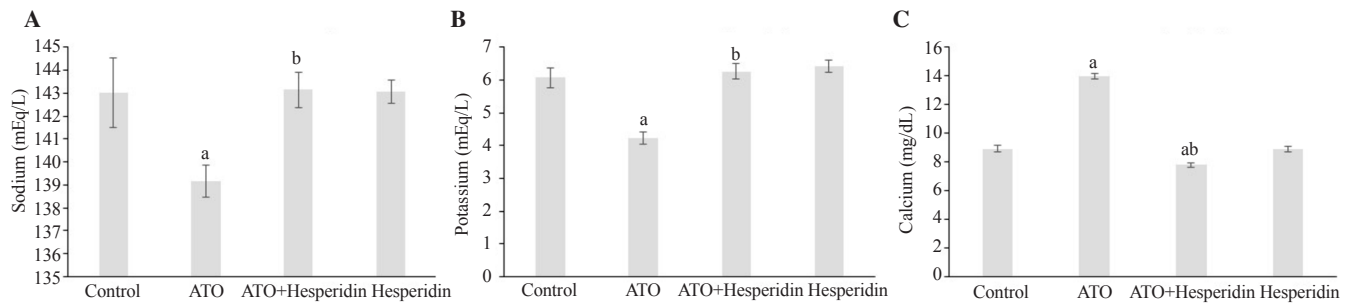

Figure 1. Effect of hesperidin on the electrolyte balance (A: sodium, B: potassium, C: calcium) in ATO-intoxicated rats. Data are presented as mean ± SD (*n*=6) and analyzed by one way ANOVA analysis followed by the Dunnett *post hoc* test. [a]*P*<0.05 compared with the control group, [b]*P*<0.05 compared with the ATO group. ATO: arsenic trioxide.
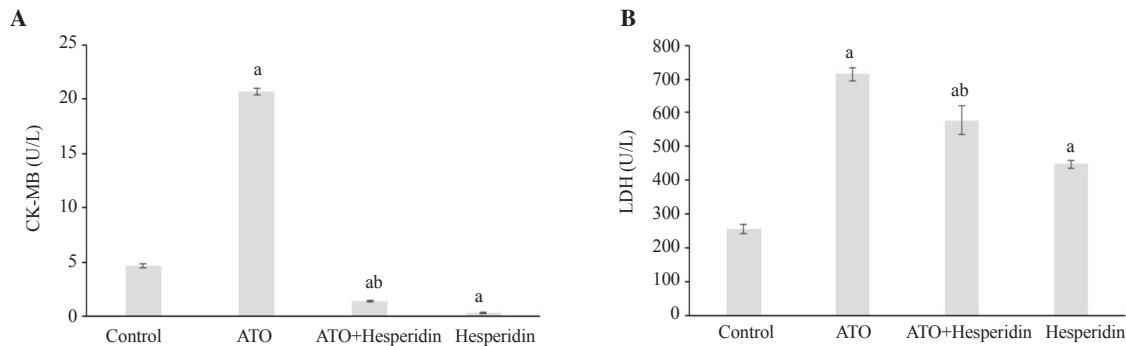

Figure 2. Effect of hesperidin on cardiac biomarkers (A: CK-MB, B: LDH) in ATO-intoxicated rats. Data are presented as mean ± SD (*n*=6) and analyzed by one way ANOVA analysis followed by the Dunnett *post hoc* test. [a]*P*<0.05 compared with the control group, [b]*P*<0.05 compared with the ATO group. CK-MB: creatine kinase-myoglobin binding; LDH: lactate dehydrogenase.

and antioxidant status (SOD and catalase activity) in cardiac tissues of the control and experimental groups. TBARS activity was significantly increased ($P<0.05$) and SOD and catalase activities were significantly reduced ($P<0.05$) in the arsenic trioxide-treated group compared to the control. Hesperidin significantly improved the antioxidant status (Table 2). Moreover, IL-6 level was increased significantly ($P<0.05$) in the arsenic trioxide-treated group (Table 2) compared to the control. Hesperidin downregulated serum IL-6 levels in the arsenic trioxide-treated rats.

3.5. Effect of hesperidin on ECG

ECG of different groups is presented in Figure 3. There was no significant change in RR interval, PR interval, P duration, and QRS interval in the arsenic trioxide-treated group compared to the control group. However, heart rate was significantly decreased ($P<0.05$), as well as QT and corrected QT intervals were significantly prolonged ($P<0.05$) compared to control rats (Table 3). The co-administration of hesperidin improved the heart rate compared to the arsenic trioxide-intoxicated group, which was not significantly different from the control group with no significant change in other parameters. Control rats treated with only hesperidin revealed no significant difference in RR interval, heart rate, PR interval, P duration, QRS interval, as well as QT and corrected QT interval when compared to the control group (Table 3).

