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Research Article

The effect of Cynara scolymus L. on cardiac functions in rats with experimental cardiac injury induced by total circulatory arrest

Total sirkülatuar arrest ile deneysel kardiyak hasar oluşturulan ratlarda Cynara scolymus L.'nin kardiyak fonksiyonlar üzerine etkisi

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Abstract

Aim: In this study, the potential protective effects of Cynara scolymus L. (CSL) extract on cardiac tissue and the underlying mechanisms mediating this effect were evaluated using a total circulatory arrest model in rats.

Material and Methods: A total of 24 adult male Wistar Albino rats were used in this study. The animals were randomly and equally divided into four groups (n=6): Group I (Sham), Group II (Total Circulatory Arrest – TSA), Group III [Treatment Group 1 (TSA + Cynara scolymus L. extract)], and Group IV [Treatment Group 2 (Cynara scolymus L. extract)]. After the completion of the experimental protocol, blood and cardiac tissue samples were collected from the animals. Biochemical analyses were performed to measure the levels of malondialdehyde (MDA), total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), creatine kinase-MB (CK-MB), troponin I, tumor necrosis factor-alpha (TNF-α), hypoxia-inducible factor 1-alpha (HIF-1α), and nuclear factor erythroid 2–related factor 2 (NRF2). Additionally, the harvested cardiac tissues were examined histopathologically to evaluate structural changes.

Results: According to the findings, there were no statistically significant differences between the groups in terms of MDA ($p = 0.71$), TOS ($p = 0.21$), and OSI ($p = 0.45$) levels. However, statistically significant differences were observed in TAS, TNF-α, HIF-1α, and NRF2 levels ($p < 0.01$). Histopathological examination of the heart revealed that the treatment groups had fewer areas of focal necrosis compared to Group II.

Conclusions: The findings of this study indicate that CLS extract exhibits significant anti-inflammatory and antioxidant protective effects against ischemia-reperfusion-induced cardiac injury. These results support the potential of CSL extract as a cardioprotective agent. However, further studies are needed to elucidate the underlying molecular mechanisms of these protective effects in more detail.

Keywords: Cynara scolymus L. extract, total circulatory arrest, antioxidant, tumor necrosis factor-alpha, hypoxia-inducible factor 1-alpha, nuclear factor erythroid 2-related factor 2

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Öz

Amaç: Bu çalışmada, Cynara scolymus L. (CSL) ekstraktının kalp dokusu üzerindeki potansiyel koruyucu etkileri ve bu etkiyi aracılık eden altta yatan mekanizmalar, ratlarda total sirkülatuar arrest modeli kullanılarak değerlendirilmiştir.

Gereç ve Yöntemler: Bu çalışmada toplam 24 adet yetişkin erkek Wistar Albino rat kullanıldı. Hayvanlar rastgele ve eşit olarak dört gruba ayrıldı (n = 6): Grup I (Sham), Grup II (Total Sirkülatuar Arrest - TSA), Grup III [Tedavi Grubu 1 (TSA + Cynara scolymus L. ekstresi)] ve Grup IV [Tedavi Grubu 2 (Cynara scolymus L. ekstresi)]. Deneysel protokolün tamamlanmasının ardından hayvanlardan kan ve kalp dokusu örnekleri toplanmıştır. Malondialdehit (MDA), total antioksidan düzeyi (TAS), total oksidan düzeyi (TOS), oksidatif stres indeksi (OSI), kreatin kinaz-MB (CK-MB), troponin I, tümör nekroz faktörü-alfa (TNF-α), hipoksi ile indüklenebilir faktör 1-alfa (HIF-1α) ve nükleer faktör eritroid 2 ile ilişkili faktör 2 (NRF2) seviyelerini ölçmek için biyokimyasal analizler yapıldı. Ayrıca, alınan kardiyak dokular yapısal değişiklikleri değerlendirmek için histopatolojik olarak incelenmiştir.

Bulgular: Bulgulara göre, gruplar arasında MDA (p = 0,71), TOS (p = 0,21) ve OSI (p = 0,45) düzeyleri açısından istatistiksel olarak anlamlı bir fark yoktu. Ancak TAS, TNF-α, HIF-1α ve NRF2 düzeylerinde istatistiksel olarak anlamlı farklılıklar gözlendi (p < 0,01). Kalbin histopatolojik incelemesi, tedavi gruplarının Grup II'ye kıyasla daha az fokal nekroz alanına sahip olduğunu ortaya koymuştur.

Sonuçlar: Bu çalışmanın bulguları, CLS ekstraktının iskemi-reperfüzyon kaynaklı kalp hasarına karşı önemli anti-enflamatuar ve antioksidan koruyucu etkiler sergilediğini göstermektedir. Bu sonuçlar, CSL ekstraktının kardiyoprotektif bir ajan olarak potansiyelini desteklemektedir. Bununla birlikte, bu koruyucu etkilerin altında yatan moleküler mekanizmaların daha ayrıntılı olarak aydınlatılması için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Cynara scolymus L. ekstraktı, total sirkülatuar arrest, antioksidan, tümör nekroz faktör-alfa, hipoksi ile indüklenen faktör 1-alfa, nükleer faktör eritroid 2-ilişkili faktör 2

Intraduction

Myocardial ischemia-reperfusion injury (MIRI) is a clinical condition that occurs following the restoration of blood flow to previously ischemic myocardial tissue and is often associated with severe and irreversible cellular damage. Currently, there is no definitive method available to completely prevent or effectively treat MIRI [1]. Myocardial ischemia-reperfusion (I/R) injury is a common cardiovascular issue in clinical practice. During ischemia, the interruption of myocardial blood flow followed by subsequent reperfusion fails to fully restore tissue integrity; on the contrary, the reperfusion process may exacerbate the damage, leading to more extensive tissue lesions and necrosis [2].

Cardiovascular diseases (CVDs) remain a major global health problem and are not yet fully understood due to their complex and multifactorial pathogenesis. The incidence of CVDs continues to rise worldwide, driven by the increasing prevalence of common risk factors [3]. Currently, pharmacological agents such as ampules, nitrates, statins, beta-adrenergic receptor blockers, clopidogrel, and aspirin are widely used in the treatment of CVDs. However, the long-term use of these medications may lead to serious side effects, including renal failure, rhabdomyolysis, and hemorrhagic complications [4,5]. Therefore, the need for safer and more effective therapeutic approaches is growing. In recent years, plant-based products with proven efficacy and fewer side effects, either as alternatives or adjuncts to existing treatments have gained prominence and have been the focus of intensive research [6].

Cynara scolymus L. (CSL) is recognized as a significant medicinal plant in the field of herbal therapy. Its edible parts include the flower head, fleshy bract tips, flower base, flower stalk, and the terminal part of the stem. Particularly in mature buds, typically only the flower base is consumed, which accounts for approximately 30–40% of the plant's total biomass. The stem and leaf parts are mostly used as animal feed or fertilizer [7].

Various studies have reported that CSL and its derivatives possess numerous biological activities due to their rich chemical composition [8]. Research conducted on both animal and human models has demonstrated the cardioprotective potential of CSL extracts and infusions [9]. In the present study, the protective effects of CSL extract, known for its high antioxidant capacity, were evaluated in a Wistar Albino rat model of experimental cardiac injury induced by the total circulatory arrest (TSA) method.

