*Research Article*

# Role of bioflavonoids, quercetin and catechin in attenuation of isoproterenol induced myocardial infarction in rats

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**Abstract**

**Objective:** Myocardial infarction (MI) increasingly contributing towards mortality statistics of CVD. The present study was aimed to investigate the cardioprotective effect of quercetin and catechin in isoproterenol induced myocardial infarcted rats. **Material and methods:** The cardioprotective effect was assessed by determining the effects of quercetin (30 and 60 mg/kg, p.o.,) and catechin (20 and 40 mg/kg, p.o.,) in isoproterenol induced myocardial infarction on the activities of cardiac markers, antioxidant enzymes and histopathological examination. **Results:** Administration of isoproterenol (150 mg/kg, s.c.) in rats at 24 h interval for 2 days showed significant ($p<0.001$) increase in cardiac markers (LDH, SGOT and SGPT), increase in MDA levels; significant decrease in antioxidant enzymes (SOD, GPX and CAT). Pretreatment with quercetin (30 and 60 mg/kg, p.o.,) and catechin (20 and 40 mg/kg, p.o.,) to isoproterenol treated rats for 42 days showed significant ($p<0.001$) protective effect and quercetin and catechin alone treated groups showed less significant effect. **Conclusion:** The study revealed that cardioprotective effect of quercetin and catechin in isoproterenol induced MI may be due to attenuation of antioxidant enzymes and inhibition of lipid peroxidation of membrane and decrease in cardiac marker levels, which was further confirmed by histopathological studies.

***Keywords*:** Cardiovascular diseases, myocardial infarction, Isoproterenol, Quercetin, Catechin

## Introduction

Myocardial infarction (MI) is one of the most life threatening malady globally. In developing countries, MI increases the influence of mortality and morbidity statistics due to their lifestyle modifications most commonly in urban regions. MI occurs as a consequence of protracted imbalance between coronary blood supply and demand of myocardium. The reinforced evidence reveals that ischemic tissue produces oxygen free radicals which comprise odd numbered electrons that makes chemically reactive and often leads to chain reaction causing cell death (Devika and Panneerselvam, 2017).

Age-standardized cardiovascular disease (CVD) mortality of 272 per 100 000 in habitants is reckoned by the Global Burden of disease study where, India has 235 per 100 000 inhabitants which is higher than the global average (Prabhakaran et al., 2016).

Isoproterenol {4-[1-hydroxy-2-(1-methylethylamino) ethyl] benzene-1, 2-diol} (ISO) is a synthetic catecholamine and β adrenergic agonist which leads to severe stress in myocardium ensuing infarct-like necrosis of the heart muscles. Earlier studies revealed that exposure of the heart to higher concentrations of catecholamines causes necrotic lesions resulting in myocardial infarction in experimental animals (Tiwari et al., 2009). MI with ISO is performed in experimental studies to investigate several cardiac dysfunctions and to study the efficacy of various natural and synthetic cardioprotective agents. Hence, similar to those observed in human myocardial infarction, many metabolic and morphological changes may occur in cardiac tissues of experimental animals (Mert et al., 2018).

Flavonoids are a subgroup of the more extended family of polyphenols (Foshati, 2017). Flavonoids existing from ancient times exhibit an extensive range of pharmacological activities which may have a positive impact on CVD.

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Previous studies revealed that high intake of flavonoids reduce the risk of CVD (Parasuraman et al., 2016). Flavonoids are potent antioxidants and have free radical scavenging abilities which safeguard against cardiovascular death (Zhang et al., 2015).

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a naturally occurring flavonoid copiously found in various fruits and vegetables. It possess anti-inflammatory, antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic, antiatherosclerotic (Parasuraman et al., 2016), anti-carcinogenic, antiviral, antioxidant, and psycho stimulant activities, as well as the ability to inhibit lipid peroxidation, platelet aggregation and capillary permeability, and to stimulate mitochondrial biogenesis (Li et al., 2016).

