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Cardioprotective effects of total and anthocyanin-rich extracts of *Opuntia ficus-indica* fruit on epinephrine-induced cardiac injury in rats

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ABSTRACT

Objective: To examine the effect of fruit extracts of *Opuntia ficus-indica* on epinephrine-induced myocardial injury in rats.

Methods: Rats were pre-treated with hydroalcoholic and anthocyanin-rich extracts (250, 500, and 1000 mg/kg) orally for 16 days. Epinephrine (2 mg/kg) was injected subcutaneously on days 15 and 16. Then ECG, heart rate, RR interval, cardiac histopathology, and serum parameters of cardiac damage and oxidative stress were evaluated.

Results: Pretreatment with the hydroalcoholic and anthocyanin-rich extracts of *Opuntia ficus-indica* at doses of 500 and 1000 mg/kg reversed epinephrine-induced changes in ECG, heart rate, RR interval, and cardiac histopathology, and attenuated creatine phosphokinase-MB activity and malondialdehyde level. The extracts at all doses diminished heart weight, lactate dehydrogenase, and aspartate aminotransferase, and also elevated total antioxidant capacity. However, 1000 mg/kg of the anthocyanin-rich extract was more effective than the hydroalcoholic extract.

Conclusions: Supplementation with fruit extracts of *Opuntia ficus-indica*, especially anthocyanin-rich extract, could protect against epinephrine-induced cardiac injury in rats by alleviating electrocardiographic, biochemical, histopathological, and oxidative stress parameters, and may be used in the treatment of cardiovascular diseases.

KEYWORDS: *Opuntia ficus-indica*; Antioxidant; Cardiovascular diseases; Epinephrine; Cardiac injuries; Lipid peroxidation

1. Introduction

Cardiovascular diseases (CVD) encompass a range of conditions that impact the functions of the heart and blood vessels. The World Health Organization's data indicates that 32% of global fatalities stem from CVD[1]. Among these, a heart attack is a crucial event that may lead to cardiac failure or cardiac death[2]. The myocardial damage occurs due to the lack of balance between the heart's oxygen

Significance

Opuntia ficus-indica is a widely used cactus with some cardiovascular benefits. In this animal study, fruit extracts of *Opuntia ficus-indica*, especially anthocyanin-rich extract, improved electrocardiographic, biochemical, histopathological, and oxidative stress parameters in rats with epinephrine-induced cardiac damage. However, further studies are required to elucidate the molecular mechanisms of its protective effect and confirm its efficacy.

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supply and demand, leading to the death of cardiac muscle cells[3]. Various factors contribute to cardiac injuries including arrhythmias, myocardial inflammation, microvascular obstruction, and disrupted myocardial perfusion. Myocardial damage is attributed to the excessive production of reactive oxygen species, lipid peroxidation, and disruption of myocardial cell membranes due to oxidative stress[4].

Finding strategies to prevent heart attacks and minimize heart tissue injury is of great interest to medical researchers worldwide[5]. In this context, the positive impact of plant-derived polyphenolic compounds and anthocyanins with robust antioxidant properties has been established on heart health[6].

Opuntia ficus-indica (L.) Mill. (OFI), commonly known as the Indian prickly pear, is a species of cactus that belongs to the Cactaceae[7]. OFI possesses anti-inflammatory, neuroprotective, anti-diabetic, diuretic, sedative, and anti-anxiety effects[8–12]. Studies have shown that this plant can regulate heart rate, reduce blood lipid levels, and lower blood pressure, thereby mitigating cardiovascular risk factors[13,14]. Fresh juice from OFI fruits has a high antioxidant capacity with the ability to oppose oxidative stress and benefit cardiovascular health[15]. The fruits of OFI contain essential nutrients and bioactive compounds like anthocyanins, phenolics, and flavonoids which exert potent antioxidant actions[16,17]. Toxicological investigations have reported a low toxicity for OFI[18,19]. In an acute toxicity study, oral administration of OFI hydroalcoholic extract at doses of 500–2000 mg/kg per day for 1 week showed no toxicity in various behavioral, body weight gain, biochemical, and hematological tests[19]. A previous study has exhibited renoprotective activity of OFI in an animal model of lithium carbonate-induced toxicity through biochemical, histopathological, and antioxidative evidence[20]. Moreover, an herbal mixture containing *Cactus grandiflorus* has revealed cardioprotective and anti-hyperlipidemic properties against salbutamol-induced cardiotoxicity in rabbits[21].

