



Research article

Cardioprotective effect of taurine and β -alanine against cardiac disease in myocardial ischemia and reperfusion-induced rats



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ABSTRACT

Background: The present study analyzed the synergistic protective effect of β -alanine and taurine against myocardial ischemia/reperfusion. Myocardial infarct size, lipid peroxidation, and levels of glutathione peroxidase (Gpx), superoxide dismutase (SOD), reduced glutathione (GSH), catalase, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), reactive oxygen species (ROS), apoptosis, and the mRNA and protein expression of Janus kinase 2 (JAK2) and signal transducer and activator 3 of transcription (STAT3) were determined. The molecular docking was carried out by using AutoDock 4.2.1.

Results: Combined treatment with β -alanine and taurine reduced myocardial infarct size, lipid peroxidation, inflammatory marker, ROS levels, and apoptosis and increased Gpx, SOD activity, GSH, and catalase activity. Furthermore, combined treatment significantly reduced JAK2 and STAT3 mRNA and protein expression compared with the control. The small molecule was docked over the SH2 domain of a STAT3, and binding mode was determined to investigate the inhibitory potential of β -alanine and taurine. β -Alanine bound to SH2 domain with ΔG of -7.34 kcal/mol and KI of 1.91 μ M. Taurine bound to SH2 domain with ΔG of -7.38 kcal/mol and KI of 1.95 μ M.

Conclusion: Taken together, these results suggest that the combined supplementation of β -alanine and taurine should be further investigated as an effective therapeutic approach in achieving cardioprotection in myocardial ischemia/reperfusion.

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1. Introduction

Myocardial ischemia and reperfusion are known to play a key role in the development of myocardial infarction [1]. Ischemia arises from blood flow reduction to the myocardium, as it occurs in pathological conditions such as thrombosis, atherosclerosis, and vascular spasm [2]. The imbalance between oxygen demand and supply leads to damage in cardiac tissues and necrosis [3]. Increased levels of reactive oxygen species (ROS) and tissue damage resulting from thrombosis become evident during reperfusion [4]. Thus, inhibiting ROS production and enhancing antioxidant levels may be an effective therapeutic approach for the prevention and management of myocardial ischemia/reperfusion-induced damage [5].

β -Alanine is a nonessential, nonproteogenic amino acid formed as the end product of carnosine, balenine, anserine, and dihydrouracil synthesis in humans and animals. β -Alanine is a well-known

ingredient in sports supplements, based on its ability to increase anaerobic endurance and athletic performance, and is a rate-limiting precursor of carnosine [6,7]. In addition, β -alanine plays a central role in intracellular buffering and delays the accumulation of lactic acid [8]. The anticancer activity of β -alanine has been demonstrated in several studies [9,10].

Taurine is an essential non-proteogenic amino acid and a nutritional source supporting brain cell growth, development, and differentiation [11,12]. Abdel-Daim et al. [13] have reported the hepatorenal protective impacts of taurine and N-acetylcysteine against fipronil-induced injury in rats. Abdel-Daim et al. [14] have reported the protective effect of allicin against doxorubicin-induced cardiotoxicity in rats. Abdel-Daim et al. [15] have reported the protective effect of lycopene against tulathromycin and diclofenac sodium-induced cardiotoxicity in the mice model. Neuroprotective and cardioprotective effects of taurine have been reported [16,17]. Researchers have reported the cardioprotective effect of phytochemicals against doxorubicin-induced cardiotoxicity [3]. Abdel-Daim et al. [18] have reported the protective effect of ascorbic acid

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and mirazid against tilmicosin-induced cardiotoxicity in mice. Researchers have reported the importance of antioxidants in the prevention of myocardial infarction and coronary heart disease [19,20]. Taurine was also shown to act as an antioxidant against cadmium-induced oxidative renal dysfunction [21]. In addition, taurine protects against harmful effects of apolipoprotein B100 and lipid secretion in cancer [22]. Thus, we evaluated the synergistic protective effects of β -alanine and taurine in a rat model of myocardial ischemia/reperfusion.

