

Original Research Article

Protective effect of huperzine A against isoproterenol-induced myocardial damage in rats via regulation of PI3K/Akt signaling pathway

Lili Liu^{1*}, Fengnian Wang²

¹Department of Ultrasound, Xingtai People's Hospital, Xingtai, Hebei 054000, ²The Second Department of Surgery, Neiqiu People's Hospital, Xingtai, Hebei 054200, China

*For correspondence: **Email:** 67464209@qq.com; **Tel/Fax:** 0086-13469757561

Sent for review: 24 December 2020

Revised accepted: 21 April 2021

Abstract

Purpose: To investigate the cardioprotective effect of huperzine A (Hup A) against isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Methods: Forty healthy male rats were divided into 4 groups. Control rats received only saline. Rats in Hup A group were injected with Hup A (0.5 mg/kg) only; ISO-treated MI rats were injected with ISO, while ISO + Hup A rats were pre-treated with Hup A prior to ISO exposure and again treated with Hup A. Lipid levels, antioxidant status, cardiac markers, as well as the proteins associated with the PI3K/Akt signaling pathway were determined.

Results: Pre- and post-treatment with Hup A resulted in marked reductions in cardiac markers, heart weight, lipid peroxidation product (MDA), and lipid levels, along with improved antioxidant enzyme activities ($p < 0.01$). Rats treated with Hup A displayed normal cardiac fibrillar structure, relative to ISO-treated rats. Furthermore, the protein expression levels of Akt and PI3k (pAkt/Akt and pPI3k/PI3k ratios) were significantly upregulated ($p < 0.01$) in Hup A treated rats.

Conclusion: Pre- and post-treatment of rats with Hup A exerted potent cardioprotective effect against ISO-induced cardiac failure in rats. Thus, Hup A can potentially be developed as adjuvant therapy in clinical practice for alleviating MI-related problems.

Keywords: Myocardial infarction, Huperzine A, Lipid profile, Antioxidant, PI3k, Akt

This is an Open Access article that uses a fund-ing model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Cardiovascular diseases (CVDs) are responsible for almost 30 % of death globally. Heart attack/failure or MI, one of the common types of CVDs, accounts for most of total deaths related to CVDs [1]. Myocardial infarction (MI) is mainly caused by insufficient blood supply (due to blockage of blood vessels especially coronary

artery-atherosclerosis) and subsequently results sequentially in oxidative stress, inflammation, and myocardial cell death (necrosis) [2]. At present, various medications are available for treating MI. These are anti-thrombotic, anti-platelet agents, β adrenergic/angiotensin receptor blockers, and vasodilators. However, these medications trigger various serious side effects such as hyper- or hypotension and

heartburn [3]. Therefore, there is immense interest in the development of a natural cardioprotective drugs free of any adverse effects for suppressing the clinical symptoms and damage caused by MI.

Huperzine A (Hup A) is a bioactive lycopodium alkaloid isolated from a popular Chinese herb-*Huperzia serrata*. *Qian Ceng Ta* belongs to Huperziaceae, which has been reported as a safe and potent inhibitor of acetylcholinesterase (AChE), and is highly recommended for treating various neurological diseases/disorders [4]. An extensive number of studies demonstrated that Hup A possesses a panoply of therapeutic properties including anti-inflammatory, antinociceptive, anti-convulsant, anti-apoptotic, and neuroprotective effects [5-7].

Studies have indicated that Hup A possesses AChE activity, and improves cognitive function and behavioural changes, thereby mitigating Alzheimer's disease (AD) in various animal models [8-10]. A study showed that Hup A was effective against transient cerebral ischemia and reperfusion via enhancement of memory (neurotropic factor) and lowering of oxidative stress in mice [11]. Sui and Guo demonstrated that Hup A exerted potent cardioprotective effect in a rat model owing to its antioxidant and anti-inflammatory effects [12]. Recently, Li and his colleagues also reported that Hup A exhibited cardioprotective effect through modulation of the expression of the Nrf2/HO-1 signal pathway an ISO-induced MI in rats [13].

