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Original Article

Cardioprotective effects of *Ganoderma lucidum* on isoproterenol–induced heart failure

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ABSTRACT

Introduction: *Ganoderma lucidum (G. lucidum)*, a medicinal mushroom, exerts protective effects on cardiovascular diseases but, it's effect in isoproterenol-induced heart failure has not been studied. Therefore, the aim of the present study was whether *G. lucidum* has protective effects in isoproterenol-induced heart failure.

Methods: Thirty male Wistar rats were assigned into five groups (n=6) of control, heart failure (HF) and *G. lucidum* (50, 100 and 200mg/kg). For induction of HF in rats, isoproterenol (5mg/kg) was injected subcutaneously for two weeks. In *G. lucidum* treated groups, *G. lucidum* was orally gavaged for three weeks and on day 8 isoproterenol was injected for two weeks. Then, Electrocardiogram pattern and cardiodynamic parameters, as well as myeloperoxidase activity, malondialdehyde level, cardiac remodeling and apoptosis were studied.

Results: *G. lucidum* improved hemodynamic factors such as mean arterial blood pressure as well as electrocardiogram pattern. Pre-treatment with *G. lucidum* also decreased myeloperoxidase activity, malondialdehyde level and apoptosis in cardiac tissue. Histopathologic results showed a decrease in cardiac necrosis and fibrosis. However, it had no significant effect on cardiac hypertrophy.

Conclusion: Our results show that pre-treatment with *G. lucidum* demonstrates protective effects against HF, and thereby suggest that *G. lucidum* can be considered as a possible clinical use for preventive and adjuvant treatment in heart failure.

Keywords:
Ganoderma lucidum
Heart failure
Isoproterenol
Inflammation
Oxidative stress
Apoptosis

Introduction

Heart failure (HF) is one of the most important health concerns in the world (Karagöz et al., 2016). Important features of HF, are left ventricular dysfunction, ventricular hypertrophy and remodeling, as well as reduced cardiac output (Johnson, 2014). Moreover, arrhythmias also are associated with heart failure which can be attributed to cardiac structure and fibrosis, as well as changes in membrane excitability (Sedmera et al., 2016).

Oxidative stress has been reported to play a major

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role in heart failure. Reactive oxygen species (ROS) can stimulate cardiac fibroblast cells and activate extracellular matrix metalloproteinases (MMPs), which eventually lead to cardiac myocardial remodeling (Tsutsui et al., 2011) and apoptosis in heart cells (Giordano, 2005). However, suppression of free radical generation could reduce the infarct size and improve cardiac function (Nakamura et al., 2000). As mentioned, apoptosis is involved in the pathophysiology of heart failure and is effective in heart cells damage and cardiac remodeling. Therefore, one of the therapeutic targets in heart failure is to reduce apoptosis (Kim and Kang, 2010). The inflammatory response after myocardial infarction is important for the healing process in the cardiac tissue, but excessive inflammatory response is harmful and also plays an important role in left ventricular remodeling which ultimately, can lead to chronic heart failure. Neutrophils directly damage cardiomyocytes by secreting various substances such as myeloperoxidase. Myeloperoxidase as a marker of neutrophil activity is reported as a biomarker of inflammation in cardiovascular diseases (Jefferson and Topol, 2005; Loria et al., 2008; Anzai, 2013).

Medicinal fungi have long been used to improve health status and treat various disorders. One of these fungi is *Ganoderma lucidum (G. lucidum),* known as a medicinal fungus for the treatment of various disorders (Bishop et al., 2015). This fungus is very popular in traditional medicine and reduces the risk of cardiovascular disease, cancer and also strengthens the immune system (Wachtel-Galor et al., 2004). The *G. lucidum*, through its antioxidant activity, has been shown to protect the heart from acute ethanol-induced cardiac toxicity (Wong et al., 2004) and shows cardioprotective effects in type 2 diabetic rats (Xue et al., 2010). Additionally, triterpene acids and sterols that isolated from *G. lucidum* demonstrated anti-inflammatory and anti-tumor effects (Akihisa et al., 2007). Some research has reported that *G. lucidum* through a reduction in apoptosis, oxidative stress and inflammation can be considered a protective agent against doxorubicin-induced cardiotoxicity (Xu et al., 2017; Veena et al., 2018). Since oxidative stress and inflammation play an important role in the pathophysiology of heart failure and the reducing effects of *G. lucidum* have been reported on these two factors, it was hypothesized that *G. lucidum* can play a protective role in heart failure and it can be used for prevention and adjuvant treatment of heart failure. Therefore, this study was designed to evaluate the effects of *G. lucidum* on electrocardiogram pattern and cardiodynamic parameters, as well as myeloperoxidase activity, malondialdehyde level, cardiac remodeling and apoptosis in isoproterenol-induced heart failure model in rats.

Material and methods

Animals

Thirty male Wistar rats weighting (250±30g) were used in this study. The animals were kept in polycarbonate cages in an environmentally controlled condition (22±2°C with 50±10% relative humidity and a 12h light/12h dark cycle) with free access to food and water. During the present study all procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, 8th Edition, 2011). All animal protocols were reviewed and approved by the Ethics Committee of Urmia University of Medical Sciences. (details omitted for double-blind reviewing) (Code of Ethics: IR.UMSU.REC.1397.330).

