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Ameliorative potential of astaxanthin in isoproterenol-induced heart failure in rats via the regulation of the renin-angiotensin system.

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Keywords: Angiotensin-Converting Enzyme;Arterial Pressure; Endomyocardial Fibrosis; Lactate Dehydrogenase; Renin

Abstract. Heart failure (HF) is a condition in which the heart cannot pump blood effectively to the body. Isoproterenol (ISO) induces HF in rodents by affecting the renin-angiotensin system (RAS). Astaxanthin (AST) is known to have protective effects on the cardiovascular system. However, clear evidence showing that AST improves HF through RAS regulation has not yet been reported. This study aimed to investigate the role of AST in ISO-induced HF in rats. HF was induced by intraperitoneal (i.p.) injection of ISO (5 mg/kg/day) for seven consecutive days. AST (25 and 50 mg/kg), aliskiren (30 mg/kg), ramipril (4 mg/kg), and telmisartan (8 mg/kg) were administered orally for 21 days, starting from the last dose of ISO (day 8). Changes in systolic and diastolic blood pressure and heart rate associated with HF were measured on days 0, 7, 14, 21, and 28. Additionally, changes in heart-to-body weight ratio, serum creatine kinase-MB (CK-MB), serum angiotensin-converting enzyme (ACE) activity, plasma renin activity (PRA), tissue hydroxyproline, and lactate dehydrogenase (LDH) activity, along with histopathological alterations, were evaluated. The administration of AST and RAS-modulating agents reduced ISO-induced changes in cardiac function and biochemical markers. It also demonstrated cardioprotective effects. Therefore, AST may be useful for treating cardiotoxic HF due to its RAS-regulatory actions. However, further studies are needed to confirm this therapeutic potential across different HF models and animal species.

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Potencial mejorador de la astaxantina en la insuficiencia cardíaca inducida por isoproterenol en ratas a través de la regulación del sistema renina-angiotensina.

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Palabras clave: Enzima Convertidora de Angiotensina; Presión Arterial; Fibrosis Endomiocárdica; Lactato Deshidrogenasa; Renina.

Resumen. La insuficiencia cardíaca (IC) es una alteración de la capacidad funcional del corazón para bombear sangre al organismo. El isoproterenol (ISO) induce IC en roedores mediante la alteración del sistema renina-angiotensina (SRA). Se sabe que la astaxantina (AST) ejerce una acción reguladora sobre el sistema cardiovascular. Sin embargo, aún no se ha descrito evidencia clara de que la AST mejore la IC mediante la regulación del SRA. El presente estudio fue diseñado para investigar el papel de la AST en la IC inducida por ISO en ratas. La IC se indujo mediante administración intraperitoneal (i.p.) de ISO (5 mg/kg/día) durante siete días consecutivos. La AST (25 y 50 mg/kg), aliskiren (30 mg/kg), ramipril (4 mg/kg) y telmisartán (8 mg/kg) se administraron por vía oral durante 21 días consecutivos, desde la última dosis de ISO (día 8). Los cambios asociados a la IC en la presión arterial sistólica y diastólica y en la frecuencia cardíaca se evaluaron en diferentes puntos temporales, es decir, los días 0, 7, 14, 21 y 28. También se evaluaron los cambios en la relación entre el peso corporal y el corazón, la creatinquinasa sérica-MB (CK-MB), la actividad sérica de la enzima convertidora de angiotensina (ECA) y la actividad plasmática de la renina (PRA); y la actividad tisular de la hidroxiprolina y la lactato deshidrogenasa (LDH) junto con los cambios histopatológicos. La administración de agentes moduladores de AST y SRA atenúa los parámetros bioquímicos y funcionales cardíacos inducidos por ISO. También demuestra efectos cardioprotectores. Por lo tanto, la AST puede utilizarse para la IC asociada a cardiotoxinas debido a sus acciones reguladoras del SRA. Sin embargo, se requieren estudios más exhaustivos para demostrar su eficacia terapéutica en diferentes IC y especies animales.

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INTRODUCTION

Heart failure (HF) is also known as congestive heart failure (CHF), which indicates impairment of the pumping of blood by the heart. The symptoms of HF are fatigue, shortness of breath, and leg swelling [1]. The global prevalence rate of HF is rising to 24% and affects one person out of four in their lifetime. Furthermore, it is expected to increase by 8.5% among persons aged 65 to 70 years [2]. Isoproterenol (ISO) is widely used to treat bradycardia in patients with arrhythmias. Structurally, it is similar to epinephrine and an agonist of non-selective beta-adrenergic receptors [3]. Experimentally, ISO causes myocardial fibrosis, raises diaphragmatic contractility, and mimics the pathogenesis of cardiomyopathy and human heart failure [4,5] and the mechanism of the effects,

of isoproterenol on diaphragmatic contractility and fatigue in septic peritonitis in vitro. Furthermore, the administration of ISO is known to induce HF by modulating the renin-angiotensin system (RAS) [6–8] changes in circulating and tissue renin-angiotensin system (RAS). Moreover, ISO is known to increase heart rate and systemic arterial blood pressure [9,10]. Even a single dose of ISO also induces type 2 diabetes mellitus-associated myocardial infarctions [11]. Besides, the multiple doses of ISO cause chronic heart failure in mice [12]. A one-week administration of ISO at 5 mg/kg induces changes in hemodynamic parameters in rats by increasing angiotensin II levels and angiotensin-converting enzyme 2 (ACE2) activity [6,13] 5 mg/kg/day, intraperitoneally. The activation of ACE2 may be due to the fact that raising cardiac angiotensin-II peptide concentrations is associated with increased ACE2 expression in normal rats [14]. However, these effects are absent in other conditions and other species [15]. In most cases, ISO does not affect ACE2 expression. An experimental study also demonstrated that ISO increases the cardiac renin-angiotensin system function in a rat model of cardiac hypertrophy [6,16].

Astaxanthin (AST) is a type of keto-carotenoid belonging to the terpene compound group. AST is a metabolite of canthaxanthin and zeaxanthin. It contains hydroxyl and ketone groups, which have key antioxidant properties and act as free radical scavengers and chelators of metal ions [17]. It improves cardiac function and exercise tolerance in HF patients[18]. Additionally, AST provides myocardial protection against steroid-induced cardio-renal dysfunction and related hypertensive complications [19]. Moreover, AST also has modulating effects on the RAS system and enhances vascular function against cardiotoxins [20,21]. However, further research is necessary to fully understand the role of AST in ISO-induced HF and RAS regulation under these conditions. This study examined the effects of specific RAS modulators and AST on cardiovascular dysfunction caused by ISO in rats. Therefore, the impact of AST was evaluated in this research regarding its effects on ISO-induced HF and its possible modulation of the RAS.