Material and Methods

Formation of Study Groups and Experimental Cardiac Injury Model

A total of 24 adult male Wistar Albino rats weighing between 250 and 300 grams were used in this study. The animals were randomly divided into four groups: Group 1: SHAM (n = 6), Group 2: Control-TSA (n = 6), Group 3: Treatment 1 (TSA + CSL, n = 6), and Group 4: Treatment 2 (CSL, n = 6). Prior to the experiment, the rats were acclimated to environmental conditions for five days without any intervention under standard laboratory conditions. During this period, the ambient temperature was maintained at 22 ± 2 °C, relative humidity at 50%, and a 12-hour light-dark cycle was observed. The animals were provided with ad libitum access to standard laboratory feed and clean tap water. All rats were housed in three separate transparent and observable cages. Food was withheld 12 hours prior to surgical procedures, but water was provided ad libitum.

In the study, rats in Group 1 were fed only standard laboratory chow and tap water for 14 days, with no pharmacological or surgical interventions applied. On day 15, the animals were sacrificed under deep anesthesia [Ketamine (90 mg/kg) and Xylazine (10 mg/kg), intraperitoneal (IP)] and blood and tissue samples were collected. Rats in Group 2 were similarly followed for 14 days on a standard diet; however, on the 15th day, under deep anesthesia, TSA was induced to establish an experimental cardiac injury model, followed by collection of biological samples. Rats in Group 3 received the same standard diet for 14 days, supplemented with oral/gavage administration of CSL extract at a dose of 1500 mg/kg/day. On day 15, TSA was applied to induce cardiac injury, and blood and tissue samples were subsequently collected. Rats in Group 4 were administered CSL extract alone (1500 mg/kg/day, oral gavage) for 14 days. On day 16, the animals were sacrificed under deep anesthesia, and blood and tissue samples were collected and stored under appropriate conditions (Figure 1).

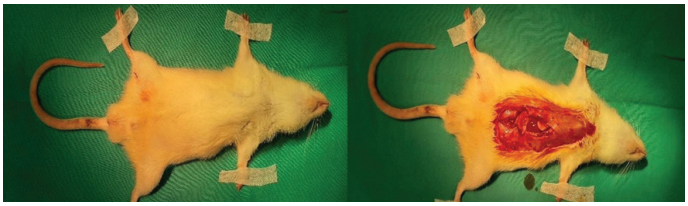


Figure 1. Experimental protocol for blood and tissue collection on day 16.

Experimental Cardiac Injury Model by Total Circulatory Arrest (TSA) in Rats

To induce experimental cardiac injury, deep anesthesia was achieved in rats by IP administration of a combination of Ketamine (90 mg/kg) and Xylazine (10 mg/kg). Following anesthesia, the left inguinal-femoral area was shaved and the surgical site was prepared and sterilized using alcohol and an alcohol-iodine solution. After the surgical incision, the femoral vein was isolated and a 24-gauge (yellow) intravenous catheter was inserted. Immediately prior to cardiac arrest induction, all rats received 0.1 mL of heparin intravenously. In Groups 2 (TSA group) and 3 (CSL + TSA group), cardiac arrest was induced by intrapericardial administration of 0.2 mL potassium chloride following anesthesia, thereby establishing the TSA model. After TSA application, a 20-minute waiting period was observed, after which blood and tissue samples were collected [10].

Preparation of CLS Extract

The CSL powder used in this study was provided by Naturalya Kimya İlaç Ltd. Şti (Naturalya Chemistry & Pharmaceuticals Ltd. Türkiye)®. Fifty grams of the obtained CSL samples were incubated overnight at 40°C in a 1:1 mixture of methanol (MeOH) and water. After incubation, the mixture was divided into smaller volumes and homogenized for 15 minutes using an ultrasonic processor. The homogenized suspension was filtered through Whatman filter paper, and the solvent was completely removed using a rotary evaporator at a temperature not exceeding 40°C. Finally, the crude MeOH extracts obtained were freeze-dried using a lyophilizer to obtain a powdered form. Prior to the experiment, the powder was dissolved in water and prepared for oral administration.

Plasma Collection and Experintal Procedure

Blood samples were collected from the heart and major veins of rats subjected to TSA under deep anesthesia and transferred into biochemical analysis tubes. The blood samples were centrifuged at 4000 rpm for 10 minutes to separate the plasma, which was then transferred into Eppendorf tubes and stored at -80°C. On the day of analysis, plasma samples were thawed at room temperature and prepared for further biochemical assessments.

In the first phase, oxidative stress parameters including malondialdehyde (MDA), total antioxidant capacity (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were analyzed in tissue and plasma samples. TAS and TOS levels were

measured using commercial kits from Rel Assay. TAS results were expressed in Trolox Equivalent/L, while TOS results were expressed in μmol H_2O_2 Equivalent/L. OSI values were calculated and reported as Arbitrary Units (AU) [11,12].

In the second phase, the levels of Tumor Necrosis Factor 2 (TNF2), Hypoxia-Inducible Factor 1-alpha (HIF-1α), and Nuclear Factor Erythroid 2-Related Factor 2 (NRF2) were measured in plasma and homogenized tissue samples using the ELISA (Enzyme-Linked Immunosorbent Assay) method, with commercial kits from BT LAB (Cat. No: E0031Ra).

Histopathological Examination of Cardiac Tissue

Cardiac tissue samples obtained from rats were fixed in 10% formaldehyde solution. Following fixation, the tissues were processed in a Leica Bond-Max immunohistochemistry device for 4 hours and subsequently embedded in paraffin blocks after a total of 12 hours of tissue processing. Sections of 3–5 μm thickness were cut from the paraffin blocks and stained with hematoxylin and eosin (H&E). The stained sections were examined under a microscope to assess histopathological changes such as necrosis, inflammation, and edema in the cardiac tissue. For the quantitative evaluation of focal necrosis areas, all sections were systematically scanned at 60x magnification using a NiU model microscope. Signs of inflammation and edema were also observed during the evaluations.

This study was conducted with the approval of the Local Animal Ethics Committee of Harran University (File No: 395481, Decision No: 2024/008/06, dated 09.12.2024). Adult male Wistar Albino rats used in the experiments were obtained from the Harran University Experimental Animal Research Center. Housing, feeding, and all experimental procedures were carried out in the same facility.

Statistical Analysis

Statistical analyses were performed using two different platforms. Multivariate metabolomic analyses were conducted using MetaboAnalyst (version 6.0, www.metaboanalyst.ca), a web-based tool for metabolomic data processing and interpretation. Prior to analysis, the data were log-transformed for normalization and standardized using the auto-scaling method (mean-centering and division by standard deviation). Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) were used to evaluate overall differences between groups. Variable Importance in Projection (VIP) scores were calculated to identify potential biomarkers with discriminatory power between groups. Correlation analysis and hierarchical clustering (heatmap) were applied to visualize the relational patterns among parameters. A significance level of $p < 0.05$ was considered as the threshold. In addition, the obtained data were also analyzed using IBM SPSS Statistics version 27.0. The normality of distribution was assessed with the Kolmogorov-Smirnov test. For comparisons between groups, one-way analysis of variance (ANOVA) was used for parametric data, followed by Tukey's HSD post-hoc test where significant differences were found. For non-normally distributed data, the Kruskal-Wallis test was applied, and pairwise comparisons were performed using Dunn-Bonferroni correction when appropriate. The data are presented as mean ± standard deviation, and a p-value < 0.05 was considered statistically significant in all analyses.