However, the molecular mechanism underpinning the cardioprotective property of Hup A and the involvement of the PI3k/Akt signaling pathway have not yet been studied. The present study was aimed at investigating the molecular mechanism underpinning the cardioprotective property of Hup A against ISO-induced MI in the rat model by assaying lipid profile, antioxidant status, morphological changes, as well as expressions of proteins involved in the PI3k/Akt signaling pathway.

EXPERIMENTAL

Rats

Forty healthy adult male albino Sprague Dawley (SD) rats of mean weight 220 ± 5 g were raised under normal laboratory conditions (22 ± 2 °C at 55 – 65 % relative humidity). The rats were handled with utmost care based on the guidelines stipulated by International Research Council [9]. The animal study was approved by the institutional animal ethical board of Xingtai

People's Hospital, China (approval no. XPH-HU/12/2019).

Establishment of isoproterenol-induced myocardial infraction

In this study, the most reliable ISO-induced MI model was used since this method mimics the pathophysiology of human MI, and it is also an easy and less time-consuming procedure for inducing MI in the rat model. The MI was induced by injecting the rats intraperitoneally (i.p) with isoproterenol hydrochloride (ISO-HCl) at a dose of 85 mg/kg body weight (bwt). The heart rate was monitored before, during, and after ISO administration.

Grouping of animals and treatment

Forty healthy male SD rats were randomly assigned to 4 groups, with 10 rats per group: control rats (group I) received only saline for 16 days; Hup A rats (group II) were injected with only Hup A at a dose of 0.5 mg/kg (i.p); ISO-induced MI rats (group III) were injected (i.p) with ISO as indicated above on the 8th and 9th days. The ISO + Hup A rats (group IV), were pretreated with Hup A (0.5 mg/kg bwt.) for 7 days, followed by ISO exposure on the 8th and 9th days, and another treatment with Hup A after 7 days. Hup A (98 % HPLC grade) was purchased from Shanghai Tauto Biotech, Co. Ltd; Shanghai, China).

Sample collection

After 16 days of the treatment, all rats were fasted overnight. On the morning of the 17th day, the rats were weighed and sacrificed under ether anesthesia, and blood samples were collected and processed. The hearts were removed immediately and weighed. A portion of the heart was fixed in 10 % formaldehyde for histopathological examination. The remaining heart samples were chopped, homogenized and centrifuged at 15,000 g for 10 min at 4 °C). The homogenate was used for various biochemical assays.

Determination of lipid profiles

Serum lipid profiles i.e., low-density lipoprotein cholesterol (LDL-c), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and triglycerides (TGs) were measured using commercial lipid profile assay kits bought from Biosino Biotechnology and Science Inc. (Beijing, China), based on manufacturers protocol.

Assessment of antioxidants and lipid peroxidation product

Cardiac lipid peroxidation product malondialdehyde (MDA) was determined using commercial kits from Beijing Zhongshan Goldenbridge Biotechnology Co. Ltd. (Beijing, China). The cardiac antioxidant enzyme activities [superoxide dismutase (SOD) and catalase (CAT)] were assayed using antioxidant assay kits purchased from Sangon Biotechnology (Shanghai, China), in accordance with the supplier's procedure.

Determination of cardiac markers

The concentration of various serum cardiac markers such as creatine kinase isoform (muscle/brain, CK-MB); cardiac troponin I (cTn I), lactate dehydrogenase (LDL), and cardiac troponin T (cTnT) were measured using rat-specific ELISA kits supplied by CUSABIO Technology LLC (TX, USA).

