

Cardioprotective Effect of Daidzein Against Isoproterenol-Induced Myocardial Infarction Injury in Rats

Vadivelan R (✉ vadivelanr@jssuni.edu.in)

JSS College of Pharmacy Ooty <https://orcid.org/0000-0001-7927-4554>

Vikash Sundaram

JSS College of Pharmacy Ooty

Tharani Mohanasundaram

JSSCP Ooty: JSS College of Pharmacy Ooty

RUCHI TIWARI

PSIT: Pranveer Singh Institute of Technology

Meenatchisundaram Subramani

Shri Nehru Maha Vidyalaya College of Arts and Science: SNMV College of Arts and Science

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Abstract

Purpose

Daidzein (DZ) is an isoflavone derived from soy plants that has a wide range of nutritional benefits. The cardioprotective effect of DZ on isoproterenol-induced myocardial infarction (MI) injury in rats was investigated in this work.

Methods

DZ (10 and 20 mg/kg) was given to adult male Wistar Albino rats on a daily basis for 6 weeks. After the treatment period, the rats were subcutaneously injected with isoproterenol (ISO) (85 mg/kg) at 24-hour intervals for two days. Myocardial infarct size, aspartate transaminase (AST), alanine transaminase (ALT), creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) activities, as well as malondialdehyde (MDA) levels, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (GSH) catalase (CAT) activities, and the histological alterations in the heart were observed using haematoxylin and eosin staining. Tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6) levels in heart tissues were detected with ELISA kits.

Results

In ISO-induced rats, DZ administration reduced myocardial infarct area, improved histological abnormalities in the myocardium, and decreased the activities of myocardial injury marker enzymes. Furthermore, DZ effectively reversed ISO-induced lipid peroxidation and antioxidant depletion, as well as considerably lowering cardiac pro-inflammatory cytokine levels in this animal model.

Conclusion

This research shows that DZ reduces ISO-induced myocardial infarction injury and is linked to a reduction in oxidative stress and inflammation.

Introduction

Myocardial infarction (MI) is a kind of ischemic heart disease in which the heart muscle is characterised by an imbalance of ischemia and necrosis [1, 2]. Despite considerable prognostic improvements over the last decade, acute myocardial infarction remains the most devastating manifestation of coronary artery disease, affecting over 7 million individuals globally and contributing for over 4 million deaths in Europe and Northern Asia each year [3, 4]. The main pathophysiological processes involved in myocardial infarction have been thoroughly defined: oxidative stress and inflammation [5, 6]. Antioxidant treatment appears to have the capacity to reduce ISO-induced myocardial damage [7–9].

When given in significant amounts, the synthetic catecholamine L-isoproterenol (L-ISO) induces cardiac cell death. It's a common paradigm for producing infarct-like myocardial lesions in rats [10]. An improved understanding of the mechanisms involved in MI has motivated the hunt for a drug that might minimise myocardial harm. A drug that protects the myocardium from toxic substances may help to prevent or postpone myocardial infarction. Free radical scavengers (antioxidants) such as β -carotene, vitamin C, vitamin E, and selenium, among other nutritional advantages, have high antioxidant effects because free radical generation causes significant injury in MI.

A polyphenol-rich diet has been demonstrated in several studies to help protect against chronic illnesses, particularly those that are inflammatory and create reactive species. Soybeans are recognised to constitute a complete diet since they include proteins, lipids, essential amino acids, and beneficial secondary metabolites such as isoflavones and phenolic compounds. Soybean isoflavones are thought to be responsible for at least some of the health benefits associated with soy consumption.

In epidemiological research, consumption of soybeans and soy products has been shown to lower the incidence of human cancer, osteoporosis, and cardiovascular disease [11–13]. The three isoflavone aglycones genistein, DZ, and glycitein are all found in four glucosidic forms in soybeans and soy meals. DZ has lately been found to offer a number of health advantages [14–17], including chemoprevention of cardiovascular diseases and cancer, as well as the ability to prevent and cure osteoporosis in postmenopausal women with bone loss without the need of oestrogen replacement medication.

As a result, the goal of this investigation was to see if oral DZ medication might protect rats' hearts against oxidative myocardial damage caused by ISO.

