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Research Article
Asiatic Acid Enhances Antioxidant and Anti-inflammatory Activity to Suppress Isoproterenol Induced Cardiotoxicity

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Abstract

Background and Objective: Myocardial infarction (heart attack) owing to ischemia is the primary contributor to most of the death caused by cardiovascular diseases (CVDs). This pre-clinical study was framed to investigate the beneficial efficacy of Asiatic acid (AA) against isoproterenol (ISO)-induced myocardial infraction (MI) in experimental rats. **Materials and Methods:** Healthy male rats (n = 32), were separated into four groups with 8 rats in each group. Group I rats were given only saline (control), group II rats were orally administrated with AA (20 mg kg^{-1}) for 7 days (AA alone), group III rats were induced with ISO (100 mg kg^{-1}, s.c) for 2 consecutive days (MI model), group IV rats were pre-treated (5 days) and co-treated (6th and 7th day) with AA (20 mg kg^{-1} via orally) and followed by induction of ISO (AA+ISO). **Results:** Rats pre and co-treated with AA for 7 days and followed by ISO induction (group IV rats)results in considerable increase in the activities of ATPases ($Na^{2+}/K^{+}$and Mg^{2+}) and endogenous antioxidants (CAT, SOD, GPx) as well as substantial decrease in the levels of heart weight, heart to body weight ratio, lipid peroxidation product (MDA), Ca^{2+} ATPases, cardiac markers (cTnT, CK-MB, LDH) and inflammatory markers (IL-6, IL-1β, TNF-α).Moreover, administration with AA greatly reduced the pathological changes (edema, necrosis, neutrophil infiltration) in cardiac tissue and lookalike as a control group. **Conclusion:** Taken together, that treatment with AA considerably attenuated the ISO-induced cardiotoxicity or MI by exhibiting potent antioxidant and anti-inflammatory activity. However, further studies (clinical trials) are required to support its importance against Myocardial infarction.

Key words: Isoproterenol, myocardial infarction, antioxidant, asiatic acid, ATPases

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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

Acute myocardial infarction (MI) or heart attack is the main reason of mortality and morbidity globally and hence a major health concern[1]. The China Heart Failure Symposium reported that the mortality of severe MI patients was almost 50% in China[2]. Similarly, other global studies have indicated that around 40-60% of cases of clinical manifestation of coronary heart disease (CHD) is MI. MI is caused due to the reduced blood supply to the myocardium (hypoxia)due to abrupt occlusion owing to disrupted atherosclerotic plaque[3,4]. The physio-pathological of MI is still unknown but researchers had demonstrated that oxidative stress, inflammation, hypoxia, necrosis, mitochondrial dysfunction (altered energetics) and apoptosis are the crucial contributor for MI[5,6]. Isoproterenol (ISO, 1-3,4 dihydroxyphenyl-2-isopropyl aminoethanol hydrochloride) is a β-adrenergic agonist and synthetic catecholamine in excess dose would cause intense stress (oxidative stress due to auto-oxidation of catecholamines) to cardiac tissue (cardiomyocytes) and eventually results in necrosis and hence ISO-induced MI is the best non-invasive model used to explore the cardioprotective function of various synthetic and natural drugs[1,7]. The pathophysiology of human MI is similar to ISO-induced MI, both share similar biochemical conditions including oxidative stress, inflammation, necrosis, apoptosis, mitochondrial dysfunction and loss of bioenergetic are the key events contribute for ISO-induced MI in rat and mice model[8,9]. In the last decade, many scientists had shown immense interest on a natural product with antioxidant and anti-inflammatory properties to restrain the extent of myocardial ischemic injury[10,11].

Asiatic acid (AA, 2,3,23-trihydroxy-ur-12-ene-28-oic acid) is a naturally occurring pentacyclic triterpene and considered as one of the active phytocomponent of herb *Centellaasiatica* (belong to family Apiaceae) which has been used as folk medicine to treat various neurological disorders especially in China and India[12]. AA possesses a broad range of pharmaceutical activities including anti-inflammatory, anti-cancer, antioxidant, anti-diabetic as well as neuroprotective, hepatoprotective, cardioprotective actions[13,14]. Previously, Asiatic acid has been reported to protect the cardiac tissue against myocardial ischemia/reperfusion injury in H9c2 cell model[15]. Moreover, asiatic acid (one of the three triterpenes) could protect myocytes (H9c2) against glucose-induced injury by abolishing oxidative stress[16]. Also, Huo *et al.*[17], demonstrated that asiatic acid could efficiently inhibit left ventricular remodeling in myocardial infarction rat model. Therefore, asiatic acid would be the suitable candidate to investigate the cardioprotective action against ISO-Induced MI in a rat model.

