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Cardioprotective Effect of Mumie (Shilajit) on Experimentally Induced Myocardial Injury

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Abstract This study assessed the effects of mumie (shilajit) pre-treatment, a traditional drug which is well known in the ancient medicine of both east and west, on cardiac performance of rats subjected to myocardial injury. Animals were divided into control, M250, and M500 (received mumie at dosages of 250 and 500 mg/kg/day, orally for 7 days, respectively) main groups each consisting of two subgroups—with and without heart injury. On the 6th and 7th days, isoproterenol (ISO) (85 mg/kg i.p.) was injected (s.c.) to half of the animal subgroups to induce myocardial damage. On the 8th day, after hemodynamic parameter recordings, hearts were removed for further evaluation. Mumie pre-treatment had no significant effects on hemodynamic and cardiac indices of normal animals. When the cardiac injury was induced, mumie maintained the $\pm dp/dt$ maximum, attenuated the serum cardiac troponin I, and reduced the severity of cardiac lesions. Despite the mild positive effects of mumie on total antioxidant capacity and lipid proxidation index, no significant difference was observed among animal groups. The findings suggest the prominent cardioprotective effect of mumie against destructive effects of ISO. It seems that other mechanisms than reinforcements of antioxidant system are involved in this beneficial effect.

Keywords Mumie (shilajit) · Myocardial injury · $\pm dp/dt$ maximum · Cardiac troponin I · Total antioxidant capacity · Lipid proxidation index

Introduction

Cardiovascular diseases (CVDs) are the number one well-known causes of mortality and accounting for 30 % of total deaths. They also are the major cause of morbidity worldwide [1]. The World Health Organization (WHO) reported that heart attacks are responsible for 7.6 million deaths annually in the world [2]. Despite it peaked and its gradual decline in many developed countries, it is still rising in developing countries [3] and over 80 % of CVD deaths occur in low- and middle-income countries [2]. CVD(s) is associated with high economic cost; accordingly in 2010, Americans spent a staggering amount of $503.2 billion dollars on health care expenses to treat and prevent CVDs [4].

Traditional medicine with 1000 years of history and valuable experience can be promising to the prevention and treatment of various diseases [5]. WHO estimates that around 80 % of the world's population relies on traditional medicine for their health needs [6].

Mumie, also known as *Shilajit, Salajit, Shilajatu, Mumijo,* or *Mummiyo* is a pale brown to blackish-brown exudation of variable consistency extracted from layers of rocks in many mountain ranges of the world [7]. As an

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ancient traditional drug, it has been used for ages as a rejuvenator [8, 9] and for treating a number of pathological conditions including tumors and pimples, bone fractures, diseases of skin, neuralgia, arthritis, inflammation, asthma, angina, gastrointestinal, and genitourinary diseases [9]. Shilajit comprises of 60–80 % humus along with other organic components such as benzoic acid, hippuric acid, fatty acid, ichthyol, ellagic acid, resin, triterpenes, sterol, aromatic carboxylic acid, 3,4-benzocoumarins, amino acids, and phenolic lipids [10, 11]. The approximate composition of Asian mumie is 20 % minerals, 15 % protein, 5 % lipids, 5 % steroids, and also some carbohydrates, alkaloids, and amino acids [12].

Experimental studies have demonstrated that shilajit has beneficial effects on lipid profile, and this is comparable with that of simvastatin [13]. Additionally, it improves the blood glucose level and lipid profile in diabetic rats [14]. Also worth mentioning is that Gaikwad et al. [15] have recently indicated that low and high concentrations of shilajit have negative and positive chronotropic effects on Daphnia hearts, respectively.

Despite the abundant traditional evidence at hand in favor of protective and therapeutic effects of mumie on different human diseases and also its unique place in traditional texts such as Ayurveda, Siddha, and Unani medicine [6], the cardiovascular outcome of mumie administration has received less attention. Thus, the present study was conducted to investigate whether mumie has beneficial cardiovascular effects on rats or not—especially for those whose hearts are exposed to vulnerable conditions.

Materials and Methods

This study was conducted according to the national guidelines for animal studies (Ethic committee permission No 90/12KA-Kerman University of Medical Sciences).

