JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/44997
RESEARCH ARTICLE

Evaluation of Cardioprotective Activity of *Corchorus aestuans* L. Leaves

Carol P. Macwan[1*], B. N. Suhagia[1], Tejal G. Soni[1], Shahbaz S. Ahmed[1] and Jaya Patel[2]

[1]Faculty of Pharmacy, Dharmsinh Desai University, Nadiad - 387001, Gujarat, India; carolpalejkar@gmail.com
[2]Parul Institute of Pharmacy and Research, Parul University, Vadodara – 391760, Gujarat, India

Abstract

Background: The effectiveness of several medicinal herbs with antioxidant qualities in MI has been assessed and demonstrated. *Corchorus aestuans* L. is one such medicinal plant that has been shown to have hypocholesteremic and antioxidant properties. *C. aestuans* L. is also well-known for its bitter tonic, cardiotonic, and stomach relieving properties. **Aim:** In order to assess the impact of *C. aestuans* methanol extract against ISO-induced changes in myocardial enzymes, antioxidant status, and cardiac pathology in experimentally induced myocardial infarction, this study was conducted. **Methods:** Male wistar albino rats were used to create an Isoproterenol (ISO) induced Myocardial Infarction (MI) in order to study the possible cardiovascular preventative effects of the methanol extract of the medicinal plant *C. aestuans*. Six groups of eight animals each were created from the 180-250 g range of animals, and during the study, the animals in groups one (normal control) and two (model control) were given distilled water. Group three received ascorbic acid (250 mg/kg), while groups four to six received methanol extract of *C. aestuans* (MECA; 50, 100, and 150 mg/kg, respectively) once a day for 28 days. Animals in group's two to six received the first dosage of ISO (100 mg/kg, S.C.) on day 27 and the second dose 24 hours later. Rats were anesthetized using chloroform, then their hearts were isolated and serum was collected. Twelve hours following the second large dose, the rats anesthetized, killed, and samples of their hearts and serum were taken. After the heart was cleaned with ice-cold saline, it was weighed. A histology study was conducted on heart tissue, while biochemical markers were analyzed in serum and heart homogenate. **Results:** When isoproterenol was administered, the concentrations of CK-MB (Creatinine Kinase - Myocardial Band), LDH (Lactate Dehydrogenase), and NA^+ (Sodium) increased dramatically, but the concentrations of K+ (Potassium) and MDA (Malondialdehyde) dropped. The incidence of these alterations was dramatically decreased by ascorbic acid and MECA (Methanol extract of *C. aestuans*) treatment. Consequently, the cardioprotective effect can be explained by decreased levels of cardiac marker enzymes. **Conclusion:** MECA (50 mg / kg, 100 mg / kg and 150 mg /kg p.o. once for 28 days) attenuated serum enzymes and marker, increased tissue calcium and sodium levels, decreased potassium levels, and inhibited lipid peroxidation to significantly counteract the effect of experimentally induced MI.

Keywords: Cardioprotective, *Corchorus aestuans* L., Isoproterenol, Myocardial Infarction

1. Introduction

Myocardial Infarction (MI) is the term used to describe myocardial necrosis or death[1]. One of the primary causes of morbidity in Ischemic Heart Disease (IHD) is myocardial ischemia reperfusion damage, in which oxidative stress is a major factor[2]. IHD is a disorder where the myocardium's supply and demand for oxygen are out of balance, resulting myocardial hypoxia and the accumulation of waste products, most commonly from coronary atherosclerosis[3].

Oxygen derived free radicals or oxygen radicals are oxygen molecules with an odd number of electrons, making them chemically reactive and frequently triggering chain events that contribute to death of cell. There is convincing evidence that ischemic tissue creates these radicals[4].

*Author for correspondence

Article Received on: 16.07.2024 Revised on: 05.09.2024 Accepted on: 12.09.2024

The investigation of medicinal plants potential as remedies for a range of illnesses has gained more and more interest lately. Plant extracts seem to be a more cost-effective, inexpensive, and safe way to treat disease. Numerous physiologically active compounds, such as vitamins, flavonoids, and polyphenols, are responsible for the biological activity of these plants. Oxidative stress and hyperlipidaemia have also been connected to the development of myocardial infarction. Thus, antioxidant and hypolipidemic therapeutic approaches may be useful in the management of cardiovascular diseases, including ischemic heart problems[5] when claims on effectiveness of mega doses of vitamin C proved unfounded. The main role of antioxidants is to liquidate the uncontrolled production of reactive oxygen species (ROS,[6] particularly, reactive oxygen species play a cardinal role in the pathogenesis of oxidative myocardial damage with consequential cardiac malfunction. This review presents a comprehensive account of the present day knowledge regarding the oxygen free radicals involved in the genesis of ischemic heart disease, the mechanism(s.

