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Research Article

Cardioprotective Effects of Fisetin Against Sodium Arsenite-Induced Toxicity in Experimental Rats: Roles of the Nrf2/HO-1 and Bax/Bcl-2/Caspase-3 Pathways

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Abstract

Background and Objective: Sodium arsenite, a pesticide, is well known to induce cardiotoxicity via myocardial apoptosis. Fisetin, a plant, has antioxidant, anti-inflammatory and anti-apoptotic potential. This study aimed to evaluate the putative mechanism of action of fisetin against sodium arsenite-induced cardiotoxicity in experimental rats. **Materials and Methods**: Cardiotoxicity was induced in male Sprague-Dawley rats (200–230 g, n = 15, in each group) using sodium arsenite (5 mL/kg, p.o., 28 days) and concomitantly treated with either coenzyme Q10 (10 mg/kg) or fisetin (5, 10 and 25 mg/kg, p.o.) orally for 28 days. Various biochemical, molecular, and histopathological analyses were performed to evaluate the efficacy of fisetin against cardiotoxicity. Data were analyzed by one-way Analysis of Variance (ANOVA), while Tukey's multiple range tests were applied for *post hoc* analysis. **Results**: Chroni carsenite administration promoted a significant ($p < 0.001$) increase in relative heart weight and alterations in electrocardiographic, hemodynamic, and left ventricular function parameters, which were effectively and dose-dependently attenuated ($p < 0.01$ and $p < 0.001$) by fisetin (10 and 25 mg/kg). Moreover, fisetin treatment also markedly decreased elevated serum creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and lipid levels. Arsenite-induced elevated cardiac oxido-nitrosative stress was also efficiently and dose-dependently decreased ($p < 0.01$ and $p < 0.001$) by fisetin. Following arsenite exposure, the mRNA expressions of cardiac nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase 1 (HO-1) and B-cell lymphoma 2 (Bcl-2) were downregulated, and Bax and Caspase-3 mRNA were up-regulated; these expressions were likewise effectively and dose-dependently ($p < 0.01$ and $p < 0.001$) inhibited by fisetin. Histopathological observations of the heart suggested that fisetin attenuated arsenite-induced myocardial aberrations. **Conclusion**: Fisetin effectively mitigates sodium arsenite-induced cardiotoxicity in experimental rats. The protective effects of fisetin are associated with antioxidant (Nrf2/HO-1) and apoptotic (Bax/Bcl-2 and caspase-3) pathways in experimental rats. Thus, fisetin can be considered a potential phytoconstituent in managing pesticide-induced cardiotoxicity.

Keywords: apoptosis; cardiotoxicity; fisetin; heme oxygenase 1; nuclear factor erythroid 2-related factor-2; sodium arsenite

1. Introduction

Sodium arsenite is a common inorganic arsenic compound used as a pesticide in dyeing and soap industries [1]. It's a listed hazardous substance by the Occupational Safety and Health Administration (OSHA) and is well known to induce cardiotoxicity [2]. Arsenic exposure, whether through environmental contamination, occupational exposure or intentional ingestion, has been associated with adverse cardiovascular effects resulting from alteration of ion channel function and intracellular calcium equilibrium, leading to electrocardiographic abnormalities to irreversible degenerative cardiomyopathy and congestive heart failure [3,4]. The escalating incidence of cardiovascular diseases in China led to 4 million fatalities in 2016, with reported hospitalization costs for acute myocardial infarction reaching 19 billion Renminbi (RMB) (approx. 3 billion United States Dollar (USD)) [5–7]. Furthermore, the annual hospitalization costs from cardiovascular disease (CVDs) in other Southeast Asian countries, such as South Korea, Taiwan, Thailand and Malaysia, are $10,714, $4790, $7181 and $1776, respectively [8]. Thus, sodium arsenite-induced cardiotoxicity is associated with a significant economic burden and poor quality of life of patients.

Studies have well-documented the pathophysiology of sodium arsenite-induced cardiotoxicity [1,2]. Prolonged exposure to sodium arsenite generates reactive oxygen species (ROS) through various mechanisms, including the inhibition of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) [9,10]. Excessive ROS production can lead to oxidative stress, causing damage to cellular components such as lipids, proteins and deoxyribonucleic acid (DNA) within cardiac cells [11]. Additionally, arsenic exposure can disrupt mitochondrial function and impair oxidative phosphorylation, decreasing adenosine triphosphate (ATP) production and mitochondrial membrane potential [12]. Mitochondrial dysfunction further contributes to cellular energy depletion, calcium dysregulation and increased production of ROS,

exacerbating oxidative stress and cardiotoxicity [13]. Researchers suggested chronic exposure to sodium arsenite induces myocardial apoptosis through various pathways, including activating caspases, mitochondrial-mediated apoptosis and oxidative stress-induced cell death [14,15]. Excessive apoptosis can lead to cardiomyocyte loss, myocardial damage and impaired cardiac function.

Pharmacological interventions for sodium arsenite-induced cardiotoxicity include cardioprotective agents such as β-blockers (such as metoprolol or carvedilol) and angiotensin-converting enzyme (ACE) inhibitors (such as lisinopril or enalapril) that used to manage hypertension, reduce cardiac workload and improve myocardial function [16,17]. These agents exert their potential via vasodilatory effects, improve endothelial function and attenuate cardiac remodeling. Additionally, the efficacy of β-blockers in enhancing left ventricular function has been attributed to their antioxidant activity [18]. Drugs commonly utilized to mitigate sodium arsenite-induced cardiotoxicity may manifest adverse effects, including low blood pressure, allergic reactions and bronchospasms or shortness of breath [19]. Consequently, it is critically imperative to explore alternative approaches, such as phytotherapies exhibiting fewer or negligible side effects [20]. Therefore, alternative phytoconstituents of natural origin may represent viable candidates for such therapeutic interventions.

Animal models and isolated cardiomyocytes serve as commonly employed models for exploring the toxic effects induced by pesticides, including cadmium, arsenic, atrazine, paraquat, etc. [21]. The widely used animal models provide valuable insights into the pathophysiology of pesticide-induced toxicity, such as sodium arsenite-induced cardiotoxicity and serve as platforms for testing novel cardioprotective agents [1,2,12]. Apart from these models, cell lines like H9c2 exhibiting many characteristics of cardiomyocytes are utilized to explore the protective efficacy of various compounds against pesticide-induced cardiotoxicity [22]. *In-vitro* investigations have demonstrated that arsenic elicits hypertrophic responses in adult H9c2 cells [22].

