Original Article

An Evaluation of Antioxidant Potential of Flavonoid Eriodictyol in Isoproterenol-Induced Myocardial Infarction in Rats

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ABSTRACT

Aim: The study was designed to evaluate the antioxidant potential of eriodictyol on lipid peroxidation caused due to isoproterenol induced myocardial infarction in albino male wistar rats. **Methods:** Myocardial Infarction was induced by subcutaneous injection of isoproterenol, 85 mg/Kg body weight after a pretreatment period of 45 days with eriodictyol in various doses 50 mg, 100 mg and 200 mg per Kg body weight through intragastric intubation. The standard drug metoprolol succinate was administered orally at a dose of 2.5 mg per Kg body weight for 45 days followed by ISO induced myocardial infarction. **Results:** The study showed significant increase in the levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes both in plasma and heart tissue with considerable decrease in the levels of enzymatic and non-enzymatic antioxidants in ISO induced myocardial infracted rats. The oral administration of eriodictyol showed significant decrease in lipid peroxidation products and with significant increase in the levels of antioxidants. The cardio protective role of eriodictyol was further assessed by histopathological studies. **Conclusion:** The results indicate that the oral administration of metoprolol succinate also modulates the lipid peroxidation and the antioxidant status in the ISO induced myocardial infracted rats.

Key words: Myocardial infarction (MI), Isoproterenol (ISO), Eriodictyol (E), Lipid peroxidation, Antioxidants, Acute Myocardial Infarction (AMI).

INTRODUCTION

Cardiovascular diseases is the leading cause of death both in men and women affecting 7 million people every year worldwide.[1] Myocardial infarction is the most important form of Ischemic Heart Disease (IHD), in which ischemia causes the death of heart muscles, characterized by necrotic cell death because of the breakdown of cellular energy metabolism.[2] Isoproterenol is a synthetic catecholamine and β1 -adrenergic agonist that induces severe stress in the cardiac muscles leading to the development of MI.[3] Myocardial necrosis induced by ISO can be accounted to adenyl cyclase activation of Ca and Na channels causing an exaggerated calcium inflow, coupled to excess contraction of the cardiac muscles leading to energy consumption and cellular death.[4] Oxygen radicals have been associated with a variety of pathological process such as ischemic injury to the heart , respiratory distress syndrome in adults, atherosclerosis, aging , ethanol induced liver injury and cancer.[5] Oxidation of the catecholamines can increase the rate of production of free radicals. Oxidative corrosion of membrane polyunsaturated fatty acids by these free radicals within the myocardium is associated with increased levels of thiobarbituric acid reactive substances (TBARS), lipid radicals (L); lipid peroxy radical (LOO) and lipid hydroperoxides (LOOH) as observed during the initial stages of MI influenced by oxidative stress.[6]

Eriodictyol is 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-2,3-dihydro-4H-chromen-

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4-one (Figure 1) is a flavanone extracted from *Yerba Santa* (*Eriodictyon californicum*)[7] and the twigs of *Millettia duchesnei*.[8] Eriodictyol present in lemon fruit, is found to suppress oxidative stress in serum, liver, and kidney of diabetic rats in combination with hesperidin.[9] Experiments shows that eriodictyol has antioxidant properties[10,11] anti-parasitic[12] and even anticancer properties .[13] Metoprolol succinate (Figure 2) is a β 1 selective adrenoceptor blocking agent are hemodynamically and energetically beneficial in the treatment of myocardial failure.[14] Metoprolol also reduces the forces of contraction of heart muscle and thereby lowers the blood pressure, by reducing the heart rate and the force of muscle contraction, reduces the need for oxygen by heart[15] and is recommended for treatment of myocardial infarction. In the present study an attempt has been made to elucidate the antioxidant property of eriodictyol in comparison with metoprolol succinate as standard.

MATERIALS AND METHODS

Drugs and chemicals

Eriodictyol, Isoproterenol hydrochloride, polyethylene glycol, nitrobluetetrazolium, glutathione, and nicotinamide adenine dinucleotide were purchased from Sigma Aldrich chemical company, St.louis, USA. All other chemicals used in the study were of analytical grade.

Formulation and administration of the drug

Eriodictyol dissolved in 5% Tween 80, 20% polyethylene glycol and 75% saline was orally administered to the animals[16] by intragastric intubation. Each animal belonging to different groups were administered with 1.0ml of the drug suspension at a dose of 50 mg per kg body weight for group 4 and 7, 100 mg per kg body weight for group 5 and 8, 200 mg per kg body weight for group 6 and 9 for 45 days. The standard drug metoprolol succinate is dissolved in distilled water and administered in a single dose of 2.5 mg[17] per Kg body weight for group 3 and 10 for 45 days.