Through methodical and scientific investigations, the effectiveness of several medicinal herbs with antioxidant qualities in MI has been assessed and demonstrated. *C. aestuans* L. is one such medicinal plant that has been shown to have hypocholesteremic and antioxidant properties. *C. aestuans* L. is also well-known for its bitter tonic, cardiotonic, and stomach relieving properties. It can also help treat pneumonia. Quercetin, a flavonoid found in *C. aestuans* L. leaves, has the ability to protect the myocardium from ischemia-reperfusion injury. This protective effect is partially mediated by quercetin's own antioxidant qualities and down regulation of nitric oxide production[7,8]. In order to assess the impact of *C. aestuans* L. methanol extract against ISO induced changes in myocardial enzymes, antioxidant status, and cardiac pathology in experimentally induced myocardial infarction, this study was conducted.

2. Materials and Methods

2.1 Identification and Collection of Plant Materials

In September, fresh and mature *C. aestuans* L. plants were collected from Nadiad, and their authenticity was verified by Dr. A.M. Patel from the Department of Botany at J and J College of Science. A herbarium specimen (DDU/FOP/16-17/F-1) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, DDU, Nadiad, for future reference.

2.2 Preparation of Methanol Extract of *C. aestuans* L., (MECA)

After collecting the plants, the leaves were separated and completely dried in the sun after being carefully cleaned with tap water. Using a grinder, the dried leaves were coarsely powdered. Hexane was used to defatted leaf powder for a full day. Following drying, 250 g of the defatted powder were collected and placed in 1000 ml volumetric flasks. A two to one powder to solvent ratio was used in multiple step maceration extraction, with methanol serving as the solvent. For seven days, the filtrate was collected every 24 hours. Using a rotary evaporator, all the filtrates were mixed and concentrated. Sunlight was used to dry the final concentration.

2.3 Drugs and Chemicals

The United States Sigma Chemical Co. in St. Louis, Missouri, provided the Isoproterenol (ISO) hydrochloride. Adenosine triphosphate, magnesium chloride, sodium chloride, and Trichloroacetic Acid (TCA) were acquired from Atul Co. The remaining chemicals used in the study were all analytical in nature.

2.4 Animals

One week before to the experiment, male wistar albino rats (weighing 180-250 gm and aged 12–14 weeks) were kept in cages with unlimited access to water and a conventional rat chow (diet) to enable them to acclimate to their new environment. The experimental protocol was approved by the institutional animal ethical committee (Protocol No. DDU/FOP/03/17), in accordance with the guidelines issued by the Ministry of Social Justice and Empowerment, Government

of India's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.5 Isoproterenol Induced Myocardial Infarction

Six sets of eight male wistar albino rats (180-250 g, born 12–14 weeks ago) were randomly assigned. Throughout the course of the trial, the animals in group one normal control and group two model control were given in distilled water. Group four to six administered MECA at three dose levels, *i.e.,* 50 mg/kg, 100 mg/kg and 150 mg/kg. Animals from Group II to Group VI received the first dose of ISO (100 mg/kg S.C.) on the 27th day, and then they received a second dose of ISO a day later. This caused MI in the animals[9].

2.6 Biochemical Estimations in Serum

Following the sacrifice of the animals, each one's blood was collected and allowed to stand at room temperature for one to two hours. The hearts were removed right away, weighed, blotted, and frozen at 80°C until needed. They were then washed with ice cold phosphate buffered saline (PH 7.4). Centrifugation was used to recover the serum for 15 minutes at 4000 rpm. It was then used to analyze several biochemical parameters (CKMB and LDH)[10].

