Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Ameliorative Effects of Capsaicin on Biochemical, Molecular, and Histopathological Changes in Experimentally-induced Cardiac Infarction in Rats

Aya A. Abo Alhamd[1], Omnia Mahmoud Abdel El-Hamid[1], Yakout A. Elsenosi[1], Mohammed A. Marzouk[1] and Demiana H. Hanna[2]*

[1]*Department of Biochemistry, Faculty of Vet.Med. Moshtohor, Benha University, Egypt.*

[2] *Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt.*

Abstract

MYOCARDIAL infarction (MI) is among the major reasons for passing away in the developed globe with a prevalence of about three million cases yearly. Capsaicin (Cap) exhibits antioxidant, anti-inflammatory, anti-carcinogenic, and heart-protective impacts. **Aim:** This trial aimed to investigate the ameliorative actions of Cap administration against rats with triggered myocardial infarction using assessment of biochemical, molecular, and histopathological changes.

Method: Rats were split up into three groups. Group I (n=15); Normal control group. Group II (n=15); Isoproterenol was administered subcutaneously to rats (20 mg/kg/day). Group III (n=15); Isoproterenol was administered subcutaneously to rats (20 mg/kg/day) and then administrated orally with capsaicin daily (1mg/kg.bw) for 30 days. Parameters Creatin Kinase-MB (CK-MB**),** Lactate dehydrogenase (LDH), pro-B-type natriuretic peptide (pro-BNP), Troponin-T (trop-T), Myoglobin, hs-C reactive protein (hs-CRP), Tumor Necrosis Factor Alpha (TNF-α) and Interleukine-6 (IL-6) were measured in the three groups using ELISA method. Also, Through the use of real-time Quantitative PCR, the expression levels of Matrix Metalloproteinase-9 (MMP-9), Hypoxia-inducible factor-1-alpha (hif1-α), and sirtuin-1 (Sirt-1) were measured in the three groups. Additionally, Histological examination were carried out in the heart tissue in all groups.

Results: The findings showed that rats with MI caused by isoproterenol had significantly higher blood levels of CK-MB, LDH, pro-BNP, Trop-T, myoglobin, hs-CRP, IL-6, and TNF-α in group II contrasted with the group I. Also, Cardiac tissue of isoproterenol-induced MI rats in group II indicated significant up-regulation in MMP-9 and hif1-α gene expression levels contrasted with the group I ($P<0.05$). Conversely, significant down-regulation in Sirt-1 expression level in group II in contrasted to group I. Various histopathological alterations were detected in heart tissue of rats treated with isoproterenol in group II as opposed to Normal control group I. Interestingly, rats treated with capsaicin in group III demonstrated marked reduction ($P<0.05$) in all measured biological parameters CK-MB, LDH, pro-BNP, trop-T, myoglobin, hs-CRP, IL-6 and TNF-α II in contrasted to group II. Additionally, group III indicated significant down-regulation in MMP-9 and hif1-α gene expression levels compared to group II ($P<0.05$). Conversely, significant up-regulation in Sirt-1 expression level in group III compared to group II. Also, Heart tissue showed improvement in pathological alterations in Cap treated group III in comparison to isoproterenol rats in group II.

Conclusion: These findings suggest that supplementation of capsaicin counteracts the negative effects of Isoproterenol, and could be used as a new alternative cardio-treated strategy at Infarction of the heart.

Keywords: Myocardial infarction, Isoproterenol, Capsaicin, Pro-B-type natriuretic peptide.

Introduction

Worldwide, cardiovascular illnesses are the primary reason for illness and death, accounting for over 17.9 million deaths. Since Infarction of the heart (MI) is among the most prevalent of these conditions, it is expected that 23.6 million deaths worldwide will be caused by MI by 2030 [1].

*Corresponding authors: Demiana H. Hanna, Email.: dhelmy@sci.cu.edu.eg Tel.: 01015181160
(Received 27 November 2024, accepted 02 February 2025)
DOI: 10.21608/EJVS.2025.339750.2521

The pathological definition of myocardial infarction (MI), is also referred to as the permanent death of cardiac cells resulting from ischemia, sometimes known as a heart attack. A variety of symptoms are able be used to identify MI, with the most common sign being chest pain. These symptoms can also be confirmed by changes in biochemical laboratory tests, electrocardiograms (ECGs), or results from imaging modalities that can identify myocardial infarction and myocardial death [2]. Persistent ischemia results in myocardial infarction, which kills cardiac cells irreversibly (necrosis). When a thrombus develops because there is no blood supply to the myocardium that is distal to the obstruction, necrosis takes place. The heart stops contracting in the necrotic area. The magnitude of the infarction and the affected cardiac region determine how much function is changed [3].

Isoproterenol (ISO) is a synthetic catecholamine and a β-adrenergic receptor agonist. When rats are injected with ISO subcutaneously (S.C.), they eventually develop MI and irreversible cellular damage [4]. One common model used to study how medications affect heart function is isoproterenol (ISO), a synthesized catecholamine that is essential for controlling cardiac contractility and metabolism [5]. Similar to patients with MI, Rats with ISO-induced MI also experience immediate hemodynamic and electrocardiographic changes. As a result, A reliable non-invasive technique for examining the effects of several potential cardioprotective drugs is the rat model of ISO-induced MI [6]. It has been discovered that ISO exerts significant stress on the myocardium, causing the heart muscle to necrotize in an infarct-like manner. A few of the theories to explain how ISO causes harm to cardiac myocytes are calcium overload, myocardial hyperactivity-induced hypoxia, coronary hypotension, loss of energy reserves, and increased production of free radicals due to improper catecholamine metabolism [7].

In the field of medication research and discovery, naturally-developed products are considered to be among the most crucial and indispensable sources of data. These products and/or herbal formulations have been used by humans for ages to promote mental and physical well-being, prevent sickness, and maintain health [8].

The prevention and treatment of cardiovascular disease (CVD) frequently involve the use of conventional drugs; however, their use is decreasing because of worries about potential long-term negative effects. Medicinal plant preparations, which have been demonstrated to treat and safeguard the cardiovascular system, are attracting increased attention as prospective substitutes for contemporary medications [9].

One of the naturally spicy ingredients of chili peppers, which are several types of plants in the genus Capsicum, is capsaicin (Cap), often referred to as 8 methyl N vanillyl-trans 6 nonenamide [10]. The strong alkaloid found in spicy red peppers, capsaicin, has a range of biological impacts, including thermogenic, analgesic, anti-inflammatory, anti-lithogenic, and cardioprotective properties [11]. It has been demonstrated that dietary capsaicin can slow the onset of atherosclerotic plaques in a number of species, including hamsters, rats, mice, and guinea pigs [12]. Habitual chili eaters have lower rates of cardiovascular disease morbidity and mortality than non-eaters, according to three sizable epidemiological studies from various nations (the USA, China, and Italy) [13].