MATERIALS AND METHODS

Experimental animals: Thirty-two healthy male Wistar strain rats (n = 32), weighing 240±10 g were bought from animal supplier (Animal life, Nantong, China). The experimental rats were housed in a cage and were maintained at 23±1°C with 55-65% humidity with 12 h day and light cycle at the animal center. Animals have full access to food (rat pellet) and water. All the procedure related to animals and the protocols employed in this animal study were based on the guidelines put forth by NIH (MD, USA). This animal study was conducted from March-April, 2017. This animal experiment was approved by the ethical committee board members of The First Hospital of Nantong, China (FHN-16/023/B234).

Induction of ISO (MI model): ISO was mixed with physiological saline (0.89%) and injected subcutaneously (s.c) at a dose of 100 mg kg^{-1} bt.wt., on 6th and 7th day (with 24 h interval)[18].

Experimental design: Healthy male Wistar strain rats (n = 32), were separated into four groups with 8 rats in each group. Group I rats were given only saline (control), group II rats were orally administrated with AA (20 mg kg^{-1}) for 7 days (AA alone), group III rats were induced with ISO (100 mg kg^{-1}, s.c) for 2 consecutive days and served as MI model, group IV rats were pre-treated (5 days) and co-treated (6th and 7th day) with AA (20 mg kg^{-1} via orally) and followed by induction of ISO (6th and 7th day) and served as treatment group (AA+ISO).

Sample collection: Rats were fasted overnight and weighed (8th day) followed by injecting with pentobarbital sodium (35 mg kg^{-1}, i.p) and the blood samples (vena cava) were collected in non-heparinized tube and sacrificed by cervical decapitation. The cardiac tissue was removed immediately and rinsed with chill saline and weighed (dry). A portion of cardiac tissue was homogenized (10%) using a Tris-HCL buffer (0.1 M, 7.4 pH). The homogenate was centrifuged at 10000 rpm for 20 min at 4°C and the separated supernatant portion was used for biochemical analysis. Remaining cardiac tissue was fixed in 10% formalin to assess morphological changes. A collected blood sample was allowed to clot

(placing the tube in slanting position) and serum samples were obtained by centrifuging at 3500 rpm for 10 min at 4°C. All the samples were stored at -80°C until the usage.

Biochemical analysis

Evaluation of cardiac markers: Activities of serum creatine kinase-MB (CK-MB, isoform), lactose dehydrogenase (LDH) and troponin T (cTn T) were assayed by commercial ELISA kitin accordance to supplier's procedure(Biosino Biotechnology and Science Inc., Beijing, China).

Measurements of lipid peroxidation products and endogenous antioxidants: The levels of lipid peroxidation product-malondialdehyde (MDA) as well as endogenous enzymic antioxidant activities including catalsae (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in cardiac tissue homogenate were assessed using standard commercial kit method provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China) based on manufacturers protocol. The protein content of cardiac tissue homogenate was estimated by the method of Lowry *et al.*[19].

Assay of membrane-bound ATPases: The membrane-bound ATPases like sodium/potassium (Na^{2+}/K^{+}) ATPase, magnesium (Mg^{2+}) ATPase and calcium (Ca^{2+}) ATPase were assayed using the methods of Bonting[20], Hjerken and Pan[21], Ohinishi *et al.*[22], respectively. Whereas, the liberated inorganic phosphate was determined by the method of Fiske and Subbarow[23] as described previously.

Determination of inflammatory markers: From the cardiac homogenate, the cytosolic fraction was extracted using Nuclear/Cytosolic fractionation kit brought from Cell Biolabs Inc., (CA, USA). The extracted cytosolic fraction of cardiac homogenate was used for determining the concentration of Tumour necrosis factor (TNF-α), Interleukins 6 (IL-6), Interleukins 1 (IL-1β) using commercial ELISA kit from Neobioscience Technology, Co., Ltd (Beijing, China) in accordance to manufacturers instruction.