Results

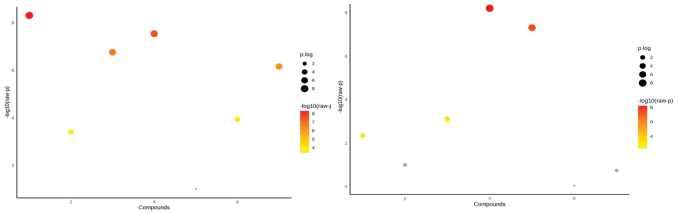
The findings obtained in this study indicate that CSL administration significantly modulates ischemia-induced inflammatory and oxidative stress responses. According to the ANOVA results presented in Table I, statistically significant differences were identified between the groups in terms of certain hematological parameters. Notably, there were significant intergroup differences in mean corpuscular volume (MCV) ($p = 0.0023$), hemoglobin (HGB) ($p = 0.0062$), and hematocrit (HCT) ($p = 0.0029$) values. Regarding the MCV parameter, a significant difference was observed among the groups ($p = 0.002$). Post-hoc analysis revealed that group IV (CLS) had significantly higher MCV values compared to group II (TSA), and group I (SHAM) had significantly higher MCV values compared to group III (TSA+CLS). Similarly, significant differences were also found in HGB and HCT parameters between the groups (HGB: $p = 0.006$; HCT: $p = 0.002$). In the groups that received CLS treatment (groups III and IV), HGB levels were significantly higher only in comparison to group II (TSA). Conversely, in group III (TSA+CLS), HGB levels were found to be significantly decreased only when compared to group II (TSA). These findings suggest that CSL administration may exert a regulatory effect on HGB levels, potentially influencing both the oxygen-carrying capacity and the viscosity of blood. Significant differences were also observed in the leukocyte parameters eosinophil (EOS) and basophil (BASO) (EOS: $p = 0.015$; BASO: $p = 0.004$). The decrease observed in group III

(TSA+CLS) may indicate a regulatory effect of CLS on certain inflammatory cell types. However, no statistically significant differences were found in parameters such as WBC, RBC, and LYM ($p > 0.05$). These findings suggest that CSL treatment or ischemic injury did not significantly affect these cell groups.

Table 2 presents the statistical analysis of blood test results among the groups. According to these results, a marked increase in TNF-α, HIF-1α, and oxidative stress markers was observed in group II (TSA) compared to the control group. The increases in TNF-α and HIF-1α levels were found to be statistically significant ($p < 0.001$). Similarly, the levels of antioxidant defense parameters NRF2 and TAS were significantly decreased in group II ($p < 0.001$). CSL administration (groups III and IV) produced favorable changes in TNF-α, HIF-1α, and NRF2 levels, demonstrating its effectiveness in suppressing inflammation and oxidative stress. Notably, group IV showed the highest levels of TAS and NRF2, which reflect antioxidant capacity. Although differences in MDA ($p = 0.71$), TOS ($p = 0.21$), and OSI ($p = 0.45$) levels were observed among the groups, these differences were not statistically significant.

According to the findings of heart tissue homogenization presented in Table 3, TNF-α levels measured in group II (TSA) were significantly higher compared to group I (SHAM) ($p < 0.001$). Conversely, in the CSL-treated groups (groups III and IV), a significant reduction in TNF-α levels was detected. MDA levels showed a marked increase in group II (TSA), which was significantly reduced with CSL treatment ($p < 0.001$). HIF-1α levels were also significantly elevated in group II (TSA) compared to group I (SHAM), but this increase was significantly diminished in the CSL-treated groups (groups III and IV). NRF2 levels were suppressed in group II (TSA), while CSL administration led to a significant increase in this parameter. Although there was no statistically significant difference in TAS levels among the groups ($p = 0.07$), a trend toward increased levels was notable in group IV (CSL). Among the oxidative stress markers, TOS and OSI were significantly elevated in group II (TSA), and CSL treatment significantly reduced these increases ($p < 0.05$–0.001).

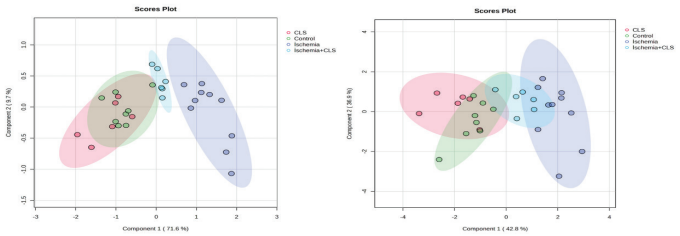
Graph 1, the significance plot visually represents the statistical significance levels of the analyzed compounds based on both blood and heart tissue samples. The size of each dot reflects the logarithmic transformation of the p-value (p.log), while the color scale indicates the -log10 (raw p) value. Larger, dark red dots signify a higher level of statistical significance. When both tissue types are considered, the most significantly altered compounds were NRF2, TAS, HIF-1α, and TNF-α, representing the biomarkers with the strongest group-wise differences. Notably, TNF-α showed an exceptionally high level of significance in blood samples, indicating a prominent involvement in the modulation of the inflammatory response. Following TNF-α, NRF2 and HIF-1α suggest that CLS treatment plays a key role in reducing oxidative stress and regulating hypoxic adaptation mechanisms. Data from heart tissue exhibited a similar trend, with significant increases in NRF2 and HIF-1α levels, supporting the contribution of CLS to cardiac oxidative stress modulation. Additionally, classical oxidative stress markers such as OSI, TOS, and MDA were also found to be statistically significant in both tissues, though their effect sizes appeared relatively lower. Taken together, these findings strongly suggest that CLS treatment significantly modulates both systemic (blood) and local (cardiac tissue) inflammatory and oxidative responses, predominantly through key molecular biomarkers such as NRF2, HIF-1α, and TNF-α. In this respect, the study provides important molecular-level evidence supporting the cardioprotective effects of CLS.



Graph 1. Bubble plot representation of the statistical significance of the compounds evaluated in blood and heart tissue based on -log10(p) and -log10(raw-p) values.