In the current study, capsaicin's effects on myocardial infarction were examined using an in vivo animal prototype in order to assess if capsaicin, a natural substance, might prevent myocardial infarction in rats that isoproterenol induces.

Material and Methods

Standards and Reagents

All of the supplies, including capsaicin and isoproterenol, were purchased from Sigma-Aldrich (St. Louis, MO, USA), along with chemicals, reagents, solvents, buffers, and other items.

Experimental animals

A total of forty-five male albino rats, with an average weight of 150–200 grams, were obtained from "The Laboratory Animals Research Center" at the Moshtohor Faculty of Veterinary Medicine, Benha University. The ages of the rats varied from 12 to 16 weeks. During the entire trial, the animals were housed in metal cages that provided ideal environmental and nutritional conditions. The rats were allowed two weeks to adjust to their new environment prior to the start of the experiment

Experimental design

Following two weeks of acclimation, rats were split into three groups randomly (15 rats per each group). The groups were then housed in different cages and given the following classifications:

Group I (normal control): Rats were used as the control in all experimental groups and were not given any medication.

Group II (Isoproterenol group): Rats were given two subcutaneous injections of isoproterenol (20 mg/Kg.BW) separated by a 24-hour period over the course of two consecutive days [14].

Group III (Capsaicin group): Isoproterenol (20 mg/Kg.BW) was injected subcutaneously into rats twice a day, separated by 24 hours. After that, capsaicin intragastric tube injection was administered to rats at a rate of 1 mg/kg BW/day thirty days later.

Sampling

Blood Samples

Blood samples were taken from the retro orbital plexus of the eyes under general anesthesia and

placed in sterile, dry screw-capped containers. After that, it was centrifuged for 15 minutes at 3000 r.p.m. and left to coagulate for 30 minutes at room temperature. Using a Pasteur pipette, the clear, clean serum was aspirated and put into a dry, sterile sample tube. After that, it was kept in a deep freezer at -20° C until it was needed for the biochemical analysis that followed. Every serum underwent analysis for the subsequent parameters: LDH, trop-T, and IL-6 were assessed by Rat ELISA kits purchased from Cusabio, Catalog Number (CSB-E11324r), (CSB-E16443r) and (CSB-E04640r) respectively. In addition to CRP, myoglobin, CK-MB, and TNF-α were investigated using an ELISA kit which was supplied via Abcam with Catalog Numbers (ab256398), (ab260068), (ab285275), and (ab46070) respectively. Also, pro-BNP was evaluated using Novus Biologicals biotech brand ELISA kits with Catalog Number (NBP2-68140) in compliance with the manufacturer's instructions.

Tissue samples

Following the collection of blood, Rats were cervical decapitated and sacrificed, and the heart tissues from each experimental animal were removed, cleaned with saline, and split into two sections as follows:

Real-time PCR of Heart tissues

Matrix metalloproteinase-9 (MMP-9), sirtuin-1 (Sirt-1), and inducible-hypoxiafactor-1-alpha (hif1-α) gene degrees of expression were relatively quantified by RT-PCR[15]. The initial cardiac tissue segment, in short, each experimental animal's 0.5 g left ventricle base was placed in an Eppendorf tube and maintained at -80°C until The RNA was taken out. Using the RNeasy Mini kit (Catalogue no. 74104) and the manufacturer's instructions, total RNA was extracted from the specimen. Thermo Fisher Scientific RevertAid cDNA synthesis kit (catalog # EP0441) was then used to synthesize first-strand cDNA from the acquired RNA specimens in compliance with the manufacturer's instructions. PCR Master Mix preparation with the Quantitect SYBR green PCR kit (Cat. No. 204141). Real-time PCR using a real-time PCR machine was the next step (Stratagene MX3005P) employing particular primers supplied from Metabion (Germany) for the genes under investigation, as indicated in Table (1).

Heart tissues for histopathological examination

In the second part of the cardiac tissue, Rats in various groups had their hearts autopsied, and the samples were kept in 10% formol saline for a whole day. The samples were dehydrated using methyl, ethyl, and 100% ethyl alcohol dilutions in order to be cleaned with tap water. Specimens were immersed in paraffin for 24 hours at 56 degrees in a hot air oven after being cleaned with xylene. Bee paraffin wax was used to form tissue blocks for sectioning at a thickness of 4 microns using a revolving LEITZ microtome. The tissue sections that were obtained were gathered onto glass slides, deparaffinized, and stained with hematoxylin and eosin [16-21] to be examined using a light microscope, X40.

Statistical Analysis

A one-way analysis of variance (ANOVA) with the Least Significant Difference (LSD) was used to estimate the differences in variable means between groups. With the results expressed as mean ± Standard Error (SE), The statistical program utilized for data analysis was the Statistical Package for Social Science (SPSS) version 20 for Windows (SPSS® Chicago, IL, USA). The probability was deemed significant when it was less than 0.05.

Results

Biomarkers of myocardial dysfunction or Stress (Natriuretic peptides)

The effect of ISO on myocardial stress is demonstrated in the current investigation by a statistically significant increase in myocardial Pro-BNP in the ISO group (II) compared to the Normal control group (I). while, Rats treated withCAP in group (III) showed significantly decreased BNP in contrast to ISO group (II), as stated in (table 2).

Biomarkers of myocardial injury (Cardiac troponin, Myoglobin, CK-MB and LDH)

Significant elevation of cardiac enzymes was recorded in ISO groups in comparison to the control group. Meanwhile, a significant decline of heart-related enzymes was shown in treated groups as shown in (table 2).

While, the obtained findings in Table (2) revealed that the treatment with CAP in group (III) exhibits a significant decline in serum levels of these heart-related enzymes including CK-MB, LDH, troponin, and Myoglobin in contrast to ISO group (II).

Biomarkers of inflammation

As represented in Table (2), the current study's ISO-induced MI group (II) significantly elevated marker that reduces inflammation, such as hs-CRP, TNF-α, and IL-6, in comparison to the normal control group (I). However, in this work blood markers (hs-CRP, TNF-α and IL-6 greatly declined in CAP treated group (III) as compared to the MI group (II) as shown in Table (2).