Western blotting

The protein expression levels of Akt and PI3k (and its phosphorylated form ratios) in cardiac homogenate were assayed using western blot method. Cardiac tissue was lysed with different proteases, and the protein concentrations of the lysates were determined with BCA protein assay kit (BioVision Inc., CA, USA). Then, the cardiac proteins were separated using 8 % SDS-PAGE and transferred onto the PVDF membranes. Then, the membranes were probed overnight at 4 °C with primary antibodies [rabbit polyclonal anti-pPI3k and PI3k antibody (1:1200); rabbit polyclonal anti-pAkt and Akt antibody (1:1500); and housekeeping anti-GADPH (1:1500)]. Thereafter, the membranes were washed with PBS and probed with horse radish peroxidase-conjugated secondary antibody at room temperature for 2 h. The intensities of the protein bands were measured using an image analyzer software (ImagQuant 400) from Healthcare LifeScience, Inc, (NJ, USA). All the antibodies were bought from Santa Cruz Biotechnology, TX, USA].

Histopathological examination

Portions of the cardiac tissue were fixed in 10 % formaldehyde and embedded in paraffin wax. The embedded cardiac tissues were sectioned using ultra-microtome at 4 - 5 μ m thickness and mounted on a microscopic slide. Each cardiac section was de-paraffinized and stained with hematoxylin and eosin stain (H & E). The stained sections were mounted on slides and examined for any morphological changes using a computerized digital optical microscope attached with an Olympus Digital camera (Olympus Co, Tokyo, Japan).

Statistical analysis

Data are expressed as mean \pm standard error of mean (SEM). Group comparison was done with one-way ANOVA, followed by Dunnett's multi-comparison test. All statistical analyses were done using GraphPad Prism (ver 6; GraphPad Software Inc, CA, USA). Values of $p < 0.01$ were taken as indicative of statistically significant differences.

RESULTS

Table 1 shows the effect of Hup A on heart and body weights, as well as its ratio in control and ISO-exposed rats. Significant increases ($p < 0.01$) in the heart weight as well as heart-to-body weight ratio were observed in ISO-exposed rats. Rats injected with ISO and treated with Hup A exhibited marked decreases ($p < 0.01$) in heart weight and heart-to-body weight ratio, when compared to ISO-exposed rats. However, no significant changes were seen in body weight.

Table 2 shows the effect of Hup A on lipid profiles (TG, TC, LDL-c, and HDL-c) in control and ISO-exposed rats. Significant increases in the levels of TGs, TC, and LDL-c, and marked decreases in the levels of HDL-c ($p < 0.01$) were observed in rats administered ISO alone, when compared with control rats given saline.

Table 1: Effect of HupA on body and heart weights as well as heart-to-body weight ratio

Parameter	Control	Hup A	ISO	ISO +Hup A
Body weight (g)	240.5 \pm 8.00	241.0 \pm 7.20	236.5 \pm 6.80	239.4 \pm 8.10
Heart weight (g)	0.590 \pm 0.05	0.599 \pm 0.06	0.775 \pm 0.08**	0.684 \pm 0.07##
Heart-to-body weight ratio (%)	0.240 \pm 0.02	0.242 \pm 0.02	0.327 \pm 0.02**	0.285 \pm 0.02##

Values are expressed as mean \pm standard error of mean (SEM). * $P < 0.05$, ** $p < 0.01$, control vs ISO group; # $p < 0.05$, ## $p < 0.01$, ISO vs ISO + Hup A group

Table 2: Effect of Hup A on lipid profiles

Parameter	Control	Hup A	ISO	ISO +Hup A
TC (mg/dL)	75.52 ± 5.10	74.8 ± 6.20	113.5 ± 9.05**	98.0 ± 7.19##
LDL-c (mg/dL)	21.80 ± 2.00	22.00 ± 1.80	65.75 ± 4.50**	39.90 ± 3.30##
HDL-c (mg/dL)	38.70 ± 3.10	38.20 ± 3.00	22.72 ± 1.90**	30.40 ± 2.60##
TGs (mg/dL)	49.20 ± 3.60	49.50 ± 4.00	83.45 ± 6.50**	62.60 ± 5.10##

Values are expressed as mean ± standard error of mean (SEM). * $p < 0.05$, ** $p < 0.01$, control vs ISO group; # $p < 0.05$, ## $p < 0.01$, ISO vs ISO + Hup A group

