Effect of Erythropoietin Pre and Post-Therapy on Serum Marker Enzymes of Cardiac Damage and Inflammation in Isoproterenol Induced Myocardial Infarcted Rats

T. HARI PRASAD[1,2*], S. SENTHILKUMAR[2]R. VIJAYARAGHAVAN[2]

P. PRAVEEN KUMAR[2], DEVIKA S KUMAR[2]

[1]Department of Physiology, MNR Medical College & Hospital

Fasalwadi - 502294, Sangareddy Dist., Telangana, India.

[2]Department of Research and Development, Saveetha Institute of Medical and Technical Sciences,

Chennai - 602 105, Tamil Nadu, India

Address for correspondence: Dr. T. HARI PRASAD

drhari.talari@gmail.com

ABSTRACT:

In the present work, the therapeutic potential of both pre and post-treatment of rhEPO (recombinant human Erythropoietin) in isoproterenol- (ISO-) induced myocardial infarction was evaluated through measurement of serum markers of myocardial infarction and inflammation. Adult male Wistar rats were divided into four groups (control, ISO induced MI, ten days rhEPO pre-treatment + ISO induction, and ISO induction + single dose rhEPO post-treatment) respectively. The therapeutic efficacy of rhEPO (5000 IU/kg b.w) i.e ten days pretreatment before ISO (75mg/kg b.w) induction, as well as the potential of a single dose of rhEPO (5000 IU/kg) post-therapy afterISO-induced myocardial damage, was investigated by measuring the serum levels of markers of cardiac injury viz., MCP-1 (Monocyte Chemoattractant Protein-1), enzyme markers such as AST (aspartate transaminase), IL6 (Interleukin -6) and LDH (Lactate dehydrogenase). Taken altogether, the research findings suggest that post-treatment of erythropoietin is more effective than pre-treatment in ameliorating the cardiac markers of MI including the inflammatory marker IL-6.

KEY WORDS: Isoproterenol; Myocardial infarction; Erythropoietin; Wistar rats; Serum cardiac markers; inflammation

1. INTRODUCTION:

Myocardial Infarction (MI), also referred to as a "Heart attack", is caused by a reduction or total suspension of blood supply to a section of the myocardium[1], which results in a limited oxygen supply[2]. The available oxygen supply cannot meet oxygen demand, resulting in cardiac

ischemia[3]. As a result of the ischemia, many changes occur at the molecular, cellular, and tissue levels of the myocardium[4]. Hypoxia, death of cardiomyocytes, inflammation, ventricular dilation and adverse remodeling, tissue necrosis, interstitial fibrosis, and contractile dysfunction are some of the main features that may present themselves during the progression of MI[5]. Myocardial infarction (MI) is a major cause of mortality and morbidity which is anticipated to take around 23.3 million lives by 2030[2]. Myocardial infarction is becoming more common in poor countries after previously being seen primarily in developed countries[6]. Isoproterenol induced animal models are commonly used to research MI. ISO is a synthetic nonselective-adrenergic agonist that is often used to cause experimental acute MI in rats. This effect is accomplished by causing myocardial oxidative stress, inflammation, and calcium overload via activation of 1-adrenergic receptors in the heart[7]. Exploring pharmaceutical strategies to treat ISO-induced cardiac anomalies could be beneficial in avoiding the onset and progression of MI[8].

Cardiac biomarkers are useful in the diagnosis of a myocardial infarction. The ideal biomarker needs to be expressed at relatively high levels within cardiac tissue, with high clinical sensitivity and specificity that is detectable in the blood early after the onset of symptoms, such as chest pain[9]. As there are numerous cardiac biomarkers, AST was the first biomarker used in the diagnosis of MI. In myocardial infarction, AST rises 6 to 8 hours after symptom onset, peaks at 24 to 36 hours, and recovers to normal in 3 to 7 days[10]. LDH (Lactate dehydrogenase) is a tissue specific biomarker released during tissue damage. In myocardial infarction, LDH-1 isoenzyme remains elevated from the second day up to the 4th day. []. LDH-1 is found to be greater than LDH-2 in myocardial infarction, with a ratio of LDH-1/LDH-2 greater than 1. The increase in LDH persists for approximately ten days. It increases at 12 hours and peaks at 24-48 hours[11]and returns to normal values within 8–14 days[12]. Interleukin-6 (IL-6) is another indicator of early atherosclerosis, as it plays a key role in the recruitment and activation of inflammatory cells in response to ischemia and subsequent reperfusion of injured myocardium[13]. Elevated levels of IL-6 have been detected in patients with myocardial infarction, especially during the period of reperfusion, indicating a role for IL-6 in the pathogenesis of IHD Ischemic heart disease[14]. Monocyte Chemoattractant Protein-1 (MCP-1) is one of the well-studied CC chemokines involved in the pathophysiology of MI. It plays a role in the early stages of atherosclerosis or in the subacute phase of MI. Another investigation had demonstrated that MCP-1 plays a significant role in the process of myocardial healing and remodeling after MI occurrence[15]. Elevation of MCP-1 in the circulation of patients with

atherosclerosis-associated complications implicates this CC chemokine ligand (CCL)2 in inflammatory processes, which contribute to the pathogenesis of myocardial infarction and ischemic stroke[16]. Our research focuses on the diagnosis of MI using serum indicators in Isoproterenol-induced and RhEPO-treated MI.

2. MATERIALS AND METHODS

2.1. Animals

Male Wistar rats (250-300gm) were obtained from Biogen Laboratory Animal Facility, Bengaluru and maintained in polypropylene cages at 23 ± 2°Cwith a relative humidity of 40–60%. A natural light and dark cycle was maintained in the rooms where the animals were housed. A commercial chow pellet diet (Krishna Valley Agrotech, LLP, Sangli, Maharashtra) was provided, and filtered water *ad libitum*. The Institutional Animal Ethics Committee of Saveetha Medical College approved this study (SU/CLAR/RD/003/2022) and the experiments were performed as per the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA, India).

2.2.Drugs and Chemicals

The therapeutic drug rhEPO was procured from Intas Pharmaceuticals Ltd., Ahmedabad, India. Each prefilled syringe of 1.0 mL contained 4000 IU of recombinant human Erythropoietin injection IP(RENOCEL 4000) . The isoproterenol (Isolin – 2mg/mL vial) was purchased from Samarth Life Sciences Pvt Ltd., Mumbai, India. All other reagents and chemicals used in the study were of analytical grade.