2.7 Biochemical Estimations in Heart Homogenate

Using EIE (Electronic and Instrumentation Engineering) Instruments Pvt Ltd, 0603121, a known weight of cardiac tissue was homogenized in 5.0 ml of 0.1 M Tris-HCl buffer pH 7.4. For fifteen minutes, the homogenate was centrifuged at 10,000 rpm (Pasto Craft Industries Private Ltd., R4RV/FA). Several biochemical parameters, such as Na^+, K^+, and Ca^{+2}, SOD, and MDA, were calculated from the supernatant. Estimating biochemical parameters such as Na^+, K^+, Ca^{+2}, SOD, and MDA provides critical insights into cellular and physiological functions. Sodium (Na^+) and potassium (K^+) are essential electrolytes involved in maintaining fluid balance, nerve function, and muscle contractions, with particular importance in cardiac health. Calcium (Ca^{+2}) plays a vital role in muscle contractions, nerve signaling, and bone health, making its assessment crucial for understanding metabolic and cardiovascular conditions. Superoxide Dismutase (SOD) is an enzyme that protects cells from oxidative stress by neutralizing harmful superoxide radicals, indicating the body's antioxidant defense capability. Malondialdehyde (MDA), a marker of lipid peroxidation, helps evaluate the extent of oxidative damage in cells, reflecting the impact of oxidative stress and its association with various diseases and aging[10].

2.8 Preparation of Heart Homogenate

After quickly removing the cardiac tissue and washing it in ice-cold normal saline, the complete heart was weighed. Grind and homogenize a whole heart in 25.0 ml of phosphate buffer (pH 7.5) with 1 mM/l Na_2EDTA. After centrifuging the homogenate for four minutes at 4000 rpm, the supernatant was used to determine biochemical parameters, including Na^+, K^+, and Ca^{+2}, SOD (Superoxide Dismutase), and Malondialdehyde (MDA) is a marker of lipid peroxidation and oxidative stress that can indicate damage in the heart[10].

2.9 Histopathology of Heart

After being fixed in 10% formalin for 24 hours, the heart pieces which were 3-5 micrometers thick were rinsed under running water for another 24 hours. In order to eliminate absolute embedding, samples were first dehydrated in an auto technicon after cleared in benzene. This process involved passing the cleared samples over three cups filled with melted paraffin at 50°C before placing them in a cubical block of paraffin created using "L" molds. Following that, the slides were microscopically examined and stained with hematoxylin and eosin using a microtome[11].

2.10 Statistical Analysis

The findings were displayed as mean ± SEM. One way ANOVA was used for the statistical analysis of several biochemical data, and Turkey's multiple comparison test was then performed.

When $P<0.05$, the data were deemed statistically significant[12].

3. Results

3.1 MECA Effect on the Heart Weight of Normal and ISO Induced MI in Rats

Heart weight rise considerably (P<0.05) in model control animals as compared to normal control animals. Pre treatment with ascorbic acid (250 mg/kg) and MECA (50, 100, and 150 mg/kg) reduced this rise in heart weight in comparison to the model control animals (Figure 1).

3.2 Effect of MECA on Cardiac Marker Enzymes

MECA's effects on the cardiac marker enzymes LDH and CKMB were significantly (P<0.05) higher in model control animals than in normal control animals. Pre treatment with ascorbic acid (250 mg/kg) and MECA (50, 100, and 150 mg/kg) was considerably (P<0.05) beneficial in reducing this rise in CKMB and LDH activity (Figures 2 and 3).

3.3 Effect of MECA on Electrolytes

Figures 4, 5, and 6 show MECA effects on electrolytes like Na^+, K^+, and Ca^{+2}. In comparison to normal control animals, model control animals displayed a significant (P<0.05) decrease in K^+ levels and a significant (P<0.05) rise in Na^+ and Ca^{+2}. When compared to model control animals, pretreatment with ascorbic acid (250 mg/kg) and MECA (50, 100, and 150 mg/kg) significantly (P<0.05) avoided these alterations (Figure 4, 5 and 6).

3.4 Effect of MECA on Antioxidant Enzyme SOD and Lipid Peroxidation MDA Status

Model control animals showed significant (P<0.05) decrease in SOD activity as compared to normal control SOD levels. Ascorbic acid (250 mg/kg) treatment significantly (P<0.05) prevented this decrease in SOD levels as compared to model control animals. Similarly, MECA (50 mg/kg), MECA (100 mg/kg) and MECA (150 mg/kg) treated animals also significantly (P<0.05) prevented this decrease in SOD levels as compared to model control animals (Figure 7).