2. Materials and methods

2.1. Materials

β -Alanine and taurine were purchased from Sigma-Aldrich (Shanghai, China). Primers were synthesized by Macrogen (China). Primary antibodies against Janus kinase 2 (JAK2; ab39636) and signal transducer, and activator 3 of transcription (STAT3; ab5073) were purchased from Abcam (UK).

2.2. Rats

Male albino Wistar rats weighing 180–200 g were purchased from the Animal House of The Fourth Hospital of Harbin Medical University, China, for use in this study. The rats were kept in polypropylene cages under standard conditions at a temperature of $25 \pm 0.5^\circ\text{C}$, relative humidity of $61 \pm 4\%$, and a standard light photoperiod (12 h light/12 h dark). All animals were handled according to internationally accepted ethical committee procedures (Medical Ethics Committee of The Fourth Hospital of Harbin Medical University, China, 2019–20).

2.3. Ischemia/reperfusion model

The rat model of acute myocardial ischemia/reperfusion was established as previously described [23]. Briefly, rats were anesthetized by the administration of chloral hydrate (5%). Then, rats were placed in the lateral position, and a tube was inserted into the trachea. Then, a proper ventilation was provided to rats at a rate of 105 cycles/min. The chest of rats was opened with the upper margin of third rib. Then, the coronary artery occlusion in rats lasted for 30 min, and the chest was closed. Finally, the rats were removed from the experimental setup and kept warm.

2.4. Groups and treatment

Male rats were grouped into five groups ($n = 6$ each): group I: sham; group II: control; group III: 100 mM β -alanine; group IV: 100 mM taurine; and group V: 100 mM β -alanine + 100 mM taurine. The dose was administered for 30 consecutive days through the oral gauge.

2.5. Myocardial infarct size determination

Myocardial infarct size was calculated as previously described [24].

2.6. Determination of biochemical marker levels

Lipid peroxidation, glutathione peroxidase (Gpx), superoxide dismutase (SOD) activity, reduced glutathione (GSH), and catalase activity levels were measured as previously described [25].

2.7. Determination of inflammatory markers

Serum tumor necrosis factor (TNF)- α and interleukin-6 (IL-6) levels were determined as previously described [26].

2.8. Determination of intracellular ROS

A heart tissue homogenate was prepared and stained with 2',7'-dichlorodihydrofluorescein diacetate in the dark for 45 min at room temperature. Samples were then viewed for fluorescent intensity under a fluorescence microscope for ROS determination [27].

2.9. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

Heart tissues were fixed in formaldehyde, embedded in paraffin, deparaffinized, and sliced into 4 μm -thick sections. The sections were treated with 4% H_2O_2 for 10 min and stained, and the images were viewed under a confocal microscope [Olympus, Japan; 28].

2.10. Reverse transcription polymerase chain reaction (RT-PCR)

The cDNA was prepared using oligo (dT) primers from total RNA isolated from tissue homogenates. Primers specific for JAK2 and STAT3 were used to perform qRT-PCR (Table 1). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as internal control. Relative expression ratios were calculated as previously described [29].

2.11. Immunohistochemistry

Formaldehyde-fixed cardiac tissues were embedded in paraffin, deparaffinized, and sliced into 4- μm -thick sections. The sections were treated with 4% H_2O_2 for 10 min and then stained overnight with the anti-JAK2 and anti-STAT3 antibodies described above. After that, the cells were incubated with fluorescein isothiocyanate-conjugated goat anti-rat antibody (ab6840, Abcam) for 1 h [30] and analyzed under a confocal microscope.