Experimental animals

Male Albino Wistar rats weighing 130-160 g were purchased from Sri Venkateswara Enterprises, Bangalore, India. Animals were housed in polypropylene cages with 6 rats per cage. Animals were fed on standard pellets and water was provided *ad libitum*. Cages were maintained under standard condition of temperature, 25 $\pm 2^0$C. The study was approved by the Animal Ethical Committee of Periyar University, Salem, Tamilnadu. (1085/ac/07/PUIAEC/OCT-2012/02).

Experimental Design

Experimental Design: The experimental rats were randomly divided into ten groups consisting of 6 rats each.

Group 1 (control). Animals received standard diet and drinking water *ad libitum* and served as control group.

Group 2 (ISO). Animals received standard laboratory diet and drinking water *ad libitum* for 45 days and was treated with ISO in two doses (85 mg/kg body weight)[18] by subcutaneous injection on 46 and 47th day at an interval of 24 hs.

Group 3 (Metoprolol Succinate positive)- Animals received metoprolol succinate via intragastric intubation at a daily dosage of 2.5 mg/Kg body weight for a period of 45 days.

Group 4 (Eriodictyol 50 positive) - Animals received eriodictyol via intragastric intubation at a daily dosage of 50 mg/Kg body weight for a period of 45 days.

Group 5 (Eriodictyol 100 positive) - Animals received eriodictyol via intragastric intubation at a daily dosage of 100 mg/Kg body weight for a period of 45 days.

Group 6 (Eriodictyol 200 positive). Animals received eriodictyol via intragastric intubation at a daily dosage of 200 mg/Kg body weight for a period of 45 days.

Group 7 (Eriodictyol 50 AMI). Animals received eriodictyol via intragastric intubation at a daily dosage of 50 mg/Kg body weight for a period of 45 days. Animals where was treated with ISO in two doses (85 mg/kg body weight) by subcutaneous injection on 46 and 47th day at an interval of 24 hs to induce AMI

Group 8 (Eriodictyol 100 AMI). Animals received eriodictyol via intragastric intubation at a daily dosage of 100 mg/Kg body weight for a period of 45 days. Animals where was treated with ISO in two doses (85 mg/kg body weight) by subcutaneous injection on 46 and 47th day at an interval of 24 hs to induce AMI

Group 9 (Eriodictyol 200 AMI). Animals received eriodictyol via intragastric intubation at a daily dosage of 200 mg/Kg body weight for a period of 45 days. Animals where was treated with ISO in two doses (85 mg/kg body weight) by subcutaneous injection on 46 and 47th day at an interval of 24 hs to induce AMI

Group 10 (Metoprolol Succinate AMI). Animals received metoprolol succinate via intragastric intubation at a daily dosage of 2.5 mg/Kg body weight for a period of 45 days. Animals were treated with ISO in two doses (85 mg/kg body weight) by subcutaneous injection on 46 and 47th day at an interval of 24 hs to induce AMI

The animals were anaesthetized and were sacrificed by cervical dislocation. Blood was withdrawn by retro orbital puncture in an anticoagulant coated tube and the

plasma separated after centrifugation was used for biochemical investigation. Heart was dissected out, cleared of blood were transferred in ice cold saline. Heart tissue was homogenized in a suitable buffer, centrifuged and used for various biochemical analysis.

Preparation of hemolysate

Blood was collected in heparinized tubes, plasma separated by centrifuged at 3000x g for 5 minutes was collected and used for the biochemical estimations.

Preparation of tissue homogenate

The excised heart tissue was removed and was immediately washed with ice cold saline. The tissue was homogenized in an appropriate buffer using a tissue homogenizer.

Biochemical Estimations

Lipid peroxidation was measured by estimating the level of thiobarbituric acid reactive substances in tissues by the method of Ohkawa (1976)[19] and the plasma levels were estimated by the method of Yagi (1976).[20] Lipid Hydro peroxides was measured by the method of Jiang (1992)[21] and conjugated dienes by the method of Rao (1968).[22]Antioxidant enzymes SOD and Catalase was estimated by Kakkar (1984)[23] and Sinha (1972)[24] respectively. Glutathione peroxidases, Reduced Glutathione, and Glutathione -s-transferase by Rotruck (1973),[25] Boyne & Ellman (1972)[26] and Habig (1977).[27] Non Enzymatic antioxidants (Vitamin C and Vitamin E) in the plasma and heart tissue was estimated by Omaye *et al*.(1979)[28] and Baker *et al*.(1951)[29]

Histopathological Studies

The rats were sacrificed at the end of the experimental period, the dissected heart were washed in ice cold saline. The heart tissues were fixed in 10% buffered neutral formalin solution. After fixation the heart tissue was processesed by embedding in paraffin. Serial sections of the tissues were cut and each section was stained with hematoxylin and eosin. The slides were observed under a light microscope and photomicrographs were taken.