Gene expression studies

The results obtained, as shown in Fig.1, indicated that the ISO group (II) had down-regulated Sirt-1 gene expression levels compared to the control group (I), whereas MMP-9 and hif1-α gene expression levels were up-regulated. Comparing the CAP group (III) to the myocardial infarction group (II), the latter demonstrated up-regulation in the expression level of the Sirt-1 gene and down-regulation in the expression levels of MMP-9 and hif1-α genes.

Histopathological findings

The microscopic examination of the rats' hearts in the control group (I) was displayed in Fig. 2.a, showing that the myocardial bundles' normal histological structure was present, and there were no histopathological changes. Further, the microscopic examination of the rats' hearts in the isoproterenol-treated group (II) demonstrated that the myocardial bundles showed a focal area of coagulative necrosis infiltrated by leucocyte inflammatory cells (Fig. 2.b) and replaced by fibroblastic cell proliferation (Fig. 2.c). Also, the microscopic examination of the heart of rats in the capsaicin-treated group (III) revealed that there was focal extravasation of red blood cells with an Oedema and a little infiltration of inflammatory cells between the myocardium (Fig. 2.d).

Discussion

Biomarkers of myocardial dysfunction or Stress (Natriuretic peptides)

The effect of ISO on myocardial stress is demonstrated in the current investigation by a statistically significant increase in myocardial Pro-BNP in the ISO group (II) relative to the control group (I). Artificial catecholamine and beta-adrenergic agonist isoprenaline/isoproterenol (ISO) triggers extreme myocardial stress, leading to an infarct-like death of the cardiac muscle in experimental animals [22]. Natriuretic Peptide of Type B The ventricular myocardium is the main organ responsible for secreting BNP in response to wall stressors such pressure overload and volume expansion [23]. Rats treated with CAP in group (III) of the current investigation, however, significantly decreased BNP in contrast to ISO group (II). This result may be attributed to that CAP improve the viability of cardiomyocytes [12].

Biomarkers of myocardial injury (Cardiac troponin, Myoglobin, CK-MB and LDH)

The current study found that, in contrasted to the normal control group (I), the ISO-induced MI in group (II) significantly elevated blood levels of cardiac enzymes, such as CK-MB (a marker for acute myocardial damage), LDH, troponin, and myoglobin.

It is possible that this was caused by changes in membrane permeability and disintegration brought on by MI triggered by ISO [24]. Increasing these lysosomal enzymes' serum concentration was caused by the discharge of enzymes into the circulation following cardiomyocyte damage [25]. These findings demonstrated ISO-induced necrotic myocardial injury and were in line with former investigations [26-28]. While, the treatment with CAP in group (III) exhibits a significant decline serum level of these heart-related enzymes including CK-MB, LDH, troponin and Myoglobin in contrast to ISO group (II). The decrease observed in serum biomarker findings in animals fed CAP may be attributed to its ability to scavenge free radicals, hence reducing enzyme leakage into the bloodstream and stabilizing membrane permeability. According to these results, CAP shields the heart by stopping the cardiac enzymes from leaking into the bloodstream while maintaining the plasma membrane and contractile mechanism of the myocyte's anatomical and functional integrity.

The reversible conversion of creatine and adenosine triphosphate (ATP) to creatine phosphate and adenosine diphosphate is catalyzed by the enzyme CK, which is located in heart muscle. The dimeric enzyme, which consists of the subunits M and B, is found in the heart. The MB form makes up around 20% of all cardiac CK, which helps in MI diagnosis specificity and sensitivity [29-31].

Anaerobic metabolism occurs across numerous tissues, including the heart and skeletal muscles, and is facilitated by the enzyme LDH. An important MI indicator, the isoenzyme LDH1 is primarily found in myocardium that has been injured or necrotic [30,32]. An abundant protein that binds iron and oxygen is called myoglobin, and it is found in large amounts in animal hearts and skeletal muscles. It is an AMI sensitive marker. During the damage, it is quickly liberated from the myocardium [33]. The greatest significant heart proteins implicated within the diagnosis of AMI are cardiac troponins (cTn) (TnC, TnI, and TnT) [34]. From heart muscle, they are produced and released [35]. The primary structure of the striate cardiac muscle is formed by the interaction of these proteins with tropomyosin [36].

Biomarkers of inflammation

The current study's ISO-induced MI group (II) significantly elevated marker that reduces inflammation, such as hs-CRP, TNF-α, and IL-6, in comparison to the normal control group (I). A plasma protein called CRP drivers from the hepatic cells.

The synthesis of CRP is generally regulated by IL-6, which in turn is upregulated TNF-α [37]. Furthermore, CRP is also produced by smooth muscle cells (SMCs) and monocytic cells in atherosclerotic plaques; in particular, it is produced in the endothelium's tunica intima, where it co-localizes with lipoproteins, inflammatory monocytes, and macrophages supplied by monocytes. This location guarantees a substantial atherosclerosis-related impact. Moreover, CRP immediately promotes and intensifies innate immunity, which in turn triggers the onset of coronary heart disease (CHD) [38]. Thus, elevated levels of high-sensitivity CRP (hs-CRP) are strongly correlated with inflammatory coronary events [39]. In addition to being a cause, a reliable indicator of peripheral artery disease, MI, stroke, and other heart events is CRP [40]. Myocardial cell inflammation incidence is

facilitated by CRP. In the initial stage of MI, cytokines play a cytoprotective effect by reducing cell apoptosis [41].

However, in this work blood markers (hs-CRP, TNF-α and IL-6), were greatly decline in CAP treated group (III) as compared to group (II). Receptor-independent and receptor-dependent mechanisms are two different ways that capsaicin can work. Its impact on inflammation is one of the more significant instances of non-receptor capsaicin activity. Because using capsaicin can lower inflammation and inflammatory chemicals, which are involved in the development of many diseases. A healthy lifestyle may be maintained because pepper consumption and non-receptor function regulate the manufacturing pathway of cellular pro-inflammatory chemicals. Capsaicin reduces the synthesis of inflammatory cytokines including TNFα and IL-6 by blocking NF-kB activation [42]. In reaction to stress and heart damage, the innate immune system is triggered. Tumor necrosis factor and interleukin-6 are examples of proinflammatory cytokines that are generated and mediate remodeling of the heart injury [43] which was in harmony with the result in the current trial.

Gene expression studies

The results obtained in this study indicated that the ISO group (II) had down-regulated Sirt-1 gene expression levels compared to the control group (I), whereas MMP-9 and hif1-α gene expression levels were up-regulated. Comparing the capsaicin group (III) to the myocardial infarction group (II), the latter demonstrated up-regulation in the expression level of the Sirt-1 gene and down-regulation in the expression levels of MMP-9 and hif1-α genes.