2.3.Experimental Animals

The study animals were divided into four groups, and six animals were used in each group. While group 1 rats served as normal control, the group 2 rats were administered with single intraperitoneal (i.p) injection of ISO-isoproterenol (75 mg/kg b.w) on the last day of experimentation (i.e day of sacrificing animals). The group 3 rats (therapeutic drug pre-treated) received rhEPO (5000 IU/kg i.p) injections continuously once daily for 10 days. After receiving the last dose of rhEPO injection on the 10^{th} day, a single dose of isoproterenol (75 mg/kg i.p) was injected the next day and then sacrificed after 2hr duration. On the day of sacrificing all rats (11^{th} day), the group 4 rats received a single injection of ISO (75 mg/kg i.p) first followed by a single injection of rhEPO (5000 IU/kg i.p) as post-treatment at 2 hrs time interval. One hr after rhEPO injections, the group 4 rats were also sacrificed.

2.4.Induction of Experimental MI

2.4.1.Experimental Design

The rats were divided into the following study groups:

Group I: Normal control (No treatment);

Group II: Myocardial infarction via induction with isoproterenol (ISO) (75 mg/kg i.p)[14]

Group III: rhEPO (5000 IU/kg i.p) pre-treatment for 10 days + ISO (75mg/kg i.p)[14]

Group IV: ISO (75mg/kg i.p) + rhEPO post-treatment (5000 IU/kg i.p)[14]

After completion of the experimental protocol, the rats were anaesthetized using isoflurane (Raman and weil, Mumbai), and blood samples were withdrawn following retro orbital puncture using hematocrit capillary tubes. The blood was collected into vacutainers (Clot activator) and subsequently, the serum was collected by allowing the blood to clot for 15-30 min at RT followed by centrifugation at 3,500 rpm for 10 min in a cooling centrifuge (REMI CPR-24PLUS, India) and preserved for biochemical investigations (which are not included in this paper).

The animals were trans-cardially perfused with normal saline followed by a neutral buffered formalin solution. The heart tissues were carefully removed, and processed for histo-pathological and immunohistochemical examination.

2.5.Assay of Aspartate transaminase (AST)

To assay serum AST, the commercially available kit was used (Elab Science, USA). About 800 µl of reagent-1 (R1: TRIS (pH 7.8; 80 mmol/l): L- Aspartate (240 mmol/l), MDH (malate dehydrogenase) (≥600 U/l), LDH (lactate dehydrogenase) (≥600 U/l)) was mixed with 200 µl of reagent-2 (R2: 2-Oxaloglutarate (12 mmol/l): NADH (0.18 mmol), Pyridoxal-5-Phosphate FSGood buffer (pH 9.6; 0.7 mmol/l), Pyridoxal-5-Phosphate (0.09 mmol/l)) in a 5 ml test tube and 100 µl serum was added. It was mixed well and reading was taken using a UV-Visble spectrophotometer (Labomed, UV-2700, Los Angeles, USA) .

2.6.Determination of MCP-1 protein

MCP-1 protein levels in serum were measured using MCP-1 commercially available ELISA kit (Thermo Fischer Scientific, Massachusettes, US). About 100 µL of serum samples were incubated for 2 hours at room temperature in the wells pre-coated with primary antihuman MCP-1 antibody. After incubation, wells were washed three times, and horseradish peroxidase-conjugated polyclonal antibodies against MCP-1 were added and again incubated for 2 hours

at room temperature. Then tetramethylbenzidine substrate was added for 30 minutes and, after that stopping reagent 2M sulfuric acid was added, the absorbance was measured at 450 nm using a microplate reader (Mindray, MR96A model).

2.7.Determination of IL-6

IL-6 levels were assayed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Thermo Fischer Scientific, Massachusettes, US). This assay uses monoclonal anti–IL-6 antibodies, bound to microtiters plates. Unbound protein is removed by washing, and a polyclonal anti–IL-6 antibody conjugated to horseradish peroxidase is added, with any excess conjugated antibody removed by further washing. The IL-6 bound is quantified by the addition of substrate solution hydrogen peroxide and tetramethylbenzidine. The reaction is stopped after 20 minutes of incubation by adding 2M sulphuric acid at room temperature, and the color intensity is quantified by using a microtiter plate reader (Mindray, MR96A model) at a wavelength of 450 nm.

2.8. Assay of LDH

The levels of LDH in serum were measured using an LDH ELISA assay kit (R&D Systems, Minneapolis, USA), according to the manufacturer's instructions. The serum were then incubated with the reagents included in the kits. Finally, the absorbance values were measured using a microplate reader (Mindray, MR96A model) at 450 nm. All experiments were performed independently at least 3 times and the LDH level was expressed as U/l.

2.9. Statistical analysis

The data were expressed as mean, standard deviation and standard error. The mean values were compared by one way analysis of variance and once it was found statistically significant, multiple comparison test was done by Bonferroni 't' test. A probability of 0.05 and less was considered statistically significant. SigmaPlot 14.5 version (Systat Software Inc., San Jose, USA) was used for statistical analysis and for graph plotting.

3. RESULTS

3.1 Assessment of serum markers of myocardial injury

The results of serum markers viz., AST, MCP-1, IL-6 and LDH are depicted in Figures 1 to 4 respectively. The mean, standard deviation and standard error of the various markers (AST, MCP-1, IL-6, and LDH) are given in Table 1.

The mean values of serum AST levels in control, isoproterenol, pre-administration of erythropoietin (10 days) + isoproterenol, and isoproterenol followed by erythropoietin administration (2 hrs after ISO) are 124.67, 170.80, 132.26 and 111.43 respectively. Compared to the control group, the isoproterenol group and pre-administration of erythropoietin + isoproterenol groups showed 1.37 and 1.06 fold increase in the enzyme levels ($P < 0.001$ and $P = 1.0$ respectively). The isoproterenol followed by the erythropoietin administration group showed a 0.89 fold decrease ($P = 0.385$) in AST levels compared with the control. This shows that both pre and post-administration of EPO have a beneficial effect in reducing the AST levels. However, a better effect was seen in the EPO post-treatment group.