MDA levels were found to be significantly (P<0.05) higher in model control animal as compared to normal control animals. MDA levels were significantly (P<0.05) decreased in Ascorbic acid (250 mg/kg) treated animals as compared to model control animals. MDA levels

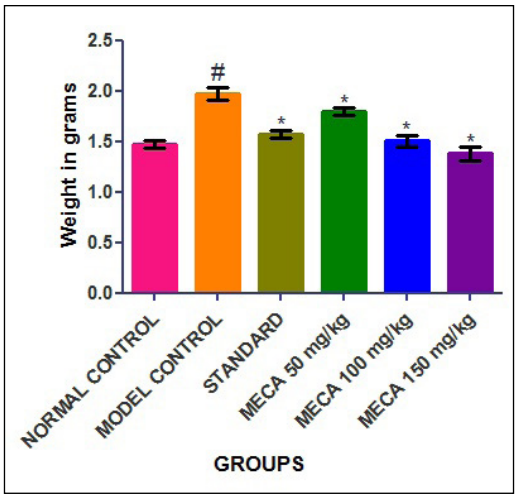


Figure 1. Effect of MECA on Heart weight.

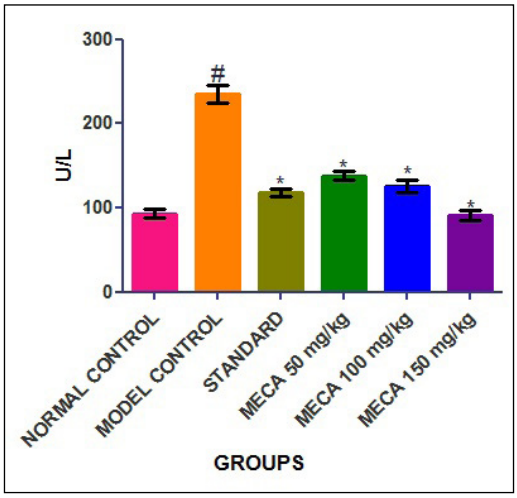


Figure 2. Effect of MECA on CKMB.

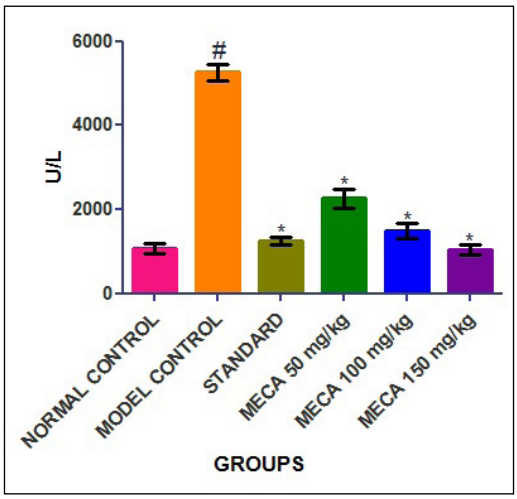


Figure 3. Effect of MECA on LDH.

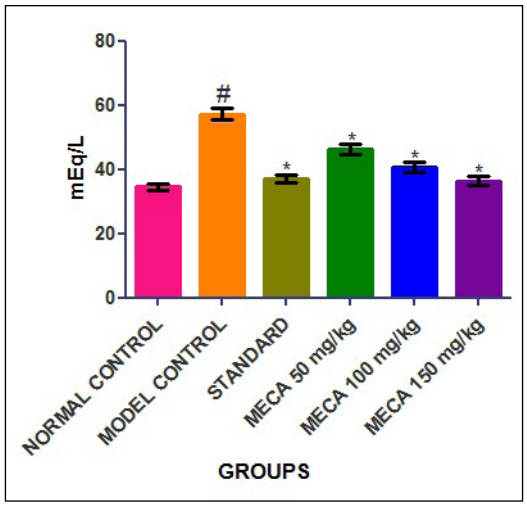


Figure 4. Effect of MECA on sodium levels.

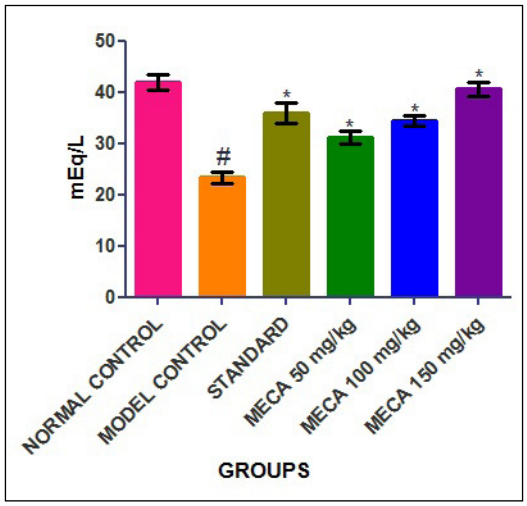


Figure 5. Effect of MECA on potassium levels.

