https://doi.org/10.48047/AFJBS.1.3.2019.50-58

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

N-Acetylcysteine: The potential protective role in myocardial infarction in rats, focus on the antioxidant and anti-inflammatory markers

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Article History

Volume 1, Issue 3, Apr-Aug 2019

Received: 5 June 2019

Accepted: 20 June 2019

Published: 25 June 2019

Doi: *10.48047/AFJBS.1.3.2019.50-58*

Abstract: Background: Acute myocardial infarction (AMI) is caused by the interruption of blood supply to a portion of the heart, which causes heart cells to die. **Aim of the present study** is to investigate the potential role of NAC in MI and detect the role of oxidative stress markers, and inflammatory markers (IL-6 and TNF-α. **Materials and methods:** 32 male Wistar rats were divided into four groups, each comprising eight animals (n=8): a control group (Group 1) was maintained in typical control circumstances and given a balanced diet; NAC group (group II), Rats will be orally treated with NAC (10 mg/kg) dissolved in saline daily for a period of 14 days. ISO group (group III) The animals will be subcutaneously injected with ISO (100 mg/kg) at an interval of 24 h for 2 days (on 15th and 16th day). NAS+ISO group (group 4) Rats will be orally treated with NAC (10 mg/kg) dissolved in saline daily for a period of 14 days. On the 15th day the animals will be S.C injected with ISO and a 2nd dose will be administrated on the 16th day. **Result:** Isoproterenol induced myocardial infarction with notably increase in inflammatory mediators TNF-α and IL-6. Furthermore, SOD is markedly decreased. NAC has a protective effect on cardiac muscle and improve cardiac hemodynamics

Keywords: *N-acetylcysteine, cardiac infarction, oxidative stress, Isoprenaline*

Abbreviations:

NAC: N-acetylcysteine, ISO: isoproterenol

TNF-α: tumor necrotic factor alpha, IL-6: interlukin-6

1.Introduction

It is estimated that congestive heart failure affects over 23 million individuals globally (1). Additionally, it is anticipated that the prevalence of heart failure would rise globally. According to WHO predictions, ischemic heart disease will rank as the second most common illness endangering human health by 2030 (2).

The development of heart failure, which is typified by contractile dysfunction, is significantly influenced by oxidative stress (3). Excessive myocardial generation of reactive oxygen species (ROS) and systemic

inflammation have been noted in heart failure patients and in vivo models (4). Additionally, it has been shown that the degree of oxidative stress correlates with the grade of cardiac function and the severity of heart failure (5). Myocardial cell apoptosis brought on by oxidative stress may cause damage to cardiac tissue and a consequent decline in its hemodynamics (6). Free radicals produced from oxygen play a significant part in tissue damage during myocardial ischemia and reperfusion. There is strong evidence that cardiac damage during ischemia-reperfusion is caused by reactive oxygen, such as superoxide anion, hydrogen peroxide, and hydroxyl radicals (7). Lipid peroxidation produces malonaldehyde (MDA), which is a byproduct that indirectly indicates the amount of damage to cardiac tissues and the production of free radicals (8). Superoxide dismutase enzyme (SOD) is a superoxide radical scavenger that plays a crucial protective function against myocardial cell injury (9). At the same time, myocardial cell membrane lipid peroxidation increases cell membrane permeability and a large amount of LDH in cells is leaked into the intercellular space and body fluid (10).

The acetyl molecule of L-cysteine with an active mercapto group is called N-acetylcysteine (NAC) (11). NAC has been shown in more recent research to dilate blood vessels, prevent oxidative damage, boost immunity, suppress apoptosis and the inflammatory response, and encourage cells to synthesize glutathione (12). NAC has the potential to enhance cardiac function and myocardial cell systolic function, prevent myocardial apoptosis, preserve ventricular and vascular remodeling, lower serum opiomelanocortin levels, and increase serum nitric oxide (NO) content, all of which would enhance vascular endothelial function (13).