Graph 2, In this analysis, the biochemical and metabolic profiles derived from heart tissue and blood samples of the four experimental groups (SHAM, TSA, TSA+CLS, CLS) were evaluated using two-dimensional Principal Component Analysis (PCA). The PCA score plots visually demonstrate that the biomarkers effectively discriminate between groups and reflect systemic and tissue-level changes induced by experimental interventions. Samples from group I (SHAM)

occupied a stable position on the PCA plane, as expected, and showed a similar distribution to those from the CLS-only group. Group II (TSA) samples were clearly separated from all other groups on the far right of the PCA plane in both heart and blood samples, indicating pronounced and dominant biochemical and inflammatory disturbances associated with ischemia at both systemic and tissue levels. Group III (TSA+CLS) was positioned near Group II, though it showed partial divergence, especially in heart tissue samples. This suggests that CLS application moderately modulated ischemia-induced alterations but did not fully restore normal profiles. Group IV (CLS) samples showed a distribution closely resembling the control group (SHAM) in both heart and blood datasets, indicating that CLS treatment did not significantly alter systemic or tissue biomarker profiles in healthy subjects. Overall, the PCA results successfully distinguished the experimental groups based on prominent biochemical and metabolic differences. Ischemia-related biomarker alterations persisted despite CLS treatment, while CLS alone did not induce significant systemic changes in non-ischemic subjects. The partial separation of Group III (TSA+CLS) from Group II (TSA), despite not fully reverting to baseline, highlights the therapeutic potential of CLS.



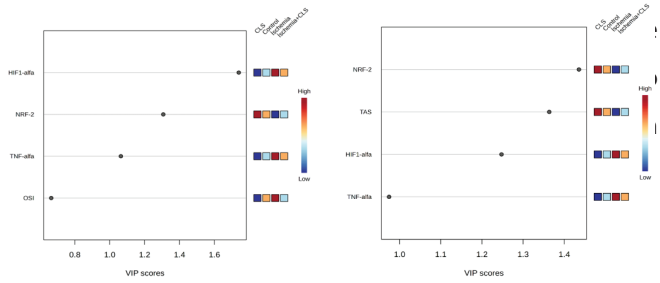
Graph 2. Representative PCA score plots derived from heart tissue and blood sample.

Graph 3, this 3D Principal Component Analysis (PCA) plot illustrates the distribution of the four experimental groups (SHAM, TSA, TSA+CLS, CLS) based on their metabolic and biochemical profiles. PCA analysis of both sample types (heart tissue and blood) clearly demonstrates group separation: Group II (TSA) shows the most distinct segregation in both tissues, particularly along the first and third principal components, indicating that ischemia leads to pronounced systemic and tissue-specific biochemical alterations. Group III (TSA+CLS) exhibits less deviation compared to the ischemic group, suggesting that CLS application partially mitigates the ischemia-induced changes. However, this mitigation does not result in full normalization, implying that while CLS exerts a therapeutic effect, its impact is limited yet meaningful. Groups I (SHAM) and IV (CLS) occupy similar positions across both sample types, indicating that CLS does not induce systemic stress or disturb metabolic balance under physiological conditions. Notably, the third principal component appears to reflect a more specific differentiation in heart tissue, sharply distinguishing the ischemic group, and possibly representing the unique effects of certain critical biomarkers. Overall, PCA analysis confirms that the selected biomarkers effectively discriminate among groups, with ischemic conditions showing clear divergence even after CLS treatment. CLS reduces this divergence to a certain extent, supporting its therapeutic potential.

Graph 3. Representative visualization of three-dimensional PCA (principal component analysis) score plots derived from cardiac tissue and blood samples.

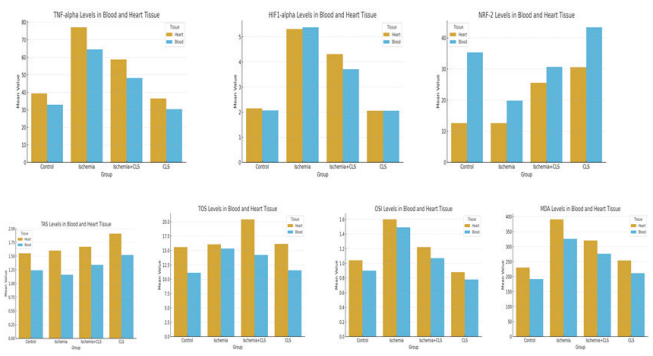
Graph 4, this figure presents a representative visualization of Variable Importance in Projection (VIP) scores derived from Partial Least Squares Discriminant Analysis (PLS-DA) performed on heart tissue and blood samples. VIP scores indicate the importance of each biomarker in explaining group separation. The figure shows parameter levels for the four experimental groups (SHAM, TSA, TSA+CLS, CLS) using color-gradient boxes, where red denotes high levels and blue indicates low levels. HIF-1α exhibits the highest VIP score (~1.4) in both tissues, with elevated levels particularly in Group II (TSA) and reduced levels in Group IV (CLS). As a marker of hypoxic response, this suggests that CLS treatment may suppress hypoxic stress. NRF-2 stands out as a key determinant of antioxidant defense, showing high VIP scores (~1.3) in both heart tissue and blood, with the highest levels in Group IV (CLS) and lowest in Group II (TSA), indicating a protective response induced by CLS against oxidative stress. TAS also demonstrates a high VIP score (~1.3), especially in blood samples, with maximum levels

in Group IV (CLS) and minimum levels in the ischemia group, reflecting increased systemic antioxidant capacity following CLS treatment. TNF-α, an inflammatory marker, has VIP scores around 1.0 in both tissues, showing elevated levels in Group II (TSA) and reduced levels in Group IV (CLS), suggesting that CLS reduces inflammatory responses. OSI exhibits VIP scores below 1.0, indicating a lesser contribution to the model's classification performance. When results from heart tissue and blood samples are evaluated together, parameters such as HIF-1α, NRF2, TAS, and TNF-α collectively reflect the therapeutic effects of CLS at both local (heart tissue) and systemic (blood) levels. Particularly, the regulatory effects on hypoxia (HIF-1α)



Graph 4. Representative visualization of PLS-DA analysis based on VIP scores derived from cardiac tissue and blood samples.

Graph 5, significant increases in TNF-α levels were observed in both heart tissue and blood samples of Group II (TSA), reflecting the activation of the proinflammatory response during ischemic processes. CLS treatment significantly suppressed TNF-α levels in both tissues, clearly demonstrating its anti-inflammatory effect. This suggests that CLS may act as a potential regulator in controlling inflammatory cytokines. MDA levels, an indicator of lipid peroxidation, reached their highest values in the ischemic group, supporting ischemia-induced oxidative membrane damage. A significant reduction in MDA levels was observed in Group III (TSA+CLS), indicating CLS's protective role in reducing lipid peroxidation and cellular damage. HIF-1α, a key regulator of the hypoxic response, was significantly elevated in the ischemic group, reflecting an adaptive response to tissue oxygen deprivation. CLS treatment significantly decreased HIF-1α levels in both heart tissue and blood, suggesting attenuation of hypoxia-related stress responses and potential indirect support of tissue oxygenation. NRF2, a principal regulator of antioxidant defense, showed marked increases in Group IV (CLS) and Group III (TSA+CLS). This finding provides strong evidence that CLS activates cellular antioxidant responses and enhances the capacity to cope with oxidative stress. TAS levels reached maximum values in the CLS group, indicating that CLS increases antioxidant capacity at both systemic and tissue levels, thereby strengthening free radical scavenging mechanisms. This rise in TAS is a significant indicator of CLS's protective effects. Although TOS levels were highest in heart tissue of Group III (TSA+CLS), the increase was noticeably lower in blood samples. CLS monotherapy led to a significant reduction in TOS levels, suggesting that CLS's inhibitory effect on oxidant production may vary depending on ischemic conditions. OSI values were markedly elevated in the ischemia group, indicating an imbalance between antioxidant and oxidant status. CLS treatment suppressed OSI levels and restored redox balance. This supports CLS's homeostatic role in improving oxidative status. Collectively, CLS treatment suppresses ischemia-induced proinflammatory (TNF-α), hypoxic (HIF-1α), and oxidative stress (MDA, TOS, OSI) responses while activating antioxidant defense mechanisms (NRF-2, TAS). These results strongly support CLS as an effective protective agent at both local (heart tissue) and systemic (blood) levels with the potential to modulate ischemic injury.