2.12. Molecular docking studies

The docking parameter and grid were prepared by AutoDockTools. The molecular docking was carried out by using AutoDock 4.2.1. The grid box ($80 \times 80 \times 80 \text{ \AA}$) with spacing (0.375 \AA) was selected, which includes the SH2 dimerization domain of STAT3. The one hundred independent docking runs were carried out for each molecule. The PyMOL 0.99 was used for analyzing docking results [31].

2.13. Statistical analysis

All experimental data are reported as means \pm standard deviation. Comparisons were made using analysis of variance followed by

Table 1

List of real-time polymerase chain reaction primers used for the amplification of JAK-2 STAT3.

S.NO	Gene name	Forward primer	Reverse primer
1	JAK2	5'-TTTGAAGACAGGGACCCTACACAG-3'	5'-TCATAGCGGCACATCTCCACA-3'
2	STAT3	5'-TGGAAGAGGCGGCAGCAGATAGC-3'	5'-CACGGC CCCCATTCCACAT-3'
3	GAPDH	5'-GGTACCAGGGCTGCTTTT-3'	5'-ATCTCGTCTCGGAAGATGGT-3'

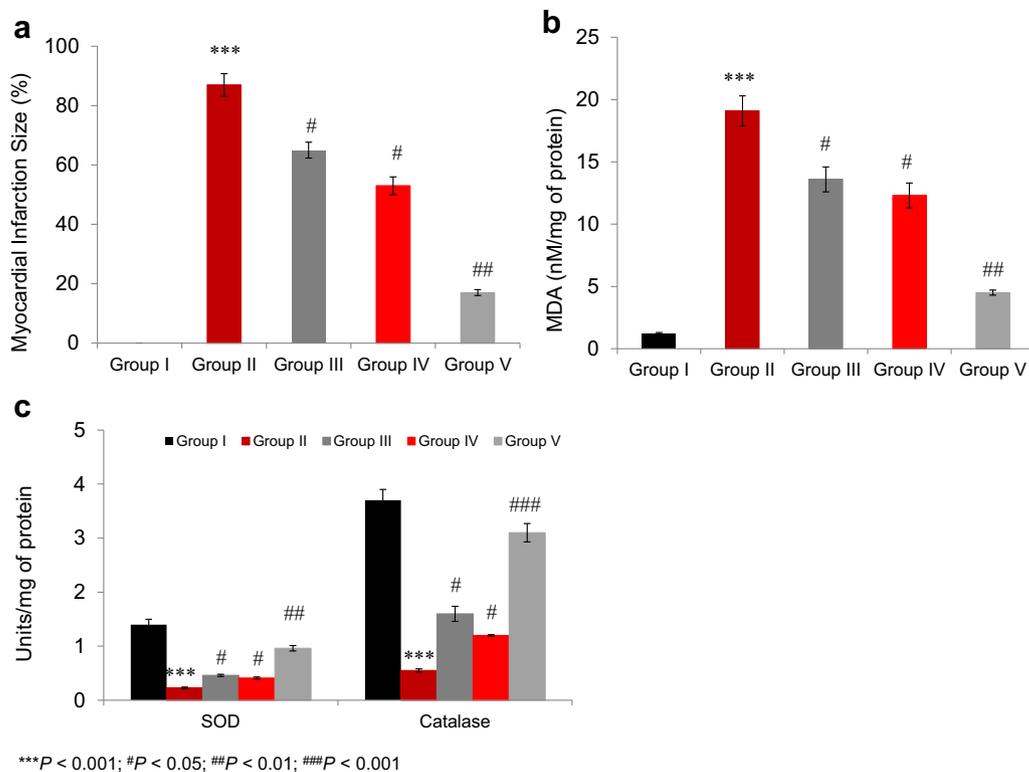


Fig. 1. Synergistic neuroprotective effect of β -alanine and taurine on infarction size (a), lipid peroxidation (b), and SOD and catalase activities (c) in a rat model of myocardial ischemia/reperfusion. * $P < 0.05$ vs. sham and # $P < 0.05$ vs. control, $n = 6$.