Regarding the reported antioxidant and cardiovascular advantages of OFI, this investigation aimed to explore the impact of total and anthocyanin-rich extracts from the fruits of OFI on epinephrine-induced heart damage in rats.

2. Materials and methods

2.1. Plant material and extract preparation

The fruits of OFI were purchased from a local market in Isfahan city (Isfahan province of Iran) in June 2023 and acknowledged by a botanist. A voucher specimen (SAM5002) was stored in the Herbarium of Department of Pharmacognosy, Isfahan University of

Medical Sciences. To obtain the hydroalcoholic extract (OFI-HE), the dried fruit materials were first pulverized and then subjected to a maceration process with a solution of ethanol:water (70:30) at room temperature for 72 h, repeated three times. For preparation of anthocyanin-rich extract (OFI-AE), a solution of methanol:acetic acid (97:3) was used[22]. After filtration, the resulting extracts were concentrated by removing the solvent using a vacuum rotary evaporator under reduced pressure at 45 °C. The extract powders were obtained through freeze-drying and stored at –20 °C.

2.2. Determination of total phenolic and anthocyanin contents

To measure the quantity of total phenolic compounds and standardize the extracts of OFI, the Folin-Ciocalteu assay was conducted[23]. In this colorimetric method, a mixture of phosphomolybdate and phosphotungstate solution served as the Folin-Ciocalteu reagent. In summary, extracts or standard samples were mixed with 20% sodium carbonate and subsequently incubated with diluted Folin-Ciocalteu reagent for 120 min. The UV absorbance was recorded using a spectrophotometer at 765 nm. The total phenolic content of the samples was determined using a standard curve generated from different concentrations of gallic acid and expressed in terms of milligrams of gallic acid equivalent (GAE) per gram of dried OFI extract.

To measure anthocyanin content, the dried fruit extract was dissolved in a solution of 1% hydrochloric acid in methanol and then centrifuged at 13000 rpm for 10 min. The absorbance of the supernatant was measured at 530 and 657 nm and the amount of extract anthocyanin was calculated with $A = A_{530} - (0.25 \times A_{657})$ formula[24].

2.3. Animals and experimental design

Wistar male rats, weighing (180 ± 25) g, were obtained from the animal house of the School of Pharmacy and Pharmaceutical Sciences. These rats were kept under controlled conditions of 20–25 °C and a 12-hour light/12-hour dark cycle with free access to water and standard food. Before the experiment, the animals were acclimatized to the experimental environment for one week.

The rats were divided into 10 groups with 6 animals per group. Normal saline was administered by oral gavage to the normal control group for 16 d. Epinephrine (Iran Hormone Pharmaceutical Co., Iran) 2 mg/kg was injected subcutaneously (*s.c.*) at a 24-hour interval on days 15 and 16 in the epinephrine control group[25]. The extract control groups received 1000 mg/kg of OFI-HE or OFI-AE orally alone for 16 d. The therapeutic groups received OFI-HE or OFI-AE orally at doses of 250, 500, and 1000 mg/kg for 16 d

and epinephrine on days 15 and 16. The doses of OFI extracts were selected based on a previous study[26].

The rats' weights were recorded at the beginning of the experiment and subsequently every other day. Twenty-four hours after the last epinephrine injection, a lead II electrocardiogram (ECG) was recorded in anesthetized animals (ketamine 60 mg/kg and xylazine 6 mg/kg) by a computerized data acquisition eWave system and evaluated with eProbe software (Science Beam; Parto Danesh Co., Iran). The RR interval and heart rate (HR) were detected, and ST-segment changes were assessed.