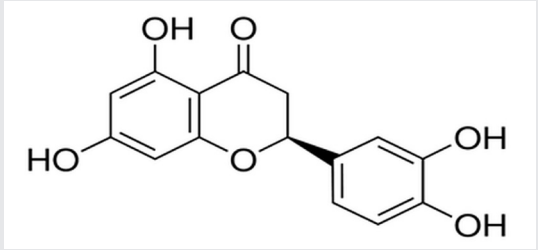

Figure 1: Structure of Eriodictyol, its molecular formula is: C15H12O6. Molecular weight is 288.5 g/mol..

Statistical Analysis

The results are tabulated and graphically represented as the mean ±SD. Statistical analysis was performed using one way analysis of variance. All data were analyzed by Duncan's multiple range test for group mean comparison using statistical program SPSS 16.00 for windows. Values not sharing a common superscript (a-h) differ significantly with each other. The findings were considered statistically significant if p value < 0.05.[30]

RESULTS

Histopathological Observations

Figure 3 shows the cardiac architecture for various experimental groups. Histopathological analysis of the control (group 1), metoprolol succinate alone (group 3) and eriodictyol alone treated groups (group 4, 5, 6) showed normal cardiac fibers. It is observed that in ISO induced (group 2), myocardial infracted heart sessions showed severe cardiac damage with inflammatory cell infiltration. The pretreatment with eriodictyol showed concentration dependent decrease in the degree of the

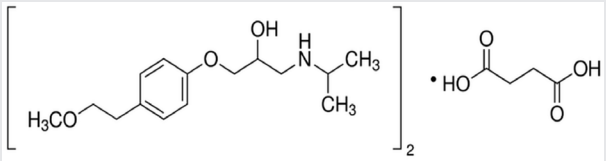

Figure 2: Structure of Metoprolol succinate, its molecular formula is: C34H56N2O10. Molecular weight is 652.8g/mol.

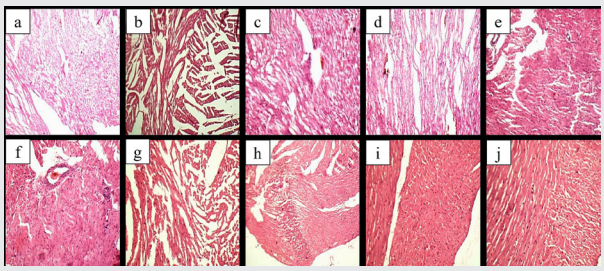

Figure 3: Histopathology changes in the myocardial infarction of control and experimental rats. a) Group I Normal appearance of the myocardial tissue in the control rats. b) Group II shows severe cardiac necrosis with the splitting of cardiac myocytes. c) Group III treated with metoprolol succinate alone and d), e) & f) Group IV ,V and VI treated with various doses of eriodictyol (50,100,200 kg per body weight)shows no changes in cardiac architecture. g) GroupVII shows necrosis and seperation of the muscle fibers. h) Group VIII shows very mild degree of necrosis. i) Group IX and j) Group X shows cardiac muscle cell with very less degree of infiltration of inflammatory cells and no necrosis.(200X).

infarct as seen in group 7, 8 and 9. Metoprolol succinate pretreatment also decreased the severity of the infarct when induced with ISO as compared to the ISO alone induced group.

Effect of eriodictyol and metoprolol succinate on lipid peroxidation in myocardial infarction rats

Figure 4 and Figure 5 shows the effect of eriodictyol and metoprolol succinate on plasma and tissue levels of lipid peroxidation in the control and the experimental animals. Rats induced with ISO alone (group 2) showed significant increase ($p<0.05$) in the level of TBARS, lipid hydroperoxides and conjugated dienes in the plasma and tissue compared to the normal control rats (group 1). Oral supplementation of eriodictyol (50 mg, 100 mg and 200 mg/kg body weight) daily for a period of 45 days has helped in reversing the effect of lipid peroxides. The levels of TBARS, lipid hydro peroxides and conjugated dienes considerably reduced in the plasma and heart in group 9 and group 10 rats as compared with the ISO alone treated group. Pretreatment with eriodictyol and metoprolol succinate (group 10) significantly restored the plasma and tissue levels of TBARS, lipid hydro peroxides and conjugated dienes to near normal levels comparable to those of control rats.