With the limited availability of effective treatments for pharmaceutical drugs and their side effects, research focuses increasingly on understanding etiopathological mechanisms and exploring traditional medicinal plant resources with fewer side effects for alternative or complementary strategies [23,24]. The World Health Organization (WHO) also approximates that 3.5 to 4 billion individuals worldwide continue to depend on pharmaceuticals sourced from medicinal plants [25]. Fisetin, a plant flavonoid, exists in various fruits and vegetables, such as strawberries, apples, onions and cucumbers [26]. It has a wide range of potential health benefits and pharmacological effects, including antioxidant, anti-inflammatory and neuroprotective in various neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease, anti-cancer, anti-diabetic, anti-aging, anti-allergic, anti-asthmatic and bone repair [27–29]. Researchers documented the anti-cancer potential of fisetin via inhibition of multiple signaling pathways involved in cancer progression, including protein kinase B (PI3K or Akt), mitogen-activated protein kinase (MAPK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and wingless-type MMTV integration site family β (Wnt/β)-catenin pathways [30]. Fisetin inhibited NF-κB and MAPK pathways, reducing inflammation and inflammatory responses [31]. Furthermore, fisetin ameliorates apoptosis by alleviating the elevated levels of Caspase-3, -8, -9 and B-cell lymphoma 2 (Bcl-2) [30]. Considering these modulating potentials, the present investigation aimed to evaluate the putative mechanism of action of fisetin against sodium arsenite-induced cardiotoxicity in experimental rats.

2. Materials and Methods

2.1 Study Area

This study was conducted from January to March, 2024 at the Animal House of Qingdao Municipal Hospital, Qing Dao, China.

2.2 Animals

Male rats (strain: Sprague-Dawley, weight: 200–230 g) were procured from the animal house of Qingdao Municipal Hospital. Rats were housed at normal maintenance conditions, including temperature: 24 $\pm$ 1 °C, normal dark/light cycle and relative humidity: 45–55%.

2.3 Induction of Sodium Arsenite-Induced Cardiotoxicity and Treatment Schedule

According to the previously reported method, sodium arsenite (Merck Ltd, Beijing, China; 5 mg/kg, p.o., 28 days) was administered in rats to induce cardiotoxicity [1]. The rats were randomly divided into various groups contain 15 rats in each group namely, arsenite control (As control, treated with 5 mL/kg, p.o. distilled water), coenzyme Q10 (Merck Ltd, Beijing, China; As+CoQ 10, treated with Coenzyme Q10 10 mg/kg, p.o.), fisetin (Merck Ltd, Beijing, China; F 5, F 10 and F 25 mg/kg, treated with fisetin 5, 10 or 25 mg/kg, p.o.) [26,31] for 28 days. Another group of aged-matched healthy rats named as normal were also maintained and treated with distilled water (5 mL/kg, p.o.) for 28 days.

2.4 Behavioral Analysis

On the 29th day, electrocardiographic, hemodynamic changes and left ventricular contractile function were measured using an eight-channel recorder Power lab data acquisition system (AD Instruments Pvt. Ltd., with software LABCHART version 7.3 pro software, Bella Vista NSW, Australia) [2,32].

Table 1. Effect of fisetin on sodium arsenite-induced alteration in body weight, heart weight and serum biochemistry and serum lipid profiles of rats.

Parameter	Normal	As control	As+CoQ (10)	As+F (5)	As+F (10)	As+F (25)
Body weight (gm)	236.00 ± 4.58	240.80 ± 4.50	230.30 ± 3.17	233.00 ± 2.75	240.70 ± 2.79	244.80 ± 2.15
Heart weight (gm)	0.19 ± 0.03	0.89 ± 0.03$^{###}$	0.40 ± 0.03***	0.82 ± 0.03	0.61 ± 0.03**	0.55 ± 0.02***
Heart weight/body weight ($\times 10^{-3}$)	0.79 ± 0.12	3.70 ± 0.18$^{###}$	1.75 ± 0.11***	3.52 ± 0.12	2.53 ± 0.10**	2.26 ± 0.07***
Serum CK-MB (IU/L)	1081 ± 41.29	2474 ± 48.28$^{###}$	1365 ± 33.41***	2486 ± 14.86	1946 ± 48.77**	1527 ± 22.11***
Serum LDH (IU/L)	1375 ± 73.50	2819 ± 74.17$^{###}$	1754 ± 83.35***	2797 ± 64.13	2465 ± 63.65**	2004 ± 41.29***
Serum ALP (IU/L)	123.50 ± 3.72	349.60 ± 2.26$^{###}$	151.50 ± 4.99***	329.30 ± 4.61	277.00 ± 4.07**	196.30 ± 4.85***
Total cholesterol (mg/dL)	13.72 ± 0.81	54.52 ± 0.48$^{###}$	19.59 ± 0.48***	49.31 ± 0.59	35.54 ± 0.84**	27.22 ± 0.56***
Triglycerides (mg/dL)	60.16 ± 1.35	159.90 ± 2.35$^{###}$	73.71 ± 3.08***	146.40 ± 2.70	120.70 ± 2.60**	97.53 ± 1.60***
LDL-C (mg/dL)	1.06 ± 0.15	7.01 ± 0.08$^{###}$	1.46 ± 0.16***	6.34 ± 0.17	4.05 ± 0.03**	2.42 ± 0.16***
HDL-C (mg/dL)	66.02 ± 2.55	21.09 ± 2.16$^{###}$	50.64 ± 2.35***	24.66 ± 2.01	42.27 ± 2.63**	50.23 ± 2.33***
VLDL-C (mg/dL)	12.03 ± 0.27	31.97 ± 0.47$^{###}$	14.74 ± 0.62***	29.27 ± 0.54	24.14 ± 0.52**	19.51 ± 0.32***

Data are expressed as Mean ± SEM (n = 6) and analyzed by One-way Analysis of Variance (ANOVA) followed by Tukey's multiple range test. **$p < 0.01$ and ***$p < 0.001$ as compared to the As-control group, $^{###}p < 0.001$ as compared to normal group, As, Sodium arsenite control group; As+CoQ10 (10), Sodium arsenite+Coenzyme Q10 (10 mg/kg); As+F (5), Sodium arsenite+fisetin (5 mg/kg); As+F (10), Sodium arsenite+fisetin (10 mg/kg); As+F (25), Sodium arsenite+fisetin (25 mg/kg); CK-MB, Creatine Kinase-MB; ALP, Alkaline Phosphatase; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; LDH, Lactate Dehydrogenase; VLDL, Very Low-Density Lipoprotein.