Among the primary causes of illness and death globally is myocardial infarction, which is mostly caused by a mismatch between the heart's capacity to pump oxygen-rich blood and its requirement for it due to coronary artery blockage [44,45]. The heart cannot handle the loss of blood, nutrients, and oxygen during MI because of its low capacity for anaerobic metabolism [46] causing pathological alterations that ultimately lead to heart failure [47]. Hypoxia brought on by myocardial hyperactivity, coronary hypotension, calcium overload, energy reserve depletion, and excessive formation of free radicals from catecholamine oxidative metabolism are among the damage caused by ISO to cardiac myocytes [48].

A transcription factor known as hypoxia-inducible factor 1 (HIF-1) controls oxygen homeostasis in all metazoan species like a master regulator. It regulates vascular remodeling and angiogenesis to control oxygen supply, and it regulates glucose metabolism and redox homeostasis to govern oxygen usage. According to an analysis of animal models, HIF-1 plays a crucial protective function in the etiology of ischemic heart disease and pressure-overload heart failure by activating these homeostatic mechanisms. This might be the cause for the current study's HIF-1 upregulation in ISO group (II) [49].

In the current trial the decrease in HIF-1α in the capsaicin-treated group (III) may be attributed to that Cap can lower HIF-1α accumulation and raise intracellular oxygen levels by inhibiting mitochondrial respiration [50]. In previous study, In the ISO group, there was a considerable rise in the expression levels of HIF 1α and MMP 9. Both the pairwise correlation and the correlation with atrial fibrosis were positive. Moreover, HIF-1α may aid in the growth of cardiac fibrosis by controlling the degree of MMP-9 expression. In the same way, the current study did [51].

The most well-researched of the eleven MMPs that have been quantified after MI is MMP-9. MMPs are zinc-dependent enzymes that are produced in response to cardiac injury. They function by cleaving substrates of the extracellular matrix to eliminate necrotic and damaged tissue [52].

MMPs are crucial proteolytic enzymes that perform a crucial part in the remodeling of the heart, a process that results in structural changes to cardiomyocytes in the myocardium, both infarcted and non-infarcted and the breakdown of extracellular matrix. This remodeling serves as the anatomic basis in the event that congestive heart failure develops and sudden heart failure [53]. Matrix metalloproteinases (MMPs) are essential for altering the matrix of the heart tissue [54].

Preceding research revealed that, in comparison to the control group, isoproterenol caused an increase in MMP-9 mRNA of more than eight times, with a p-value of less than 0.0001 [55], as the same way, the current study did.

It was discovered that TNF-α, NF-κb, and oxidative stress all promoted the expression of MMP-9. ROS have the ability to modify MMP activity by influencing transcriptional and post-translational pathways. Additionally, activating NF-κB (a transcriptional regulator), they can also stimulate Pro-MMP. It is thought that medications that block MMP-9 can provide a unique enhancement in in the treatment of coronary heart disorder [56].

In the current research, Cap increase the expression level of MMP-9, similar to earlier research demonstrated that CAP significantly attenuated the expression of MMP 9 expression in cardiomyocytes [57]. In addition to prior research demonstrated that Capsaicin administration markedly downregulated the expression of MMP-9 in cardiac tissue of ISO-induced rats [58].

Silent information regulator 1 (SIRT1) is a widely expressed protein with a complicated role in the etiology, course, and treatment of several illnesses [59]. Numerous experimental investigations have indicated the potential involvement of sirtuins in a range of various heart-related conditions, including atherosclerosis, cardiac hypertrophy, heart failure, and endothelial dysfunction [60,61]. Sirtuins 1, 3, and 6 have been shown in experimental tests to exhibit protective properties against inflammation, endothelial dysfunction, oxidative stress, atherosclerosis, and dyslipidemia [60]. Systemic oxygen delivery and blood circulation depend on cardiac activity. To preserve efficient contractility, the heart has inherent oxygen requirements that must be satisfied. Capsaicin causes a rise in SIRT6 (Sirtuin 6), which subsequently promotes the deacetylation and degradation of Hif1α [62].

Conclusion

Cardiovascular diseases, such as myocardial infarction, are a leading cause of mortality in humans, and the incidence of myocardial infarction increases every year worldwide. the findings of the present study implicate that Capsaicin administration provided an effective treatment to myocardial infarction through its ameliorating role of serum biochemical, molecular parameters and attenuated the histopathological changes via its free radical scavenging and antioxidant properties in rats with MI caused by ISO. So, Capsaicin could be used as a new alternative cardio-treated strategy at myocardial infarction.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

The experiment was conducted in compliance with the protocols for the use and care of laboratory animals that were authorized by the ethical animal committee of Benha University. (Approval no. BUFVTM 12-12-22).

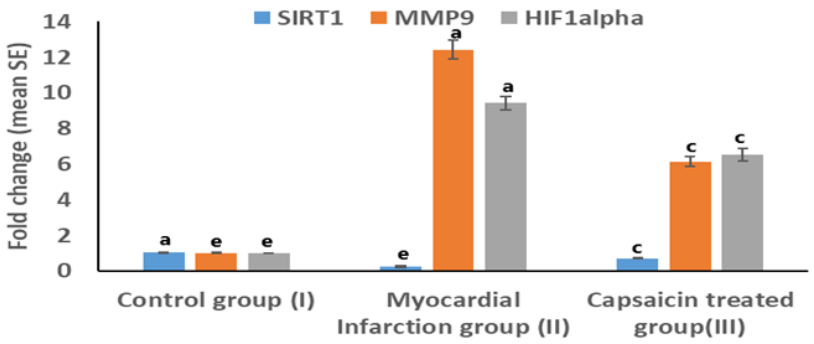

Fig. 1. Treatment impact of capsaicin administration on some genes expression level in isoproterenol- triggered heart dysfunction experimentally in rats.

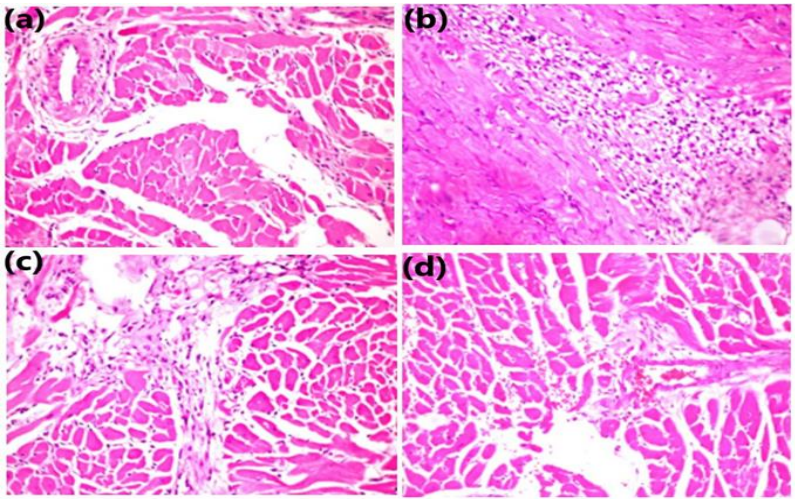

Fig. 2. The microscopic examination of the heart tissues of rats in the different studied groups. (a): control group (I), (b) and (c): isoproterenol treated group (II), (d): capsaicin treated group (III).