Effect of eriodictyol and metoprolol succinate on plasma and tissue enzymatic antioxidants in myocardial infarction rats:

Figure 6 and Figure 7 represents the effect of eriodictyol and metoprolol succinate on SOD, catalase and glutathione peroxidase in the plasma and tissue of the normal and the ISO induced rats. ISO alone induced rats (group 2) showed significant decrease in the activities of enzymatic antioxidants in the plasma as compared to the control rats (group 1). Pretreatment with eriodictyol (group 7-9) considerably increased the levels of antioxidant enzymes compared to the ISO induced group. The levels of antioxidants enzymes also showed significant increase in animals pretreated with the standard drug, metoprolol succinate (group 10). Collating the concentration of antioxidant enzymes in group 9 and group 10, it is evident that eriodictyol is more effective in increasing the amount of both circulatory and tissue antioxidant enzymes compared to metoprolol succinate.

Effect of eriodictyol and metoprolol succinate on plasma and tissue non enzymatic antioxidants in myocardial infarction rats:

Figure 8 and Figure 9 depicts the effect of eriodictyol and metoprolol succinate on non enzymic antioxidants such as reduced glutathione, ascorbic acid and tocoph-

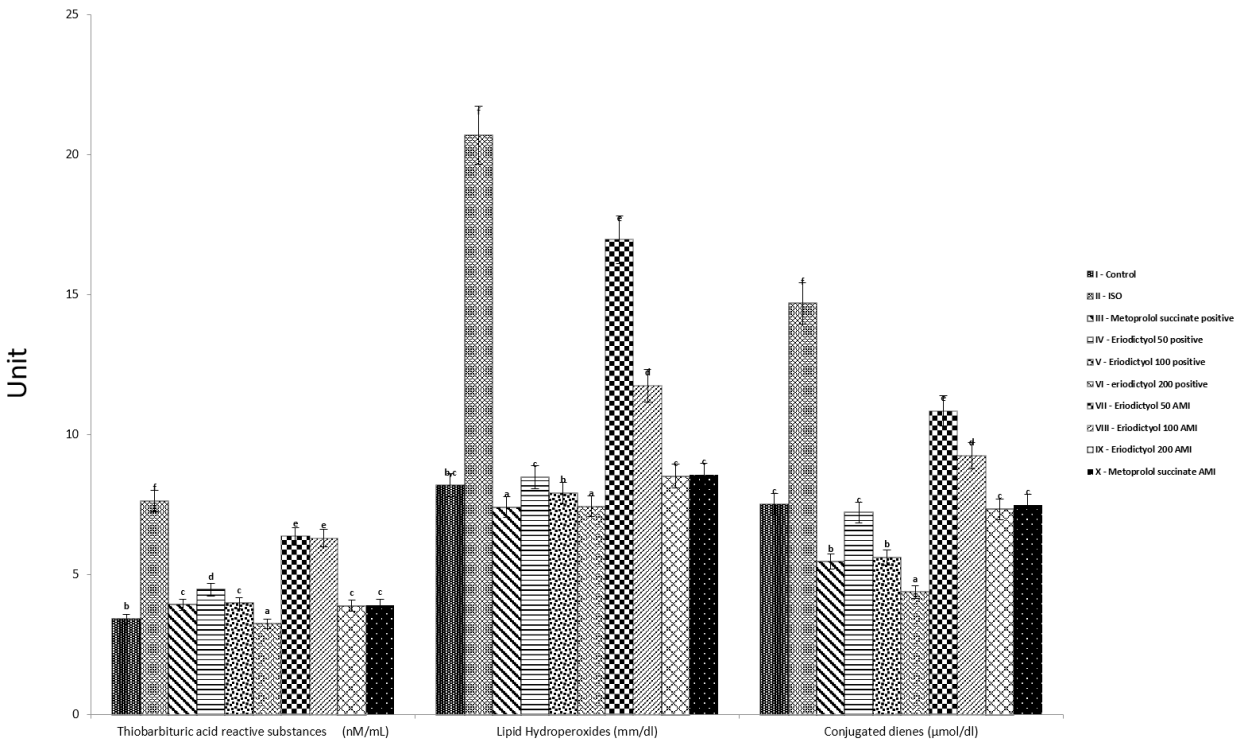

Figure 4: Effect of Eriodictyol on plasma lipid peroxidation in control and experimental rats. Each column is mean +SD for six rats in each group. Values not sharing a common superscript letter (a-h) differ significantly at P<0.05 (Duncans Multiple Range Test).