Chemicals

Chemical materials were prepared as sodium thiopental from Sandoz (Austria), isoproterenol (ISO) from Sigma (UK), Troponin I assay kit from BioMerieux (France), total antioxidant capacity (TAC) assay kit from Randox Laboratory Ltd. (UK), and Mumie from the Pharmacy of Traditional Medicines (Kerman, Iran).

The mumie was suspended in tap water and then passed through a clean filter to remove the possible precipitated impurities. During the treatment period, suspension was prepared every day with the required concentrations (250 or 500 mg/ml).

Animal Groups

Experimental procedures were performed on Wistar rats which were 3 months old and weighed 250–300 g. The animals were housed in a temperature-controlled room and were allowed free access to rat regular diet and water. Then, they were randomly divided into three main groups: the control, mumie250, and mumie500. Each main group consisted of two subgroups within which the animals were exposed to or not heart injury: (CTL and ISO), (M250 and M250 + ISO), and (M500 and M500 + ISO). For 7 days, the control subgroups received 1 ml/kg of tap water by gavages daily. M250 and M250 + ISO subgroups were gavaged with the aqueous mumie suspension the dosage of 250 mg/kg/day, while M500 and M500 + ISO subgroups were gavaged with the aqueous mumie suspension the dosage of 500 mg/kg/day, respectively, for 7 days. These dosages of mumie were selected upon previous studies [16] and researches which showed that even the consumption of 5,000 mg/kg/day of shilajit in rat had no significant histopathological effects on the heart and other organs, and also studies had shown that the LD50 of mumie is >5,000 mg/kg of body weight [17, 18]. Two hour after receiving the doses of mumie or vehicle on the 6th and 7th days, ISO 85 mg/kg was injected (s.c.) to half of the subgroups in order to induce cardiac damage [19]. Then, 2 h after ISO injection in all animals on the 7th day, 1.5 ml of blood sample was taken under light ether anesthesia by retro-orbital puncture and centrifuged. The serum was stored at −20 °C for measurement of troponin I, a biochemical marker of myocardial injury, by VIDAS troponin I ultra assay [20].

Surgical Preparation and Experimental Protocol

On the 8th day, animals were anesthetized with sodium thiopental (50 mg/kg i.p.). Surgical preparation and hemodynamic parameters were recorded by the method described in previous studies [21, 22]. Briefly, the trachea was cannulated and animals had spontaneous breathing throughout the experiment. The left common carotid artery was cannulated with a filled catheter (saline with 15 IU/ml heparin) and connected to a pressure transducer and also a PowerLab analog to digital converter (AD Instruments, Australia) which recorded the heart rate (HR) and arterial blood pressure (BP). The other cannula was inserted into the left ventricle through the right carotid artery, and accordingly, the left ventricular pressure (LVP) was recorded. The mean arterial pressure (MAP) was calculated by "$\mathrm{MAP} = P_d + (P_s - P_d)/3$ formula," where P_d is the diastolic arterial pressure and P_s is the systolic arterial pressure. The maximum velocity of contraction ($+dp/dt$ max) and maximum velocity of relaxation ($-dp/dt$ max)

were calculated from the LVP trace [22]. At the end of the experiment, the animals were killed, their hearts were removed and washed with cold saline, and later a piece of heart apex was dissected, weighed, and homogenized in 5 ml of 0.1 M Tris–HCl buffer (pH 7.4) in ice-cold condition. After centrifuging, the clear supernatant solution was taken for biochemical analysis. The amount of total protein was measured by using the Lowry et al. [23] method. Malondialdehyde (MDA) levels, an index of lipid peroxidation which produced by oxidative elements activation, were estimated by the concentration of thiobarbituric acid reactive substances (TBARS) in heart tissue [24]. TAC in the heart tissues was determined by using Randox assay kit (according to the manufacturer's protocol) [25]. The remaining portions of the hearts were fixed using 10 % buffered formalin. After paraffin molding of the tissues, 5-μm-thick sections were prepared, stained with hematoxylin and eosin (H&E), and later examined microscopically by two pathologists blindfolded of animal grouping. The lesions were graded as follows: (0) nil; (1) minimum (focal myocytes damage); (2) mild (small multifocal degeneration with slight degree of inflammatory process); (3) moderate (extensive myofibrillar degeneration and/or diffuse inflammatory process); (4) severe (necrosis with diffuse inflammatory process) [22].