TABLE 1. Primers sequences, target genes for SYBR green real time PCR:

Gene	Forward primer	Reverse primer	Accession number
Rat ß-actin	TCCTCCTGAGCGCAAGTACTCT	GCTCAGTAACAGTCCGCCTAGAA	V01217
MMP9	TCGAAGGCGACCTCAAGTG	TTCGGTGTAGCTTTGGATCCA	NM_031055
hif1 alpha	GGACGATGAACATCAAGTCAGCA	GGAATGGGTTCACAAATCAGCAC	AH006789.2
SIRT1	CAC-CAG-AAA-GAA-CTT-CAC-CAC-CAG	ACC-ATC-AAG-CCG-CCT-ACT-AAT-CTG	NM_001414959.1

TABLE 2. The impact of using capsaicin on certain serum parameters during the experimental induction of myocardial infarction in rats using isoproterenol.

Animal groups	ProBNP (pg/Ml)	TropT (pg/Ml)	Myoglobin (ng/mL)	CK-MB (U/L)	LDH (U/L)	CRP (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
Control group (I)	39.35±2.78^{c}	9.66±1.08^{d}	33.80±2.36^{c}	0.30±0.03^{c}	315.77±25.38^{c}	3.31±0.35^{c}	1.53±0.15^{d}	15.80±2.46^{c}
Myocardial Infarction group (II)	119.03±13.24^{a}	78.06±5.67^{a}	87.30±4.82^{a}	0.79±0.05^{a}	915.49±64.65^{a}	36.88±6.78^{a}	7.74±0.50^{a}	62.14±5.01^{a}
Capsaicin treated group (III)	82.54±4.43^{b}	33.27±4.99^{bc}	70.39±2.72^{b}	0.57±0.07^{b}	641.22±25.14^{b}	14.79±1.23^{b}	3.80±0.27^{b}	41.01±2.67^{b}

* Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ($P<0.05$).

References

1. World Health Organization http://www.who.int/cardiovascular_diseases/about_cvd/en/. 2021. Accessed 11 June 2021.
2. Chapman, R., Adamson, D., Shah, S.,Anand, A., Strachan, E.; Ferry, V. and Cruikshank, A. *High-Sensitivity Cardiac Troponin and the Universal definition of Myocardial Infarction. Circulation*, **141**(3), 161–171(2020):.
3. Lewis, S. L., Bucher, L., Heitkemper, M. M., Harding, M. M., Kwong, J. and Roberts, D. *Medical-surgical nursing-ebook: assessment and management of clinical problems, single volume, Elsevier Health Sciences*, **46**(6), 1111-1113 (2019).
4. Elshourbagy, N., The effect of carvedilol and Q10 on isoprenaline-induced cardiotoxicity: Electrocardiographic, biochemical and histopathological evaluation in rats, *Al-Azhar J. Pharm. Sci*., **54**, 148–158(2016).
5. Saravanan, G., Ponmurugan, P., Sathiyavathi, M., Vadivukkarasi, S. and Sengottuvelu, S., Cardioprotective activity of Amaranthus viridis Linn: effect on serum marker enzymes, cardiac troponin and antioxidant system in experimental myocardial infarcted rats, *Int. J. Cardiol*., **165**(3), 494-498(2013).
6. Khalifa, A.A., El Sokkary, N.H., Elblehi, S.S., Diab, M.A. and Ali, M.A., Potential cardioprotective effect of octreotide via NOXs mitigation, mitochondrial biogenesis and MAPK/Erk1/2/STAT3/NF-K_ pathway attenuation in isoproterenol-induced myocardial infarction in rats. *Eur. J. Pharmacol*., **925**, 174978(2022).
7. Patel, V., Upaganlawar, A.; Zalawadia, R. and Balaraman, R., Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: A biochemical, electrocardiographic and histoarchitectural evaluation, *European Journal of Pharmacology*; **644** (1-3),160-168 (2010).
8. Bello, U., Agu, C.M., Ajiya, D.A.; Mahmoud, A.A., Udopia, L., Lawal, N.M., Abubakar, A.A. and Muhammad, M., Biodiesel, in a quest for sustainable renewable energy, a review on its potentials and production strategies, *J. Chem. Rev*., **4**, 272-287 (2022).
9. Sheida, A., Taghavi, T., Shafabakhsh, R., Ostadian, A., Razaghi Bahabadi, Z., Khaksary Mahabady, M., Hamblin, M.R. and Mirzaei, H., Potential of natural products in the treatment of myocardial infarction: focus on molecular mechanisms, *Critical Reviews Food Science and Nutrition*. **1**,18(2022).
10. Hernández Pérez, T., Gómez García, M.D.R., Valverde, M.E. and Paredes López, O., Capsicum annuum (hot pepper), An ancient Latin American crop with outstanding bioactive compounds and nutraceutical potential, *A review Comprehensive Reviews in Food Science and Food Safety*, **19**, 2972-2993 (2020).
11. Srinivasan, K., Biological activities of red pepper (Capsicum annuum) and its pungent principle capsaicin: a review, *Crit. Re.v Food Sci. Nutr.*, **56**, 1488-1500(2016)
12. Huang, W.,Cheang, W.S.,Wang, X., Lei, L., Liu, Y., Ma, K.Y., Zheng, F., Huang, Y. and Chen, Z.-Y.,Capsaicinoids but not their analogue capsinoids lower plasma cholesterol and emodelin beneficial vascular activity, *J. Agric. Food Chem*., **62**, 8415–8420(2014).
13. Bonaccio, M., Di Castelnuovo, A., Costanzo, S.,Ruggiero, E., De Curtis, A., Persichillo, M., Tabolacci, C., Facchiano, F., Cerletti, C., Donati, M.B., de Gaetano, G., Iacoviello, L., and Moli-sani Study Investigators, Chili pepper consumption and mortality in Italian adults. *J. Am. Coll. Cardiol*., **74**, 3139–3149 (2019).
14. Govindasami, S., Veera Venkata Sathibabu Uddandrao, Raveendran, N., and Sasikumar, V. (Therapeutic Potential of Biochanin-A Against Isoproterenol-induced Myocardial Infarction in Rats. *Cardiovascular & Hematological Agents in Medicinal Chemistry*, **18**(1), 31-36 (2020).
15. Ismail, K.M., Hassan, S.S., Hanna, D.H., Palladium-Imidazole Nanoparticles' Cytotoxic Effects on Colon Cancer Cells: Induction of Cell Cycle Arrest and Apoptosis Mediated via Mitochondria. *Applied Organometallic Chemistry*, 39 (1), e7908.
16. Moussa, A.K., Abd El-Rahman, H.A., Mohamed, R.R. and Hanna, D.H. Hyaluronic Acid-Based pH-Sensitive Nanogel, Synthesis,Characterization, and Assessment for Acyclovir Delivery In Vitro and In Vivo, *Biomacromolecules*, **26**(1), 341–362 (2025). https://doi.org/10.1021/acs.biomac.4c01189
17. Hanna, D.H. and Saad, R. G. Induction of mitochondria mediated apoptosis in human ovarian cancer cells by folic acid coated tin oxide nanoparticles. *PLOS ONE*, **16**(10), e0258115(2021). https://doi.org/10.1371/journal.pone.0258115
18. Hanna, D. H., Beshay, S. N., El. Shafee, E. and El-Rahman, H. A. A., The protective effect of aqueous extract of Stevia rebaudiana against tartrazine toxicity in male Wistar rat. *Cell Biochem. Funct.*, **41**(8), 1462–1476(2023). https://doi.org/10.1002/cbf.3886
19. Sayed, A. A., Abdel Moteleb, M. M., El Shafee, E. and Hanna, D.H., Oral drug delivery vehicle for insulin and curcumin based on egg albumin-dextran conjugate, In vitro and in vivo studies, *Journal of Drug Delivery Science and Technology*, **100**,106052(2024). https://doi.org/10.1016/j.jddst.2024.106052.
20. Hanna, D.B., Al-Habashy, Y.M., El Desouky, M.A.A. and Abd El Rahman, H.A,. Impact of Energy Drinks on Hepatic and Renal Tissues in Pregnant Wistar Rats, *Egypt. J. Vet. Sci.*, **1**(12), 2145 (2024). DOI: 10.21608/ejvs.2024.295316.2145
21. Hanna, D.B., Salama, F.E., El Desouky, M.A.A. and Abd El Rahman, H.A., Assessment of the Potential Toxic Consequences of Energy Drinks on the Brain