3.6. Echocardiographic findings

Echocardiographic findings are presented in Supplementary Figure 1. Vital Doppler's ultrasonographic indices like peak systolic velocity (PSV), end-diastolic velocity (EDV), resistive index (RI), and pulsatility index (PI) were found to be affected by arsenic trioxide administration. The PSV and EDV were significantly higher in arsenic trioxide-treated rats [(34.2 ± 6.57) cm/s, $P<0.05$; (11.97 ± 1.87) cm/s, $P<0.05$] compared to the control [(15.43 ± 2.26) cm/s; (7.07 ± 0.97) cm/s] (Supplementary Figure 1). In addition, PI and RI were moderately higher after administration of arsenic trioxide [(12.29 ± 2.88) and (0.91 ± 0.16)] compared to control [(9.86 ± 2.58) and (0.53 ± 0.27)]. Hesperidin treatment reduced PSV [(32.18 ± 8.62) cm/s], EDV [(8.50 ± 1.26) cm/s, $P<0.05$], and RI (0.74 ± 0.10) values

Table 2. Effect of hesperidin (100 mg/kg) on lipid peroxidation, SOD, catalase and IL-6 in ATO-intoxicated rats.

Parameter	Control	ATO	ATO+Hesperidin	Hesperidin
TBARS (nM/mg protein)	0.77 ± 0.03	1.720 ± 0.095^a	0.81 ± 0.09^b	0.63 ± 0.08
SOD (U/mg protein)	22.40 ± 1.15	15.78 ± 1.22^a	19.64 ± 1.68	21.64 ± 1.87
Catalase (mmol H_2O_2/min/mg protein)	14.60 ± 1.22	7.89 ± 0.49^a	9.02 ± 1.12^a	13.10 ± 1.39
IL-6 (pg/mL)	35.83 ± 2.05	68.66 ± 3.41^a	50.33 ± 1.92^{ab}	38.66 ± 2.26

Data are presented as mean ± SD ($n=6$) and analyzed by one way ANOVA analysis followed by the Dunnett *post hoc* test. [a]$P<0.05$ compared with the control group, [b]$P<0.05$ compared with the ATO group. TBARS: thiobarbituric acid reactive substances, SOD: superoxide dismutase.
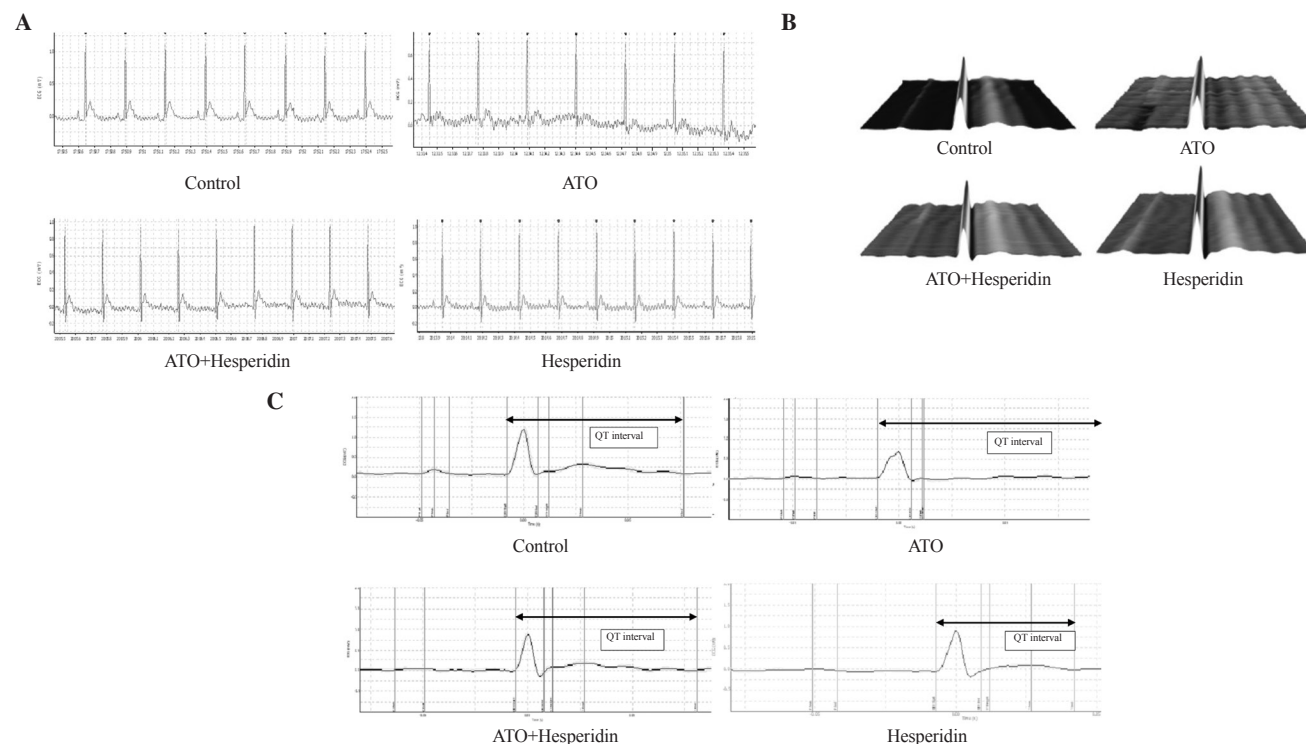

