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The potential effect of irisin in endotoxemia induced cardiac injury in mouse model: role of AMPK

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Abstract:

Polymicrobial sepsis is a life-threatening situation characterized by multiorgan dysfunction resulting from the body's abnormal response to microbial invasion. The assessment of irisin cardio protective potential against experimentally sepsis-caused endotoxic cardiac damage in mice was the aim of research**.** 24 Mice were enrolled in separated four groups (n = 6): Sham group, CLP group, ddH_2O group, Irisin-treated group 10µg/kg IP, 1hr before CLP. 24 hr later, all mice were sacrificed and a cardiac sample was taken for measurement of TNF-α, MPO, caspase-11, F2 – isoprostane and serum troponin by ELISA and gene expression of AMPK by qpcr and histopathological study. **Results**: irisin treated group showed significant changes as compared with CLP group regarding TNF-α, MPO, CASPASE-11, F2 – ISOPROSTANE and (Ctn-I) as well as affect tissue mRNA expression of AMPK gene ($p<0.05$). We conclude that Irisin has cardio protective effects attributable to its anti-inflammatory and anti-oxidative action. Also, Irisin showed a cardio-protective effect as they affect tissue mRNA expression of AMPK gene.

Keywords: Sepsis, Irisin & AMPK.

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Introduction:

Sepsis is characterized as a global healthcare concern, stemming from a systemic inflammatory response triggered by bacterial infection [1, 2] It stands as a leading cause of mortality after infections [3,4]. The pathogenesis of cardiomyopathy in the context of sepsis involves intricate alterations in structural, molecular and metabolic aspects within the cardiac tissue [5].

Impairment of cardiac function stands as a significant outcome of sepsis, contributing to escalated mortality rates. This phenomenon has been linked to heightened inflammation, inhibition of both fatty acid and glucose oxidation, depletion of adenosine triphosphate (ATP), and impairment of the cardiac adrenergic response, which exacerbates cardiac function [6].

In other words, sepsis may decrease cardiac work via a rise expression level of pro-inflammatory cytokines such as interleukins and TNF-α which act as cardio depressant pro-inflammatory mediators resulting in cardiac contractile dysfunction, cardiac hypertrophy, and heart failure [7,8].

Functioning as a detector of intracellular energy levels, AMPK serves as a crucial focal point for the regulation of inflammation. Emerging insights indicate that activating AMPK can mitigate oxidative stress and counteract inflammation [9].

Thus far, the mechanistic links between AMPK and inflammation have primarily revolved around their relationship with the NF-κB pathway. Evidence shows that chemical activators of AMPK can diminish NF-κB-mediated transcription [10].

The signaling pathways included in the inflammatory response are arranged in to anti-inflammatory pathways and pro inflammatory pathways [11,12] and the former involved Nrf2 pathway and the latter includes NF-κB pathway. Irisin is the out membrane part of fibronectin type 3 domain which consists of five proteins. It activated during exercise in skeletal muscles and its concentration is elevated in healthy persons [13]. Irisin level was found to be low in patients with cardiovascular diseases [14] and supplementation of irisin (recombinant) or exercise-activated irisin might be a successful strategy to fight obesity and CVDs [15]. Irisin exerts physiological actions by promoting mitochondrial quality control, diminishing the production of ROS, mitigating inflammation, enhancing energy metabolic balance, and optimizing cellular homeostasis through improved autophagy [16,17].

This effect hypothesized to assess the cardioprotective potential of irisin against experimentally sepsis-caused endotoxic cardiac injury in mice**, which is the aim of this research.**

Materials and methods

The University of Kufa & Department of Pharmacology and Therapeutics was the site of this study. Animal Care and Research Committee of the University of Kufa gave approval for this study.

Study Design

Twentyfour adult males of Swiss white mice (weighting 20 - 30 g, aged 4-8 weeks) were purchased from the animal resource center, under conditions of (24°C ± 2°C) with alternative 12-hrlight/12-hr dark cycles. The mice were also allowed to a free access to the water and diet until the start of experiment. Mice randomized into 4 groups (n=6): Sham group: laparotomy without CLP, CLP group, ddH_2O group and irisin treated group10µg/kg IP, 1hr before CLP, then the animals were sacrificed 24hr after CLP.