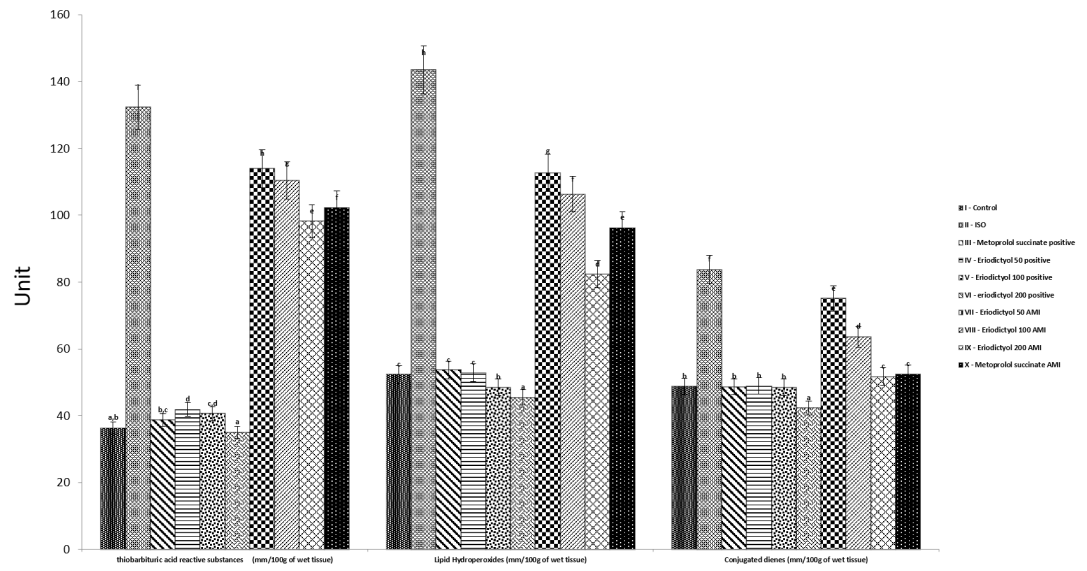

Figure 5: Effect of eriodictyol on tissue lipid peroxidation in control and experimental rats. Each column is mean +SD for six rats in each group. Values not sharing a common superscript letter (a- i) differ significantly at P<0.05 (Duncans Multiple Range Test).

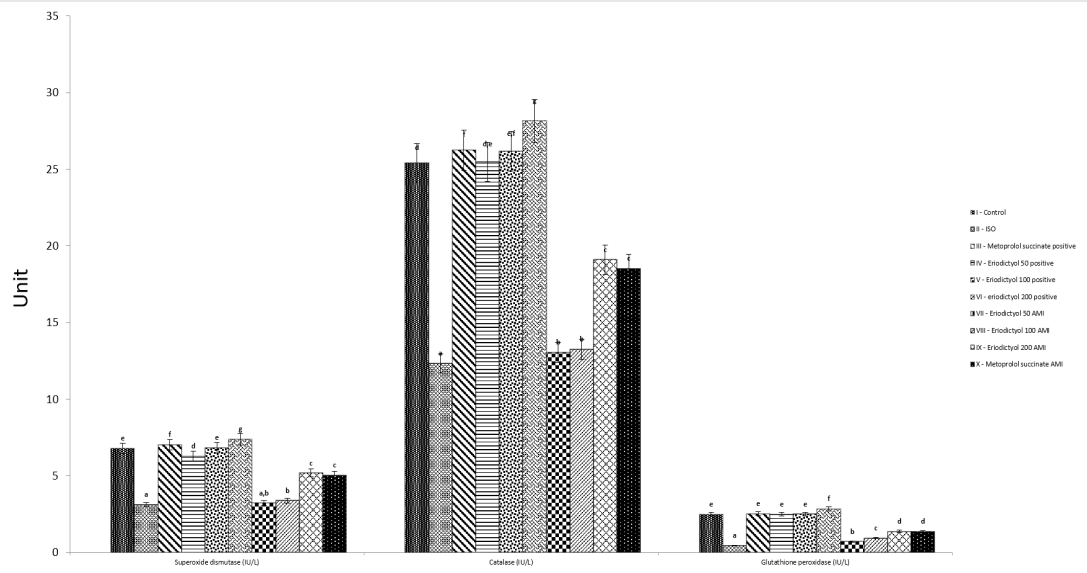

Figure 6: Effect of Eriodictyol on plasma antioxidants in control and experimental rats.Each column are mean +SD for six rats in each group. Values not sharing a common superscript letter (a-g) differ significantly at *P* < 0.05 (Duncans Multiple Range Test).

erol in the plasma and tissue of normal and ISO induced rats. The ISO induced rats exhibited significant decrease in the levels of vitamin C, vitamin E and reduced glutathione compared with the normal control rats. Pretreatment with eriodictyol significantly increased the plasma and tissue levels of non enzymic antioxidants in group 7- group 9 compared to the ISO alone induced group .Similar results were obtained when pretreated with the metoprolol succinate. The value of plasma and tissue non enzymatic antioxidants in group 9 and group 10 shows that eriodictyol was more functional in boosting the non enzymatic antioxidants compared to metoprolol succinate.

