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Research Article
Cardioprotective Efficacy of Hispidulin on Isoproterenol-induced Heart Failure in Wistar Rats

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Abstract

Background and Objective: Heart attack or myocardial infarction (MI) is a serious global issue due to the high mortality rate than other heart diseases. The current animal study was framed to examine whether hispidulin (HIS) exhibit cardioprotective activity in isoproterenol (ISO) induced rat model. **Materials and Methods:** Totally forty Wistar male rats were segregated into four different groups. Control rats were administered with saline, Hispidulin (HIS) group rats were intraperitoneally injected with HIS (50 mg kg^{-1} b.wt.) for 28 days, MI induced group (ISO) rats were injected with 100 mg kg^{-1} of ISO for last 2 days. Whereas, HIS and ISO-induced rats (HIS+ISO) group rats were injected with HIS (50 mg kg^{-1}, i.p.) for 28 days and exposed to ISO (100 mg kg^{-1}). **Results:** Rats injected with ISO significantly increased the heart to body weight ratio, heart weight, cardiac diagnostic markers (LDH, cTnC, CPK), lipid peroxidation products, lipid profile (TC, TG and LDL-c), inflammatory markers as compared with control rats. However, rats pre-treated with HIS for 28 days considerably abolished those elevation caused by ISO induction and revert all the biochemical values to near normal. A marked upregulation in the protein expression of NF-κB p65 subunits and TNF-α were noted in ISO-induced (MI) group. But on supplementation with HIS the levels of protein expression of NF-κB p65 subunits and TNF-α were exponentially downregulated. **Conclusion:** The outcome of this study implied that HIS possess potent cardioprotective activity against ISO-induced MI model by enhancing the oxidative status and suppressing hyperlipidemia and inflammatory response.

Key words: Heart attack, hispidulin, isoproterenol, peroxidation products, hyperlipidemia, myocardial infarction, heart weight, cardioprotective activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

For several years natural compounds (herbs/phytocomponents) have been used in traditional or folk medicine (Complementary or alternative medicine) for treating various ailments as they are considered as safe than most of the synthetic compounds[1]. Therefore, most of the researchers are focusing on natural materials like polyphenols and flavonoids for treating various dysfunctions and disorders or disease conditions[2]. Recently, flavonoids are gaining more attention for treating ischemic/coronary heart disease owing to its potent anti-inflammatory, anti-oxidant and anti-lipidemic properties[3]. One such popular flavonoid (flavone) is Hispidulin (HIS; 6-methoxy-5,7,4'-trihydroxyflavone). The HIS is a natural flavonoid (active compound) extracted from the different Traditional Chinese Medicinal (TCM) plants/herbs especially *Salvia* species, *Artemisia* species, *Centaurea* species as well as in *Eupatorium litoralle, Grindelia argentina, Saussurea involucrate*[4,5]. The HIS display array of biological activities including anti-tumor, anti-microbial, anti-inflammatory, anti-oxidant, anti-thrombosis as well as hepatoprotective and osteoprotective properties[4,6]. Furthermore, hispidulin can penetrate the blood-brain barrier (BBB) and act as allosteric modulator at the benzodiazepine receptor and thereby demonstrate its neuroprotective function[7].

Heart attack or Myocardial infarction (MI) is the prime most reason for the mortality and morbidity globally as compared to other types of cardiovascular diseases (CVDs). Therefore, MI is considered as a major health issue and need great attention[8]. The MI comes under ischemic heart disease and caused mainly due to blockage of blood flow (atherosclerosis-narrowing of blood vessels) and results in hypoxia and eventually myocardial tissue damage (cardiomyocytes death)[9]. The main pathophysiology of MI is the excessive production of free radicals, which outplays the numbers of antioxidants and results in oxidative stress, followed by increased inflammatory response and myocardial necrosis and apoptosis (cardiac damage) as well as trigger mitochondrial dysfunction and ultimately end up in MI[10]. Isoproterenol (ISO) is a β-adrenergic agonist and a synthetic catecholamine which induced heart failure similar to MI at the supramaximal dosage (100 mg kg^{-1} b.wt.). The ISO results in cardiotoxicity via triggering increased free radical production owing to catecholamine (auto-oxidation) which leads to oxidative stress, followed by the elevated inflammatory response, mitochondrial dysfunction and myocyte apoptosis (Ca^2+overload) and eventually results in cardiac damage alike MI[11].