The mean values of serumMCP-1 levels (pg/mL) in control, isoproterenol, pre-administration of erythropoietin (10 days) + isoproterenol, and isoproterenol followed by erythropoietin administration (2 hrs after ISO) are 10.5, 25.86, 20.96 and 12.00 respectively. Compared to the control group, the isoproterenol group pre-administration of erythropoietin + isoproterenol group showed 2.46 and 1.99 fold increase in the MCP-1 levels ($P < 0.001$ and 0.001, respectively). The isoproterenol followed by erythropoietin post-administration group showed no significant change (1.14 fold increase) in comparison with the control group ($P = 1.02$). This shows that erythropoietin post-treatment has a significant beneficial effect as compared with pre-treatment.

The mean values of serumIL-6 levels (pg/mL) in control, isoproterenol, pre-administration of erythropoietin (10 days) + isoproterenol, and isoproterenol followed by erythropoietin administration (2 hrs after ISO) are 23.93, 61.63, 55.63 and 30.23 respectively. Compared to the control group, the isoproterenol group and pre-administration of erythropoietin + isoproterenol group showed a 2.57 and 2.32 fold increase in the IL-6 levels ($P < 0.01$ and 0.01, respectively). The isoproterenol followed by erythropoietin post-administration group showed no significant change (1.26 fold increase) in comparison with the control group ($P = 1.0$). This shows that erythropoietin post-treatment has a significant beneficial effect as compared with pre-treatment.

The mean values of serum LDH levels (U/mL) in control, isoproterenol, pre-administration of erythropoietin (10 days) + isoproterenol, and isoproterenol followed by erythropoietin post administration (2 hrs after ISO) are 66.90, 207.6, 160.5 and 73.4 respectively. Compared to the control group, the isoproterenol group, pre-administration of erythropoietin + isoproterenol group and the isoproterenol followed by erythropoietin post-administration group showed 3.1, 2.39, and 1.09 fold increase in the levels of LDH levels ($P < 0.001$, 0.05 and P=1respectively).

This shows that post-treatment with rhEPO has rendered a better protective effect than the pre-administration of erythropoietin.

Figure 1

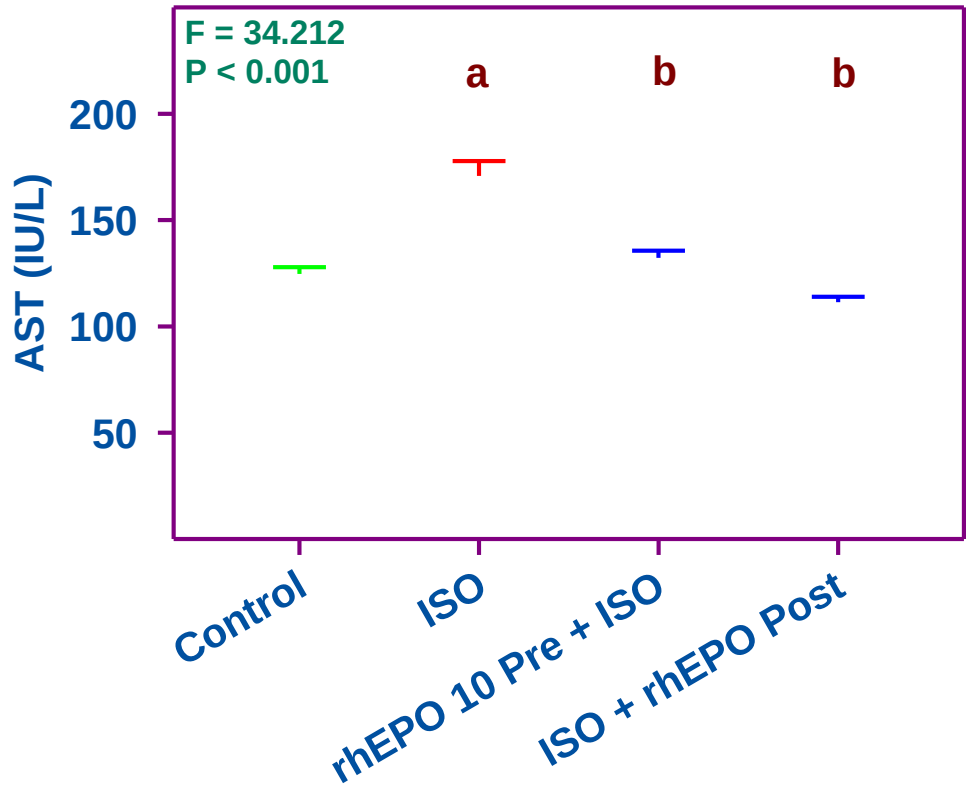

Figure 1: Comparative effect of erythropoietin (rhEPO) pre-treatment and post-treatment on isoproterenol (ISO) induced changes in AST levels in Wistar rats.

Values are mean ± SE (n = 5 each)

Pre = 10 days pre-treatment with erythropoietin followed by isoproterenol on the next day.

Post = Isoproterenol followed by erythropoietin treatment after 1 hr.

The 'F' and 'P' values are by one way ANOVA with Bonferroni 't' test for multiple comparison.

[a]Significantly different from control.

[b]Significantly different from isoproterenol.

[c]Significantly different from erythropoietin pre-treatment + isoproterenol.

Figure 2:

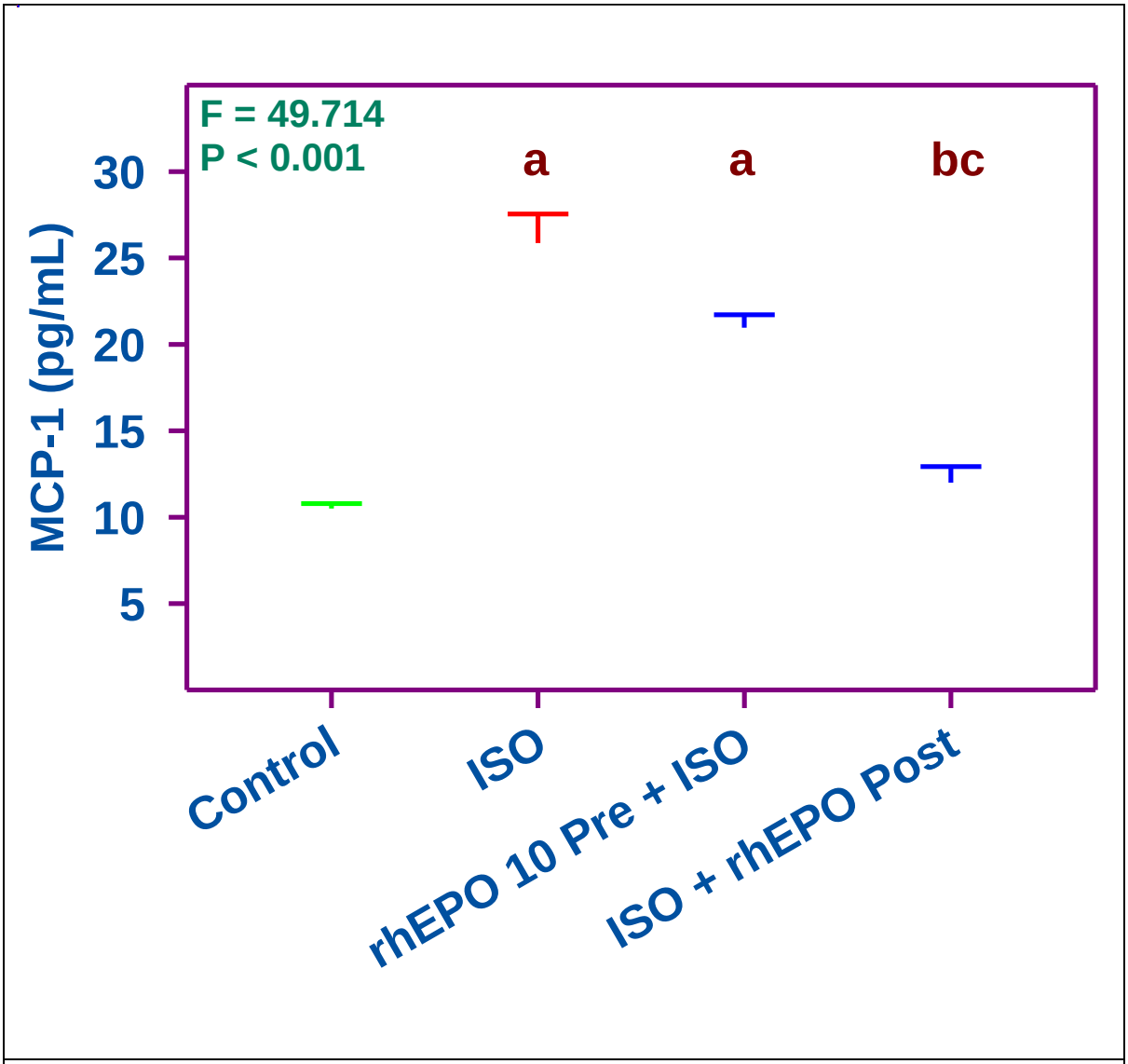

Figure 2: Comparative effect of erythropoietin (rhEPO) pre-treatment and post-treatment on isoproterenol (ISO) induced changes in MCP-1 levels in Wistar rats.

Values are mean ± SE (n = 5 each)

Pre = 10 days pre-treatment with erythropoietin followed by isoproterenol on the next day.

Post = Isoproterenol followed by erythropoietin treatment after 1 hr.

The 'F' and 'P' values are by one way ANOVA with Bonferroni 't' test for multiple comparison.

[a]Significantly different from control.

[b]Significantly different from isoproterenol.

[c]Significantly different from erythropoietin pre-treatment + isoproterenol.

Figure 3:

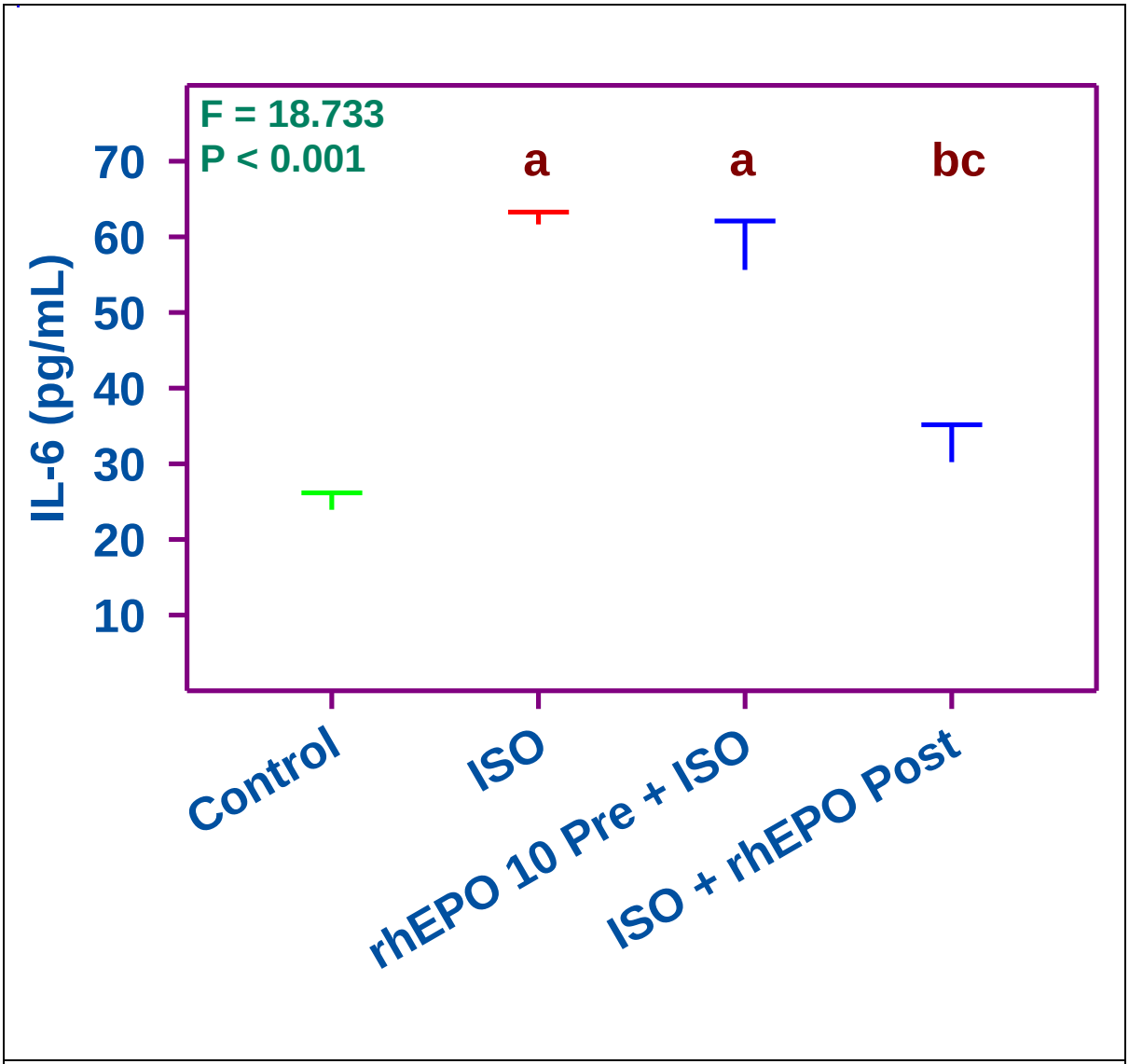

Figure 3: Comparative effect of erythropoietin (rhEPO) pre-treatment and post-treatment on isoproterenol (ISO) induced changes in IL-6 levels in Wistar rats.