DISCUSSION

Lipid peroxidation is a progression of deterioration of the cell membranes[31] due to the increased rates of free radical production on oxidative stress[32]. Cell injury induced by free radical particularly reactive oxygen species is an important mechanism of cell damage in

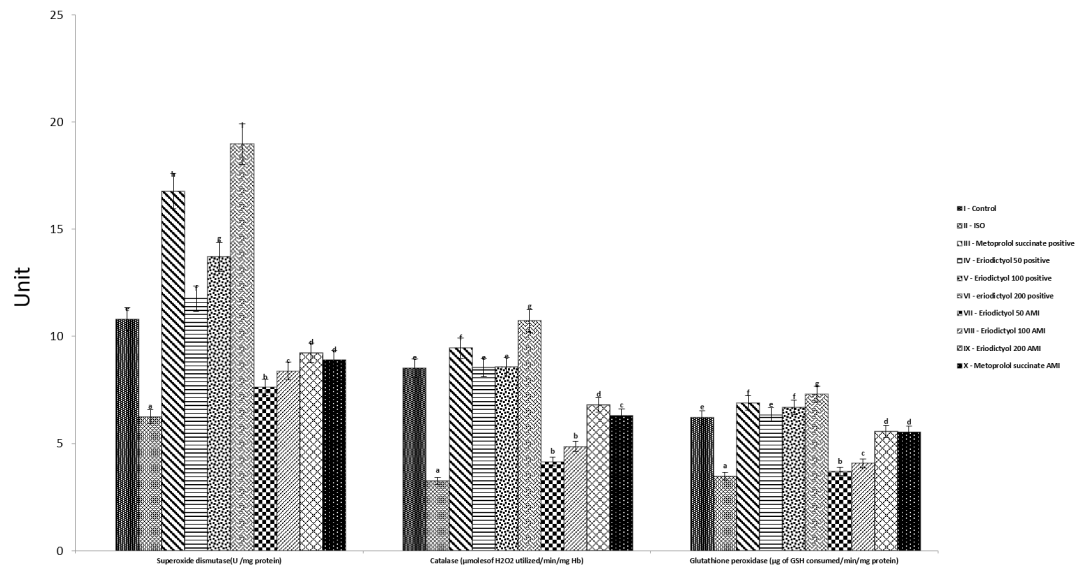

Figure 7: Effect of Eriodictyol on tissue antioxidants in control and experimental rats. Each column is mean +SD for six rats in each group. Values not sharing a common superscript letter (a- i) differ significantly at P<0.05 (Duncans Multiple Range Test).

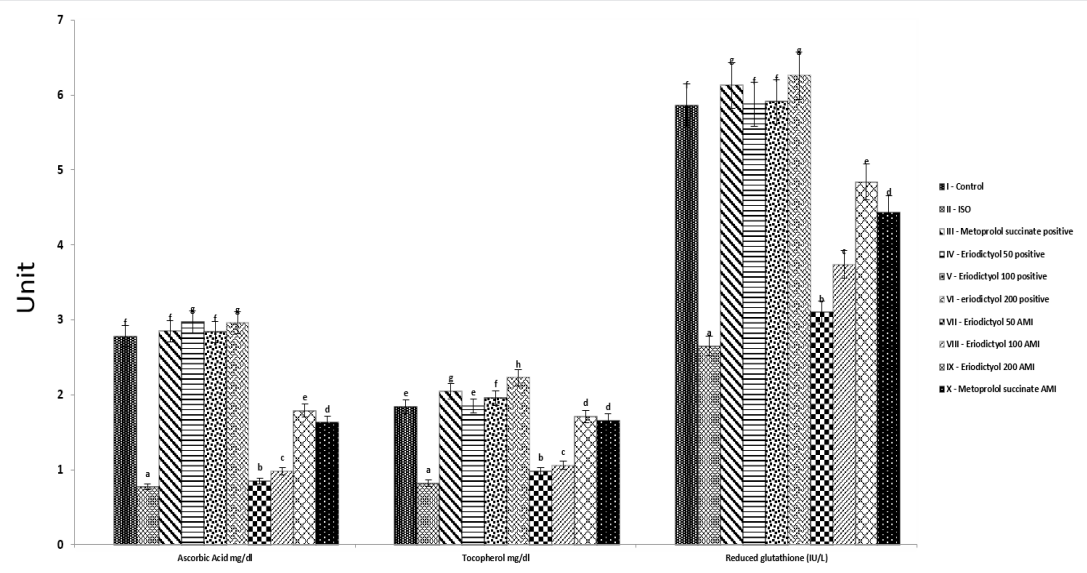

Figure 8: Effect of Eriodictyol on plasma non enzymatic antioxidants in control and experimental rats. Each column is mean +SD for six rats in each group. Values not sharing a common superscript letter (a-h) differ significantly at P<0.05 (Duncans Multiple Range Test).