Assessment of morphological changes: Formalin-fixed cardiac tissue was dehydrated, fixed and embedded in liquid paraffin and made as a block. The cardiac tissue (block)was sliced into4-5 μm diameter using microtome and bound to microscopic slide. Subsequently, the cardiac tissue slides were stained with hematoxylin and eosin (H and E) stain and assessed for any morphological changes (edema, necrosis and inflammatory cells infiltration) using a light microscope (Olympus Co., Tokyo, Japan) at 100× magnification.

Statistical analysis: Values were expressed as the average Mean±Standard deviation. The difference between the experimental group (Control vs ISO or AA+ISO vs ISO) was analyzed by One-Way ANOVA followed by *post-hoc* Dunnett's multi-comparison test using SPSS software (Ver 17) from IBM Inc., (USA). The $p<0.05$ was deemed as minimal statistical difference.

RESULTS

Efficacy of AA on body weight, heart weight and heart to body weight ratio in experimental animals were shown in Table 1. In case of body weight, no significant changes were observed in any of the groups. Whereas, heart to body weight ratio and the heart weight were considerably escalated ($p<0.05$) in ISO administered rats. In contrary, the pre- and co-treated with AA (AA+ISO) markedly reduced ($p<0.05$) the heart to body weight ratio and the heart weight as compared with ISO-induced MI model rats.

The table 2 represented the efficacy of AA on serum cardiac markers in experimental animals. A pronounced increase ($p<0.01$) in the activities of serum cardiac markers like LDH, CK-MB and cTn T were noted in ISO injected rats than control rats. However, the levels of these serum cardiac markers were concomitantly declined ($p<0.01$) after treatment with AA on equivalence with the ISO-induced group.

The efficacy of AA on cardiac lipid peroxidation product and antioxidants in experimental animals were optimized in Table 3. In comparison with control rats, the levels of lipid peroxidation product (MDA)were exponentially inclined ($p<0.01$) with the substantial decline ($p<0.01$) in the activities of various endogenous antioxidants like CAT, GPx, SOD in ISO-induced rats. Interestingly, pre- and co-treated rats with AA for 7 days (AA+ISO) significantly reverted the levels of lipid peroxidation product (MDA, $p<0.05$) as well as the activities of various endogenous antioxidants like CAT ($p<0.01$), SOD ($p<0.05$) and Gpx ($p<0.05$)to near normal as compared with ISO-induced rats.

As shown in Table 4 the activities of cardiac membrane-bound ATPases like Na^{2+}/K^{+} ATPase, Mg^{2+} ATPase was remarkably decreased ($p<0.01$) in the ISO administered rats. In contrast, the activities of Ca^{2+} ATPase was significantly increased ($p<0.01$) in the ISO-induced rat vs. control rat.

Table 1: Represents the efficacy of AA on heart weight, body weight and heart to body weight ratio in experimental animals

Groups	Body weight (g)	Heart weight (g)	Heart to b.wt. ratio (%)
Control	254.82±9.82	0.605±0.07	0.237±0.02
AA	256.77±8.07	0.612±0.09	0.238±0.03
ISO	251.25±9.90[a]	0.829±0.11[a$]	0.329±0.03[a$]
AA+ISO	252.63±10.05[b]	0.710±0.08[b$]	0.281±0.04[b$]

Values are expressed as the average (mean)±standard deviation for 8 rats in each group, p-value: $p<0.05, #p<0.01, Where "a" denotes the comparison between ISO and control group, "b"denotes the comparison between AA+ISO and ISO group

Table 2: Represents the efficacy of AA on serum cardiac markers in experimental animals

Groups	LDH (IU L^{-1})	CK-MB (IU L^{-1})	cTn T (ng mL^{-1})
Control	90.92±8.44	77.63±9.12	0.46±0.05
AA	89.13±9.30	79.91±8.50	0.49±0.05
ISO	156.70±11.05[a#]	145.74±12.62[a#]	1.61±0.12[a#]
AA+ISO	118.88±13.35[b#]	106.30±10.83[b#]	0.95±0.11[b#]