Experimental design

In this study, the animals were randomly divided into five groups consisting of 6 rats in each group (Jannesar et al., 2020). Rats in Group-I (Control) received normal saline for three weeks and on day 8, normal saline (0.5ml) was injected subcutaneously (SC*)* for two weeks. Group-II (HF): Rats were received normal saline for three weeks and on day 8, isoproterenol (5mg/kg) was injected SC for two weeks. Rats in groups III, IV and V were treated orally with *G. lucidum* (50, 100 and 200mg/kg, respectively) for three weeks and on day 8, isoproterenol (5mg/kg; SC) was injected for two weeks. Heart failure was induced by daily isoproterenol administration (5mg/kg, SC) for two weeks. Normal saline was used to dissolve isoproterenol (Sigma Chemicals Co. USA, cat no: I5627) and injected based on body weight. Isoproterenol, a non-selective β adrenoceptor agonist, induces loss of cardiac myocytes, cardiac hypertrophy associated with arrhythmias and fibrosis which can progress heart failure disease (Abbaszadeh et al., 2018; Benjamin et al., 1989). All treatments were administered orally and started one week before induction of heart failure. The duration of the study was 21 days and the dose of *G. lucidum* (Iran ganoderma com-

pany, Iran) was selected based on our pilot study.

Hemodynamic assessment

On day 22, the animals were anesthetized with ketamine and xylazine (Alfasan, Netherlands) with doses of 60 and 10mg/kg respectively, and limb lead II electrocardiography was performed to detect myocardial ischemia. Then, invasive hemodynamic was measured as previously described (Soraya et al., 2012). Briefly, the left carotid artery cannulated with a polyethylene tube and mean arterial pressure (MAP), arterial blood pressure, developed pressure and heart rate were recorded continuously after stabilization period. Electrocardiography (ECG) and pressure signals were measured by a Power lab system (AD Instruments, Australia).

Myeloperoxidase assay

Activity of myeloperoxidase (MPO) in the myocardial tissue was measured as previously described (Mullane et al., 1985; Rameshrad et al., 2016), with slight modification. Briefly, heart tissues were homogenized in 0.5% hexadecyl-trimethyl ammonium bromide prepared in potassium phosphate buffer (PH=6, 5mM; Merck Millipore). The homogenates were frozen immediately and three freeze and thaw cycles were done. After that, the samples were centrifuged at 4000rpm for 45min and 100µl of supernatant was mixed with 2.9ml of O-dianisidine dihydrochloride (0.167mg/ml) and 0.0005% H_2O_2 prepared in phosphate-buffered saline. Then, the reaction was stopped by adding 100µl of 1.2M HCl (Merck Millipore) after 5min and the absorbance was measured at 460nm. The concentrations were calculated by calibration curves and the activity of MPO were reported as units of MPO in milligram tissue (U/mg tissue).

Malondialdehyde assay

Cardiac tissues were removed and homogenized in 1.5% potassium chloride to make a 10% homogenate. Then, 0.25ml of supernatant was combined with 3ml of phosphoric acid 1%. Then 1ml of thiobarbituric acid was added and the mixture was heated for 45min at 90°C. After cooling, 3ml of n-butanol was added and centrifuged at 3000rpm for 15min. The absorbance change of butanol phase was measured at 532nm. The results were reported as nanomol/mg protein (Young and Trimble, 1991).

Tissue weights

After hemodynamic assessment, the rat's chest was opened and the heart was rapidly excised and weighted. The wet heart weight to body weight ratio was calculated to assess the degree of cardiac hypertrophy (Soraya et al., 2012).

Histopathological examinations

At the end of experiment and after weighting of heart, the cardiac apex was placed in 10% formalin. After cutting the heart into 5µm sections, hematoxylin and eosin and Gomori trichrome staining were used to evaluate myocardial necrosis and fibrosis, respectively. Two trained persons (at least one pathologist) were quantified histological changes by scoring and given a number for each change as follows: 1, 2, 3 and 4 for low, moderate, high and intensive pathological changes, respectively (40x magnification) (Abbaszadeh et al., 2018).

Quantitative real-time PCR (Q-PCR) analysis

We used Q-PCR for the monitoring of the transcription levels of Bax and Bcl-2 genes involved in apoptosis. RNA isolation kit (Yekta Tajhiz Azma, Iran) was used for total RNA extraction from different groups. Total RNA concentration was quantified by a spectrophotometer (eppendorf) instrument, then cDNA synthesis kit (cat: A101161; Parstous, Iran) was used for synthesis of cDNA. The mRNA levels of Bax and Bcl-2 genes were measured by MIC Real time PCR system (LABGENE) using a SYBR Green dye-based PCR Master Mix (Yekta Tajhiz Azma, Iran). Results were normalized against the housekeeping GAPDH gene and measurable calculations were prepared by applying formula 2-ΔΔCT analysis. The list of the primers is available in Table 1 (Ikeguchi et al., 2002).

Statistical analysis

All data are expressed as mean± SEM. Statistical analysis was performed using GraphPad Prism 8. Comparisons between groups were made using one-way analysis of variance (ANOVA) and if there were a significant difference, Tukey post-test was performed. Differences between means were considered significant at $P<0.05$ (95% confidence interval (CI)).