Isoproterenol (ISO) has been shown to produce MI in quantities greater than the recommended dosage by oxidizing and producing more free radicals, which in turn causes cytotoxicity (14). ISO-induced myocardial necrosis leads to fluctuating membrane permeability, which in turn causes the membrane and myocardial tissues to lose their structural and functional integrity. It has been demonstrated to seriously harm cardiac myocytes due to hypoxia, coronary hypotension, calcium excess, energy reserve depletion, and excessive radical oxidative species generation (15). Increases in cardiac enzyme, changes in ECG, lipid peroxide buildup, and disturbed cardiac function are indicators of necrotic cardiac tissue (16).

2. Material and methods:

2.1. Chemical used:

N-Acetylcysteine (NAC) was purchased from Sigma Aldrich. It was freshly prepared by dissolving in distilled water before use. **Urethane**: Was purchased from Urethane was sourced from Sigma-Aldrich (St. Louis, MO, USA). It was dissolved in saline before use. **Isoproterenol (ISO)** Was purchased from (Sigma Aldrich). It was freshly prepared by dissolving in distilled water before use. **Creatine Kinase - MB** kits: Spinreact (Spain). **LDH** kits: Biosystem Egypt (Spain). malondialdehyde (MDA) assay kit (cat no. A003-1), superoxide dismutase (SOD) assay kit (cat no. A001-3), lactic dehydrogenase (LDH) assay kit (cat no. A020-2)

2.2. Experimental animals: Thirty-two mature male albino rats, each weighing 180–220 grams, were acquired from the Animal House at Benha University's Faculty of Veterinary Medicine in Egypt. The animals were housed in stainless-steel cages with unrestricted access to food and water and carefully regulated ambient conditions.

An even 12-hour light/dark cycle and a precise 23 ± 1°C ambient temperature was maintained. These conditions were continued for 10 days before the experiment for acclimatization. The study period lasted for 3 weeks. Experimental rats should be under complete healthy conditions all over the experiment and under care of a professional technician and a qualified researcher. No rats were died throughout the experiment. At the end of the study the rats were incinerated at Benha university hospital incinerator.

2.3. Protocol of animal study:

Randomly, the rats were divided into four equal groups after a week of acclimation (n=8). The randomization process made sure that, there were no statistically significant variations in body weight between the groups. Following that, the groups were put through the following therapy procedures:

Control group: Animals of this group received no medication rather than intra peritoneal saline injection in a dose of 2.5 ml/kg/ day for 14 days (17)

NAC group: Rats will be orally treated with NAC (10 mg/kg) dissolved in saline daily for a period of 14 days (18).

ISO group: rats were injected with saline (0 mL/kg i.p daily, for 8 days and subcutaneously received ISO (85 mg/kg) at the 15^{th} and 16^{th} days (19).

AS+ISO group: NAC (10 mg/kg) dissolved in saline will be given orally to the rats every day for 14 days. The animals will get an ISO S.C. injection on day 15, and a second dose will be given on day 16.

2.4. Procedure of the experiment:

Twelve hours after treatment and following an overnight fast, rats were anesthetized using urethane at a dose of 1.5 g/kg, then fixed in the supine position on a wood table. Mean arterial pressure (MAP) of each rat are recorded. Electrodes were inserted under the skin and ECG test. The changes in T-wave were observed and a strip of lead II was recorded for each animal. After the animals were placed on an operating table, a craniocaudal incision was made about 2 cm from the sternum, parallel to and slightly to the left. Blood samples were then extracted from the aorta, placed in non-heparinized tubes, and allowed to clot. The resulting sera were separated using automated pipettes and kept in Eppendorf tubes at -20°C for further biochemical analysis. Finally, the hearts were removed. the heart tissue was washed with saline and patted dry using filter paper, frozen in liquid nitrogen and stored at -80°C in order to assess antioxidant and anti-inflammatory markers.

2.5. Tissue homogenate preparation:

The heart specimens were subjected to homogenization along with sufficient volume of tissue lysis buffer. The liquid supernatant was centrifuged to cryo-centrifugation for 10 min at 4 °C and 12,000 g. Bicinchoninic acid protein kit was employed for measurement of protein content.

3. Parameters assay:

3.1.ABP measurement:

At the end of the experiment, MAP was measured by carotid artery cannulation through using Software Lab Chart 8 power lab recorder and analyzer (AD Instruments, Mountain View, CA, USA).

