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Evaluation of the cardioprotective activity of summer savory (*Satureja hortensis* L.) extract in experimental rats with Isoproterenol-induced myocardial infarction

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ABSTRACT

Background: Myocardial infarction (MI) is a serious heart ailment that requires cutting-edge remedies for effective treatment. This study examines the possible cardioprotective effects of a hydroalcoholic extract from *Satureja hortensis* L. (HASH) in rats with isoproterenol (ISO) induced myocardial infarction.
Methods: The rats were pretreated orally with hydroalcoholic extract of *Satureja hortensis* low (200 mg/kg), high dose (400 mg/kg), and metoprolol (10 mg/kg), in their respective groups for 30 days, followed by two subcutaneous doses of isoproterenol (ISO). Blood was obtained to measure biochemicals such as creatine kinase-MB (CK-MB), Troponin I, Alanine transaminase (ALT), Aspartate transaminase (AST), and lactate dehydrogenase (LDH), and an electrocardiogram (ECG) of a rat was recorded after the experiment. Myocardial integrity was examined histopathologically, and malondialdehyde (MDA) levels and antioxidant enzyme concentrations were assessed using heart tissue homogenate.
Results: Rats with myocardial infarction caused by ISO displayed significantly ($P < 0.001$) augmented CK-MB, Troponin I, LDH, AST, and ALT levels with aberrant ECG patterns, and alteration in cardiac mass as well as enhaced oxidative stress marker levels. Besides, biochemical results were validated by myocardium histology. Pre-treatment of animals with a high dose (400 mg/kg) of HASH or standard cardioprotective agent (metoprolol) prevented the ISO-induced alterations in the above parameters to a significant ($P < 0.01$) level as well as metabolic derangements and functional alterations.
Conclusions: Our research suggests that HASH pre-treatment has cardioprotective action and can prevent myocardial toxicity caused by ISO. Therefore, *Satureja hortensis* L. extract might be a promising therapeutic plant that can be further investigated for potential cardioprotective properties.

1. Introduction

Ischemic heart disease (IHD), characterized by a significant reduction in myocardial blood supply, leads to myocardial necrosis. This condition is reported to impact the heart's mechanics, electrical properties, structure, and biochemistry (Del Buono et al., 2022; Asdaq et al., 2021; Beshel et al., 2022). On a global scale, cardiovascular ailments, including heart attacks and strokes, accounted for a substantial 17.9 million mortality in 2019, representing 32 % worldwide. Additionally, coronary artery disease (CAD) affects approximately 1.72 % of the world's population (Del Buono et al., 2022). Moreover, IHD is emerging as a significant health issue in the Gulf Council nations including Saudi Arabia, where it is thought to be the primary cause of more than 45 % of fatalities (Tash et al., 2023). The INTERHEART and INTERSTROKE

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investigations found that smoking, inadequate physical activity, poor diet, alcohol use, diabetes, obesity, dyslipidemia, and hypertension were the most frequent risk factors for IHD (Dagenais et al., 2020). Consequently, the Gulf population has high rates of chronic non-communicable diseases and IHD risk factors (Alhabib et al., 2020). World Health Organization (WHO) projections show that MI will become the leading cause of mortality worldwide over the next decade (Chen et al., 2023). The increasing prevalence of IHD and its impact on the global population emphasize the importance of focused efforts in the prevention, early detection, and management of cardiovascular diseases (Noushida et al., 2024).

Due to its detrimental effects on heart functioning, oxidative stress is a major factor in the pathophysiology of many illnesses. Free radicals and reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, and hydroxyl radicals are consistently produced within the failing myocardium, as shown by numerous studies (Khalifa et al., 2021; Kim et al., 2022). Therefore, oxidative stress has been linked to cardiovascular diseases, especially MI, leading to extensive research into treatment options incorporating antioxidants with powerful free radical scavenging capacities (Kim et al., 2022).

Isoproterenol (ISO) induces MI and is a well-established animal model (Bachheti et al., 2022; Meeran et al., 2020) to evaluate the cardioprotective properties of the potential therapeutic agent. This model replicates the pathology observed in humans, particularly concerning the ischemic necrosis affecting the interventricular septum and ventricular subendocardial region. The mechanism behind ISO-induced myocardial infarction involves the activation of the heart's ß-adrenergic receptor, leading to a series of adverse effects, including myocardial oxidative stress, inflammation, and calcium overload (Lin et al., 2022; Aguilera-Rodríguez et al., 2023; Wu et al., 2021). Notably, high doses of ISO can undergo autoxidation, generating highly cytotoxic ROS. These contribute to increased membrane permeability, cardiac damage, reduced coronary blood flow, and impaired myocardial perfusion (Beldjoud et al., 2023; Hareeri et al., 2023). Mechanisms involving ISO-mediated activation of cytochrome-c, c-Jun NH2-terminal kinase (JNK), extracellular signal-regulated kinases, adenyl cyclase, protein kinase A, phosphorylated L-type calcium channels leading to death of myocytes through necrosis as well as apoptosis have also been reported in the literature (Motiejunaite et al., 2021; Yoganathan et al., 2023; Zhang et al., 2021; Moore et al., 2021). Therefore, the ISO-induced acute myocardial infarction model in rats (at the dose 85 – 150 mg/kg) reported to provide valuable insights into the pathophysiological processes underlying myocardial infarction in humans and is a significant tool for research in this area (Neto et al., 2022).