Currently, many scientists have involved in developing a potent novel natural cardioprotective agent against MI as the present treatment regimen were not that effective and it's too expensive[12]. Therefore, the search for the less expensive and effective natural cardioprotective agent with less or no adverse effect is highly required to combat the mortality and morbidity related to MI[13]. The HIS could be the better choice to develop into a potent cardioprotective agent as it exhibits numerous beneficial activities including anti-inflammatory, anti-oxidant and anti-hyperlipidemia. Till date, no experimental studies have conducted to check the cardioprotective activity of hispidulin. Therefore, the current animal study was planned to examine whether hispidulin exhibit cardioprotective activity against ISO-induced cardiotoxicity (heart failure) in a rat model.

MATERIALS AND METHODS

Experimental rats and ethical approval: Totally forty healthy albino Wistar male rats, weighing 240±10 g were purchased and housed in polycarbonate cage at standard laboratory condition (21±2°C with 60-65% humidity) under 12 dark/12 light condition. All rats have free access to food (standard rat pellet) and water. All the experimental procedure or protocol used in this animal experiment were approved by Ethical clearance board members of Fujian Medical University (FMU-ECB-2018/13-12) and by sticking to the guidelines indicated by NSAG (China) for the use and care of experimental animals.

Experimental design: All the forty rats were segregated into 4 different groups after a week of assimilation period. Control rats were administered with only saline for 28 days (Con, n = 10), Hispidulin (HIS, n = 10) group rats were intraperitoneally (i.p.) injected with 50 mg kg^{-1} b.wt., of HIS for 28 days. The MI induced group (ISO; n = 10) rats were intraperitoneally injected with 100 mg kg^{-1} b.wt., of isoproterenol-hydrochloride for last 2 days without any treatment (29th and 30th day). While HIS and ISO-induced rats (HIS+ISO, n = 10) treatment group rats were intraperitoneally injected with HIS (50 mg kg^{-1}, i.p.) for 28 days and exposed to ISO (100 mg kg^{-1}).

Sample collection: On the 31st day all the rats were weighed using laboratory animal weighing scale (Beijing, China) and then rats were euthanized under 2% ethyl ether and the blood sample was immediately collected. The collected blood sample was centrifuged at 3500×g for 15 min at 4°C to separate serum sample and stored at -80°C until biochemical

analysis was carried out. Heart (organ) was excised immediately from all sacrificed rats and weighed using a weighing scale. The excised heart tissue was nicely chopped and homogenized with tris-HCl buffer solution and centrifuged at 15000×g for 15 min at 4°C and the resultant supernatant was utilized for all the biochemical and molecular analysis.

Biochemical analysis

Lipid peroxidation products and antioxidants: Cardiac homogenate (supernatant) was used to assess the levels of lipid peroxidation product like malondialdehyde (MDA) and the activity of antioxidants like superoxide dismutase (SOD) and catalase (CAT) using commercial enzymatic assay kit brought from Kangchen Biotechnology (Shanghai, China) based on supplier protocol.

Cardiac diagnostic marker enzymes: Activities of serum cardiac dysfunction diagnostic marker enzymes like cardiac troponin T (cTn T), creatine kinase-MB (CK-MB isoform) and lactate dehydrogenase (LDH) were measured to check the grade of cardiac dysfunction or integrity. The CK-MB (ELISA kit) and LDH (Colorimetric assay kit) were both assayed using commercial assay kits bought from Abcam (Cambridge, UK). While, the activity of cTn T were assayed using commercial ELISA assay kit (Thermo Fisher Scientific; MA, USA).