3.2.ECG study:

Rats under anesthesia were put in a supine position to assess heart function. An artifact-free lead II arrangement was used for continuous ECG monitoring. To record ECG signals, subcutaneous needle electrodes were placed on the left forelimb, right forelimb, and left thigh. For accurate data collection and analysis, the electrodes were interfaced with a Power Lab 4/20 data collecting system (AD Instruments Pty Ltd, Australia). Using voltage calibration (in millivolts), ECG data were automatically calculated. The ECG output was used to capture parameters such heart rate (measured in beats per minute), R-R interval (measured in seconds), R and T wave amplitudes (measured in millivolts), and ST segment depression.

3.3. Measurement of Biochemical Parameter:

3.3.1 Assessment of cardiac enzymes: serum CK-MB a marker for detection of MI was analyzed using Rat CKMB ELISA Kit (catalog #E4608-100; BioVision, Milpitas, CA, USA)

3.3.2 Determination of SOD, MDA and LDH:

Cardiac tissue homogenate was homogenized 15 times at 4˚C prior to centrifugation at 1,0625 x g for 15 min at 4˚C. The supernatant was kept. Following the manufacturer's instructions, SOD activity and MDA content were assessed using SOD and MDA assay kits, respectively, and a UV visible light photometer. Serum was obtained by centrifuging blood at 1,0625 x g for 15 minutes at 4˚C two hours. As directed by the LDH test kit, the LDH level was determined using a UV visible light photometer.

3.3. 4 Estimation of Inflammatory Mediators:

The serum samples were subjected to measurement of tissue necrosis factor-α (TNF-α) and interleukin-6 (IL-6) expressions. Commercially available kits were employed to detect the expression of inflammatory mediators – TNF-α and IL-6 by following procedure indicated in user manual of the detection kit.

3.4 Hematoxylin and eosin staining of cardiac tissues: to detect pathological alterations: Each group's left ventricular anterior wall tissue was immersed in 4% paraformaldehyde for 24 hours at room temperature, followed by dehydration using alcohol made in a specific gradient (75, 85, 90, 95, and 100%), and finally embedded in paraffin blocks. 4 μm thick sections were deparaffinated in dimethylbenzene, embedded in xylene I and xylene II for 20 minutes each, absolute ethyl alcohol I and II for 10 minutes each, 95% alcohol for 5 minutes, 90% alcohol for 5 minutes, 80% alcohol for 5 minutes, and 70% alcohol for 5 minutes each, before being washed with water. Hematoxylin was used to stain the cell nuclei, and cytoplasm was used to be stained. Using a light microscope (magnification, x200; Nikon Corporation, Tokyo, Japan), the myocardial tissue structures of each group were examined.

Statistical Analysis

The data was statistically analyzed using SPSS software (Version 20; SPSS Inc., Chicago, USA). Group differences were evaluated using a one-way ANOVA, and post-hoc analysis was conducted using the Least Significant Difference (LSD) test. A p-value of less than 0.05 was considered statistically significant, and the data were presented as mean ± SD.

4. Results:

4.1 Effect of N-acetylcysteine on cardiac hemodynamics

Our results observed a significantly lower MAP levels in ISO-treated rats as compared with the control groups ($P<0.05$) Following NAC treatment, the MAP levels all returned to those observed in the control group Thus, these results indicate that NAC significantly improved cardiac function.

4.2 Changes of T-wave voltage:

When compared to the control group, ISO caused a notable rise in T wave voltage ($P<0.05$). When NAC was administered with ISO, the T wave voltage increased although it was still noticeably higher than in the control and NAC groups.