Tissue of Maternal Wister Rats Throughout Gestation and Lactation. *Egypt. J. Vet. Sci*., **1**(11), 2282 (2024). DOI: 10.21608/ejvs.2024.307621.2282

22. Mohd Aftab Siddiqui, Usama Ahmad, Ahmed Abdullah Khan, Mohammad Ahmad, Badruddeen, Mohammad Khalid and Juber Akhtar: Isoprenaline, a tool for inducing myocardial infarction in experimental animals.; *Int, J. Pharm*., **6**(2), 138-144 (2016).
23. Sabatine, M.S., Morrow, D.A., de Lemos, J.A., Omland, T., Desai, M.Y., Tanasijevic, M., Hall, C., McCabe, C.H., and Braunwald, E., Acute changes in circulating natriuretic peptide levels in relation to myocardial ischemia. *J. Am. Coll. Cardiol*.; **44**,1988–1995 (2004).
24. Khalaf, H. M., Abdalla, A. M., Ahmed, A. F. and Abdel-Aziz, A. M., Role of Nitric Oxide in Mediating the Cardioprotective Effect of Agomelatine against Isoproterenol-Induced Myocardial Injury in Rats. Naunyn-schmiedeberg's Arch. *Pharmacol*., **393**, 1809–1823 (2020).
25. Chen, Y. J., Serfass, R. C., and Apple, F. S.: Loss of Myocardial CK-MB into the Circulation Following 3.5 Hours of Swimming in a Rat Model. *Int. J. Sports Med.* , **21**, 561–565 (2000).
26. Mohamed I. Ahmed , Heba M. A. Abdelrazek , Yasser M. Moustafa ,Samar Z. Alshawwa , Maysa A. Mobasher , Basel A. Abdel-Wahab, Fathy Elsayed Abdelgawad and Dina M. Khodeer . Cardioprotective Effect of Flibanserin against Isoproterenol-Induced Myocardial Infarction in Female Rats: Role of Cardiac 5-HT2A Receptor Gene/5-HT/Ca+2 pathway, *Pharmaceuticals*, **16**, 502 (2023).
27. Saqib, F., Ali, A., Ahmedah, H.T., Irimie, C.A., Toma, S.I., Popovici, B.E., Moga, M. and Irimie, M., Cardioprotective, Hypotensive and Toxicological Studies of Populus emodel (Wall. Ex Royle), *Biomed. Pharmacother*., **142**, 112065 (2021).
28. Cinar, I., Yayla, M., Tavaci, T., Toktay, E., Ugan, R.A.; Bayram, P. and Halici, H., In Vivo and In Vitro Cardioprotective Effect of Gossypin Against Isoproterenol-Induced Myocardial Infarction Injury, *Cardiovasc. Toxicol*., **22**, 52–62 (2022).
29. Mythili, S. and Malathi, N. Diagnostic Markers of Acute Myocardial Infarction (Review), *Biomed. Rep*., **3,** 743- 748 (2015).
30. Aydin, S., Ugur, K..,Aydin, S., Sahin, ˙I. and Yardim, M., Biomarkers in Acute Myocardial Infarction: Current Perspectives, *Vasc. Health Risk Manag*., **15**, 1–10 (2019)
31. Danese, E. and Montagnana, M., An Historical Approach to the Diagnostic Biomarkers of Acute Coronary Syndrome, *Ann. Transl. Med*., **4**, 194(2016).
32. Maleki Dizaji, N., Garjani, A., Mousavi, S., Mohammadi, M. and Vaez, H. ,Time-Dependent Influence of Infliximab on Hemodynamic Responses and Cardiac Injuries of Isoproterenol-Induced Myocardial Infarction in Rats, *Eur. J. Pharmacol*., **903**, 174122 (2021).
33. Mair J, Artner-Dworzak E, Lechleitner P, Morass, B., Smidt, J., Wagner, I., Dienstl, F., and Puschendorf, B., Early diagnosis of acute myocardial infarction by a newly developed rapid immunoturbidimetric assay for myoglobin. *Heart*.; **68**(11),462–468 (1992).
34. Park, K.C., Gaze, D.C., Collinson, P.O. and Marber, M.S., Cardiac troponins from myocardial infarction to chronic disease, *Cardiovasc Res*., **113**(14),1708–1718(2017).
35. Wei, B., Jin, J.P. and Troponin, T. Isoforms and posttranscriptional modifications: evolution, regulation and function, *Arch Biochem Biophys*., **505**(2),144–154(2011).
36. Anderson, P.A., Malouf, N.N., Oakeley, A.E., Pagani, E.D., Allen, P.D.,Troponin, T. Isoform expression in humans. A comparison among normal and failing adult heart, fetal heart, and adult and fetal skeletal muscle, *Circ. Res*., **69**(5), 1226–1233 (1991).
37. Paffen, E. and demaat, M.P.M., C-reactive protein in atherosclerosis, a causal factor, *Cardiovasc Res*., **71**, 30-39 (2006).
38. Gu, L., Tseng, S.C. and Rollins, B.J., Monocyte chemoattractant protein-1, *Chem. Immunol.*, **72**, 7(1999).
39. Bucova, M., Bernadic, M. and Buckingham, T., C-reactive protein, cytokines and inflammation in cardiovascular diseases, *Bratisl. Lek Listy*., **109**(8), 333–340(2008).
40. Wang, Z. and Nakayama, T., Inflammation, a link between obesity and cardiovascular disease. *Mediators Inflamm* , **2010**,535918 (2010).
41. Swiatkiewicz, I., Kozinski, M., Magielski, P., Fabiszak, T., Sukiennik, A., Navarese, E. P., Odrowaz-Sypniewska, G., and Kubica, J., Value of C-reactive protein in predicting left ventricular emodeling in patients with a first ST-segment elevation myocardial infarction. *Mediators Inflamm*., **2012**, Article ID 250867, 11 pages (2012).
42. Cho, S.C., Lee, H. and Choi, B.Y., An updated review on molecular mechanisms underlying the anticancer effects of capsaicin. *Food Sci. Biotechnol*., **26(**2),1-13(2017).
43. Heymans, S., Hirsch, E., Anker, S. D., Aukrust, P., Balligand, J. L., Cohen-Tervaert, J. W., Drexler, H., Filippatos, G., Felix, S. B., Gullestad, L., Hilfiker-Kleiner, D., Janssens, S., Latini, R., Neubauer, G., Paulus, W. J., Pieske, B., Ponikowski, P., Schroen, B., Schultheiss, H. P., Tschöpe, C., Shah, A. M., Inflammation as a therapeutic target in heart failure ?A scientific statement from the Translational Research Committee of the Heart Failure Association of the European Society of Cardiology. *Eur. J. Heart Fail*., **11**, 119–129 (2009).
44. Pamukçu, B., Myocardial Infarction, BoD—Books on Demand: *Norderstedt, Germany*, 2019, ISBN 978-1-78984-8687 (2019).
45. Ferri, F.F., Ferri's Clinical Advisor 1030-1040.e1 2022, *Elsevier-Health Science*: Philadelphia, PA, USA(2022).