METHODS

Animals used

Male Sprague Dawley rats weighing 180–200 g (12–14 months old) were used in this study. The animals had unlimited access to water and a standard laboratory diet. They were housed in the central animal facility, which maintained a 12-hour light:12-hour dark cycle. This experimental protocol was approved by the institutional animal ethics committee (IAEC approval number: IAEC/05/2025). The IAEC guidelines were followed during the trials.

Experimental design

The experimental design consisted of seven groups of eight rats each.

Group I: Animals served as naive controls and did not receive any drug administration.

Animals served as a negative control in Group II. For seven consecutive days, this group of animals received intraperitoneal (i.p.) treatment of ISO (5 mg/kg/day) to induce heart failure (HF). Approximately 0.05% ascorbic acid in 0.9% sodium chloride (NaCl) was used to prepare the ISO solution.

Groups III and IV: Animals served as test subjects for the drug treatments. These groups of animals were administered AST (25 and 50 mg/kg) orally for 21 consecutive days after the last dose of ISO.

Group V-VII: Animals were used as reference drug treatments. These groups of animals received oral administration of specific drugs: a direct renin inhibitor, aliskiren (30 mg/kg); an angiotensin-converting enzyme (ACE) inhibitor, ramipril (4 mg/kg); and an angiotensin II receptor antagonist, telmisartan (8 mg/kg), for 21 consecutive days following the last dose of ISO administration.

At 0, 7, 14, 21, and 28 days, HF-related changes in heart rate (HR), diastolic blood

pressure (DAP), and systolic blood pressure (SAP) were recorded. Blood samples were collected on the twenty-eighth day for biochemical analysis of plasma renin activity (PRA), ACE activity, and serum creatine kinase isoenzyme-MB (CK-MB). Afterwards, all animals were weighed and euthanized with diethyl ether. To assess the heart-to-body weight ratio, the heart was removed and weighed. Immediately, the heart was perfused prior to tissue homogenization, and the cardiac chambers were opened to remove residual blood. Then, cardiac tissue was homogenized in ice-cold phosphate buffer (pH 7.4). Centrifugation at 769 x g was performed to obtain a clear supernatant, which was used to measure lactate dehydrogenase (LDH) activity and hydroxyproline content, both of which are tissue biomarkers. Additionally, ISO-induced histopathological changes were examined using eosin-hematoxylin staining.

Assessment of Isoproterenol-induced functional changes in the heart

The ISO induced the HF with alteration of functional changes of the heart, such as blood pressure and heart rate, using the non-invasive tail-cuff method described by Wang et al. [22]. Briefly, the animal was placed in appropriate holders based on its body size (CODA-HT8, CODA high-throughput noninvasive blood pressure system, Kent Scientific Corporation, Torrington, United States). The tail was positioned into the rear side of the tail port with the screw of the rear hatch without pinching it. The animals were given five minutes to acclimate to the holder. Frequent contact with the animals was avoided to reduce stress and irritation. Without applying any force, the tail occlusion cuff was placed at the base of the tail. The distance between the tail occlusion cuff and the volume pressure recording (VPR) sensor cuff was 2 mm. Body temperature was measured with an infrared thermometer, and it was maintained between 34 and 35°C using a rodent heating pad. Data were recorded as averages of multiple measurements using CODA Data Acquisition Software.

Assessment of Isoproterenol-induced changes in serum and plasma biomarkers

Serum biomarkers, including CK-MB, ACE activity, and PRA, were measured in rat samples. Serum CK-MB was assessed using the rat CK-MB ELISA kit (NBP2-75313, Novus Biologicals, Kuala Lumpur, Malaysia) according to the manufacturer's protocol. Briefly, 100 μL of serum and 100 μL of biotinylated detection antibody working solution were combined in a well plate and incubated at 37 °C for 90 minutes; the plate was then washed with 350 μL of wash buffer. Subsequently, 100 μL of the horseradish peroxidase conjugate working solution was added, and the mixture was incubated at 37 °C for 30 minutes. Importantly, 90 μL of substrate reagent was added after the plate had been washed. After 15 minutes of incubation at 37 °C, 50 μL of stop solution was added to halt the reaction. A microplate reader (Bio-Tek Microplate Instruments, Penang, Malaysia), operating at 450 nanometers, was used to measure the chromogen changes. The reference standard curve was prepared using standards from 31.25 to 2000 pg/mL for CK-MB activity.

Serum ACE levels were measured using the rat ACE ELISA kit (CSB-E04490r; Cusabio Technology LLC, Houston, United States) following the manufacturer's instructions. Briefly, microplate wells were filled with 100 μL of serum and incubated at 37°C for two hours. Afterward, 100 μL of biotinylated detection antibody working solution was added, and the plate was incubated again at 37°C for 60 minutes. The plate was washed with 200 μL of wash buffer solution. Next, 100 μL of horseradish peroxidase conjugate working solution was added, followed by incubation at 37°C for 60 minutes. After washing, 90 μL of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate reagent was added. The reaction was stopped after 15 to 30 minutes of incubation at 37°C in the dark by

adding 50 μL of stop solution. The change in chromogen was measured at 450 nm using a microplate reader (Bio-Tek Microplate Instruments, Penang, Malaysia). A standard curve was prepared with standard concentrations ranging from 31.25 to 2000 ng/mL of ACE activity.

Plasma renin activity (PRA) was measured using the rat PRA ELISA kit (IB59131; Immuno-Biological Laboratories Inc., Minneapolis, United States) following the manufacturer's instructions. Briefly, 500 μL of plasma was added to the microplate wells and incubated for 2 hours at 37°C. Then, 5 μL of phenylmethylsulfonyl fluoride (PMSF) solution was added and vortexed, followed by the addition of 50 μL of the generation buffer. Next, 60 μL of the subtract solution was combined with 250 μL of the sample and transferred to another well plate. After incubating the plate at 37°C for 15 to 30 minutes, 50 μL of stop solution was added to halt the reaction. Changes in chromogen were measured using a microplate reader (Bio-Tek Microplate Instruments, Penang, Malaysia) at 450 nm. The reference standard curve was prepared with standards ranging from 0.2 to 60 ng/mL of PRA activity.