2.5 Biochemical Analysis

Post behavioral assessment, the blood of anesthetized rats (ketamine (50 mg/kg; Pfizer Inc., New York City, NY, USA) and diazepam (5 mg/kg; Kern Liebers India, Tumkur, India), intramuscular) was collected using retro-orbital puncture. Serum was separated was used for the estimation of Creatine Kinase-MB (CK-MB), Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP), total cholesterol, low-density lipoprotein, triglyceride and high-density lipoprotein using respective kits according to manufacturer's instructions (Accurex Biomedical Pvt. Ltd., Mumbai, India) [33].

Then, animals were euthanized by carbon dioxide (3–7 liters/min flow for 10 L chambers) asphyxiation, the heart was isolated to estimate the levels of total protein, lipid peroxidation (Malondialdehyde (MDA) content), nitric oxide (NO content), superoxide dismutase (SOD) and reduced Glutathione (GSH) as described by Kandhare *et al*. [34] and Visnagri *et al*. [35]. The mRNA expressions of Nuclear Factor Erythroid 2-Related Factor 2 (*Nrf2*, *NFE2L2*, forward primer: CCTCACCTCTGCTGCCAGT, reverse primer: GGGAGGAATTCTCCGGTCTC, base pair: 316), Heme Oxygenase 1 (*HO-1*, *HMOX1*, forward primer: TTGTAACAGACTTGCCAGAG, reverse primer: CACTCACTGGTTGTATGCG, base pair: 202), Bcl-2 Associated X (*Bax*, forward primer: CTACAGGGTTTCATCCAG, reverse primer: CCAGTTCATCTCCAATTCG, base pair: 96), B-cell lymphoma 2 (*Bcl-2*, forward primer: CGGAGGCTGGGATGCCTTTG, reverse primer: TTTGGGGCAGGCATGTTGAC, base pair: 231) and Caspase-3 (*CASP3*, forward primer: GCTGGACTGCGGTATTGAGA, reverse primer: TAACCGGGTGCGGTAGAGTA, base pair: 108) were analyzed in cardiac tissue using using quantitative reverse transcription polymerase chain reaction (RT-PCR) as described by Visnagri *et al*. [36] and Kandhare *et al*. [37]. Single-stranded cDNA was synthesized from 5 mg total cellular RNA using a commercially available RT-PCR kit (MP Biomedicals India Private Ltd., Mumbai, India). Amplification of β-actin mRNA (forward primer: GTCACCCACACTGTGCCCATCT, reverse primer: ACAGAGTACTTGCGCTCAGG AG, base pair: 764) served as a control for sample loading and integrity. The PCR products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. The size of the amplicons was confirmed using a 100-base pair ladder as a standard size marker. The amplicons were visualized and images were captured using a gel documentation system (Alpha Innotech Inc., San Leandro, CA, USA). Gene expression was assessed by generating densitometry data for band intensities in different sets of experiments by analyzing the gel images semi-quantitatively using the Image J program Version 1.33 (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The intensity of mRNAs was standardized against that of the β-actin from each sample and the results were expressed as PCR-product/β-actin mRNA ratio. As described previously, another portion of cardiac tissue was fixed for histopathological evaluation using Hematoxylin and Eosin (H&E) stain [38].

2.6 Statistical Analysis

Data was expressed as a Mean ± Standard Error Means (SEM). Data analysis was performed using Graph Pad Prism 5.0 software (Graph Pad, San Diego, CA, USA). Data was analyzed by One-way Analysis of Variance (ANOVA) and Tukey's multiple range tests were applied for *post hoc* analysis. A value of $p < 0.05$ was considered to be statistically significant.

Table 2. Effect of fisetin on sodium arsenite-induced alteration in electrocardiographic, hemodynamic changes and left ventricular function in rats.

Parameter	Normal	As control	As+CoQ (10)	As+F (5)	As+F (10)	As+F (25)
Heart rate (BPM)	345.50 ± 8.04	260.70 ± 8.28$^{###}$	316.50 ± 7.76***	262.50 ± 8.71	296.00 ± 9.99**	331.30 ± 9.40***
QRS interval (ms)	12.67 ± 1.23	36.5 ± 0.67$^{###}$	17.33 ± 0.92***	33.00 ± 0.52	24.50 ± 0.67**	22.67 ± 0.99***
QT interval (ms)	48.00 ± 2.46	89.33 ± 2.81$^{###}$	56.00 ± 2.28***	87.67 ± 2.50	78.00 ± 2.62**	59.00 ± 0.58***
QTc interval (ms)	131.50 ± 1.23	185.50 ± 2.29$^{###}$	141.20 ± 2.41***	175.70 ± 1.52	165.30 ± 1.65**	147.70 ± 2.04***
RR interval (ms)	142.30 ± 5.35	228.70 ± 5.74$^{###}$	156.50 ± 4.07***	220.30 ± 4.49	202.30 ± 4.13**	174.30 ± 4.51***
PR interval (ms)	14.00 ± 0.97	31.17 ± 0.65$^{###}$	15.67 ± 0.80***	30.00 ± 1.00	24.67 ± 0.67**	21.50 ± 1.29***
ST interval (ms)	19.00 ± 1.46	38.83 ± 1.14$^{###}$	22.83 ± 0.60***	37.00 ± 1.10	29.83 ± 0.87**	26.00 ± 1.32***
SBP (mmHg)	146.00 ± 4.49	94.33 ± 3.60$^{###}$	136.00 ± 5.07***	98.83 ± 5.42	127.30 ± 3.24**	145.80 ± 1.47***
DBP (mmHg)	115.00 ± 3.77	96.00 ± 3.34$^{###}$	115.80 ± 1.82***	92.67 ± 2.62	98.67 ± 3.94**	110.50 ± 2.57***
MABP (mmHg)	120.20 ± 2.79	91.17 ± 1.68$^{###}$	113.20 ± 2.09***	89.33 ± 3.12	101.70 ± 1.69**	104.30 ± 1.69***
LVEDP (mmHg)	5.19 ± 0.44	10.72 ± 0.30$^{###}$	5.67 ± 0.24***	10.27 ± 0.41	8.16 ± 0.32**	6.01 ± 0.27***
LVESP (mmHg)	86.54 ± 2.71	128.40 ± 4.37$^{###}$	85.95 ± 3.33***	132.00 ± 2.02	116.40 ± 2.78**	97.07 ± 2.81***
dp/dt_{Max}	3145 ± 48.38	2534 ± 81.45$^{###}$	3216 ± 46.70***	2636 ± 69.54	3152 ± 90.32**	3222 ± 43.81***
dp/dt_{Min}	–827 ± 86.33	–1343 ± 27.54$^{###}$	–1057 ± 47.04***	–1283 ± 78.44	–1084 ± 31.18**	–1046 ± 57.47***
Systolic duration (ms)	47.20 ± 1.06	69.84 ± 4.30$^{###}$	51.16 ± 1.88***	65.13 ± 1.86	66.01 ± 2.49**	51.64 ± 0.69***
Diastolic duration (ms)	146.50 ± 2.10	109.90 ± 1.27$^{###}$	159.50 ± 2.57***	113.40 ± 3.34	127.40 ± 3.24**	148.10 ± 3.48***
Pressure time index	16.35 ± 1.12	28.95 ± 0.97$^{###}$	19.61 ± 0.90***	28.49 ± 0.85	26.61 ± 0.74**	18.59 ± 1.17***
Contractility index	58.01 ± 2.01	40.25 ± 2.50$^{###}$	60.96 ± 1.14***	41.83 ± 1.95	47.83 ± 1.66**	58.93 ± 2.04***
Tau (ms)	4.80 ± 0.23	9.04 ± 0.47$^{###}$	5.19 ± 0.33***	8.59 ± 0.35	5.54 ± 0.41**	4.57 ± 0.43***

