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Cardioprotective effects of silymarin in heart failure for adjunctive therapy: Role of Nrf2 signal transduction pathway

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In the present study, the potential of silymarin (SIL) administration as an adjuvant therapy in heart failure (HF) model of rats was investigated. HF induction was done by injecting 85 mg/kg/day isoproterenol and silymarin (SIL), trimetazidine (TMZ) and SIL+TMZ were administered to HF rats for 2 weeks. Then, echocardiography parameters such as ejection fraction (EF) and fractional shortening (FS) were evaluated. The anxiety and depression like behaviours were evaluated by elevated plus maze (EPM) and sucrose preference test (SPT). Furthermore, the plasma levels of BNP, Galectin-3 and Nrf2, as well as the serum levels of cytokines IL-1β, IL-6 and TNF-α were measured. Finally, the expression levels of *SOD1*, *GPx1* and *NQO1* genes were measured using RT-PCR technique. Administration of SIL, TMZ and SIL+TMZ to HF rats for 2 weeks was associated with improvement of EF and FS parameters. Also, all treatments improved animals function in both EPM and SPT tests. Furthermore, decreases in the plasma levels of BNP and Galectin-3 and an up-regulation of Nrf2 were observed in HF rats receiving SIL, TMZ and SIL+TMZ. In addition, downregulation of IL-1β, IL-6 and TNF-α cytokines and overexpression of *SOD1*, *GPx1* and *NQO1* genes were seen in HF rats after administration of SIL, TMZ and SIL+TMZ. Silymarin has the potential to be used as an adjunctive therapy in heart failure.

Keywords: Gene, Parameter, Cytokine, Galectin-3, B-type natriuretic peptide

Heart failure (HF) is a serious health complication that is associated with many problems such as reducing the quality of life, social functioning and job performance[1]. In this disease, due to the reduction of blood pumping to the body as a result of the cardiac dysfunction, symptoms of the disease such as extreme fatigue, frequent hospitalization are manifested, which is associated with high mortality[1]. HF is characterised by the imbalance of left ventricular function and myocardial energy consumption (lack of mechano-energetic coupling)[2] and it seems that oxidative stress plays a central role in the development of this phenomenon, and patients have high levels of reactive oxygen species in blood and pericardial fluid. In addition, high levels of malondialdehyde (MDA) in HF patients have been found to have a negative correlation with the ejection fraction (EF) of the left ventricle and a positive correlation with the severity of the disease[3]. Therapeutic approaches include administration of beta-blockers and angiotensin-converting enzyme inhibitors[4]. However, HF progresses rapidly over time and current treatments have failed to stop it.

Silymarin is a standardised extract of *Silybum marianum* and a member of flavonolignans, which has strong antioxidant and anti-inflammatory properties[5]. This compound inhibits free radicals, reduces lipid peroxidation and improves the activity of antioxidant enzymes such as superoxide dismutase (SOD) in erythrocytes[6]. The cardioprotective effects of this compound have also been reported in various studies. For example, silymarin showed protective effects in heart damage induced by doxorubicin[7], and the administration of this natural compound to thalassemia patients improved cardiac outcomes due to the reduction of iron overloading due to the chelating properties of silymarin[8]. Also, cardiac dysfunction induced by liver cirrhosis was reduced by silymarin administration[9]. In addition, this natural product with antioxidant activity has shown protective effects in diseases such as atherosclerosis[10] and hypertension[11]. However, cardioprotective effects in heart failure conditions have not been studied so far. Therefore, based on the above-mentioned studies, our hypothesis was that silymarin could have cardioprotective effects in HF conditions. Thus, in the present study, the cardioprotective effects of silymarin against heart failure were studied in the rat model.

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Materials and Methods

Animals

50 male Wistar rats were prepared. The weight range of the animals was 220-265 g. The animals were kept in laboratory conditions with a light/dark cycle of 12/12 hours, temperature of 22 to 24 °C and free access to water and food in the animal house and in the cages. Ethical issues were based on the instructions of the Medical Ethics Committee of Bushehr University of Medical Sciences, and before the experiment, an ethical code was obtained.

Experimental design and grouping

The current research was an experimental study, in which the therapeutic effects of silymarin on rat model of heart failure were evaluated. For this purpose, animals were randomly grouped into five groups as follows: Control (n=10): healthy rats that received normal saline (0.5%); HF (n=10): animals that received subcutaneous injection of 85 mg/kg/day isoproterenol for 2 consecutive days[12]; HF+SIL (n=10): rats that received 100 mg/kg silymarin (SIL) intraperitoneal (i.p.) everyday immediately after HF induction for 2 weeks; HF+TMZ (n=10): Animals that received 10 mg/kg Trimetazidine (TMZ) every day by gavage after HF induction for 2 weeks; HF+SIL+TMZ (n=10): Rats received 10 mg/kg Trimetazidine (TMZ) by gavage and 100 mg/kg SIL i.p. every day for two weeks after HF induction.