Assessment of Isoproterenol-induced changes in tissue biomarkers

Cardiac tissue biomarkers, such as hydroxyproline content and LDH, were measured. Hydroxyproline, an amino acid, results from the hydrolysis of connective tissue proteins like collagen; therefore, it plays a key role in stabilizing collagen. The tissue hydroxyproline content was determined following the method of Stegemann and Stalder[23], with slight modifications from Cissell et al.[24]. Briefly, about 30 μL of tissue sample was diluted to 100 μL with papain solution to extract maximum collagen, then centrifuged at 1968 G for 10 minutes. Next, 30 μL of this aliquot was mixed with 100 μL of 4 N sodium hydroxide (NaOH), and the mixture was incubated at 120°C for 15 minutes. After cooling to room temperature, 100 μL of 4 N hydrochloric acid (HCl) was added to neutralize the alkaline solution. A mixture containing 0.625 mL of chloramine-T (0.05 M in 74% v/v distilled water), 2-propanolol (26% v/v), NaOH (0.629 M), citric acid (0.140 M), sodium acetate (0.453 M), and glacial acetic acid (0.112 M) was used to oxidize hydroxyproline into pyrrole-2-carboxylate. This mixture was incubated at room temperature for 20 minutes. Each sample was then vortexed immediately after adding 0.625 mL of p-dimethylaminobenzaldehyde (DMAB; 1 M, 15% w/v in 2-propanol plus concentrated acid; Ehrlich's solution). After another 20-minute incubation at 65°C, the samples were cooled to room temperature. The resulting red chromophore was measured with a spectrophotometer (DU 640B, Beckman Coulter Inc., Brea, CA, USA) at 558 nm. A standard curve was prepared using 0.5, 0.75, 1, and 1.5 μg of pure hydroxyproline per milliliter. The hydroxyproline concentration was multiplied by a factor of 8.2 to estimate collagen content. Results were expressed as micrograms of hydroxyproline per milligram of tissue protein.

Lactate dehydrogenase (LDH, EC 1.1.1.27) is a major oxidoreductase enzyme involved in the anaerobic metabolism pathway. When β-nicotinamide adenine dinucleotide (NAD+) is reduced to nicotinamide-adenine dinucleotide hydrogen (NADH), lactate reversibly converts to pyruvate. The tissue LDH content was assessed as described in the method of Singh et al. [25], with a slight modification from Dewi et al. [26]. Briefly, about 50 μL of tris-(hydroxymethyl)-aminomethane hydrochloride (200 mM, pH 8), 50 μL of lithium lactate (50 mM; 49 mg lithium lactate in 2.5 mL water), and 50 μL of phenazine methosulfate (PMS), iodonitrotetrazolium chloride (INT), and NAD solutions (PIN) mixture were combined with 50 μL of sample (adding all reagents first, then the aliquot). The PIN mixture was prepared by mixing 100 μL of PMS (0.9 mg in 100 μL of water), 100 μL of INT (3.3

mg in 100 μL of DMSO), and 2.3 mL of NAD (8.6 mg NAD in 2.3 mL of water). The microplate was incubated at room temperature for 5 minutes. Changes in chromogen were measured using a microplate reader (Bio-Tek Microplate Instruments, Penang, Malaysia) at 490 nm via the endpoint assay method. The standard curve was prepared using 50 μL of standards at 0, 2.5, 5, 7.5, 10, and 12.5 nmoles of NADH. LDH activity was calculated using the following equation:

$$LDH\ Activity\ (mU/mL) = \frac{\Delta A_{Final} - \Delta A_{Initial}\ (B)}{Reaction\ Time\ x\ V}\ x\ DF$$

In this case, B represents the amount of NADH produced (nmole). The sample volume (mL) injected into the well is represented by V, the sample dilution factor by DF, and absorbance changes by ΔA. The amount of LDH enzyme that catalyzes the conversion of lactate to pyruvate, producing 1.0 μmole of NADH per minute at 37°C, is defined as 1 unit of LDH activity. The LDH activity results are expressed as units per gram of protein (U/g protein).

Estimation of tissue total proteins

The total tissue proteins were estimated using the method described by Lowry et al.[27]. The results were expressed as milligrams of total protein per gram of tissue.

Evaluation of histopathological changes

The ISO-induced cardiac histopathological changes were evaluated using eosin-hematoxylin techniques, as described by Grimm [7], with minor modifications by Nikam et al. [28]. Briefly, tissue was fixed in 10% formalin and sectioned into 4 μm transverse slices. Cardiac tissue alterations were observed, and images were captured using an Olympus EP50 microscope camera (Olympus Corporation, Tokyo, Japan). Microscopic analyses were performed with a 400x light microscope, including a 35 μm scale bar.

Statistical analysis

All of the data were represented as mean ± standard deviations (SD). Data on systolic and diastolic blood pressure and heart rate were examined using a two-way analysis of variance (ANOVA) with a Bonferroni post hoc test. Data for CK-MB, ACE & PRA activities, hydroxyproline content, and LDH activity levels were examined using one-way ANOVA and Tukey's Multiple Range tests in GraphPad Prism version 5.0 software. A probability (p) value < 0.05 was judged statistically significant.

RESULTS

Effect of astaxanthin on isoproterenol -induced cardiac functional changes

Administration of ISO (5 mg/kg/day) for seven consecutive days in rats caused a significant ($p<0.05$) decrease in SAP and DAP and a significant increase in HR compared to the normal group. This suggests that ISO may induce potential HF through strong, nonselective β-adrenergic (β1) receptor agonist activity, which enhances inotropic and lusitropic effects in cardiac muscle. Oral administration of AST (25 and 50 mg/kg for 21 consecutive days) alleviates ISO-induced cardiac functional changes in a dose-dependent manner compared to the ISO group. This effect is similar to that seen with aliskiren (30 mg/kg), ramipril (4 mg/kg), and telmisartan (8 mg/kg) in the 21-day treatment groups. The impact of AST on ISO-induced changes in cardiac function, such as SAP, DAP, and HR, is shown in Fig. 1.

Effect of astaxanthin on isoproterenol -induced changes in serum and plasma biomarkers

Administering ISO (5 mg/kg/day) to rats for seven days caused significant ($p<0.05$) increases in CK-MB, ACE, and PRA levels compared to the control group. ISO may induce heart failure by promoting cardiac muscle injury and degeneration,