Satureja hortensis L., also known as *Satureja postii Arzn.*, is a fragrant plant that is a member of the *Lamiaceae* family and is known by a variety of common names, including the following: Winter savory, garden savory, summer savory, and bean herb (Fierascu et al., 2018). *Satureja hortensis* extract, well-known for its abundant concentration of phenolic acids, exhibits promising potential in counteracting oxidative damage caused by various species of free radicals and non-free radical species (Ejaz et al., 2023). These phenolic acids act as natural antioxidants and have been found to offer protective effects against several severe diseases, including Alzheimer's disease, oxidative stress dysfunction, cancer, diabetes, and cardiovascular disease (Emre et al., 2021). They are reported to be protective against cisplatin-induced liver, renal, and testicular toxicity in experimental animals (Boroja et al., 2018). Another study also validates the protective effect of the plant extract against testicular injury induced by cyclophosphamide in animals (Asadipour and Amirghofran, 2019).

Given that *Satureja hortensis* has the potential to have antioxidant properties, and that antioxidants play an important role in the prevention and treatment of cardiovascular diseases, there is a need to supplement the therapies currently used to manage IHD. Therefore, the purpose of this study was to examine the possible cardioprotective effects of *Satureja hortensis* extract in protecting against isoproterenol-induced cardiac dysfunction and oxidative stress in experimental rats.

2. Materials and Methods

2.1. Plant extract

Satureja hortensis L. hydroalcoholic extract (HASH) was used for the research. The HASH was produced by applying the Soxhlet extraction process, as was previously stated (Popovici et al., 2019). *Satureja hortensis* was extracted using a Soxhlet apparatus with 50 g of homogenized, crushed aerial parts and 400 ml of 70 % ethanol over the course of 48 h. After a vacuum-operated rotary evaporator was used to extract the solvent, filter paper was used to filter the final extract. The pellet was then lyophilized and stored at −20 °C in a dark glass tube until further analysis was conducted.

2.2. Drugs and reagents

The cTn I assay kit (MBS280167)was acquired from Isha Diagnostic in Bangalore. As for ALT (MBS2022291), LDH (MBS6384009), AST (MBS2019147), and CK-MB (MBS173052) assay kits were obtained from Agappe Diagnostic, a reputable Bangalore-based company. The SOD (CS0009) and CAT (MAK381) assay kits were sourced from Sigma, Aldrich, Germany. All supplies and kits were obtained through standard suppliers located in Bangalore, India. Furthermore, the ISO and 1,1,3,3-tetra ethoxy propane were provided by TCL Pvt Ltd in Bangalore. It is important to note that all the chemicals and reagents utilized in this investigation were of analytical quality.

2.3. Experimental animals

Thirty, male adult Wistar rats weighing 170–190 g (8–10 weeks) were used in this investigation. They were raised at Al-Ameen College of Pharmacy in India, in the Central Animal House. The rodents spent their days and nights in plastic cages with wood-chip bedding on a 12-hour day/night cycle. They had access to the usual pellet food and water provided in a laboratory setting. This experimental work was approved by the institutional animal ethics committee (AACP/IAEC/SEP2021/05).

2.4. Induction of MI

Except for the normal control group, the rats were given subcutaneous injections of 85 mg/kg/body weight of isoproterenol hydrochloride for two days in a row (the 29th and 30th) with a 24-hour interval between injections. Based on the results of a previous investigation and a pilot study for ISO dose determination, an ISO dose of 85 mg/kg/body weight was established (Moore et al., 2021).

2.5. Experimental design

Rats were categorized into five groups. The weights and ECGs of the rats were recorded prior to the conduct of the study.

- Group 1 (n = 6) – Control group, free of treatments
- Group 2 (n = 6) – Isoproterenol hydrochloride (85 mg/kg, b.w, s.c) on 29th and 30th day.
- Group 3 (n = 6) – Metoprolol (10 mg/kg, b.w, p.o) for 30 days followed by Isoproterenol hydrochloride (85 mg/kg, b.w, s.c) on 29th and 30th day
- Group 4 (n = 6) – HASH (200 mg/kg, b.w, p.o) for 30 days followed by Isoproterenol hydrochloride (85 mg/kg, b.w, s.c) on 29th and 30th day
- Group 5 (n = 6) – HASH (400 mg/kg, b.w, p.o) for 30 days followed by Isoproterenol hydrochloride (85 mg/kg, b.w, s.c) on 29th and 30th day

*b.w. – body weight; s.c. – subcutaneously; p.o. – per oral.

Rats showed decreased water intake and activity post-ISO administration, which made the situation worse following a second ISO dosage. Diethyl ether was given to the rats 50 h after the initial ISO dosage. Each animal was placed into a gallon glass tank with an environment saturated with ether; the rats were all given ether anesthesia before being decapitated. After the blood samples were taken, the serum was separated and refrigerated between 4 and 8 degrees Celsius until biochemical analysis. As previously mentioned (Asdaq and Inamdar, 2011), the heart tissue homogenate was produced in a 10 % sucrose solution and kept between 4 and 8 degrees until analysis.