Results

Pre-treatment with G. lucidum improves electrocar-

TABLE 1: List of primers

Genes	Sequences	Tm (°C)
Bax	F: AGGATCGAGCAGAGAGGATGG R: AGCTCCATGTTGTTGTCCAGT	62
Bcl-2	F:CCGGGAGAACAGGGTATGATAA R: CCCACTCGTAGCCCCTCTG	60
GAPDH	F: ATGATTCTACCCACGGCAAG R:CTGGAAGATGGTGATGGGTT	60

TABLE 2: Effects of *G. lucidum* on hemodynamic parameters and R-amplitude of ECG

Groups	MAP (mm Hg)	ABP (mm Hg)	Heart rate (bpm)	SP (mm Hg)	DP (mm Hg)	R-amplitude (μV)
Control	157±4	170±7.1	221±10.7	169±7.5	149±4.3	0.93±0.04
HF	111±7.6$^{\#}$	120±13^{a}	191±7.7^{a}	126±13.2^{a}	103±5.7$^{\#}$	0.46±0.06$^{\#}$
GL 50 mg/kg	147±6.7**	165±13.7	222±6.3**	179±15*	130±4.4**	0.83±0.07***
GL 100 mg/kg	137±7.8*	157±13.4	220±5**	162±12.6	123±6.8*	0.78±0.05**
GL 200 mg/kg	143±3.6**	157±7.7	213±9.7**	161±9.3	135±2.4***	0.92±0.05***

Data are expressed as mean±SEM (n=6). $^{\#}P<0.001$; $^{a}P<0.05$ vs control group. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ vs HF group using one-way ANOVA with Tukey post test. GL: *Ganoderma lucidum*; HF: Heart failure; MAP: Mean arterial pressure; ABP: Arterial blood pressure; SP: Systolic pressure; DP: Diastolic pressure.

diogram pattern and hemodynamic parameters in heart failure

The pattern of electrocardiogram in control group was normal, whereas HF group demonstrated significant decrease in R-amplitude and ST segment depression (indication of myocardial ischemia). Pre-treatment with *G. lucidum* (50, 100 and 200mg/kg) increased R-amplitude ($P<0.001$, $P<0.01$ and $P<0.001$, respectively) as shown in Table 2 and improved ECG pattern specially at dose of 200mg/kg as can be seen in Figure 1. Overall, it was observed that hemodynamic parameters improved by *G. lucidum* treatment. MAP significantly reduced from 157±4 mmHg in the control group to 111±7.6 mmHg in the HF group. Treatment with *G. lucidum* (50, 100 and 200mg/kg) demonstrated a profound increase in MAP in comparison to HF group ($P<0.01$, $P<0.05$ and $P<0.01$, respectively). Other hemodynamic parameters also improved in *G. lucidum* treated groups as shown in Table 2.

Effects of pre-treatment with G. lucidum on myeloperoxidase activity in heart failure

Myocardial neutrophil infiltration was also investigated in order to determine whether *G. lucidum* is associated with anti-inflammatory effect. It was found that MPO activity (U/mg tissue) increased from 4.34±0.29 in control group to 6.94±0.62 in HF group ($P<0.01$). Pre-treatment with *G. lucidum* at doses of 50 ($P<0.01$), 100 and 200 mg/kg/day ($P<0.001$) which was started 7 days before induction of HF, decreased MPO activity in the cardiac tissue to 4.47±0.44, 3.25±0.37 and 3.5±0.3 respectively (Fig. 2).

Effects of pre-treatment with G. lucidum on lipid peroxidation in heart failure

Malondialdehyde levels in cardiac tissues were measured to determine lipid peroxidation. It was found that malondialdehyde level (nMol/mg of protein) significantly increased from 6.28±0.43 in control group to 22.4±6.3 in HF group ($P<0.01$). Pre-treatment with *G. lucidum* at doses of 50, 100 and 200 mg/kg/day which was started 7 days before induction of HF, reduced malondialdehyde level to 8.6±0.97 ($P<0.05$), 9.39±0.56 ($P<0.05$) and 6.83±0.53 ($P<0.01$) respectively (Fig. 3).

Effects of pre-treatment with G. lucidum on cardiac hypertrophy in heart failure

To determine the cardiac weight gain, the heart weight to body weight ratio was measured. This ratio was increased from 3.06±0.01 in control group to 5.48±0.14 in HF group rats ($P<0.001$). Pre-treatment with *G. lucidum* at all three doses had not significant effect on cardiac hypertrophy (Fig. 4).