Then, blood was collected from the neck region and serum was separated by centrifugation at 4000 rpm for 10 min and used for assessment of biochemical markers related to cardiac injury and oxidative stress. After euthanasia *via* CO₂ exposure, the hearts were removed, weighed, and fixed in 10% formalin solution for histopathology.

2.4. Biochemical assay

The activities of markers associated with myocardial injury including lactate dehydrogenase (LDH), creatine phosphokinase-MB (CK-MB), and aspartate aminotransferase (AST) were measured in serum samples according to the manufacturer's instructions by colorimetric method (at 340 nm) using enzymatic kits (Pars Azmoon Co., Iran) *via* an auto-analyzer (Nihon Kohden, Japan).

2.5. Total antioxidant capacity assay

The ferric-reducing antioxidant power (FRAP) test was carried out according to the instruction of a standard kit (Hakiman Pajooh Co., Iran) in plasma samples. The FRAP reagent comprising ferric-tripyridyl triazine complex was used and the absorbance of the samples was read at 570 nm (540-590 nm). The total antioxidant capacity as FRAP value was calculated using a standard curve of FeSO₄ and expressed as millimoles (mM) of FeSO₄ equivalents[27].

2.6. Lipid peroxidation assay

To measure lipid peroxidation, the malondialdehyde (MDA) level

was determined in plasma samples through the thiobarbituric acid reactive substances test using a standard kit (Hakiman Pajooh Co., Iran). The absorbance of the samples was read at 532 nm and the MDA value was calculated using a standard curve and expressed in micromoles (μM)[28].

2.7. Histopathological examination

The cardiac apex samples were paraffinized and sectioned into 5 μm thickness. Hematoxylin and eosin (H&E) staining was performed and slides were visualized by a standard Zeiss® light microscope for histopathological examination (×400 magnification).

2.8. Statistical analysis

All data were presented as mean ± standard error of the mean (SEM) and were subjected to one-way analysis of variance (ANOVA) followed by Tukey *post-hoc* test using SPSS software version 25.0. A *P*-value of <0.05 was considered statistically significant.

2.9. Ethical statement

The experimental procedures adhered to the international guidelines for the use and care of laboratory animals and were approved by the Institutional Research Ethics Committee of Isfahan University of Medical Sciences (Ethical approval ID: IR.MUI.AEC.1402.028, 11 July, 2023).

3. Results

3.1. Total phenolic and anthocyanin contents

The content of total phenolic compounds in OFI-HE was (102.54 ± 43.00) mg GAE/g of dried plant extract and in OFI-AE (83.88 ± 42.00) mg GAE/g of dried plant extract. The amount of anthocyanins in OFI-HE was 40.4 mg, and in OFI-AE, it was 55.2 mg per 100 g of dried fruit extract.

Table 1. Effect of *Opuntia ficus-indica* hydroalcoholic and anthocyanin-rich extracts on body weight and relative heart weight changes in rats with epinephrine-induced cardiac injury.

Parameters	Normal control	EPN control	OFI-HE control 1 000 mg/kg	OFI-AE control 1 000 mg/kg	EPN (2 mg/kg) + OFI-HE			EPN (2 mg/kg) + OFI-AE		
					250 mg/kg	500 mg/kg	1 000 mg/kg	250 mg/kg	500 mg/kg	1 000 mg/kg
Body weight changes (%)	10.26 ± 1.98	9.55 ± 1.72	8.55 ± 0.22	4.83 ± 0.31 [#]	6.91 ± 0.33	5.17 ± 0.25 [#]	5.32 ± 0.29 [#]	5.18 ± 1.24 [#]	4.65 ± 0.75 [#]	4.74 ± 0.66 [#]
Relative heart weight (%)	0.38 ± 0.02	0.52 ± 0.03 ^{###}	0.39 ± 0.02	0.38 ± 0.03	0.44 ± 0.02 ^{***}	0.40 ± 0.01 ^{***}	0.39 ± 0.02 ^{***}	0.39 ± 0.02 ^{***}	0.38 ± 0.01 ^{***}	0.40 ± 0.02 ^{***}

Values are expressed as mean ± SEM (*n*=6). [#]*P* < 0.05 and ^{###}*P* < 0.001 versus the normal control; ^{***}*P* < 0.001 versus the EPN control. EPN: epinephrine; OFI-HE: *Opuntia ficus-indica* hydroalcoholic extract; OFI-AE: *Opuntia ficus-indica* anthocyanin-rich extract.