2.6. ECG recording

An electrocardiogram (ECG) was taken 24 h following the second ISO injection. All rats were anesthetized with a combination of xylazine (13 mg/kg) and ketamine (87 mg/kg). Once stabilized, the front and left hind limbs were attached to a subcutaneous ECG needle electrode, while the right hind limb was grounded. ECG was recorded for 5 min using a dual bio-amplifier (AD Instruments, Power Lab) and analyzed using Lab Chart software (Shin et al., 2011; Birari et al., 2020; Pullaiah et al., 2021).

2.7. Cardiac biomarkers estimation

Levels of cTn I in serum were gauged with an enzyme microplate kit and analyzed via an ELISA microplate reader. Additionally, serum evaluations for CK-MB, LDH, AST, and ALT were carried out using a spectrophotometer (Kavsak et al., 2017).

2.8. Myocardial oxidative stress estimation

The lipid peroxidation products were evaluated in cardiac tissue by measuring Malondialdehyde (MDA) levels. Also identified as a thiobarbituric acid-reactive substance (TBARS), MDA was quantified (O'donnell et al., 2010), and its absorbance was measured at 532 nm. TBARS concentrations were thus denoted in terms of nanomoles per milligram of protein. Utilizing a high-precision Eppendorf 5415D centrifuge, heart tissue homogenates underwent centrifugation at 12,000 revolutions per minute for ten minutes at a precisely regulated temperature of 4 degrees Celsius. The supernatants derived from the cardiac tissue post-centrifugation were subsequently employed in the determination of the activities of several endogenous antioxidative enzymes, namely, superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) (Asdaq et al., 2021).

2.9. Histology

Post-sacrifice, the heart was quickly removed, cleaned with a saline solution, and conserved in a 10 % formalin solution. Sections of the heart tissue embedded in paraffin were prepared with a thickness of 5 μm each. These sections were treated with hematoxylin and eosin (H&E) staining and underwent histopathological examination.

2.10. Analysis of data

One-way ANOVA and Dunnett's *t*-test were employed to enable statistical comparisons across groups. A P value of less than 0.05 defines the comparison's significance level. P values < 0.001,<0.01,<0.05, and > 0.05 are expressed in the results section tables. A comparison of the results obtained after different treatments were done between groups. GraphPad Prism 9 was utilized for conducting all statistical analyses.

3. Results

3.1. Body weight and heart weight ratio

Notable disparities were discerned in the heart-to-body weight ratios among the distinct experimental cohorts (as depicted in Table 1). MI control rats exhibited a significant augmentation in cardiac mass relative to normal control rats, with a value of 4.6 ± 0.15 ($p < 0.001$). Prophylactic administration of HASH at low dose (200 mg/kg) (HASHLD) (3.92 ± 0.07; $p < 0.05$) and at high dose (400 mg/kg) (HASHHD) (3.7 ± 0.13; $p < 0.01$), as well as the metoprolol regimen (3.4 ± 0.16; $p < 0.01$) before ISO challenge, significantly mitigated the heart-to-body weight ratio (mg/g) when juxtaposed with the MI control cohort. Interestingly, no significant ($p > 0.01$) change in the heart-to-body weight ratio was observed in groups of animals that received low or high doses of HASH when compared to the normal control group.

3.2. ECG recording

An ECG was conducted 24 h after the second isoproterenol dose. The MI control group evidenced a notable surge ($p < 0.001$) in both heart rate (HR) and the ST segment when contrasted with the normal control group. Conversely, these variables were significantly ($p < 0.01$) diminished in the standard group (metoprolol, 10 mg/kg) and the HASHHD group (400 mg/kg). Further, treatment with a low dose (200 mg/kg) of HASH produced a significant ($p < 0.05$) decline in the heart rate, while no significant change was noticed in the ST segment elevation when compared to the MI control group (Table 2).

In the ECG tracings presented in (Table 3 and Fig. 1), the following observations are recorded:

A. The Normal group shows a regular ECG with a normal pattern.
B. The ISO-treated rats display extreme ST-segment elevation.
C. The group pretreated with the standard group (metoprolol, 10 mg/kg) shows a significant improvement in the elevated ST segment, with the ECG pattern regaining normalcy.
D. The group pretreated with HASH-LD (200 mg/kg) slightly elevates the ST segment.
E. The group pretreated with HASH-HD (400 mg/kg) demonstrates a significant improvement in the elevated ST segment, almost regaining the normal ECG pattern.

These findings suggest that pretreatment with the standard (metoprolol) and high doses of HASH positively affect the ST-segment elevation induced by ISO treatment, leading to improved ECG patterns.

3.3. The effect of HASH on liver Enzymes, cardiac biomarkers, and myocardial oxidative stress biomarkers.

In the serum of both NC and ISOMI rats, Table 4 shows the effect of

Table 1
Effect of HASH on Heart to Body weight ratio.

