e-ISSN: 2248-9126
Vol 7|Issue 1| 2017 |8-13. Print ISSN: 2248-9118

Indian Journal of Pharmaceutical Science & Research

www.ijpsrjournal.com

CARDIOPROTECTIVE EFFECTS OF RUTIN VIA ALTERATION IN CK-MB AND TNF-A LEVELS COUPLED WITH ANTIOXIDANT EFFECT IN ISOPROTERENOL INDUCED MYOCARDIAL NECROSIS IN RATS

Deepali Soni*, Suresh Kumar Gupta

Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), University of Delhi, Pushp Vihar Sec-3, New Delhi 110017, India.

ABSTRACT

To study the cardioprotective effect of Rutin in Isoproterenol induced Myocardial necrosis in rats. Cardiovascular disease (CVDs) is the single largest cause of death worldwide and now become the leading cause of mortality in India with a death rate of 272 per 100 000 population in India which is higher than the global average of 235 per 100 000 population. But unfortunately, its pharmacotherapy is still incomplete. Rutin is a naturally occurring flavonoid having a long history of use in nutritional supplements for its action against oxidative stress, inflammation, and hyperglycemia but remains unexplored for its role in MI. This study was conducted to address this lacuna. In this study rats were treated with Rutin (10 mg/kg, per orally [p.o.]), Lisinopril (10 mg/kg, p.o.) and isoproterenol control group (normal saline) for thirty days, with concurrent subcutaneous administration of isoproterenol (ISO, 85 mg/kg) at 24 h interval on last two consecutive days whereas control group was administered with vehicle only. Isoproterenol significantly attenuated cardiac antioxidant enzymes and increased plasma cardiac injury biomarkers Creatine kinase MB. Isoproterenol also altered cardiac activity as evidenced by a decrease in blood pressure and increase in heart rate. The damage due to oxidative stress was revealed by histopathology alterations such as myocyte necrosis, myofibrillar degeneration and pyknotic nucleus. However, pre-treatment with Rutin demonstrated restoration of hemodynamic alterations along with significant preservation of antioxidants and myocyte injury specific marker enzymes. Furthermore, the protective effect of Rutin was reconfirmed by the histopathological salvage of myocardium. Present study demonstrated the cardioprotective potential of Rutin, as evidenced by favourable improvement in ISO-induced hemodynamic, plasma cardiac biomarkers and tissue antioxidant status along with maintenance of the integrity of myocardium. Rutin treatment significantly ameliorated these above-mentioned changes with a decrease in blood reduced expression of TNF-α ($p<0.001$) and CK-MB ($p<0.05$) compared to ISO rats. Also, provided significant protection against oxidative stress ($p<0.001$) in the myocardium, prevented degenerative changes in the heart and improved Blood pressure ($p<0.01$), and Heart rate ($p<0.05$) compared to ISO rats.Theheart-to-body weight ratio(Hw/Bw) was significantly reduced in Rutin treatment group compared to isoproterenol control rats($P<0.05$). Our data suggest the possible cardioprotective effects of Rutin in Isoproterenol-induced myocardial necrosis in rats, and that protection might be in part due to the attenuation of oxidative stress and moderate increment in antioxidant reserves.

Keywords: Cardioprotective, Flavonoids, Isoproterenol, Myocardial infarction, Rutin.

INTRODUCTION

Cardiovascular disease (CVDs) is a dreadful disease worldwide and now become the leading cause of mortality in India. A quarter of the total mortality is attributable to CVDs. Ischemic heart disease, myocardial infarction and stroke are the predominant causes and are responsible for >80% of CVDs deaths. Some aspects of the

Corresponding Author:-**Deepali Soni** Email: deepalianjali11@gmail.com

CVD epidemic in India are particular causes of concern, including its accelerated buildup, the early age of disease onset in the population, and the high case fatality rate [1]. In India, the epidemiological transition from predominantly infectious disease conditions to non-communicable diseases has occurred over a rather brief period of time. Premature mortality in terms of years of life lost because of CVD in India increased by 59%, from 23.2 million (1990) to 37 million (2010). Despite wide heterogeneity in the prevalence of cardiovascular risk factors across different regions, CVD has emerged as the leading cause of death in all parts of India, including poorer states and rural areas [2]. Overall, cardiovascular diseases (CVDs) accounted for around one-fourth of all deaths in India in 2008. CVDs are expected to be the fastest growing chronic illnesses between 2005 and 2015, growing at 9.2% annually. A more worrying fact is that the incidences of CVDs have gone up significantly for people between the ages 25 and 69 to 24.8%, which means losing more productive people to these diseases.