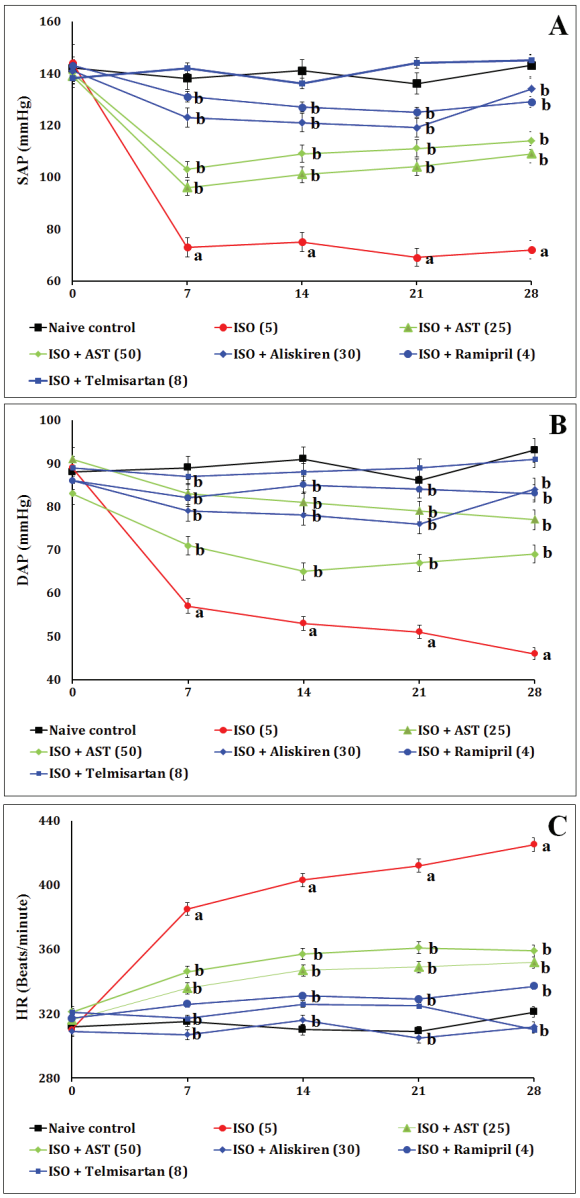

Fig. 1. Effect of AST on ISO-induced cardiac functional changes. Fig. 1A shows changes in SAP; Fig. 1B shows changes in DAP; and Fig. 1C shows changes in heart rate. Values in parentheses indicate the dose in mg/kg. Results are presented as mean ± standard deviation (SD), with n = 8 rats per group, analyzed using a two-way ANOVA with Bonferroni post hoc test. *p<0.05 vs. control group; #p<0.05 vs. ISO group. Abbreviations: AST, astaxanthin; DAP, diastolic blood pressure; HR, heart rate; ISO, isoproterenol; mmHg, millimeter of mercury (blood pressure units); and SAP, systolic blood pressure.

along with elevated circulating RAS mediators. Oral administration of AST (25 and 50 mg/kg) for 21 days decreased ISO-induced changes in serum and plasma biomarkers in a dose-dependent fashion, compared to the ISO group. This effect was comparable to results seen in groups treated for 21 days with aliskiren (30 mg/kg), ramipril (4 mg/kg), and telmisartan (8 mg/kg). Table 1 illustrates how AST influences ISO-induced changes in serum and plasma biomarkers, including CK-MB, ACE, and PRA levels.

Effect of astaxanthin on isoproterenol -induced changes in heart and body weight ratio

Administration of ISO (5 mg/kg/day) for 7 days in rats resulted in a significant ($p<0.05$) increase in the heart-to-body weight ratio compared to the control group. This indicates that ISO may induce potential heart failure (HF) through degeneration of cardiac muscle. Oral administration of AST (25 and 50 mg/kg) for 21 days reduced the ISO-induced changes in the heart-to-body weight ratio in a dose-dependent manner compared to the ISO group. The effect was similar to that observed in the 21-day treatment groups with aliskiren (30 mg/kg), ramipril (4 mg/kg), and telmisartan (8 mg/kg). The impact of AST on the ISO-induced heart and body weight ratio is summarized in Fig. 2.

Effect of astaxanthin on isoproterenol -induced changes in tissue biomarkers

Administering ISO (5 mg/kg/day) for 7 days to rats caused significant ($p<0.05$) increases in hydroxyproline and LDH activity levels compared to the control group. This suggests that ISO may induce potential HF by affecting diastolic function, myocardial structure, muscle mass, and energy metabolism. Oral treatment with AST (25 and 50 mg/kg for 21 days) reduces ISO-induced changes in tissue biomarkers in a dose-dependent way, similar to the effects seen in the groups treated for 21 days with aliskiren

Table 1. Effect of astaxanthin on isoproterenol - induced changes in serum and plasma biomarkers.

Groups	CK-MB (pg/mL)	ACE (ng/mL)	PRA (ng/mL)
Naive control	212 ± 13.5	3.22 ± 1.52	10.3 ± 0.9
ISO (5)	456 ± 14.9*	14.72 ± 1.14*	43.4 ± 1.3*
ISO + AST (25)	293 ± 12.7#	7.48 ± 0.93#	17.5 ± 0.8#
ISO + AST (50)	264 ± 8.6#	5.61 ± 1.04#	11.9 ± 1.2#
ISO + Aliskiren (30)	241 ± 13.1#	4.35 ± 1.47#	9.7 ± 0.4#
ISO + Ramipril (4)	227 ± 11.2#	4.39 ± 0.95#	15.4 ± 0.7#
ISO + Telmisartan (8)	217 ± 9.7#	3.45 ± 1.13#	13.7 ± 0.8#

The values in parentheses represent a dose in mg/kg. The results are presented as mean ± SD, with eight rats in each group. *$p<0.05$ vs. control group; #$p<0.05$ vs. ISO group. ACE, angiotensin-converting enzyme; AST, astaxanthin; CK-MB, creatine kinase isoenzyme-MB; ISO, isoproterenol; PRA, plasma renin activity. Groups were analyzed using a one-way ANOVA and Tukey's Multiple Comparisons test.

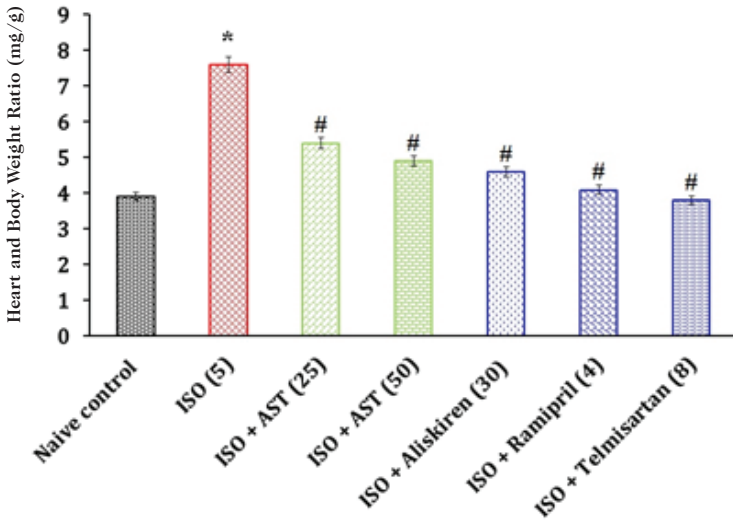

Fig. 2. Effect of AST on ISO-induced changes in the heart and body weight ratio. The values in parentheses indicate a dose in mg/kg. Results are shown as mean ± standard deviation (SD), with n = 8 rats per group, analyzed using a one-way ANOVA and Tukey's Multiple Comparisons test. *p<0.05 compared to the control group; #p<0.05 compared to the ISO group. Abbreviations: AST, astaxanthin; ISO, isoproterenol.