Graph 5. Comparative evaluation of key biochemical markers in heart tissue and blood samples across experimental groups.

Figure 2 presents correlation heatmaps illustrating the relationships among biochemical parameters obtained from

heart tissue and blood samples. Correlation coefficients are represented according to the color scale as follows:

- Red tones: Positive correlations (approaching +1)
- Blue tones: Negative correlations (approaching –1)
- White/pale tones: Weak or nonsignificant relationships.

Positive correlations: Strong positive correlations were observed between OSI, TOS, MDA, HIF-1α, and TNF-α in both tissue types. This indicates that oxidative stress increases concurrently with hypoxia and inflammation, reflecting the interconnected nature of these pathological processes. Notably, in Group II (TSA), these parameters were simultaneously elevated, while CLS treatment showed a trend toward reduction.

Negative correlations: NRF-2 and TAS showed significant negative correlations with OSI, TOS, MDA, HIF-1α, and TNF-α in both heart tissue and blood samples. This suggests that activation of the antioxidant system (NRF-2, TAS) suppresses stress and inflammatory markers. TAS, in particular, was inversely correlated with MDA, TOS, and OSI, highlighting its crucial role in balancing oxidative stress.

Within heart tissue, very strong correlations were evident between HIF-1α and TNF-α as well as TOS. CLS treatment attenuated these associations and activated the antioxidant system (NRF-2, TAS). In blood samples, the correlation patterns resembled those in heart tissue, with a more pronounced systemic reflection. The negative correlation of TAS with TNF-α and OSI further suggests that CLS stimulates systemic antioxidant responses. Overall, these correlation analyses clearly demonstrate that oxidative stress, inflammation, and hypoxia processes are positively interrelated, while antioxidant defense systems show inverse relationships with these processes in both heart tissue and blood. Importantly, CLS modulates these interactions positively by suppressing oxidative and inflammatory responses via NRF-2 and TAS pathways. These findings imply that evaluating CLS's therapeutic effects through systemic interaction networks rather than isolated biomarkers provides a more comprehensive understanding.

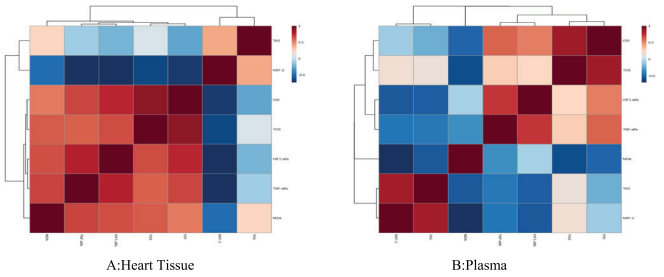


Figure 2. Combined evaluation of heatmap data derived from cardiac tissue and blood samples.

Figure 3, presents heatmaps illustrating the clustering and differentiation of four experimental groups (SHAM, TSA, TSA+CLS, CLS) based on biochemical parameter levels. The color gradients represent biomarker expression levels across groups, with red tones indicating high levels and blue tones indicating low levels. In both blood and heart tissue samples, markers such as HIF-1α, TNF-α, OSI, TOS, and MDA are notably elevated in group II (TSA), while remaining lower in other groups. This clustering reflects the concurrent activation of inflammatory (TNF-α), hypoxic (HIF-1α), and oxidative stress (TOS, OSI, MDA) pathways. These parameters stand out as key discriminators separating Group II (TSA) from the other groups. Both heart tissue and blood heatmaps show TAS and NRF2 clustered together, indicating a coordinated antioxidant defense system. These parameters are suppressed (low) in Group II (TSA) but elevated in CLS and SHAM groups, supporting findings that CLS treatment enhances antioxidant responses. MDA, a lipid peroxidation marker, is positioned close to the inflammation and oxidative stress cluster in both heart and blood heatmaps, suggesting that while it is related to oxidative stress, it may act somewhat independently as a biomarker. Group II (TSA) exhibits a distinct biochemical profile in both tissue types characterized by elevated oxidative stress and inflammatory burden. CLS treatment modulates this profile by reducing individual parameter levels and shifting the overall group profile closer to that of the control group. Notably, increases in TAS and NRF2 levels demonstrate CLS's activation of antioxidant defense systems, thereby attenuating oxidative, hypoxic, and inflammatory damage. The heatmap analyses reveal not only individual biomarker levels but also their interrelations and clustering patterns both within and between groups. The results

from heart tissue and blood samples show high consistency, clearly indicating that pathological processes (oxidative stress, inflammation, hypoxia) are co-activated, while CLS treatment suppresses these processes and exerts a protective effect. The antioxidant system emerges as a critical regulatory mechanism in these pathways.

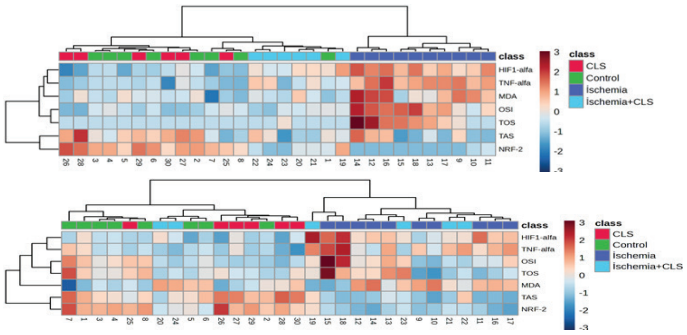


Figure 3. Heatmap representing group-based clustering according to biochemical parameters in cardiac tissue and blood samples.

Histopathological Evalution of Cardiac Tissue

In Groups I and IV, the myocardial tissue preserved its regular histological architecture (blue arrow). This finding indicates that Group I functioned as a physiological (SHAM) control, while the preserved structure in Group IV suggests that the applied treatment strategy may be effective in maintaining cardiac tissue integrity (Figures 4,7). In contrast, Group II exhibited severe histopathological alterations, such as prominent vacuolar degeneration and congestion in the cardiomyocytes (Figure 5). However, these pathological findings were markedly reduced in Group III, where the cardiomyocytes displayed a more organized morphological structure (green arrow) (Figure 6). The structural improvement observed in Group III supports the potential cardioprotective effects of CLS extract on myocardial tissue.