Table 3: Effect of Hup on cardiac antioxidants and lipid peroxidation product

Parameter	Control	HupA	ISO	ISO + HupA
SOD (U/mg)	5.51 ± 0.38	5.38 ± 0.42	3.21 ± 0.25**	4.68 ± 0.39##
CAT (U/mg)	12.35 ± 1.00	12.50 ± 1.20	8.25 ± 0.95**	10.84 ± 1.10##
MDA (nmol/mg)	0.58 ± 0.05	0.60 ± 0.07	1.24 ± 0.11**	0.82 ± 0.08##

Table 4: Effect of Hup A on serum levels of cardiac markers

Parameter	Control	HupA	ISO	ISO+HupA
cTn T (ng/mL)	0.51 ± 0.32	0.52 ± 0.35	1.20 ± 0.10**	0.79 ± 0.09##
cTn I (ng/mL)	0.29 ± 0.04	0.30 ± 0.05	1.10 ± 0.11**	0.63 ± 0.06##
CK-MB (IU/L)	70.00 ± 8.10	69.70 ± 7.50	123.10 ± 11.00**	92.00 ± 10.00##
LDH (IU/L)	80.20 ± 7.00	81.00 ± 8.40	140.40 ± 13.00**	106.35 ± 10.00##

Values are expressed as the mean ± standard error of mean (SEM). * $P < 0.05$, ** $p < 0.01$, control vs ISO group; # $p < 0.05$, ## $p < 0.01$, ISO vs ISO + Hup A group

However, seven days of pre-treatment, and post-treatment with Hup A (before/after exposure of ISO; ISO + Hup A) significantly reduced the levels of TGs, TC, and LDL-c, while enhancing HDL-c to levels comparable with the control group value ($p < 0.01$).

The effect of Hup A on cardiac antioxidants (SOD and CAT) and lipid peroxidation product (MDA) in control and ISO exposed rats is presented in Table 3. Rats exposed to ISO at a dose of 85 mg/kg for 2 days showed marked decreases in the activities of SOD and CAT, while MDA level was elevated ($p < 0.01$). However, treatment with Hup A led to marked decrease in the level of MDA, and significant increases in the activities of CAT and SOD, when compared with the ISO-treated rats.

As shown in Table 4, the serum concentrations of various cardiac markers (cTn T, cTn I, CK-MB and LDH) were markedly raised ($p < 0.01$) in ISO-exposed rats, relative to control rats. However, in rats injected i.p. with Hup A for 14 days (pre- and post-treatment), there were significant decreases in the serum levels of these cardiac markers to near-normal values similar to those of the control group. Figure 1 shows the effect of Hup A on the protein expression levels of pPI3K and pAkt in cardiac tissue of experimental rats. Marked downregulations ($p < 0.01$) in the protein expressions of pPI3K (pPI3k/pI3k ratio) and pAkt (pAkt/Akt ratio) were seen in ISO-exposed rats, relative to control rats. However, treatment with

Hup A produced significant upregulations in the protein expressions of pPI3K (pPI3k/pI3k ratio) and pAkt (pAkt/Akt ratio), when compared with ISO-alone exposed rats ($p < 0.01$).

Figure 2 shows the effect of Hup A on cardiac histopathological changes. The cardiac slide of control (2 A) and Hup A (2 B), reveal normal myocardial architecture (myofibrils) without any pathological changes. In contrast, the cardiac slide of ISO-induced MI (2C) shows dense inflammatory infiltration and disoriented myofibrils, while the cardiac slide for rats in ISO + Hup A group (2D) reveals less inflammatory infiltration and no other pathological changes.

DISCUSSION

Many researchers have reported that huperzine A is a potent cardioprotective agent in a rat model of MI [12,13]. However, the molecular mechanism underpinning the cardioprotective property of Hup A, and the involvement of the PI3k/Akt signaling pathway have not been determined in previous studies. Therefore, the current study was designed to investigate the molecular mechanism underpinning the cardioprotective property of Hup A against ISO-induced MI in rats via measurement of heart weight, lipid profiles, antioxidant status, cardiac markers, and morphological changes, as well as expression levels of proteins involved in PI3k/Akt signaling pathway.