46. Madigan, M. and Atoui, R., Therapeutic Use of Stem Cells for Myocardial Infarction, *Bioengineering*, **5**-**28**(2018).

47. Fishbein, G.A., Chapter 10. Myocardial Ischemia and Its Complications. In Cardiovascular Pathology, *Academic Press, London, UK*,, p. **39** (2022).

48. Patel, V., Upaganlawar, A., Zalawadia, R. and Balaraman, R., Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats, a biochemical, electrocardiographic and histoarchitectural evaluation, *Eur. J. Pharmacol*. **644** (1-3), **160–168** (2010).

49. Gregg L. Semenza , Hypoxia-Inducible Factor 1 and Cardiovascular Disease, *Annu Rev Physiol*., **76**, 39–56(2014).

50. Srinivasan K., Antioxidant potential of spices and their active constituents, *Crit. Rev. Food Sci. Nutr*., **54**(3),352-372. (2011)

51. Fangju Su, Weize Zhang, Yongqing Chen, Ling Ma, Hanping Zhang and Fei Wang: significance of hypoxia-inducible factor 1α expression with atrial fibrosis in rats induced with isoproterenol. *Experimental and Therapeutic Medicine,* **8**, 1677-1682(2014).

52. Iyer, R.P., Jung, M. and Lindsey, M.L., MMP-9 signaling in the left ventricle following myocardial infarction, *Am. J. Physiol. Heart Circ. Physiol*., **311**(2),H190-H198(2016).

53. Reinhardt, D., Sigusch, H. H., Hensse, J., Tyagi, S. C., Körfer, R. and Figulla, H. R. Cardiac emodeling in end stage heart failure upregulation of matrix metalloproteinase (MMP) irrespective of the underlying disease, and evidence for a direct inhibitory effect of ACE inhibitors on MMP," *Heart*, **88**,(5), 525–530 (2002).

54. Thenappan, T., Chan, S. Y. and Weir, E. K.., Role of extracellular matrix in the pathogenesis of pulmonary arterial hypertension. *Am. J. Physiol, Heart Circ. Physiol.*, **315**, H1322–H1331(2018).

55. Marwa Medhet, Wesam M. El-Bakly, Amira M. Badr, Azza Awad and Ebtehal El-Demerdash, Thymoquinone attenuates isoproterenol-induced myocardial infarction by inhibiting cytochrome C and matrix metalloproteinase-9 expression. *Clin. Exp. Pharmacol. Physiol*.; **49**,391–405(2022).

56. DeLeon-Pennell, K.Y., Meschiari, C.A., Jung, M. and Lindsey, M.L., Matrix metalloproteinases in myocardial infarction and heart failure. *Prog. Mol. Biol. Transl. Sci*., **147**,75-100(2017).

57. Yuan Hu, Xianzhe Dong, Tianyi Zhang, Hongming Ma, Wenshan Yang, Yichen Wang, Ping Liu and Yibang Chen, Kai Xin San suppresses matrix metalloproteinases and myocardial apoptosis in rats with myocardial infarction and depression ,*Molecular Medicine REPORTS*, **21**, 508-516 (2020).

58. Radhiga, T., Senthil, S., Sundaresan, A. and Pugalendi, K.V. Ursolic acid modulates MMPs, collagen-I, α-SMA, and TGF-β expression in isoproterenol-induced myocardial infarction in rats, *Human and Experimental Toxicology*, **38**(7) 785–793(2019).