Materials And Methods

Drugs and Chemicals

Sigma Chemical Co. provided the ISO and DZ (St. Louis, MO, USA). Agappe Diagnostics provided the aspartate transaminase (AST), alanine transaminase (ALT), creatine kinase (CK-MB), and lactate dehydrogenase (LDH) kits (Kerala, India). Commercially available kits (Qualigens Diagnostics) were used to test the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), as well as reduced glutathione (GSH) and malondialdehyde (MDA) in cardiac tissues (Mumbai, India). R&D Systems provided ELISA kits for tumour tissue necrosis factor (TNF- α) and interleukin-6 (IL-6) research (Minneapolis, MN, USA).

Animals

The JSS College of Pharmacology sold 32 male Wistar-Albino rats weighing between 180 and 200 grammes. The animals were kept under typical laboratory settings, which included a 12 hr light/dark cycle with a temperature of 25 \pm 2°C and a humidity of 50 \pm 15%. They were given a week to acclimate to the circumstances of the animal home before the experiment and were given unlimited access to regular

laboratory food and water. The experiment followed the rules set out by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), based in New Delhi, India. The Institutional Animal Ethics Committee of JSS College of Pharmacy in Ooty gave its approval to the experimental protocol (Approval No.06, dated,20-06-2019).

Induction of Experimental MI

To produce experimental MI, ISO was dissolved in normal saline and subcutaneously administered into rats (85 mg/ kg) at 24-hour intervals for two days. The ISO dosage was determined based on prior investigations [18, 19] and a pilot trial for fixation.

Experimental Design

Animals were randomly assigned into four groups (each with eight rats) and treated as follows after a one-week acclimatisation period:

Sham: animals received distilled water (2 ml/kg) for 6 weeks, followed by injection with normal saline (1 ml) on the 43rd and 44th days.

ISO: animals received distilled water (2 ml/kg) for 6 weeks, followed by injection with ISO (85 mg/kg) on the 43rd and 44th days.

DZ10 + ISO: animals were orally pre-treated with DZ (10 mg/kg/ kg) by gastric gavage needle for 6 weeks, followed by injection with ISO (85 mg/kg) on the 43rd and 44th days.

DZ20 + ISO: animals were orally pre-treated with DZ (20 mg/kg/ kg) by gastric gavage needle for 6 weeks, followed by injection with ISO (85 mg/kg) on the 43rd and 44th days.

Previous investigations [20-22] were used to determine the DZ dosage. Rats were anaesthetized and slaughtered when the animal experiment was completed. The serum was isolated from blood samples using centrifugation. The heart samples were isolated from the surrounding tissues and cleaned twice with ice-cold phosphate buffer saline immediately after blood collection (PBS). To create about 10% w/v homogenates, the samples were homogenised in phosphate buffer (25 mM, pH 7.4). After centrifuging the homogenates at 1700 rpm for 10 minutes, the supernatant was collected and kept at 20°C until biochemical analysis. For histological investigation, some of the heart samples were kept in 10% formalin.

Histopathological Studies

The cardiac apex was kept in 4% paraformaldehyde, processed in ethanol, and embedded in paraffin wax as soon as the blood was collected. The cardiac apex was stained with haematoxylin and eosin (H&E) and examined under a light microscope at a magnification of 100 times (Olympus, Tokyo, Japan).

Evaluation of Lipid Peroxidation and Antioxidant Enzyme Levels

After the experimental treatment, the homogenates of heart tissues were centrifuged at 16,000 rpm for 10 minutes. The supernatant was utilised to test MDA levels, SOD, CAT, and GPX activity, as well as GSH concentrations, using a microplate reader set at 560 and 532 nm, as directed by the manufacturer. The commercially available test kits were provided by Qualigens Diagnostics (Mumbai, India).

Measurement of MI markers in Serum

According to the manufacturer's instructions, commercial kits from Agappe Diagnostics (Kerala, India) were used to test the activities of AST, ALT, CK-MB, and LDH.

Determination of Pro-Inflammatory Cytokines in Heart

According to the manufacturer's instructions, ELISA kits were used to conduct an enzyme immunoassay of tumour tissue necrosis factor (TNF- α) and interleukin-6 (IL-6) in cardiac homogenate (Minneapolis, MN, USA). The colour intensity was measured at 450 nm using a microplate reader, and the cytokines levels were expressed as pg/ml of tissue (Infinite M200 Pro, Tecan, Switzerland).

Statistical Analysis

All data were expressed as mean SD and analysed using one-way ANOVA followed by a post-hoc test to determine the significant difference between groups. The statistical analysis was carried out using GraphPad Prism 8. (GraphPad Software, Inc., San Diego, CA, USA). A p value of 0.05 or less was considered statistically significant in all tests.