Myocardial infarction (MI) or acute myocardial infarction (AMI), commonly known as a heart attack, occurs when blood flow stops to a part of the heart causing damage to the heart muscle. The most common symptom is chest pain or discomfort which may travel into the shoulder, arm, back, neck, or jaw. Often it is in the center or left side of the chest and lasts for more than a few minutes. Most MIs occur due to coronary artery disease. Risk factors include high blood pressure, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor diet, and excessive alcohol intake, among others [2]. Oxidative stress, in the heart, is a major contributing factor in the development and progression of Myocardial infarction and long-term excessive reactive oxygen species (ROS) generation leads to cellular dysfunction. An imbalance between increased ROS generation and impaired antioxidant defences contributes to oxidative stress in hearts [3]. Inflammation plays an important role in the pathogenesis of cardiovascular diseases which ultimately leading to fibrosis causing increasein the size of heart and weight as reported in experimental models.

Similarly, In the present study, a significant increase in index of cardiac hypertrophy in ISO-induced Myocardial necrotic rat hearts compared to normal rat hearts was observed. However, Rutin-treated rat hearts showed significant improvement in index of cardiac hypertrophy and cardiac myocardium histopathology. The results of the present study demonstrate the myocardial salvaging effects of Rutin in the setting of Myocardial necrosis. Beneficial effects are attributed to the antioxidant and anti-inflammatory effects of Rutin along with a protective effect on Hemodynamic parameters and pathology. Thus, cardioprotective effects of Rutin are mediated via alteration in TNF-α, and CK-MB levels coupled with antioxidant effect in Isoproterenol-induced myocardial necrosis rats. The results clearly emphasize the potential of Rutin as adjunctive therapy in MI along with conventional drugs.

MATERIALS AND METHODS

Chemicals procurement

Rutin (purity-98.5%) was obtained from Loba chemicals, India and isoproterenol hydrochloride was purchased from TCI chemicals, India. Lisinopril was purchased from TCI Chemical, India. The biochemical kits Creatine kinase MB and Tumor Necrosis Factor-alpha (CK-MB and TNF-α) for cardiac biomarker and another Enzyme-linked immunosorbent assay (ELISA) kits were purchased from Krishgen Biosystems, India. All other chemicals used in the study were of analytical grade purchased from Merck Chemicals India unless otherwise mentioned.

Animals

Male Wistar rats with body weight: “180–200g”; age “6 weeks”, were obtained from Central Animal House, DIPSAR, New Delhi. Ethical clearance for handling the animals was obtained from the Institutional All experimental procedures were performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. Animals were housed under controlled laboratory conditions at 21 ±2^{o}C, relative humidity 50±15 %, and on a12-h light/dark cycle. The rats were fed a standard rat chowdiet and filtered drinking water ad libitum.

Experimental design

Wistar albino rats were randomly assigned into 4 groups each containing 8 animals. In all groups, theanimal was treated with vehicle or drug per orally (2ml/kg, 1% Sodium CMC suspension in normal saline) for 7 days. In MI rats, ISO was in distilled water and administered subcutaneously (s.c.) for last 2 consecutive days of treatment. The treatment includes normal control group (normal saline), ISO control (normal saline and ISO), lisino 10 + ISO (Lisinopril 10mg/kg and ISO), Rutin 10 + ISO (Rutin10mg/kg and ISO).

Induction of myocardial injury

ISO (85 mg/kg, s.c.) in a normal saline solution injected into rats for last 2 consecutive days at an interval of 24 h (i.e., on 30th and 31st day of treatment) to induce experimental myocardial infarction having a similar physiological alteration to a human being [4-7].

Experimental Studies

Hemodynamic Measurement

After 15 days training of BP measurement, rats were undertaken for recording BP with minimal stress and restraint. A hemodynamic measurement such as SAP, MAP, DAP, and HR were measured by using non-invasive

rat's tail cuff method using CODA NIBP recorder (KENT Scientific Instruments, USA) on 31st day after 24 h of second ISO injection [5, 8].