Statistical Analysis

Quantitative data are expressed as mean ± SEM, and comparisons were performed by one-way ANOVA followed by Tukey's post hoc test. Histopathological changes are reported qualitatively as the number of animals with different grades of myocardial lesions in each group. Also, statistical analyses were performed by using the nonparametric Kruskal–Wallis and pairwise differences by the Mann–Whitney *U* test (*F*). *p* values <0.05 were considered as statistically significant.

Results

Heart Function and Hemodynamic Findings

The 1-week administration of mumie with dosages of 250 and 500 mg/kg/day did not show any significant changes in systolic, diastolic, MAP, HR, left ventricular end-diastolic pressure (LVEDP), max d*p*/d*t*, and min d*p*/d*t* (±d*p*/d*t* max), in normal mumie subgroups compared with CTL subgroup (Table 1; Fig. 1). ISO-induced cardiac injury was associated with only a non-significant decreasing trend in the BP of all groups (Table 1). However, positive and negative max d*p*/d*t* significantly dropped in ISO group compared with CTL group ($p < 0.01$ and $p < 0.05$, respectively) (Fig. 1). Mumie pre-treatment prevented the reduction in these parameters in the presence of the cardiac injury in such way that there were no significant differences between the values of ±d*p*/d*t* max in M250 + ISO and M500 + ISO subgroups whenever compared with the values of the intact corresponding mumie and control subgroups (Fig. 1). Moreover, the level of LVEDP was greater in ISO and M250 + ISO subgroups but not statistically significant (Table 1).

Biochemical Findings

Mumie consumption alone had no significant effects on the serum level of cardiac troponin I (cTnI). As shown in Fig. 2, the serum level of cTnI significantly increased in control animal subgroups (ISO) which were exposed to ISO-induced heart injury ($p < 0.001$ vs. CTL, M250, and M500 subgroups). The pre-treatments with the above-mentioned doses of mumie decreased the cTnI level in animals which were exposed to ISO-induced heart injury that it only was significant in M500 + ISO whenever compared with ISO subgroup ($p < 0.05$) (Fig. 2). The hearts of the animals with myocardial lesions showed some degree of TAC reduction and MDA elevation, but the

Table 1 Cardiovascular indices in different animal groups

Groups	Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)	Mean arterial pressure (mm Hg)	Heart rate (beat/min)	LVEDP
CTL	119 ± 6	92 ± 4	101 ± 4	377 ± 13	4.5 ± 1.5
ISO	105 ± 5	84 ± 4	91 ± 4	387 ± 6	8.6 ± 1.4
M250	122 ± 6	97 ± 8	105 ± 8	390 ± 15	3.7 ± 1
M250 + ISO	115 ± 5	98 ± 6	104 ± 6	403 ± 12	8 ± 1.5
M500	123 ± 7	96 ± 6	105 ± 6	382 ± 13	4.3 ± 1.7
M500 + ISO	105 ± 4	89 ± 4	94 ± 4	354 ± 41	4.4 ± 0.9

Values are mean ± SEM, $n = 7$–9

n Number of animals; *CTL* control; *ISO* isoproterenol; *M250* animal subgroups which received 250 mg/kg/day of mumie; *M500* animal subgroups which received 500 mg/kg/day of mumie; *LVEDP* left ventricular end-diastolic pressure

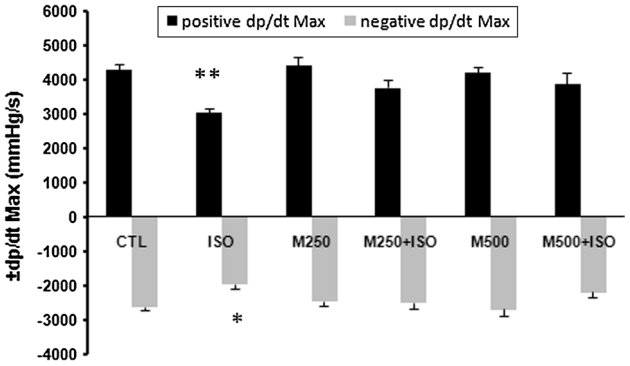

Fig. 1 Maximum contraction (+d*p*/d*t* max) and relaxation velocity (−d*p*/d*t* max) in different animal groups. Values are mean ± SEM, $n = 7$–9. *n* number of animals; *CTL* control; *ISO* isoproterenol; *M250* animal subgroups which received 250 mg/kg/day of mumie; *M500* animal subgroups which received 500 mg/kg/day of mumie. +d*p*/d*t* max, maximum velocity of heart contraction over time; −d*p*/d*t* max, maximum velocity of heart relaxation over time.*$p < 0.05$ and **$p < 0.01$ versus CTL group