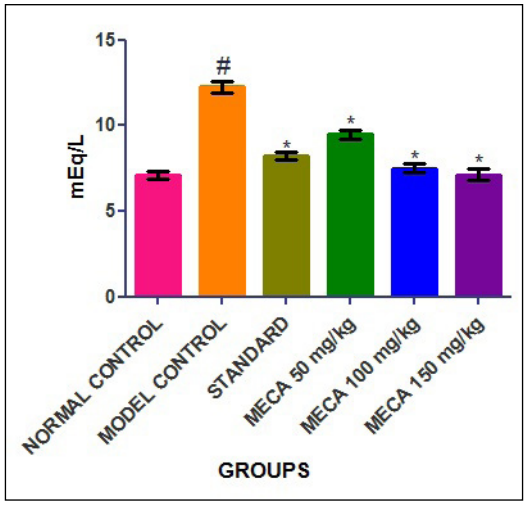


Figure 6. Effect of MECA on calcium levels.

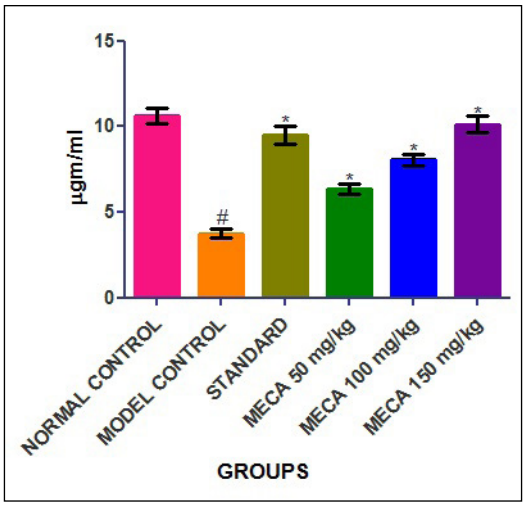


Figure 7. Effect of MECA on SOD levels.

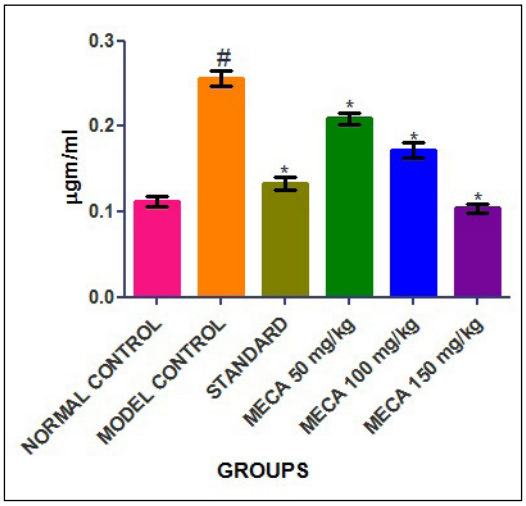


Figure 8. Effect of MECA on MDA level.

The values were expressed as mean ± SEM.
Significantly different from Normal control group at $P < 0.05$.
Significantly different from Model control group at $P < 0.05$.

were also significantly ($P<0.05$) decreased in MECA (50 mg/kg), MECA (100 mg/kg) and MECA (150 mg/kg) treated animals as compared to model control animals (Figure 8).

3.5 Effect of MECA on Histopathology

Figure 9 displays tissue sections of normal controls with normal myofibrillar structure, which includes striations, a branching appearance, and continuity with adjacent myofibrils (Grade 0). The cardiac tissue of rats

