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The protective and therapeutic effects of agmatine on isoproterenol-induced myocardial injury in rats

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Abstract

Chronic inflammation and oxidative stress can contribute to the development of cardiovascular diseases. Isoproterenol (ISO), a synthetic catecholamine, is used to study the effects of drugs on cardiotoxicity. Agmatine (AGM) is a type of biogenic amine produced through the decarboxylation of arginine. The purpose of the current study was to evaluate the effects of AGM against ISO-induced cardiotoxicity due to the described roles of AGM in cardiovascular disease. Four groups of thirty-two Wistar Albino rats were divided equally as control, ISO, AGM+ISO, and ISO+AGM. ISO was administered intraperitoneally (i.p.) twice at a dose of 150 mg/kg, at 24-hour intervals. Prior to and after ISO injection, 20 mg/kg of AGM was injected i.p. Hemodynamic measurements and serum and tissue biochemical analyses were evaluated at the end of the experiment. The levels of glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) in the tissue were measured. In the ISO group, levels of lactate dehydrogenase (LDH) and creatine kinase (CK) were increased significantly ($p<0.05$). Co-administration of ISO and AGM significantly reduced CK and LDH levels ($p<0.05$). MDA levels increased in the ISO-treated group but decreased in the AGM-treated groups ($p<0.05$). Notably, there was a reduction in the CAT level in the ISO treatment group ($p<0.05$). CAT was found to be significantly increased ($p<0.05$) in the groups that received AGM compared to the ISO group. AGM+ISO group had a reduced density of degenerated cardiomyocytes and granulation tissue compared to the ISO group ($p<0.05$). Although granulation tissue demonstrated a significant reduction in the ISO+AGM group in comparison to the ISO group ($p<0.05$). In this study, the results indicate that AGM treatment can potentially inhibit ISO-induced myocardial injury and vascular dysfunction by preserving vascular integrity. Notably, the protective effects of AGM on cardiac damage appear to outweigh its therapeutic benefits, as shown by histopathological analysis.

Keywords: Agmatine, isoproterenol, myocardial injury, oxidative stress, rat

Introduction

Ischemic heart disease is one of the leading causes of sudden death in the world. Complications that may develop after myocardial infarction (MI) may cause irreversible damage to myocardial functions [1]. For this reason, there is a need for new preventive and therapeutic agents before or after MI. The most basic feature of MI is cardiomyocyte necrosis. Along with the increase in cardiac biomarkers, ischemic electrocardiography (ECG), changes also guide the diagnosis. Imaging of regional myocardial movement disorder also supports the diagnosis of MI [2].

Isoproterenol (ISO) is a synthetic sympathomimetic catecholamine that stimulates β_1 and β_2 receptors. Significant histopathological and biochemical alterations are brought about by the prolonged activation of β-adrenergic receptor agonists with a high dosage of ISO [3]. ISO-induced MI is a recognized procedure and is frequently applied to examine the effects of many drugs on cardiac function [4]. ISO causes rapid autoxidation and the formation of oxidative products in the myocardium [5]. However, ISO depletes the energy reservoir within the cells of the heart muscle. Necrosis is the ultimate result of several metabolic

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and structural alterations caused by ISO. [5]. In a study, it was emphasized that high-dose ISO administration to rats was similar to the biochemical, physiopathological, and histopathological changes of MI in humans [6].

Agmatine (AGM) (4-[aminobutyl]guanidine) is an imidazoline receptor agonist and can interact with many receptors. AGM is a vasoactive metabolite of L-arginine. It causes various pharmacological effects by binding to imidazoline and α_2-adrenoceptors. AGM has been known to have anti-depressant, anti-inflammatory, and neuroprotective activities in recent years [7].

There have been some studies examining the impact of AGM on the cardiovascular system. In the ischemia rat model, administration of AGM before ischemia was able to provide hemodynamic improvement due to its vasodilator feature [8]. AGM also showed a protective effect against oxidative stress [9]. It has been reported that AGM activates endothelial nitric oxide synthase. It was also emphasized that AGM regulates Ca^{+2} reuptake and cardiac inotropy [10].

As a result, we proposed that AGM could have cardioprotective benefits by reducing oxidative stress and suppressing MI-related myocardial and vascular remodeling. The ISO-induced MI model was developed for the current study to determine the impacts and underlying processes of AGM on cardiac damage in rats.