Lipid profile: The serum lipid profile including triacylglycerol or triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c), were analyzed using commercial assay kit from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) by following supplier's procedure.

Inflammatory markers: The Nuclear/Cytosolic fractionation kit was bought from BioVision Inc., (CA, USA) and used to extract the cytosolic and nuclear fraction from the heart tissue homogenate (supernatant). Then the levels of various cardiac inflammatory markers like Interleukin one beta (IL-1β), Tumor necrosis factor alpha (TNF-α) and Interleukin six (IL-6) were measured in cytosolic fraction using commercial rat specific Quantikine ELISA kit (R and D Systems, Inc., MN, USA).Thermo Fisher Scientific, Inc., (MA, USA). Whereas, the concentration of Nuclear factor kappa b p65 subunit (NF-κB p65) an active NF-κB subunit was measure in cerebral nuclear fraction using from ELISA NF-κB p65 transcription factor assay kit from Abcam (Cambridge, UK).

Molecular analysis

Western blot analysis: The western blot technique[11] was employed to quantify the protein expression of TNF-α from cytosolic fraction and NF-κB p65 subunit from nuclear fraction of heart homogenate. The protein levels were estimated using BCA protein assay kit from BioVision Inc., (CA, USA) by treating cardiac tissue homogenate (supernatant) with lytic RIPA buffered solution containing various proteinase. Fifty micrograms of protein from each group (sample) were uniformly separated using 12% SDS-PAGE apparatus and electro-transferred onto PVDF membrane. Then the membrane was blocked and probed with primary antibodies for overnight at 4°C. Primary antibodies: Rabbit polyclonal anti-NF-κB p65 (1:1000 dilution; Abcam, Cambridge, UK), rabbit monoclonal anti- TNF-α (1:800 dilution; Abcam, Cambridge, UK) and rabbit monoclonal anti-β actin (1:1200 dilution; Abcam, Cambridge, UK) a standard/control. Then followed by exposing to secondary antibody-rabbit polyclonal anti-horseradish peroxidase (HRP) antibody (1:12000 dilution from Abcam, Cambridge, UK) and incubated for 1 h at 37°C. The protein bands in the PVDF membrane was developed using Enhanced Chemiluminescence (ECL) detection system (Bio Rad Laboratories, Inc., CA, USA) and quantified using image analysing software (Image Plus software; Ver 7) from the Media Cybernectics, Inc., (MD, USA).

Statistical analysis: Data are expressed as the mean±standard error of mean (SEM) with ten rats in each group (n = 10). The p-value (statistical difference) among the groups (ISO vs Control and HIS+ISO vs ISO) was tested using One-way analysis of variance (ANOVA) followed by Least significance difference (*post hoc* test) using GraphPad Prism (ver:5; Graphpad Software Inc., CA, USA). A p value less than 0.05 is considered as the statistical difference.

RESULTS

Effect of HIS on the change in body weight, heart weight and heart to body weight ratio: The data in Table 1 represents the effect of HIS change in body weight, heart weight and heart to body weight ratio in control and ISO-induced rats. As compared with control rats, the ISO-induced rats showed a significant increase ($p<0.01$) in the heart weight and heart to body weight ratio. Whereas, rats pre-treated with HIS for 28 days and followed by ISO-induction showed a remarkable decline ($p<0.01$) in the levels of heart weight and heart to body weight ratio. Nevertheless, no significant alteration was observed in the case of body weight in any of the group.