Cardiac biomarker estimated in plasma

At the end of experimental period (after 24 h of second ISO injection or last drug/vehicle treatment), blood was collected from the retro-orbital plexus of ether-anesthetized rat treatment using K2EDTA as an anticoagulant in centrifuged tube. It was centrifuged for 15 minutes at 1000g at 2-8˚ C within 30 minutes of collection. The clear plasma was separated out and stored at -20˚ C until determination of myocardial injury markers such as CK-MB [8, 9] by spectrophotometrically as per methods in specific kits.

Heart weight to body weight Index

The rats were euthanised by carbon dioxide asphyxiation and decapitated after 31st day and the heart was rinsed with ice-cold normal saline and blotted by filter paper, and weighed. The ratio of heart weight to body weight (HW/BW) was calculated [10, 11].

Antioxidant parameter estimation

On the31st day of treatment, rats were decapitated followed by excision of heart for biochemical estimation. Hearts were stored at -80^{o}C which were brought to room temperature and weighed. The homogenate was centrifuged at 7000 rpm for 15 minutes and the supernatant was used for the estimation of the Total Antioxidant Capacity assay.

Histopathological Examination

Rat heart tissues were washed with normal saline and then kept in 10% buffered neutral formalin solution (phosphate buffered; pH 7.4). The fixed tissues were embedded in paraffin and serial sections were cut. 4 µm thin sections were cut from the left ventricle and stained with haematoxylin and eosin (H&E). The sections were examined under a light microscope at x40 magnification to investigate the histological changes in the necrotic heart in the presence and absence of Rutin treatment [10, 11]. Histological evaluation was performed by an independent clinical pathologist in a blinded fashion.

Statistical Analysis

All data are expressed as mean and standard error of mean (SEM) were calculated for all variables in each group. One-way analysis of variance (ANOVA) was applied for statistical analysis with post hoc analysis using Dunnett'st-test. Ap-value< 0.05 was considered as statistical significant.

RESULTS

Mortality rate during the study was 20% in the ISO-treated group only.

Effect of Rutin on hemodynamic parameters

ISO administration induced significantly decreased SAP, MAP and DAP and raised HR in ISO group (75±3.255, 65.2±3.006, 60.8±2.887) as compared to control group (126.6±1.913, 114±1.140, 107.4±1.777). Lisino10mg+ISO groups (115.4±0.748, 108.8±0.734, 102. ±1.881) significantly restored hemodynamic parameter as compared to ISO group (P<0.01). Rutin 10mg+ISO group (105.6±2.063, 93.6±1.029, 75±3.93) significantly restored BP parameters except HR as compared to control group (Fig.1).

Effect of Rutin on plasma cardiac biomarker

The significant raised plasma biomarkers were observed in ISO group (17.346±0.358) as compared to control group (7.3±0.221) (P<0.01). Lisinopril 10mg+ISO (13.51±0.095) was found significant as compared to control group. Rutin 10mg+ISO groups (11.18±0.554) significantly restored (p<0.05) plasma cardiac biomarkers as compared to ISO group (Fig. 2).

Effect of Rutin on theantioxidant parameter

Heart of control group animals showed high Total Anti-oxidant antioxidant capacity. A significant (p<0.001) fall was observed in TAC in Isoproterenol control group (229.2±8.771mmol) as compared to control group (347.2±9.840mmol). However, treatment with Rutin and Lisinopril significantly (p<0.05) protected the decrease in TAC (297.2±27.550mmol) and (292.6±8.570mmol) as compared to Isoproterenol animals (Fig. 3).

Effect of Rutin on hypertrophy index of heart weight

ISO group (3.903±1.129) significantly increased in HW and HW/BW ratio compared to control group (3.769±1.09). Lisino10mg+ISO groups (3.348±1.116) significantly (P<0.05) attenuated HW/BW ratio as compared to ISO group. Rutin 10mg+ISO group (3.836±1.112) showed a significant alteration in heart weight (Fig. 4).