Echocardiographic parameters

Transthoracic echocardiography parameters 1 were used to evaluate heart function. For this purpose, the animals were initially anesthetized by subcutaneous injection of 80 mg/kg of ketamine and 8 mg/kg of xylazine. Then, the chest area was shaved and the animals were placed on their backs on the bed next to the machine. In the next step, the transducer was placed directly on the chest area by the operator to record the echocardiographic parameters. Echocardiography was recorded blindly by the operator using a 6 to 12 MHz transducer system. Two-dimensional images were taken in the mid-paracentral section from the perspective of the left ventricular short-axis, and later M-mood images were taken from the perspective of the parasternal long-axis, at the level of the papillary muscles of the left ventricle to measure the thickness parameters of the interventricular septal thickness during systole (IVSs) and diastole (IVSd), LV end-systolic posterior wall thickness during diastole (LVDd), and systole (LVDs), left ventricular posterior wall thickness at the end of systole (PWTs) and diastole (PWTd) were obtained. Finally, ejection fraction (EF) and shortening fraction (FS) were calculated.

Behavioral tests

Elevated Plus Maze

This test is used to measure the level of animal anxiety. The device was made of wood and had four arms in the shape of plus (+). The dimensions of the open and closed corridors were 50×10cm, and the two sides and ends of the closed corridor had walls 40 cm high. All four corridors led to a central area measuring 10×10cm. The maze was supported by pedestals 50 cm above the surface. Rats were placed gently in the central area of the maze and free movement was measured for 5 min. After counting the number of entries into the open and closed arms and the time the animal spent there, the percentage of open arm entries (OAE%) and open arm time (%OAT) were calculated. Locomotor activities were measured based on the total number of entries into the open and closed arms of the maze.

Sucrose preference test

This test was performed over a 48-hour period. For this purpose, two bottles (one containing 2% sucrose and the other containing plain water) were placed on the animal's cage, and the position of both bottles was changed every 12 hours to prevent psychological bias. The weight of the bottles containing water and sucrose solution was recorded at the beginning and after the test to calculate the amount of consumption. Sucrose preference was calculated as the percentage of sucrose solution consumed relative to the total amount of fluid consumed. It is worth noting that reluctance to consume the bottle containing sucrose solution is considered an indicator of depressive behaviour.

Biochemical parameters

At first, blood was collected from the animals, and the plasma and serum were prepared. The plasma levels of B-type natriuretic peptide (BNP), Nuclear factor erythroid 2-related factor 2 (Nrf2) and Galectin-3 were measured by Mybiosource (MBS9380631), Mybiosource (MBS752046) and ThermoFisher (ERLGALS3) kits, respectively. Also, serum levels of interleukins (ILs) -1β, -2, -6, -7, -10, as well as tumor necrosis factor alpha (TNF-α) were measured by Abcam (UK) kits.

Table 1 — The sequences of primers of *NQO1*, *SOD1*, *HO-1* and *GR* genes

Genes	Forward primer sequences [5'-3']	Reverse primer sequences [5'-3']
NQO1	GCATTGGCCACAATCCACCAG	ATGGCCCACAGAGAGGCCAAA
SOD1	TTTTGCTCTCCCAGGTTCCG	CCCATGCTCGCCTTCAGTTA
GPx1	AGTTCGGACATCAGGAGAATGGCA	TCACCATTCACCTCGCACTTCTCA
β-actin	TCTTCCAGCCTTCCTTCCTG	CACACAGAGTACTTGCGCTC

Gene expression

We measured the important genes in Nrf2 pathway such as *NQO1*, *SOD1* and *GPx1* using RT-PCR technique. For this purpose, at first, RNA was extracted from blood cells using QIAamp RNA Blood Mini (QIAGEN, Cat# 52304, Germany), and after ensuring the quantity and quality of the extracted RNA, cDNA synthesis was performed using the relevant kit (QIAGEN, Cat# 205311, Germany). Primer design was done using Primer 3 software, and to ensure the correctness of the primer sequences, the sequences were blasted on the NCBI-Primer blast site. The sequences of primers of *NQO1*, *SOD1*, *HO-1* and *GPx1* genes are given in Table 1 and *β-actin* gene was used as the internal control gene.

The RT-PCR reaction mixture included 1 µL of each of F and R primers, 10 µL of SYBR Green Supermix, 2 µL of cDNA, and 6 µL of sterilized double distilled water in a final volume of 20 µL. The temperature-time program of the device consisted of one cycle of 95°C for 30 s followed by 40 cycles of 95°C for 15 s and 60°C for 30 s.

Statistical analysis

The results were reported as mean ± standard deviation. Analysis was performed in GraphPad Prism V.8 software using one-way analysis of variance (ANOVA) after ensuring the normal distribution of the data and considering $P<0.05$ as the significance level. Tukey's multiple range test was used for the post hoc test.