Data are expressed as Mean ± SEM (n = 6) and analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple range test. **$p < 0.01$ and ***$p < 0.001$ as compared to the As-control group, $^{###}p < 0.001$ as compared to normal group, As, Sodium arsenite control group; As+CoQ10 (10), Sodium arsenite+Coenzyme Q10 (10 mg/kg); As+F (5), Sodium arsenite+fisetin (5 mg/kg); As+F (10), Sodium arsenite+fisetin (10 mg/kg); As+F (25), Sodium arsenite+fisetin (25 mg/kg); SBP, Systolic blood pressure; DBP, Diastolic blood pressure; MABP, Mean Arterial Blood Pressure; LVEDP, Left Ventricular End-Diastolic Pressure; LVESP, Left Ventricular End-Systolic Pressure; BPM, Beats Per Minute; ms, millisecond; mmHg, millimeters of mercury; dp/dt_{Max}, maximum derivative of pressure change with respect to time; dp/dt_{Min}, minimum derivative of pressure change with respect to time.

3. Results

3.1 Effect of Fisetin on Sodium Arsenite-Induced Alteration in Body Weight and Relative Heart Weight

The body weight of normal rats and rats in the AS control and treatment groups did not differ significantly. However, Table 1 illustrated the impact of sodium arsenite on heart weight (absolute) and heart weight to body weight ratio (relative heart weight). Compared to the normal group, administration of sodium arsenite caused a significant increase ($p < 0.001$) in absolute and relative heart weights in the AS control group. On the other hand, treatment of CoQ significantly attenuated absolute and relative heart weights compared with the AS control group. When compared with AS control rats, fisetin (10 and 25 mg/kg) treated rats also showed a significant and dose-dependent decrease ($p < 0.01$ and $p < 0.001$) in the absolute and relative heart weights. Administration of fisetin (5 mg/kg) did not show any significant protection against sodium arsenite-induced increased cardiac weights (Table 1).

3.2 Effect of Fisetin on Sodium Arsenite-Induced Alteration in Electrocardiographic Parameters

Table 2 depicted a notable alteration in the cardiac parameters, encompassing electrocardiography and hemodynamics, observed in rats following sodium arsenite administration. The heart rate in AS control group showed a significant ($p < 0.001$) decrease, whereas QRS, QT, QTc, RR, PR and ST intervals significantly ($p < 0.001$) increased when compared to the normal group. On the other hand, treatment with CoQ showed a significant increase ($p < 0.001$) in heart rate and a significant ($p < 0.001$) decrease in QRS, QT, QTc, RR, PR and ST intervals when compared to AS control group. Treatment with fisetin (10 and 25 mg/kg) showed significant and dose-dependent attenuation ($p < 0.01$ and $p < 0.001$) in sodium arsenite-induced electrocardiographic parameter alterations compared to AS control group (Table 2).

3.3 Effect of Fisetin on Sodium Arsenite-Induced Alteration in Hemodynamic and Left Ventricular Function Parameters

There was a significant decrease ($p < 0.001$) in the Systolic blood pressure (SBP), Diastolic blood pressure (DBP) and Mean Arterial Blood Pressure (MABP) after administration of the sodium arsenite in AS control rats compared to normal rats. Treatment with CoQ significantly ($p < 0.001$) increased SBP, DBP and MABP compared to AS control rats. Treatment with fisetin (10 and 25 mg/kg) also

Table 3. Effect of fisetin on sodium arsenite-induced alteration in oxido-nitrosative stress activity of rats.

Parameter	Normal	As control	As+CoQ (10)	As+F (5)	As+F (10)	As+F (25)
SOD (U/mg of protein)	9.25 ± 0.54	4.42 ± 0.58$^{###}$	8.08 ± 0.53***	4.73 ± 0.47	6.31 ± 0.63**	7.80 ± 0.39***
GSH (µg/mg protein)	0.39 ± 0.01	0.26 ± 0.01$^{###}$	0.36 ± 0.01***	0.31 ± 0.01	0.29 ± 0.01**	0.37 ± 0.01***
MDA (nmol/L/mg of protein)	2.32 ± 0.37	7.16 ± 0.27$^{###}$	2.93 ± 0.28***	7.01 ± 0.29	5.24 ± 0.15**	3.36 ± 0.15***
NO (µg/mL)	6.52 ± 0.48	15.39 ± 0.33$^{###}$	6.74 ± 0.49***	13.31 ± 0.51	12.38 ± 0.54**	9.76 ± 0.56***
Total protein (mg/mL)	20.83 ± 1.3	62.64 ± 1.80$^{###}$	30.94 ± 1.32***	54.37 ± 1.57	45.60 ± 1.56**	39.01 ± 1.84***

Data are expressed as Mean ± SEM (n = 6) and analyzed by One-way Analysis of Variance (ANOVA) followed by Tukey's multiple range test. **$p < 0.01$ and ***$p < 0.001$ as compared to the As-control group, $^{###}p < 0.001$ as compared to normal group, As, Sodium arsenite control group; As+CoQ10 (10), Sodium arsenite+Coenzyme Q10 (10 mg/kg); As+F (5), Sodium arsenite+fisetin (5 mg/kg); As+F (10), Sodium arsenite+fisetin (10 mg/kg); As+F (25), Sodium arsenite+fisetin (25 mg/kg); SOD, Superoxide dismutase; GSH, Glutathione; MDA, Malondialdehyde; NO, Nitric oxide.

increased the SBP, DBP and MABP significantly ($p < 0.01$ and $p < 0.001$) and dose dependently compared to AS control rats. Compared to AS control rats, fisetin (5 mg/kg) failed to inhibit sodium arsenite-induced alteration in SBP, DBP and MABP (Table 2).