Values are expressed as the average (mean)±standard deviation for 8 rats in each group, p-value: $p<0.05, #p<0.01, Where "a" denotes the comparison between ISO and control group, "b"denotes the comparison between AA+ISO and ISO group

Table 3: Represents the efficacy of AA on cardiac antioxidants and lipid peroxidation product in experimental animals

Groups	Gpx (μg mg^{-1} pro)	CAT (U mg^{-1} pro)	SOD (U mg^{-1} pro)	MDA (nmols mg^{-1} pro)
Control	10.64±0.72	13.56±1.60	7.79±0.60	0.57±0.07
AA	10.92±0.91	14.28±1.45	8.01±0.82	0.52±0.06
ISO	6.60±0.80[a#]	8.90±1.05[a#]	4.56±0.34[a#]	0.85±0.11[a#]
AA+ISO	8.87±0.97[b$]	11.79±1.07[b#]	6.61±0.82[b$]	0.69±0.10[b$]

Values are expressed as the average (mean)±standard deviation for 8 rats in each group, p-value: $p<0.05, #p<0.01, Where "a" denotes the comparison between ISO and control group, "b"denotes the comparison between AA+ISO and ISO group

Table 4: Represent the efficacy of AA on the membrane-bound ATPases (heart tissue) in experimental animals

Groups	Na^{2+}/K^{+} ATPase	Ca^{2+} ATPase	Mg^{2+} ATPase
Control	0.315±0.06	0.830±0.09	0.081±0.001
AA	0.330±0.08	0.870±0.08	0.078±0.002
ISO	0.228±0.04[a#]	1.575±0.02[a#]	0.042±0.001[a#]
AA+ISO	0.291±0.02[b$]	1.111±0.10[b$]	0.066±0.001[b$]

Values are expressed as the average (mean)±standard deviation for 8 rats in each group, p-value: $p<0.05, #p<0.01, Where "a" denotes the comparison between ISO and control group, "b"denotes the comparison between AA+ISO and ISO group. Unit: The enzyme activity of μmole Pi (inorganic phosphate) liberated per minutes per milligram protein

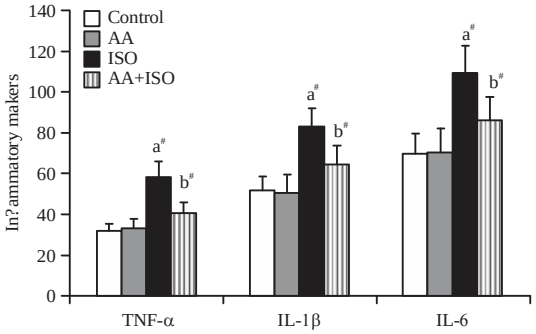

Fig. 1: Efficacy of AA on various inflammatory markers in experimental animals. Values are expressed as the average Mean±Standard deviation for 8 rats in each group. The p-value: $p<0.05, #p<0.01, Where "a" denotes the comparison between ISO and control group, "b"denotes the comparison between AA+ISO and ISO group

AA-treated rats followed by 2 days administration of ISO rats showed (AA+ISO) a significant restoration (normalcy, p<0.05) of the activities of cardiac membrane-bound ATPases (Na^{2+}/K^{+}, Mg^{2+}, Ca^{2+}) on comparison with MI model (ISO injected) rats.

In Fig. 1, it illustrated the efficacy of AA on various inflammatory markers in experimental animals. The mean concentration of various inflammatory markers including IL-6, IL-1β and TNF-α in the cytosolic fraction of cardiac homogenate was substantially escalated (p<0.01) in MI model rats. Nevertheless, the treatment with AA the levels of these inflammatory markers (IL-6, IL-1β and TNF-α) were considerably decreased (p<0.01) than in that of ISO administered MI group.