Figure 3. Effect of hesperidin on ECG of ATO-intoxicated rats. A: Electrocardiogram of different groups; B: Waterfall plot of ECG taken in different groups of animals; C: Averaging view of ECG taken in different groups of animals.

in arsenic trioxide-intoxicated rats. Hesperidin induced no significant changes in the above parameters [PSV: (16.66±3.08) cm/s; EDV: (6.98±0.93) cm/s; PI: (8.66±1.78); RI: (0.66±0.13)] in the normal rats.

3.7. Histopathological findings

In the control group, the cardiac tissue (Figure 4A) revealed a normal histological structure. Rats treated with arsenic trioxide showed that the capillaries adjacent to myocardial fibers were congested. The myocardial fibers showed a moderate degree of coagulative necrosis, a loss of cross striation, and a distortion in the pattern of cross striation. The nuclei of the cardiac muscle cells were atrophied with a dense accumulation of chromatin material. Pyknotic nuclei were frequently seen (Figure 4B). Rats treated with arsenic trioxide and hesperidin showed a normal histoarchitecture of the myocardial fibers with mild interstitial edema and weakly congested blood capillaries (Figure 4C). Most of the nuclei had euchromatin and the euchromatic nuclei were frequent. The cross-striation of the muscle was normal (Figure 4C). Rats treated with hesperidin alone showed normal cardiac histoarchitecture with densely packed myocardial fibres, euchromatic nuclei, and normal capillary bed (Figure 4D).

4. Discussion

This study showed that arsenic trioxide caused no significant effect on the values of Hb but total RBC count and PCV were dropped significantly compared to the normal control group, which is in partial agreement with the findings of Ghosh *et al*[25]. Hesperidin elevated total RBC count and PCV in arsenic trioxide-intoxicated rats. Normal rats administered with hesperidin showed no substantial change in Hb and PCV levels compared to the control group, which is in agreement with the findings of Saleh *et al*[26]. Arsenic trioxide also caused a significant fall in total platelet count and a significant rise in total WBC count, which is in congruence with the earlier findings of Wu *et al*[27]. Parmar *et al*. demonstrated that there was a decrease in RBC and platelet counts and an increase in WBC counts in male Charles Foster rats administered with sodium arsenite[28]. Ghosh *et al*. reported hematological abnormalities such as reduced erythrocyte count, poikilocytosis, neutropenia, thrombocytopenia, and lymphocytopenia in Wistar rats treated with arsenic trioxide for 28 d[25]. Amsat *et al*. demonstrated that exposure to sublethal concentrations of arsenic trioxide in *Channa punctatus* fish resulted in a significant reduction in RBC count and an elevated WBC count[29]. According to Erslev and Gabuzda, there was a release of thrombopoietin due to arsenic trioxide exposure, which may be responsible for an elevated WBC count[30]. High levels of neutrophils and low levels of lymphocytes together represent an elevated NLR ratio, which is indicative of severe infection, inflammatory disorders, or stress[31]. Hesperidin possesses anti-inflammatory properties in many disease models and has been reported to inhibit oxidative stress and inflammation in rodent models[8,15]. In this study, hesperidin improved hematological parameters and thereby decreased the NLR ratio in arsenic trioxide-intoxicated rats, which

Table 3. Effect of hesperidin (100 mg/kg) on ECG in ATO-intoxicated rats.