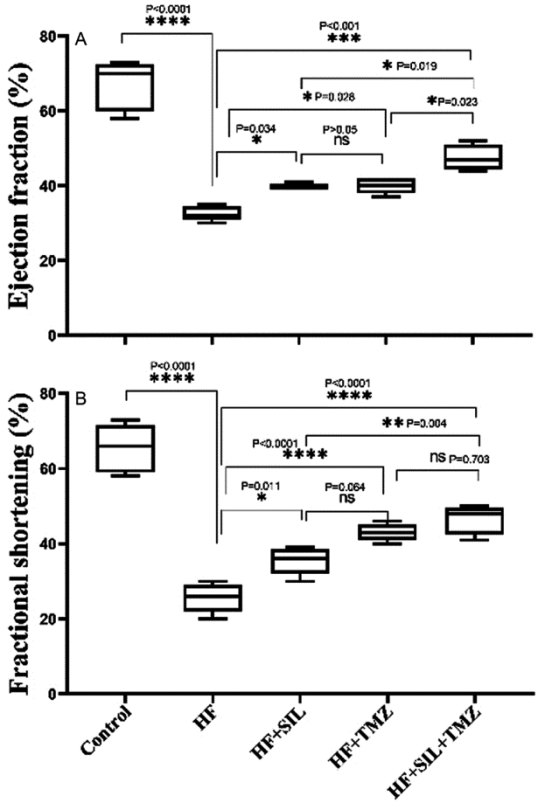

Fig. 1 — The effect of administration of silymarin (SIL), trimetazidine (TMZ) and TMZ+SIL on ejection fraction (EF, A) and fractional shortening (FS, B) of heart failure model rats (n=10). 130 mg/kg/day isoproterenol for 2 consecutive days was used to induce HF. Rats received 100 mg/kg SIL i,p. and 10 mg/kg TMZ orally for 2 weeks every day after HF induction, and echocardiographic parameters were measured one day after receiving the last dose.

Results

Ejection fraction and fractional shortening

Administration of isoproterenol for two consecutive days led to a significant decrease in both EF (CI95%: 27.61-41.19, $P<0.0001$) and FS (CI95: 31.87-47.73, $P<0.0001$) compared to healthy rats, so that EF and FS were decreased 34.40%, and 39.80%, respectively, in HF rats (EF: 32.60±1.94%; FS: 25.6 ± 3.84%) compared to healthy rats (EF: 67.3 ± 6.63%; FS: 65.4 ± 6.38%). Nevertheless, the administration of SIL ($P=0.011$), TMZ ($P<0.0001$) and SIL+TMZ ($P<0.0001$) to HF rats was associated with significant improvement of echocardiographic parameters (EF and FS) compared to HF rats (Fig. 1A & B). Interestingly, the combined administration of SIL+TMZ to HF rats resulted in a significant improvement of EF (47.60±3.36%) compared to TMZ-treated HF rats (40.00±2.12%, $P=0.023$, Fig. 1A). This suggests that adjunctive therapy with SIL in HF condition can be associated with potential therapeutic effects.

Behavioral tests

Elevated plus maze

A significant decrease in the percentage of time spent in the open arm ($P<0.001$, Fig. 2A) and the

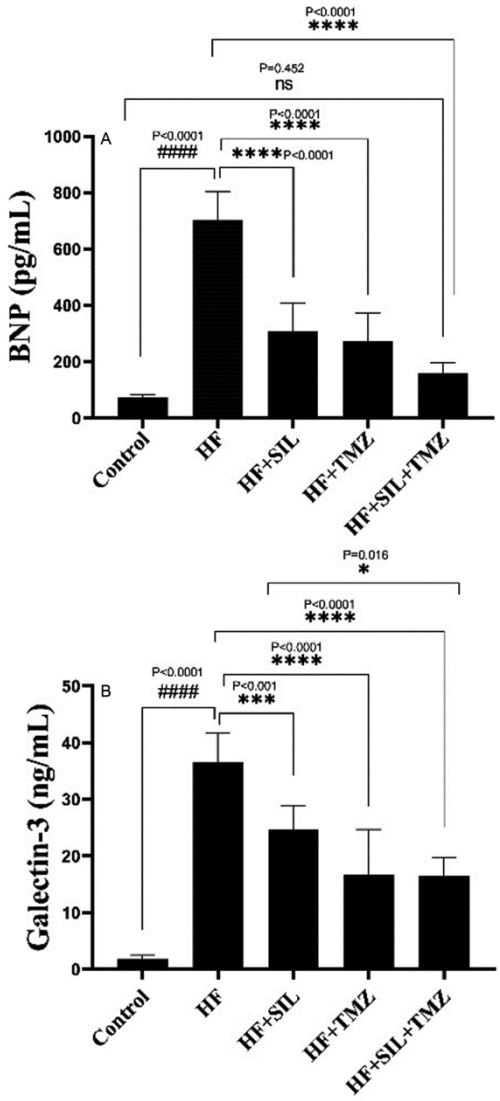

Fig. 2 — The effect of administration of silymarin (SIL), trimetazidine (TMZ) and TMZ+SIL on the time spent on open arm (A), number of entries in open arm (B) and locomotor activity (C) of HF rats in elevated plus maze (n=10). 130 mg/kg/day isoproterenol for 2 consecutive days was used to induce HF. Rats received 100 mg/kg SIL i,p. and 10 mg/kg TMZ orally for 2 weeks every day after HF induction, and EPM test were conducted one day after receiving the last dose. * P <0.05, ** P <0.01,*** P <0.001,**** P <0.0001 compared with control; $ P <0.05, $$ P <0.01, $$$ P <0.001 compared with HF animals.