Parameter	NC	ISOMI	MP	HASHLD	HASHHD
Hw/Bw (mg/g)	3.0 ± 0.10	4.6 ± 0.15*	3.4 ± 0.16^{B}	3.9 ± 0.07^{A§}	3.7 ± 0.13^{B§}

The test data are presented as the mean ± SEM, derived from an analysis of n = 6 samples. Normal control; ISOMI: Isoproterenol-induced myocardial infarction; MP: Metoprolol; HASHLD: low dose of hydroalcoholic extract of *Satureja hortensis* (200 mg/kg); HASHHD: high dose of hydroalcoholic extract of *Satureja hortensis* (400 mg/kg). Differences from the normal control group are denoted with *($p < 0.001$) when they are significant. The notations ^{B}($p < 0.01$) and ^{A} ($p < 0.05$) highlight significant divergences from the ISOMI control group, § ($p > 0.05$) indicates the lack of significant variation when compared with the control group. Statistical comparisons among these groups were facilitated using one-way Analysis of Variance (ANOVA) and the Dunnett *t*-test.

Table 2
Cardioprotective Effect of HASH on Electrocardiogram (ECG) in ISO-induced MI in rats.

ECG parameters	NC	ISOIMI	MP	HASHLD	HASHHD
Heart Rate (BPM)	283.5 ± 6.48	368.9 ± 16.25*	298.0 ± 10.50^{B}	320.7 ± 7.11^{A}	302.3 ± 6.69^{B}
ST (mV)	0.039 ± 0.00	0.133 ± 0.01 *	0.0412 ± 0.05^{B}	0.096 ± 0.02	0.062 ± 0.01^{B}

The test data are presented as the mean ± SEM, derived from an analysis of n = 6 samples. Normal control; ISOMI: Isoproterenol induced myocardial infarction; MP: Metoprolol; HASHLD: low dose of hydroalcoholic extract of *Satureja hortensis* (200 mg/kg); HASHHD: high dose of hydroalcoholic extract of *Satureja hortensis* (400 mg/kg). Differences from the normal control group are denoted with *($p < 0.001$) when they are significant. The notations ^{B}($p < 0.01$) and ^{A} ($p < 0.05$) highlight significant divergences from the ISOMI control group. Statistical comparisons among these groups were facilitated using one-way Analysis of Variance (ANOVA) and the Dunnett *t*-test.

Table 3
ECG pattern and ST segments difference on HASH.

Parameters	NC	ISOIMI	MP	HASHLD	HASHHD
ST Segments	R	SE	IE	SE	IE
ECG Pattern	NP	IP	RNP	SRNP	RNP

Symbols were indicated: R-Regular, NP-Normal Pattern SE- Striking & Elevation, IP Irregular Pattern, IE- Improvement, SNP- Slighly Returning, and RNP-Returning to Normal Pattern.

SH therapy on the functioning of three important liver marker enzymes: LDH, ALT, and AST. The activity of the aforementioned hepatic enzymes in the serum significantly ($p < 0.001$) increased in rats with ISO-induced myocardial infarction. On the other hand, rats that received low ($p < 0.05$) and high doses ($p < 0.01$) of HASH prior to the ISOMI challenge showed a significant reduction in the activities of ALT, whereas, only a high dose of HASH was able to significantly decline AST ($p < 0.01$) and LDH ($p < 0.05$) levels when compared to ISO control group (ISOMI).

Table 5 presents the serum levels of cardiac troponin I (cTn I) and CK-MB in normal and ISOMI-treated rats. There was a notable increase ($p< 0.001$) in serum cTn I levels in the ISOMI-treated rats when compared to the normal control group. However, ISOMI rats that received a daily high dose of HASH treatment for 6 weeks exhibited a significant reduction ($p< 0.01$) in the serum levels of both cTn I and CK-MB when contrasted with rats subjected to ISOMI treatment alone. Although a low dose of HASH treated also resulted in a decline in the CK-MB and cTn I serum levels, the change was not statistically significant.

This study endeavored to determine the potential protective effects of HASH on cardiac myocytes by evaluating the impacts of its oral administration on the levels of SOD, GSH, CAT, and MDA in rat heart tissues. A significant increase ($p< 0.001$) in MDA levels was noted in rats subjected to ISO injection. However, pre-administration of HASHHD (400 mg/kg) significantly mitigated ($p<0.01$) this alteration, as delineated in Table 6. Further, ISO administration caused a significant ($p< 0.001$) decline in the endogenous antioxidant activities especially, SOD, GSH, and CAT, when compared to the normal control group. Pretreatment of animals with high doses of HASH and a standard drug (metoprolol) exhibited significant ($p < 0.01$) protection from ISO-induced fall in endogenous antioxidant levels. Prior administration of low doses of HASH for six weeks also offered protection by keeping a better level of these antioxidants in serum, especially, GSH ($p< 0.05$), but the effect was mostly modest.