Parameter	Control	ATO	ATO+Hesperidin	Hesperidin
RR interval (s)	0.227±0.045	0.257±0.026	0.224±0.026	0.217±0.018
Heart rate (bpm)	273.620±47.801	236.350±21.560[a]	271.500±33.840[b]	278.750±24.137
PR interval (s)	0.046±0.004	0.049±0.003	0.052±0.006	0.051±0.009
P duration (s)	0.016±0.003	0.018±0.002	0.018±0.004	0.015±0.003
QRS interval (s)	0.016±0.003	0.017±0.003	0.015±0.002	0.017±0.001
QT interval (s)	0.068±0.012	0.087±0.003[a]	0.079±0.006	0.074±0.011
QTc (s)	0.142±0.013	0.172±0.005[a]	0.168±0.014	0.161±0.025

Data are presented as mean ± SD (*n*=6) and analyzed by one way ANOVA analysis followed by the Dunnett *post hoc* test. [a]*P*<0.05 compared with the control group, [b]*P*<0.05 compared with the ATO group. QTc: corrected QT interval.
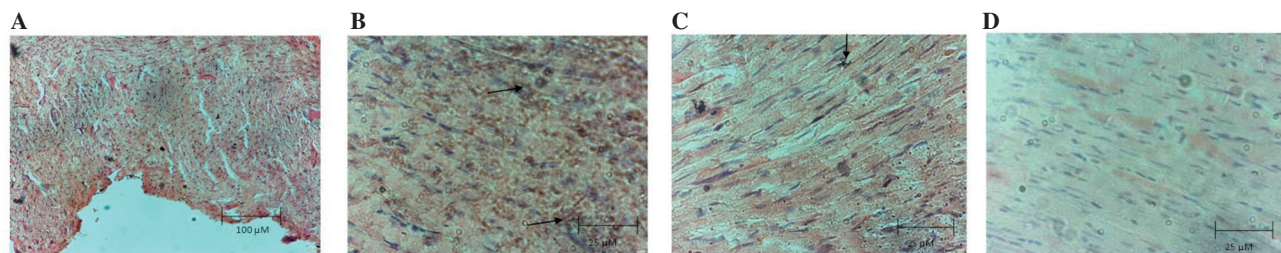

Figure 4. Histopathological results of cardiac tissue after treatment with hesperidin (H&E, ×100). (A) The control group shows a normal histological structure; (B) the arsenic trioxide-intoxicated group shows the coagulative necrosis in myocardial fibers (arrow); (C) the arsenic trioxide-intoxicated group treated with hesperidin shows improved cardiac cytoarchitecture and normal cross-striation pattern in myofibers (arrow); (D) the group treated with hesperidin alone shows a normal cytoarchitecture of the myocardium.

may be attributed to its anti-inflammatory and antioxidant effect that improved the hematopoiesis[8].

In this study, there was a significant decrease in sodium concentration in the arsenic trioxide-treated group when compared to the control group. Anand and Saxena reported that arsenic trioxide intoxication causes significant hyponatremia, which could be related to the depletion of antioxidant status and ROS release, possibly because of its binding with proteins of the renal tubular epithelium[32]. A significant decrease in potassium concentration was observed in the arsenic trioxide-treated group, which is in agreement with Varghese *et al*[33]. Hypokalemia induced by arsenic trioxide can cause a prolonged QT interval in the heart. Additionally, arsenic trioxide administration caused an elevated serum calcium concentration. The result is in agreement with the finding of Raghu *et al*. in which exposure to arsenic trioxide is associated with increased accumulation of calcium in cardiac tissue, leading to ROS-induced apoptosis[34]. Elevated levels of calcium concentration alter the normal electrical activities of cardiac muscle and cause significant myocardial damage by activating calcium channels in cardiomyocytes. Zhang *et al*. reported that arsenic trioxide significantly affects calcium homeostasis, which is notably responsible for cardiac toxicities[35]. Thus, arsenic trioxide may cause electrolyte imbalance. The present study shows that hesperidin restored sodium, potassium, and calcium homeostasis.

CK-MB and LDH are two important cardiac marker enzymes. These enzymes are predominantly present in myocardial tissues, and they are released into the circulation when there is any injury to cardiac tissue in the form of myocardial ischemia, infarction, necrosis, inflammation, myocardial membrane damage, and myocardial cell death. An increased concentration of these enzymes in plasma is directly proportional to the number of necrotic and damaged cells available in cardiac tissue. In the present study, CK-MB and LDH levels were significantly high in the arsenic trioxide-treated group compared with the control group. Consistent with the current findings, Wang *et al*. reported the significant release of cardiac marker enzymes CK-MB and LDH in response to arsenic trioxide administration; these enzymes indicate the occurrence of myocardial cell damage and necrosis, which exude such enzymes into the systemic circulation[36]. Arsenic trioxide-intoxicated rats treated with hesperidin significantly lowered CK-MB and LDH levels, which may be attributed to its free radical scavenging properties. The present results are in agreement with several other pieces of literature[12,37].