percentage of open arm entries (P<0.0001, Fig. 2B) in the EPM test was observed in HF rats compared to control rats. However, administration of SIL, TMZ, and SIL+TMS to HF rats significantly improved both OAT% and OAE% compared to control rats. It was found that the combined administration of SIL+TMZ caused the greatest improvement in both OAT% and OAE% in this test. In addition, SIL, TMZ, and SIL+TMZ significantly improved the locomotor

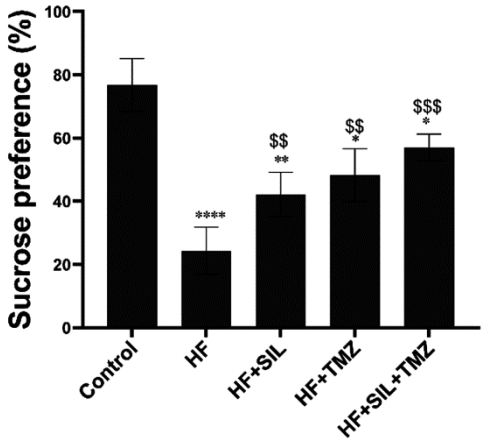

Fig. 3 — The effect of administration of silymarin (SIL), trimetazidine (TMZ) and TMZ+SIL on the sucrose preference of HF rats (n=10). 130 mg/kg/day isoproterenol for 2 consecutive days was used to induce HF. Rats received 100 mg/kg SIL i,p. and 10 mg/kg TMZ orally for 2 weeks every day after HF induction, and SPT were conducted one day after receiving the last dose. * P <0.05,** P <0.01,**** P <0.0001 compared with control; $$ P <0.01, $$$ P <0.001 compared with HF animals.

activity of HF rats compared to HF ones (Fig. 2C). Importantly, no significant difference in locomotor activity was observed between HF rats receiving the combined treatment (SIL+TMZ) and HF animals. This indicates that these compounds are able to exert protective effects against HF-induced anxiety.

Sucrose preference test

The percentage of sucrose preference was significantly reduced in HF rats compared to healthy control animals, which could indicate the occurrence of depression in the animals. However, the percentage of sucrose preference was significantly improved in rats receiving SIL, TMZ, and SIL+TMZ compared to HF rats. However, no significant difference was observed in the percentage of sucrose preference among HF rats receiving SIL, TMZ, and SIL+TMZ (Fig. 3).

BNP and Galectin-3

The ANOVA table results showed that there were significant differences in both BNP (F (4, 20) = 46.3, P<0.0001) and Galectin-3 (F (4, 20) = 33.51, P<0.0001) among groups. Plasma levels of BNP (103.5±701.6 pg/mL, P<0.0001, CI95%: -780.1 to -479.1) and Galectin-3 (36.52±5.26 ng/mL, P<0.0001, CI95%: -43.99 to -25.41) were significantly increased in HF rats compared to control rats. However, a significant decrease in BNP plasma levels was observed in rats receiving SIL (97.81±309.2 pg/mL), TMZ (98.81±274.2 pg/mL) and SIL+TMZ (37.88±157.8 pg/mL) compared to HF rats (P<0.0001, Fig. 4A). Similarly, rats receiving