Procedure

The induction of sepsis was done via the cecal ligation and puncture model (CLP) based on previous studies [18-20].

Preparation of Irisin:

The product irisin is lyophilized from a 0.2 micromillimeter filtered solution of PBS, pH 7.4 (clear solution), according to MedChemExpress package insert 10µg irisin dissolved in 1ml of ddH_2O [21].

Collection of tissue samples

At the end of the procedure (24 hours), the mice were re-anesthetized with xylazine (20 mg/kg) and ketamine (100 mg /kg). A blood sample was collected immediately from the heart [22-24].

The heart tissue was rinsed with ice-cold saline to remove any red blood cells or clots and divided into 3 parts: for homogenization & ELISA study, qRT-PCR and histopathology.

Tissue homogenization for Elisa.

All blood cells or clots washed from heart by washing with ice salin and then with a high-intensity ultrasonic liquid processor homogenized in 1:10 (w/v) PBS that contained 1%Triton X-100 and a protease inhibitor cocktail, The homogenates were centrifuged at 3000 rpm for 20 min at 4°C [25].

Tissue preparation for histopathology

The heart tissue histopathology and scoring were performed according to Zingarelli protocol [26].

Expression of AMPK heart tissue by qRT-PCR

The mRNA expression of AMPK is determined using a quantitative real-time PCR, as specified by the manufacturer Real-time quantitative (RT-q) PCRTotal RNA was extracted using special chemicals &instruments according to the previous paper of [27] The primer sequences used for q RT-PCR Gene primer sequence is:

AMPKr:5-GGTCCTGGTGGTTTCTGTTG-3'

AMPKf:5-CTCTATGCTTTGCTTTGCTGTGTGG-'3

Statistical analysis

A SPSS 24.0 for window was performed. ANOVA was used for the multiple comparisons among all groups followed by Bonferroni's test. The Mann-Whitney U and Kruskal -Wallis tests were used to assess the histopathological changes which were determined as scores from 0 to 4.

Results

Effect of irisin on cardiac tissue level of inflammatory markers TNF-α, CASPASE-11, MPO& on oxidative biomarkerF2-ISOPROSTANE

The result of this study reveals that there is elevation in TNF-α, CASPASE-11, MPO&F2-ISOPROSTANE, in CLP and DdH_2O groups as compared with the sham group (significant difference P value <0.05). Additionally, irisin group showed markedly decreased levels ($p<0.05$) of these markers if compared with the CLP & DdH_2O groups. More interestingly, level of serum troponin was significantly higher ($p<0.05$) in CLP and DdH_2O groups as compared with the sham group. Also, irisin group showed markedly reduced levels ($p<0.05$) of serum troponin when compared with CLP and DdH_2O groups (significant difference P value <0.05) (figure 1 - 6).