Catechin is a polyphenolic compound belongs toflavonoids class naturally found in plant-based foods. (+) -catechin and (−) -epicatechin are the specific types of catechins which are most commonly refer to exhibit cardioprotective effect. The cardioprotective effects of catechins are diverse which include thrombus formation, platelet activation, atherosclerosis, and reductions in systemic blood pressure. This review signifies the existing literature on the rapport between cardiovascular health and catechins, which may prove to have implications for pharmaceutical progress (Mangelsand Mohler, 2017).

The present study was aimed to investigate whether oral administration of bioflavonoids, quercetin and catechin for 42 days has any protective effect against ISO-induced myocardial infarction. Biochemical and histopathological changes induced by ISO were observed and their changes with quercetin and catechin were assessed.

## Material and methods

### Materials and Chemicals

The drugs, isoproterenol hydrochloride, quercetin and catechin were procured from Sigma Aldrich Chemicals Ltd, Bangalore. All other chemicals and reagents used were of analytical grade.

### Experimental Animals

After obtaining permission from Institutional Animal Ethical Committee (IAEC), Albino wistar rats (150-200 g) of either sex were obtained from IISC, Bangalore. All the experiments were performed in accordance with the guideline established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPSCEA), New Delhi, India. They were acclimatized for one week prior to experiment. Rats were housed in polypropylene cages lined with husk in fully ventilated room. Animals were maintained in 12:12 h light and dark cycle and were housed at temperature of 25±2ºC. They had free access to a standard chow diet and water *ad libitum* (IAEC/Ph.cology/02/2010-11).

### Preparation of drug solutions

Quercetin and catechin are sparingly soluble in aqueous solutions. To solubilise these drugs 80% dimethyl sulphoxide (DMSO) was used as a vehicle and two concentrations of drugs, quercetin (30, 60 mg/kg) and catechin (20, 40 mg/kg) were prepared.

### Dose Selection

Based on previously reported articles (Arias et al., 2015; AnandhBabu and Liu, 2008) the acute dose of the drugs, quercetin and catechin was found to be 50 mg/kg and 30 mg/kg respectively. Hence in the present study, we selected the doses of 30 mg/kg and 60 mg/kg for quercetin and 20 mg/kg and 40 mg/kg for catechin andthe dose of ISO (150mg/kg) was also selected as per previously reported articles (Keshtkar et al., 2017).

### Assessment of Cardioprotective effect

Rats were randomly divided into ten groups of 10 each. Animals of group I served as control group administered with 0.5 ml of vehicle (80% DMSO). Group II animals were injected with ISO dissolved in saline (150 mg/kg s.c.) once daily for 2 days (Keshtkar et al., 2017). Animals of group III and IV were treated with quercetin 30 mg/kg and 60 mg/kg p.o., respectively using rat oral feeding needle for 42 days. Animals of group V and VI were administered with quercetin 30 mg/kg and 60 mg/kg p.o., respectively for 42 days and then treated with ISO dissolved in saline (150 mg/kg s.c.) once daily for 2 days. Animals of group VII and VIII were treated with catechin 20 mg/kg and 40 mg/kg p.o., respectively for 42 days. Animals of group IX and X were treated with catechin 20 mg/kg and 40 mg/kg p.o., respectively for 42 days and then treated with ISO dissolved in saline (150 mg/kg s.c.) once daily for 2 days.

After 24 h of second dose of ISO, all the rats were anaesthetized with thiopental sodium (45 mg/kg, i.p.). Blood samples were collected and serum was separated by centrifugation at 2500 rpm at 30ºC for 15 min and estimation of marker enzymes viz., serum glutamic oxaloacetic transaminase (SGOT), *lactate dehydrogenase (*LDH), serum glutamic pyruvic transaminase (SGPT) and serum malondialdehyde (MDA)was performed. The heart was dissected out, washed immediately in ice-chilled physiological saline, blotted and weighed. A known weight of the heart tissue homogenized in 0.1M Tris-HCL (pH 7.4) buffer solution to produce 10% w/v homogenate. The homogenate was centrifuged at 3000 rpm, 4ºC for 5min, and the supernatant subjected for assays of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and tissue MDA. Similarly rat hearts were subjected for histopathological studies.