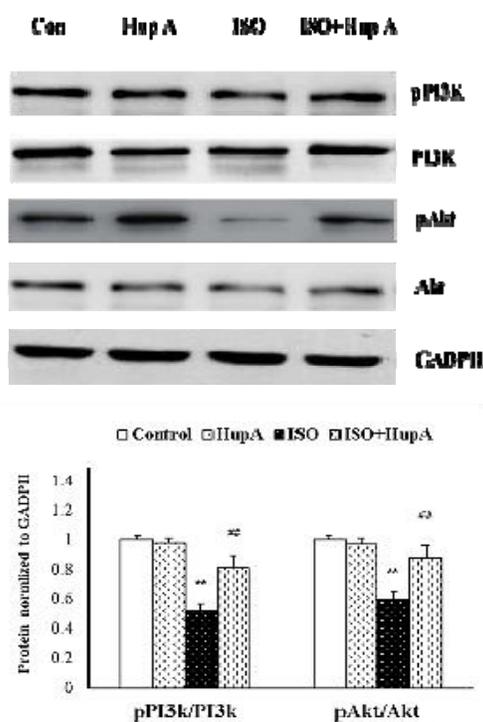


Figure 1: Effect of Hup A on protein expressions of PI3k and Akt in cardiac tissue. Values are expressed as mean \pm standard error of mean (SEM). * $P < 0.05$, ** $p < 0.01$, control vs ISO group; # $p < 0.05$, ## $p < 0.01$, ISO vs ISO + Hup A group

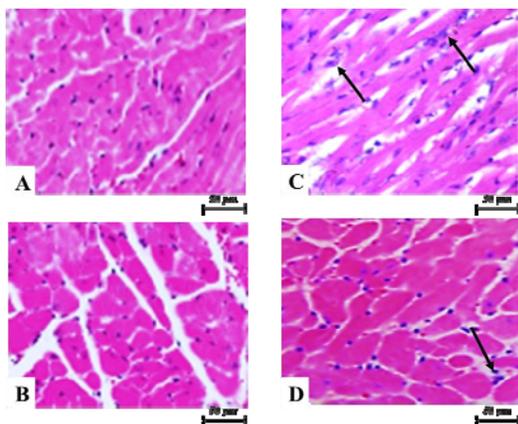


Figure 2: Effect of Hup A on cardiac histopathological changes. Slides A and B represent control (2A) and Hup A (drug control; 2B), respectively, showing normal myocardial architecture (myofibrils) without any pathological changes. In contrast, cardiac slide C for ISO-HI model (2C) displays ISO-induced MI condition with dense inflammatory infiltration (crowding of inflammatory cells, arrowed) and disoriented myofibrils. In contrast, cardiac slide D (2D) from ISO-induced MI rats treated with HupA shows less inflammatory infiltration without any pathological changes. Scale bar: 50 μ m

The outcome of the current study showed that pre- and post-treatments of rats with Hup A resulted in significant cardioprotective effect via significant reductions in heart weight, cardiac markers, lipid peroxidation product (MDA), and lipid profiles, along with increased antioxidant enzyme activities. Besides, the protein expression levels of Akt and PI3k (pAkt/Akt and pPI3k/PI3k ratios) were markedly upregulated in Hup A-treated rats. Exposure of rats to ISO triggered enhancement of oxidative stress and inflammatory responses, which resulted in significant damage in the myocardium and eventually edema, leading to increased heart weight [14,15].

Heart-to-body weight ratio and heart weight were significantly increased in ISO-treated rats. However, rats administered (pre- and post-treatments) with Hup A showed significant decreases in the values of heart-to-body weight ratio and heart weight, when compared to ISO-treated rats. These effects might be due to the anti-oxidative and anti-lipid peroxidation activities of Hup A [5], which may decrease myocardial damage, thereby decreasing edema and lowering heart weight. Likewise, the activities of various antioxidants were elevated in the Hup A+ISO group through enhancement of free radical-scavenging and anti-lipid peroxidation effects [16]. Moreover, recently, Li and his colleagues demonstrated that Hup A lowered oxidative stress by positively modulating the protein expressions of the Nrf2/HO-1 signaling pathway [13]. However, the connection between Hup A and the PI3K/Akt and Nrf2/HO-1 signaling pathways needs to be determined in the future.