Values are mean ± SE (n = 5 each)

Pre = 10 days pre-treatment with erythropoietin followed by isoproterenol on the next day.

Post = Isoproterenol followed by erythropoietin treatment after 1 hr.

The 'F' and 'P' values are by one way ANOVA with Bonferroni 't' test for multiple comparison.

[a]Significantly different from control.

[b]Significantly different from isoproterenol.

[c]Significantly different from erythropoietin pre-treatment + isoproterenol.

Figure 4

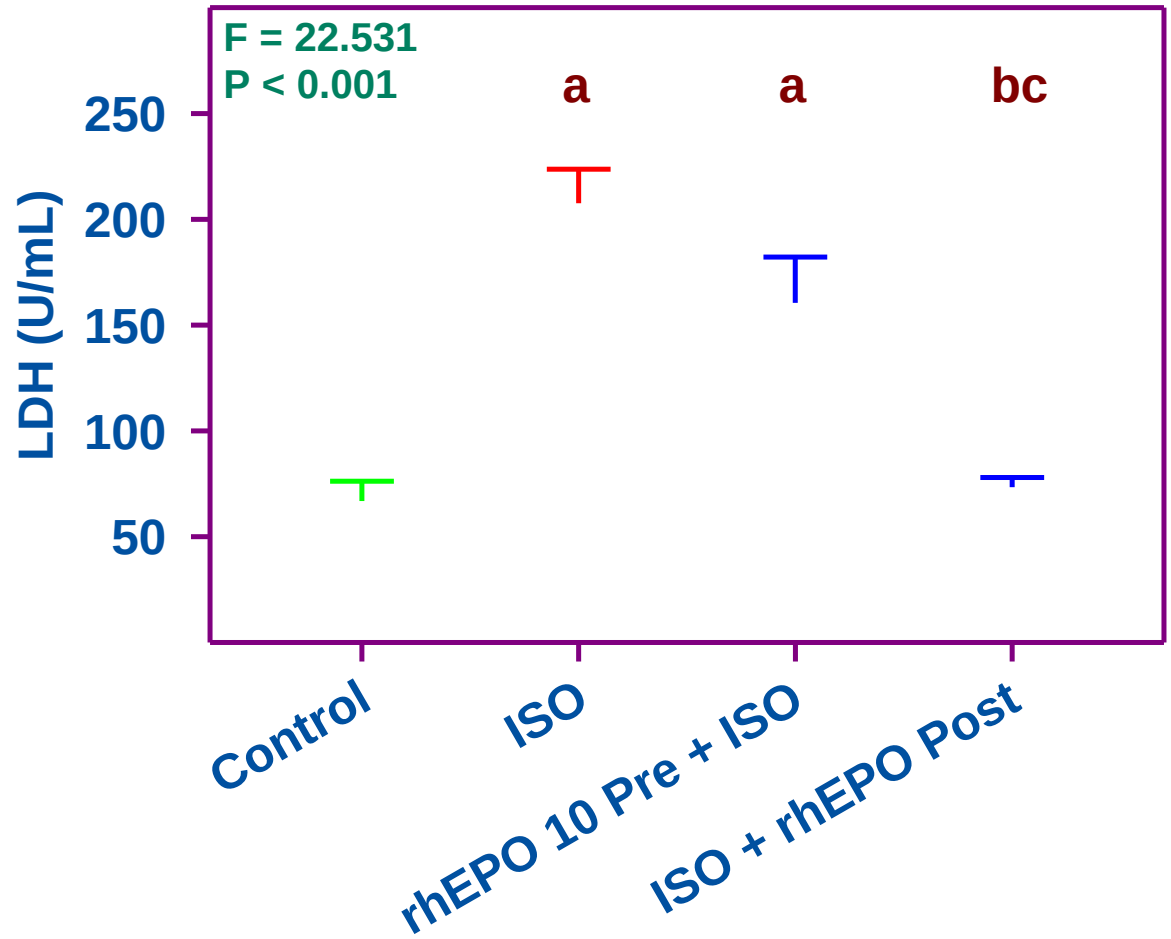

Figure 4: Comparative effect of erythropoietin (rhEPO) pre-treatment and post-treatment on isoproterenol (ISO) induced changes in LDH levels in Wistar rats.

Values are mean ± SE (n = 5 each)

Pre = 10 days pre-treatment with erythropoietin followed by isoproterenol on the next day.

Post = Isoproterenol followed by erythropoietin treatment after 1 hr.

The 'F' and 'P' values are by one way ANOVA with Bonferroni 't' test for multiple comparison.

[a]Significantly different from control.

[b]Significantly different from isoproterenol.

[c]Significantly different from erythropoietin pre-treatment + isoproterenol.

Table 1:

Table 1: Comparative effect of erythropoietin (rhEPO) pre-treatment and post-treatment on isoproterenol (ISO) induced changes in Wistar rats.