59. Vivek Singh and Saba Ubaid. Role of Silent Information Regulator 1 (SIRT1) in Regulating Oxidative Stress and Inflammation, *Inflammation* , **43**(5),1589-1599.Springer science(2020).

60. Winnik, S., Auwerx, J., Sinclair, D.A. and Matter, C.M., Protective effects of sirtuins in cardiovascular diseases from bench to bedside, *Eur. Heart J*.; **36**(48),3404–3412(2015).

61. Sosnowska, B., Mazidi, M., Penson, P., Gluba-Brzozka, A., Rysz, J. and Banach, M., The sirtuin family members SIRT1, SIRT3 and SIRT6,Their role in vascular biology and atherogenesis, *Atherosclerosis*; **265**,275-282 (2017).

62. Dongling Luo, Wenxin Li, Changming Xie, Li Yin, Xiaoyan Su, Jie Chen and Hui Huang, Capsaicin Attenuates Calcification Through Promoting SIRT6-Mediated Deacetylation and Degradatioqwan of Hif1α (Hypoxic-Inducible Factor-1 Alpha), *Anticalcification Mechanism of Capsaicin. Hypertension*.,**79**,906–917 (2022).

التأثيرات التحسينية للكابسيسين على التغيرات البيوكيميائية والجزيئية والنسيجية المرضية في احتشاء القلب المستحث لدى الفئران

أيه على أبو الحمد[1] ، أمنيه محمود عبد الحميد[2] ، ياقوت احمد السنوسى [2] ، محمد احمد مرزوق[2] ودميانه بدرى حلمى[3]

[1] قسم الكيمياء الحيوية - كلية الطب البيطري- جامعة بنها - مصر.

[2] قسم الكيمياء الحيوية - كلية الطب البيطري- جامعة بنها - مصر..

[3] قسم الكيمياء - كلية العلوم- جامعة القاهرة - مصر.

الملخص

الخلفية: يعد احتشاء عضلة القلب (MI) من بين الأسباب الرئيسية للوفاة في العالم المتقدم حيث يبلغ معدل انتشاره حوالي ثلاثة ملايين حالة سنويًا. يُظهر الكابسيسين (الكاب) تأثيرات مضادة للأكسدة ومضادة للالتهابات ومضادة للسرطان وواقية للقلب.

الهدف: تهدف هذه التجربة إلى دراسة الإجراءات التحسينية لإدارة Cap ضد الفئران المصابة باحتشاء عضلة القلب باستخدام تقييم التغيرات البيوكيميائية والجزيئية والنسيجية المرضية.

الطريقة: تم تقسيم الفئران إلى ثلاث مجموعات. المجموعة الأولى (ن = 15)؛ مجموعة التحكم العادية. المجموعة الثانية (ن = 15)؛ تم إعطاء الأيزوبروتيرينول تحت الجلد للفئران (20 ملغم / كغم / يوم). المجموعة الثالثة (ن = 15)؛ تم إعطاء الأيزوبروتيرينول تحت الجلد للفئران (20 ملجم / كجم / يوم) ثم تم إعطاؤه عن طريق الفم باستخدام الكابسيسين يوميًا (1 ملجم / كجم من وزن الجسم) لمدة 30 يومًا. المعلمات الكرياتين كيناز-MB (CK-MB)، نازعة هيدروجين اللاكتات (LDH)، الببتيد المدر للصوديوم من النوع B (pro-BNP، trop-T) Troponin-T)، الميوجلوبين، البروتين التفاعلي (hs-CRP) hs-C)، وتم قياس عامل نخر الورم ألفا (TNF-α) والإنترلوكين-6 (IL-6) في المجموعات الثلاث باستخدام طريقة ELISA. أيضًا، من خلال استخدام PCR الكمي في الوقت الحقيقي، تم تحديد مستويات التعبير عن (MMP-9 Matrix Metalloproteinase-9)، والعامل المحفز لنقص الأكسجة-1- alpha (hif1-α))، و(Sirt-1 sirtuin-1). تقاس في المجموعات الثلاث. بالإضافة إلى ذلك، تم إجراء الفحص النسيجي لأنسجة القلب في جميع المجموعات.

النتائج: أظهرت النتائج أن الفئران المصابة باحتشاء عضلة القلب الناجم عن الأيزوبروتيرينول كان لديها مستويات دم أعلى بكثير من CK-MB، LDH، pro-BNP، Trop-T، الميوجلوبين، hs-CRP، IL-6، وTNF-α في المجموعة الثانية. مع المجموعة الأولى. أيضًا، أشارت الأنسجة القلبية لفئران MI المستحثة بالأيزوبروتيرينول في المجموعة الثانية إلى زيادة كبيرة في التنظيم في مستويات التعبير الجيني MMP-9 وhif1-α المتناقضة مع المجموعة الأولى. المجموعة الأولى (P <0.05). على العكس من ذلك، تم اكتشاف انخفاض كبير في مستوى التعبير Sirt-1 في المجموعة الثانية مقارنة بالمجموعة الأولى. تم اكتشاف تغيرات نسيجية مختلفة في أنسجة القلب لدى الفئران المعالجة بالإيزوبروتيرينول في المجموعة الثانية بدلاً من المجموعة الضابطة الطبيعية الأولى. ومن المثير للاهتمام أن الفئران التي عولجت بـ أظهر الكابسيسين في المجموعة الثالثة انخفاضًا ملحوظًا (P <0.05) في جميع المعايير البيولوجية المقاسة CK-MB، LDH، pro-BNP، trop-T، الميوجلوبين، hs-CRP و IL-6 و TNF-α II على النقيض من المجموعة الثانية. بالإضافة إلى ذلك، أشارت المجموعة الثالثة إلى انخفاض كبير في مستويات التعبير الجيني MMP-9 وhif1-α مقارنة بالمجموعة الثانية (P <0.05). على العكس من ذلك، هناك زيادة كبيرة في مستوى التعبير Sirt-1 في المجموعة الثالثة مقارنة بالمجموعة الثانية. كما أظهرت أنسجة القلب تحسنا في التغيرات المرضية في المجموعة الثالثة المعالجة بالكاب مقارنة بفئران الأيزوبروتيرينول في المجموعة الثانية.

الاستنتاج: تشير هذه النتائج إلى أن مكملات الكابسيسين تتصدى للآثار السلبية للأيزوبروتيرينول، ويمكن استخدامها كاستراتيجية بديلة جديدة لعلاج احتشاء القلب.

الكلمات الدالة : احتشاء عضلة القلب، الأيزوبروتيرينول، الكابسيسين، الببتيد المدر للصوديوم من النوع B.