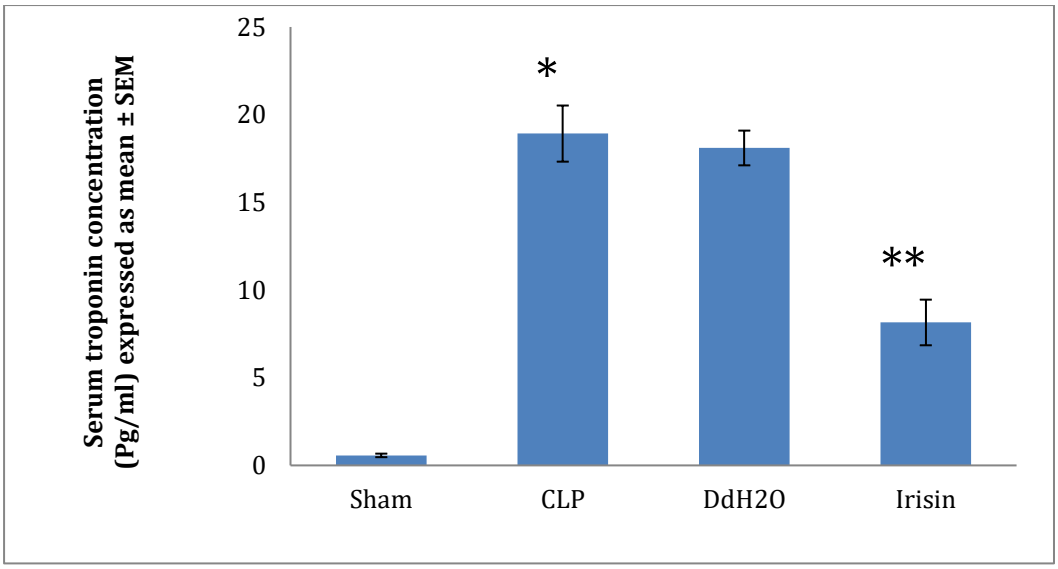

Figure 1: mean serum troponin level.

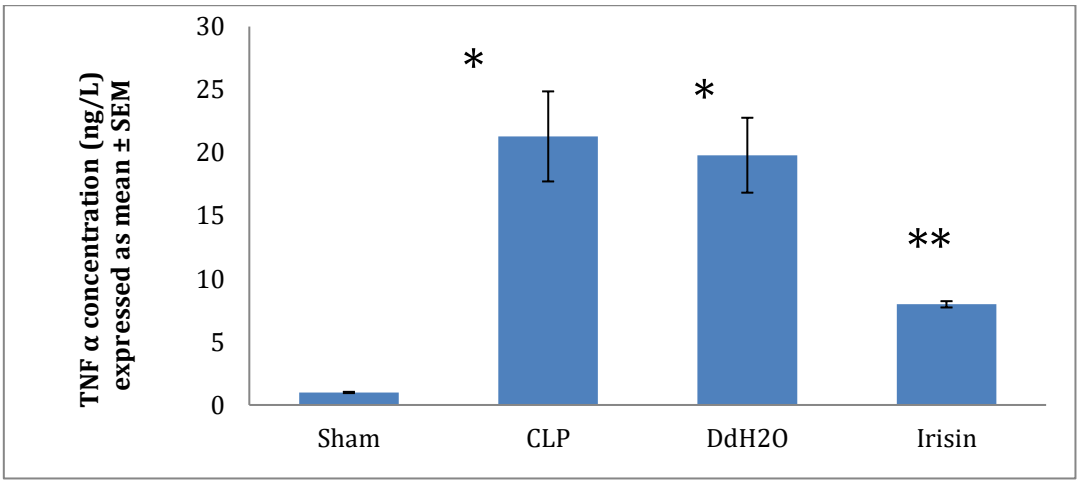

Figure 2: Mean cardiac tissue TNF level.