There was a significant ($p < 0.001$) increase in Left Ventricular End-Diastolic Pressure (LVEDP), Left Ventricular End-Systolic Pressure (LVESP), systolic duration, pressure time index and tau. In contrast, there was a significant ($p < 0.001$) decrease in dp/dt_{Max}, dp/dt_{Min}, diastolic duration and contractility index in AS control rats after sodium arsenite administration compared to normal rats. However, treatment with CoQ significantly ($p < 0.001$) inhibited sodium arsenite-induced alteration in left ventricular functions compared to AS control rats. Treatment with fisetin (10 and 25 mg/kg) showed a significant and dose-dependent ($p < 0.01$ and $p < 0.001$) decrease in LVEDP, LVESP, systolic duration, pressure time index and tau, whereas an increase in dp/dt_{Max}, dp/dt_{Min}, diastolic duration and contractility index as compared to AS control rats (Table 2).

3.4 Effect of Fisetin on Sodium Arsenite-Induced Alteration in Serum Biochemistry

The levels of CK-MB, LDH, ALP, total cholesterol, triglycerides, Low-Density Lipoprotein (LDL)-C and Very Low-Density Lipoprotein (VLDL)-C increased significantly ($p < 0.001$). In contrast, HDL-C level was significantly ($p < 0.001$) decreased in AS control group compared to the normal group. On the other hand, treatment with CoQ significantly ($p < 0.001$) decreased CK-MB, LDH, ALP and total cholesterol, triglycerides, LDL-C and VLDL-C levels, whereas significantly ($p < 0.001$) increased HDL-C levels compared to AS control group. Treatment with fisetin (10 and 25 mg/kg) significantly and dose-dependently ($p < 0.01$ and $p < 0.001$) inhibited sodium arsenite-induced alteration in CK-MB, LDH, ALP and lipid profile compared to AS control group. However, there was no change in elevated levels of CK-MB, LDH, ALP, total cholesterol, triglycerides, LDL-C and VLDL-C and decreased level of HDL-C by fisetin (5 mg/kg) treated groups compared to AS control group (Table 1).

3.5 Effect of Fisetin on Sodium Arsenite-Induced Alteration in Oxido-Nitrosative Stress Level

There was an effective reduction ($p < 0.001$) in GSH and SOD levels in the cardiac tissue of AS control rats than normal rats. However, treatment with CoQ effectively attenuated ($p < 0.001$) this sodium arsenite-induced reduced GSH and SOD levels in cardiac tissue than AS control rats. Administration of fisetin (10 and 25 mg/kg) effectively and dose-dependently increased ($p < 0.01$ and $p < 0.001$) level of SOD and GSH as compared to AS control rats (Table 3).

The levels of total protein, nitric oxide and MDA in cardiac tissue was markedly elevated ($p < 0.001$) in AS control rats than normal rats. However, CoQ treatment effectively ($p < 0.001$) reduced cardiac total protein, nitric oxide and MDA levels than AS control rats. Fisetin (5 mg/kg) treatment failed to significantly decrease total protein, MDA and NO levels compared to AS control rats. However, administration of fisetin (10 and 25 mg/kg) showed significant and dose-dependent ($p < 0.01$ and $p < 0.001$) decreased levels of total protein, MDA and NO as compared to AS control rats (Table 3).

3.6 Effect of Fisetin on Sodium Arsenite-Induced Alteration in Cardiac Nrf2, HO-1, Bax, Bcl-2 and Caspase-3 mRNA Expressions

The impact of fisetin on the alteration of cardiac Nrf2, HO-1, Bax, Bcl-2 and Caspase-3 mRNA expressions induced by sodium arsenite is detailed in Fig. 1. The significant down-regulation ($p < 0.001$) in Nrf2, HO-1 and Bcl-2 mRNA expressions, whereas Bax, Bax:Bcl-2 and Caspase-3 mRNA expressions were significantly up-regulated ($p < 0.001$) after administration of sodium arsenite in AS control rats as compared to normal rats. The CoQ significantly attenuated this sodium arsenite-induced alteration in cardiac Nrf2, HO-1, Bax, Bcl-2 and Caspase-3 mRNA expressions ($p < 0.001$) compared to AS control rats. Treatment with fisetin (10 and 25 mg/kg) also significantly and dose-dependently ($p < 0.01$ and $p < 0.001$) down-regulated the Bax, Bax:Bcl-2 and Caspase-3 mRNA expressions, whereas significantly and dose-dependently ($p < 0.01$ and $p < 0.001$) up-regulated Nrf2, HO-1 and Bcl-2 mRNA ex-