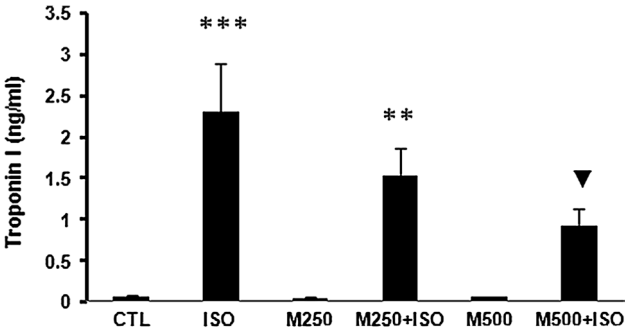

Fig. 2 Serum levels of cardiac troponin I in different animal groups. Values are mean ± SEM, $n = 7$–9. *n* number of animals; *CTL* control; *ISO* isoproterenol; *M250* animal subgroups which received 250 mg/kg/day of mumie; *M500* animal subgroups which received 500 mg/kg/day of mumie.***$p < 0.001$ versus CTL, M250, and M500 subgroups, **$p < 0.01$ versus CTL, M250 and M500 subgroups, ▼$p < 0.05$ versus ISO subgroup

difference was not statistically significant when compared with corresponding subgroups and other groups (Figs. 3, 4).

Histopathological Findings

The myocardial tissue examination revealed normal microscopic appearance in CTL, M250, and M500 subgroups (Fig. 5). ISO induced moderate-to-severe cardiac damage so that in the ISO group, 44.5 % of the animals' hearts showed extensive myofibrillar degeneration and/or diffused inflammatory process, 44.5 % of them showed necrosis with diffused inflammatory process, and only 11 % of the hearts were associated with mild lesions. Mumie consumption attenuated the destructive effects of

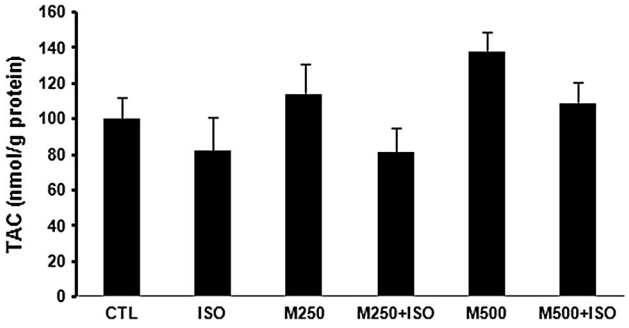

Fig. 3 Total antioxidant capacity (TAC) of heart tissue. Values are mean ± SEM, $n = 7$–9. *n* number of animals; *CTL* control; *ISO* isoproterenol; *M250* animal subgroups which received 250 mg/kg/day of mumie; *M500* animal subgroups which received 500 mg/kg/day of mumie

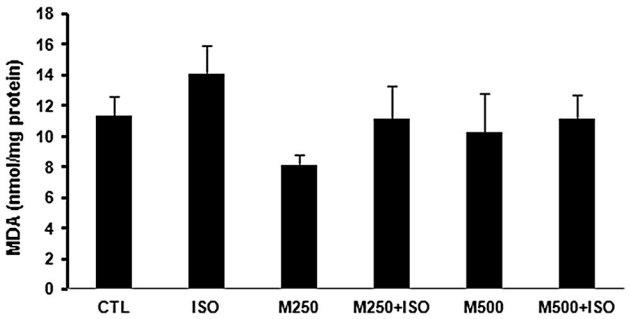

Fig. 4 Malondialdehyde (MDA) levels of heart tissue in different animal groups. Values are mean ± SEM, $n = 7$–9. *n* number of animals; *CTL* control; *ISO* isoproterenol; *M250* animal subgroups which received 250 mg/kg/day of mumie; *M500* animal subgroups which received 500 mg/kg/day of mumie

ISO on heart in such way that the severity of the injuries reduced significantly in M500 + ISO group versus the ISO group ($p < 0.05$) (Fig. 5; Table 2).