Tukey's post hoc test. A P value < 0.05 was considered to indicate statistical significance.

3. Results

The myocardial infarction size was substantially larger in the control than in the sham group (Fig. 1a, $P < 0.05$). In rats treated with β -alanine or taurine, the infarction size was reduced. However, in rats treated with a combination of β -alanine and taurine, the myocardial infarction size was reduced significantly, by 80.4%, compared with the control (Fig. 1a, $P < 0.05$). The Malondialdehyde (MDA) content was substantially increased in control compared with that in sham rats (Fig. 1b, $P < 0.05$). Although the MDA content in cardiac tissues was reduced by β -alanine and taurine when administered individually, combined treatment resulted in a reduction of 76.4% compared with the control group ($P < 0.05$; Fig. 1b). SOD and catalase activities were substantially reduced in the control group when compared with that in the sham group (Fig. 1c, $P < 0.05$). Both β -alanine and taurine increased SOD and catalase activities, but their combined treatment resulted in significant increases in SOD (317.3%) and catalase (463.6%) activities when compared with the control ($P < 0.05$; Fig. 1c) group. GSH and Gpx levels were substantially lower in control than in sham rats (Fig. 2a and b, $P < 0.05$) and were increased in rats treated with β -alanine or taurine. However, combined treatment with β -alanine and taurine significantly increased both GSH (178.6%) and Gpx (188.2%) levels when compared with the control group (both $P < 0.05$, Fig. 2a and b). Tumor necrosis factor- α (TNF- α) and IL-6 levels were substantially higher in the control than in the sham group ($P < 0.05$, Fig. 2c). In β -alanine- or taurine-treated rats, levels of both markers were reduced, whereas the combined treatment resulted in significant decreases in TNF- α (58%) and IL-6 (59.7%) levels compared with the control ($P < 0.05$, Fig. 2c).

Intracellular ROS levels were substantially higher in control than in sham rats ($P < 0.05$, Fig. 3) and were reduced in rats treated with β -

alanine or taurine. However, ROS levels in rats treated with both β -alanine and taurine were significantly reduced (71.8%) compared with control levels ($P < 0.05$, Fig. 3). Apoptosis, as determined by the TUNEL assay, was substantially increased in control compared with sham rats (Fig. 4, $P < 0.05$) and significantly reduced in β -alanine- and taurine-treated rats (75.8%, $P < 0.05$) when compared with control rats (Fig. 4). Similarly, combined β -alanine and taurine treatment significantly reduced JAK2 and STAT3 mRNA and protein expression compared with the control ($P < 0.05$; Fig. 5). The small molecule was docked over SH2 domain of a STAT3, and binding mode was also determined to investigate the inhibitory potential of β -alanine and taurine. β -Alanine bound to SH2 domain with ΔG of -7.34 kcal/mol and KI of 1.91 μM . Taurine bound to SH2 domain with ΔG of -7.38 kcal/mol and KI of 1.95 μM (Table 2).

4. Discussion

The present study evaluated synergistic protective effects of β -alanine and taurine in a rat model of myocardial ischemia/reperfusion injury. As the rate-limiting precursor of carnosine, β -alanine is widely used in sports supplements to increase anaerobic endurance and athletic performance [6,7]. Hoffman et al. [32] have reported the increased military performance following β -alanine supplementation. Smith et al. [33] have reported the antioxidant potential of β -alanine against exercise-induced oxidative stress in women. β -Alanine also exhibits anticancer activity through carnosine formation [9,10]. Taurine has cardioprotective and neuroprotective effects [17], and its acts as an antioxidant in cadmium-induced toxicity. Niu et al. [34] have reported the protective effect of taurine against apoptosis, inflammation, and oxidative stress in brain injury by renormalizing the level of lipid peroxidation, GSH, Gpx, SOD, catalase, TNF- α , and IL-6.