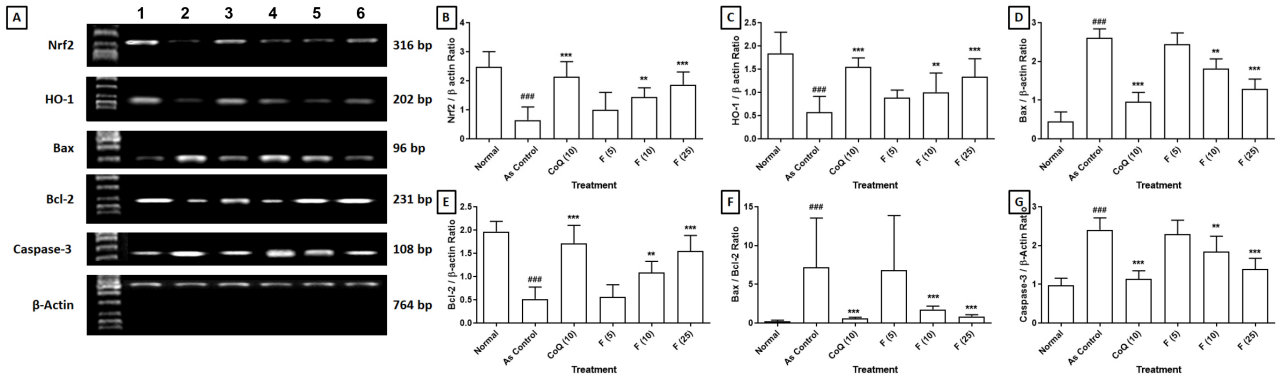

Fig. 1. Effect of fisetin on sodium arsenite-induced alteration in cardiac. (a) Nrf2, HO-1, Bax, Bcl-2 and Caspase-3 mRNA expression in rats, (b) Quantitative representation of the mRNA expression of Nrf2, (c) HO-1, (d) Bax, (e) Bcl-2, (f) Bax:Bcl-2 and (g) Caspase-3. Data are expressed as Mean ± SEM (n = 6) and analyzed by One-way Analysis of Variance (ANOVA) followed by Tukey's multiple range test. $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to the As-control group, $^{\#\#\#}p < 0.001$ as compared to normal group, Lane 1: mRNA expression of normal group, Lane 2: mRNA expression of Sodium arsenite control group, Lane 3: mRNA expression of As+CoQ10 (10) treated group, Lane 4: mRNA expression of As+fisetin (5 mg/kg) treated group, Lane 5: mRNA expression of As+fisetin (10 mg/kg) treated group, Lane 6: mRNA expression of mRNA expression of As+fisetin (25 mg/kg) treated group, As, Sodium arsenite control group; As+CoQ10 (10), Sodium arsenite+Coenzyme Q10 (10 mg/kg); As+F (5), Sodium arsenite+fisetin (5 mg/kg); As+F (10), Sodium arsenite+fisetin (10 mg/kg); As+F (25), Sodium arsenite+fisetin (25 mg/kg); Nrf2, Nuclear Factor Erythroid 2-Related Factor 2; HO-1, Heme Oxygenase 1; Bax, Bcl-2 Associated X; Bcl-2, B-cell lymphoma 2.

Table 4. Effect of fisetin on sodium arsenite-induced alteration in heart histology.

Parameter	Normal	As control	As+CoQ (10)	As+F (5)	As+F (10)	As+F (25)
Myocardial necrosis	–	++++	+	++++	+++	++
Congestion	+	+++	+	+++	++	+
Edema	–	++++	+	+++	++	+
Vacuolization	–	+++	+	+++	++	–
Inflammatory infiltration	+	++++	++	++++	++	+

As, Sodium arsenite control group; As+CoQ10 (10), Sodium arsenite+Coenzyme Q10 (10 mg/kg); As+F (5), Sodium arsenite+fisetin (5 mg/kg); As+F (10), Sodium arsenite+fisetin (10 mg/kg); As+F (25), Sodium arsenite+fisetin (25 mg/kg); –, No abnormality detected; +, Damage/active changes up to less than 25%; ++, Damage/active changes up to less than 50%; +++, Damage/active changes up to less 75%; ++++, Damage/active changes up to more than 75%.

pressions compared to AS control rats. Rats treated with fisetin (5 mg/kg) failed to significantly attenuate cardiac Nrf2, HO-1, Bax, Bcl-2 and Caspase-3 mRNA expressions compared to AS control rats (Fig. 1).

3.7 Effect of Fisetin on Sodium Arsenite-Induced Alteration in Heart Histology

Fig. 2a depicted histopathological observations of the heart from the normal group, revealing a well-maintained architecture with normal myocardial fibers and muscle bundles, well-defined boundaries, mild congestion and inflammatory infiltration. The cardiac tissue from the AS control rats showed severe myocardial degeneration and necrosis (++++), congestion and vacuolization (+++), edema (++++) and infiltration of inflammatory cells (++++) with the disorganized arrangement of muscle bundles with no well-defined boundaries (Fig. 2b). Administration of CoQ showed protection against sodium arsenite-induced myocardial damage reflected by mild inflammatory infiltration (++), edema and congestion and myocardial necrosis (+) (Fig. 2c). Administration of fisetin (5 mg/kg) did not show any reduction in myocardial necrosis (++++), inflammatory infiltration (++++), congestion (+++) and edema (+++) when compared with AS control group (Fig. 2d). Cardiac tissue from fisetin (10 and 25 mg/kg) treated groups showed a reduction in myocardial aberrations induced by sodium arsenite reflected by the presence of moderate myocardial necrosis (+++ and ++), inflammatory cell infiltration (++ and +), congestion (++ and +) and edema (++ and +) (Fig. 2e,f) and (Table 4).

4. Discussion

In developed and developing countries, pesticides are widely used in agriculture, public health and household