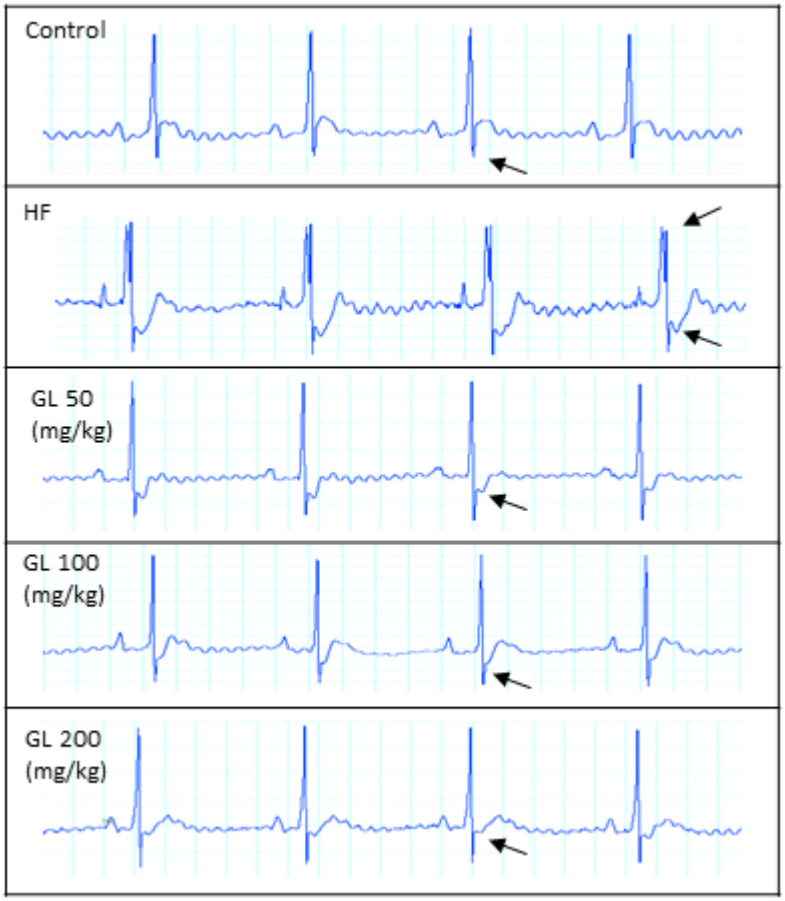

FIGURE 1. Images of electrocardiogram pattern and changes in control, HF and GL treated groups. *GL: Ganoderma lucidum*; HF: Heart failure. Arrows show ST segment depression and R-amplitude suppression.

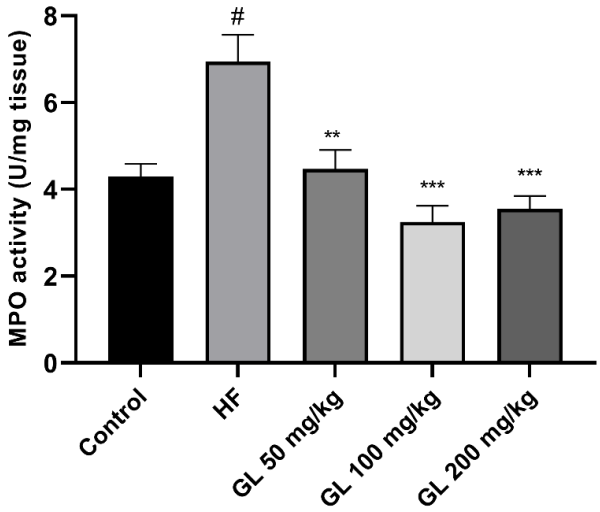

FIGURE 2. The effects of pre-treatment with G. lucidum (50, 100 and 200 mg/kg/day) on myeloperoxidase (MPO) activity in the cardiac tissues. Data are expressed as mean±SEM (n=6). $^{\#}P<0.001$ vs control group. $^{**}P<0.01$ and $^{***}P<0.001$ vs HF group. For analysis, one way ANOVA was used with Tukey post test. GL: Ganoderma lucidum; HF: Heart failure.

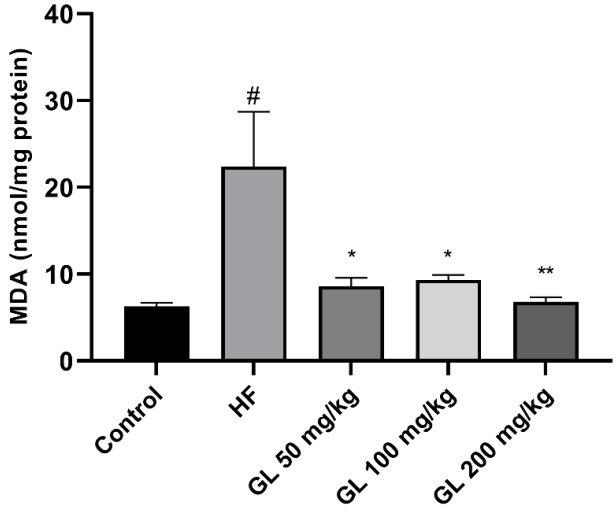

FIGURE 3. The effects of pre-treatment with G. lucidum (50, 100 and 200 mg/kg/day) on malondialdehyde (MDA) level in the cardiac tissues. Data are expressed as mean±SEM (n=6). $^{\#}P<0.01$ vs control group. $^{*}P<0.05$ and $^{**}P< 0.01$ vs HF group. For analysis, one way ANOVA was used with Tukey post test. GL: Ganoderma lucidum; HF: Heart failure.