pathologic conditions, ischemia and in cellular ageing.[33] Catecholamines are also known to cause increased reactive oxygen species generation through oxidation and subsequent oxidative stress[34] which leads to myocardial necrosis.[35,36] The formation of free radicals and the accumulation of lipid peroxides is one of the most major biochemical changes associated with myocardial damage induced by catecholamines.[37] Isoproterenol is widely used model to produce infarct like lesion of myocardium in rats.[38]

In the present study, increased levels of lipid peroxidation (TBARS), lipid hydroperoxides and conjugated dienes in heart and plasma of ISO induced rats were observed compared to the control rats. The cells when exposed to inherently damaging agents, results in irreversible cell injury characterized by the depletion of energy stores in the form of ATP, cellular swelling caused by changes in ion concentration and water influx.[39,40] Decrease in cellular ATP, slows down the activity of cellular enzymes bringing in irreversible damage to intracellular organelles and production of ROS increases.

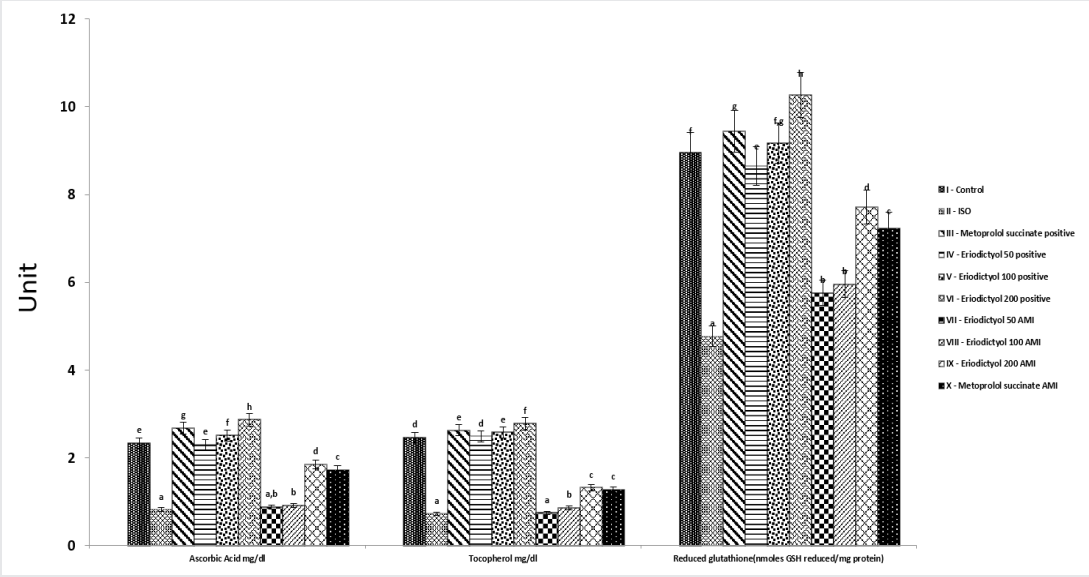

Figure 9: Effect of Eriodictyol on tissue non-enzymatic antioxidants in control and experimental rats. Each column is mean ± SD for six rats in each group. Values not sharing a common superscript letter (a-h) differ significantly at P<0.05 (Duncans Multiple Range Test).

The decreased levels of lipid peroxidation products (TBARS, LOOH, CD) were observed in rats pretreated with eriodictyol compared with the ISO alone induced rats. The deleterious effects of the free radicals are kept under control by a delicate balance between the rate of production and the rate of elimination of the free radicals by the defense mechanism. Antioxidants are the natural defense mechanism existing in our body, which are capable of scavenging free radicals.[41] The decreased levels of lipid peroxidation products can be attributed to the increased activity of antioxidant enzymes or compounds that deactivates these free radicals. Various *in vitro* studies have demonstrated that flavanol, flavones and anthocyanin have considerable radical scavenging properties.[42,43] This activity is attributed to their hydrogen donating ability. The phenolic groups of flavonoids serve as a source of readily available 'H'atoms such that the subsequent radicals produced can be delocalized over the flavonoid structure.[44] Earlier studies on flavonoids have undoubtedly established the role of flavonoids in inhibiting lipid peroxidation *in vitro* and *in vivo* at an early stages by acting as scavengers of superoxide anion and hydroxyl radicals by terminating the propagation of free radical chain reactions.[45,46] Studies have showed that eriodictyol has free radical scavenging potential[47] contributing to defense against lipid peroxidation.