Table 1: Effect of HIS change in body weight, heart weight and heart to body weight ratio in control and ISO-induced rats

	Control	HIS	ISO	HIS+ISO
Heart weight (g)	0.580±0.05	0.585±0.04	0.825±0.05$^{a\#}$	0.655±0.04$^{b\#}$
Body weight (g)	256.500±6.50	258.250±5.00	254.400±5.50^{NS}	255.800±7.00^{NS}
Heart to body weight ratio (%)	0.226±0.01	0.226±0.01	0.324±0.02$^{a\#}$	0.256±0.01$^{b\#}$

Data are expressed as the mean±standard error of mean (SEM), p-value: $^{\#}p<0.01$, 'a' shows the significant difference between ISO vs. control and 'b' shows the significant difference between HIS+ISO vs. ISO. NS: Non-significant

Table 2: Effect of HIS on the heart lipid peroxidation products and antioxidant enzymes in control and ISO-induced rats

Parameters	Control	HIS	ISO	HIS+ISO
MDA (nmol mg^{-1} pro)	0.62±0.08	0.60±0.07	1.24±0.14$^{a\#}$	0.75±0.09$^{b\#}$
SOD (U mg^{-1} pro)	4.95±0.40	5.01±0.50	3.26±0.29$^{a\#}$	4.11±0.42^{b*}
CAT (U mg^{-1} pro)	16.36±1.50	15.98±1.45	9.77±1.00$^{a\#}$	13.22±1.42$^{b\#}$

Data are expressed as the mean±standard error of mean (SEM), p-value: $^{\#}p<0.01$, $^{*}p<0.05$, 'a' shows the significant difference between ISO vs. control and 'b' shows the significant difference between HIS+ISO vs. ISO

Table 3: Effect of HIS on serum cardiac markers enzymes in control and ISO-induced rats

Parameters	Control	HIS	ISO	HIS+ISO
CK-MB (IU L^{-1})	67.70±8.00	69.78±9.50	145.20±13.00$^{a\#}$	83.80±9.00$^{b\#}$
LDH (IU L^{-1})	92.10±10.00	91.00±11.40	162.33±14.70$^{a\#}$	109.21±10.90$^{b\#}$
cTn T (ng mL^{-1})	0.58±0.06	0.59±0.05	1.43±0.15$^{a\#}$	0.81±0.09$^{b\#}$

Data are expressed as the mean±standard error of mean (SEM), p-value: $^{\#}p<0.01$, 'a' shows the significant difference between ISO vs. control and 'b' shows the significant difference between HIS+ISO vs. ISO

Table 4: Effect of HIS on the serum lipid profile in control and ISO-induced rats

Parameters	Control	HIS	ISO	HIS+ISO
TC (mg dL^{-1})	66.81±5.20	67.10±6.00	102.20±10.00$^{a\#}$	78.80±8.40$^{b\#}$
TG (mg dL^{-1})	41.55±6.00	42.00±4.05	72.98±9.50$^{a\#}$	52.60±4.69$^{b\#}$
LDL-C (mg dL^{-1})	34.50±4.10	33.85±4.00	73.92±8.10$^{a\#}$	49.10±5.30$^{b\#}$
HDL-C (mg dL^{-1})	23.35±2.75	24.97±3.40	18.05±2.00$^{a\#}$	21.50±3.00$^{b\#}$

Data are expressed as the mean±standard error of mean (SEM), p-value: $^{\#}p<0.01$, 'a' shows the significant difference between ISO vs. control and 'b' shows the significant difference between HIS+ISO vs. ISO

Effect of HIS on the heart lipid peroxidation products and antioxidant enzymes: Effect of HIS on the heart lipid peroxidation products and antioxidant enzymes in control and ISO-induced rats are shown in Table 2. The mean value of lipid peroxidation product like MDA was greatly inclined ($p<0.01$) in ISO-induced rat. Upon supplementation with HIS, the mean value of MDA was concomitantly decreased ($p<0.01$) as compare to the ISO-induced group. In the case of the heart, endogenous antioxidant enzymes like SOD and CAT were significantly attenuated ($p<0.01$) in ISO-induced rats as compared with control rats. However, the activity of these heart antioxidant enzymes was considerably ameliorated ($p<0.01$) upon HIS intervention.