Results

Effect of DZ on AST, ALT, CK-MB and LDH Enzyme Activities in ISO induced rats

We looked at the myocardial damage marker activity in heart tissue from all groups to see if DZ may help with ISO-induced myocardial injury. Two subcutaneous injections of ISO substantially increased blood activities of AST, ALT, CK-MB, and LDH in comparison to the sham group ($p < 0.05$), as shown in Fig. 1. In this animal model, however, DZ treatment significantly reduced serum AST, ALT, CK-BA, and LDH activity ($p < 0.05$).

Effect of DZ on Histopathological Assessments in Heart in ISO Induced Rats

In compared to the sham group ($p < 0.05$), ISO caused a substantial infarction region (Fig. 2). The area of myocardial infarction in DZ-treated groups was significantly reduced ($p < 0.05$) as compared to the model group (Fig. 2). The preventive effect of DZ in ISO-induced myocardial damage was also validated by histological investigation. As seen in (Fig. 3), sham animals had normal histoarchitecture, but ISO rats' heart tissue had visible cardiac muscle fibres with muscle separation and inflammatory cells. DZ greatly reduced these alterations, resulting in hyperplastic muscle fibres with localised hyalinized muscle bundles and the lack of inflammatory cells.

Effect of DZ on Lipid Peroxidation and Oxidative Stress Parameters in ISO Induced Rats

DZ was tested for its effect on ISO-induced lipid peroxidation and oxidative stress in rats. A considerable rise in MDA, a lipid peroxidation by-product, was identified in the ISO group's heart, as seen in Fig. 4. Furthermore, animals administered ISO displayed decreased antioxidant activity, including SOD, CAT, and GPx, as well as lower non-enzymatic antioxidant (GSH) concentrations, when compared to sham rats. DZ, on the other hand, dramatically decreased cardiac MDA levels, enhanced SOD, CAT, and GPx activities, and elevated GSH concentrations in ISO-treated rats ($p < 0.05$). These results show that ISO injection causes oxidative stress in the heart of rats, which is reduced by DZ therapy.

Effect of DZ on Pro-Inflammatory Cytokines in ISO Induced Rats

Figure 5 depicts the production of pro-inflammatory cytokines such as TNF- α and IL-6 in cardiac tissues. Injection of ISO substantially raised the production levels of pro-inflammatory cytokines in the heart ($p < 0.05$) as compared to the sham group. In this animal model, DZ treatment decreased ISO-induced increases in cardiac TNF- α and IL-6 release.

Discussion

In this study, the cardioprotective activities of DZ against ISO-induced myocardial infarction damage in rats were discovered. DZ therapy reduced myocardial infarct area, enhanced myocardial histoarchitecture, and lowered blood levels of myocyte marker enzymes in rats with ISO-induced myocardial injury. In addition, DZ greatly decreased lipid peroxidation and restored the antioxidant increase caused by ISO.

ISO-induced myocardial injury [23, 24] is a synthetic non-selective-adrenergic agonist that has been routinely used to assess the effect of drugs on myocardial infarction. The current work effectively created a rat model of myocardial damage, as demonstrated by drastically elevated blood levels of AST, ALT, CK-MB, and LDH, as well as aberrant cardiac microstructure detected on histological inspection. These findings are in line with previous *in vivo* studies [25, 26]. Furthermore, as indicated by considerably lower blood levels of myocardial damage indicators and visibly decreased histological alterations, DZ can attenuate ISO-induced myocardial infarction injury.

Myocardial infarction damage and oxidative stress appear to be connected [27]. There has been a change in oxidant and antioxidant metabolism in individuals with acute myocardial infarction [28, 29]. The initial line of defence against oxidative damage in cells is free radical scavenging enzymes including CAT, SOD, and GPx, as well as GSH [30]. MDA levels increased in ISO-treated rat cardiac tissues, but antioxidants including SOD, CAT, GPx, and GSH decreased. As a consequence, oxidative stress may be involved in ISO-induced myocardial infarction damage in rats. DZ treatment, on the other hand, decreased ISO-induced oxidative stress by increasing antioxidant activity and decreasing lipid peroxidation.

The emergence of high inflammatory cytokine levels has long been linked to acute MI [31]. The levels of these cytokines can be used to predict the severity and duration of an acute MI event [32]. As a result, the

identification of cytokines in the blood can be utilised to identify acute MI. Inflammatory cytokines overexpression can result in scar tissue or an infarct, both of which can compromise heart function.