3.2. Effect of OFI extracts on body and heart weight changes

As shown in Table 1, the weight of rats receiving 500 and 1000 mg/kg of OFI-HE or OFI-AE simultaneously with epinephrine as well as 1000 mg/kg of OFI-AE alone for 16 d significantly decreased compared to the normal control rats ($P < 0.05$). Rats with epinephrine-induced myocardial injury showed significantly raised heart weight. However, OFI extracts at all doses were able to significantly prevent the increase in heart weight.

3.3. Effect of OFI extracts on ECG parameter

As seen in Figure 1 and Table 2, administration of epinephrine significantly caused ECG abnormalities such as elevation in ST segment and reduction in R wave amplitude, with a notable increase in RR interval by 18.9% and a reduction in HR by 23.03%, as compared to the control group ($P < 0.001$).

These ECG changes were significantly reversed in animals pre-treated with 500 and 1000 mg/kg of OFI-HE and OFI-AE, as demonstrated by declined ST-segment elevation and augmented R wave amplitude as well as decreased RR interval ($P < 0.001$ at 1000 mg/kg of OFI-AE) and improved HR ($P < 0.001$ at 1000 mg/kg of OFI-AE).

3.4. Effect of OFI extracts on biochemical parameters

The epinephrine-challenged group exhibited a marked rise in the serum level of LDH activity [(1546 ± 120) IU/L] when compared to the normal control group [(801 ± 72) IU/L] ($P < 0.001$). Pretreatment with all doses of OFI-HE and OFI-AE attenuated the activity of this enzyme, especially at the dose of 1000 mg/kg of OFI-AE [(632 ± 56) IU/L, $P < 0.001$] when compared to the epinephrine control group (Figure 2A).

Following epinephrine injection, the activity of CK-MB was significantly elevated ($P < 0.001$). OFI-AE and OFI-HE at all doses could significantly decline CK-MB activity (Figure 2B).

A significant elevation in the serum activity of AST was observed after exposure to epinephrine ($P < 0.001$), which was significantly prevented by administration of OFI-HE and OFI-AE at all doses. However, lower efficacy was observed at the 1000 mg/kg dose (Figure 2C).

Treatment with 1000 mg/kg of OFI-HE or OFI-AE alone in normal rats did not cause any alteration in the levels of cardiac biochemical markers.

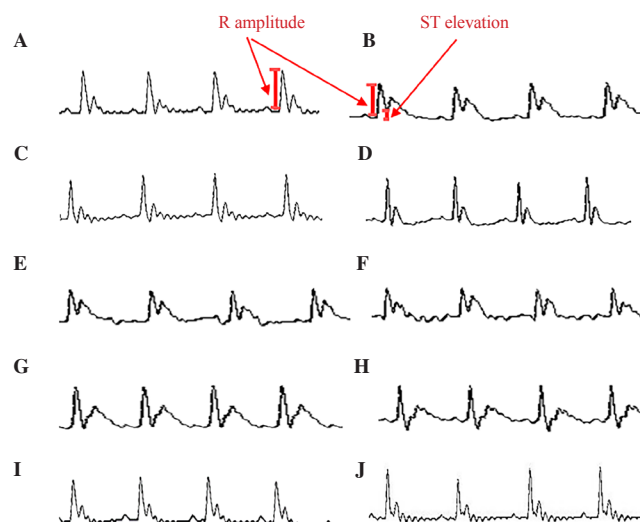


Figure 1. Representative electrocardiograph traces of lead II for normal control (A); epinephrine control (B); *Opuntia ficus-indica* hydroalcoholic (C) and anthocyanin-rich extracts (D); epinephrine-challenged groups treated with 250, 500, 1000 mg/kg of *Opuntia ficus-indica* hydroalcoholic (E, G, I), or anthocyanin-rich extract (F, H, J).