3.4. Histological examination

The results from the histopathological tests showed varying levels of harm to the myocardium in the rat groups that were examined (Fig. 2). The control group of rats displayed a healthy myocardial membrane with no indications of inflammation. In contrast, the group of rats that had experienced an infarction showed a necrotic zone with mononuclear cell infiltration, lymphocytes, and edema in cardiomyocytes, as well as an increase in interstitial fibroblast and connective tissue proliferation. However, administering standard medication (metoprolol − 10 mg/kg) before the onset of symptoms resulted in a better heart condition, with fewer mononuclear cell infiltrations and no fibrosis. Meanwhile, administering HASHLD (200 mg/kg) before the onset of symptoms led to a reduction in the degree of mononuclear cell infiltration and interstitial edema, but there was a marked increase in fibroblast and connective tissue proliferation. Finally, administering HASHHD (400 mg/kg) before the onset of symptoms resulted in significant restoration of myocardial integrity, with very few inflammatory cell infiltrations and no fibrosis.

4. Discussion

The purpose of this research was to explore the cardioprotective efficacy of the hydroalcoholic extract of *Satureja hortensis* on rats that had undergone an isoproterenol-induced myocardial infarction. The injection of ISO triggers a number of processes that eventually cause harm to the myocardium. The study discovered that pre-treatment with a standard drug (metoprolol, 10 mg/kg) and HASH (particularly 400 mg/kg) exhibited a substantial drop in biomarker levels and improved physical and metabolic parameters when compared to the MI control group. The strong antioxidant capacity of the extract can be attributed to the presence of phenolic acid and flavonoids, and the extract showed a dose-dependent cardioprotective effect.

The results of our investigation show that HASH prevents ISO-induced myocardial infarction in rats. The administration of ISO caused the heart to enlarge, as seen by higher heart weights, but body weights stayed mostly unchanged, leading to an increased ratio of heart weight to body weight. HASH pre-treatment, on the other hand, lessened these effects and helped to maintain heart weights at levels that were nearly normal. These findings are in accordance with the previous study reported in the literature (Asdaq and Inamdar, 2011). Nevertheless, pre-treatment with HASH in the present study might have mitigated these effects by keeping heart weights close to normal. When compared to the normal group, the control group's heart rates increased substantially and their ST-segment was elevated. As reported in the literature, The increased stimulation of β1-adrenoreceptors by ISO may have caused the tachycardia in the control group (Beshel et al., 2022). According to an earlier study, such effects could have resulted in abnormalities in the heart's sympathetic and parasympathetic nervous systems as well as intracellular Ca2 + overload (Asdaq et al., 2021). The myocardium may have experienced acute ischemia tissue damage if the ST segment is elevated.

Another observation of the current study suggested that The heart might be shielded from ISO-induced alterations by pre-treatment with metoprolol and a high dose of HASH (400 mg/kg), suggesting a possible protective action of these treatments on the myocardium. The finding is supported by earlier research where metoprolol is reported to work by altering the beta-1 adrenergic receptor's intracellular structure. This alteration allows the receptor to interact with surrounding proteins that suppress hyperactivated neutrophils, preventing the inflammatory damage linked to ISO-induced myocardial infarction (Clemente-Moragón et al., 2020). Furthermore, cardioprotection provided by a high dose of *Satureja hortensis* could be related to its high polyphenolic content, primarily rosmarinic acid, which acts through two separate mechanisms, namely reactive oxygen species filtering and inflammatory process modulation (Ejaz et al., 2023). Lower doses of HASH likely lacked an adequate concentration of these polyphenolic chemicals, which is why they did not show significant cardioprotection.

The electrophysiological results were validated by examining the biochemical markers in the serum. According to recent scientific research, cTn I function as a very sensitive and precise marker for