DZ appears to lower TNF- α and IL-6 levels in rats treated with ISO thereafter, according to research. ISO treatment causes TNF- α and IL-6 levels to rise in group II animals. In rats with ISO-induced MI, DZ treatment has an anti-inflammatory effect that is dose-dependent.

Conclusion

According to the current study, DZ exhibited a cardioprotective effect in rats by lowering infarct size. TNF- α and IL-6 expression is also reduced, which has an anti-inflammatory effect. There was a decrease in oxidative stress in rats given DZ. Cardioprotective qualities of DZ are thought to be due to a boost in the cellular antioxidant system and a decrease in inflammatory mediators in myocardial cells.

Abbreviations

MI, Myocardial Infarction, ISO, Isoproterenol, AST, Aspartate transaminase, ALT, Alanine transaminase, CK-MB, Creatinine kinase myocardial band, LDH, Lactate dehydrogenase, MDA, Malondialdehyde, GPx, Glutathione peroxidase, GSH, Glutathione, CAT, Catalase, SOD, Superoxide dismutase, TNF- α , Tumour necrosis factor alpha, IL, Interleukin, ELISA, Enzyme linked immunoassay, CPCSEA, Committee for the purpose of control and supervision of experiments on animals, DZ, Daidzein

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose

Authors Contributions

All authors contributed to research conception and design. Material preparation, data collection and analysis were prepared by [Vadivelan Ramachandran], [Vikash Sundaram] and [Tharani M]. The first draft

was written by [Vadivelan Ramachandran], reviewed and edited by [Ruchi Tiwari] and [Meenatchisundaram Subramani] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

The experimental methodology followed the requirements of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) and was approved by the JSS College of Pharmacy's IAEC (Institutional Animal Ethics Committee) (Approval No.06, dated,20-06-2019).

Availability of data and materials

The data supporting the findings of the article will be provided by Dr Vadivelan Ramachandran, Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, The Nilgiris, Tamilnadu, India (vadivelanr@jssuni.edu.in) on reasonable request.