Effect of Rutin on Tumor Necrosis Factor-alpha (TNF-α) in Heart

A significant rise (p<0.001) was observed in the expression of TNF-α levels in Heart of Isoproterenol group (3262±76.166) as compared to normal control group (471.66±136.536). However, Rutin (2277.66±227.166) and Lisinopril (1747.33±47.792) showed significant protection (p<0.001) against the rise in inflammatory expression of TNF-α in the treatment group as compared to the Isoproterenol group (Fig. 5).

Effect of Rutin on heart histopathology

Histological analysis of myocardium showed disorientation of myofibril, pyknotic nucleus, necrosis, and extravagation of blood cells in ISO group. Lisino10mg+ISO groups showed normal myocardium as

compared to control group. Rutin 10mg+ISO group demonstrated marked improvement with less disorientation of myofibril, leukocytes infiltration and absence of pyknotic nucleus as compared with ISO group (Fig. 6).

Fig 1

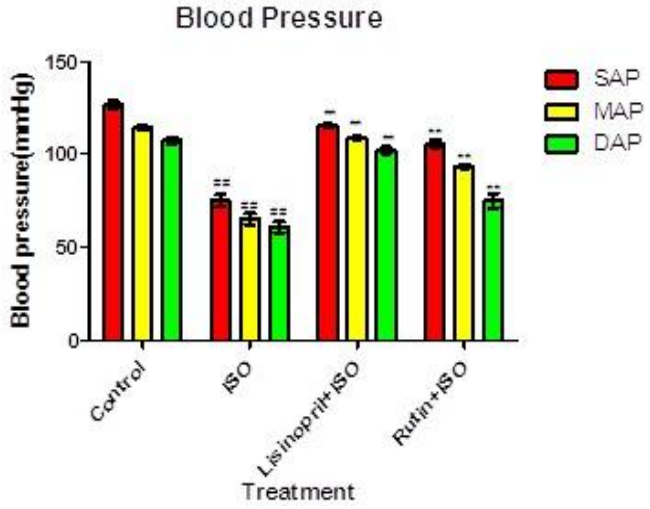


Fig 2

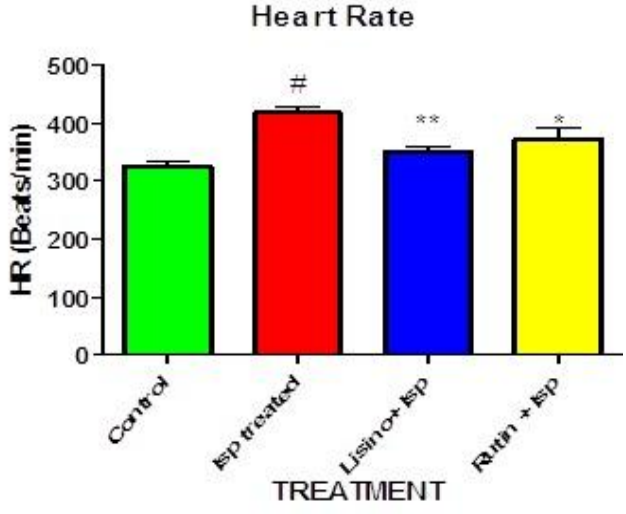


Fig 3

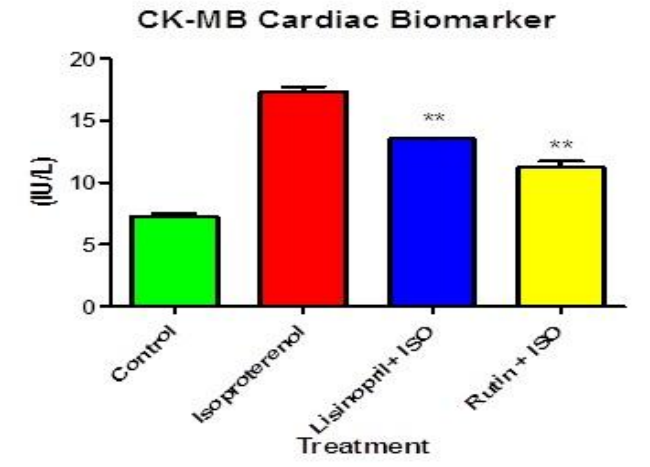


Fig 4

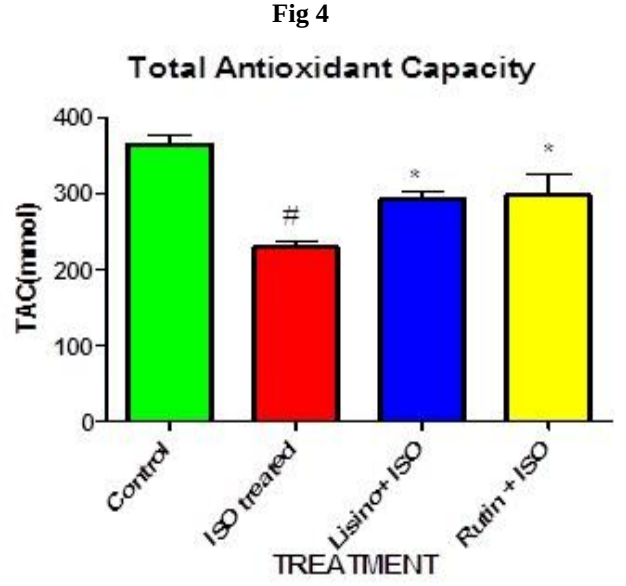


Fig 5

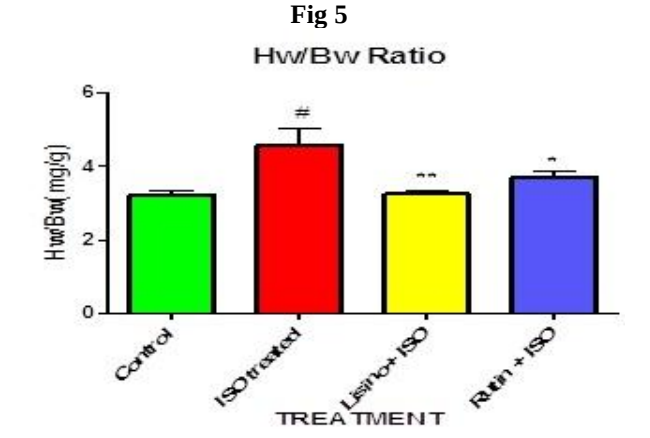


Fig 6

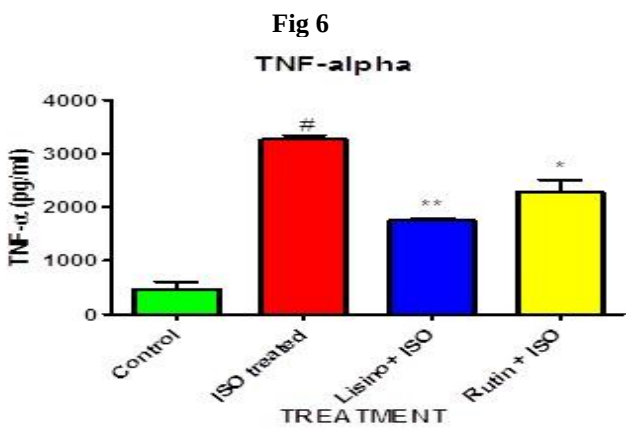
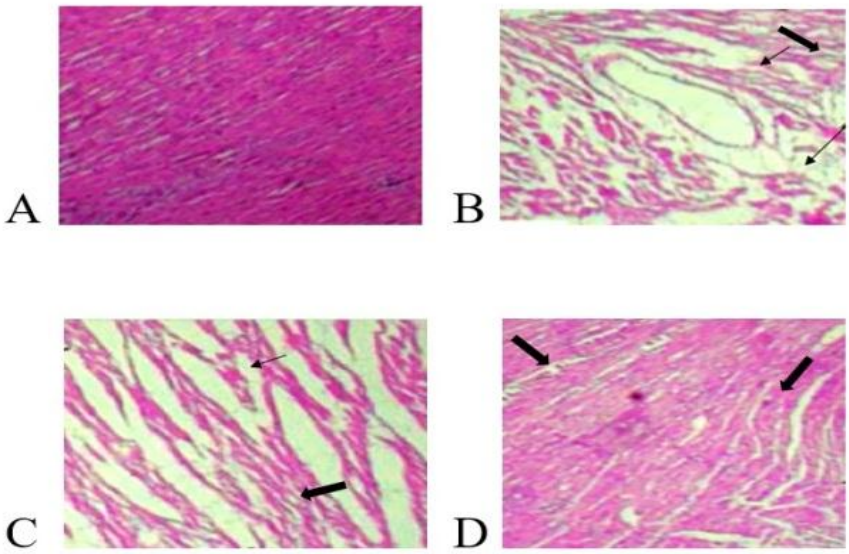