(30 mg/kg), ramipril (4 mg/kg), and telmisartan (8 mg/kg). The impact of AST on ISO-induced alterations in tissue biomarkers, such as hydroxyproline and LDH levels, is illustrated in Table 2.

Effect of astaxanthin on isoproterenol -induced histopathological changes

The histological changes in rat cardiac tissue in naive control animals showed no alterations in rat cardiomyocytes, i.e., branched cardiomyocytes with a central oval vesicular nucleus and mild separation of the connective tissue. In contrast, ISO (5 mg/kg/day; for 7 days) caused potential microscopic changes in cardiac tissue, such as cell destruction, pyknotic and karyolytic nuclei, hemorrhage, edema, and myocyte degeneration. Oral administration of AST (25 and 50 mg/kg) for 21 days alleviated ISO-induced histological alterations in cardiac tissue. These findings were comparable to those seen with reference RAS modulators, i.e., aliskiren (30 mg/kg), ramipril (4 mg/kg), and telmisartan (8 mg/kg) treatment groups. This indicates that AST exhibits cardioprotective effects against ISO-induced myocardial damage and dysfunction (Fig. 3). The changes were observed at 400× magnification (scale bar: 35 μm).

Table 2. Effect of astaxanthin on isoproterenol - induced changes in tissue biomarkers.

Groups	Hydroxyproline (μg/mg of tissue protein)	LDH activity (U/g protein)
Naive control	1.67 ± 0.92	1176.81 ± 12.73
ISO (5)	6.91 ± 1.02*	1492.18 ± 17.62*
ISO + AST (25)	2.29 ± 0.43#	1232.25 ± 9.04#
ISO + AST (50)	2.13 ± 0.67#	1213.94 ± 11.14#
ISO + Aliskiren (30)	2.03 ± 1.05#	1199.07 ± 10.46#
ISO + Ramipril (4)	1.82 ± 0.87#	1204.63 ± 11.06#
ISO + Telmisartan (8)	1.94 ± 1.03#	1191.04 ± 9.83#

The values in parentheses indicate a dose in mg/kg. The results are shown as mean ± SD, with eight rats in each group. *$p<0.05$ versus control group; #$p<0.05$ versus ISO group. AST, astaxanthin; ISO, isoproterenol; and LDH, lactate dehydrogenase.

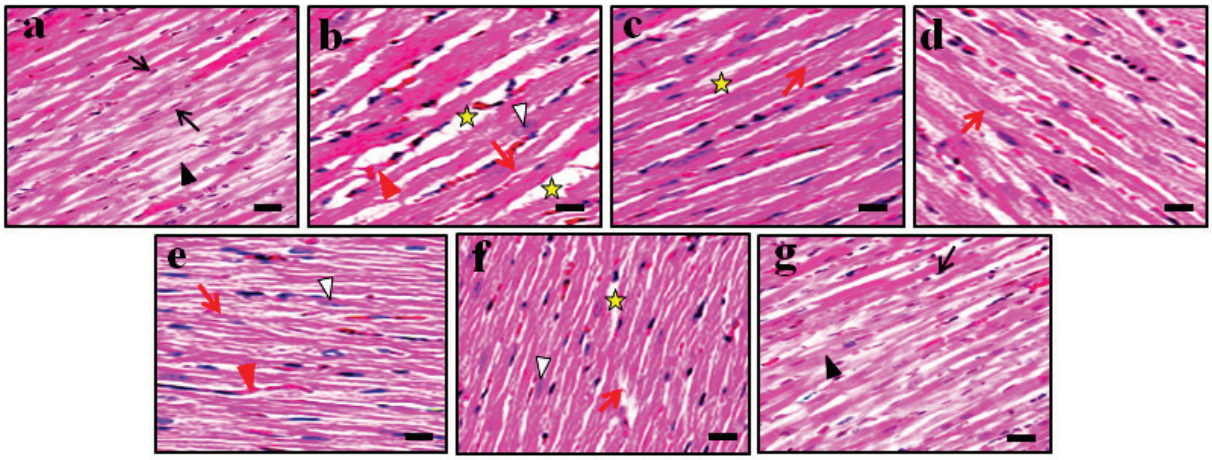

Fig. 3. Effect of AST on ISO-induced histopathological changes in rat cardiac tissue. In each group, two rats were used to evaluate cardiac tissue histopathology. Tissue sections were stained with eosin and hematoxylin. Figures 3a to 3g display histological changes in cardiac tissue from naïve control, ISO (5 mg/kg/day for 7 days), AST (25 mg/kg for 21 days), AST (50 mg/kg for 21 days), aliskiren (30 mg/kg for 21 days), ramipril (4 mg/kg for 21 days), and telmisartan (8 mg/kg for 21 days) groups, respectively. Fig. 3a shows normal cardiac tissue structure. Fig. 3b depicts ISO-associated myocardial damage. Fig. 3c and 3g demonstrate that AST and RAS modulators have cardioprotective effects against ISO toxicity. In this figure, the black arrow points to a normal pyknotic and karyolytic nucleus; the black arrowhead indicates normal cardiac cell structure; the white arrowhead shows cell destruction; the red arrow highlights abnormalities in myocardial fiber arrangement; the red arrowhead signifies hemorrhage and edema; and the yellow star indicates myocyte degeneration. Microscopic examinations were performed at 400× magnification; scale bar, 35 μm.

DISCUSSION

The i.p. administration of ISO (5 mg/kg/day for seven days) in rats demonstrated a significant ($p<0.05$) development of cardiac dysfunction through alterations in hemodynamic parameters, such as reductions in SAP and DAP, along with increases in HR levels and elevated serum CK-MB, ACE, and PRA levels. Furthermore, there was an increase in the heart-to-body weight ratio and in tissue hydroxyproline and LDH activity lev-

els. ISO was also shown to destroy myocytes, resulting in pyknotic cells, karyolytic nuclei, hemorrhage, edema, and myocyte degeneration. These findings indicate that ISO causes potential HF through multiple pathway abnormalities, including free radical-associated cardiac damage, altered energy demands, and RAS pathway disruptions [29,30]. Similar results were observed in ISO-induced HF in rats, which showed changes in the same hemodynamic parameters and myocardial function. These outcomes are consistent with those reported in other studies [31]. Oral AST therapy at 25 and 50 mg/kg for 21 days significantly reduced ISO-related cardiac dysfunctions, biomarker alterations, and histological abnormalities. This effect was comparable to that seen in the RAS modulator treatment groups (aliskiren, ramipril, and telmisartan).