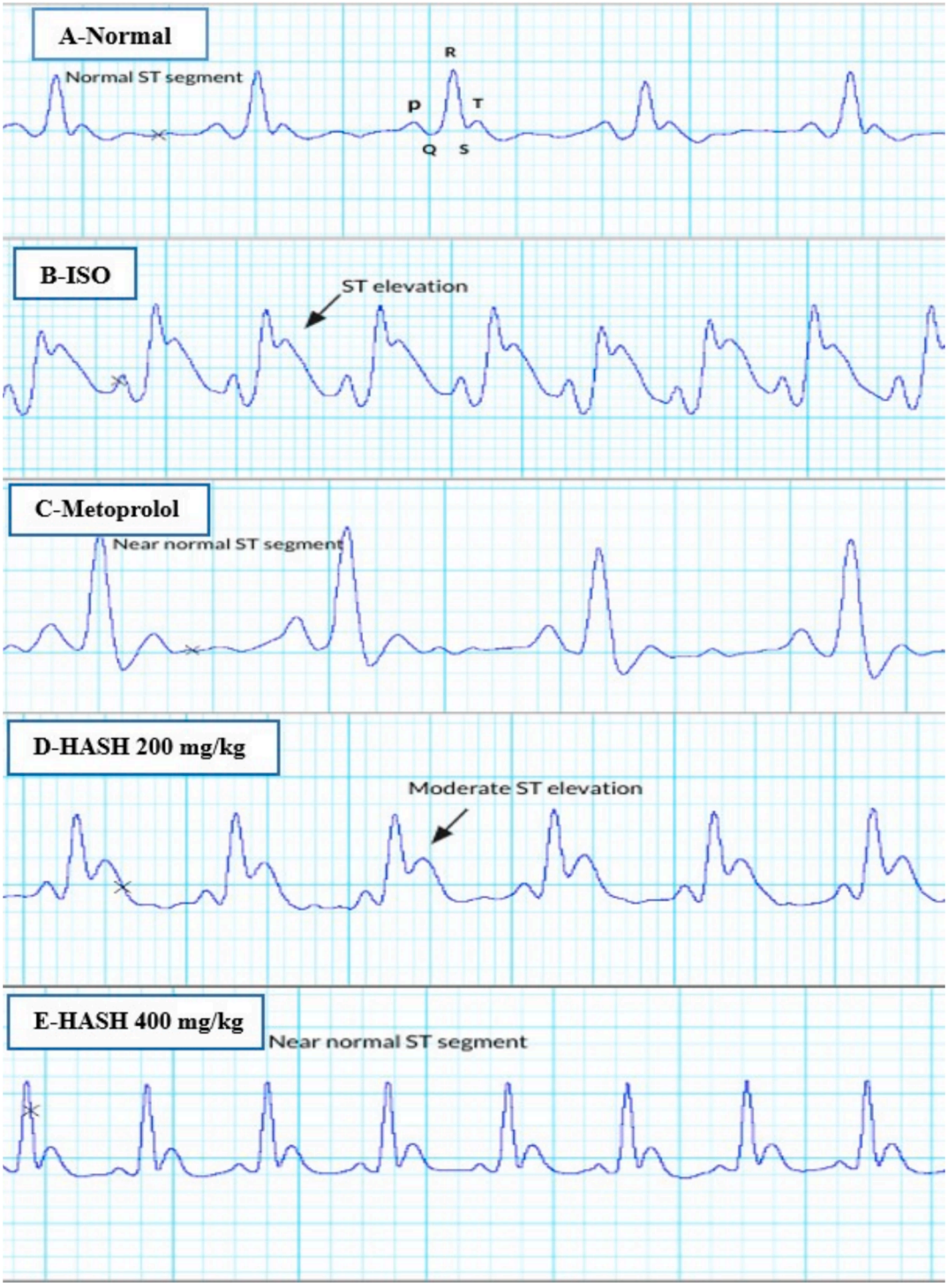

Fig. 1. Effect of HASH on ECG tracings in rats Representative sampel of ECG from an analysis of n = 6 samples.

myocardial damage (Clasen et al., 2023). The results of our study show that rats given ISO had higher serum cTn I levels than the normal control group, which may imply that ISO causes cardiac injury (Moore et al., 2021). But in contrast to the ISO control group, the HASH-treated group showed noticeably lower levels of cTn I, suggesting that HASH may be able to lessen the severity of cardiac injury. Serum CK-MB activity test is a widely used diagnostic test that is rare in other tissues and abundant in myocardial tissue. Our investigation demonstrated that rats exposed to ISO exhibited elevated levels of these marker enzymes (Moore et al., 2021), a sign of greater heart injury and membrane permeability. However, rats were given HASH before ISO had decreased enzyme activity, indicating that HASH's phenolics and flavonoids can preserve membrane integrity and stop enzyme leakage, thus safeguarding the heart (Beyhoff et al., 2020).

The results of our study indicate that HASH may protect the heart against oxidative injury. When applied as a pretreatment, HASH markedly reduced the levels of lipid peroxide in rats that had been given ISO. This shows that the anti-oxidant properties of HASH can successfully prevent the generation of reactive oxygen species brought on by the administration of ISO. Furthermore, the HASH pre-treatment enhanced

Table 4
Effects of HASH on Liver enzyme activity.

Liver enzymes activity	NC	ISOMI	MP	HASHLD	HASHHD
LDH (U/L)	33.42 ± 2.90	81.49 ± 11.41*	40.08 ± 2.92^{B}	69.47 ± 10.48§	48.09 ± 7.46^{A}
ALT (U/L)	33.66 ± 1.70	55.26 ± 3.16 *	35.19 ± 2.9^{B}	41.59 ± 4.12 ^{A}	39.11 ± 2.89^{Bb}
AST (U/L)	84.65 ± 1.79	112.60 ± 4.58 *	88.08 ± 2.74^{B}	102.40 ± 4.67§	92.22 ± 4.70^{B}

The test data are presented as the mean ± SEM, derived from an analysis of n = 6 samples. Normal control; ISOMI: Isoproterenol-induced myocardial infarction; MP: Metoprolol; HASHLD: low dose of hydroalcoholic extract of *Satureja hortensis* (200 mg/kg); HASHHD: high dose of hydroalcoholic extract of *Satureja hortensis* (400 mg/kg). LDH: Lactate dehydrogenase; ALT: Alanine transferases; AST: aspartate aminotransferase. Differences from the normal control group are denoted with *($p < 0.001$) when they are significant. The notations ^{B}($p < 0.01$) and ^{A} ($p < 0.05$) highlight significant divergences from the ISOMI control group, § ($p > 0.05$) indicates the lack of significant variation when compared with control group. Statistical comparisons among these groups were facilitated using one-way Analysis of Variance (ANOVA) and the Dunnett *t*-test.

Table 5
Effect of HASH on the Cardiac biomarkers of the ISOIMI rats.