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Figures

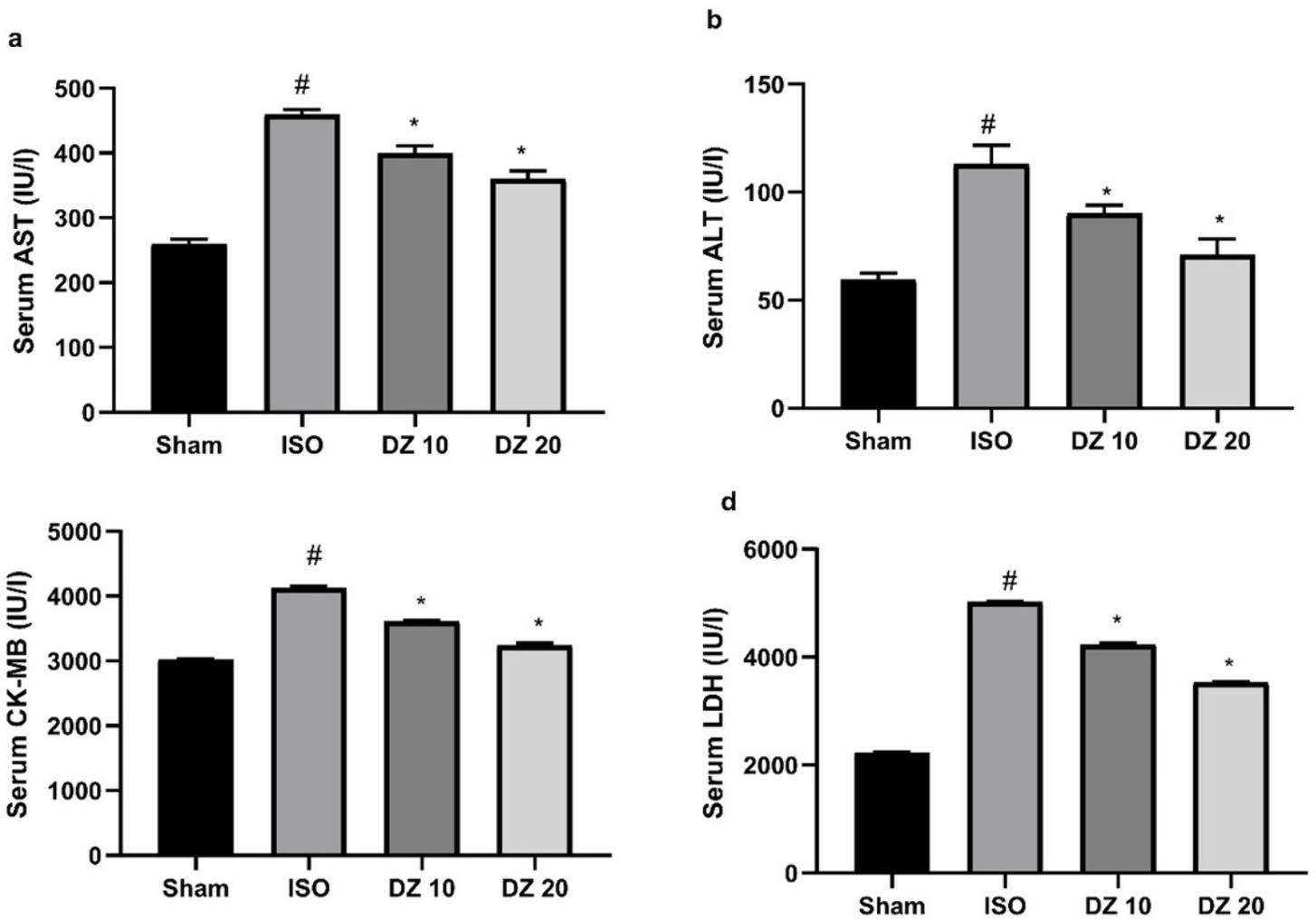


Figure 1

(a) Serum aspartate transaminase (AST), (b) alanine transaminase (ALT), (c) creatine kinase-MB (CK-MB) and (d) lactate dehydrogenase (LDH) levels between the groups. Results were expressed as mean \pm SD, $n=8$. # $p < 0.05$ compared with Sham group, * $p < 0.05$ compared with ISO group.