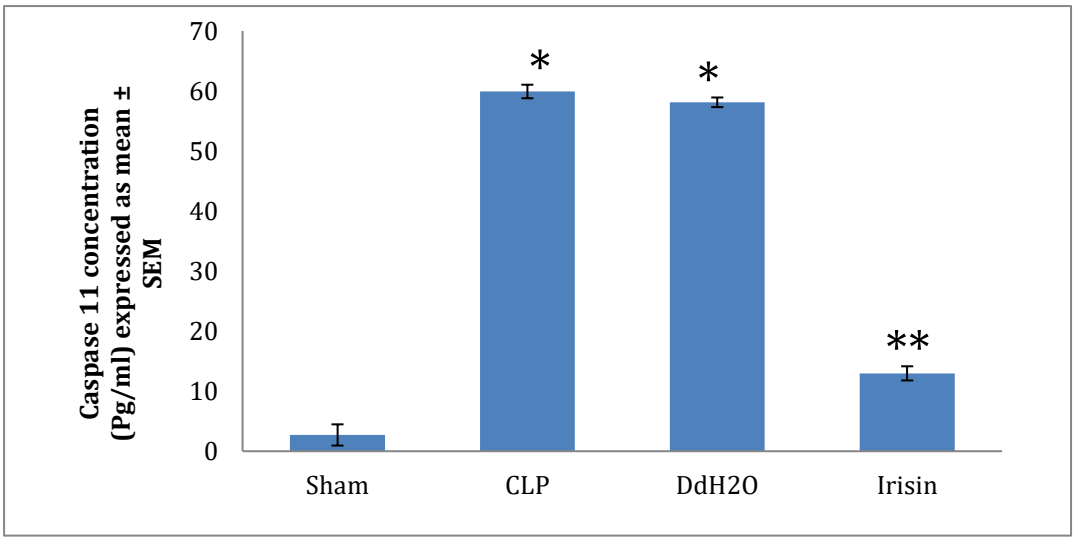

Figure 3: Mean caspase 11 tissue level.

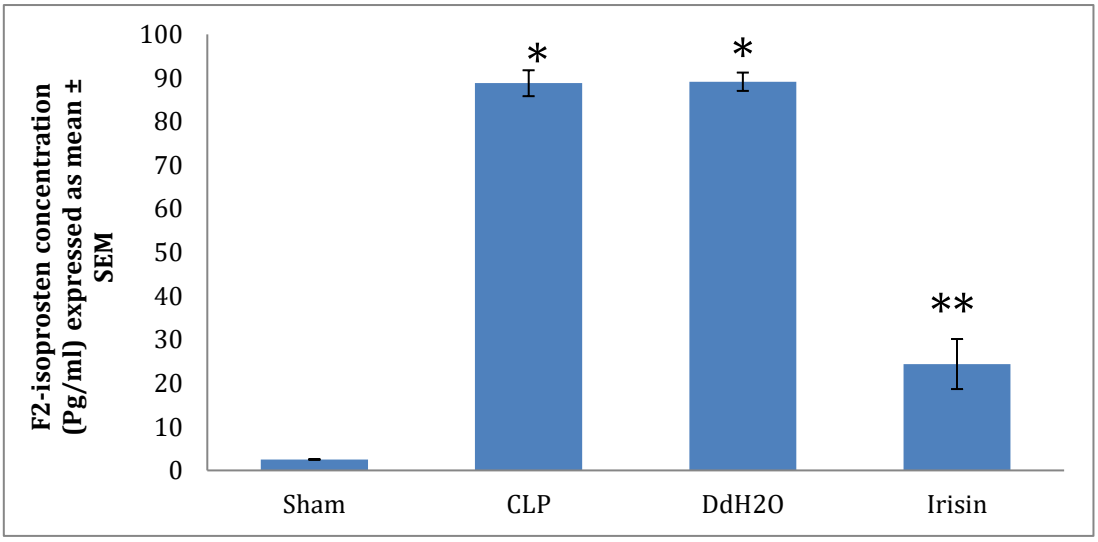

Figure 4: Mean F2 isoprostane cardiac tissue level.