Circulatory and local ACE activity, plasma renin content, and the renin-angiotensin system in the heart and aorta contribute to the development of cardiovascular issues, including myocardial infarction and heart failure [32,33]. Proteomic analysis showed that ISO increases myocardial tissue weight by 55% in hypertrophied rat hearts [34]. Additionally, ISO raises left ventricular tissue weight by 33% and decreases systolic and diastolic blood pressure by 13%. Administering ISO (1, 10, 100, and 500 μg/kg) increases plasma renin activity in a dose-dependent manner [33]. Inhibitors of the renin-angiotensin system are known to improve heart failure in experimental animals [35,36]. RAS modulators, such as spironolactone and captopril, reduce ISO-induced cardiac remodeling in rats[37]. Aliskiren has been shown to lower serum cardiac enzymes like LDH and CK-MB 38, decrease cardiac biomass [39], and also reduce oxidative stress and apoptosis [38,40]. In this study, the AST treatment group exhibited results similar to those of the control group. However, the ACE inhibitor ramipril worsens ISO-induced myocardial dysfunctions by regulating hydroxyproline content in rats[41,42]. The current research demonstrates that AST alleviates ISO-induced cardiac damage by enhancing mitochondrial function and scavenging free radicals in rats [43]. AST is also known to decrease ACE protein expression, mitigate hypertensive conditions [21], prevent ISO-induced loss of cardiac muscle mass [43], and reduce hydroxyproline content [44].

Normally, ISO does not influence ACE2 expression. However, some studies have reported elevated ACE2 levels in Sprague-Dawley rats [6]. Another study indicated that increased cardiac angiotensin-II peptide levels are linked to higher ACE2 expression in normal rats [14]. Nonetheless, these effects are not observed in other conditions or species[15]. Therefore, the impact of ISO on the renin-angiotensin system is complex and varies depending on the specific tissue and animal model. Experimentally, ISO is known to raise plasma angiotensin-II peptide levels, which can lead to left ventricular hypertrophy and worsen heart failure progression[16]. Additionally, angiotensin II directly influences cardiac tissue via the angiotensin II type 2 receptor, resulting in myocardial damage [45]. Furthermore, administering an angiotensin II receptor antagonist, such as telmisartan, can reduce ISO-induced cardiac dysfunction[46]. Similar findings are observed in this study: telmisartan (8 mg/kg) and AST administration normalize elevated blood pressure and modify biomarker levels, including serum CK-MB, ACE, PRA, hydroxyproline, and LDH activity. Moreover, AST has been shown to lower blood pressure in spontaneously hypertensive rats [47] by inhibiting ACE activity and regulating the RAS [48]. Additionally, AST supplementation has demonstrated the ability to decrease blood pressure in humans by suppressing the RAS [49]. Our study also indicates that AST improves ISO-induced cardiac dysfunction and blood pressure control through RAS regulatory mechanisms.

The current study revealed that oral treatment with AST reduces ISO-induced cardiac dysfunction by regulating SAP and DAP, decreasing elevated HR, CK-MB, ACE,

and PRA levels, and lowering the heart-to-body weight ratio, tissue hydroxyproline, and LDH activity levels. Therefore, AST provides cardioprotection against ISO-induced toxicity by regulating the RAS system. It can potentially be used for various heart failure conditions. However, further research using diverse preclinical animal models and human subjects is necessary.

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Conflict of interest

The authors declare no conflicts of interest regarding the present study.

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Contribution of the authors

LC: performed the experiments, collected data, conceived the study, and drafted the manuscript. FQ: supervised the project, secured funding, and critically revised the intellectual content.

REFERENCES

1. **He X, Huang S, Yu C, Chen Y, Zhu H, Li J, et al.** Mst1/Hippo signaling pathway drives isoproterenol-induced inflammatory heart remodeling. Int J Med Sci. 2024;21(9):1718-1729. *https://doi.org/10.7150/ijms.95850.*
2. **Bozkurt B, Ahmad T, Alexander K, Baker WL, Bosak K, Breathett K, et al.** HF STATS 2024: Heart Failure Epidemiology and Outcomes Statistics an Updated 2024 Report from the Heart Failure Society of America. J Card Fail. 2025;31(1):66-116. *https://doi.org/10.1016/j.cardfail.2024.07.001.*
3. **Perez-Bonilla P, LaViolette B, Bhandary B, Ullas S, Chen X, Hirenallur-Shanthappa D.** Isoproterenol induced cardiac hypertrophy: A comparison of three doses and two delivery methods in C57BL/6J mice. PLoS One. 2024;19(7):e0307467. *https://doi.org/10.1371/journal.pone.0307467.*
4. **Fujimura N, Sumita S, Narimatsu E, Nakayama Y, Shitinohe Y, Namiki A.** Effects of isoproterenol on diaphragmatic contractility in septic peritonitis. Am J Respir Crit Care Med. 2000;161(2 Pt 1):440-446. *https://doi.org/10.1164/ajrccm.161.2.9904044.*
5. **Rebrova TY, Korepanov VA, Stepanov I V, Afanasiev SA.** Modeling of isoproterenol-induced chronic heart failure in 24-month-old rats. Bull Exp Biol Med. 2024;178(1):30-33. *https://doi.org/10.1007/s10517-024-06277-8.*
6. **Nadu AP, Ferreira AJ, Reudelhuber TL, Bader M, Santos RA.** Reduced isoproterenol-induced renin-angiotensin changes and extracellular matrix deposition in hearts of TGR(A1–7)3292 rats. J Am Soc Hypertens. 2008;2(5):341-348. *https://doi.org/10.1016/j.jash.2008.04.012.*
7. **Grimm D, Elsner D, Schunkert H, Pfeifer M, Griese D, Bruckschlegel G.** Development of heart failure following isoproterenol administration in the rat: Role of the renin–angiotensin system. Cardiovasc Res. 1998;37(1):91-100. *https://doi.org/10.1016/S0008-6363(97)00212-5.*
8. **Keihanian F, Moohebati M, Saeidinia A, Mohajeri SA, Madaeni S.** Therapeutic effects of medicinal plants on isoproterenol-induced heart failure in rats. Biomed Pharmacother. 2021;134:111101. *https://doi.org/10.1016/j.biopha.2020.111101.*
9. **Driscoll DJ, Gillette PC, Lewis RM, Hartley CJ, Schwartz A.** Comparative hemody-

namic effects of isoproterenol, dopamine, and dobutamine in the newborn dog. Pediatr Res. 1979;13(9):1006-1009. *https://doi.org/10.1203/00006450-197909000-00011.*