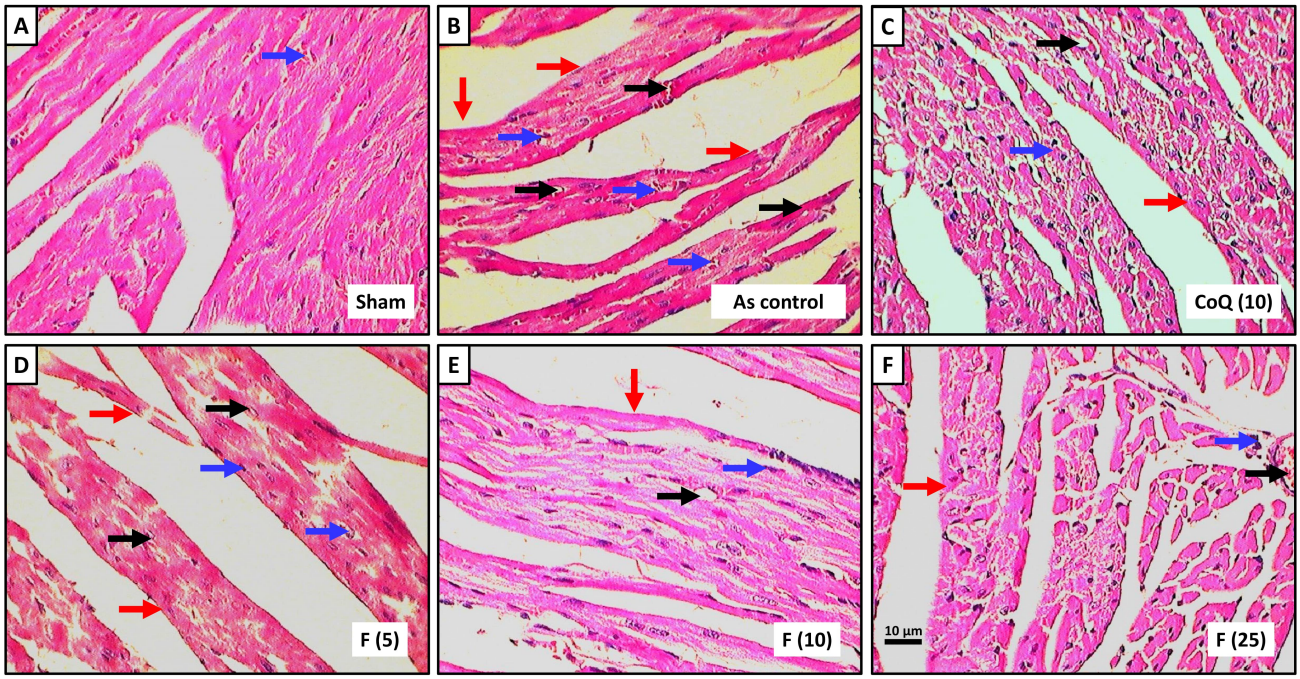

Fig. 2. Effect of fisetin on sodium arsenite-induced alteration in heart histology of rats stained with H&E staining. Photomicrograph of sections of the heart of normal group, (a) Sodium arsenite control group, (b) Sodium arsenite+Coenzyme Q10 (10 mg/kg), (c) Sodium arsenite+fisetin (5 mg/kg), (d) Sodium arsenite+fisetin (10 mg/kg), (e) Sodium arsenite+fisetin (25 mg/kg) and (f) Treated group. Images at 40×. Scale bar = 10 µm. Myocardial necrosis (red arrow), edema (black arrow) and inflammatory infiltration (blue arrow). H&E, Hematoxylin and Eosin.

settings. However, chronic exposure to these pesticides through occupational, environmental or dietary routes can lead to long-term health effects, including cardiovascular complications [39]. Many pesticides, including sodium arsenite, induce oxidative stress, promote inflammation, endothelial dysfunction, etc., which are key mechanisms underlying the development of cardiotoxicity [1,40]. Thus, this study investigated the potential of fisetin on sodium arsenite-induced cardiotoxicity via evaluating the expression of key genes involved in apoptosis, including Bax, Bcl-2 and Caspase-3, providing valuable insights into potential mechanisms of action.

Evaluating relative heart weight is essential for detecting cardiotoxicity, particularly in preclinical assessments. Changes in heart weight relative to body metrics offer insights into cardiac hypertrophy or atrophy, common in cardiotoxicity induced by drugs or toxins [41,42]. While heart weight alone may not definitively diagnose cardiotoxicity, it improves understanding of cardiac pathology and identifies potential cardiotoxic agents when coupled with histological, biochemical and functional assessments [38]. Integrating heart weight evaluations into comprehensive protocols aids early detection and safety assessment of pharmaceuticals and environmental exposures [43,44]. The substantial rise in heart weight and heart/body weight ratio in the arsenite control group corroborates prior findings on arsenic's cardiotoxic effects [39]. The observed alteration in relative heart weight is a crucial indicator of arsenite-induced cardiotoxicity. Notably, rats treated with fisetin adverted these alterations, demonstrating its cardioprotective potential. Current study results aligned with the findings from previous investigators where fisetin exhibited its cardioprotective effects against cardiotoxicity induced by myocardial ischemia injury and doxorubicin via mitigating the elevation in heart weight and relative heart weight in rodents [45].

Furthermore, this organoprotective potential was further demonstrated by the favorable effects of fisetin on hemodynamic parameters, including systolic, diastolic and mean arterial blood pressure [46,47]. Heart rate and blood pressure are the fundamental hemodynamic parameters, whereas stroke volume, cardiac output and total peripheral resistance are the advanced hemodynamic measures [48–50]. These improvements in blood pressure profiles indicate enhanced cardiac output and peripheral vascular function, possibly mediated by the vasodilatory and anti-inflammatory properties of fisetin [51]. The observed enhancements in the contractility index and pressure-time index further support the cardioprotective effects of fisetin against arsenite-induced myocardial dysfunction. Notably, the fisetin also positively affected tau [45], reflecting improved ventricular relaxation and diastolic function. The mechanisms underlying the cardioprotective effects are multifactorial and may involve antioxidant, anti-

inflammatory and calcium-modulating properties [45,52–54]. The alteration in electrocardiographic and hemodynamic parameters observed in this investigation offers valuable insights into fisetin's cardioprotective potential against arsenite-induced cardiotoxicity.

Creatine Kinase MB (CK-MB) is an enzyme predominantly found in the myocardium and has elevated levels in the bloodstream, suggesting myocardial injury [55]. Researchers documented a link between sodium arsenite-induced cardiotoxicity and elevated CK-MB, reflecting myocardial injury and dysfunction [56,57]. The LDH is another crucial intracellular enzyme in energy production that catalyzes pyruvate to lactate in anaerobic conditions [58–60]. Initially, LDH elevation indicates cardiac damage, diagnosing acute myocardial infarction and is also elevated in valve heart disease, heart failure and coronary artery disease [61–63]. Furthermore, changes in ALP levels observed during sodium arsenite-induced cardiotoxicity may be secondary to liver dysfunction, inflammation or other systemic effects [17]. In line with these studies, our results demonstrate a parallel pattern, where rats administered with arsenite displayed increased serum markers (CK-MB, LDH and ALP). However, treatment with fisetin prevented these changes, suggesting protection against arsenite-induced cardiac injury. The decrease in CK-MB and LDH levels following intervention with fisetin implies preserved cardiomyocyte integrity and reduced myocardial damage, which may be potentially attributed to the fisetin's antioxidative properties, lipid peroxidation inhibition and prevention of membrane damage [26,31]. Recent research reinforces the cardioprotective attributes of fisetin via averting the rise in CK-MB and LDH levels in rats experiencing myocardial ischemia-reperfusion injury.