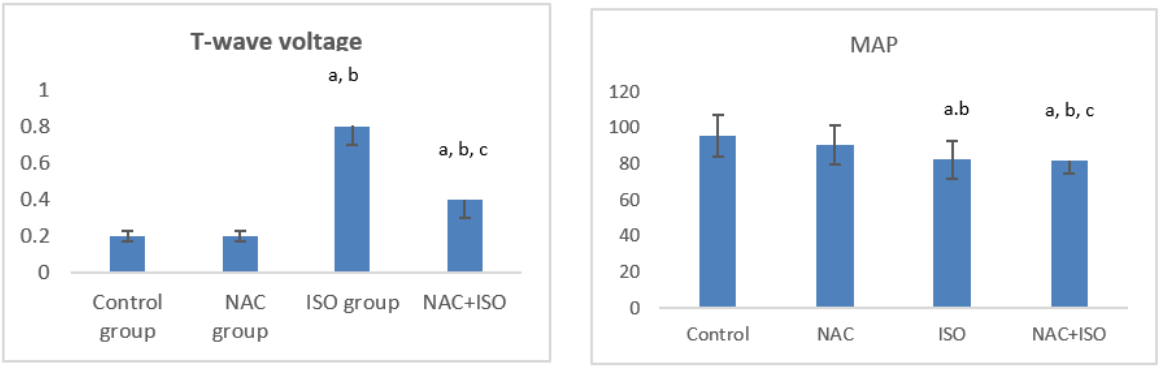


Figure (1): NAC; N-acetylcysteine, ISO; isopropanol, MAP; mean arterial blood pressure

Data are represented as Mean ± SD., n=8. P , < 0.05 is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method. a: P < 0.05 vs. Control group; b: P < 0.05vs. NAC group. c: p < 0.05 ISO group. a: significant vs control group, b: significant vs NAC group, c significant vs NAC+ISO group.

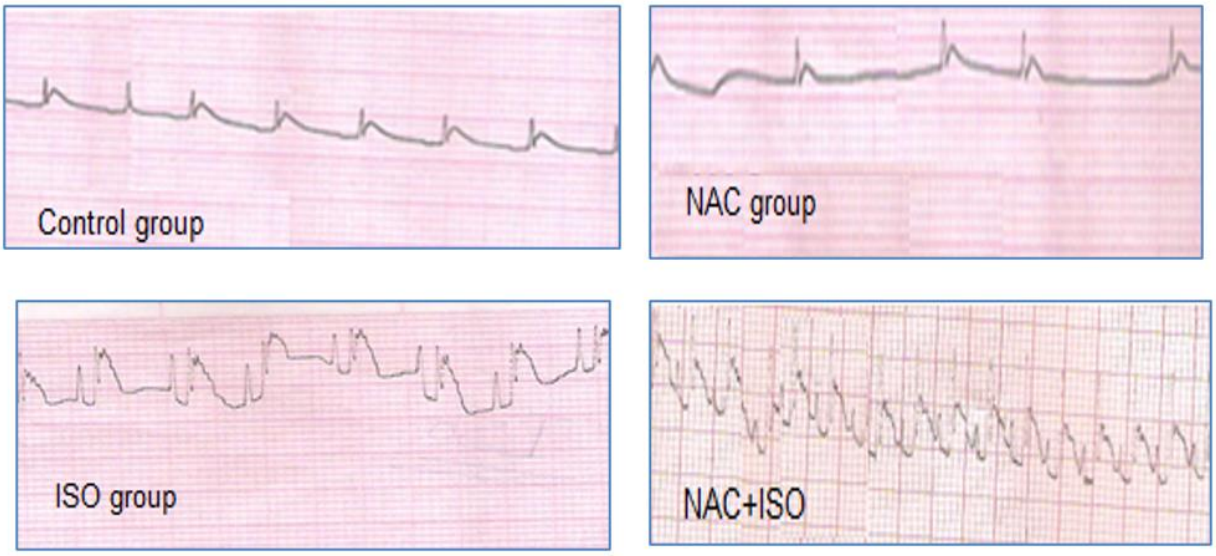


Figure (2): ECG recording in different groups detect abnormal T waves in ISO-induced myocardial infarction

4.3. Assessment of serum CK-MB and LDH: There was no significant change in CK-MB level, and LDH level in group II (NAC group) when compared with control group. On the other hand, there was dramatic increase in CK-MB level, LDH and T wave voltage in ISO group compared to control group. Both the LDH level and the CK-MB level had significantly increased in NAC+ISO group in comparison to control group.

4.4. Evaluation of TNF-α and IL-6

Inflammatory markers TNF- α and IL-6 were assessed and the results showed no change in NAC treated rats compared to control rats. While ISO treated animals showed remarkable significant increase in TNF- α and IL-6 compared to control and NAC groups. Combination of NAC with ISO in group IV caused significant decrease in TNF- α and IL-6 compared to ISO-treated animals.