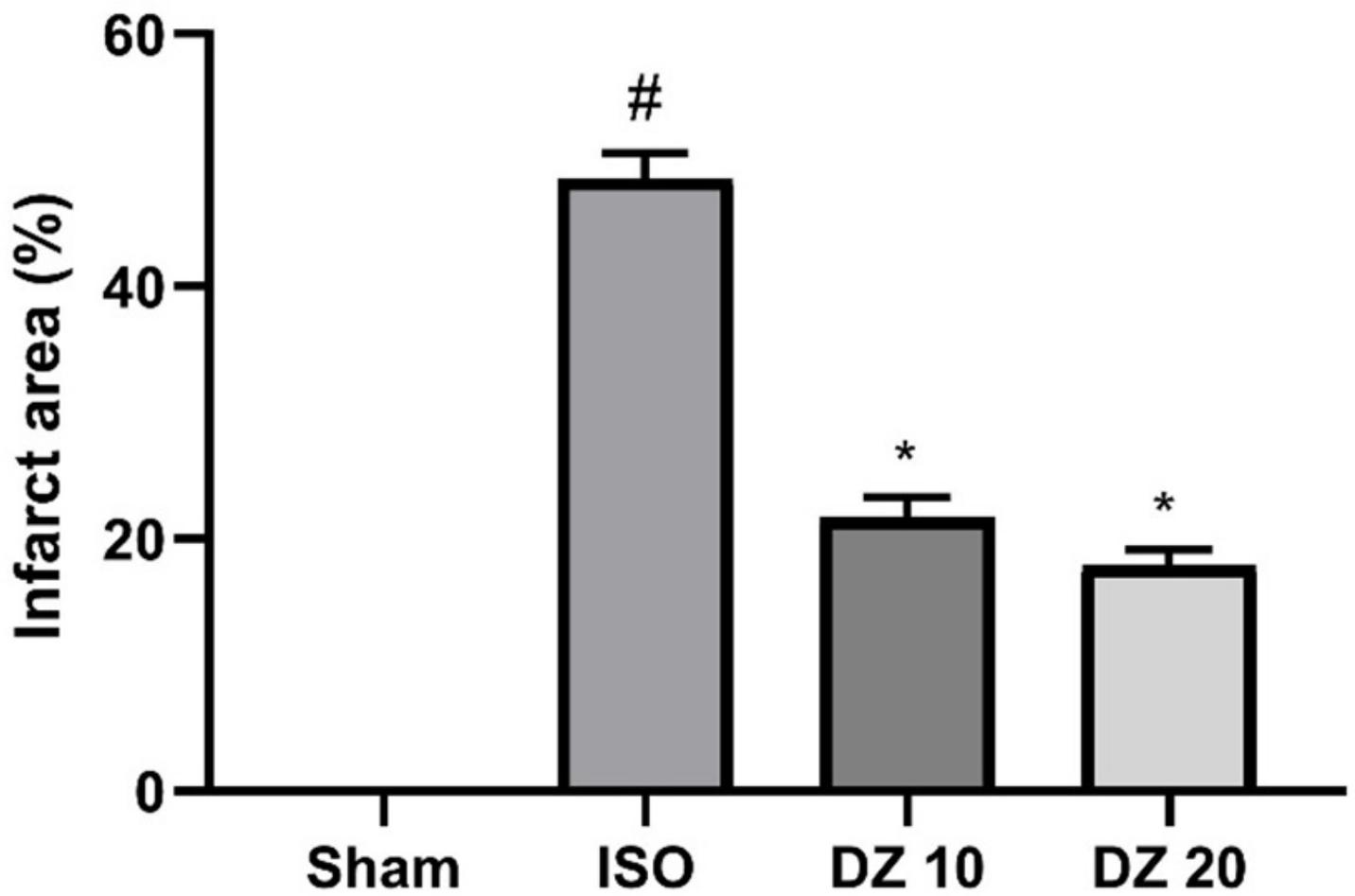


Figure 2

Effect of DZ on myocardial infarct area. Results were expressed as mean \pm SD, n=8. # $p < 0.05$ compared with Sham group, * $p < 0.05$ compared with ISO group.