Many pesticides, including organophosphates, pyrethroids and organochlorines, induce oxidative stress by generating ROS and disrupting antioxidant defense mechanisms [26,64,65]. Oxidative stress in the cardiovascular system can lead to lipid peroxidation, protein damage and DNA oxidation, contributing to endothelial dysfunction, inflammation and atherosclerosis [66–68]. Consequently, arsenite-induced cardiotoxicity is characterized by an imbalance in endogenous antioxidant enzymes and oxidative stress markers. The levels of SOD and GSH were reduced due to the excessive generation of arsenite-induced ROS [1] which further alter the functions of proteins, lipids and DNA within the cardiac tissue. The Nrf2 has been reported for its cellular defense potential against oxidative stress [69–72]. Overall, Nrf2 signaling plays a crucial role in protecting against sodium arsenite-induced cardiotoxicity by orchestrating antioxidant, anti-inflammatory and cytoprotective responses in cardiac cells. Numerous researchers reported that antioxidant treatments such as ellagic acid, catechin, eugenol, genistein, naringin, phloretin, resveratrol, α-lipoic acid, etc., protected arsenite-induced mitochondrial damage via their antioxidant potential [73]. In the current study also, the antioxidant efficacy of fisetin during arsenite-induced cardiotoxicity was supported by a reduction in cardiac MDA and NO levels along with an increase in cardiac SOD, GSH, Nrf2 and HO-1 levels. The results of the present investigation are by findings from previous researchers [45,52–54].

Nitric oxide (NO) is critical in regulating cardiomyocyte contractility, with inducible NO synthase implicated in cardiomyopathy and heart failure [74,75]. Dysregulated NO synthesis contributes to arsenite-induced cardiotoxicity, as demonstrated in endothelial cell studies linking arsenite-induced redox activation with endothelial NO synthesis and apoptosis. The NO is also proposed as a marker of subclinical cardiac dysfunction in pediatrics. While physiologically acting as a vasodilator, elevated NO levels contribute to nitrosative stress and tissue damage [76,77]. Current study findings depicted that fisetin modulated the NO levels, potentially by restoring NO homeostasis and reducing nitrosative stress in cardiac tissues of arsenite-induced rats. This was consistent with a study demonstrating that treatment with fisetin reduced the elevation of NO levels during myocardial ischemia injury and doxorubicin-induced myocardial damage in rodents [45,52–54]. The cardioprotective effects of fisetin against arsenite-induced oxide-nitrosative stress may be attributed to hydroxyl groups in ring B that enhance the efficacy of flavonoids as potent free radical scavengers. Substituting phenolic hydroxyl groups onto this ring elucidates a mechanism involving hydrogen atom transfer or single-electron transfer, followed by sequential electron transfer-proton transfer, leading to significant antioxidant properties [78]. Fisetin, a flavonoid compound, features a B-ring arrangement with hydroxyl substitutions at C-3' and C-4' positions, which might contribute to its antioxidant potential.

Sodium arsenite-induced cardiotoxicity involves various mechanisms, one of which is apoptosis, a programmed cell death process that plays a significant role in cardiac injury and dysfunction [1,2]. Arsenite exposure activates both intrinsic and extrinsic apoptotic pathways in cardiac cells. The intrinsic pathway involves the release of cytochrome c from mitochondria and subsequent activation of caspase cascades, including Caspase-9 and Caspase-3, leading to apoptotic cell death [36,79]. Furthermore, arsenite exposure downregulates anti-apoptotic pathways, such as Bcl-2 family proteins, which normally promote cell survival and inhibit apoptosis. Overall, apoptosis plays a significant role in sodium arsenite-induced cardiotoxicity by mediating cardiac cell death, impairing myocardial function and contributing to the development of cardiotoxicity [1,2]. Thus, the researcher targeted apoptotic pathways as a potential therapeutic strategy for mitigating the adverse effects of arsenic exposure on the myocardium. Current results also demonstrated a reinstatement of Bcl-2 mRNA expression and a decline in Bax and Caspase-3 mRNA expres-

sion levels after fisetin treatment, culminating in a reduced Bax/Bcl-2 ratio. These observations suggest that fisetin confers cardioprotective effects by modulating apoptotic pathways, thereby alleviating arsenite-induced cardiotoxicity.

Histological analysis of cardiac tissue is vital in evaluating drug-induced cardiotoxicity and understanding the underlying mechanisms of heart pathology. It offers valuable insights into structural alterations within the myocardium, including myocardial degeneration, inflammation, fibrosis and vascular changes indicative of cardiac injury [11]. This analysis serves as a gold standard for assessing cardiotoxicity induced by chemotherapy agents like arsenic, enabling the detection of early signs of cardiac injury and guiding timely intervention to prevent irreversible damage [2]. Moreover, histopathological evaluation provides detailed morphological information, characterizing arsenite-induced cardiac lesions and elucidating their pathophysiological mechanisms. In current study, histological examination revealed significant myocardial degeneration, interstitial inflammation and myocardial cell hemorrhage in rats administered with arsenic, consistent with previous reports of arsenite-induced cardiotoxicity. However, administration of fisetin attenuated these histopathological alterations, indicating its potential cardioprotective effects against arsenite-induced cardiac damage. Current study findings aligned with prior research demonstrating the cardioprotective properties of fisetin against myocardial ischemia injury and doxorubicin-induced cardiotoxicity [45,52–54].

The present study has several limitations. First, this study reported the cardioprotective potential of fisetin via its antioxidant and antiapoptotic potential however, future study is needed to explore another possible mechanism for the beneficial effects of fisetin against cardiotoxicity. Second, more comprehensive studies are needed to clarify the mechanism of fisetin using advanced techniques like echocardiography and TUNEL staining. Third, this study evaluated the effect of fisetin on a rat model, however, future studies focusing on isolated rat heart models using the Langendorff methods are needed to confirm the findings of the present study.

5. Conclusion

The findings of this study demonstrate that fisetin effectively mitigates sodium arsenite-induced cardiotoxicity in experimental rats. The protective effects of fisetin are associated with antioxidant (Nrf2/HO-1) and antiapoptotic pathway (Bax/Bcl-2 and Caspase-3) in experimental rats. Fisetin treatment dose-dependently attenuated cardiac dysfunction, reduced oxidative stress, and modulated key molecular markers involved in these pathways. These results suggest that fisetin may have potential as a therapeutic agent for preventing or treating arsenite-induced cardiotoxicity.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

TZ conceived and designed the research study. LX performed the data acquisition and drafted the manuscript. TZ analyzed the data. Both (TZ and LX) authors contributed to editorial changes in the manuscript. Both (TZ and LX) authors read and approved the final manuscript. Both (TZ and LX) authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The Animal Ethical Committee of Qingdao Municipal Hospital approved (approval no. 202188726D) all the experimental protocols. All procedures involving animals were conducted in accordance with the ARRIVE (Animal Research: Reporting of *In-Vivo* Experiments) guidelines and National Institute of Health Guide for Care and Use of Laboratory Animals.

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Conflict of Interest

The authors declare no conflict of interest.

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