Taurine was also shown to be effective against apolipoprotein B100 and lipid secretion in cancer [22]. Synergism between β -alanine and taurine in significantly reducing lipid peroxidation in aged rats,

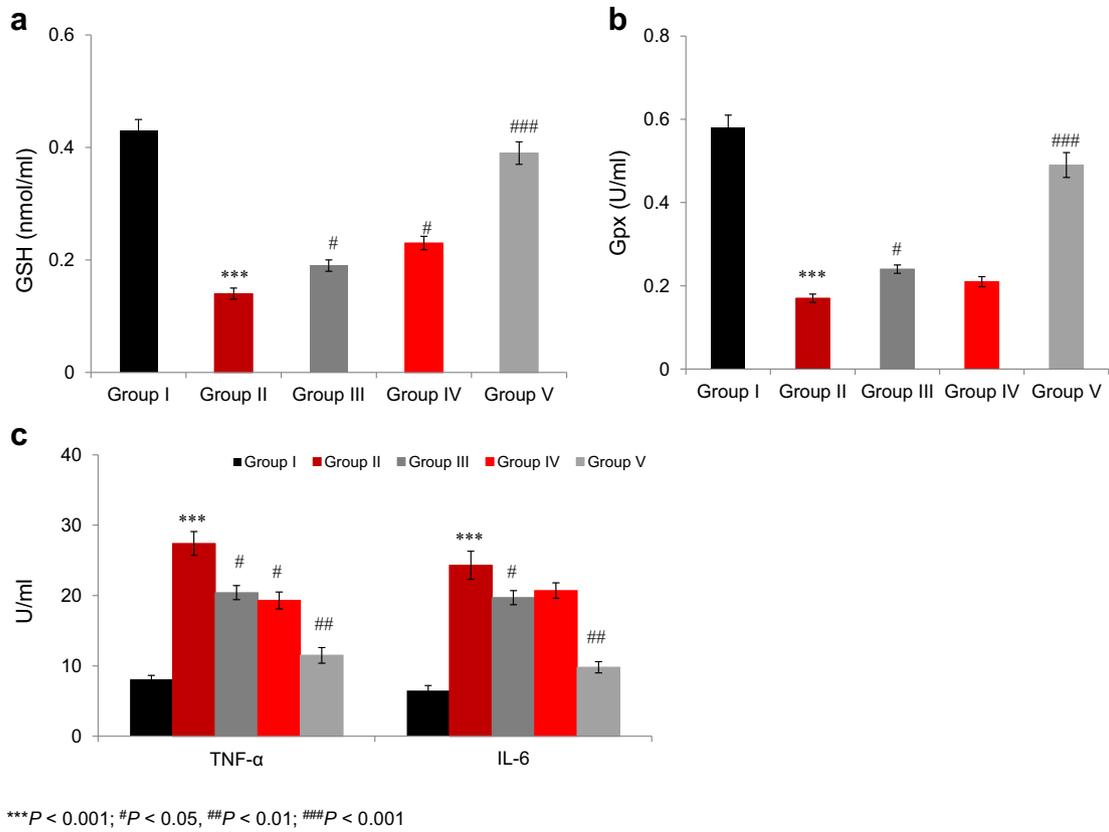


Fig. 2. Synergistic neuroprotective effect of β-alanine and taurine on GSH (a) and Gpx (b), and TNF-α and IL-6 levels (c) in a rat model of myocardial ischemia/reperfusion. **P* < 0.05 vs. sham and #*P* < 0.05 vs. control, n = 6.