Material and Methods

The study was approved by the İnönü University Animal Experiments Local Ethics Committee (protocol number: 2015/A-74). The rats were kept in an environment with a temperature of 20-25°C and a humidity of 60% under a 12-hour/12-hour light/dark cycle. Thirty-two three month-old *Wistar albino* adult rats weighing 250-300 g were acquired from Inonu University Experimental Animal Production and Research Center for this study. The experimental groups were randomized simply as previously determined.

Control Group: No drugs were administered, only vehicle solutions were administered intraperitoneally (i.p.) (n=8).

ISO Group: ISO at a dose of 150 mg/kg was administered i.p. in two doses at 24-hour intervals (n=8).

AGM+MI Group: After 20 mg/kg AGM administration i.p. for 3 days, ISO was administered i.p. in two doses at 24-hour intervals (n=8).

MI+AGM Group: ISO was administered i.p. in two doses at 24-hour intervals. Then 20 mg/kg AGM was administered i.p. for 3 days (n=8)

The recommended dosages outlined in the ISO and AGM literature were administered i.p. [11,12]. To conclude the experiment, rats were anesthetized with 1.2 g/kg of ethyl carbamate (also referred to as urethane) i.p. 48 hours after the second ISO treatment. Three limb electrodes were placed on the right anterior limb, left anterior limb, and left lower limb. ECG recordings were obtained from lead D2. Heart rates (HR) were evaluated from ECG. Blood pressure (BP) was measured through carotid artery cannulation. The transducer was employed to capture information from the automated support system for the MP100 Data Acquisition Module produced by Biopac Systems. At the end of the experiment, blood, heart and descending aorta tissues were collected from the rats under high-dose anesthesia.

Serum biochemical analysis

After centrifuging the collected blood at 2500 rpm for 10 minutes, the serum was separated. We analyzed the serum biochemistry by purchasing the services of the Central Laboratory of İnönü University Turgut Özal Medical Center.

Heart biochemical analysis

Malondialdehyde (MDA) analysis

Using the Mihara and Uchiyama technique [13], tissue MDA levels were measured spectrophotometrically at 535 nm and 520 nm. MDA concentrations were determined by using the standard graph of 1,1,3,3-tetramethoxypropane. The results were presented as nmol/gram wet tissue (gwt).

Glutathione (GSH) analysis

The Ellman technique [14] was employed to ascertain GSH levels. The method relies on the response of reduced GSH with 5,5'-dithiobis-(2-nitrobenzoic acid). The sample from the rat heart was spectrophotometrically analyzed at 410 nm, and the results were interpreted from the glutathione standard graph and reported as μmol/gwt.

Superoxide dismutase (SOD) analysis

The Sun et al. technique was used to determine CuZn-SOD activity, and samples were read spectrophotometrically at 560 nm [15]. As U/g protein, enzyme activity was reported.

Catalase (CAT) analysis

The Luck technique was employed to quantify catalase activity in samples of cardiac tissue. The spectrophotometric assessment of CAT activity in heart tissue samples was performed by breaking down 30 mmol/L of H_2O_2 in 50 mmol/L of potassium phosphate buffer (PH 7.0) at 240 nm at intervals of 15 s over a duration of 150 s. The evaluation was carried out on the time interval of linear absorbance decline [16]. Enzyme activity measurement was determined as K/g protein.

Protein assay

Protein determination in tissue was performed by Biuret protein assay using bovine serum albumin as standard [17].

Histopathological analysis

At the end of the experiment, tissues from the heart and descending aorta were removed and preserved in 10% formaldehyde. Following fixation, specimens were dehydrated in an alcohol series of 70%, 80%, 90%, 96%, and 99%. The

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tissue samples were then embedded in paraffin after being cleaned with xylene. After tissue tracing, paraffin blocks were prepared and sections of 4-5µm thickness were cut from those blocks. Hematoxylin-eosin (H-E) staining was used to identify the general morphological structure of the sections.

Congestion-hemorrhage, granulation tissue, interstitial edema, and cardiomyocyte degeneration (myofibril loss, vacuolization, dense eosinophilic cytoplasm, and pyknotic nucleus) were all evaluated in heart sections. Ten randomly chosen regions were inspected, and the areas were graded as follows: 0: no change, 1: mild change, 2: moderate change, and 3: severe change.