3.5. Effect of OFI extracts on total antioxidant capacity and lipid peroxidation

Figure 3A shows the effect of OFI extracts on oxidative stress markers in rats with epinephrine-induced cardiac injury. Epinephrine markedly reduced the FRAP value from (0.89 ± 0.05) mM in normal rats to (0.47 ± 0.03) mM ($P < 0.001$). Pretreatment with all doses of OFI-HE and OFI-AE significantly increased FRAP levels as compared with the epinephrine-challenged group. It is noteworthy that administration of OFI-HE or OFI-AE (1000 mg/kg) alone obviously elevated FRAP value in normal rats.

Regarding lipid peroxidation, a high content of MDA was observed in the epinephrine-challenged group when compared to the normal control rats ($P < 0.001$). Treatment with 500 and 1000 mg/kg of OFI-HE and OFI-AE noticeably lessened the serum MDA content (Figure 3B).

3.6. Effect of OFI extracts on cardiac histopathology

Microscopic examination exhibited normal histological structure of

Table 2. Effect of *Opuntia ficus-indica* hydroalcoholic and anthocyanin-rich extracts on ECG parameters in rats with epinephrine-induced cardiac injury.

Parameters	Normal control	EPN control	OFI-HE control	OFI-AE control	EPN (2 mg/kg) + OFI-HE			EPN (2 mg/kg) + OFI-AE		
					1000 mg/kg	500 mg/kg	250 mg/kg	1000 mg/kg	500 mg/kg	250 mg/kg
Heart rate (BPM)	325.1 $\pm$ 9.9	250.2 $\pm$ 13.4 ^{###}	309.4 $\pm$ 8.3	322.3 $\pm$ 4.6	263.1 $\pm$ 7.7	306.7 $\pm$ 2.8 [*]	290.1 $\pm$ 7.8 [*]	227.8 $\pm$ 8.6	310.9 $\pm$ 5.2 ^{***}	341.2 $\pm$ 3.1 ^{***}
RR-interval (ms)	191.3 $\pm$ 5.2	227.4 $\pm$ 3.3 ^{###}	204.2 $\pm$ 7.7	199.2 $\pm$ 5.6	219.8 $\pm$ 4.5	195.4 $\pm$ 2.7 [*]	208.0 $\pm$ 5.2	228.5 $\pm$ 1.4	195.3 $\pm$ 6.4 [*]	176.7 $\pm$ 6.2 ^{***}

Values are expressed as mean $\pm$ SEM ($n=6$). ^{###} $P < 0.001$ versus the normal control; ^{*} $P < 0.05$, and ^{***} $P < 0.001$ versus EPN control. BPM: beat per minute; ms: millisecond.

heart tissue in the control rats (Figure 4A) and in rats that received OFI extracts (1000 mg/kg) alone (Figure 4C and 4D). In contrast, degeneration of myocytes (rupture and vacuolization), infiltration of inflammatory cells, and focal hemorrhage were observed in epinephrine-challenged rats (Figure 4B). Administration of OFI

extracts at the dose of 250 mg/kg did not cause any significant effect (Figure 4E and 4F). However, heart sections obtained from the groups pre-treated with 500 (Figure 4G and 4H) and 1000 mg/kg (Figure 4I and 4J) of OFI extracts, showed fewer histopathological alterations.