**Histopathological examination**

Hearts were removed from sacrificed rats, washed, immediately fixed in 10% neutral buffered formalin, and carefully embedded in molten paraffin. Cross section of (5-mm-thick) of fixed myocardial tissues was cut and stained them with hematoxylin and eosin (H&E), and then visualized under light microscopic for histopathological changes. The investigators performed histopathological examinations were blind to biochemical estimations (Wei et al., 2017).

**Statistical analysis**

The results are expressed as mean ± S.D from n=6 rats in each group, statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's Multiple comparison test. $p<0.001$ was considered significant.

**Results**

**Cardiac markers**

ISO treated rats showed a significant ($p<0.001$) increase in activity of serum LDH, SGOT, SGPT and MDA levels when compared with vehicle group rats. Pretreatment with Quercetin (30 and 60 mg/kg) and catechin (20 and 40 mg/kg) significantly ($p<0.001$) reduced the serum LDH, SGOT, SGPT and MDA levels. Pretreatment with Quercetin (30 and 60 mg/kg) and catechin (20 and 40 mg/kg) in ISO treated rats significantly ($p<0.001$) reduced the activities of enzymes compared with the ISO-treated rats. All the values of treatment groups were compared with those of vehicle group and ISO treated group (Table 1)

**Biochemical Parameters**

ISO treated rats showed a significant ($p<0.001$) increase in activity of serum MDA and decrease in SOD, GPX and CAT levels when compared with vehicle group rats. Pretreatment with Quercetin (30 and 60 mg/kg) and catechin (20 and 40 mg/kg) significantly ($p<0.001$) reduced the MDA levels whereas increased the SOD, GPX and CAT levels. Pretreatment with Quercetin (30 and 60 mg/kg) and catechin (20 and 40 mg/kg) in ISO treated rats significantly ($p<0.001$) reduced the activities of SOD, GPX and CAT and increased the MDA levels. All the values of treatment groups were compared with those of vehicle group and ISO treated group (Table 2).

**Histopathological examination**

In histopathological examination, DMSO treated rats showed some congested vascular spaces whereas, ISO group rats showed coagulative necrosis of cardiocytes, mixed inflammatory infiltration and damaged vascular spaces. The other groups with quercetin and catechin treated animals showed comparatively less inflammation and necrosis of cardiocytes (Figure 1).

**Discussion**

MI is an acute condition of myocardial necrosis, prevails as consequence of imbalance between myocardial demand and coronary blood supply. Generation of toxic reactive species such as hydroxyl radicals, superoxide radicals and hydrogen peroxide leads to damage the myocardial cells (Manjunatha et al., 2011).

ISO causes severe stress in the myocardium, resulting in infarct like necrosis of the heart muscle (Panda et al., 2017). ISO induced myocardial infarction was reported to enhance adenyl cyclase activity, leads to increased cAMP formation, that sequentially causes higher lipid accumulation in the myocardium and consequently it generate free radical to stimulate lipid peroxidation, and therefore causes irreversible damage to the myocardial membrane (Upaganlawar et al., 2011).