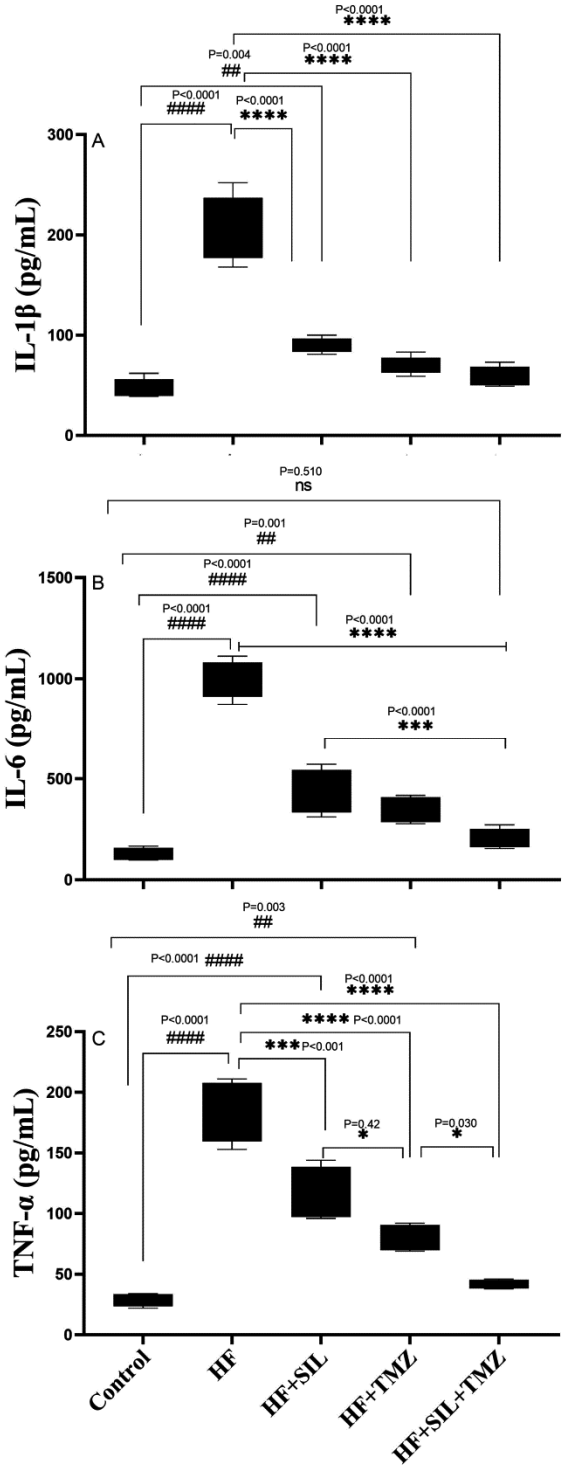

Fig. 4 — The effect of administration of silymarin (SIL), trimetazidine (TMZ) and TMZ+SIL on the plasma levels of B-type natriuretic peptide (BNP, A) and Galectin-3 (B) of rats (n=5). 130 mg/kg/day isoproterenol for 2 consecutive days was used to induce HF. Rats received 100 mg/kg SIL i,p. and 10 mg/kg TMZ orally for 2 weeks every day after HF induction, and the plasma levels of BNP and Galectin-3 were measured one day after receiving the last dose.

SIL (24.72±4.22 ng/mL), TMZ (16.68±8.0 ng/mL) and SIL+TMZ (16.46±3.23 ng/mL) showed a significant decrease in Galectin-3 plasma levels compared to HF rats (Fig. 4B).

Inflammatory cytokines

The analysis of variance indicated significant differences in serum levels of IL-1β (F (4, 20) = 72.79, P<0.0001), IL-6 (F (4, 20) = 104.6, P<0.0001) and TNF-α (F (4, 20)= 62.02, P<0.0001) among groups. Induction of HF with the administration of isoproterenol for two consecutive days was associated with the significant increases in serum levels of inflammatory cytokines IL-1β, IL-6 and TNF-α compared to control healthy rats. However, the administration of SIL, TMZ and SIL+TMZ decreased the serum levels of the aforementioned cytokines in HF rats (Fig. 5A, 5B & 5C). These findings indicate strong anti-inflammatory effects of both SIL and TMZ in HF condition.

Gene expression

The ANOVA results showed significant differences in *SOD1* (F (4, 10) = 40.0, P<0.0001), *GPx1* (F (4, 10) = 64.16, P<0.0001) and *NQO1* (F (4, 10) = 77.69, P<0.0001) genes expressions among groups. HF induction was associated with approximately 4-fold decreases in *SOD1* gene (0.071±0.240), 2-fold in *GPx1* (0.05±0.48) and 2.5-fold in *NQO1* (0.07±0.54) compared to healthy control rats, indicating a decrease in the expression of Nrf2 pathway genes (Fig. 6A, 6B & 6C). However, the administration of SIL, TMZ and TMZ+SIL was associated with a significant improvement in the expression of *SOD1*, *GPx1* and *NQO1* genes in HF rats. Interestingly, the expression of *NQO1* gene in the HF rats receiving TMZ and SIL+TMZ was significantly higher than in the healthy rats (Fig. 6C), which indicates the over-activation of the Nrf2 pathway in these rats.