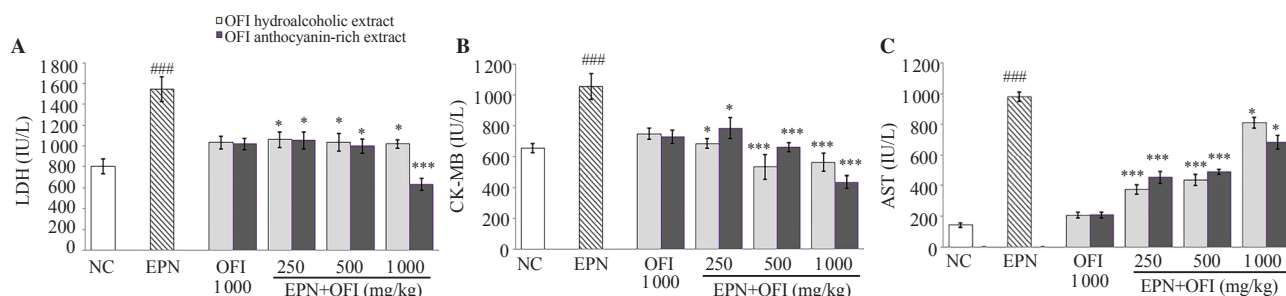


Figure 2. Effects of *Opuntia ficus-indica* hydroalcoholic and anthocyanin-rich extracts on serum lactate dehydrogenase (LDH) (A), creatine kinase-MB (CK-MB) (B), and aspartate aminotransferase (AST) (C) in rats with epinephrine-induced cardiac injury. Values are expressed as mean $\pm$ SEM ($n=6$). ### $P<0.001$ versus normal control; * $P<0.05$ and *** $P<0.001$ versus the epinephrine control. NC: normal control; EPN: epinephrine; OFI: *Opuntia ficus-indica* extract.

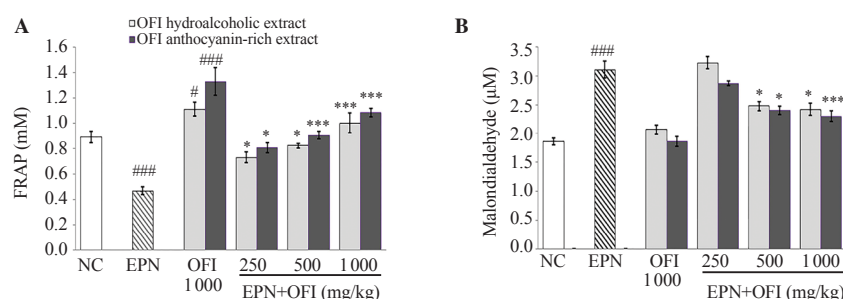


Figure 3. Effects of *Opuntia ficus-indica* hydroalcoholic and anthocyanin-rich extracts on serum ferric-reducing antioxidant power (FRAP) (A) and malondialdehyde level (B) in rats with epinephrine-induced cardiac injury. Values are expressed as mean $\pm$ SEM ($n=6$). # $P<0.05$ and ### $P<0.001$ versus normal control; * $P<0.05$ and *** $P<0.001$ versus the epinephrine control.