Effect of HIS on serum cardiac markers enzymes: The results in Table 3 epitomized the activity of serum cardiac diagnostic marker enzymes like LDH, CK-MB, cTn T in control and ISO-induced rats. An exponential elevation ($p<0.01$) in the activity of serum cardiac diagnostic marker enzymes like LDH, CK-MB, cTn T were observed in ISO-induced rats as compared with control rats which are only treated with saline. While rats pre-treated with HIS for 28 days and exposed to ISO injection showed a dramatic decline ($p<0.01$) in the activity of those serum cardiac diagnostic marker enzymes than ISO-induced rats.

Effect of HIS on the serum lipid profile: Table 4 indicated the effect of HIS on the serum lipid profile in control and ISO-induced rats. A pronounced increase ($p<0.01$) in the levels of lipid profile like TC, TG and LDL-c with a significant decrease ($p<0.01$) in HDL-c level in ISO-induced group. In contrary, the levels of TC, TG and LDL-c were considerably decreased ($p<0.01$) with significant improvement ($p<0.01$) in the levels of HDL-c in rats injected with HIS for 28 days, before ISO induction as equivalent with ISO-induced (MI model) rats.

Effect of HIS on heart inflammatory markers: As shown in Fig. 1, the concentration of various pro-inflammatory cytokines like IL-1β, IL-6, TNF-α and NF-κB p65 in heart tissue homogenate were significantly enhanced ($p<0.01$) in rats injected with ISO. While rats injected with 50 mg kg^{-1} of HIS for 28 days before ISO induction could significantly suppress ($p<0.01$) the concentration of IL-1β, IL-6, TNF-α and NF-κB p65 on comparison with ISO-induced rats.

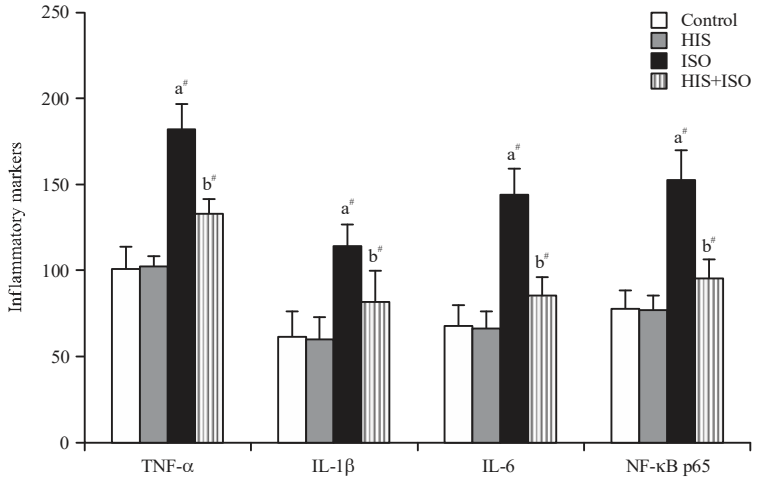

Fig. 1: Effect of HIS heart inflammatory markers in control and ISO-induced rats

Data are expressed as the mean±standard error of mean (SEM), p-value: #p<0.01, 'a' shows the significant difference among ISO vs. control and 'b' shows the significant difference between HIS+ISO vs. ISO

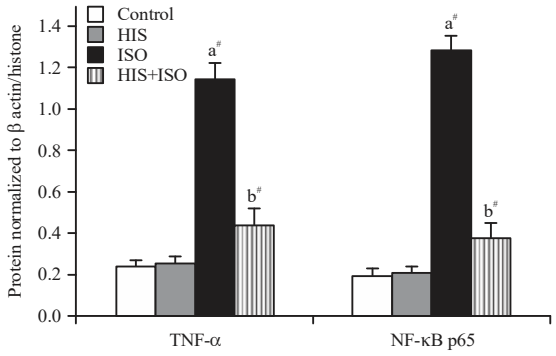

Fig. 2: Effect of HIS on the protein expression of TNF-α and NF-κb p65 in heart tissue of control and ISO-induced rats

Data are expressed as the mean±standard error of mean (SEM), p-value: #p<0.01, 'a' shows the significant difference among ISO vs. control and 'b' shows the significant difference among HIS+ISO vs. ISO