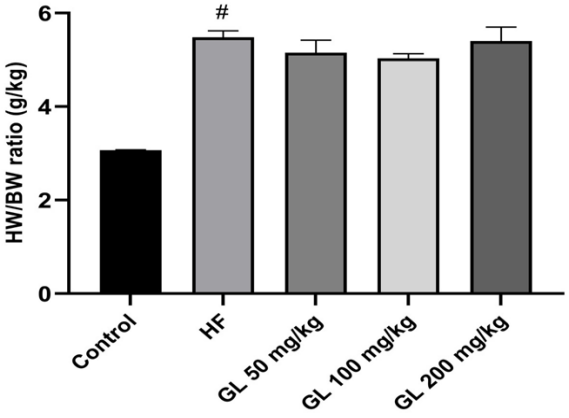

FIGURE 4. The effects of pre-treatment with G. lucidum (50, 100 and 200 mg/kg/day) on heart weight to body weight ratio in heart failure. Data are expressed as mean±SEM (n=6). #P<0.001 vs control group. One way ANOVA was performed with Tukey post test. GL: Ganoderma lucidum; HF: Heart failure.

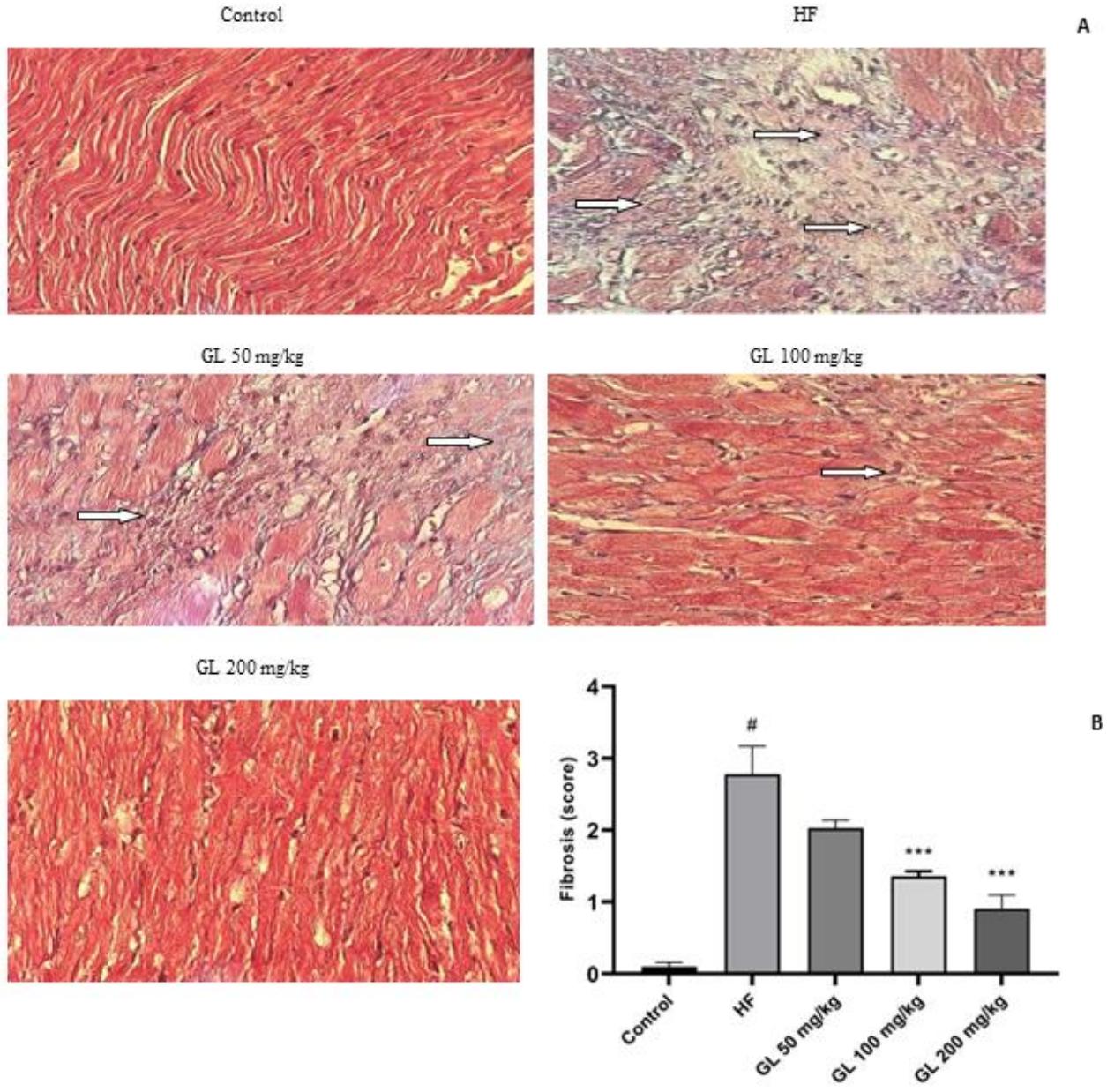

FIGURE 5. (A) Heart tissue of a rat with HF shows intensive cardiomyocyte fibrosis. G. lucidum treatment showed significant reduction in fibrosis. (B) Grading of histopathological changes in the rat's cardiac apex tissues. Data are expressed as mean±SEM. $^{\#}P<0.001$ vs control group. $^{***}P<0.001$ vs HF group using one way ANOVA with Tukey post test (n=6). GL: *Ganoderma lucidum*; HF: Heart failure.