The Fig. 2 displayed the efficacy of AA on cardiac tissue morphological alterations in experimental animals after staining with Haematoxylin and eosin stain. The slides of control rats portrait the normal architecture with prominent

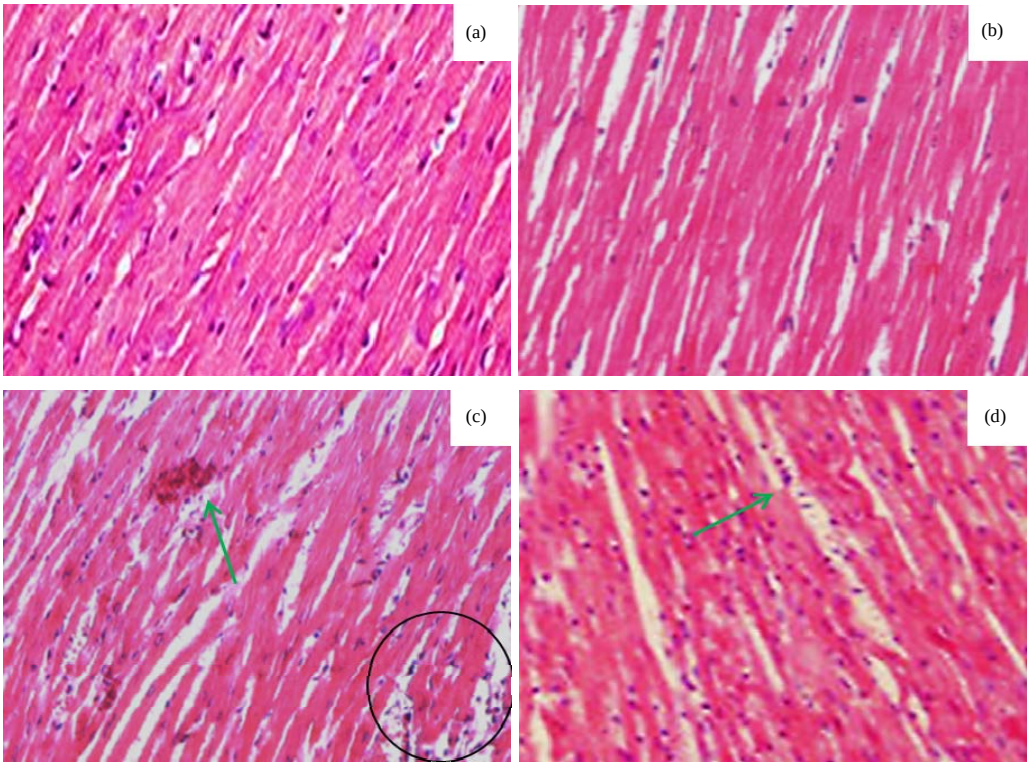

Fig. 2(a-d): Displays the efficacy of AA on cardiac tissue morphological alterations in experimental animals after staining with Haematoxylin and eosin stain. The cardiac tissue slides of control rats showed normal architecture with prominent myofibrillar structure (a). Likewise, AA alone treated rat cardiac tissue slides portrait the presence of normal myofibrillar structure without any evidence of pathological changes (b). Meanwhile, the slides of ISO-induced rats revealed the presence of disrupted or ruptured myofibrillar structure (arrow mark) with notable amount of neutrophil granulocytes infiltration (encircled), necrosis and edematous intracellular space (c). However, AA pre- and co-treated rats cardiac tissue slides illustrate the lesser presence of disrupted or ruptured myofibrillar structure (arrow mark) with least amount of neutrophil granulocytes infiltration, necrosis and edema (d)

myofibrillar structure (Fig. 2a). Likewise, AA alone treated rat slides showed the presence of normal myofibrillar structure without any evidence of pathological changes (Fig. 2b). Meanwhile, the slides of ISO-induced rats revealed the presence of disrupted or ruptured myofibrillar structure (arrow mark) with a notable amount of neutrophil granulocytes infiltration (encircled), necrosis and edematous intracellular space (Fig. 2c). However, AA pre- and co-treated rats slides illustrated the lesser presence of disrupted or ruptured myofibrillar structure (arrow mark) with least amount of neutrophil granulocytes infiltration, necrosis and edema (Fig. 2d).