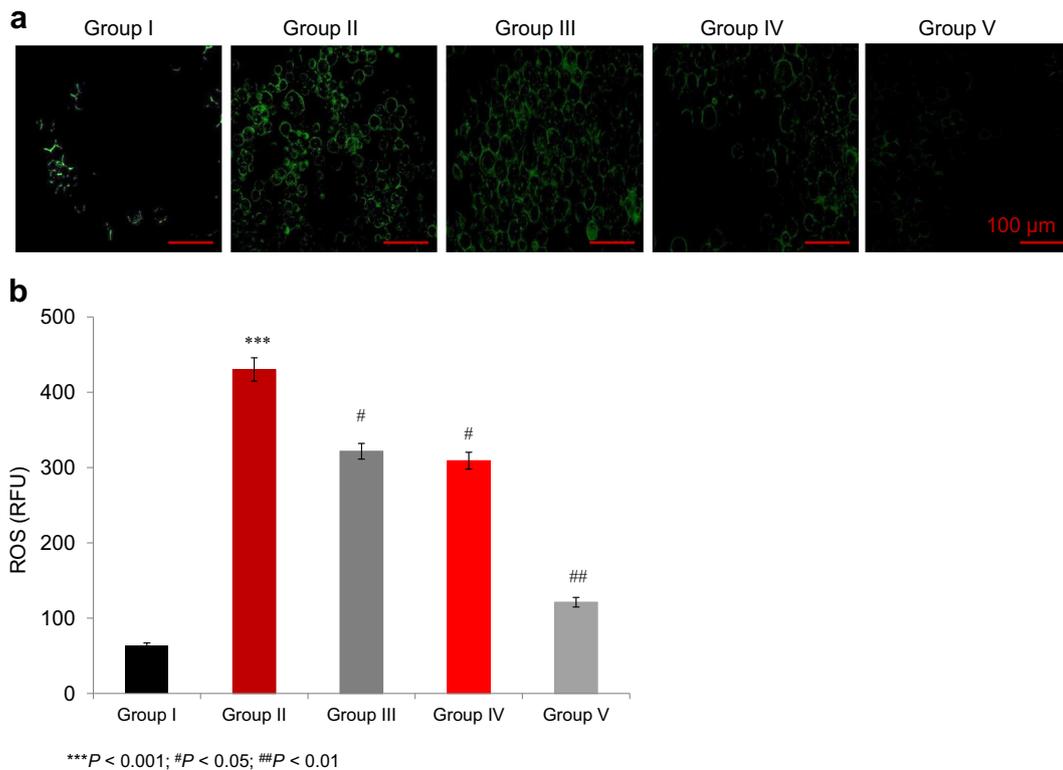


Fig. 3. Synergistic neuroprotective effect of β-alanine and taurine on ROS levels in a rat model of myocardial ischemia/reperfusion. **P* < 0.05 vs. sham and #*P* < 0.05 vs. control, n = 6.