**Table 1.** Effects of quercetin and catechin on cardiac markers in the isoproterenol induced myocardial infarction in rats

| Groups | LDH (IU/L) | SGOT (IU/L) | SGPT (IU/L) | MDA (n.mol/g wet tissue) |
|---|---|---|---|---|
| DMSO | 154.4 ± 0.97 | 135.2 ± 1.19 | 122.1 ± 1.13 | 17.9 ± 0.37 |
| ISO (150mg/kg) | 452.6 ± 3.56 | 302.4 ± 2.59 | 254.5 ± 3.02 | 45.8 ± 2.45 |
| Quercetin (30mg/kg) | 146.0 ± 3.07*** | 129.8 ± 0.73*** | 119.6 ± 0.79*** | 17.5 ± 0.69*** |
| Quercetin (60mg/kg) | 134.9 ± 1.31*** | 115.9 ± 2.71*** | 107.9 ± 1.28*** | 16.8 ± 0.98*** |
| Quercetin (30mg/kg) + ISO (150mg/kg) | 345.8 ± 2.31*** | 162.4 ± 2.55*** | 150.6 ± 3.32*** | 38.3 ± 1.34*** |
| Quercetin (60mg/kg) + ISO (150mg/kg) | 280.8 ± 4.38*** | 143.8 ± 4.17*** | 134.7 ± 2.25*** | 30.2 ± 1.43*** |
| Catechin (20mg/kg) | 143.7 ± 0.96*** | 136.7 ± 2.73*** | 119.7 ± 1.18*** | 17.2 ± 0.73*** |
| Catechin (40mg/kg) | 134.2 ± 1.61*** | 126.1 ± 1.39*** | 112.6 ± 1.79*** | 16.5 ± 0.84*** |
| Catechin (20mg/kg) + ISO (150mg/kg) | 376.4 ± 1.39*** | 169.7 ± 3.78*** | 156.7 ± 1.20*** | 39.0 ± 0.73*** |
| Catechin (40mg/kg) + ISO (150mg/kg) | 265.9 ± 3.60*** | 166.4 ± 0.96*** | 138.6 ± 1.94*** | 32.7 ± 1.78*** |

Values are expressed in mean ± S.D. n = 6; ***p < 0.001 significant when compared with ISO treated group. All data were subjected to one-way ANOVA followed by Dunnett's test.

**Table 2.** Effects of quercetin and catechin on biochemical parameters in the isoproterenol induced myocardial infarction in rats

| Groups | SOD (Units/mg protein) | CAT (µ moles of $H_2O_2$ metabolized/mg protein/min) | GPX (µ moles of glutathione oxidized/min/mg protein) | MDA (n.mol/ml) |
|---|---|---|---|---|
| DMSO | 37.7 ± 0.79 | 32.2 ± 0.96 | 33.9 ± 1.44 | 4.4 ± 0.87 |
| ISO (150mg/kg) | 8.57 ± 0.37 | 11.5 ± 0.18 | 8.8 ± 0.35 | 12.6 ± 0.81 |
| Quercetin(30mg/kg) | 38.2 ± 1.39*** | 34.4 ± 0.66*** | 34.5 ± 1.79*** | 4.1 ± 0.55*** |
| Quercetin (60mg/kg) | 41.2 ± 1.57*** | 36.2 ± 0.66** | 36.2 ± 0.48*** | 3.8 ± 0.27*** |
| Quercetin (30mg/kg) + ISO (150mg/kg) | 14.34 ± 0.89*** | 22.73 ± 0.76*** | 14.2 ± 0.41*** | 9.3 ± 0.59*** |
| Quercetin (60mg/kg) + ISO (150mg/kg) | 17.03 ± 1.53*** | 24.2 ± 0.73*** | 19.5 ± 1.06*** | 8.8 ± 0.49*** |
| Catechin (20mg/kg) | 37.8 ± 1.20*** | 33.3 ± 1.20*** | 34.9 ± 0.33*** | 4.0 ± 0.42*** |
| Catechin (40mg/kg) | 40.6 ± 0.77*** | 35.9 ± 1.10ns | 36.2 ± 1.17*** | 3.9 ± 0.53*** |
| Catechin (20mg/kg) + ISO (150mg/kg) | 13.6 ± 1.11*** | 19.5 ± 0.11*** | 15.5 ± 0.29*** | 9.65 ± 0.80*** |
| Catechin (40mg/kg) + ISO (150mg/kg) | 18.1 ± 0.54*** | 22.1 ± 0.82*** | 20.9 ± 1.27*** | 7.74 ± 0.90*** |

Values are expressed in mean ± S.D. n = 6, ***p < 0.001 significant when compared with ISO treated group. All data were subjected to one-way ANOVA followed by Dunnett's test.