When the production of ROS increases or the scavenging system are ineffective, it results in an excess of these free radicals leading to a condition called oxidative stress. Antioxidants are molecules which can reduce the oxidative stress, by scavenging the ROS/RNS generated by various metabolic processes. ROS and RNS derived from oxygen and nitrogen are converted into free radicals which are highly unstable and are capable of damaging biologically relevant molecules such as protein, lipid and DNA can trigger a number of human diseases.[48] The most important antioxidant enzymes are superoxide dismutase, catalase and glutathione peroxidase. Superoxide dismutase can act on superoxide anion converting it into hydrogen peroxide.[49] The hydrogen peroxide thus generated is detoxified by the catalase by converting it into O_2 and H_2O.[5O] Another important enzyme which has potential to remove H_2O_2 from the system is glutathione peroxidase.[51] Glutathione peroxidase removes H_2O_2 by using it to oxidize reduced glutathione to oxidized glutathione. Glutathione peroxidases can also catalyze the reduction of unstable hydro peroxides at the expense of GSH.[52] Hence the level of reduced glutathione available in the system can moderates the activity of antioxidant enzymes.

In the present study, ISO induced myocardial damage was evident from the decreased level of antioxidant enzymes SOD, catalase and glutathione peroxidase in ISO alone treated group. The result obtained where in agreement with similar finding in various studies concluding that catecholamine administration can lead to the depletion of energy levels in the cardiac muscle cells, leading to complex structural and biochemical changes that results in cell damage and necrosis.[53] Pretreatment

with eriodictyol has resulted in an increase in the levels of antioxidant enzymes which can be attributed to the free radical scavenging potential of eriodictyol thereby protecting the antioxidant system. Vitamin C and Vitamin E are the major non enzymatic antioxidants in aqueous and lipophilic phase respectively. Both vitamin C and E are capable of protecting the cells from oxidative cell damage.[54] In our study, decreased levels of vitamin C and E which were observed in ISO induced rats were reversed on pretreatment with eriodictyol.

The Histopathological result obtained supports the finding of the study. The damage to the heart tissue in the ISO treated group can be seen as confluent necrosis of the muscle fibers with inflammatory cell infiltration and edema. Eriodictyol pretreated myocardium (50 mg, 100 mg)showed decrease degree of infarct as compared to the ISO treated group, whereas the myocardium pretreated with 200 mg/kg body weight showed near normal cardiac architecture. Restoration of normal heart architecture and protection of the myocardium when administered with eriodictyol in ISO induced rats reveal the protective effect of eriodictyol.

CONCLUSION

Findings from our results showed that eriodictyol protected myocardium from ISO induced myocardial infarction and structural injury via improved histopathology and biochemical parameters. In the present study, eriodictyol pretreatment prevented the ISO induced increase in lipid peroxidative parameters, showing maximum antioxidant potential at 200 mg/kg body weight. The values obtained for eriodictyol was such more significant compared to metoprolol succinate which is a standard drug generally used in the treatment of angina pectoris and cardiac failure. Hence due to its antioxidant property eriodictyol will provide an accessible medicine for heart in future. The precise molecular mechanism of eriodictyol against ISO induced myocardial infarction is underway.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

ABBREVIATION USED

IHD: Ischemic Heart Disease; **MI:** Myocardial Infarction; **ISO:** Isoproterenol; **TBARS:** Thiobarbituric Acid Reactive Substances; **LOOH:** Lipid Hydroperoxides; **SOD:** Superoxide Dismutase; **CD:** Conjugated Dienes; **ROS:** Reactive Oxygen Species; **RNS:** Reactive Nitrogen Species; **GSH:** Reduced Glutathione.

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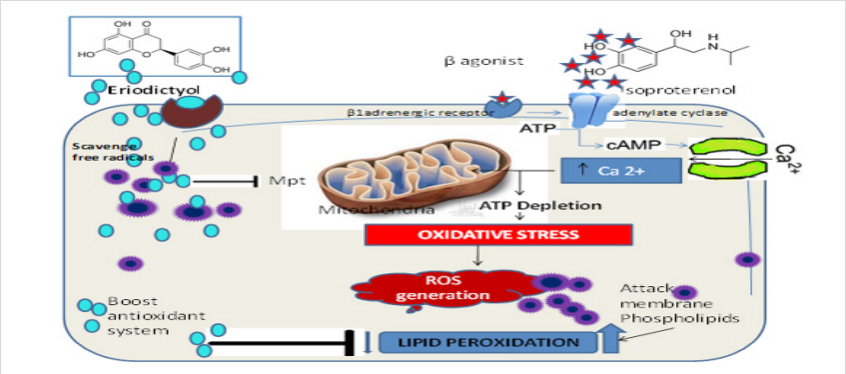
PICTORIAL ABSTRACT



SUMMARY

- The study showed significant increase in the levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes both in plasma and heart tissue with considerable decrease in the levels of enzymatic and non-enzymatic antioxidants in ISO induced myocardial infracted rats.
- The oral administration of eriodictyol showed significant decrease in lipid peroxidation products and with significant increase in the levels of antioxidants.
- The cardio protective role of eriodictyol was further assessed by histopathological studies.

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