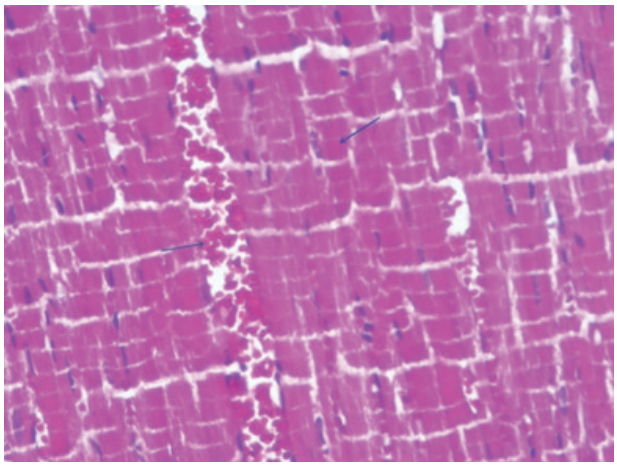


Figure 4. GroupI (SHAM).

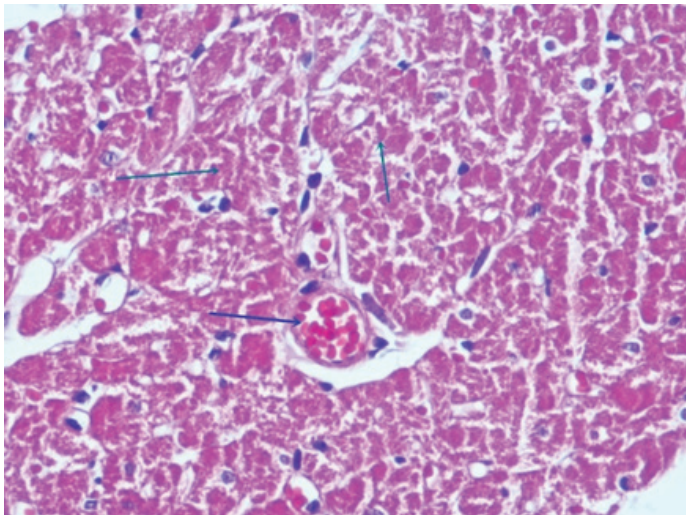


Figure 5. Group II (TSA).

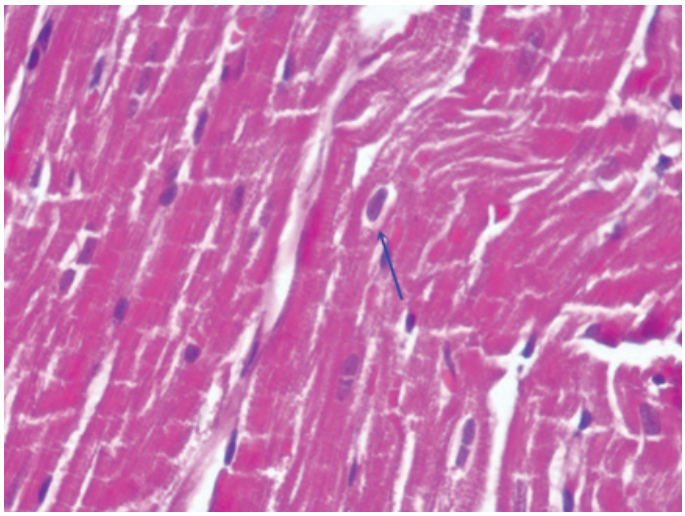


Figure 6. Group III (TSA+CLS).

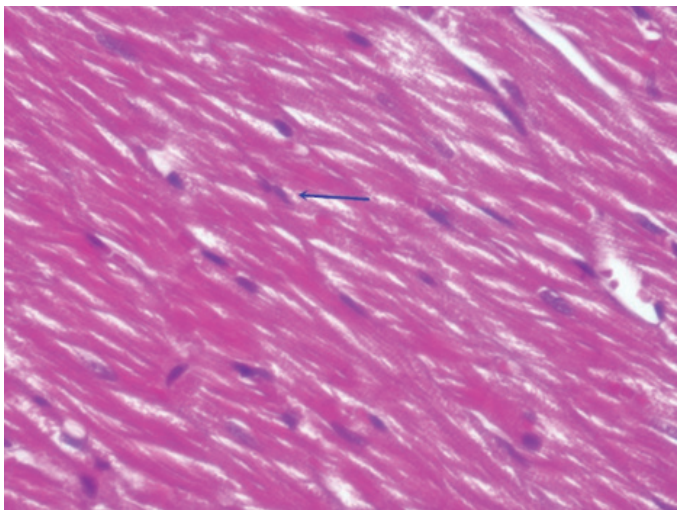


Figure 7. Group IV (CLS).

Table 1. Comparison of hematological parameters in blood between groups (Mean ± SD).

	GROUP I (SHAM)	GROUP II (TSA)	GROUP III (TSA+CLS)	GROUP III (CLS)		
Parameter	(Mean±SD)	(Mean±SD)	(Mean±SD)	(Mean±SD)	p-valuea	Posthocb
MCV	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.02	0,002*	A-B, B-C, D-B
WBC	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.02	0,1221	n.s.
RBC	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.02	0,0559	n.s.
HGB	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.02	0,006*	B-C, D-B
HCT	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.02	0,002*	B-C
LYM	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.02	0,2414	n.s.
EOS	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.02	0,015*	A-C, B-C
BASO	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.03 ± 0.02	0,004*	B-C

a: Anova Test, **: p <0.001, *: p <0,05, b: Tukey Test.

Table 2. Comparison of biochemical parameters in blood between groups (mean ± SD) and levels of statistical significance.

	GROUP I(SHAM)			GROUP II (TSA)			GROUP III (TSA+CLS)			GROUP IV (CLS)				
	Min	Max	Ort ±SS	Min	Max	Ort ±SS	Min	Max	Ort ±SS	Min	Max	Ort ±SS	pa	host hocb
TNF-alfa	31.50	34.50	32.93 ± 1.12	51.85	71.40	64.52 ± 7.53	9.16	63	48.18 ± 9.16	10.25	31.50	30.35 ± 5.66	<0.001**	B-D
MDA	128.00	236.00	192 ± 34.99	224	468.00	325.9 ± 77.59	55.68	320.5	276.47 ± 55.68	0.71	128.00	211.13 ± 11.65	0.71	
HIF1-alfa	1.43	2.54	2.07 ± 0.41	3.40	6.50	5.38 ± 0.92	0.81	5.6	4.31 ± 0.81	1.43	1.43	2.06 ± 0.68	<0.001**	B-D. C-D. B-A
NRF-2	20.50	42.50	35.35 ± 7.32	10.20	29.40	18.96 ± 5.47	4.21	35.2	30.17 ± 4.21	20.50	20.50	43.43 ± 8.27	<0.001**	A-D. C-D. A-B. C-A. C-B
TAS	1.14	1.45	1.24 ± 0.1	0.87	1.31	1.12 ± 0.15	0.17	1.5646724	1.34 ± 0.17	1.14	1.14	1.52 ± 0.19	<0.001**	A-D. C-D. A-B. C-A. C-B
TOS	10.25	13.20	11.12 ± 1.04	11.598298	22.29	16.5 ± 3.62	4.05	22.150742	14.22 ± 4.05	10.25	71.40	11.55 ± 1.48	0.21	
OSI	0.71	1.08	0.9 ± 0.12	0.9954659	2.16	1.49 ± 0.35	0.27	1.5642671	1.07 ± 0.27	0.71	468.00	0.78 ± 0.22	0.45	

a: Anova Test. **: p <0.001. *: p <0.05. b: Tukey Test.