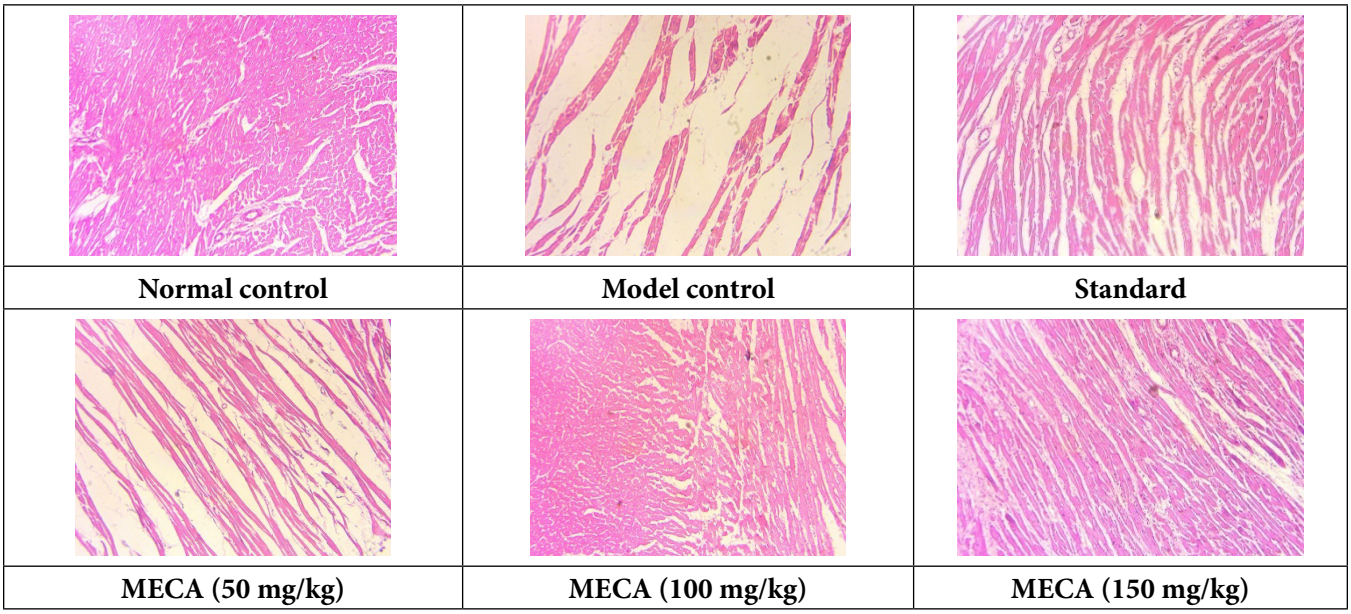


Figure 9. Photographs of hematoxyline and eosin-stained paraffin section of rat hearts.

treated with isoproterenol showed leucocytic infiltration (Grade 3) and diffuse and severe vascular degeneration of the myocardium bundles under microscopic examination. Grade 2 and Grade 1 myofibrillar tissue degradation and decreased leucocytic infiltration were the dose-dependent protective effects seen in rats administered 50 mg/kg, 100 mg/kg, and 150 mg/kg of MECA extract.

4. Discussion

The permanent damage and ensuing necrosis in wave front from the subendo-cardium to the subepicardium caused by a significant and protracted decrease in coronary perfusion is known as an acute myocardial infarction. A rise in heart weight could be brought about by a higher water content, oedematous intramuscular spaces, and widespread cardiac muscle fiber necrosis, which would be followed by inflammatory cells invading the injured tissues. Enzymes are the best indicators of tissue damage of all the macromolecules that leak from injured tissues. Elevated blood levels of these marker enzymes are suggestive for cellular injury, the degree of necrotic damage, and the disruption of the functional integrity from the cell membrane. There is a clear correlation between the quantity of necrotic cells in the cardiac tissue and the levels of these enzymes in the serum. Numerous studies have also documented increased LDH and CKMB levels in MI brought on by ISO. When compared to ISO model control animals, treatment with MECA dramatically reduced the levels of CKMB and LDH, indicating a reduction in the necrotic damage brought on by ISO.

Normal heart function, blood coagulation, and the preservation of cell membrane integrity all depend on calcium. Adenylate cyclase activity is elevated in ISO-induced MI, leading to an increase in cAMP production. Furthermore, the administration of ISO inhibits the glycolytic pathways in the heart muscles, which results in the breakdown of glycogen, the loss of pyridine nucleotides and ATP, and an increase in intracellular Ca^{+2}. This could be the cause of the higher Ca^{+2} content in the cardiac tissue seen in the study's ISO induced MI animals. The production of ROS (Reactive Oxygen Species) can be initiated by intracellular Ca^{+2} overload, which implies that both the generation of ROS and the Ca^{+2} surge may contribute to the contractile dysfunction of the ischemic myocardium.