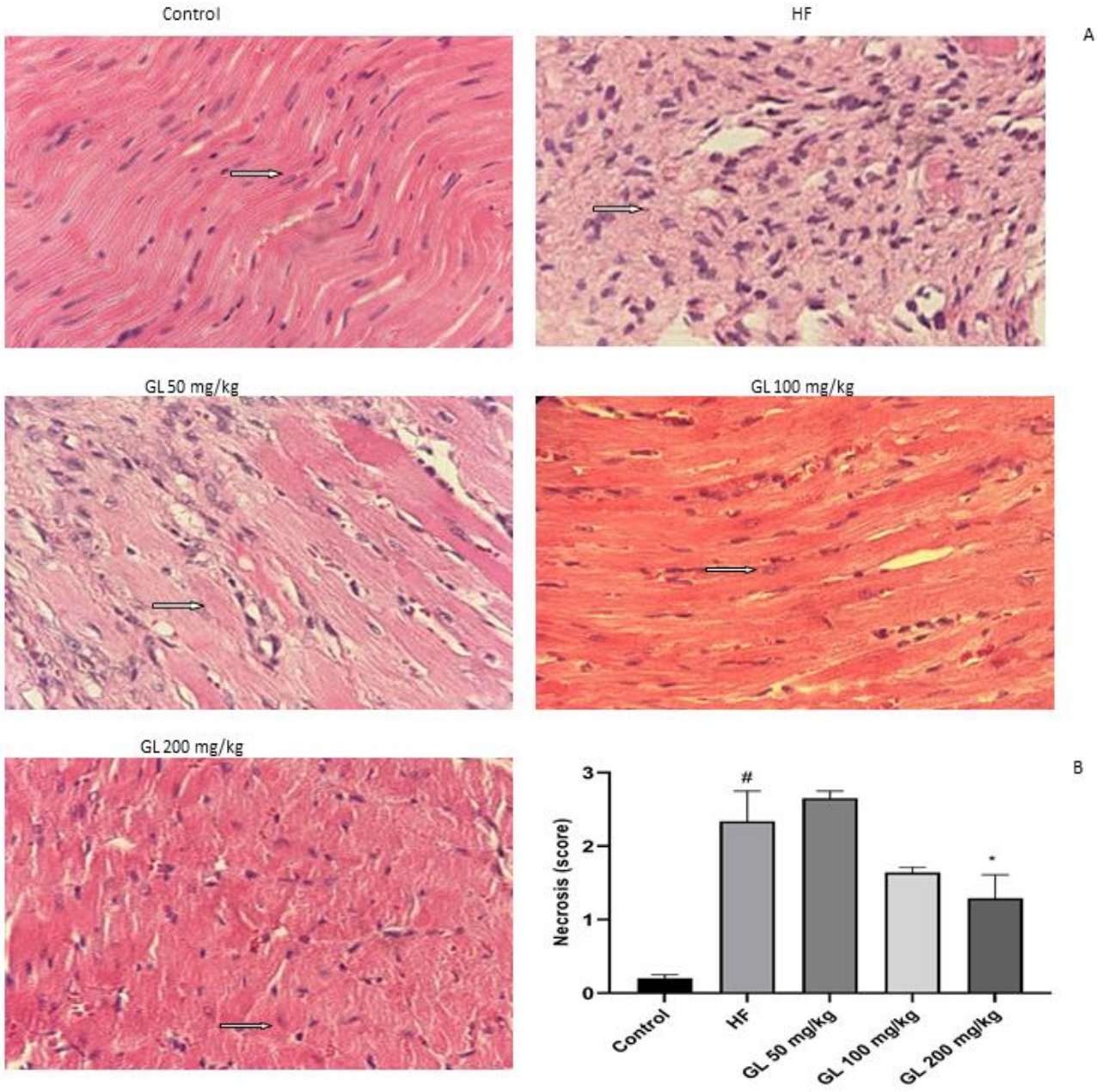

FIGURE 6. (A) Cardiac tissue of a rat with HF shows intensive cardiomyocyte necrosis and increased edema in myocardial tissue. G. lucidum pre-treatment showed significant reduction in necrosis. (B) Grading of histopathological changes in the rat's cardiac apex tissues. Data are expressed as mean±SEM. $^{\#}P<0.001$ vs control group. $^{*}P<0.05$ vs HF group using one way ANOVA with Tukey post test (n=6). GL: *Ganoderma lucidum*; HF: Heart failure.

Effects of pre-treatment with G. lucidum on histopathology

In histopathological examination, normal control group did not have necrosis and fibrosis and showed a clear intact structure of the cardiac tissue (Figs. 5A and 6A). Rats in HF group demonstrated extensive necrosis ($P<0.001$, Fig. 6A) and fibrosis ($P<0.001$, Fig. 5A) along with increased edema in the myocardial tissue. Treatment with *G. lucidum* at 100 and 200mg/kg doses markedly decreased myocardial fibrosis ($P<0.001$, Fig. 5B). Also *G. lucidum* at dose of 200mg/kg prevented inflammatory responses and myocardial necrosis ($P<0.05$) as shown in Figure 6B.