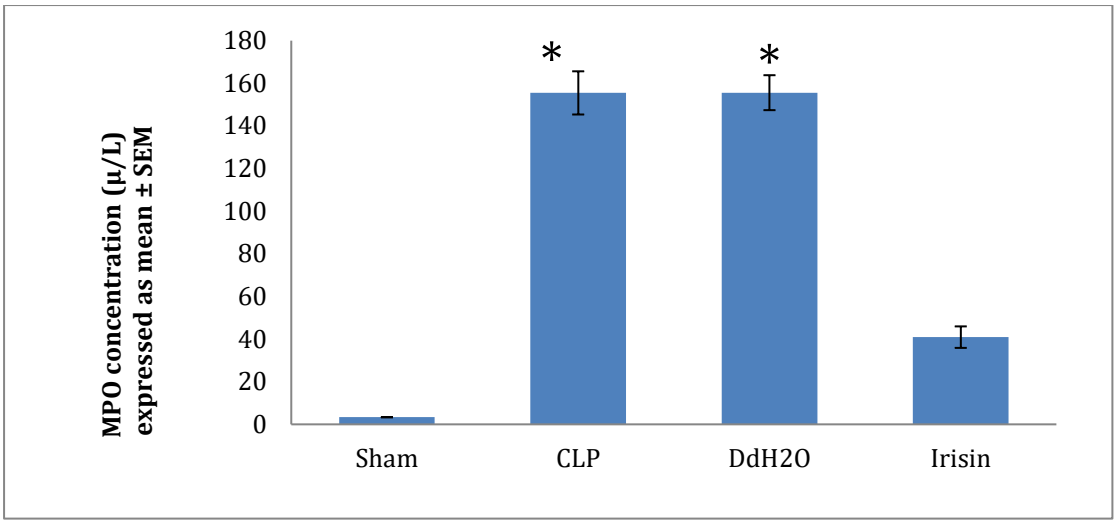

Figure 5: mean MPO cardiac tissue level.

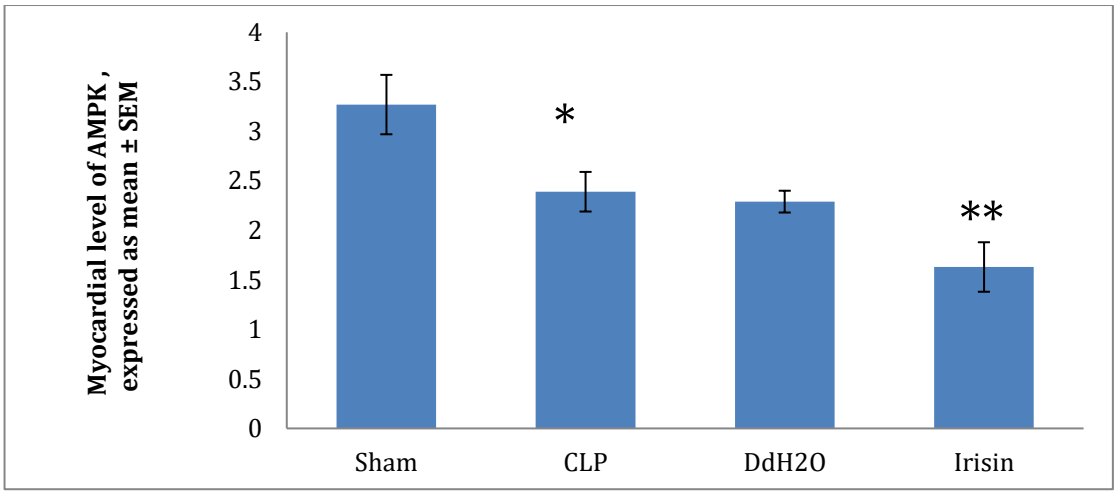

Figure 6: Mean AMPK cardiac tissue expression. *significant difference as compared with the sham group, ** significant difference as compared with the clp group, in all figures.

Histopathological findings

Data showed that CLP caused a significant tissue damage which was represented as scores from 0 – 4 and characterized by Sham group (A) showing normal cardiomyocyte, CLP & DdH_2O groups (B&C) score 4 showing necrotic cardiomyocyte. Irisin group (D) score 2 showing reduction in the tissue damage by reducing the necrosis in the cardiomyocyte (Figure 7)

- Sham group: all animals in this group had normal histopathological findings of 100 %, as shown in figure 7.
- Cecal ligation and puncture (CLP) group: score 4 damaged cardiac tissue (Myocardial tissue sections of mice in the CLP group: showed congested blood vessels (black arrow) & extravasation of blood cells (red arrow), H&E, 10X) in figure 7.
- DdH2O group: score 4 damaged cardiac (tissue Myocardial tissue sections of mice in the Vehicle DMSO group: showed congested blood vessels (black arrow) & extravasation of blood cells (red arrow). H&E, 10X) in figure 7.

- Irisin group: histological changes arranged from mild to moderate changes with a different number of mice, as shown in the Figure 7 (Myocardial tissue sections of mice in the Irisin group).