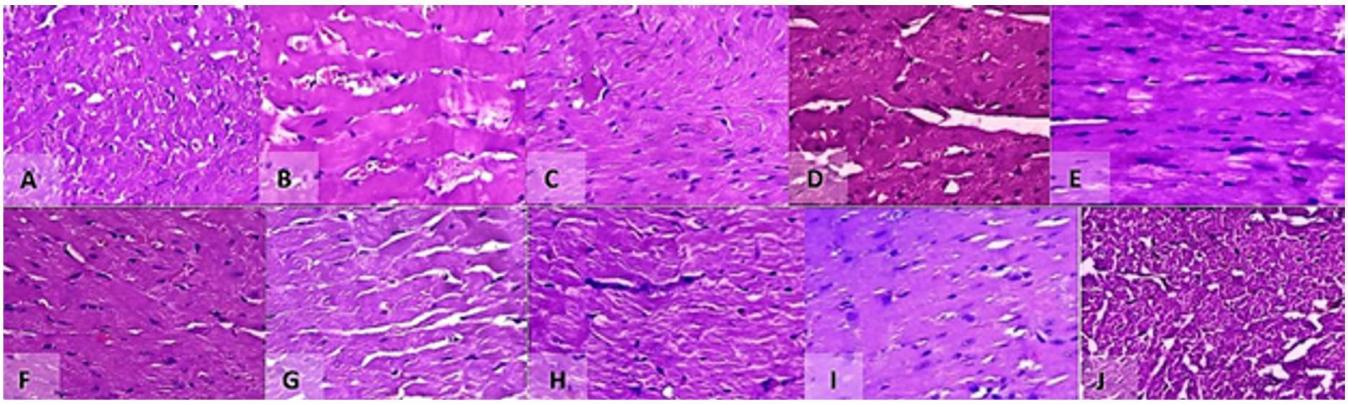


**Figure 1.** Effect of quercetin and catechin treatment on histopathological changes insections of cardiac muscle. (A) DMSO group rats showed some congested vascular spaces; (B) ISO group showed some areas of coagulative necrosis of cardiocytes, mixed inflammatory infiltration and damaged vascular spaces; (C) Quercetin (30 mg/kg$^{-1}$) group showed few congested vascular spaces; (D) Quercetin (60 mg/kg$^{-1}$) group showed some proliferating vascular spaces; (E) Quercetin (30 mg/kg$^{-1}$) + ISO group showed focal areas of necrosis of cardiocytes, mild inflammation, few congested vascular spaces; (F) Quercetin (60 mg/kg$^{-1}$) + ISO group showed proliferating and congested vascular spaces; (G) Catechin (20 mg/kg$^{-1}$) group showed few proliferating vascular spaces; (H) Catechin (40 mg/kg$^{-1}$) group showed normal architecture; (I) Catechin (20 mg/kg$^{-1}$) + ISO group showed few areas of coagulative necrosisof cardiocytes, moderate stromal inflammation and congested vascular spaces; (J) Catechin (40 mg/kg$^{-1}$) + ISO group showed focal areas of coagulative necrosis of cardiocytes, mild stromal inflammation and proliferating vascular diseases.

The cardiac marker enzymes such as LDH, SGOT and SGPT serve as sensitive index to measure the severity of MI (Raju et al., 2008). Leakage of enzymes (LDH, SGOT and SGPT) from heart tissue to serum will takes place when myocardial membrane gets ruptured (Rao and Viswanath, 2007). This leads to increased activity of these enzymes in rat serum with MI induced by ISO. In the current study, a significant increase in serum cardiac markers LDH, SGOT and SGPT activity was observed in ISO treated rats when compared with vehicle treated rats. Pretreatment with doses of quercetin and catechin, significantly (p<0.001) reduces the levels of serum cardiac markers LDH, SGOT and SGPT. This suggests the cardioprotective potential of quercetin and catechin.