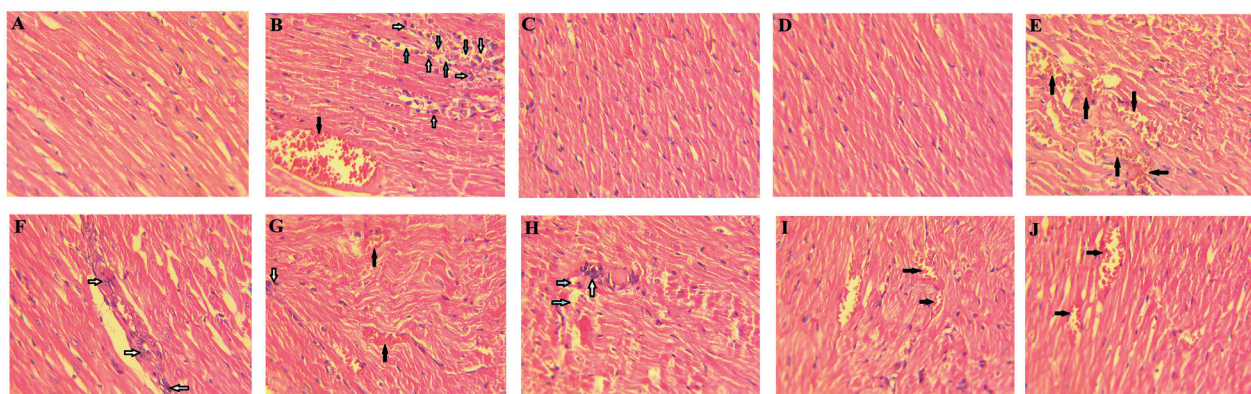


Figure 4. Histological sections of heart tissue of normal control (A); epinephrine control (B); *Opuntia ficus-indica* hydroalcoholic (C) and anthocyanin-rich extracts (D); epinephrine-challenged groups treated with 250, 500, 1000 mg/kg of *Opuntia ficus-indica* hydroalcoholic (E, G, I), or anthocyanin-rich extract (F, H, J). Black arrows: areas of hemorrhage and congestion; white arrows: infiltration of inflammatory cells; blue arrows: degeneration of myocytes, (Hematoxylin and eosin staining; $\times 400$ magnification).

4. Discussion

In the present study, we found that OFI fruit extracts, especially OFI-AE, exerted a potent protective effect against epinephrine-induced myocardial injury in rats through augmentation of antioxidants defense, inhibition of lipid peroxidation, and restoration of the changed ECG pattern, biochemical markers, and histopathological architecture.

Previous studies have reported the protective effects of OFI on various cellular and tissue injuries such as liver, brain, and kidney damage caused by various harmful factors *via* antioxidant, anti-apoptotic, and anti-inflammatory activities[20,29–31]. In this study, epinephrine-induced myocardial damage led to the abnormalities in ECG pattern evidenced by raised ST segment, depressed R wave amplitude, prolonged RR interval, and dropped HR which may be related to the interruption of membrane integrity and subsequently dysfunction of myocardial cell membrane and cardiac ischemia[32,33]. Epinephrine induces hyperactivity of myocardial and spasm of coronary artery and subsequent heart ischemia by stimulating beta- and alpha-adrenergic receptors, respectively[25]. Besides the ischemic and necrotic changes, the over-activity of catecholamine is associated with the production of deleterious free radicals, which harms the structure of heart cells and weakens the antioxidant defense system[33]. As our data confirmed, epinephrine resulted in cardiac weight gain, histopathological alterations, release of LDH, CK-MB, and AST into the circulation, decreased antioxidant capacity, and augmented lipid peroxidation.

In this investigation, OFI extracts pretreatment at the doses of 500 and 1000 mg/kg protected the rats' hearts against epinephrine-induced cardiac injury by suppressing the ST-segment, enhancing R wave amplitude, and normalizing heart rate. There was an increase of 36.4% in HR and a reduction of 22.3% in RR interval after administration of 1000 mg/kg of OFI-AE. Pretreatment of epinephrine-challenged rats with OFI extracts, especially OFI-AE, also reversed the heart weight gain, serum cardiac parameters, and cardiac pathological alterations. Regarding serum AST activity, OFI extracts showed lower efficacy at their highest dose. During myocardial damage or necrosis, release of AST from the cytoplasm and mitochondria may occur depending on the severity of the injury[34]. Moreover, decreased hepatic arterial flow may happen when cardiac output is decreased following myocardial injury[35]. Although higher doses of extracts were safe in normal animals, they might lead to toxicity in the condition of liver stress.

We also found that OFI extracts decreased body weight gain in animals receiving doses of 500 and 1000 mg/kg. In the study of Grube *et al*, treatment with a natural fiber complex derived from OFI (3 g/day, for 12 week) led to the weight loss in overweight and obese adult men and women[36]. However, the data from a

meta-analysis of randomized clinical trials displayed that OFI consumption reduces body fat percentage and body mass index without significant impact on body weight[14]. The growing body of evidence suggests the valuable cardiovascular protective effects of OFI consumption[14,37–39]. Clinical studies have revealed the anti-hyperglycemic and antihyperlipidemic properties of OFI[14,37]. The data obtained from a meta-analysis of available clinical trials displayed the impact of OFI supplementation on decreases in total cholesterol and blood pressure[14]. Moreover, the diuretic effect and decreased potassium excretion in the urine have been reported for OFI cladodes[38].