Table 3. Comparison of biochemical parameters in cardiac tissue among experimental groups.

	GROUP I(SHAM)	GROUP II (TSA)	GROUP III (TSA+CLS)	GROUP III (CLS)		
	Ort ±SS	Ort ±SS	Ort ±SS	Ort ±SS	p	host hoc
TNF-alfa	39.35 ± 1.24	77.13 ± 12.22	58.71 ± 10.54	36.42 ± 6.8	0.001**	B-D. C-D. B-A. C-B
MDA	230.4 ± 41.98	391.08 ± 93.11	320.26 ± 66.15	253.64 ± 13.63	0.001**	B-D. B-A. C-B
HIF1-alfa	2.15 ± 0.55	5.63 ± 0.92	4.31 ± 0.81	2.06 ± 0.68	0.001**	B-D. C-D. B-A. C-A. C-B
NRF-2	30.32 ± 4.82	16.02 ± 4.22	25.62 ± 4.81	36.92 ± 7.03	0.001**	B-D. C-D. B-A. C-A
TAS	1.55 ± 0.12	16.02 ± 0.19	1.67 ± 0.21	1.9 ± 0.24	0.07	
TOS	15.99 ± 1.49	16.02 ± 5.2	20.43 ± 5.82	16.61 ± 2.13	0.001**	B-D. B-A. C-B
OSI	1.04 ± 0.13	16.02 ± 0.4	1.22 ± 0.31	0.88 ± 0.25	0.006*	B-D. B-A. C-B

a: Anova Test. **: p <0.001. *: p <0.05. b: Tukey Test.

Discussion

The global incidence of CVDs continues to rise. making CVDs one of the leading causes of death worldwide [13]. Ischemia is defined as a condition in which tissues or organs receive insufficient oxygen due to reduced arterial or venous blood flow. During this process. the depletion of cellular energy reserves and the accumulation of toxic metabolites can lead to cell death [14]. Reperfusion refers to the restoration of blood flow and oxygen supply to previously ischemic tissues or organs [15]. MIRI is a complex pathophysiological process involving multiple molecular mechanisms. including excessive production of reactive oxygen species (ROS). mitochondrial dysfunction. and impaired autophagy [16].

In recent years. findings have suggested that complete blood count (CBC) parameters. a widely used and cost-effective hematological analysis method. may be associated with morbidity. mortality. and vascular calcification in various CVDs. Accordingly. CBC parameters have gained importance as valuable biomarkers in clinical research [17]. One study demonstrated that extremity I/R injury exerts not only local but also systemic effects throughout the organism. This process resulted in significant alterations in hematological and metabolic parameters. as well as micro-rheological properties such as blood fluidity and cellular deformability. The findings indicate that I/R injury induces complex and multifaceted pathophysiological changes at the systemic level [18]. According to our study. the obtained results reveal that CSL extract exerts marked effects on erythrocyte morphology and hemoglobin (HGB) metabolism. while also suggesting a possible immunomodulatory influence on certain leukocyte subpopulations. When administered in conjunction with ischemia. CSL appears to have regulatory potential on the hematological profile. Further molecular investigations are required to elucidate these effects at a mechanistic level.

Oxidative stress resulting from the excessive production of reactive oxygen species (ROS) plays a critical role in the pathogenesis of reperfusion injury. ROS. generated through the uncontrolled activity of specific enzymatic sources. can cause damage to cellular structures and severely impair tissue function [19]. Under physiological conditions. antioxidant defense systems are generally able to maintain cellular homeostasis. However. when ROS production increases—such as during immune cell activation—antioxidants may fail to adequately neutralize lipids. proteins. carbohydrates. and nucleic acids. leading to oxidative damage [20]. MDA is an aldehyde formed as a result of the peroxidation of polyunsaturated fatty acids and contains double bonds where methylene bridges are disrupted [21]. Numerous studies have demonstrated that MDA levels in biological samples serve as a reliable biomarker of oxidative damage in the body [22]. Moreover. organosulfur compounds such as alpha-lipoic acid have been reported to exert antioxidant effects by reducing MDA levels. even in individuals with impaired health status [23]. Although MDA is an oxidative stress marker and intergroup differences were observed in the blood results of our study. these changes were not found to be statistically significant. This may indicate that the effect of CSL on this parameter is limited or indirect. However. the significant reduction in MDA levels—a marker of lipid peroxidation—observed in cardiac tissue homogenates supports the protective effect of CSL against oxidative damage. MDA is an indicator of reactive oxidative products that compromise cell membrane integrity. and its increase under ischemic conditions reflects the cellular consequences of oxidative stress. The reduction of these levels following CSL treatment suggests a decrease in cellular injury.

The heart is a TNF-producing organ. with both cardiac myocytes and myocardial macrophages capable of synthesizing TNF [24]. Under ischemic and anoxic conditions. cardiomyocytes and local myocardial mononuclear macrophages become activated to produce high levels of TNF-α; concurrently. a significant upregulation of TNF receptor 2 (TNFR2) expression is observed [25]. Administration of anti-TNF-α antibodies approximately three hours prior to I/R has been shown to reduce ROS production in endothelial cells. thereby alleviating endothelial dysfunction [26]. Conversely. in a rat cardiomyopathy model induced by adriamycin. elevated serum TNF-α levels were associated with worsened cardiac function and increased mortality [27]. In a porcine model. Liakopoulos et al. demonstrated that methylprednisolone suppressed systemic and myocardial TNF-α elevations following cardiac surgery. preventing progression of myocardial dysfunction. highlighting the pivotal role of TNF-α in this process [28]. In our study. serum TNF-α levels were found to be highest in Group II (TSA) compared to Group I. Moreover. TNF-α levels in Group II were significantly elevated compared to Group IV.

These findings suggest that CSL administration may exert a protective effect by suppressing this increase in TNF-α levels. clearly indicating the activation of inflammatory responses. In cardiac tissue homogenates. the significant reduction in TNF-α levels—one of the key proinflammatory cytokines—strongly supports the anti-inflammatory effects of CSL. TNF-α is a crucial biomarker of early inflammatory processes triggered by ischemic injury. and its elevation reflects the systemic inflammatory burden. The suppression of this increase following CSL treatment suggests the therapeutic potential of CSL in mitigating the ischemic response.