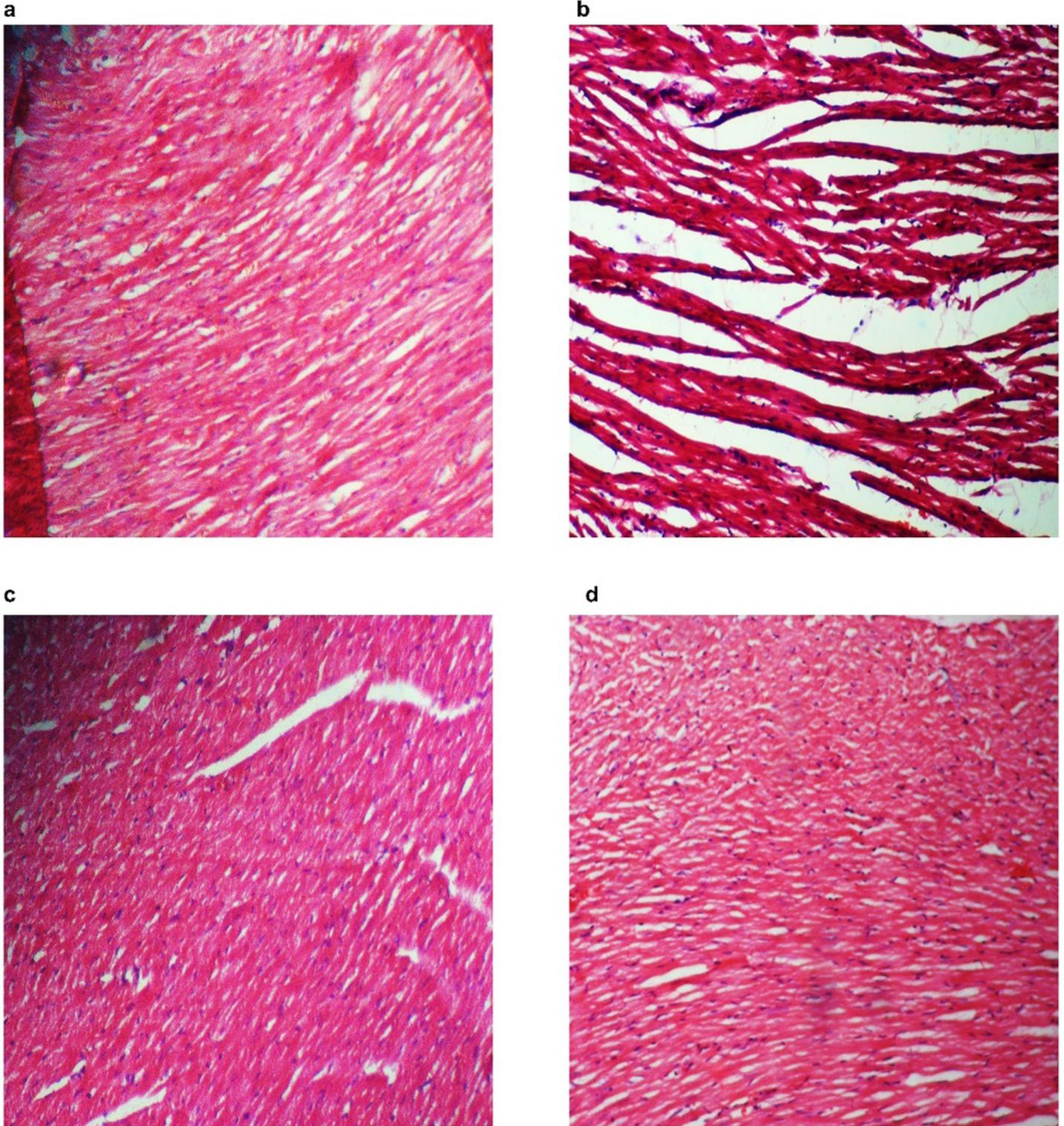


Figure 3

Representative histological photographs of myocardial tissue between the groups, (a) Group-I-Sham group showing normal cardiac muscle fibres, (b) Group-II - ISO heart showing cardiac muscle fibres with muscle separation and inflammatory cells, (c) Group-III-DZ 10 + ISO treated heart showing hyperplastic muscle fibres with focal hyalinized muscle bundles and absence of inflammatory cells, (d) Group-IV-DZ 20 + ISO treated heart showing hyperplastic muscle fibres with absence of inflammatory cells.