Tunica intima-media thickness was evaluated in ten randomly chosen regions from each of the sections for the assessment of the aorta. Additionally, the entire region was inspected, and the severity of histological alterations (thinning and rupture of elastic lamellae) seen in the vessel wall was graded as 0: no change, 1: mild, 2: moderate, and 3: severe change. Leica Micros Imaging Solutions Ltd., Cambridge, UK, provided the Leica DFC-280 research microscope and Leica Q Win Image Analysis System for use in the analyses.

Statistical analysis

The statistical software application SPSS (SPSS for Windows version 17) was used for the statistical analysis. The ANOVA test was used to assess parametrically distributed data. The Kruskal-Wallis H test was used for intergroup comparison for non-parametric distributed data. The median (minimum-maximum) and mean±standard deviation were used to express the data. The statistically significant was defined as $p<0.05$.

Results

Haemodynamics and ECG analysis results

There was no significant difference between the all groups for HR and mean BP ($p>0.05$) (Table 1).

Arrhythmia or ischaemic ECG changes were not observed in rats in the control group. In the ISO group, ST depression was observed in one rat and T negativity was observed in two rats. In the AGM+ISO group, ST depression was observed in one rat, block in one rat, and T negativity in three rats. In the ISO+AGM group, ST depression was observed in two rats, block in three rats, and T negativity in two rats (Figure 1).

Arrhythmia was not observed in any of the experimental groups (Table 2).

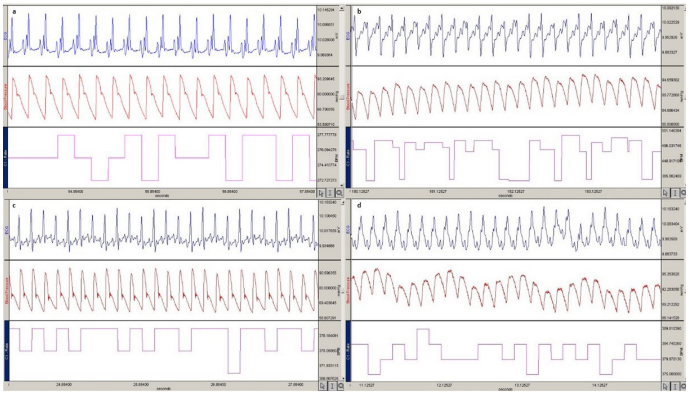

Figure 1. ECG changes observed in the experimental groups. **1a.** Normal ECG, **1b.** ST depression, **1c.** T negativity, **1e.** Bundle Brunch Block

Table 1. Heart rate and mean blood pressure values

	HR (beats/minute)	Mean BP (mmHg)
Control	340.63±69.00	80.75±9.85
ISO	367.50±21.41	84.50±15.63
AGM+ISO	345.75±54.34	76.88±4.02
ISO+AGM	338.25±69.00	79.25±8.05

There was no significant difference between all groups for HR and mean BP ($p>0.05$)

Table 2. ECG changes of the groups

	n	Arrhythmia	ST depression	Bundle brunch block	T negativity
Control	8	0	0	0	0
ISO	8	0	1	0	2
AGM+ISO	8	0	1	1	3
ISO+AGM	8	0	2	3	2

Biochemical results

When the serum biochemistry findings were evaluated, there was a significant difference for the creatine kinase (CK) parameter between the ISO group and the control group, AGM+ISO group, and ISO+AGM group ($p<0.05$). Comparing the ISO group to the other groups, the CK level increased substantially in the ISO group ($p<0.05$). Comparing the ISO group to the control

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and AGM+ISO groups, the ISO group's lactate dehydrogenase (LDH) level was substantially higher ($p<0.05$). In terms of troponin levels, there was not a significant difference between any of the groups ($p>0.05$) (Table 3).

The ISO and control, AGM+ISO, and ISO+AGM groups differed significantly in terms of the MDA parameter, according to the heart tissue biochemistry data ($p<0.05$). Comparing the ISO group to the other groups, the MDA level was considerably higher in the ISO group ($p<0.05$). Regarding the CAT parameter, there was a significant difference between the ISO group and control, the AGM+ISO, and ISO+AGM groups ($p<0.05$). Comparing the ISO group to the other groups, the CAT level was considerably lower in the ISO group ($p<0.05$). SOD and GSH levels did not significantly differ between any of the groups ($p>0.05$) (Table 4).