10. **Zinzuvadia D, Suthar J, Patel A, Shah U, Solanki N, Koria H.** *Gynostemma pentaphyllum* Makino exerts cardioprotective effects by improving hemodynamic, biochemical and histopathological changes through activation of PI3K signalling in isoproterenol-induced myocardial infarction in rats. Am J Transl Res. 2024;16(6):2290-2300. *https://doi.org/10.62347/GHIX8452.*
11. **Forte E, Panahi M, Baxan N, Ng FS, Boyle JJ, Branca J, et al.** Type 2 MI induced by a single high dose of isoproterenol in C57BL/6J mice triggers a persistent adaptive immune response against the heart. J Cell Mol Med. 2021;25(1):229-243. *https://doi.org/10.1111/jcmm.15937.*
12. **Pan Y, Gao J, Gu R, Song W, Li H, Wang J, et al.** Effect of injection of different doses of isoproterenol on the hearts of mice. BMC Cardiovasc Disord. 2022;22(1):409. *https://doi.org/10.1186/s12872-022-02852-x.*
13. **Krenek P, Kmecova J, Kucerova D, Bajuszova Z, Musil P, Gazova A, et al.** Isoproterenol-induced heart failure in the rat is associated with nitric oxide-dependent functional alterations of cardiac function. Eur J Heart Fail. 2009;11(2):140-146. *https://doi.org/10.1093/eurjhf/hfn026.*
14. **Cano IP, Dionisio TJ, Cestari TM, Calvo AM, Colombini-Ishikiriama BL, Faria FAC, et al.** Losartan and isoproterenol promote alterations in the local renin-angiotensin system of rat salivary glands. PLoS One. 2019;14(5): e0217030 *https://doi.org/10.1371/journal.pone.0217030.*
15. **Kim JE, Kang YJ, Lee KY, Choi HC.** Isoproterenol inhibits angiotensin II-stimulated proliferation and reactive oxygen species production in vascular smooth muscle cells through heme oxygenase-1. Biol Pharm Bull. 2009;32(6):1047-1052. *https://doi.org/10.1248/bpb.32.1047.*
16. **Leenen FH, White R, Yuan B.** Isoproterenol-induced cardiac hypertrophy: Role of circulatory versus cardiac renin-angiotensin system. Am J Physiol Heart Circ Physiol. 2001;281(6):H2410-6. *https://doi.org/10.1152/ajpheart.2001.281.6.H2410.*
17. **Mohammadi SG, Feizi A, Bagherniya M, Shafie D, Ahmadi A-R, Kafeshani M.** The effect of astaxanthin supplementation on inflammatory markers, oxidative stress indices, lipid profile, uric acid level, blood pressure, endothelial function, quality of life, and disease symptoms in heart failure subjects. Trials. 2024;25(1):518. *https://doi.org/10.1186/s13063-024-08339-8.*
18. **Kato T, Kasai T, Sato A, Ishiwata S, Yatsu S, Matsumoto H, et al.** Effects of 3-month astaxanthin supplementation on cardiac function in heart failure patients with left ventricular systolic dysfunction - A pilot study. Nutrients. 2020;12(6): 1896. *https://doi.org/10.3390/nu12061896.*
19. **Sarker M, Chowdhury N, Bristy AT, Emran T, Karim R, Ahmed R, et al.** Astaxanthin protects fludrocortisone acetate-induced cardiac injury by attenuating oxidative stress, fibrosis, and inflammation through TGF-β/Smad signaling pathway. Biomed Pharmacother. 2024;181:117703. *https://doi.org/10.1016/j.biopha.2024.117703.*
20. **Pereira CPM, Souza ACR, Vasconcelos AR, Prado PS, Name JJ.** Antioxidant and anti−inflammatory mechanisms of action of astaxanthin in cardiovascular diseases (Review). Int J Mol Med. 2021;47(1):37-48. *https://doi.org/10.3892/ijmm.2020.4783.*
21. **Gao C, Gong N, Chen F, Hu S, Zhou Q, Gao X.** The effects of astaxanthin on metabolic syndrome: A comprehensive review. Mar Drugs. 2025;23(1):9. *https://doi.org/10.3390/md23010009.*
22. **Wang Y, Thatcher SE, Cassis LA.** Measuring blood pressure using a noninvasive tail cuff method in mice. Methods Mol Biol. 2017;1614:69-73. *https://doi.org/10.1007/978-1-4939-7030-8_6.*
23. **Stegemann H, Stalder K.** Determination of hydroxyproline. Clin Chim Acta. 1967;18(2):267-273. *https://doi.org/10.1016/0009-8981(67)90167-2.*