Discussion

Regarding the clinical importance of myocardial infarction as the major dangerous consequence of ischemic heart disease and its following outcomes, there is urgency for the development and also for the better assessment of new drugs for controlling and improving the degree of myocardial ischemia and infarction.

In the present study, we pre-treated the rats by mumie, after which the animals' hearts were exposed to damaging conditions by the injection of high doses of ISO in order to investigate whether mumie has protective effects on myocardial injury.

The findings of this study revealed that mumie had a prominent cardioprotective effect and it also attenuated the

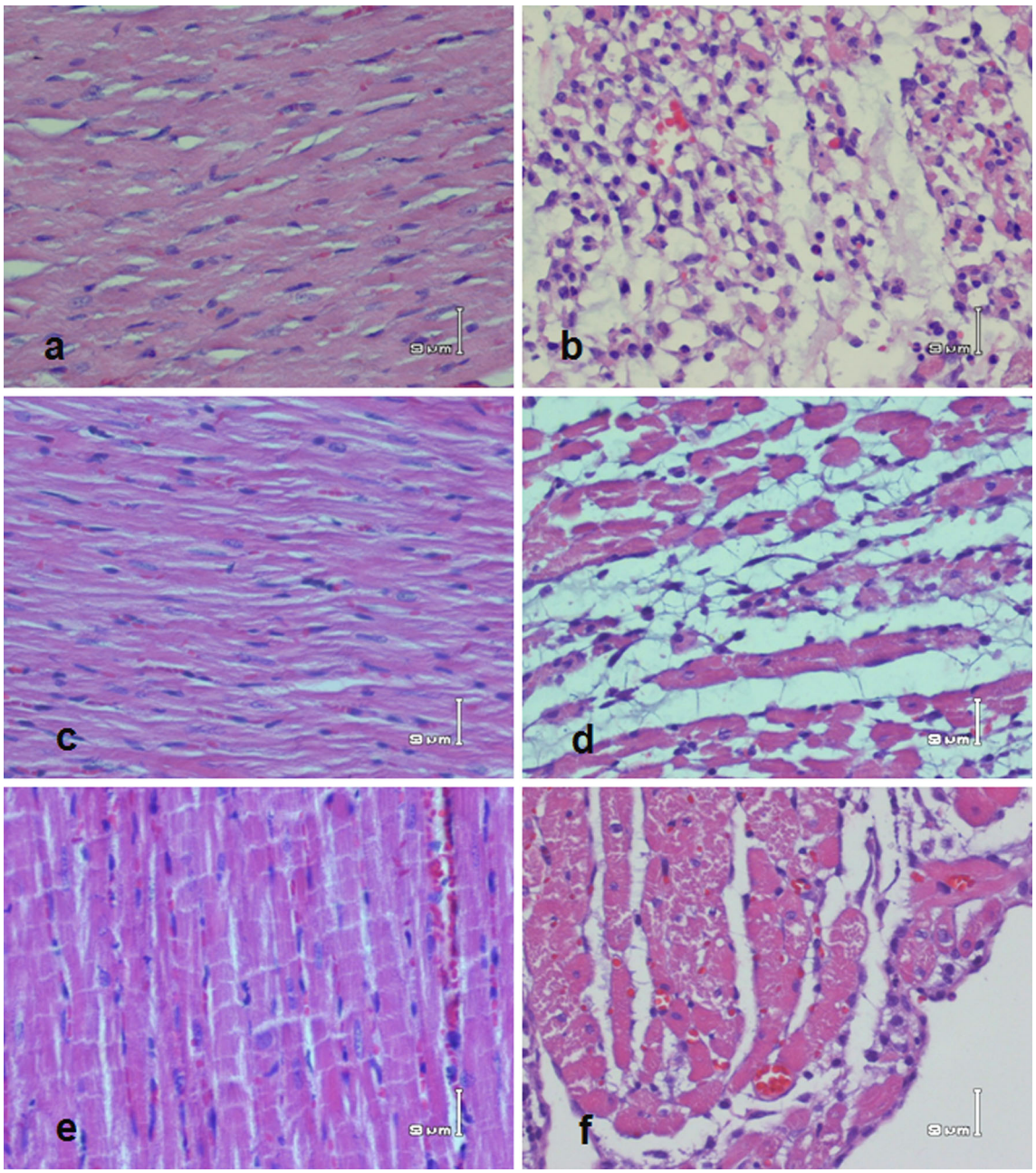

Fig. 5 H&E-stained sections of heart tissue in different animal groups. The magnification of is ×200. **a** CTL group heart sections showing normal appearance of cardiac myofibers. **c**, **e** Sections of animals' heart from mumie250 to mumie500 groups, respectively. Similar to the control group, the heart muscle appearance is normal in these two groups. **b** ISO group section showing severe myodegeneration and necrosis of muscle fibers, interstitial edema, and inflammatory cell infiltration. **d**, **f** Show sections of heart tissues from M250 + ISO to M500 + ISO groups, respectively. As it is evident, mumie consumption, especially at the dose 500 mg/kg, prominently attenuated the destructive effects of isoproterenol on cardiomyocytes. *ISO* isoproterenol

harmful effects of ISO on heart tissue and heart performance in rat.

Under normal conditions, 1-week consumption of mumie had no significant effects on BP, and $\pm dp/dt$ max as the main parameters of the cardiovascular system. On the other hand, mumie pre-treatment prevented the negative effects of ISO on max dp/dt and min dp/dt in such way that these parameters had no significant differences in comparison with the corresponding control subgroups. Low levels of serum cTnI and attenuation of myocardial lesions in mumie subgroups, which were exposed to ISO (especially M500 + ISO), confirmed the cardioprotective effects of mumie.