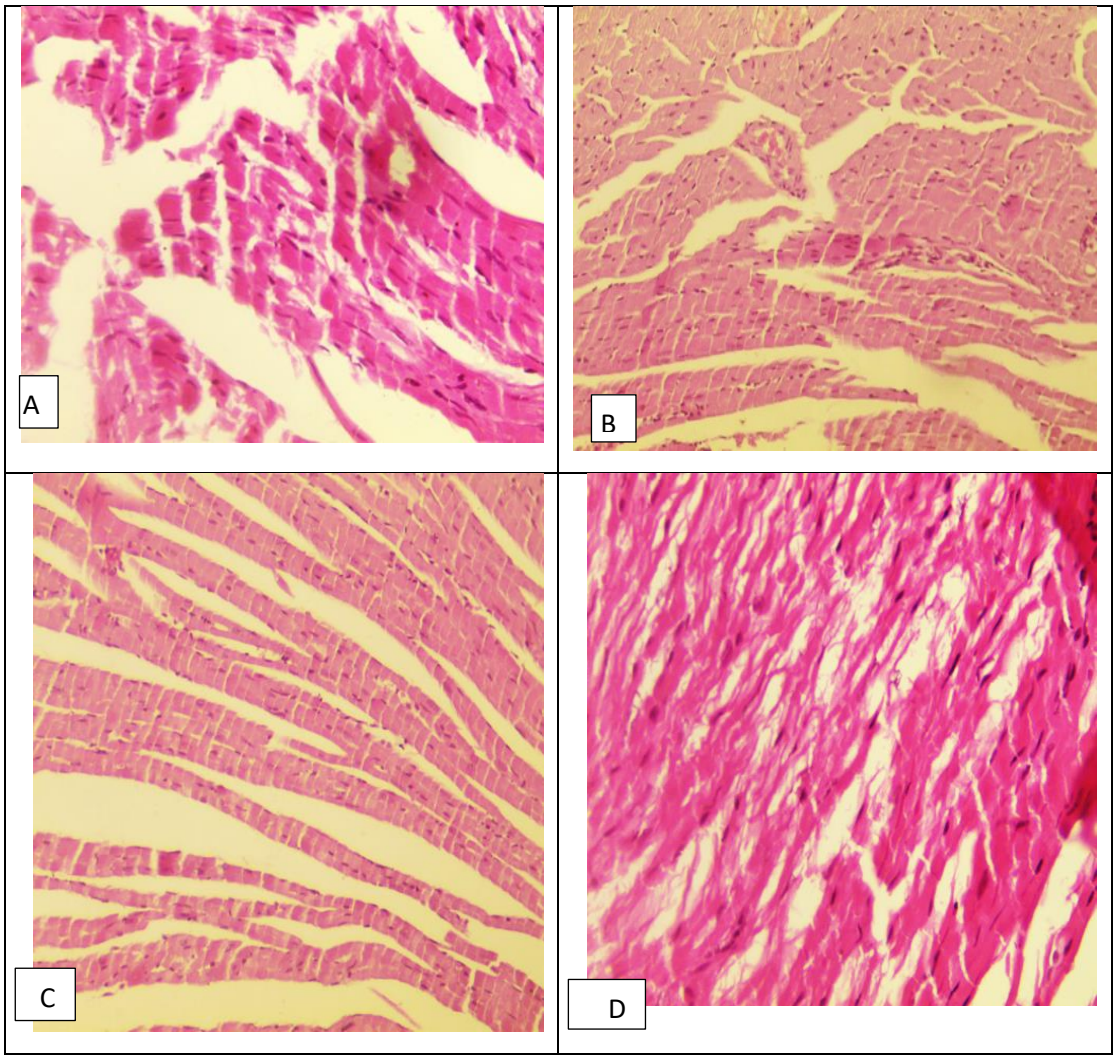

Figure 7: Data showed that CLP caused a significant tissue damage which was represented as scores from 0 – 4 and characterized by Sham group (A)showing normal cardiomyocyte, CLP& DdH_2O groups (B&C) score 4 showing necrotic cardiomyocyte. Irisin group (D) score 2 showing reduction in the tissue damage by reducing the necrosis in the cardiomyocyte.

Discussion

Sepsis is a critical condition involving the malfunction of organs due to an imbalanced immune response to infection, and it stands as a leading contributor to mortality among hospitalized individuals [28,29].