S.No.	Parameter	Groups	Mean	SD	SE	Statistics
1	AST	Control	124.667	5.550	3.204	F = 34.212 P < 0.001
		ISO	170.800	12.050	6.957	
		rhEPO 10 Pre + ISO	132.267	5.771	3.332	
		ISO + rhEPO Post	111.433	4.336	2.504	
2	MCP-1	Control	10.50	0.5	0.289	F = 49.714 P < 0.001
		ISO	25.867	2.909	1.680	
		rhEPO 10 Pre + ISO	20.967	1.290	0.745	
		ISO + rhEPO Post	12	1.609	0.929	
3	IL-6	Control	23.933	3.850	2.223	F = 18.733 P < 0.001
		ISO	61.633	2.836	1.637	
		rhEPO 10 Pre + ISO	55.633	11.169	6.448	
		ISO + rhEPO Post	30.233	8.545	4.933	
4	LDH	Control	66.90	16.146	9.322	F = 22.531 P < 0.001
		ISO	207.6	27.861	16.086	
		rhEPO 10 Pre + ISO	160.5	37.458	21.627	
		ISO + rhEPO Post	73.4	7.987	4.611	

n = 5 each

Pre = 10 days pre-treatment with erythropoietin followed by isoproterenol on the next day.

Post = Isoproterenol followed by erythropoietin treatment after 1 hr.

The 'F' and 'P' values are by one way ANOVA with Bonferroni 't' test for multiple comparison.

The significance from control and ISO groups are given in Figure 1, 2 and 3.

4. DISCUSSION

The inflammatory responses triggered during Myocardial infarction (MI) lead to the recruitment of leukocytes apart from causing damage to the myocardium, healing, and scar formation[17-19]. Chemokines are capable of regulating the leukocyte trafficking (migration and activation) and mediating the inflammatory processes; being expressed by several non-hematopoietic cells including endothelial cells, smooth muscle cells, and cardiomyocytes, the functions of chemokines are very extensive. Chemokines role in the pathophysiology of MI has been established through several experimental and clinical studies. Of several chemokines, the role of CC chemokine – monocyte chemoattractant protein-1 (MCP-1/CCL2) in the pathophysiology of MI is well indicated.

Several lines of research suggest that MI is accompanied by inflammatory responses leading to the recruitment of leukocytes which initiates myocardial damage, healing, and scar formation[20]. If the leukocytes are found to be recruited to the site of the infarcted myocardium, there will be the release of cytokines and proteinases that may elevate the inflammation reaction and left ventricular remodeling. Evidence support that chemokine cells are capable of secreting enormous levels of angiogenic factors viz., vascular endothelial growth factor (VEGF) which helps induce angiogenesis in the infarcted heart. The key role of MCP-1 is to recruit monocytes to the infarcted area as well as at the site of inflammation[21], thereby imparting its key role in the early stages of atherosclerosis or perhaps in the subacute phase of MI. As MCP-1 plays a significant role in MI during the process of myocardial healing as well as cardiac remodeling [22-24]. It is important to study this molecule in cardiac damage and repair.

In contention with our present results, increased serum MCP-1 levels have been reported in ISO induced MI in rats[25], suggesting augmentation of the inflammatory process. Elevated cardiac markers/enzymes such as MCP-1, LDH and AST were noticed in the heart tissues of

ISO induced MI rats that were reverted to near normal levels after Baobab fruit pulp (*Adansonia digitata*) therapy[26]. Indeed, during a 10-month follow-up in a large study involving patients with acute coronary syndromes, elevated serum MCP-1 levels were shown to be associated with an increased risk of death or MI[27].

Interleukin-6 (IL-6) is a pleiotropic cytokine in the initiation of the acute-phase reaction that accompanies myocardial infarction[28]. It has been associated with a broad range of humoral and cellular immune response driving inflammation, host defense, and tissue injury. In patients suffering from acute myocardial infarction (AMI), serum levels of both IL-6 levels and creatine kinase (CK) activity reached a peak in all the patients[29]. The study revealed that there was a good correlation between IL-6 levels and peak serum C-reactive protein (CRP) levels. since IL-6 is synthesized in the myocardium, serum IL-6 levels become elevated in AMI, suggesting that IL-6 could be involved in the pathogenesis of AMI.

Elevated IL-6 and LDH levels have been reported in ISO induced rodent models of MI in serum and heart tissue samples[30-33]**.** According to Ridker et al 2000[28]**,** Interleukin-6 (IL-6) plays a critical role in the events of inflammation and tissue injury. It was indicated that the increased risk of MI in subjects increased with increasing quartiles of baseline IL-6 concentration. In fact, men who were in the highest quartile showed a 2.3 times higher risk of MI than those in the lowest quartile.

Based on Kucharz and Wilk's (2000)[34] findings, elevated serum IL-6 was observed in patients with myocardial infarction, and this hike in IL-6 level was correlated to the mass of the myocardial damage. The role of LDH and proinflammatory cytokines such as IL-6 have been well studied in the ISO model of MI including the testing of therapeutic agents that attenuated LDH and IL-6 levels[35-38]. Several natural and synthetic drugs that attenuated serum/heart IL-6 and LDH levels in ISO induced MI models are also well evident[39-41]. In the present study of ISO induced MI in rats, rhEPO post-treatment (single dose) rendered better therapeutic efficacy in bringing down the levels of serum IL-6 levels as compared with 10 days pre-treatment with rhEPO before ISO induction. A study finding denotes that signaling mechanisms associated with erythropoietin preconditioning and cardioprotection functions involves NFkB activation followed by the opening of K(ATP) channels including attenuation of lactate dehydrogenase (LDH) levels[42].To the best of our knowledge, we lack studies on the measurement of serum levels of cardiac marker enzymes viz., MCP-1, LDH, IL-6, and AST to validate the therapeutic potential of EPO in ISO induced MI. From the present findings, we generate evidence to

support that EPO pre and post-therapy had an impact on the altered serum cardiac markers of MI thereby promoting cardiac repair and myocardial protection. According to Shahzad et al (2019)[43], the pre-treatment of test drug Syringic acid protected the rats from isoproterenol-induced cardiotoxicity by amelioration of serum level of marker enzymes (LDH, AST) and pro-inflammatory cytokines (IL-6 and TNF-alpha) respectively.

5. CONCLUSIONS

In the present study, the efficacy of both pre and post-therapy of rhEPO was tested in an isoproterenol-induced model of myocardial infarction in rats through evaluation of inflammatory and cardiac serum markers of MI. The altered cardiac markers due to MI-mediated cardiac damage were significantly attenuated by both EPO pre and post-therapy. However, the post-treatment with EPO following MI rendered better therapeutic efficacy than ten days pre-treatment with EPO in reversing the serum markers of myocardial infarction.

6. FUNDING ACKNOWLEDGEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

7. CONFLICT OF INTEREST

The authors declare no competing interests.