Table 3. Serum biochemistry results

	CK (U/L)	Troponin (pg/mL)	LDH (U/L)
Control	1304.75±830.35[a]	5171.00±3599.46	379.88±124.46[a]
ISO	2658.13±881.38	9551.35±15467.49	795.25±547.34
AGM + ISO	1387.25±916.61[a]	5102.74±3150.69	346.00±148.29[a]
ISO + AGM	1471.88±687.26[a]	4807.19±1573.91	380.50±180.05

CK: creatine kinase LDH: lactate dehydrogenase, [a]there is a statistically significant difference compared to the ISO group ($p<0.05$). There is a significant difference between ISO and control, AGM+ISO, ISO+AGM groups in terms of CK, there is a significant difference in terms of LDH between ISO and control, AGM+ISO groups

Table 4. Heart tissue biochemistry results

	MDA nmol/gwt	SOD U/g protein	CAT K/g protein	GSH µmol/gwt
Control	130.79±26.45[a]	261.74±29.18	489.63±64.17[a]	607.45±99.26
ISO	267.89±57.64	266.98±29.66	220.33±85.24	567.77±182.12
AGM+ISO	128.75±14.61[a]	275.65±28.39	477.26±124.10[a]	472.43±71.05
ISO+AGM	131.73±25.42[a]	274.22±42.09	484.33±76.48[a]	589.90±209.49

MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase, GSH: Glutathione, [a]There is a statistically significant difference compared to the ISO group ($p<0.05$). There is a significant difference between ISO and control, AGM+ISO, ISO+AGM groups in terms of MDA, there is a significant difference in terms of CAT between ISO and control, AGM+ISO, ISO+AGM groups

Histopathological results

The cardiac tissue in the control group showed a normal histopathological appearance with the exception of some minor changes (Figure 2A, 2B).

The myocardium in the ISO group showed significant histopathological changes. Granulation tissue was the most prominent change (Figure 3A).

In some of these areas, granulation tissue replaced myocardial tissue. Increased density of degenerating cardiomyocytes and interstitial edema were two other notable changes seen in the ISO group compared to control group (Figure 3.B) ($p<0.05$).

Granulation tissue and cardiomyocyte density decreased significantly in the AGM+ISO group compared to the ISO group ($p<0.05$) (Figure 4A, 4B).

Granulation tissue decreased more in the ISO+AGM group than in the ISO group ($p<0.05$), but other degenerative changes did not decrease statistically significantly ($p>0.05$) (Figure 5A, 5B).

Comparing the AGM+ISO and ISO+AGM groups, the ISO+AGM group had a significantly higher density of degenerated cardiomyocytes ($p<0.05$) (Table 5).

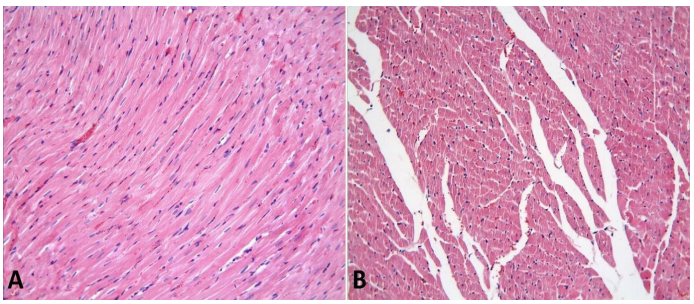

Figure 2. Normal histological appearance of myocardial tissue in longitudinal **2A.** and transverse **2B.** planes in the control group, H-Ex20

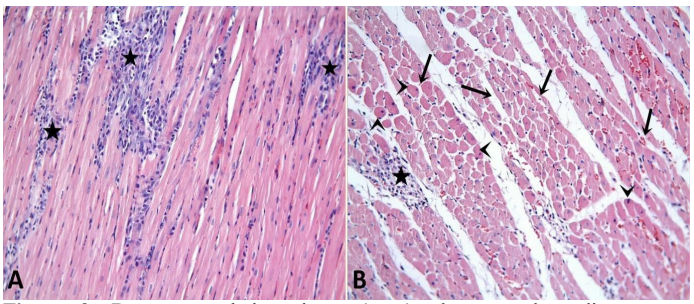

Figure 3. Dense granulation tissue (stars), degenerated cardiomyocytes (arrowheads) and interstitial edema (arrows) are observed in ISO group, H-E x20