Hyperlipidemia/hypercholesterolemia is a major risk factor for CVDs, especially MI [17]. The levels of TGs, TC, and LDL-c were significantly increased, but these increases were reverted to normalcy in rats treated with Hup A, owing to its hypolipidemic activity. Moreover, Hup A treatment lowered the serum levels of cardiac markers (cTn T, cTn I, CK-MB, and LDL) thereby decreasing cardiac damage, indicating its potent anti-lipid peroxidation property [5, 16]. Several studies have reported the importance of the PI3K/Akt signaling pathway in the cardioprotective defence mechanism based on its involvement in the suppression of free radical generation (oxidative stress) and subsequent inflammatory cascade and myocardial damage [18,19]. Rats injected with ISO had marked downregulations of the protein expressions of pPI3K (pPI3k/pi3k ratio) and pAkt (pAkt/Akt ratio).

However, upon treatment with Hup A, these protein expression levels were markedly upregulated, resulting in increased pAkt/Akt and pPI3k/pI3k ratios in the Hup A+ISO group (treatment group). These results are in agreement with the report of Mao and co-workers who showed that Hup A markedly upregulated protein expressions of pPI3k and pAkt and thus lowered oxidative stress and apoptosis in a murine HT22 cell model [20]. Thus, Hup A exerts strong cardioprotective activity through lowering of oxidative stress by positively regulating the PI3k/Akt signaling pathway. This is supported by results of histopathological studies which confirmed that the cardiac tissue of rats treated with Hup A had near-normal cardiac fibrillar structure, with minimal inflammatory infiltration and no other pathological changes.

CONCLUSION

Treatment of MI rats with Hup A results in improved cardioprotective effect via significant reduction in heart weight-to-body weight ratio, heart weight, cardiac markers, lipid peroxidation product, and lipid levels, as well as increased antioxidant enzyme activities. These findings are consistent with histopathological evidence of normal cardiac architecture. Moreover, the protein expression levels of Akt and PI3k (pAkt/Akt and pPI3k/PI3k ratios) are markedly upregulated in Hup A-administered MI rats. Therefore, Hup A can be developed as a potential adjuvant therapy for use along with standard cardioprotective drugs for MI-related disorders. However, large-scale clinical trials are needed to buttress this.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Lili Liu and Fengnian Wang designed, executed this study as well as drafted this manuscript. Lili Liu helped in histological analysis and data analysis.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or