Oxidative stress plays an important role in the pathogenesis of myocardial infarction. Antioxidant enzymes such as SOD, GPX and CAT are the first line cellular defense against oxidative stress (Karthick and Prince, 2006). In the current study, SOD activity decreased significantly (p<0.001) in the ISO treated group as compared with the vehicle group. This may be due to an excessive formation of superoxide anions and due to the decreased activity of an enzyme SOD (Manoharan et al., 2005). This reduction in SOD activity was reversed significantly (p< 0.001) dose dependently of quercetin (30 and 60 mg/kg, p.o.) and catechin (20 and 40 mg/kg, p.o.) pretreatment.

The activity of $H_20_2$ scavenging enzyme CAT was also decreased significantly (P<0.001) in ISO treatment group. The decline in the enzyme level may be explained by the

fact that excessive superoxide anions may inactivate SOD, thus resulting in an inactivation of the $H_2O_2$ scavenging enzyme (Manoharan et al., 2005). Pretreatment of Quercetin (30 and 60 mg/kg, p.o.) and Catechin (20 and 40 mg/kg, p.o.) attenuates the levels of CAT enzyme and considerably improve cellular antioxidative defense against oxidative stress.

GPX is one of the major antioxidant enzymes present in the heart, which prevents peroxidation of membrane lipids and has a protective function maintaining thiol groups of enzymes. GPX metabolizes $H_2O_2$ and lipid hydro peroxides, using GSH as an electron donor. This prevents the formation of the highly reactive hydroxyl radical. The reduced activity of GPX in heart is due to the decreased availability of reduced glutathione and due to accumulation of oxidized glutathione which in turn inactivates many enzymes containing SH groups (Ragunath et al., 2011). The level of glutathione peroxidase was decreased significantly ($p<0.001$) in the ISO treated group as compared with the vehicle group and reduction was significantly reversed by quercetin (30 and 60 mg/kg, p.o.) and catechin (20 and 40 mg/kg, p.o.) pretreatment. Doses of quercetin and catechin perse treated rats showed very less significance in all the above parameters when compared with vehicle group.

MDA is major lipid peroxidant and increased MDA content may contribute to increased generation of free radicals (Karthick and Prince, 2006). Previous studies have reported that isoproterenol produces free radicals and these free radicals are involved in membrane damage which leads to marked alteration in the structural integrity and function of cell membranes (Karthick and Prince, 2006; Manoharan et al., 2005). MDA levels in heart tissue and serum were found to be significantly ($p<0.001$) higher in ISO treated group when compared with vehicle group, while in pretreated groups with drugs quercetin (30 and 60 mg/kg, p.o.) and catechin (20 and 40 mg/kg, p.o.) showed decreased levels of MDA significantly ($p<0.001$) and dose dependently.

Histopathological findings of pretreated groups with drugs quercetin (30 and 60 mg/kg, p.o.,) and catechin (20 and 40 mg/kg, p.o.,) showed reversal of myonecrosis. Doses of quercetin and catechin perse treated rats had no significant reversal of toxic effects on cardiac architecture.

## Conclusion

The cardioprotective effect of quercetin and catechin were evaluated by measuring the levels of cardiac markers in serum, antioxidants in heart tissue and lipid peroxidation in both heart tissue and serum. Both drugs showed significant ($p<0.001$) antioxidant activity against isoproterenol induced myocardial infarcted rats. The probable mechanism involved might be antioxidant activity by attenuation of oxidative stress and moderate increment in antioxidant reserves, further confirmed by histopathological studies.

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**Conflicts of interest:** None

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