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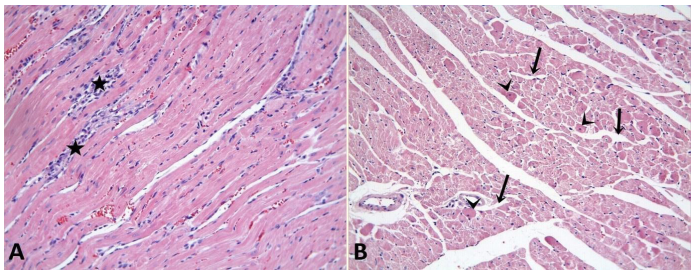

Figure 4. In the AGM+ISO group, granulation tissue (stars) and degenerated cardiomyocytes (arrowheads) were significantly reduced in the myocardial tissue (arrows indicate interstitial edema), H-E x20

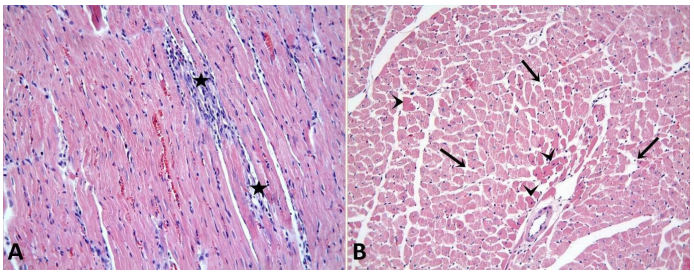

Figure 5. There is a decrease in granulation tissue (stars) in the ISO+AGM group, but other changes persist (arrows indicate interstitial edema, arrowheads indicate degenerated cardiomyocytes), H-E x20

Table 5. Histological score rezsults in myocardial tissue

Groups	Congestion-haemorrhage	Interstitial edema	Granulation tissue	Degenerate cardiomyocyte
Control	0.0 (0.0-2.0)	0.0 (0.0-2.0)	0.0 (0.0-0.0)	0.0 (0.0-3.0)
ISO	0.0 (0.0-3.0)	1.0 (0.0-3.0)[a]	1.0 (0.0-3.0)[a]	1.0 (0.0-3.0)[a]
AGM+ISO	0.0 (0.0-3.0)	1.0 (0.0-3.0)	0.0 (0.0-3.0)[b]	0.0 (0.0-3.0)[b]
ISO+AGM	0.0 (0.0-3.0)	2.0 (0.0-3.0)	0.0 (0.0-3.0)[b]	1.0 (0.0-3.0)[c]

[a]Significant increase compared to the control group ($p<0.05$), [b]Significant decrease compared to the ISO group ($p<0.05$), [c]Significant increase compared to the AGM+ISO group ($p<0.05$)

Descending aorta tissue

The vessel wall appeared in the control group's sections in a histopathologically normal pattern. (Figure 6. A). In this group, tunica intima-media thickness was 80.27±8.68 µm. In the ISO group with a tunica intima-media thickness of 69.80±4.41 µm (Figure 6B), disruption of vessel wall integrity was observed in some sections. The difference between control and ISO groups in terms of tunica intima-media thickness was statistically significant ($p<0.05$). In AGM+ISO and ISO+AGM groups, tunica intima-media thickness was 76.24±8.00 and 75.59±7.84 µ, respectively (Figure 6C and 6D).