their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. Pagidipati NJ, Gaziano TA. Estimating deaths from cardiovascular disease: a review of global methodologies of mortality measurement. *Circulation* 2013; 127(6): 749-756.
2. Raish M. *Momordica charantia polysaccharides ameliorate oxidative stress, hyperlipidemia, inflammation, and apoptosis during myocardial infarction by inhibiting the NF- κ B signaling pathway.* *Int J Biol Macromol* 2017; 97: 544-551.
3. Mnafigui K, Hajji R, Derbali F, Khlif I, Kraiem F, Ellefi H, Elfeki A, Allouche N, Gharsallah N. Protective effect of hydroxytyrosol against cardiac remodeling after isoproterenol-induced myocardial infarction in rat. *Cardiovasc Toxicol* 2016; 16(2): 147-155.
4. Zhu N, Lin J, Wang K, Wei M, Chen Q, Wang Y. Huperzine A protects neural stem cells against A β -induced apoptosis in a neural stem cells and microglia co-culture system. *Int J Clin Exp Pathol* 2015; 8(6): 6425.
5. Ferreira A, Rodrigues M, Fortuna A, Falcão A, Alves G. Huperzine A from *Huperzia serrata*: a review of its sources, chemistry, pharmacology and toxicology. *Phytochem Rev* 2016; 15(1): 51-85.
6. Damar U, Gersner R, Johnstone JT, Schachter S, Rotenberg A. Huperzine A as a neuroprotective and antiepileptic drug: a review of preclinical research. *Expert Rev Neurother* 2016; 16(6): 671-680.
7. Ma T, Gong K, Yan Y, Zhang L, Tang P, Zhang X, Gong Y. Huperzine A promotes hippocampal neurogenesis in vitro and in vivo. *Brain Res* 2013; 1506: 35-43.
8. Damar U, Gersner R, Johnstone JT, Schachter S, Rotenberg A. Huperzine A: A promising anticonvulsant, disease modifying, and memory enhancing treatment option in Alzheimer's disease. *Med Hypotheses* 2017; 99: 57-62.
9. Wu TY, Chen CP, Jinn TR. Traditional Chinese medicines and Alzheimer's disease. *Taiwan J Obstet Gynecol* 2011; 50(2): 131-135.
10. Wang R, Yan H, Tang XC. Progress in studies of huperzine A, a natural cholinesterase inhibitor from Chinese herbal medicine 1. *Acta Pharmacol Sinica* 2006; 27(1): 1-26.
11. Wang ZF, Tang LL, Yan H, Wang YJ, Tang XC. Effects of huperzine A on memory deficits and neurotrophic factors production after transient cerebral ischemia and reperfusion in mice. *Pharmacol Biochem Behav* 2006; 83(4): 603-611.

12. Sui X, Gao C. Huperzine A ameliorates damage induced by acute myocardial infarction in rats through antioxidant, anti-apoptotic and anti-inflammatory mechanisms. *Int J Mol Med* 2014; 33(1): 227-233.
13. Li Z, Liu W, Gao L, Xiang D. Down-modulation of endoplasmic reticulum stress-initiated apoptosis by huperzine A in isoproterenol provoked myocardial infarction rat model: Role of Nrf2/HO-1 signaling axis. *Trop J Pharma Res* 2020; 19(8): 1-8.
14. Afroz R, Tanvir EM, Karim N, Hossain M, Alam N, Gan SH, Khalil M. Sundarban honey confers protection against isoproterenol-induced myocardial infarction in Wistar rats. *BioMed Res Int* 2016; 2016.
15. Rathore N, Kale M, John S, Bhatnagar D. Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat erythrocytes. *Indian J Physiol Pharmacol* 2000; 44(2): 161-166.
16. Ohba T, Nakamura S, Shimazawa M, Hayashi Y, Kono H, Hara H. Protective effects of *Huperzia serrata* and its components against oxidative damage and cognitive dysfunction. *Pharma Nutr* 2020; 13: 100203.
17. Shaik AH, Shaik NR, Mohammed AK, Al Omar SY, Mohammad A, Mohaya TA, Kodidhela LD. *Terminalia pallida* fruit ethanolic extract ameliorates lipids, lipoproteins, lipid metabolism marker enzymes and paraoxonase in isoproterenol-induced myocardial infarcted rats. *Saudi J Biol Sci* 2018; 25(3): 431-436.
18. Cheng S, Zhang X, Feng Q, Chen J, Shen L, Yu P, Yang L, Chen D, Zhang H, Sun W, Chen X. Astragaloside IV exerts angiogenesis and cardioprotection after myocardial infarction via regulating PTEN/PI3K/Akt signaling pathway. *Life Sci* 2019; 227: 82-93.
19. Cui G, Shan L, Hung M, Lei S, Choi I, Zhang Z, Yu P, Hoi P, Wang Y, Lee SM. A novel Danshensu derivative confers cardioprotection via PI3K/Akt and Nrf2 pathways. *Int J Cardiol* 2013; 168(2): 1349-1359.
20. Mao XY, Zhou HH, Li X, Liu ZQ. Huperzine A alleviates oxidative glutamate toxicity in hippocampal HT22 cells via activating BDNF/TrkB-dependent PI3K/Akt/mTOR signaling pathway. *Cell Mol Neurobiol* 2016; 36(6): 915-925.