Effects of pre-treatment with G. lucidum on apoptosis in heart failure

Apoptosis in heart tissue was measured and it was found that apoptosis in isoproterenol-induced HF

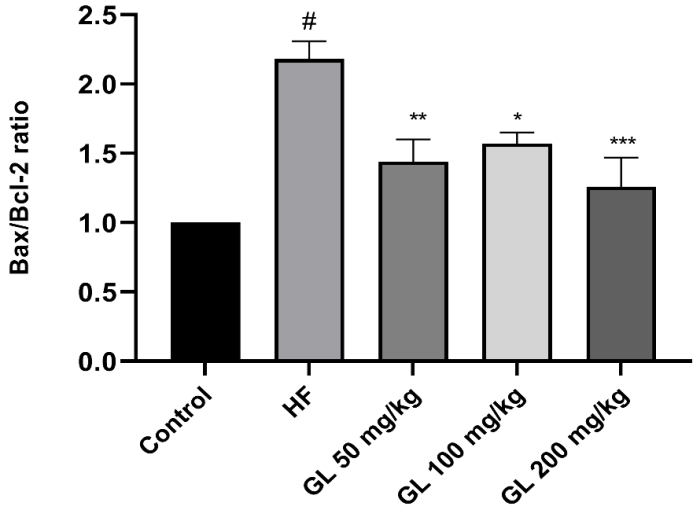

FIGURE 7. The effects of pre-treatment with *G. lucidum* at different doses on apoptosis in the cardiac tissues. Data are expressed as mean±SEM (n=6). $^{\#}P<0.001$ vs control group. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ vs HF group. For analysis, one way ANOVA was used with Tukey post test. GL: *Ganoderma lucidum*; HF: Heart failure.

group increased significantly. It was also found that pre-treamtnet with *G. Lucidum* (50, 100 and 200 mg/kg/day, $P<0.01$, $P<0.05$ and $P<0.001$, respectively) significantly reduced apoptosis in comparison to isoproterenol-induced HF group (Fig. 7).

Discussion

The present study has investigated the effects of pre-treatment with *G. lucidum* on cardiodynamic parameters, electrocardiogram, myocardial neutrophil infiltration, malondialdehyde level, cardiac remodeling and apoptosis in HF. Key findings are: (1) *G. lucidum* improved ECG pattern (increment of R amplitude and reduction of ST-segment depression) and cardiodynamic parameters, (2) pre-treatment with *G. lucidum* decrease myocardial myeloperoxidase activity and malondialdehyde level which are normally elevated in HF, (3) *G. lucidum* pre-treatment decreased myocardial apoptosis, necrosis and fibrosis with no effects on cardiac hypertrophy. The results of the present study show the protective effects of *G. lucidum* in heart failure.

Limited studies with *G. lucidum* have been performed on the electrocardiogram pattern and hemodynamic parameters. Sargowo et al. (2016) reported that β-glucan, an active component of *G. lucidum*, reduces circulating endothelial cells and improves endothelial dysfunction in atherosclerosis and stable angina patients by its antioxidant effects. A study on rats with type 2 diabetes reported cardiodynamic parameters improvement in the combined use of *G. lucidum*-derived polysaccharide and metformin. This study related the results to the down-regulation of the expression of MMP-2 (Qiai et al., 2016). Another study in isolated perfused rat hearts reported that *G. lucidum* had no effect on ventricular arrhythmias but could improve diastolic function and reduce necrotic death of cardiomyocytes as determined by a decrease in creatine kinase levels (Lasukova et al., 2008). Treatment with *G. lucidum* in a transverse aortic constriction-induced cardiomyopathy in rats attenuates cardiac dysfunction that correlates with a reduced expression of circular RNA circ-Foxo3 (Xie et al., 2016). Also, consumption of *G. lucidum* reduced blood pressure and cholesterol in rats with hypertension (Kabir et al., 1988). Considering the conflicting results, our study showed improvement of hemodynamic and electrocardiogram parameters in *G. Lucidum* pre-treated groups that can be attributed to differences in the disease being studied.

Inflammation and oxidative stress are fundamental to the pathophysiology of HF. The production of ROS during inflammation can cause heart damage by causing oxidative stress. For this reason, decreasing inflammatory responses as well as reducing oxidative stress are important therapeutic targets in heart failure (Ma et al., 2018). It was reported that, lucidenic acids and ganoderic acids isolated from *G. lucidum* demonstrate

anti-inflammatory effects (Akihisa et al., 2007). Previous studies have shown that *G. lucidum* polysaccharides have anti-oxidant, hypoglycemic, anti-inflammatory and anti-tumor activities (Zhao et al., 2012; Li et al., 2011; Lin and Zhang, 2004). Many of the *G. lucidum* polysaccharides modulate immune functions by activating the expression of cytokines (such as tumor necrosis factor-α, IL-1 and IL-6) or by antitumor activity (Chen et al., 2004). A study by Waty et al. (2015) on atherosclerotic rats received *G. lucidum* for 5 weeks has shown that the inflammatory markers such as CRP and IL-6 are significantly reduced in Ganoderma treated groups. Our results indicate that *G. lucidum* reduces myeloperoxidase enzyme levels as a marker of tissue neutrophil activity and therefore it can be suggested that the prevention of neutrophil migration to cardiac tissue may be one of the cardioprotective mechanisms of *G. Lucidum* in the heart failure.