DISCUSSION

In this study, no significant changes were observed in the body weight in all the experimental rats. However, the heart weight and heart to body weight ratio were considerably increased in ISO administered rats due to altered myocytes permeability and subsequently results in increased body weight. ISO-induced MI model is the most reliable and well accepted non-invasive method (nil or less mortality rate) to induce MI and moreover, the pathophysiology of ISO-induced MI is almost similar to the human MI[8,24]. Song and Si[2], hinted that induction of ISO increased the heart weight and thus increased in the heart to body weight is also increased. While pre- and co-treated with AA considerably reduced the heart weight and heart to body weight ratio owing to membrane protective/stability activity (antioxidant). Previously, Xu *et al.*[25], demonstrated that treatment with Asiatic acid could effectively prevent the cardiac hypertrophy and thus decrease the heart weight and heart to body weight ratio.

Ample amount of studies indicated that the major pathophysiological mechanism behind the ISO-induced MI is the auto-oxidation of catecholamines (quinones) and subsequently modify the membrane permeability and trigger inflammatory cascade and finally end up in necrosis of cardiomyocytes and MI[26,27]. The levels of different serum cardiac markers like LDH, CK-MB and cTn T were significantly increased in ISO injected rats because of overproduction of free radicals which results to loss of membrane integrity and thus enhance the movement of these cardiac markers from the damaged cardiomyocytes into the serum. Nonetheless, AA treatment considerably lowered the levels of these serum cardiac markers in owing to anti-lipid peroxidation activity (membrane stabilizing property).

The levels of lipid peroxidation product like MDA were considerably elevated with a significant decrease in the activities of enzymic antioxidants like CAT, SOD and Gpx were noted in ISO-induced rats because of increased oxidative stress. However, treatment with asiatic acid markedly suppressed the production of free radicals and thereby restored the activities of endogenous antioxidants. Another study also indicated that asiatic acid could significantly reduce the production of lipid peroxidation and thus alleviate oxidative stress by improving the activities of various enzymic antioxidants in diabetic rat model[28]. The activities of cardiac membrane-bound ATPases like Na^{2+}/K^{+} and Mg^{2+} ATPases were remarkably decreased in the ISO administered rats due to increased lipid peroxidation of cardiomyocytes which alter the movement of Na^{2+} and K^{+} as well as increased necrosis and apoptosis, contribute to decreasing the activity of both Na^{2+}/K^{+} ATPase and Mg^{2+} ATPase after ISO induction[29]. Whereas, the activities of Ca^{2+} ATPase is significantly increased in the ISO-induced rat due to altered movement of Na^{2+} and K^{+} ion which prevents the movement of Ca^{2+}ion out of the cell and results in increased accumulated in the cell (Ca^{2+} overload).The cardiomyocytes tend to increase the numbers of membrane-boundCa^{2+} ATPases to balance the levels of intra and extracellular Ca^{2+} concentration[30]. However, AA-treated rats showed a significant restoration (normalcy) of the activities of cardiac membrane-bound ATPases like Na^{2+}/K^{+}, Ca^{2+} and Mg^{2+} ATPases owing to the antioxidant, free radical scavenging and anti-apoptotic activities[12] and thus endorsing its membrane stabilizing activity.

Increased oxidative stress, could contribute to the inflammatory response and hence the concentration of various inflammatory markers including IL-6, IL-1β and TNF-α were notably increased in ISO-induce dMI rats. Nevertheless, the treatment with AA the levels of these inflammatory markers like IL-6, IL-1β and TNF-α were significantly decreased. This results are in agreement with the results of Yun *et al.*[31]. Lv *et al.*[32], hinted that asiatic acid display potent antioxidant and anti-inflammatory activities against lipopolysaccharides (LPS) and D-galactosamine induced hepatic injury by down-regulating the expression of various pro-inflammatory cytokines via NF-κB (inactivation of p65) and MAPK signaling pathway.

The slides of ISO-induced rats revealed the presence of disrupted or ruptured myofibrillar structure (cardiac hypertrophy) with a notable amount of neutrophil granulocytes infiltration, necrosis, fibrosis and edematous intracellular space. The cardiac slides of rats pre- and co-treated with AA for 7 days portrait lesser presence of disrupted or ruptured myofibrillar structure with least amount of neutrophil granulocytes infiltration, necrosis, fibrosis and edema. Likewise, Huo *et al.*[17], reported that addition of Asiatic acid would considerably prevent the cardiac hypertrophy and fibrosis and thus ensure its cardioprotective activity.