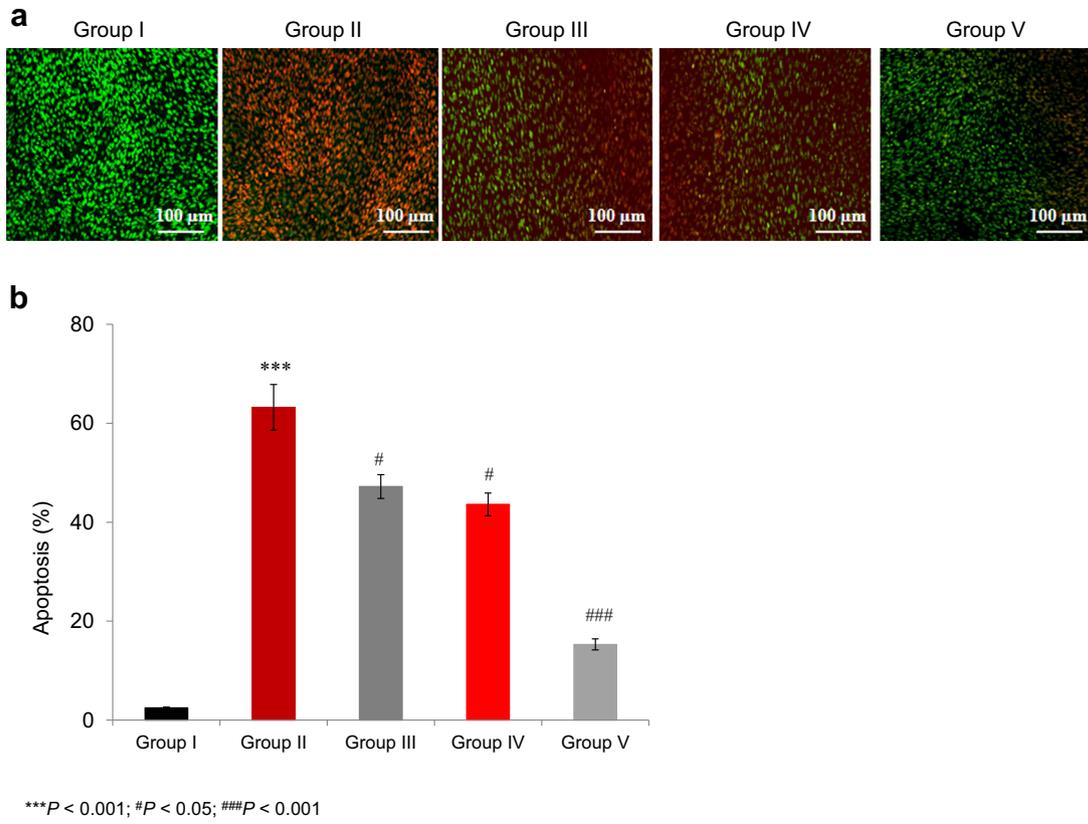


Fig. 4. Synergistic neuroprotective effect of β -alanine and taurine on the apoptosis level in a rat model of myocardial ischemia/reperfusion. * $P < 0.05$ vs. sham and # $P < 0.05$ vs. control, n = 6. Scale bar is 100 μ m.