Oxidative stress plays an important role in the development of heart failure and causes cardiac myocardial remodeling and apoptosis (Tsutsui et al., 2011; Giordano, 2005). Oxidative stress can stimulate the activation of myocardial MMP, which has a major role in LV remodeling and leads to development of heart failure (Senoner and Dicht, 2019). A study examining the *G. lucidum* effects on doxorubicin-induced cardiac toxicity showed the protective effects. They concluded that these protective effects could be through anti-apoptotic, anti-inflammatory and antioxidant effects (Xu et al., 2017). Zhang et al. (2014) showed that *G. lucidum* was implicated in ischemic stroke-induced injury in the hippocampus. They reported that *G. lucidum* reduced malondialdehyde level and increased superoxide dismutase levels in the serum and hippocampus and thereby prevents ischemic injury of the brain. The results of another study reported that crude polysaccharides extracted from Ganoderma reduced malondialdehyde and increased levels of superoxide dismutase and catalase in rat liver tissue samples, and therefore *G. lucidum* had protective effects on the liver (Susilo et al., 2019). A study showed that *G. lucidum* has cardioprotective effects because it has a dose-dependent antioxidant effect in rat heart (Wong et al., 2004). Consistent with the results of these studies, our study also showed a decrease in cardiac malondialdehyde levels in *G. lucidum* pre-treated groups which may be one of the cardioprotective mechanisms of *G. lucidum* in HF.

G. lucidum polysaccharides can decrease spontaneous apoptosis and also the fas-mediated apoptosis in human neutrophils (Hsu et al., 2002). Also *G. Lucidum* polysaccharides can be neuroprotective and reduce oxidative stress-induced apoptosis in ischemia-reperfusion injury and this effect is attributed to regulation of apoptosis-associated proteins (Sun et al., 2017). A study on mice with CTX-induced immunosuppression showed that *Ganoderma atrum* decreased oxidative stress and apoptosis (Li et al., 2017). Our study also showed that *G. lucidum* reduced Bax/Bcl-2 ratio in all treated groups which may be one of the cardioprotective mechanisms of *G. lucidum* in heart failure.

Previous studies have shown that remodeling in the left ventricle is an important factor in the pathophysiology of HF, and measuring remodeling is one of the best ways to diagnose heart failure (Konstam et al., 2011). A study by Xie et al. (2016) on the effects of *Ganoderma* in the cardiovascular system showed a decrease in left ventricular hypertrophy. A recent study on the cardioprotective effects of *G. lucidum*, has shown the efficacy of this fungus in reducing cardiac hypertrophy (Meng and Yang, 2019). In contrary, our results showed no significant reduction in cardiac hypertrophy in any of the studied groups, which may be due to differences in the intervention methods. Ganoderma on isolated perfused heart showed a decrease in the rate of necrotic cell death (Lasukova et al., 2015). Animal studies have shown a decrease in necrosis in tumor cells (Siwulski et al., 2015). In consistent, our results demonstrated that *G. lucidum* at high doses significantly reduced myocardial necrosis. A study by Chen et al. (2016) demonstrated the anti-fibrotic effect of *G. lucidum* on bleomycin-induced lung injury in rats. Another study also reported anti-fibrotic effect of *G. lucidum* in the liver that is consistent with our results. Reduction in myocardial necrosis and fibrosis can be considered as other protective mechanisms of *G. lucidum* in isoproterenol-induced HF.

Our model mimics advanced heart failure where there is chronic adrenergic stimulation and also examines the ischemic aspects of the heart that are different from previous studies. Moreover, our comprehensive study has studied essential aspects such as hemodynamic factors, electrocardiogram, cardiac remodeling in addition to inflammatory aspects and oxidative stress, and apoptosis in the heart. Overall, our results suggest that pre-treatment with *G. lucidum* can be considered as a possible

clinical use for preventive and adjuvant treatment in heart failure.

This study, although clearly revealing the cardioprotective effects of *G. lucidum*, has some limitations. We showed that protective effects of *G. lucidum* pre-treatment, can be attributed in part to a reduction in myocardial fibrosis, necrosis and apoptosis, as well as anti-inflammatory and antioxidant effects, but we did not examine all signaling pathways involved in this protective effect. We also did not study the post-treatment effects of *G. lucidum* on heart failure. Therefore, it is suggested to investigate the signaling pathways involved in the protective effects of *G. lucidum* in future studies. In addition, the preventive or therapeutic effects of *G. lucidum* and the comparison of disease severity between these two groups are suggested in clinical studies.

Conclusion

The present study for the first time demonstrated the cardioprotective effects of *G. lucidum* on isoproterenol-induced HF in rats, that can be attributed partially to reduction in myocardial fibrosis, necrosis and apoptosis as well as anti-inflammatory and antioxidant effects. According to our findings, *G. lucidum* can be considered as a preventive and adjuvant treatment for heart failure.

Conflict of interest

The authors declare that there is no conflict of interest.

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