CONCLUSION

These findings showcased the cardioprotective potential of AA against ISO-induced MI model due to antioxidant, anti-inflammatory, anti-necrotic and apoptotic properties of AA. However, more studies are needed to endorse this result as well as the underlying mechanism of cardioprotective activity of AA, must be revealed before developing to a promising therapeutic agent.

SIGNIFICANCE STATEMENTS

This pre-clinical study indicates that treatment with AA for 7 days display potent cardioprotective property which could open a door for developing a novel therapeutic agent against myocardial injury (ischemia) for MI patients along with standard MI drugs and procedure.

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REFERENCES

1. Mnafgui, K., R. Hajji, F. Derbali, I. Khlif and F. Kraiem *et al.*, 2016. Protective effect of hydroxytyrosol against cardiac remodeling after isoproterenol-induced myocardial infarction in rat. Cardiovasc. Toxicol., 16: 147-155.
2. Song, S. and L.Y. Si, 2015. Klotho ameliorated isoproterenol-induced pathological changes in cardiomyocytes via the regulation of oxidative stress. Life Sci., 135: 118-123.

3. Goyal, S.N., C. Sharma, U.B. Mahajan, C.R. Patil and Y.O. Agrawal *et al*., 2015. Protective Effects of Cardamom in Isoproterenol-Induced Myocardial Infarction in Rats. Int. J. Mol. Sci., 16: 27457-27469.
4. Hemalatha, K.L. and P.S.M. Prince, 2016. Preventive effects of zingerone on cardiac mitochondrial oxidative stress, calcium ion overload and adenosine triphosphate depletion in isoproterenol induced myocardial infarcted rats. RSC Adv., 6: 112332-112339.
5. Frangogiannis, N.G., 2015. Pathophysiology of myocardial infarction. Compr. Physiol., 5: 1841-1875.
6. Skyschally, A., R. Schulz and G. Heusch, 2008. Pathophysiology of myocardial infarction: Protection by ischemic pre- and post-conditioning. Heart Cardiovasc. Dis., 33: 88-100.
7. Fathiazad, F., A. Matlobi, A. Khorrami, S. Hamedeyazdan and H. Soraya *et al*., 2012. Phytochemical screening and evaluation of cardioprotective activity of ethanolic extract of *Ocimum basilicum* L.(basil) against isoproterenol induced myocardial infarction in rats. DARU J. Pharm. Sci., Vol. 20. 10.1186/2008-2231-20-87.
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. Hassan, M.Q., M.S. Akhtar, M. Akhtar, S.H. Ansari, J. Ali, S.E. Haque and A.K. Najmi, 2015. Benidipine prevents oxidative stress, inflammatory changes and apoptosis related myofibril damage in isoproterenol-induced myocardial infarction in rats. Toxicol. Mech. Methods, 25: 26-33.
10. Li, H., Y.H. Xie, Q. Yang, S.W. Wang and B.L. Zhang *et al*., 2012. Cardioprotective effect of paeonol and danshensu combination on isoproterenol-induced myocardial injury in rats. PloS One, Vol. 7. 10.1371/journal.pone.0048872.
11. 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.
12. Lv, J., A. Sharma, T. Zhang, Y. Wu and X. Ding, 2018. Pharmacological review on asiatic acid and its derivatives: A potential compound. SLAS TECHNOL.: Trans. Life Sci. Innov., 23: 111-127.
13. Qi, Z., X. Ci, J. Huang, Q. Liu, Q. Yu, J. Zhou and X. Deng, 2017. Asiatic acid enhances Nrf2 signaling to protect HepG2 cells from oxidative damage through Akt and ERK activation. Biomed. Pharmacother., 88: 252-259.
14. Jiang, W., M. Li, F. He, Z. Bian and Q. He *et al*., 2016. Neuroprotective effect of asiatic acid against spinal cord injury in rats. Life Sci., 157: 45-51.
15. Huang, X., L. Zuo, Y. Lv, C. Chen and Y. Yang *et al*., 2016. Asiatic acid attenuates myocardial ischemia/reperfusion injury via Akt/GSK-3β/HIF-1α signaling in rat H9c2 cardiomyocytes. Molecules, Vol. 21. 10.3390/molecules 21091248.
16. Chan, C.Y., M.C. Mong, W.H. Liu, C.Y. Huang and M.C. Yin, 2014. Three pentacyclic triterpenes protect H9c2 cardiomyoblast cells against high-glucose-induced injury. Free Radical Res., 48: 402-411.
17. Huo, L., W. Shi, L. Chong, J. Wang, K. Zhang and Y. Li, 2016. Asiatic acid inhibits left ventricular remodeling and improves cardiac function in a rat model of myocardial infarction. Exp. Ther. Med., 11: 57-64.
18. Hemalatha, K.L. and P.S.M. Prince, 2015. Preventive effects of zingerone on altered lipid peroxides and nonenzymatic antioxidants in the circulation of isoproterenol-induced myocardial infarcted rats. J. Biochem. Mol. Toxicol., 29: 63-69.
19. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
20. Bonting, S.L., 1970. Sodium-Potassium Activated Adenosine Triphosphatase and Cation Transport. In: Membranes and Ion Transport, Bittar, E.E. (Ed.), Wiley Interscience, England, pp: 257-363.
21. Hjerten, S. and H. Pan, 1983. Purification and characterization of two forms of a low-affinity Ca^{2+}-ATPase from erythrocyte membranes. Biochimica Biophysica Acta (BBA)-Biomembr., 728: 281-288.
22. Ohnishi, T., T. Suzuki, Y. Suzuki and K. Ozawa, 1982. A comparative study of plasma membrane Mg^{2+}-ATPase activities in normal, regenerating and malignant cells. Biochimica Biophysica Acta (BBA)-Biomembr., 684: 67-74.
23. Fiske, C.H. and Y. Subbarow, 1925. The colorimetric determination of phosphorus. J. Biol. Chem., 66: 375-400.
24. Prince, P.S.M. and K.L. Hemalatha, 2018. A molecular mechanism on the antiapoptotic effects of zingerone in isoproterenol induced myocardial infarcted rats. Eur. J. Pharmacol., 821: 105-111.
25. Xu, X., L. Si, J. Xu, C. Yi and F. Wang *et al*., 2015. Asiatic acid inhibits cardiac hypertrophy by blocking interleukin-1β-activated nuclear factor-κB signaling *in vitro* and *in vivo*. J. Thoracic Dis., 7: 1787-1797.
26. Khan, V., S. Sharma, U. Bhandari, S.M. Ali and S.E. Haque, 2018. Raspberry ketone protects against isoproterenol-induced myocardial infarction in rats. Life Sci., 194: 205-212.
27. Priscilla, D.H. and P.S.M. Prince, 2009. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in wistar rats. Chem. Biol. Interact., 179: 118-124.