In a recent study conducted by Khoulood *et al.*, supplementation with a flavonoid-rich OFI fresh fruit juice in healthy male athletes significantly improved cardiovascular markers including triglycerides, total cholesterol, low-density lipoprotein, LDH, creatine kinase, and MDA during the yo-yo intermittent recovery test. OFI also enhanced the performance and the rating of perceived exertion and prevented muscle damage induced by intense exercise. Their results showed a significant decrease in the maximal HR without any effect on the systolic and diastolic blood pressure[15]. In another study on healthy men, single use of OFI fruit juice declined total autonomic heart regulation and HR variability after a high-fat and high-carbohydrate meal *via* lessening the vagal tone[39].

The cardioprotective properties of pretreatment with OFI extracts against deleterious effects of epinephrine in the present investigation may be attributed to the antioxidant activities of OFI. As our findings revealed, administration of OFI extracts counteracted the epinephrine-induced oxidative stress status by decreasing lipid peroxidation and enhancing total antioxidant capacity. The great amounts of phenolic compounds in the OFI-HE and anthocyanin content in the OFI-AE may be related to the beneficial cardiovascular activities of OFI extracts. In different articles, very diverse values have been reported for the content of phenolic compounds in OFI[40]. In the study of El-Hawary *et al.*, total phenolics in the fruit peel and pulp extracts were determined as 165.20 and 53.48 mg GAE/g of extract, respectively[41]. Regarding anthocyanin content, Mansour and Ben calculated the amounts of monomeric anthocyanins in OFI peel as 15.28 mg/100 g[42]. Interestingly, our findings showed that OFI-AE (1000 mg/kg) increased plasma total antioxidant activity in normal rats. In the study of Avila-Nava and co-workers, consumption of OFI (300 g) for 3 d led to a notable rise in antioxidant activity in blood by 5% and in plasma by 20% in healthy people[43].

The presence of bioactive phytochemicals including organic acids (ascorbic, caffeic and hydroxybenzoic acid), carotenoids (β -carotene, lutein, lycopene), flavonoids (isorhamnetin, kaempferol, rutin) and pigments (betacyanins, betaxanthins, betalains) in OFI fruit likely contributes to its cardioprotective potential[40].

There is much evidence for impact of isorhamnetin on CVD

through myocardial and endothelial protective, anticoagulant, antihypertensive, antihyperlipidemic, antioxidative, anti-atherosclerotic, and anti-inflammatory effects[44]. Recent studies have revealed the beneficial activities of kaempferol against myocardial injuries caused by ischemia/reperfusion *via* inhibiting MAPK signaling and subsequent oxidative, inflammatory, and apoptotic events[45]. Kaempferol has also protected cardiomyocytes by reducing reactive oxygen species levels, preserving the mitochondrial membrane potential, and decreasing cytochrome C release[46].

The major limitations of the current investigation include the lack of assessment of the impact of OFI fruit extracts on cardiac function and also lack of evaluation of the detailed molecular mechanisms involved in the protective activities of OFI fruit extracts against cardiac injury.

In conclusion, the current findings confirmed the cardioprotective benefits of OFI fruit against epinephrine-induced myocardial injuries in rats by improving ECG pattern and myocardial architecture, dropping serum levels of lipid peroxidation and cardiotoxicity parameters, and enhancing total antioxidant capacity. There is a crucial need for further research to clarify the exact mechanisms and potential therapeutic benefits of OFI fruit, especially regarding CVD and disease conditions associated with oxidative stress.

Conflict of interest statement

The authors declared no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

LS was responsible for the conceptualization of the study, supervision, designing the animal investigation, and editing of the manuscript; ZH contributed to the histopathological examination;

MS designed the herbal studies; MF and MK were involved in the animal treatments, data acquisition, and writing original draft.

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