Cardiac biomarkers	NC	ISOIMI	MP	HASHLD	HASHHD
Troponin-I (ng/ml)	0.023 ± 0.07	0.185 ± 0.04*	0.038 ± 0.07^{B}	0.118 ± 0.07	0.073 ± 0.07^{B}
CK-MB(U/L)	7.63 ± 0.94	16.76 ± 1.56*	9.64 ± 0.85^{B}	12.08 ± 2.26	11.06 ± 1.49^{B}

The test data are presented as the mean ± SEM, derived from an analysis of n = 6 samples. Normal control; ISOMI: Isoproterenol induced myocardial infarction; MP: Metoprolol; HASHLD: low dose of hydroalcoholic extract of *Satureja hortensis* (200 mg/kg); HASHHD: high dose of hydroalcoholic extract of *Satureja hortensis* (400 mg/kg). CK-MB: Creatinine kinase-MB. Differences from the normal control group are denoted with ^{a}($p < 0.001$) when they are significant. The notations ^{b}($p < 0.01$) and ^{c} ($p < 0.05$) highlight significant divergences from the ISOMI control group, while ^{ns} ($p > 0.05$) signifies the lack of any significant disparities in normal control. Statistical comparisons among these groups were facilitated using one-way Analysis of Variance (ANOVA) and the Dunnett *t*-test.

SOD and GSH scavenging abilities, which successfully decreased ISO's production of superoxide and H2O2.

In addition, compared to hearts from rats treated with ISO, the histological results of myocardial infarcted hearts treated with HASH showed almost normal morphological features of cardiac muscle and no necrosis. These findings have similarities with previous research and suggest that pre-treating rats' hearts with HASH may protect the tissues from injury from ISO-induced myocardial necrosis (Xing et al., 2022). The results of the study indicate that the *Satureja hortensis* hydroalcoholic extract may have a cardioprotective effect, preventing the heart tissue damage caused by ISO in rats. Moreover, it is intriguing to note that several study parameters, including ST-segment elevation in ECG recordings, biochemical markers like LDH, Troponin-I, and CK-MB, and endogenous antioxidants like CAT and SOD, did not change in the groups of animals given a low dose of HASH.

Nevertheless, at high doses, all these parameters were considerably altered, suggesting that a concentration of HASH greater than 200 mg/kg is ideal for producing a therapeutic effect. Similar findings where variation in doses affecting the therapeutic outcome has been reported in the literature (Asdaq et al., 2021; Chen et al., 2023). It will therefore be crucial to ascertain the possible therapeutic advantage of HASH at various concentrations to investigate its therapeutic effective range of dosages or therapeutic index. With this in mind, we advised that future research assess the cardioprotective potential of HASH at a range of dosages, from the current study's middle ground between low and high

Table 6
Effects of HASH on Myocardial oxidative stress.

Myocardial Oxidative Stress Markers	NC	ISOMI	MP	HASHLD	HASHHD
SOD (units/mg of protein)	1.85 ± 0.11	0.83 ± 0.04*	1.39 ± 0.07^{B}	1.19 ± 0.08§	1.61 ± 0.18^{B}
GSH (µg of GSH/mg protein)	14.96 ± 1.54	4.32 ± 0.52*	9.91 ± 1.19^{A}	8.14 ± 1.52^{A}	13.09 ± 1.71^{B}
CAT (µmol of H_2O_2 decomposed/min/mg protein)	0.90 ± 0.09	0.40 ± 0.03*	0.69 ± 0.08^{A}	0.45 ± 0.03§	0.77 ± 0.06^{B}
MDA (nmoles/mg of tissue)	0.42 ± 0.09	2.4 ± 0.23 *	1.12 ± 0.23^{A}	1.95 ± 0.41§	0.84 ± 0.04^{B}

The test data are presented as the mean ± SEM, derived from an analysis of n = 6 samples. Normal control; ISOMI: Isoproterenol-induced myocardial infarction; MP: Metoprolol; HASHLD: low dose of hydroalcoholic extract of *Satureja hortensis* (200 mg/kg); HASHHD: high dose of hydroalcoholic extract of *Satureja hortensis* (400 mg/kg). SOD: Superoxide dismutase; GSH: Glutathione; CAT: Catalase; MDA: Melandialdehyde. Differences from the normal control group are denoted with *($p < 0.001$) when they are significant. The notations ^{B}($p < 0.01$) and ^{A} ($p < 0.05$) highlight significant divergences from the ISOMI control group, § ($p > 0.05$) indicates the lack of significant variation when compared with the control group. Statistical comparisons among these groups were facilitated using one-way Analysis of Variance (ANOVA) and the Dunnett *t*-test.

(300 mg/kg) to multiple higher doses, to get the full therapeutic value of HASH. As the study did not include a control group of healthy rats that received HASH treatment, it is also likely that HASH had some effect on the rats who were not afflicted. Thus, we advise future research to include a control group of healthy rats that receive HASH treatment at varying doses.

5. Conclusions

Our findings indicate that the pretreatment with the hydroalcoholic extract of *Satureja hortensis* L may have cardioprotective effects and lessen the damage to the myocardium that is caused by exposure to ISO. The action of the extract is shown to be dose-dependent, with 400 mg/kg restoring the ISO-induced elevated ST segment, reducing the leakage of cardiac markers, elevating the myocardial antioxidative activities, and impressively maintaining the myocardial integrity during periods of ISO-induced myocardial stress. As a result, we believe that the *Satureja hortensis* L. extract could be a good candidate for further research so that it can be used for therapeutic purposes.