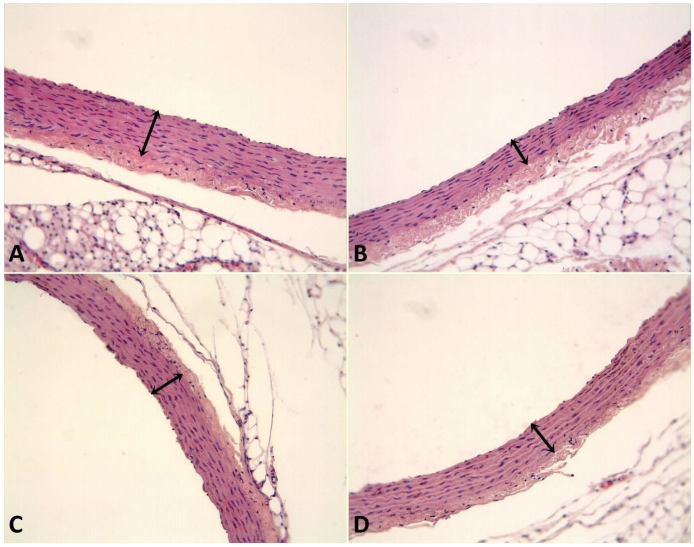

Figure 6. Normal histological appearance of the descending aorta in the control group (**6A**). Thinning of the tunica intima-media in the ISO group (**6B**) and increased tunica intima-media thickness in the AGM+ISO (**6C**) and ISO+AGM (**6D**) groups compared to the ISO group. Double-headed arrows indicate tunica intima-media thickness, H-E x20

The tunica intima-media thickness of both groups was found to be statistically significantly higher compared with the ISO group ($p<0.05$). On the other hand, no statistical difference was observed between the AGM+ISO and ISO+AGM groups ($p>0.05$) (Table 6).

Table 6. Tunica intima-media thickness and descending aorta histological score results

Groups	Tunica intima-media thickness
Control	80.27±8.68[a]
ISO	69.80±4.41
AGM+ISO	74.99±8.18[a]
ISO+AGM	75.59±7.84[a]

[a]Significant increase compared to the ISO group ($p<0.05$)

Discussion

The primary outcome of this research was that ISO caused histopathological adverse effects in both the myocardium and the aorta. ISO caused inflammation in myocardial tissue and impaired vascular integrity in the aorta. The addition of AGM to ISO improved the histopathological changes. On the other hand, the use of AGM as a protective agent was more effective than its therapeutic properties. ISO raised the level of MDA, which is one of the parameters of oxidative stress, and reduced the level of CAT, an antioxidant. Administration of AGM before or after ISO administration resulted in a decrease in MDA levels and an increase in CAT levels.

Myocardial necrosis in type 2 MI occurs when there is an imbalance between the supply and demand of oxygen to the myocardium, as opposed to coronary plaque instability. Hypotension, hypertension, tachyarrhythmias, bradyarrhythmias, anemia and hypoxemia are some of the mechanisms involved [2].

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ISO is a synthetic compound that acts as a non-selective beta-adrenergic receptor agonist. It acts primarily on beta-1 and beta-2 adrenergic receptors in the heart and other tissues. ISO is often used in experimental settings to induce cardiotoxicity in the cardiovascular system [6]. ISO-induced cardiotoxicity refers to the adverse effects on the heart that can result from excessive stimulation of beta-adrenergic receptors. Some effects associated with ISO-induced cardiotoxicity include arrhythmia, MI, cardiac hypertrophy, and impaired contractility [18]. Treatment with antioxidants may prevent ISO-induced cardiac dysfunction [19].

Decarboxylation of L-arginine produces AGM, a cationic amine with potent biological activity. AGM exhibits a strong affinity for imidazoline and two adrenergic receptors and is present in numerous mammalian tissues [20]. The highest concentrations of AGM are found in the stomach, aorta, and small intestine, while lower concentrations are found in the spleen, adrenal gland, skeletal muscle, and brain [21].

One of the risk factors for MI is atherosclerosis. AGM exhibits a defensive effect on mitochondria and regulates fatty acid metabolism. An extended treatment of agmatine has been shown to inhibit atherosclerosis in mice [22]. Prior research suggests that AGM has a beneficial inotropic effect and binds to imidazoline, α/β-adrenergic, and dopaminergic receptors. Therefore, AGM has been shown to have the potential to increase cardiac contractility [23]. The effect of AGM on vascular structure has mainly been researched, with studies showing that AGM binds to the imidazoline receptor located in endothelial cells and may induce the production of nitric oxide by increasing cytosolic calcium [24].

In a study on the effect of AGM on BP, systemically administered AGM decreased sympathetic nerve activity and arterial BP by inhibiting sympathetic ganglionic transmission. On the other hand, the central administration of the AGM had the opposite effect. In our study, we did not observe any difference between the groups in HR and BP values. We may have obtained this result due to the injection route or the dose used [25].