Effect of HIS on the protein expression of TNF-α and NF-κB p65: To confirm the anti-inflammatory activity of HIS (based on above biochemical results), the protein expression of TNF-α and NF-κB p65 in heart tissue of control and ISO-induced rats were quantified using western blot technique (Fig. 2). A marked upregulation ($p<0.01$) in the protein expression of TNF-α (cytosolic fraction) and NF-κB p65 subunit (nuclear fraction) were observed in ISO exposed rats (MI model rats) vs control rats. Meanwhile, the protein expression of TNF-α (cytosolic fraction) and NF-κB p65 subunit (nuclear fraction) were notably downregulated ($p<0.01$) in rats administered with HIS as a pre-treatment regimen for 28 days before ISO-induction.

DISCUSSION

As mentioned previously HIS exhibit numerous biological properties including anti-inflammatory, antioxidant and hepatoprotective activities. Hence, the author hypothesizes that HIS would exert cardioprotective efficacy against ISO-induced MI in a rat model as oxidative stress and inflammatory response are the main contributor for ISO-induced heart failure. The elevated heart weight in ISO-induced group is due to increased water accumulation (edematic condition-catecholamine auto-oxidation) by the loss of myocyte integrity and altered permeability[14]. But rats pre-treated with HIS showed decreased heart weight because of anti-lipid peroxidation and antioxidant activity which protect the myocyte integrity and maintain normal permeability[15]. The level of MDA was increased with a significant decrease in the activity of cardiac antioxidants like SOD and CAT after ISO induction owing to increased free radical generation (because of catecholamine auto-oxidation). However, rats administered with HIS for 28 days would improve the antioxidant activity of SOD and CAT and thereby lower the MDA levels attributing to its free radical scavenging and antioxidant activity[16]. In addition, Dabaghi-Barbosa *et al.*[17] indicated that the three hydroxyl groups present in the hispidulin could greatly contribute to free radical scavenging and antioxidant activity.

Rats induced with ISO showed elevated levels of marker enzymes like cTn T, CK-MB, LDH due to excessive free radical generation (oxidative stress) which lead to increase lipid peroxidation in cardiomyocytes and results in release (leakage) of enzymes from cardiac tissue into extracellular

fluid-serum[18]. Rats pre-treated with HIS for 28 days followed by ISO induction showed declined levels of marker enzymes like cTn T, CK-MB, LDH due to anti-lipid peroxidation/membrane protective property[16,17]. Copious amount of studies have highlighted that ISO induction could modulate the lipid metabolism and change the structural property (integrity, permeability) of myocytes and results in myocardial arrhythmias and dysfunction[19,20]. The rats injected with ISO on 29th and 30th days also showed altered serum lipid profile (increased -TC, TG and LDL-c/ decreased HDL-c). In case of rats exposed to ISO and pre-treated with HIS showcase decreased TC, TG and LDL-c with improved HDL-c and thus hinting its anti-hyperlipidemic property. Similarly, Wu and Xu[21] demonstrated that hispidulin can bind to PPAR-α and thus alter lipid metabolizing enzymes as well as improve HDL-c and lower LDL-c in a rat model.