Nrf2

The variance analysis indicated that there was significant differences in Nrf2 plasma levels between groups (F (4, 10) = 43.51, P<0.0001). A significant decrease in the expression of Nrf2 plasma levels was observed in HF rats (0.066±0.18 ng/mL) compared to healthy control rats (0.18±0.89 ng/mL). However, administration of SIL, TMZ and SIL+TMZ was associated with a significant increase in plasma levels of Nrf2 in HF rats. It is worth noting that the plasma

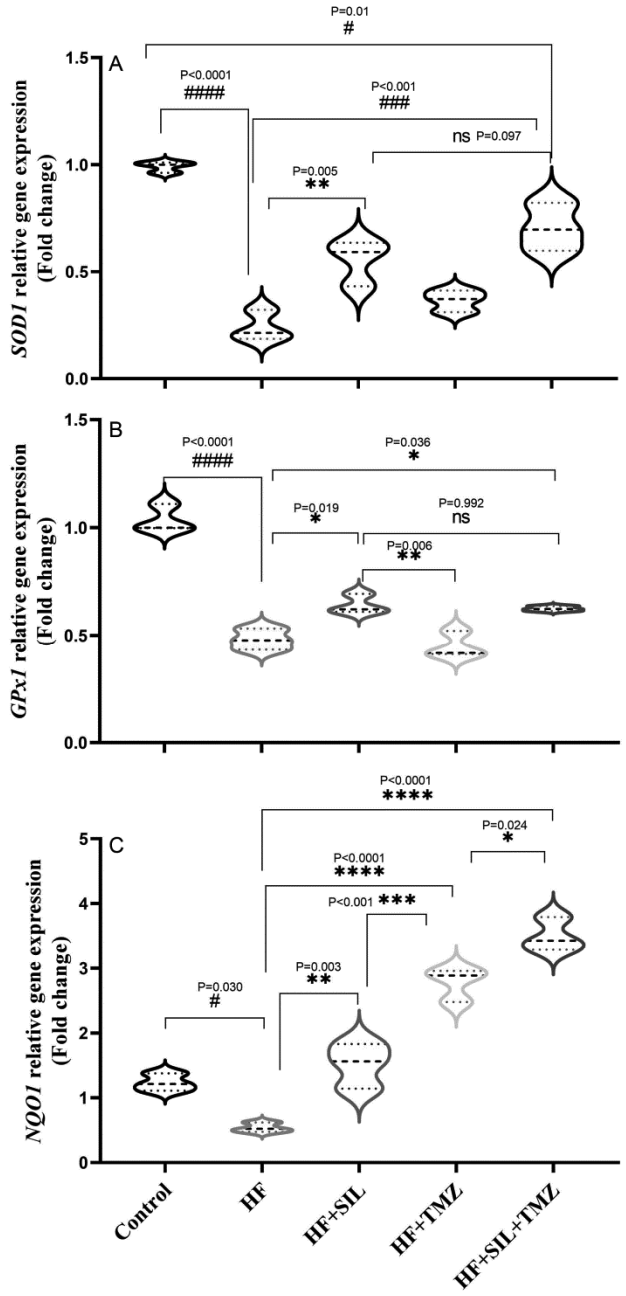

Fig. 5 — The effect of administration of silymarin (SIL), trimetazidine (TMZ) and TMZ+SIL on the serum levels of IL-1β (A), IL-6 (B) and TNF-α (n=5). 130 mg/kg/day isoproterenol for 2 consecutive days was used to induce HF. Rats received 100 mg/kg SIL i,p. and 10 mg/kg TMZ orally for 2 weeks every day after HF induction, and the serum levels of IL-1β, IL-6 and TNF-α were measured one day after receiving the last dose.

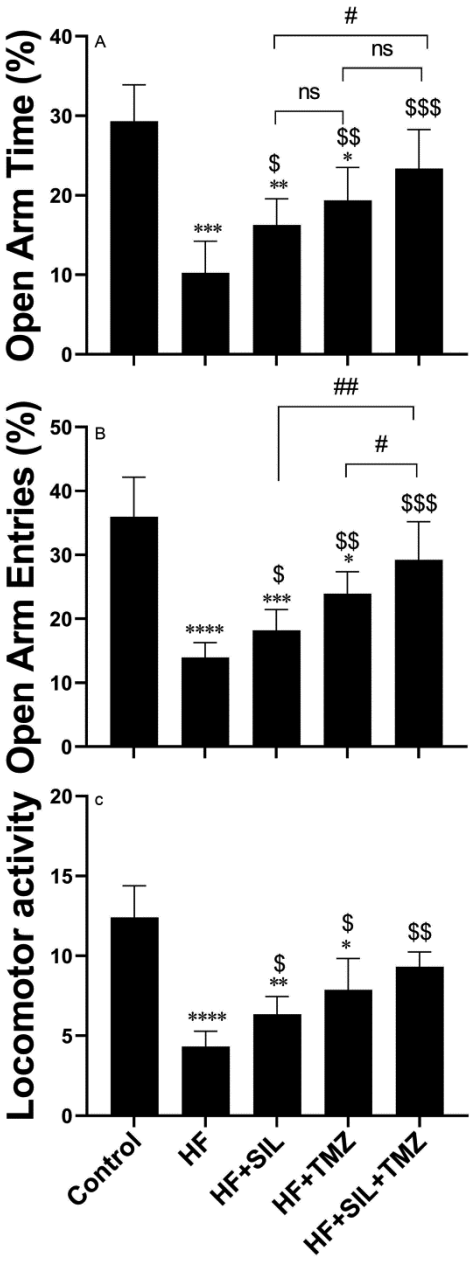

Fig. 6 — The relative expressions of super oxide dismutase 1 (*SOD1*, A), glutathione peroxidase 1 (*GPx1*, B) and NAD(P)H dehydrogenase [quinone] 1 (*NQO1*, C) in rats receiving silymarin (SIL), trimetazidine (TMZ) and TMZ+SIL. 130 mg/kg/day isoproterenol for 2 consecutive days was used to induce HF. Rats received 100 mg/kg SIL i,p. and 10 mg/kg TMZ orally for 2 weeks every day after HF induction, and the relative expression levels of aforementioned genes were measured one day after receiving the last dose by RT-PCR technique.