24. **Cissell DD, Link JM, Hu JC, Athanasiou KA.** A modified hydroxyproline assay based on hydrochloric acid in Ehrlich's solution accurately measures tissue collagen content. Tissue Eng Part C Methods. 2017;23(4):243-250. *https://doi.org/10.1089/ten.tec.2017.0018.*
25. **Singh H, Kaur P, Kaur P, Muthuraman A, Singh G, Kaur M.** Investigation of therapeutic potential and molecular mechanism of vitamin P and digoxin in I/R-induced myocardial infarction in rat. Naunyn Schmiedebergs Arch Pharmacol. 2015;388(5):565-574. *https://doi.org/10.1007/s00210-015-1103-8.*
26. **Dewi S, Ramadhani ANA, Az-zahra KA, Wardaya W.** Specific activity of lactate dehydrogenase in muscle and liver tissues of rats exposed to intermittent hypobaric hypoxia. BIO Integration. 2025; 6: 1–6. *https://doi.org/10.15212/bioi-2024-0074.*
27. **Lowry OH, Rosebrough NJ, Farr AL, Randall RJ.** Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-275. *https://doi.org/10.1016/S0021-9258(19)52451-6.*
28. **Nikam AP, Ghule AE, Piplani P, Bansal J, Bodhankar SL.** Cardioprotective and antiarrythmic activity of oxalate salt of 1-(isopropylamino)-3-(5-((isopropylamino) methyl)-2-methoxyphenoxy) propan-2-ol (PP-24): A newly synthesized aryloxypropanolamine derivative. Biomed Aging Pathol. 2011;1(2):84-89. *https://doi.org/10.1016/j.biomag.2011.06.003.*
29. **Bader Eddin L, Nagoor Meeran MF, Kumar Jha N, Goyal SN, Ojha S.** Isoproterenol mechanisms in inducing myocardial fibrosis and its application as an experimental model for the evaluation of therapeutic potential of phytochemicals and pharmaceuticals. Animal Model Exp Med. 2025;8(1):67-91. *https://doi.org/10.1002/ame2.12496.*
30. **Shakery A, Pourvali K, Shimi G, Zand H.** Isoproterenol Alters Metabolism, Promotes Survival and Migration in 5-Fluorouracil-Treated SW480 Cells with and without Beta-hydroxybutyrate. Int J Mol Cell Med. 2023;12(2):144-158. *https://doi.org/10.22088/IJMCM.BUMS.12.2.144.*
31. **Liu J, Li W, Jiao R, Liu Z, Zhang T, Chai D, et al.** Miglustat ameliorates isoproterenol-induced cardiac fibrosis via targeting UGCG. Mol Med. 2025;31(1): 288. *https://doi.org/10.1186/s10020-025-01360-w.*
32. **Busatto VC, Cunha V, Cicilini MA, Mill JG.** Differential effects of isoproterenol on the activity of angiotensin-converting enzyme in the rat heart and aorta. Braz J Med Biol Res. 1999;32(3):355-360. *https://doi.org/10.1590/s0100-879x1999000300017.*
33. **Leenen FH, McDonald RH Jr.** Effect of isoproterenol on blood pressure, plasma renin activity, and water intake in rats. Eur J Pharmacol.1974;26(2):129-135. *https://doi.org/10.1016/0014-2999(74)90218-0.*
34. **Chowdhury D, Tangutur AD, Khatua TN, Saxena P, Banerjee SK, Bhadra MP.** A proteomic view of isoproterenol induced cardiac hypertrophy: Prohibitin identified as a potential biomarker in rats. J Transl Med. 2013;11:130. *https://doi.org/10.1186/1479-5876-11-130.*
35. **Erokhina IL, Okovityĭ S V, Kulikov AN, Kazachenko AA, Emel'ianova OI.** Effects of renin-angiotensin system inhibitors on density of rat myocardial, pericardial, and pulmonary mast cells in experimental heart failure. Cell Tiss Biol. 2009;3:544-550. *https://doi.org/10.1134/S1990519X09060078.*
36. **Szymanski MW, Singh DP.** Isoproterenol. In: StatPearls [Internet]. Treasure Island (FL); StatPearls Publishing. 2025. Disponible en: https://www.ncbi.nlm.nih.gov/books/NBK526042/
37. **Gallego M, Espiña L, Vegas L, Echevarria E, Iriarte MM, Casis O.** Spironolactone and captopril attenuates isoproterenol-induced cardiac remodelling in rats. Pharmacol Res. 2001;44(4):311-315. *https://doi.org/10.1006/phrs.2001.0865.*
38. **Bin-Jaliah I, Hussein AM, Sakr HF, Eid EA.** Effects of low dose of aliskiren on isoproterenol-induced acute myocardial infarction in rats. Physiol Int. 2018;105(2):127-144. *https://doi.org/10.1556/2060.105.2018.2.11.*

39. **Weng LQ, Zhang WB, Ye Y, Yin PP, Yuan J, Wang XX, et al.** Aliskiren ameliorates pressure overload-induced heart hypertrophy and fibrosis in mice. Acta Pharmacol Sin. 2014;35(8):1005-1014. *https://doi.org/10.1038/aps.2014.45.*
40. **Zhao Z, Liu H, Guo D.** Aliskiren attenuates cardiac dysfunction by modulation of the mTOR and apoptosis pathways. Braz J Med Biol Res. 2020;53(2):e8793. *https://doi.org/10.1590/1414-431X20198793.*
41. **Keles MS, Bayir Y, Suleyman H, Halici Z.** Investigation of effects of Lacidipine, Ramipril and Valsartan on DNA damage and oxidative stress occurred in acute and chronic periods following isoproterenol-induced myocardial infarct in rats. Mol Cell Biochem. 2009;328(1-2):109-117. *https://doi.org/10.1007/s11010-009-0080-y.*
42. **Khloussy HM, Badawy AD, Mohamed YSIE, Maher M.** Role of 17-β estradiol and ramipril in OPG/RANKL pathway in a rat model of post-menopausal osteoporosis. Egypt J Hosp Med. 2023;90(1):522-527. *https://doi.org/10.21608/ejhm.2023.279677.*
43. **Mahmoud DSE, Kamel MA, El-Sayed IE, Binsuwaidan R, Elmongy EI, Razzaq MK, et al.** Astaxanthin ameliorated isoproterenol induced myocardial infarction via improving the mitochondrial function and antioxidant activity in rats. J Biochem Mol Toxicol. 2024;38(8):e23804. *https://doi.org/10.1002/jbt.23804.*
44. **Ni Y, Nagashimada M, Zhuge F, Zhan L, Nagata N, Tsutsui A, et al.** Astaxanthin prevents and reverses diet-induced insulin resistance and steatohepatitis in mice: A comparison with vitamin E. Sci Rep. 2015;5:17192. *https://doi.org/10.1038/srep17192.*
45. **Althammer F, Roy RK, Kirchner MK,** Podpecan Y, Helen J, **McGrath S,** Campos Lira **E, Stern JE.** Angiotensin-II drives changes in microglia-vascular interactions in rats with heart failure. Commun Biol. 2024;7(1):1537. *https://doi.org/10.1038/s42003-024-07229-8.*
46. **Guo BY, Li YJ, Han R, Yang SL, Shi YH, Han DR, et al.** Telmisartan attenuates isoproterenol-induced cardiac remodeling in rats via regulation of cardiac adiponectin expression. Acta Pharmacol Sin. 2011;32(4):449-455. *https://doi.org/10.1038/aps.2010.231.*
47. **Hussein G, Nakamura M, Zhao Q, Iguchi T, Goto H, Sankawa U, et al.** Antihypertensive and neuroprotective effects of astaxanthin in experimental animals. Biol Pharm Bull. 2005;28(1):47-52. *https://doi.org/10.1248/bpb.28.47.*
48. **Preuss HG, Echard B, Bagchi D, Perricone NV, Yamashita E.** Astaxanthin lowers blood pressure and lessens the activity of the renin-angiotensin system in Zucker fatty rats. J Funct Foods. 2009;1(1):13-22. *https://doi.org/10.1016/j.jff.2008.09.001.*
49. **Mokhtari E, Rafiei S, Shokri-Mashhadi N, Saneei P.** Impact of astaxanthin supplementation on blood pressure: A systematic review and meta-analysis of randomized controlled trials. J Funct Foods. 2021;87:104860. *https://doi.org/10.1016/j.jff.2021.104860.*