Fig 6. Histopathology of A. Control B. Isoproterenol C. Rutin D. Lisinopril ( ) showed disorientation of myofibrils;( ) Showed pyknotic nucleus, on ISO induced myocardial necrosis in rats.



DISCUSSION

The results of the present study demonstrate the myocardial salvaging effects of Rutin in the setting of myocardial necrosis. Beneficial effects are attributed to the antioxidant and anti-inflammatory effects of Rutin along with a protective effect on cardiac electrophysiology and pathology. Thus, cardioprotective effects of Rutin are mediated via alteration in CK-MB, and TNF-α levels coupled with antioxidant effect in Isoproterenol-induced myocardial necrosis in rats. The results clearly emphasise the potential of Rutin as adjunctive therapy in Myocardial infarction along with conventional therapy.

CONCLUSION

The data from present study strongly suggest that there is a possible cardioprotective action of Rutin in ISO-induced myocardial necrosis in rats.Rutin not only attenuates oxidative stress but a slight increment in antioxidant enzymes is also seen. Further studies are warranted to explore molecular mechanisms that contribute to the cardioprotective effects of Rutin.

DISCLOSURE OF INTERESTS

The authors declare that they have no conflicts of interest to disclose.

ACKNOWLEDGEMENT

We are grateful to the Department of Pharmacology, DIPSAR, for providing the research facilities in the present investigation.

REFERENCE

1. Prabhakaran D, Jeemon P, Roy A. Cardiovascular Diseases in India. *Circulation*, 133, 2016, 1605-1620.
2. Mendis S, Puska P, Norrving B. Global Atlas on Cardiovascular Disease Prevention and Control. World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization, 2011, 3-18.
3. Remmen HV, Williams MD, Guo Z. Knockout mice heterozygous for Sod2 show alterations in cardiac mitochondrial function and apoptosis. *Am J Physiol Heart CircPhysiol*, 281, 2001, H1422–H1432.
4. Mohanty IR, Arya DS, Dinda A, Gupta SK. Comparative cardioprotective effects and mechanisms of vitamin E and lisinopril against ischemic reperfusion induced cardiac toxicity. *Environ ToxicolPharmacol*, 35(2), 2013, 207-17.
5. Goyal S, Arora S, Mittal R. Myocardial salvaging effect of telmisartan in experimental model of myocardial infarction. *Eur J Pharmacol*., 619, 2009, 75-84.
6. Wang YF, Wang XY, Ren Z. Phyllaemblicin B inhibits Coxsackie virus B3 induced apoptosis and myocarditis. *Antivir Res.*, 84(2), 2009, 50-8.
7. Yeager JC, Iams SG. The hemodynamics of isoproterenol-induced cardiac failure in the rat. *Cir Shock*, 8, 1981, 151-63.
8. Yadav CH, Akhtar M, Khanam R. Isoproterenol toxicity induced ECG alterations in wistar rats: role of histamine H3 receptor agonist imetit. *Int J Pharmacy Pharmaceu Sci.*, 6(5), 2014, 654-8.
9. Rosalki SB. An improved procedure for serum creatine phosphokinase determination. *J Lab Clin Med.* 69, 1976, 696.

10. Bandyopadhyay D, Chattopadhyay A, Ghosh G, Datta AG. Oxidative stress-induced ischemic heart disease: protection by antioxidant. *Curr Med Chem*., 11(3), 2004, 369-87.
11. Saklani R, Gupta SK, Mohanty IR. *Mol Cell Bioche*, 2009, 2767-1.
12. Ojha S, Golechha M, Kumari S. Protective effect of *Emblicaofficinalis* (amla) on isoproterenol-induced cardiotoxicity in rats. *Toxicol Indus Health*, 28, 2012, 399-403.