28. Ramachandran, V. and R. Saravanan, 2013. Asiatic acid prevents lipid peroxidation and improves antioxidant status in rats with streptozotocin-induced diabetes. J. Funct. Foods, 5: 1077-1087.
29. Lobo, R.O., B.K.C. Sagar and C.K. Shenoy, 2017. Bio-tea prevents membrane destabilization during Isoproterenol-induced myocardial injury. J. Microsc. Ultrastruct., 5: 146-154.
30. Bhaskaran, S.K. and P. Kannappan, 2017. Protective effect of *Azolla microphylla* on biochemical, histopathological and molecular changes induced by isoproterenol in rats. Biomed. Pharm., 89: 473-481.
31. Yun, K.J., J.Y. Kim, J.B. Kim, K.W. Lee and S.Y. Jeong *et al*., 2008. Inhibition of LPS-induced NO and PGE2 production by asiatic acid via NF-κB inactivation in RAW 264.7 macrophages: Possible involvement of the IKK and MAPK pathways. Int. Immunopharmacol., 8: 431-441.
32. Lv, H., Z. Qi, S. Wang, H. Feng, X. Deng and X. Ci, 2017. Asiatic acid exhibits anti-inflammatory and antioxidant activities against lipopolysaccharide and D-galactosamine-induced fulminant hepatic failure. Front. Immunol., Vol. 8. 10.3389/fimmu.2017.00785.