Cardiac troponin I (cTnI) is released during myocardial injury from the cytosolic pool of the cardiomyocytes with degradation of actin and myosin filaments. It is a powerful

Table 2 Histopathological scores and animal number with different degrees of injury in each subgroup

Groups	Myocardial pathology scores					
	0	1	2	3	4	Mean
CTL ($n = 9$)	9	0	0	0	0	0
ISO ($n = 9$)	0	0	1	4	4	3.33**
M250 ($n = 9$)	9	0	0	0	0	0
M250 + ISO ($n = 7$)	0	0	3	3	1	2.71**
M500 ($n = 7$)	0	0	0	0	0	0
M500 + ISO ($n = 7$)	0	1	4	1	1	2.29**▼

CTL control; *ISO* isoproterenol; *M250* animal subgroups which received 250 mg/kg/day of mumie; *M500* animal subgroups which received 500 mg/kg/day of mumie; *1* minimum (focal myocytes damage); *2* mild (small multifocal degeneration with slight degree of inflammatory process); *3* moderate (extensive myofibrillar degeneration and/or diffuse inflammatory process); *4* severe (necrosis with diffuse inflammatory process)

** $p < 0.01$ versus CTL, M250 and M500 subgroups, ▼ $p < 0.05$ versus ISO subgroup

candidate in mammals as a sensitive and tissue-selective diagnostic test for cardiac injury [26]. Increase in serum cardiac troponin levels is closely correlated with the severity of myocardial damage [27]. Based on previous studies, the high doses of ISO (a non-selective beta adrenergic agonist) induce the global heart ischemia which is associated with increased levels of serum cTnI. It also reduces heart contractility and relaxation velocity, causes a rising in ventricular end-diastolic pressure, and leads to heart lesions similar to myocardial infarction [20, 28, 29]; all the above was confirmed in the current study. ISO has positive chronotropic and inotropic effects which produce markedly increased workload and oxygen demand of myocardial muscle. Moreover, it enhances heart susceptibility to injury by increasing the activation of calcium channels, and hence, it leads to cytosolic calcium overload. The other mechanisms that have been suggested for cardiotoxicity of high doses of ISO are including the oxidative stress, changes in electrolyte contents, metabolism alteration, and the coronary insufficiency [30].