8. REFERENCES

1. Thygesen K, Alpert JS, White HD, et al. Universal definition of myocardial infarction.Circulation.2007;116(22):2634-2653. doi:10.1161/CIRCULATIONAHA.107.187397
2. Alwan A. Global status report on non-communicable diseases 2010. World Health Organization 2011.
3. 3.Massberg S, Polzin A. [Update ESC-Guideline 2017: Dual Antiplatelet Therapy]. Dtsch Med Wochenschr. 2018 Aug;143(15):1090-1093. [PubMed] [Ref list]
4. K. Kurrelmeyer, D. Kalra, B. Bozkurt, F. Wang, Z. Dibbs, Y. Seta, et al., Cardiacremodeling as a consequence and cause of progressive heart failure. Clin. Cardiol. 21 (1998) 14e19.

5. J.N. Cohn, R. Ferrari, N. Sharpe, Cardiac remodelingeconcepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling, J. Am. Coll. Cardiol. 35 (2000) 569e582.
6. Goyal SN, Sharma C, Mahajan UB, Patil CR, Agrawal YO, Kumari S, Arya DS, Ojha S. Protective effects of cardamom in isoproterenol-induced myocardial infarction in rats. Int. J Mol Sci 2015; 16(11): 27457- 27469
7. Nagoor Meeran, M., Stanely Mainzen Prince, P. Protective effects of N-acetyl cysteine on membrane-bound adenosine triphosphatases and minerals in isoproterenol-induced myocardial-infarcted rats: an in vivo and in vitro study. J Biochem Mol Toxicol (2012) 26: 276–281.
8. Carey, I., DeWilde, S., Shah, S., Harris, T., Whincup, P., Cook, D. Statin use after first myocardial infarction in UK men and women from 1997 to 2006: Who started and who continued treatment? Nutr Metab Cardiovasc Dis (2012) 22: 400–408.
9. R. P. Tiwari, A. Jain, Z. Khan et al., "Cardiac troponins I and T: molecular markers for early diagnosis, prognosis, and accurate triaging of patients with acute myocardial infarction," Molecular Diagnosis & Therapy, 2012, vol. 16, no. 6, pp. 371–381.
10. Ladue JS, Wroblewski F, Karmen A. Serum Glutamic Oxaloacetic Transaminase Activity in Human Acute Transmural Myocardial Infarction. Science 1954;120:497-9. [Crossref] [PubMed]
11. Mercer RM, Corcoran JP, Porcel JM, Rahman NM, Psallidas I. Interpreting pleural fluid results Clin Med (Lond). 2019 May;19(3):213-217. [PMC free article] [PubMed]
12. Schmiechen NJ, Han C, Milzman DP. ED use of rapid lactate to evaluate patients with acute chest pain. Ann Emerg Med. 1997;30(5):571–577
13. Mythili S, Malathi N. Diagnostic markers of acute myocardial infarction. Biomed Rep (2015) , 3(6):743–748.
14. Chandrasekar B, Mitchell DH, Colston JT, Freeman GL. Regulation of CCAAT/Enhancer binding protein, interleukin-6, interleukin-6 receptor, and gp130 expression during myocardial ischemia/reperfusion. Circulation. 1999;99:427–33. [PubMed] [Google Scholar] [Ref list]

15. Morimoto H, Takahashi M, Izawa A, Ise H, et al. Cardiac overexpression of monocyte chemoattractant protein-1 in transgenic mice prevents cardiac dysfunction and remodeling after myocardial infarction. Circ. Res. 2006;99:891–899. [PubMed] [Google Scholar] [Ref list]

16. A. Arakelyan, J. Petrkova, Z. Hermanova, A. Boyajyan, J. Lukl, M. Petrek,. "Serum Levels of the MCP-1 Chemokine in Patients With Ischemic Stroke and Myocardial Infarction". Mediators of Inflammation, vol. 2005, Article ID 105164, 5 pages, 2005. https://doi.org/10.1155/MI.2005.175

17. Morimoto H, Takahashi M. Role of monocyte chemoattractant protein-1 in myocardial infarction. Int J Biomed Sci. 2007 Sep;3(3):159-67. PMID: 23675039; PMCID: PMC3614683.
18. Frangogiannis NG. Chemokines in the ischemic myocardium: from inflammation to fibrosis. Inflamm. Res. 2004; 53: 585-595.
19. Frangogiannis NG, Entman ML. Chemokines in myocardial ischemia. Trends Cardiovasc. Med. 2005; 15: 163-169.
20. Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. Cardiovasc. Res. 2002; 53: 31-47.
21. Kumar AG, Ballantyne CM, Michael LH, Kukielka GL, et al. Induction of monocyte chemoattractant protein-1 in the small veins of the ischemic and reperfused canine myocardium. Circulation. 1997;95:693–700.
22. Morimoto H, Takahashi M, Izawa A, Ise H, et al. Cardiac overexpression of monocyte chemoattractant protein-1 in transgenic mice prevents cardiac dysfunction and remodeling after myocardial infarction. Circ. Res. 2006;99:891–899.
23. Dewald O, Zymek P, Winkelmann K, Koerting A, et al. CCL2/Monocyte Chemoattractant Protein-1 regulates inflammatory responses critical to healing myocardial infarcts. Circ. Res. 2005;96:881–889.
24. Kaikita K, Hayasaki T, Okuma T, Kuziel WA, et al. Targeted deletion of CC chemokine receptor 2 attenuates left ventricular remodeling after experimental myocardial infarction. Am. J. Pathol. 2004;165:439–447.
25. Sangeethadevi G, V V SU, Jansy Isabella RAR, Saravanan G, Ponmurugan P, Chandrasekaran P, Sengottuvelu S, Vadivukkarasi S. Attenuation of lipid metabolic abnormalities, proinflammatory cytokines, and matrix metalloproteinase expression by

biochanin-A in isoproterenol-induced myocardial infarction in rats. Drug Chem Toxicol. 2022 Sep;45(5):1951-1962. doi: 10.1080/01480545.2021.1894707. Epub 2021 Mar 15. PMID: 33719799.