During ISO induction (auto-oxidation of catecholamine) the elevated free radicals trigger inflammatory cascade via upregulating pro-inflammatory cytokines like IL-1β, IL-6 and TNF-α via NF- κB signaling pathway[8]. This animal experiment also indicated that rats injected with ISO (100 mg kg^{-1}) displayed the increased concentration of cardiac pro-inflammatory cytokines like IL-6, IL-1β, TNF-α and NF-κB p65 subunit. Whereas, rats pre-treated with HIS before ISO induction would increase oxidative status and thus lower ISO-induced oxidative stress and inflammatory response and hence the concentration of cardiac pro-inflammatory cytokines like IL-6, IL-1β, TNF-α and NF-κB p65 subunit were significantly decreased in HIS treated rats. To support the above data, Qin *et al.*[22] also reported that treatment with hispidulin would significantly suppress the NF-κB activation (signaling pathway) and eventually downregulate the production of various pro-inflammatory cytokines like IL-6, IL-1β and thereby showcasing its potent anti-inflammatory activity. To affirm the anti-inflammatory activity of HIS, the protein expression of TNF-α and NF-κB p65 in heart tissue of control and ISO-induced rats were quantified. The protein expression of TNF-α and NF-κB p65 were greatly upregulated in ISO-induced MI model group but on supplementation, with HIS the protein expression of TNF-α and NF-κB p65 were considerably downregulated. The above results endorsed the biochemical results (ELISA test) and affirm that HIS could exhibit anti-inflammatory activity by inactivating or suppressing the movement of activated nuclear NF-κB p65 into the cytosol to exhibit inflammatory response. Likewise, Nepal *et al.*[23] highlighted that hispidulin treatment would significantly reduce or suppress the activation of NF-κB and its active subunit p65 in RAW 264.7 cell line. Few limitations of this study included the omission of apoptotic markers, infarct size and edema. However, these limitations would be minimized in upcoming experiments.

CONCLUSION

The current animal experiment concluded that pre-treatment with HIS (50 mg kg^{-1}) for 28 days before ISO-induction (MI model) can considerably lower the elevated heart weight, lipid peroxidation, cardiac markers, lipid profile, inflammatory markers with improved antioxidant status and thus hispidulin showcase its strong cardioprotective activity in MI rat model. Nevertheless, more studies are needed to cross-check its molecular mechanism behind its cardioprotective activity.

SIGNIFICANCE STATEMENT

Collectively, the results of the present study shows that HIS display strong cardioprotective activity against ISO-induced MI model by enhancing the oxidative status and suppressing hyperlipidemia and inflammatory response. The above results would help the scientist or researchers to focus in detailed mechanism of cardioprotective activity of hispidulin and in future it can be developed into the potent cardioprotective agent and can be recommended against MI.

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REFERENCES

1. Li, J.W.H. and J.C. Vederas, 2009. Drug discovery and natural products: End of an era or an endless frontier? Science, 325: 161-165.
2. Pandey, K.B. and S.I. Rizvi, 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Med. Cell. Longevity, 2: 270-278.
3. Jiang, W., H. Wei and B. He, 2015. Dietary flavonoids intake and the risk of coronary heart disease: A dose-response meta-analysis of 15 prospective studies. Thromb. Res., 135: 459-463.
4. Patel, K. and D.K. Patel, 2017. Medicinal importance, pharmacological activities and analytical aspects of hispidulin: A concise report. J. Tradit. Complement. Med., 7: 360-366.