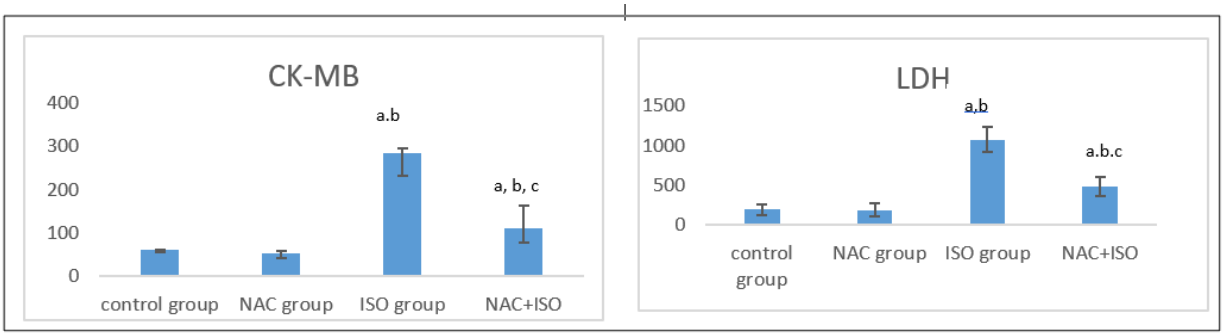
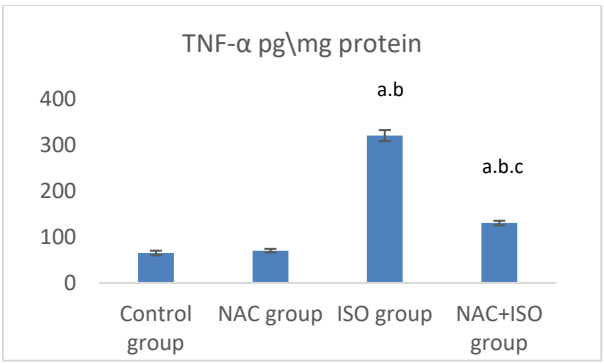
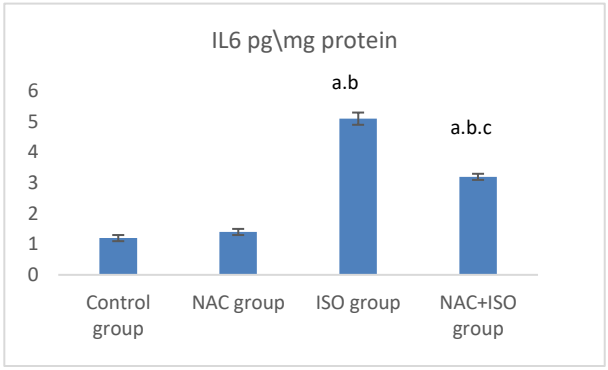
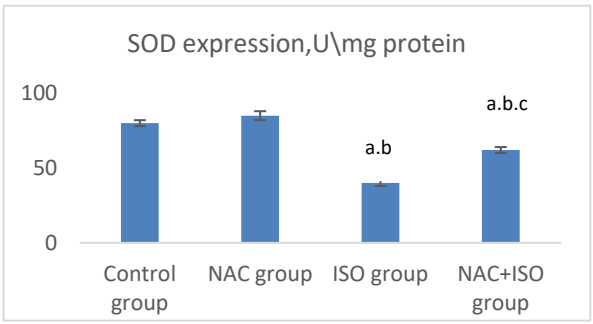


Figure (3): NAC: N-acetylcysteine, ISO; isopropanol, CK-MB: creatine kinase-MB, LDH: Lactate dehydrogenase, Data are represented as Mean ± SD., n=8. P < 0.05 is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method. a: P < 0.05 vs. Control group; b: P < 0.05vs. NAC group. c: p < 0.05 ISO group.