26. Ghoneim MA, Hassan AI, Mahmoud MG, Asker MS. Protective Effect of Adansonia digitata against Isoproterenol-Induced Myocardial Injury in Rats. Anim Biotechnol. 2016;27(2):84-95. doi: 10.1080/10495398.2015.1102147. PMID: 26913549.
27. de Lemos JA, Morrow DA, Sabatine MS, Murphy SA, et al. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation. 2003; 107: 690-695.
28. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000 Mar 23;342(12):836-43. doi: 10.1056/NEJM200003233421202. PMID: 10733371.
29. Ikeda U, Ohkawa F, Seino Y, Yamamoto K, Hidaka Y, Kasahara T, Kawai T, Shimada K.Serum interleukin 6 levels become elevated in acute myocardial infarction J Mol Cell Cardiol. 1992 Jun;24(6):579-84. doi: 10.1016/0022-2828(92)91042-4.
30. Al-Botaty BM, Elkhoely A, K El-Sayed E, Ahmed AAE. Ethyl pyruvate attenuates isoproterenol-induced myocardial infarction in rats: Insight to TNF-α-mediated apoptotic and necroptotic signaling interplay. Int Immunopharmacol. 2022 Feb;103:108495. doi: 10.1016/j.intimp.2021.108495. Epub 2021 Dec 29. PMID: 34973531.
31. Cinar I, Yayla M, Tavaci T, Toktay E, Ugan RA, Bayram P, Halici H. In Vivo and In Vitro Cardioprotective Effect of Gossypin Against Isoproterenol-Induced Myocardial Infarction Injury. Cardiovasc Toxicol. 2022 Jan;22(1):52-62. doi: 10.1007/s12012-021-09698-3. Epub 2021 Oct 1. PMID: 34599475.
32. Abukhalil MH, Hussein OE, Aladaileh SH, Althunibat OY, Al-Amarat W, Saghir SA, Alfwuaires MA, Algefare AI, Alanazi KM, Al-Swailmi FK, Kamel EM, Mahmoud AM. Visnagin prevents isoproterenol-induced myocardial injury by attenuating oxidative stress and inflammation and upregulating Nrf2 signaling in rats. J Biochem Mol Toxicol. 2021 Nov;35(11):e22906. doi: 10.1002/jbt.22906. Epub 2021 Sep 5. PMID: 34486204.
33. Abdelzaher WY, Ahmed SM, Welson NN, Alsharif KF, Batiha GE, Labib DAA. Dapsone Ameliorates Isoproterenol-Induced Myocardial Infarction via Nrf2/ HO-1; TLR4/ TNF-α Signaling Pathways and the Suppression of Oxidative Stress,

Inflammation, and Apoptosis in Rats. Front Pharmacol. 2021 May 19;12:669679. doi: 10.3389/fphar.2021.669679. PMID: 34093197; PMCID: PMC8170324.

34. Kucharz EJ, Wilk T. Dynamics of serum interleukin-6 level in patients with acute myocardial infarction. Eur J Intern Med. 2000 Oct;11(5):253-256. doi: 10.1016/s0953-6205(00)00098-4. PMID: 11025249.
35. Aodah AH, Devi S, Alkholifi FK, Yusufoglu HS, Foudah AI, Alam A. Effects of Taraxerol on Oxidative and Inflammatory Mediators in Isoproterenol-Induced Cardiotoxicity in an Animal Model. Molecules. 2023 May 15;28(10):4089. doi: 10.3390/molecules28104089. PMID: 37241830; PMCID: PMC10224333.
36. Khalifa AA, El Sokkary NH, Elblehi SS, Diab MA, Ali MA. Potential cardioprotective effect of octreotide via NOXs mitigation, mitochondrial biogenesis and MAPK/Erk1/2/STAT3/NF-kβ pathway attenuation in isoproterenol-induced myocardial infarction in rats. Eur J Pharmacol. 2022 Jun 15;925:174978. doi: 10.1016/j.ejphar.2022.174978. Epub 2022 Apr 30. PMID: 35500641.
37. Rathod S, Agrawal Y, Sherikar A, Nakhate KT, Patil CR, Nagoor Meeran MF, Ojha S, Goyal SN. Thymoquinone Produces Cardioprotective Effect in β-Receptor Stimulated Myocardial Infarcted Rats via Subsiding Oxidative Stress and Inflammation. Nutrients. 2022 Jun 30;14(13):2742. doi: 10.3390/nu14132742. PMID: 35807920; PMCID: PMC9268596.
38. Rahnavard M, Hassanpour M, Ahmadi M, Heidarzadeh M, Amini H, Javanmard MZ, Nouri M, Rahbarghazi R, Safaie N. Curcumin ameliorated myocardial infarction by inhibition of cardiotoxicity in the rat model. J Cell Biochem. 2019 Jul;120(7):11965-11972. doi: 10.1002/jcb.28480. Epub 2019 Feb 18. PMID: 30775806.
39. Zou G, Wan J, Balupillai A, David E, Ranganathan B, Saravanan K. Geraniol enhances peroxiredoxin-1, and prevents isoproterenol-induced oxidative stress and inflammation associated with myocardial infarction in experimental animal models. J Biochem Mol Toxicol. 2022 Aug;36(8):e23098. doi: 10.1002/jbt.23098. Epub 2022 May 24. PMID: 35608392.
40. Li J, Thangaiyan R, Govindasamy K, Wei J. Anti-inflammatory and anti-apoptotic effect of zingiberene on isoproterenol-induced myocardial infarction in experimental animals. Hum Exp Toxicol. 2021 Jun;40(6):915-927. doi: 10.1177/0960327120975131. Epub 2020 Nov 26. PMID: 33242989.
41. Khalifa AA, Rashad RM, El-Hadidy WF. Thymoquinone protects against cardiac mitochondrial DNA loss, oxidative stress, inflammation and apoptosis in isoproterenol-

induced myocardial infarction in rats. Heliyon. 2021 Jul 14;7(7):e07561. doi: 10.1016/j.heliyon.2021.e07561. PMID: 34355083; PMCID: PMC8322274.

42. Diwan V, Kant R, Jaggi AS, Singh N, Singh D. Signal mechanism activated by erythropoietin preconditioning and remote renal preconditioning-induced cardioprotection. Mol Cell Biochem. 2008 Aug;315(1-2):195-201. doi: 10.1007/s11010-008-9808-3. Epub 2008 Jun 5. PMID: 18528635.
43. Shahzad S, Mateen S, Naeem SS, Akhtar K, Rizvi W, Moin S. Syringic acid protects from isoproterenol induced cardiotoxicity in rats. Eur J Pharmacol. 2019 Apr 15;849:135-145. doi: 10.1016/j.ejphar.2019.01.056. Epub 2019 Feb 4. PMID: 30731086.