It is well known that oxygen radicals have the ability to cause damage by spreading lipid peroxidation in the membrane of the heart cells. Lipid peroxide is a crucial pathogenic event in myocardial infarction and the accumulation of lipid peroxides suggests the disease's progression and consequences. Blood arteries are damaged by elevated lipid peroxide levels, which increases platelet adhesion and aggregation

to the damaged areas. The current study's findings demonstrated that, in comparison to normal control animals, the model control animals levels of lipid peroxides were much higher. The generation of superoxide radicals at the site of injury affects SOD (Superoxide radicles), which causes a decrease in activity and an accumulation of superoxide radicals that harms the myocardium. Oxidative stress causes an increase in the use of antioxidant enzymes such as SOD.

4. Conclusion

MECA (50 mg/kg, 100 mg/kg and 150 mg/kg p.o. once for 28 days) attenuated serum enzymes and marker, increased tissue calcium and sodium levels, decreased potassium levels, and inhibited lipid peroxidation to significantly counteract the effect of experimentally induced MI (ISO). Its chemical component, quercetin, which has antioxidant capacity, may be responsible for the protective impact; nevertheless, concrete proof of this mechanism needs to be established.

5. Acknowledgement

The authors would like to thank the Department of Science and Technology (DST), Government of Gujarat and the Gujarat Council on Science and Technology (GUJCOST). Without their grant, this research could not have been finished. The facilities at the institute are provided by the Faculty of Pharmacy at Dharmsinh Desai University, for which the authors are equally grateful.

6. References

1. Gilski DJ, Borkenhagen B. Risk evaluation in action for cardiovascular health. Crit Care Nurse. 2005; 25(1):26–37. https://doi.org/10.4037/ccn2005.25.1.26
2. Velasco-Loyden G, Perez-Martinez L, Vidrio-Gomez S, Perez-Carreon JI, Sanchez VC. Cancer chemoprevention by an adenosine derivative in a model of cirrhosis-hepatocellular carcinoma induced by diethylnitrosamine in rats. Tumor Biol. 2017; 39(2):1–12. https://doi.org/10.1177/1010428317691190
3. Hegstad AC, Ytrehus K, Myklebust R, Jorgensen L. Ultrastructural changes in the myocardial myocytic mitochondria: Crucial step in the development of oxygen radical-induced damage in isolated rat hearts?. Basic Res Cardiol. 1994; 89:128-138. https://doi.org/10.1007/bf00788732
4. Cai Q, Rahn RO, Zhang R. Dietary flavonoids, quercetin, luteolin and genistein, reduce oxidative DNA damage and lipid peroxidation and quench free radicals. Cancer Lett. 1997; 119(1):99–107. https://doi.org/10.1016/s0304-3835(97)00261-9
5. Ginter E, Simko V, Panakova V. Antioxidants in health and disease. Bratislava Med J. 2014; 115(10):603–6.
6. Bandyopadhyay D, Chattopadhyay A, Ghosh G, Datta A. Oxidative stress-induced ischemic heart disease: Protection by antioxidants. Curr Med Chem. 2005; 11(3):369–387. https://doi.org/10.2174/0929867043456016
7. Satyavati GV, RainaMK, Sharma M. Medicinal Plants of India. Indian Council of Medical Research. 1976; 1-2:278–281.
8. Khan MSY, Bano S, Javed K MM. A comprehensive review on the chemistry and pharmacology of Corchorus species-a source of cardiac glycosides, triterpenoids, ionones, flavonoids, coumarins, steroids and some other compounds. J Sci Ind Res. 2006; 65:283–298.
9. Wang J, Bo H, Meng X, Wu Y, Bao Y, Li Y. A simple and fast experimental model of myocardial infarction in the mouse. Texas Hear Inst J. 2006; 33(3):290-293. PMID: 17041683
10. Rosalki BS. A simple colorimetric method for the determination of serum alpha-hydroxybutyric dehydrogenase activity. J Clin Pathol. 1962; 15566–568). https://doi.org/10.1136/jcp.15.6.566
11. Mohanty I, Singh AD, Dinda A, Joshi S, Talwar KK, Gupta SK. Protective effects of Curcuma longa on ischemia-reperfusion induced myocardial injuries and their mechanisms. Life Sci. Elsevier. 2004; 75(14):1701–1711.
12. Bolton S, Bon C. Pharmaceutical statistics: Practical and clinical applications. 2003; 4:1-756.