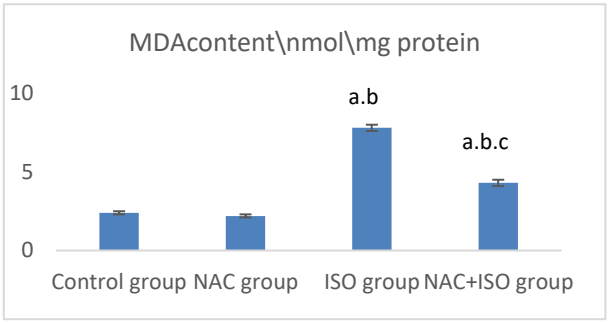


Figure (4): NAC: N-acetylcysteine, ISO; isopropanol, IL-6; Interleukin 6, TNF-α: Tumor necrosis factor alpha, SOD; superoxide dismutase, MDA: malonaldehyde

Data are represented as Mean ± SD., n=8. $P < 0.05$ is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method. a: $P < 0.05$ vs. Control group; b: $P < 0.05$vs. NAC group. c: $p < 0.05$ ISO group.

4.5. Measurement of antioxidant parameters:

SOD level was reduced in ISO-induced acute MI, the activity of SOD was found to be improved in the groups that received NAC with ISO, revealing antioxidant potential of NAC. One of the most commonly detected indicators of oxidative stress is malondialdehyde (MDA). It was significantly elevated in rats given ISO, a sign of acute oxidative stress. MDA levels were much lower in animals given NAC with ISO.

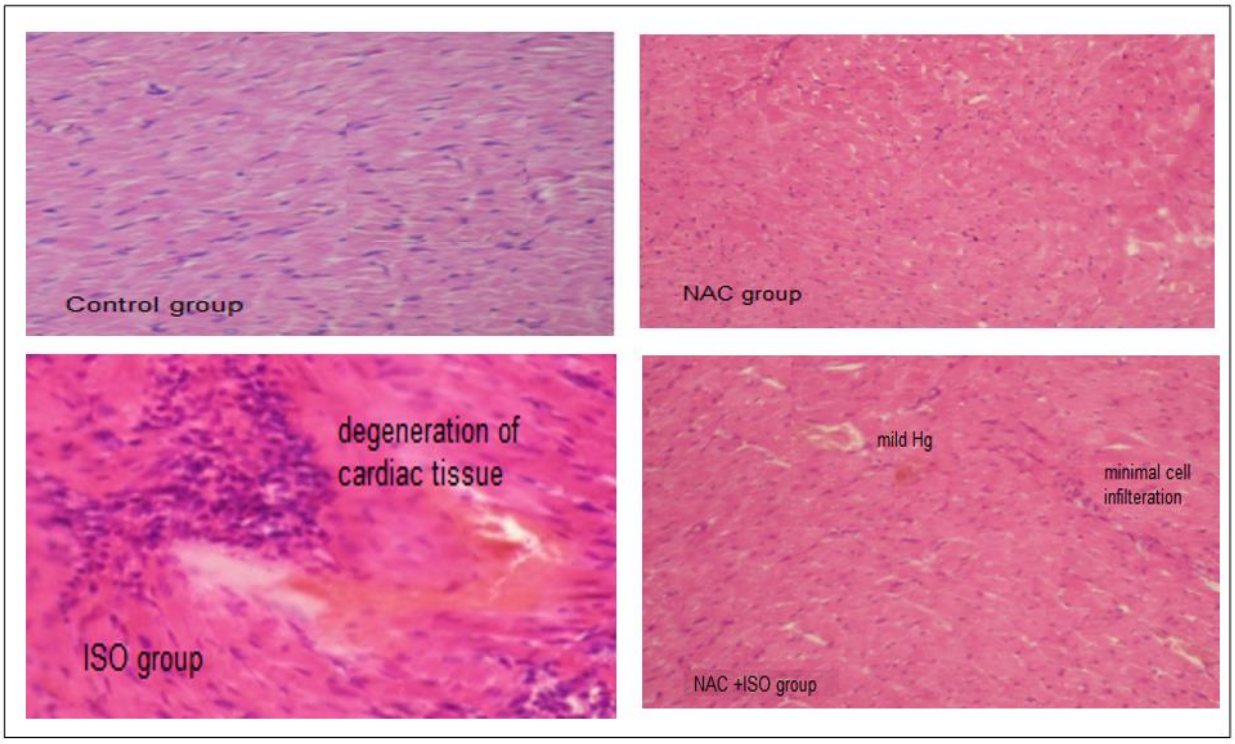


Figure (5): A photomicrograph of cardiac biopsy in all studied groups

Discussion:

Isoproterenol (ISO) a β-adrenergic agonist has been known to exert both inotropic and chronotropic cardiac effects that eventually lead to necrosis, cell infiltration. It has been found to induce significant damage to cardiac myocytes because of hypoxia, coronary hypotension, calcium overload, loss of energy reserve and excessive formation of radical oxidative species (20).