5. Atif, M., I. Ali, A. Hussain, S.V. Hyder and B. Niaz *et al*., 2015. Pharmacological assessment of hispidulin-A natural bioactive flavone. Acta Pol. Pharm.-Drug Res., 72: 829-842.
6. He, L., L. He, Y. Wu, L.L. Wang and Y. Wu *et al*., 2011. Hispidulin, a small flavonoid molecule, suppresses the angiogenesis and growth of human pancreatic cancer by targeting vascular endothelial growth factor receptor 2 mediated PI3K/Akt/mTOR signaling pathway. Cancer Sci., 102: 219-225.
7. Lin, T.Y., C.W. Lu, S.J. Wang and S.K. Huang, 2015. Protective effect of hispidulin on kainic acid-induced seizures and neurotoxicity in rats. Eur. J Pharmacol., 755: 6-15.
8. Othman, A.I., M.M. Elkomy, M.A. El-Missiry and M. Dardor, 2017. Epigallocatechin-3-gallate prevents cardiac apoptosis by modulating the intrinsic apoptotic pathway in isoproterenol-induced myocardial infarction. Eur. J. Pharmacol., 794: 27-36.
9. Lu, H., Y. Tan, L. Yang, H. Dong, Y. Liao, S. Cao and S. Fu, 2018. Cardioprotective efficiency of tangeretin against heart failure induced by isoproterenol in rats. Int. J. Pharmacol., 14: 1145-1152.
10. Liu, J., L. Chen and H. Lu, 2018. Asiatic acid enhances antioxidant and anti-inflammatory activity to suppress isoproterenol induced cardiotoxicity. Int. J. Pharmacol., 14: 1038-1045.
11. Sun, S.J., X.P. Wu, H.L. Song and G.Q. Li, 2015. Baicalin ameliorates isoproterenol-induced acute myocardial infarction through iNOS, inflammation, oxidative stress and P38MAPK pathway in rat. Int. J. Clin. Exp. Med., 8: 22063-22072.
12. Heusch, G. and B.J. Gersh, 2016. The pathophysiology of acute myocardial infarction and strategies of protection beyond reperfusion: A continual challenge. Eur. Heart J., 38: 774-784.
13. Gu, M., A.B. Zheng, J. Jin, Y. Cui and N. Zhang *et al*., 2016. Cardioprotective effects of genistin in rat myocardial ischemia-reperfusion injury studies by regulation of P2X7/NF-κB pathway. Evidence-Based Complement. Alternat. Med., Vol. 2016. 10.1155/2016/5381290.
14. Khalil, M., I. Ahmmed, R. Ahmed, E.M. Tanvir and R. Afroz *et al*., 2015. Amelioration of isoproterenol-induced oxidative damage in rat myocardium by *Withania somnifera* leaf extract. BioMed Res. Int., Vol. 2015. 10.1155/2015/624159.
15. An, P., T. Wu, H. Yu, K. Fang, Z. Ren and M. Tang, 2018. Hispidulin protects against focal cerebral ischemia reperfusion injury in rats. J. Mol. Neurosci., 65: 203-212.
16. Ferrandiz, M.L., G. Bustos, M. Paya, R. Gunasegaran and M.J. Alcaraz, 1994. Hispidulin protection against hepatotoxicity induced by bromobenzene in mice. Life Sci., 55: PL145-PL150.
17. Dabaghi-Barbosa, P., P. Dabaghi-Barbosa, A.M. Rocha, P. Dabaghi-Barbosa and A.M. Rocha *et al*., 2005. Hispidulin: Antioxidant properties and effect on mitochondrial energy metabolism. Free Radical Res., 39: 1305-1315.
18. Panda, V.S. and S.R. Naik, 2008. Cardioprotective activity of *Ginkgo biloba* phytosomes in isoproterenol-induced myocardial necrosis in rats: A biochemical and histoarchitectural evaluation. Exp. Toxicol. Pathol., 60: 397-404.
19. Prince, P.S.M., K. Dhanasekar and S. Rajakumar, 2011. Preventive effects of vanillic acid on lipids, Bax, Bcl-2 and myocardial infarct size on isoproterenol-induced myocardial infarcted rats: A biochemical and *in vitro* study. Cardiovasc. Toxicol., 11: 58-66.
20. Hemalatha, K.L. and P.S.M. Prince, 2015. Antihyperlipidaemic, antihypertrophic and reducing effects of zingerone on experimentally induced myocardial infarcted rats. J. Biochem. Mol. Toxicol., 29: 182-188.
21. Wu, X. and J. Xu, 2016. New role of hispidulin in lipid metabolism: PPARα activator. Lipids, 51: 1249-1257.
22. Qin, W., J. Xi, B. He, B. Zhang, H. Luan and F. Wu, 2016. Ameliorative effects of hispidulin on high glucose-mediated endothelial dysfunction via inhibition of PKCβII-associated NLRP3 inflammasome activation and NF-κB signaling in endothelial cells. J. Funct. Foods, 27: 392-405.
23. Nepal, M., H.J. Choi, B.Y. Choi, M.S. Yang, J.I. Chae, L. Li and Y. Soh, 2013. Hispidulin attenuates bone resorption and osteoclastogenesis via the RANKL-induced NF-κB and NFATc1 pathways. Eur. J. Pharmacol., 715: 96-104.