Recently, it has been reported that low concentrations of mumie (shilajit) have negative chronotropic effect; however, its high concentrations have positive chronotropic effect on Daphnia heart [15]. In the present study, the HR showed no significant differences among control animal subgroups, which this reflected the effect-less of the selected doses of mumie on HR. It is well known that normally about 70 % of the oxygen in the coronary arterial blood is removed as blood flows through the heart muscle [31]. Since not much oxygen is left, the blood oxygen reserve of the heart is lower than many other tissues. Under stressful conditions, for example a few moments after the injection of high doses of ISO, the heart is faced with increase HR and workload and in turn hypoxia and greater ATP utilization than production due to increase in demand, therefore the risk of myocardial injury increases. It is reported that by increasing the oxygen-carrying capacity of the blood and improving the circulation, mumie augments the tissue oxygen levels and can provide adequate oxygen during hypoxia [32, 33]. Therefore, the above-mentioned effect of mumie can be considered as a plausible reason for its cardioprotective effect which was observed in our study.

On the other hand, our findings showed that in presence of mumie, the degrees of congestion and leukocytes infiltration were both reduced in injured hearts. Previous studies demonstrated that mumie prevents degranulation of mast cells and inhibits the histamine release [32, 34] and it is able significantly to reduce the hind foot edema induced by carrageenan in rat [35]. Accordingly, the anti-inflammatory effect of mumie through reduction of histamine release and the stabilization of capillary permeability may explain the reduction of congestion and leukocytes infiltration in damaged hearts, which this was a remarkable finding of the present study.

Some studies have suggested an antioxidant role for mumie. Frolova et al. [16] indicated that mumie pretreatment (1 day and 1 h before hepatitis induction) non-significantly attenuated the increased level of MDA following tetrachloromethane-induced liver hepatitis. In agreement with our findings, Vivek et al. demonstrated that long-term shilajit pretreatments (for 90 days) significantly recovered the harmful effects of ISO on the heart (18). They showed that this positive effect was associated with the reduction of lipid proxidation and the elevation of reduced glutathione (GSH) levels along with low levels of lactate dehydrogenase (LDH) and creatinine kinase (CK) (18). In the present study, the insignificant lower level of MDA along with greater level of tissue TAC in the M500 subgroups with or without damaged hearts was observed. However, this finding in consistent with Frolova et al.'s study showed that mumie does not significantly change the oxidative stress or antioxidant capacity when measured at 1 day after treatment in the presence or absence of ISO-induced myocardial injury. On the other hand, Vivek et al. reported that long-term consumptions of mumie have an antioxidant property and provides its cardioprotective effects under heart stress conditions (18). However, they have not ruled out the other possible mechanisms involved in the cardioprotective effects of shilajit. It is noteworthy that we measured a more reliable factor (TAC) which represents the TAC of the heart, but Vivek et al. have merely measured the GSH as a part of antioxidant system of the heart tissue. Additionally, this study was conducted only on male rats, just like Frolova et al.'s study. This was Vivek et al. used both male and female rats. Also, the disparity in the

duration of mumie consumption in these studies is likely to be the other reason which can explain the different levels of MDA.

The positive effects of mumie on the cardiac performance indices (especially on max d*p*/d*t*, an important index of cardiac contractility, and min d*p*/d*t*, an important index of cardiac relaxation velocity) can be explained by its ability to protect and prevent the myocardial cells from severe injury induced by ISO through the above possible mechanisms, which results in the better functioning of the heart.

In conclusion, the present findings demonstrate that mumie has prominent protective effects against acute myocardial injuries. The plausible mechanisms for this valuable effect may be derived from some favorable physiological alterations including the increase in myocardial oxygen supply, the stabilizing of membrane capillaries and the attenuating of inflammation and congesting of the heart tissue, and to some extent inhibiting the formation of the oxygen free radical and subsequent peroxidation of lipid and consequently ameliorating the severity of cardiac damage, and systolic/diastolic dysfunction. However, in the present study, the reinforcement of the antioxidant system is likely to not be the main mechanism involved in this beneficial effect. These findings may contribute to better understandings of the beneficial effects of mumie against ISO-induced heart injury and contributes to training prospective therapeutic agent in clinical management of myocardial ischemia and infarction. However, further studies are needed to clarify the detailed mechanisms and also its clinical value.

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