CRediT authorship contribution statement

A. Muthukumar: Conceptualization, Formal analysis, Writing – original draft. **Swati Mittal:** Data curation, Formal analysis, Visualization, Writing – original draft. **Tsering Choezom:** Project administration, Software, Writing – original draft. **Keserla Bhavani:** Conceptualization, Investigation, Validation, Writing – original draft. **Kuntal Das:** Data curation, Resources, Supervision, Writing – review & editing. **Noopur Joyce:** Conceptualization, Project administration, Validation, Writing – original draft. **Mansour Almuqbil:** Conceptualization, Funding acquisition, Writing – review & editing. **Moneer E. Almadani:** Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. **Fuzail Ahmad:** Conceptualization, Resources, Visualization, Writing – original draft. **Farhana Yasmin:** Conceptualization, Data curation, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

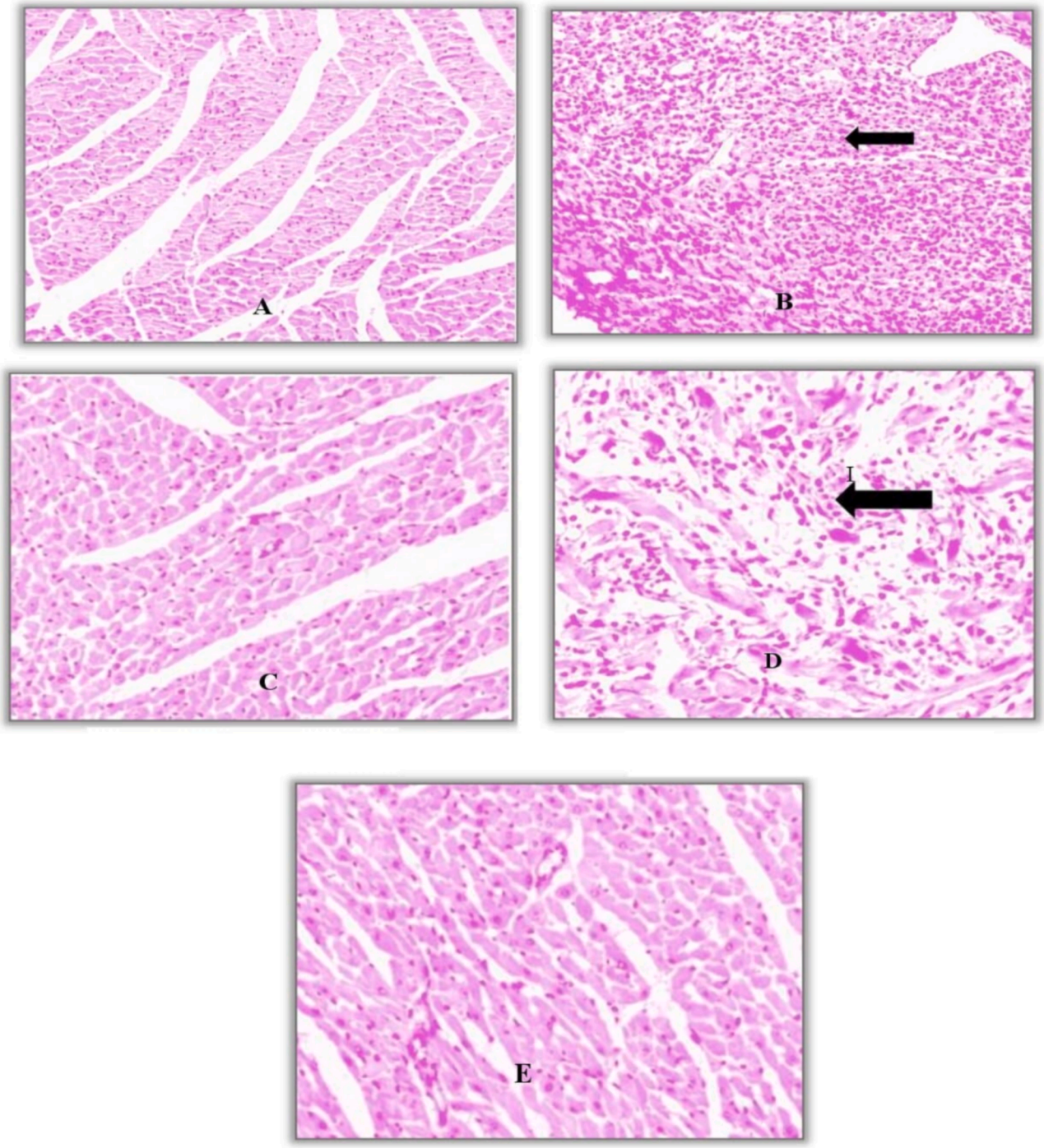

Fig. 2. Representative images of histological examinations of different treatments (n = 6). Normal control; B) MI control group; C) Metoprolol (10 mg/kg); D) HASH-LD (200 mg/kg); E) HASH-HD (400 mg/kg).

the work reported in this paper.

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