levels of Nrf2 in HF rats receiving SIL, TMZ and SIL+TMZ were not only significantly higher than HF rats, but also significantly higher than healthy control rats (Fig. 7).

Discussion

The findings of the present research indicated the cardioprotective effects of silymarin in HF condition and it significantly improved EF and FS in HF rats. In

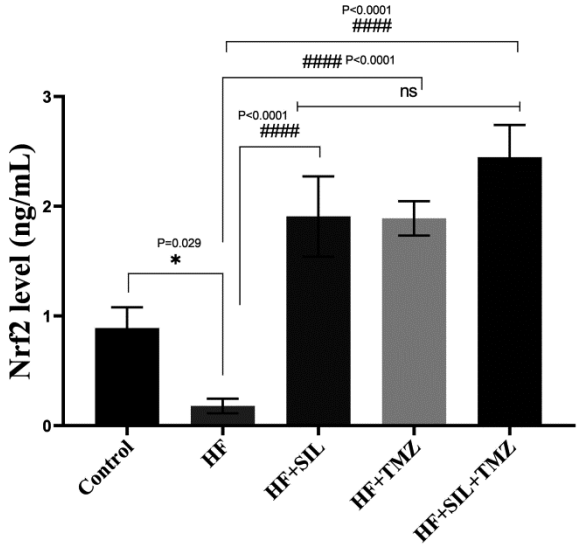

Fig. 7 — The effect of administration of silymarin (SIL), trimetazidine (TMZ) and TMZ+SIL on the plasma level of Nuclear factor erythroid 2-related factor 2 (Nrf2) (n=5). 130 mg/kg/day isoproterenol for 2 consecutive days was used to induce HF. Rats received 100 mg/kg SIL i,p. and 10 mg/kg TMZ orally for 2 weeks every day after HF induction, and the plasma level of Nrf2 was measured one day after receiving the last dose.

particular, the administration of SIL together with TMZ led to the improvement of echocardiographic EF parameters in rats, which indicates the potential of SIL as an adjunctive therapy in the treatment of HF. Furthermore, SIL, TMZ and SIL+TMZ showed protective effects against HF-induced anxiety and depression in EPM and SPT tests. Importantly, SIL was able to prevent the increase of BNP and Galectin-3 induced by HF and showed the anti-inflammatory effects by significantly reducing the serum levels of inflammatory cytokines such as IL-1β, IL-6 and TNF-α in HF rats compared to positive control rats (HF). Interestingly, the activation of the Nrf2 pathway by inducing the expression of *SOD1*, *GPx1* and *NQO1* genes was identified as the action mechanism of the cardioprotective effects of SIL in HF disease.

Decreases in both EF and FS can indicate heart failure, and in the present study, ISO administration was associated with a decrease in both echocardiographic parameters, which indicates that ISO is effective in inducing HF in animal models. Nevertheless, in the present study, it was found that SIL, TMZ and their combined treatment can be effective in preventing EF and FS reductions, which indicates the cardioprotective effects of these compounds. SIL has been shown to have cardioprotective effects in studies. For example, administration of 500 mg/kg SIL to myocardial infarction model rats resulted in an approximately 5-fold reduction in infarct size compared to control[13]. Recently, a systematic review found that SIL can prevent doxorubicin-induced cardiotoxicity and exert cardioprotective activities through anti-apoptotic, anti-inflammatory, and antioxidant effects[7].

BNP has potential as a biomarker in the diagnosis of HF[14] and is considered as an indicator for the prognosis of clinical outcomes and mortality in HF patients[15]. It has been found that HF is associated with increased plasma levels of BNP, and administration of BNP-reducing drugs is associated with a reduction in patient mortality[16]. For this reason, the plasma levels of BNP were measured in the present study and the results indicated a significant increase in HF rats compared to the control group. Importantly, both SIL and TMZ were associated with decreased BNP levels, indicating their therapeutic potential in HF. The significant reduction of BNP plasma levels in HF rats by SIL and TMZ can explain the improvement of EF and FS parameters after administration of both of them to HF rats. In addition, the pathophysiology of HF involves multiple factors, including severe oxidative stress and exacerbation of inflammatory responses, which are accompanied by upregulation of BNP[17], and it seems that SIL, with its antioxidant and anti-inflammatory properties shown in the present study, is able to prevent HF-induced oxidative stress and inflammation and subsequently reduce BNP levels.