Supression of oxidative stress is a major pathway in attenuation of cardiotoxicity in rats[38]. ROS generates MDA due to the oxidation of membrane phospholipids. TBARS is measured as a marker of lipid peroxidation *in vivo*. Lipid peroxidation increases the MDA level and is one of the characteristic features of augmented oxidative stress associated with arsenic trioxide toxicity[17]. We observed a surge in free radical formation (TBARS) and a reduction in antioxidant activities such as SOD and catalase in the arsenic trioxide-treated group. SOD is usually found in the plasma membrane, which protects cells from oxidative stress. Catalase is a tetrameric hemoprotein that acts as a catalyst for the removal of hydrogen peroxide. These enzymatic antioxidants counter lipid peroxidation. Hesperidin administration in the arsenic trioxide-intoxicated group significantly decreased lipid peroxidation, and increased SOD and catalase activities. The increased activities of these enzymes might be due to the ROS-scavenging property of hesperidin[15]. Administration of hesperidin also decreased the IL-6 level in the arsenic trioxide-intoxicated rats, which suggests its anti-inflammatory effect and is consistent with earlier findings[13,39].

ECG findings demonstrated that rats treated with arsenic trioxide produced significant changes. It was observed that there was no significant change in the RR interval, PR interval, P duration, or QRS interval in the arsenic trioxide-treated group compared to the control group. However, heart rate was significantly decreased. This finding is in agreement with Fan *et al*. who demonstrated that arsenic trioxide reduced heart rate remarkably[40]. Whereas QT interval and corrected QT interval were significantly prolonged. Treatment with hesperidin produced no significant changes in RR interval, PR interval, P duration, QRS interval, QT, and corrected QT interval, while improving the heart rate compared to the arsenic trioxide-intoxicated group.

Echocardiography was done to study the heart's function. Doppler echocardiography gives information about the blood flow within the heart chamber, across the valves, and in the great vessels. It gives a more complete, non-invasive evaluation of cardiac function. The amount of blood pumped out with each beat is an indication of the functioning of the heart. It can detect heart failure, myocardial ischemia, valve problems, and other issues. High EDV means an increase in the preload and an increase in the stroke volume. High PSV is due to narrowing of the lumen due to stenosis. PI is a non-invasive method for assessing vascular resistance[41]. Our results showed higher PSV and EDV in the arsenic trioxide-treated group compared to the control, which may be due to cardiac myopathy and vascular resistance[36]. Administration of hesperidin decreased the PSV and EDV in the cardiograph, which gave a qualitative idea about the cardioprotective effect of hesperidin.

Histopathological findings indicated that rats in the group challenged with arsenic trioxide, showed extensive tissue damage, fiber separation, congestion in capillaries, and a moderate degree of coagulative necrosis in myocardial fibers with loss of cross striations. In agreement with these findings, a moderate degree of myocardial coagulative necrosis and myofibrillar loss in Wistar rats following arsenic trioxide administration was reported[22]. Severe forms of damage to the cardiac tissue, including pericardial edema with lymphoid cell infiltration, vascular congestion, hyalinization, and focal hemorrhage of the myofibers were reported in doxorubicin-induced cardiac toxicity in rats[12]. The extent of myocardial damage

induced by arsenic trioxide in the present study agreed well with these findings. However, comparatively less degree of damage to cardiac tissue was noted by Hemmati *et al*.[22]. On the other hand, Chakraborty *et al*. found myofibrillar degeneration with marked diffuse infiltration of lymphoid cells and increased interstitial space in the hearts of rats treated with cyclophosphamide[42]. The cardioprotective effect of hesperidin was reported following challenge with different toxic agents like doxorubicin[12], and carbon monoxide[43] *etc*.

In conclusion, hesperidin could alleviate the arsenic trioxide-induced cardiac toxicity as evidenced by decreased CK-MB and LDH levels, improved heart rate, and alleviated histopathological damage to the heart, which may be attributed to its antioxidant and anti-inflammatory action. However, further investigation is required to strengthen the cardioprotective function of hesperidin for future clinical applications.

Conflict of interest statement

The authors have no conflict of interest to declare.

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Authors' contributions

GK and JRD designed the experiment and analyzed the result. BJ performed echocardiography. UM performed histopathology and SP analyzed the results.

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