The cardiac injury induced by ISO was confirmed by biochemical estimations, ECG and histopathological examination. myocardial injury, possibly due to ischemia, hypoxia, or direct myocardial hypoxia from systemic hypotension. Up to 50% of the final cardiac ischemia area is caused by oxidative stress, which is the basic mechanism underlying this biophysical event. Relative heart weight is directly impacted by inflammation brought on by oxidative stress due to the buildup of water and protein, edematous intramuscular space, and the infiltration of inflammatory cells (21).

This study found that rats given ISO had lower levels of MAP. It can be explained by stimulation of β2-adrenergic receptors causes vasodilation. This contradicts other studies that found systolic blood pressure increased in all MI-induced groups. When peripheral vascular resistance is reduced, ISO, a non-selective β agonist, has a slight impact on α receptors, which results in favorable inotropic and chronotropic effects (22)

When comparing ISO-treated animals to control and NAC rats, the amplitude of the T wave is noticeably higher. This is consistent with earlier research that finds problems in ISO electrocardiograms. Acute myocardial ischemia patients showed an elevation in ST-segment. in a patient suffering from myocardial ischemia (23) or in isoproterenol-induced myocardial infarction in rat (24). When the cell membrane ruptures or becomes permeable, the cytosolic enzymes lactate dehydrogenase, glutamic oxalacetic transaminase, and creatine kinase—which are diagnostic indicators of cardiac tissue damage—leak out of the injured tissues into the bloodstream. Changes in the integrity and/or permeability of the plasma membrane are reflected in the quantity of these cellular enzymes present in plasma (25). Our findings, which were consistent with other publications demonstrated a substantial increase in the levels of creatine kinase and lactate dehydrogenase in the plasma of rats given isoproterenol (26). One important byproduct of lipid peroxidation is malondialdehyde;

a higher concentration of this compound may lead to a rise in free radical production and/or a reduction in antioxidant system activity (27). According to pervious researches (27) the production of free radical-mediated lipid peroxidation in rats under stressful conditions may be the cause of isoproterenol-induced myocardial infarction.

The study's findings suggested that NAC pretreatment could lessen the increase in malondialdehyde content brought on by isoproterenol.

The potential protective antioxidant benefits of NAC in a variety of illnesses have concentrated on where oxidative stress is involved (28). NAC is thought to function as an antioxidant through a number of mechanisms, firstly, neutralizing ROS by acting as a direct sulfhydryl donor. Second mechanism is modifying intracellular GSH and extracellular glutamate levels. The third mechanism is by acting as a reducing agent for protein disulfides (29). The primary mechanisms for the actions of NAC are considered to relate to its antioxidative effects via increasing intracellular glutathione (GSH) levels and its anti-inflammatory effect through suppressing tumor necrosis factor-alpha (TNF-α), interleukins (IL-6 and IL-1β) (30).

It has been demonstrated that NAC protects against myocardial dysfunction brought on by ISO. This defensive mechanism's antioxidant action is identified by a drop in SOD and MDA levels. Apart from its antioxidant qualities. NAC may directly scavenge ROS to prevent oxidative damage (31). NAC also prevents the action of pro-inflammatory transcription factors like AP-1 (activator protein 1) and NF-KB (nuclear factor kappa-light-chain-enhancer of activated B cells), which are well-known precursors of oxidative stress (32). NAC has been shown to decrease the release of pro-inflammatory cytokines, such as TNF-α and IL-6.

Author contributions:

Both authors contribute equally in the study design, collection of samples, data analysis, and manuscript writing.

Conflict of interest: The authors declare no competing interests. Funding: No funding was received for preparing this manuscript. Consent to publication: All authors give their consent to publish this article.

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