It is well shown that ISO induces oxidative stress in cardiac tissue, causing a reduction of antioxidant enzymes and resulting in toxicological alterations. Furthermore, ISO leads to a deficit in oxygen supply, which leads to myocardial hypoxia and subsequent cardiac necrosis. Elevated lipid peroxidation contributes to cellular leakage and cardiac hypertrophy [26]. The ISO groups displayed ischaemic ECG changes, notably T negativity and ST segment depression. Increased levels of CK, a cardiac muscle enzyme, and LDH, an intracellular enzyme, also supported the development of cardiac injury in the ISO group. The reduction of CK level in the AGM-treated groups may predict an improvement in cardiac injury. Ergosterol reduced the severity of myocardial pathological damage and the levels of creatine kinase myocardial band (CK-MB) and LDH in the blood, as observed by our work [27].

In this study, the level of MDA - an indicator of lipid peroxidation - was noted to be higher in the ISO group. However, the addition of AGM to ISO treatment led to a decrease in the MDA level, revealing the potential of AGM in reducing oxidative stress. Interestingly, administering AGM before or after ISO resulted in a similar reduction in oxidative stress. In this investigation, the level of CAT was observed to be lower in the ISO group. CAT level increased in the groups in which AGM was added to ISO, suggesting that AGM enhances the antioxidative mechanism. However, no significant difference was observed between its preventative or therapeutic application.

Recently it was reported that ISO induces histopathological damage to the myocardium. Another study showed a significant increase in inflammation and severe cardiac damage due to ISO. In addition, various signaling pathways are activated and lead to an increase in cellular death [26]. In the current study it was found significant cardiotoxicity in the ISO group through histopathological analysis. Cardiomyocyte degeneration, interstitial edema and granulation tissue were observed in this group. In the groups where ISO was combined with AGM, an improvement in myocardial tissue was observed, evidenced by a decrease in granulation tissue. Additionally, the group pretreated with AGM showed reduced levels of cardiomyocyte degeneration. These histopathological findings demonstrate that AGM pretreatment offers enhanced protection against ISO cardiotoxicity. Consistent with the findings of the present study, in the ischemia-reperfusion rat model, low-dose AGM given before and after ischemia and high-dose AGM given only before ischemia had positive, protective effects on hemodynamic recovery of isolated rat hearts undergoing global ischemia and reperfusion. [28]. In a comparable study investigating cardiotoxicity, ISO treatment led to a decline in SOD, CAT, and glutathione peroxidase (GPx) levels and an increase in total oxidative stress. Furthermore, ISO treatment resulted in adverse morphological alterations in cardiomyocytes [29]. No previous research has assessed the impact of AGM on ISO-induced cardiac damage, but AGM treatment has been shown to alleviate myocardial damage in rats with doxorubicin-induced cardiotoxicity and also increase total antioxidant capacity [30].

In this study, the histopathological examination of the descending aorta revealed that the administration of ISO had a detrimental effect on the integrity of the vessel wall, leading to a thinning of the wall. However, this damage was improved in the groups that received ISO and AGM together. Previous research conducted on rabbits demonstrated that AGM can effectively alleviate oxidative stress, reduce nitric oxide production, and enhance vascular endothelial integrity [31]. In rats with sepsis, the administration of AGM reduced the increased MDA level in the aorta. Conversely, AGM increased the amounts of SOD and GSH [32].

To demonstrate myocardial damage, measurement of natriuretic peptide levels could have been conducted. Furthermore, ventricular function and the aorta could have been evaluated via echocardiography. These limitations should be taken into consideration in future studies.

Conclusion

AGM is a molecule with neurotransmitter properties that interact

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with many structurally different receptors. The endogenous substance AGM may be beneficial in the event of MI due to its oxidative stress-inhibiting activities and has an acceptable therapeutic potential against ISO-induced cardiac injury. AGM may be a therapeutic and protective agent in cardiovascular diseases if its efficacy and safety can be demonstrated in clinical trials.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The Scientific and Technological Research Council of Türkiye (TÜBİTAK) supported this project. (Application number:1919B011503507).

Ethical Approval
The approval of the ethics committee was obtained from the ethics committee of animal experiments of İnönü University (Protocol number: 2016/A-43).

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