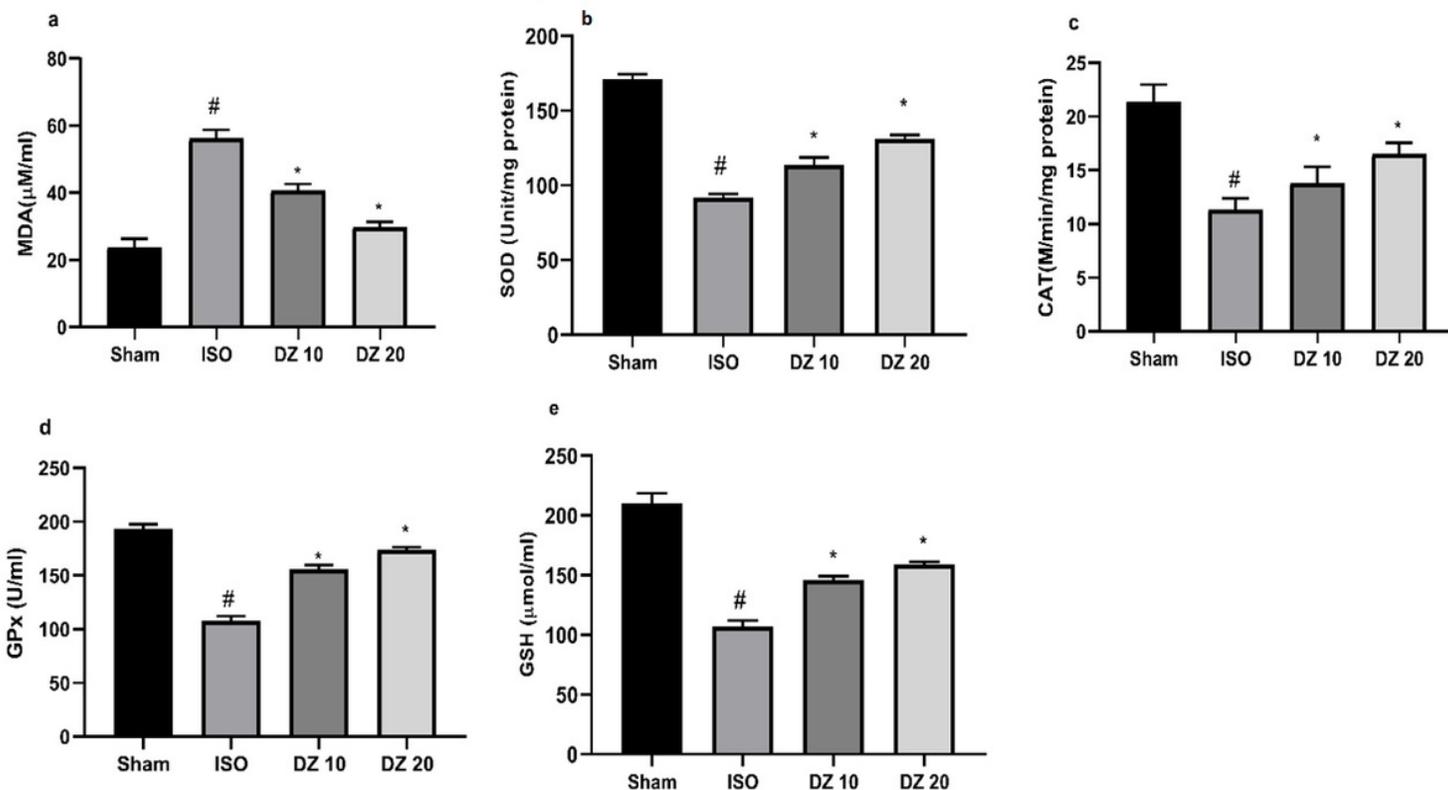


Figure 4

Effect of DZ on ISO-induced lipid peroxidation and oxidative stress in rats. (a). Cardiac malondialdehyde (MDA) levels, (b). superoxide dismutase (SOD), (c) catalase (CAT), (d). glutathione peroxidase (GPX) activities, and (e) glutathione (GSH) concentrations between the groups.

*Results are expressed as mean \pm SD, n=8. Activity is expressed as U/mg protein for SOD, μmol of H_2O_2 decomposed/second/mg protein for CAT, μmol of GSH, oxidized/min/mg of protein for GPX. # $p < 0.05$ compared with Sham group, * $p < 0.05$ compared with ISO group.

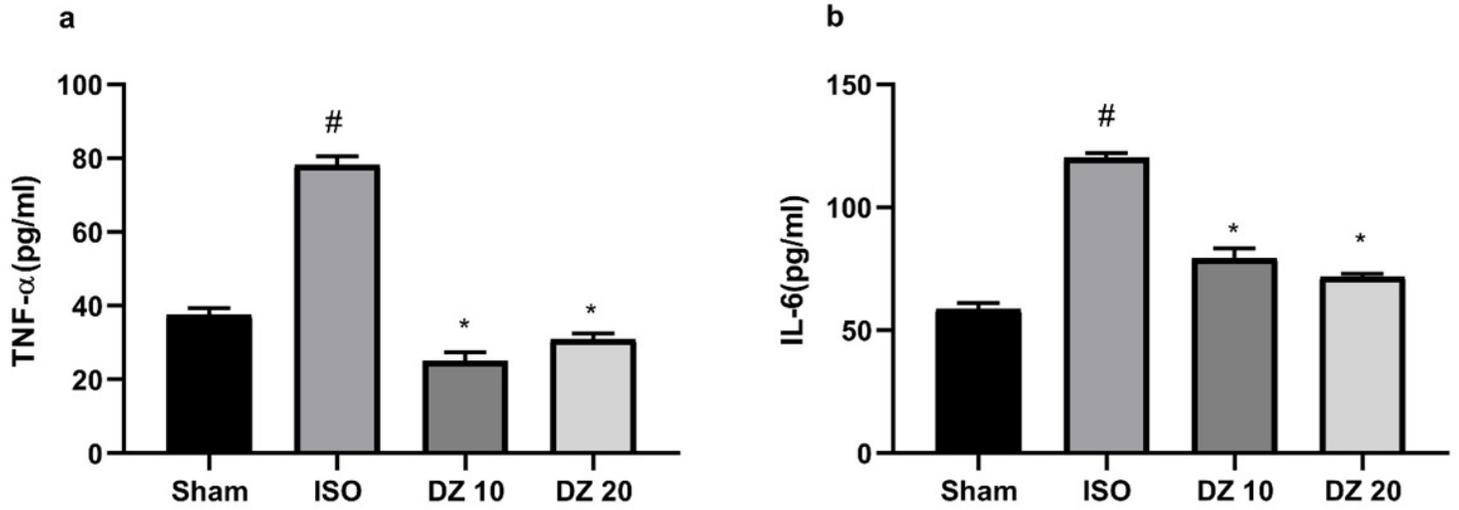


Figure 5

Effect of DZ on Pro-Inflammatory Cytokines in ISO Induced Rats. (a) Cardiac tumour tissue necrosis factor (TNF-A- α) and (b) interleukin-6 (IL-6) production levels between the groups.

*Results are expressed as mean \pm SD, n=8. [#] $p < 0.05$ compared with Sham group, * $p < 0.05$ compared with ISO group.