Recent studies have highlighted the role of the hypoxia-associated transcription factor HIF-1α in cardiac regeneration and protective mechanisms. Cerrada et al. [29] reported that transplantation of mesenchymal stem cells (MSCs) transfected with HIF-1α in a rat model of myocardial infarction (MI) led to improved cardiac function. enhanced angiogenesis. increased cardiomyocyte proliferation. and reduced fibrotic tissue accumulation. These findings suggest that HIF-1α gene therapy may enhance the efficacy of cell-based cardiac regenerative treatments. However. a significant limitation of this study is that HIF-1α expression levels within the ischemic region were not directly measured. Matsushima et al. [30] proposed that HIF-1α could serve as a potential therapeutic target by protecting cardiac fibroblasts from apoptosis and facilitating cardiac tissue remodeling after hypoxic injury. Furthermore. various preclinical studies support the cardioprotective role of HIF-1α through its preventive effects against MIRI [31]. In the present study. the highest serum HIF-1α levels were observed in Group II (TSA). indicating that this biomarker is markedly activated as part of the hypoxic stress response and plays a central role in the pathophysiology of ischemia. As a transcription factor sensitive to low oxygen levels. the increased expression of HIF-1α in response to hypoxia confirms the presence of hypoxic burden at the molecular level. Data obtained from cardiac tissue homogenates further support that this hypoxic response is also regulated at the cardiac level and that the ischemic model induced by TSA leads to significant tissue damage in cardiomyocytes. The significant reduction in HIF-1α levels observed following CSL administration demonstrates that this treatment approach exerts a balancing—and potentially suppressive—effect on adaptive responses to hypoxia. This effect of CSL suggests a reduction in hypoxic burden through improved oxygen distribution and support of mitochondrial function. Accordingly. it can be concluded that CSL not only modulates inflammatory responses but may also possess therapeutic potential in alleviating hypoxic stress by influencing cellular oxygen regulation.

Studies conducted on rat models and human endothelial cells have demonstrated that NRF2 activation is associated with a protective response against the atherosclerotic process [32]. NRF2 is reported not only to regulate antioxidant responses but also to influence atherogenesis-related processes such as plasma lipoprotein metabolism. cholesterol transport. and vascular smooth muscle cell proliferation [33]. Moreover. in a rat model of reperfusion following left anterior descending artery (LAD) occlusion. atorvastatin treatment was shown to increase NRF2 and Heme Oxygenase-1 (HO-1) protein expression. resulting in approximately a 20% reduction in infarct size [34]. These findings indicate that NRF2 functions not only as a regulator of antioxidant defense but also plays a critical role in the pathogenesis of cardiovascular diseases through mechanisms involving lipoprotein metabolism and cellular growth. In our study. the lowest levels of NRF2 were observed in Group II (TSA). while the highest levels were recorded in Group IV (CLS). clearly demonstrating the activating effect of CLS on antioxidant responses under oxidative stress conditions. This suggests that CLS not only suppresses oxidative damage but also strengthens cellular defense mechanisms. Data obtained from cardiac tissue homogenates further support the prominent role of CLS in the molecular regulation of antioxidant defense. NRF2 is one of the key transcription factors responsible for initiating the expression of antioxidant response elements and is essential for maintaining intracellular redox homeostasis. The significant increase in NRF2 levels following CLS treatment suggests that this therapeutic approach enhances endogenous antioxidant systems by supporting cellular adaptation to oxidative stress. In this regard. CLS offers a comprehensive protective effect by targeting not only the biochemical markers of oxidative stress but also the endogenous defense mechanisms developed in response to such stress.

In this context. the antioxidant properties of plant-based products are considered potential therapeutic strategies to mitigate the effects of I/R injury. CSL is a plant rich in potent

antioxidant compounds and is widely used in traditional medicine [35.36]. CSL and its derivatives contain bioactive flavonoid compounds such as dicaffeoylquinic acids (cynarin. chlorogenic acid). luteolin glucoside. and apigenin glucoside [37]. Various experimental and clinical studies have demonstrated the cardioprotective potential of CSL [38]. For instance. Roghani-Dehkordi and Kamkhah reported that CSL leaf juice reduced blood pressure in individuals with mild hypertension [39]. Wang et al. also revealed cardiovascular benefits of CSL bud extract in hypertensive rat models [40]. Additionally. CSL extracts have been reported to exert favorable effects on dyslipidemia. an important risk factor for cardiovascular diseases. In a study by Santos et al. [41]. CSL leaf extract significantly decreased LDL cholesterol. total cholesterol. and triglyceride levels. Furthermore. studies by El Morsy and Kamel in animal models demonstrated that CSL administration reduced lipid peroxidation and DNA damage while enhancing antioxidant activity [35].

A comprehensive review of the literature reveals that studies directly investigating the relationship between CSL and CVDs. especially in experimental cardiac injury models. remain limited. The 1500 mg/kg dose of CSL extract used in El Morsy and Kamel's 14-day study was taken as a primary reference for dosage determination in the present study [35]. The experimental cardiac injury protocol was adapted from reference [10]. To date. no study has evaluated the effects of CSL on cardiac injury resulting from aortic cross-clamping. In this respect. our study represents one of the few investigations assessing the potential cardioprotective effects of CSL in cardiac injury. thereby contributing valuable insights to the existing literature. Our study aims to evaluate the potential cardioprotective effects of CSL and contributes to the existing literature. In our study. CSL administration (Groups III and IV) effectively modulated inflammatory responses and oxidative stress by inducing favorable changes in TNF-α. HIF-1α. and NRF2 levels. Notably. Group IV (CSL) demonstrated the highest values in TAS and NRF2 levels. both of which reflect antioxidant capacity. Although differences were observed among groups in serum MDA. TOS. and OSI levels. these changes did not reach statistical significance. However. significant results were obtained in cardiac tissue homogenates. These findings suggest that CSL treatment may exert protective effects against inflammation and oxidative stress under ischemic conditions and provide strong evidence for its modulatory effects on both inflammatory and oxidative responses through these parameters.

In conclusion. based on the analyses conducted. the protective effects of CSL extract against induced I/R injury in the experimental groups were evaluated in the context of oxidative stress parameters (MDA. TAS. TOS. OSI). inflammatory and molecular markers (TNF-α. HIF-1α. NRF2). and histopathological findings. Ischemia was found to elevate inflammatory and hypoxic markers such as TNF-α and HIF-1α. while suppressing antioxidant defenses (NRF2. TAS). CLS administration partially reversed these adverse effects. particularly improving antioxidant capacity and inflammatory indicators. The findings demonstrate the potential cardioprotective effects of CSL in preventing I/R injury. These results may contribute to the development of novel and complementary strategies in the treatment of CVD. and further support the potential clinical application of CSL while encouraging its evaluation from a broader perspective in cardiac injury research.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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Ethics approval

This study was conducted with the approval of the Local Animal Ethics Committee of Harran University (File No: 395481. Decision No: 2024/008/06. dated 09.12.2024).

Authors' contribution

MZB: Concept, design, supervision, materials, data collection and/or processing, analysis and/or interpretation, literature search, writing manuscript, critical review. EE: Design, materials, data collection and/or processing, analysis and/or interpretation, writing manuscript. BA: Materials, data collection and/or processing, analysis and/or interpretation, literature search. IK: Methodology, analysis and/or interpretation, laboratory work, data collection. MEG: Methodology, pathological evaluation, analysis and/or interpretation. YH: Literature search, data collection and/or processing. KE: Analysis and/or interpretation, laboratory work. SG: Concept, supervision, critical review.

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