Moreover, sepsis is recognized as the foremost factor behind fatalities in intensive care units [30,31]. Among the significant complications associated with sepsis is myocardial dysfunction, often referred to as sepsis-induced cardiomyopathy or cardiotoxicity, which substantially amplifies the mortality rate [32].

The current study focused on evaluating the prophylactic effects of irisin in order to minimize the cardiotoxicity during polymicrobial sepsis in mice model which was done by cecal puncture we showed that TNF-α, CASPASE-11, MPO, F2-ISOPROSTANE levels were elevated in

the sepsis and DdH_2O groups as compared with the sham group. This finding is compatible with that obtained by Secher and others highlighted that the TNF signaling pathway plays an essential role in activating innate immunity in response to a variety of pathogen [33].

In the present study, pretreatment with the irisin before poly microbial sepsis result in significantly lower in pro-inflammatory cytokines as compared to control group TNFα. This result in agreement with that reported by the previous study [34].

The current study demonstrated a significant elevation in the tissue levels of Caspase11 in the CLP group compared to the sham group. This finding is in alignment with an earlier study which suggested that irisin decrease caspase-11, irisin protected against many diseases, due to its anti-inflammatory properties and anti-cell death, mainly pyroptosis [35]. NF-κB signaling pathways are responsible for the anti-inflammatory and anti-apoptotic effects against myocardial infarction injury [34].

Additionally, irisin causes a significant lower level of MPO in irisin treated group in comparison with the control group. This outcome is consistent with earlier research that showed irisin was effective in preventing neutrophil infiltration and suppressing apoptotic cell death in neurons within the perihematomal regions following intracerebral hemorrhage [36].

Furthermore, there is a significant decrease in heart level of F2-isoprostane for irisin pretreated group as compared to control group. These findings suggest that irisin performs a fundamental role in the protective effects on endotoxic cardiac injury through its anti- oxidative effect [35,37]. Based on our current knowledge, there is a lack of existing data concerning the influence of irisin on F2-isoprostane levels in cases of cardiac injury induced by endotoxins. This could potentially be attributed to the antioxidative effects of irisin.

The present study revealed a noteworthy increase in the serum cardiac troponin-levels within the sepsis and DdH_2O groups when compared to the sham group. This outcome aligns with the results of a prior study conducted on rabbits to assess the impact of CLP-induced sepsis on cTn-I levels, where a substantial rise in cTn-I was detected in the experimental group compared to the control group of rabbits [36].

In this study, irisin exhibited a significant reduction in the serum levels of cardiac troponin-I compared to the control group, indicating the preservation of heart function. This outcome aligns with the findings of other study which indicated that the pre-treatment with irisin result in a significant decrease in the level of cTn-I in mice model of sepsis [38]. This outcome is ascribed to enhanced mitochondrial function, the regulation of autophagy, and a decrease in apoptosis.

In this study, it was observed that irisin led to a significant increase in tissue AMPK expression compared to the sepsis group ($p < 0.05$). This finding aligns with a study by many researchers which highlighted the pivotal roles played by both the AMPK signaling pathways [39].

These pathways are critical in maintaining cellular energy balance and metabolic homeostasis by curbing inflammation and the production of reactive oxygen species (ROS), thus safeguarding cells during stressful conditions (40). Therefore, there exists a potential connection between the protective effects of irisin and the activation of AMPK signaling [7,41].

Furthermore, in the present study, the group treated with irisin exhibited a notable decrease in the extent of cardiac tissue injury. When compared with the CLP group the irisin group showed moderate architecture with less degree of histopathological changes such as a moderate degree of inflammation and necrotic area. These findings align with a prior study that showcased irisin's potential to improve myocardial function by diminishing cardiac apoptosis, pyroptosis and inflammation in the context of LPS-induced sepsis [38].

Conclusions

The present study adds to the growing body of research that irisin, has potential ameliorative impact on the cardiac injury in mice that were subjected to CLP through its role as anti-inflammatory, antioxidant and anti-apoptotic effects.

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