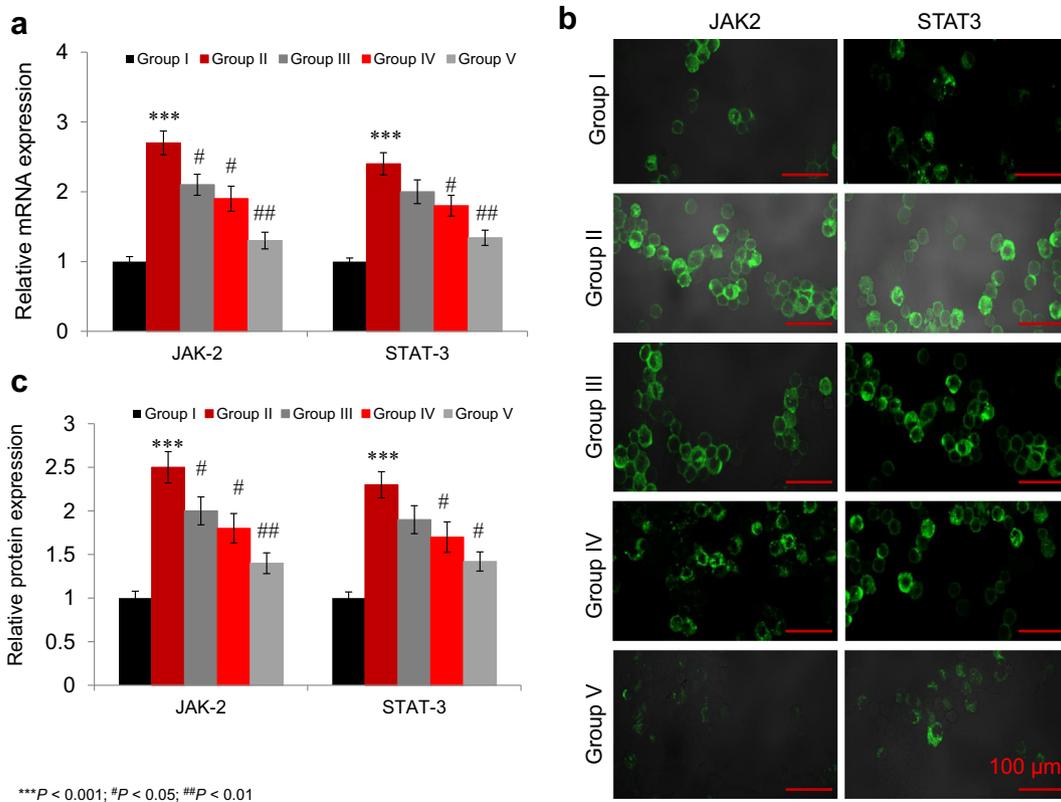


Fig. 5. Synergistic neuroprotective effect of β -alanine and taurine on JAK2 and STAT3 mRNA (a) and protein expression (b), and (c) in a rat model of myocardial ischemia/reperfusion. * $P < 0.05$ vs. sham and # $P < 0.05$ vs. control, n = 6.

Table 2Free energy of binding (ΔG) and the predicted inhibition constant (KI) determined with AutoDock 4.2.1 and interactions of β -alanine and taurine with STAT3.

Name	ΔG (kcal/mol)	KI (μM)	Polar interactions	Hydrophobic residue in 5 Å region
β -Alanine	-7.34	1.91	LYS- 591, ARG-595, GLU-612,SER-613, SER-636	PHE-610, ILE-634,VAL-637
Taurine	-7.38	1.95	LYS- 591, ARG-595, GLU-612,SER-613, SER-636	PHE-610, ILE-634,VAL-637

without affecting the cardiac antioxidant system, was also reported [35]. These results are in agreement with our report showing that β -alanine and taurine supplementation significantly reduced lipid peroxidation and improved antioxidant marker levels to nearly normal levels. In a previous study, the combined supplementation of glutamine and alanine significantly decreased TNF- α and IL-6 levels in rats [36]. This is in agreement with our finding of reduced TNF- α and IL-6 levels in rats with myocardial ischemia/reperfusion injury subsequently treated with both β -alanine and taurine.

Reduced blood flow occurs in thrombosis, atherosclerosis, vascular spasm, and other pathological conditions [2]. Both myocardial ischemia and reperfusion are features of myocardial infarction [1]. The resulting imbalances between oxygen demand and supply lead to necrosis and tissue damage [37]. The higher level of ROS leads to severe tissue damage under reperfusion condition [38]. Pandurangan et al. [39] reported an antitumor effect of β -alanine against cervical and renal tumors through ROS inhibition, whereas taurine supplementation was shown to confer a neuroprotective effect against ROS through NADPH oxidase inhibition [40]. Similarly, in our rat model of myocardial ischemia/reperfusion, the combined supplementation of β -alanine- and taurine-reduced ROS levels.

Our results also suggest the involvement of the JAK2/STAT3-signaling pathway, which was previously shown to play a vital role in cardioprotection. Duan et al. [41] reported that the cardioprotective effect of curcumin against myocardial ischemia/reperfusion was mediated by the inhibition of the JAK2/STAT3-signaling pathway. Kim et al. [42] have reported that taurine chloramine regulates the adipokine expression through downregulation of STAT-3 expression in adipocytes. Our results similarly indicated that the combined supplementation of β -alanine and taurine-reduced JAK2 and STAT3 mRNA and protein expression.

5. Conclusion

The combined supplementation of β -alanine and taurine significantly reduced levels of lipid peroxidation, ROS, inflammatory markers, and the expression of JAK2 and STAT3, whereas GSH, Gpx, SOD, and catalase levels were increased. Taking all these results together, it is suggested that the combined supplementation of taurine and β -alanine could be an effective therapeutic approach in achieving cardioprotection in myocardial ischemia/reperfusion.

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Conflict of interest

The authors declare that they have no conflict of interest.

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