Galectin-3 is one of the main regulators of inflammation and has been found to play a pivotal role in the pathophysiology of HF and its progression[18]. Overexpression of this factor is associated with the development of fibrosis in the heart and is considered a prognostic indicator for death or hospitalization in HF patients[19]. In the present study, induction of HF with ISO was associated with up-regulation of Galectin-3, which is similar to the findings of other studies[19]. Importantly, both SIL and TMZ were associated with a significant decrease in Galectin-3 expression levels in HF rats, suggesting that their protective effects are mediated through an effect on the expression of this protein. Decreased Galectin-3 expression levels by SIL have also been reported in other studies. For example, Karam *et al*. reported a decrease in the levels of this protein in liver injury model rats following the administration of silymarin, which was associated with a decrease in liver fibrosis[20]. Similarly, SIL was able to reduce liver fibrosis by reducing Galectin-3

levels in a rat liver fibrosis model[21]. In the present study, a decrease in the levels of this protein was observed after the treatment of HF rats with silymarin, which is similar to the findings of the aforementioned studies. Therefore, the reduction of Galectin-3 levels following the administration of SIL can be associated with cardioprotective effects and improvement of echocardiographic parameters. Furthermore, a decrease in Galectin-3 levels may indicate a reduction in cardiac fibrosis in HF, and several studies have reported anti-fibrotic effects of SIL in various tissues[20]. It is worth noting that there is a direct relationship between Galectin-3 levels and HF as well as cardiac fibrosis[22], and targeting it is considered as a therapeutic target for HF[23]. Therefore, SIL, which was able to reduce Galectin-3 levels in HF rats in this study, could be considered as an adjuvant therapy option in HF.

Heart failure is associated with severe inflammatory responses that are associated with increased production of inflammatory cytokines[24]. Therefore, reducing inflammation should be considered in the treatment of this disease[25]. In this study, the serum levels of inflammatory cytokines IL-1β, IL-6 and TNF-α were measured and the results indicated their overproduction in HF rats, which indicates the occurrence of inflammatory responses and is similar to the findings of other studies in this field[26]. Nevertheless, the administration of SIL, TMZ and SIL+TMZ significantly prevented the overproduction of all three studied cytokines, indicating the anti-inflammatory effects of SIL and TMZ. The anti-inflammatory effects of SIL and TMZ have been reported in various studies[27-30], and the findings of the current research confirm the anti-inflammatory effects of these drugs.

In the present study, it was observed that HF induction is associated with the downregulation of factors involved in the Nrf2 pathway, such as *SOD1*, *GPx1* and *NQO1* genes. Nrf2 is a transcription factor that activates genes related to antioxidant and anti-inflammatory defense, whose overexpressions are associated with improving the antioxidant and anti-inflammatory status of the cell[31]. Therefore, the decrease of *SOD1*, *GPx1* and *NQO1* genes after HF induction could be caused by the decrease in the expression levels of the transcription factor Nrf2, which indicates the development of oxidative stress and inflammation. However, administration of SIL, TMX, and SIL+TMX was associated with upregulation of Nrf2, which was associated with overexpression of all three genes, *SOD1*, *GPx1*, and *NQO1*. This can be attributed to the antioxidant and anti-inflammatory effects of both SIL and TMZ[32], and free radical scavenging activity of SIL has been reported in various studies[33]. Both TMZ and SIL were identified as the Nrf2 pathway activators[34], and current study results conformed this property. It is worth noting that the development of HF can be associated with the activation of other molecular pathways, such as TGF-β/Smad3, which is activated by high oxidative stress[35] and the NF-κB signaling pathway[36], which is characterised by high inflammation. Therefore, it is possible that these pathways are involved in the cardioprotective effects of silymarin observed in this study, and it is recommended that future studies investigate these signaling pathways. Nevertheless, improving antioxidant status and reducing inflammation can justify the cardio-protection and echocardiographic parameters improvement effects of SIL. Therefore, SIL with cardioprotective effects has the potential to be administered in HF patients. It is worth noting that the present study was conducted using animal model of HF, which requires caution in generalizing the findings to clinical settings. Although this study attempted to use a large sample size (n=10, each group), further studies are warranted, especially in the area of the effect of SIL on various signaling pathways in the HF model. Clinical studies are strongly recommended.

Conclusion

This study found that silymarin was able to improve antioxidant status, reduce inflammation, and consequently improve cardiac parameters in HF rats. Overall, it is concluded that silymarin has the potential to be used as an adjunctive therapy in heart failure disease and the cardioprotective effects of this natural compound are mediated by antioxidant, anti-inflammatory activities, reduction of BNP and Galectin-3 plasma levels. Clinical studies investigating the effect of silymarin administration to heart failure patients are recommended.

Conflict of interest

The authors declare no competing interests.

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