WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 9, 1502-1516. Research Article ISSN 2277– 7105

# EVALUATION OF CARDIOPROTECTIVE ACTIVITY OF MYRICETIN AND KAEMPFEROL IN ISCHAEMIA–REPERFUSION-INDUCED MYOCARDIAL INFARCTION IN BOTH NORMAL AND STREPTOZOTOCIN-INDUCED TYPE I DIABETIC RATS

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Article Received on
01 Sept 2014,
Revised on 22 Sept 2014,
Accepted on 14 Oct 2014

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## ABSTRACT

Revascularization therapy is the mainstay of treatment in the management of myocardial infarction in normal and diabetic patients. We attempted to evaluate the cardioprotective actions of myricetin and kaempferol in ischaemia–reperfusion-induced myocardial infarction in both normal and diabetic rats. Myocardial infarct size was measured using the staining agent 2,3,5- triphenyl tetrazolium chloride. Serum and tissue malondialdehyde levels, superoxide dismutase and catalase in heart tissue were estimated spectrophotometrically. A lead II electrocardiogram was monitored at various intervals throughout the experiment. Results demonstrated the larger infarct size, enhanced lipid peroxidation, partial depletion of antioxidant enzymes and drastic drop in heart rate in diabetic hearts subjected to in-vivo ischaemia–reperfusion in comparison to normal rats subjected to ischaemia–reperfusion. Furthermore, myricetin and kaempferol significantly limit the infarct size in both normal and diabetic animals in a similar fashion. However, kaempferol offered complete cardioprotection at a dose of 10 mg/kg in terms of limiting infarct size. Both flavonoids could partially but significantly attenuate the lipid peroxidation. In addition, treatment has shown moderate improvement in heart rate in both normal and diabetic rats. Our data suggest the possible cardioprotective effects of myricetin and kaempferol in ischaemia–reperfusion injury in both normal and diabetic rats, and that protection might be in part due to the attenuation of oxidative stress and moderate increment in antioxidant reserves.

**KEYWORDS:** cardioprotection; flavonoids; free radicals; reperfusion injury; myricetin; kaempferol.

## INTRODUCTION

Revascularization therapy is the mainstay of treatment of myocardial infarction in normal and diabetic patients. In the light of the poor clinical outcomes of revascularization procedures, reperfusion injury has gained more importance in cardiovascular research. Reperfusion may be considered a double-edged sword: although it is essential for the survival of the tissue, it paradoxically results in some new cellular damage that blunts the beneficial effects of reperfusion itself. One of the major therapeutic goals of modern cardiology is therefore to design strategies aimed at salvaging the myocardium from the ravages of reperfusion injury and improve the benefits of reperfusion therapy. Since diabetic patients represent a high-risk group for the development of ischaemic heart disease, they constitute a growing segment of the population undergoing coronary revascularization surgical procedures.[1–3] Recent reports indicate that patients with diabetes account for approximately 10–20% of patients currently undergoing coronary artery bypass grafting and percutaneous transluminal coronary angioplasty procedures.[1,3,4] Of numerous mechanisms implicated in the pathobiology of reperfusion injury, oxidative stress has been suggested as a central mechanism of cellular damage in ischaemia– reperfusion (I/R) injury. Formation of free radicals in a biological system includes xanthine oxidase,[5] activated neutrophils,[6] direct donation of an electron from myocardial electron transport chain,[7] catecholamine oxidation,[8] cyclo-oxygenase and lipoxygenase enzymes.[9] Since free radical mediated injury is a potential threat to a viable myocardium,[10] oxidative damage should be limited and the antioxidant defence mechanism reinforced by treating with antioxidants. Indeed, several clinical studies[11–13] and also some experimental studies[14–16] have reported that administration of antioxidants resulted in beneficial outcomes in I/R injury. Research on flavonoids has been triggered by the discovery of the ‘French paradox’ whereby populations with high red wine consumption have a relatively low cardiovascular mortality rate.[17] The flavonoids in red wine are responsible, at least in part, for this effect.[18] Furthermore, epidemiologic studies suggest a protective role of dietary flavonoids against coronary heart disease.[19] The association between flavonoid intake and the long-term effects on mortality was studied subsequently[20] and it has been suggested that flavonoid intake is inversely correlated with mortality due to coronary heart disease. The high lipid solubility of flavonoids allows them easy access into the cell, where free radicals do most damage. In addition they have wide applicability (antioxidant,

antiinflammatory, free radical scavenging and antiplatelet aggregatory effects) in cardiovascular research. These two factors prompted us to evaluate their cardioprotective activity in I/R injury. Flavonoids such as myricetin and kaempferol have received much attention in the area of nutritional biology. In spite of the tremendous potential of these two compounds to protect the heart during I/R, only a few studies have reported on such effects. In addition, the cardioprotective mechanisms of myricetin and kaempferol have not been explored so far in I/R of diabetic hearts. The objective of the present investigation was therefore to evaluate the cardioprotective actions of the flavonoids myricetin and kaempferol against I/R injury in both diabetic and normal rats. It may not always be feasible to administer flavonoids as a chronic pretreatment before revascularization procedures. An acute treatment model, just at the point of reperfusion, is therefore of greater interest and the present study used the acute treatment model.

## MATERIALS AND METHODS

Drugs and chemicals Streptozotocin (STZ), myricetin and kaempferol, 1, 1, 3, 3-tetraethoxypropane were purchased from the Sigma Chemical Company (St Louis, USA). 2, 3, 5-triphenyl tetrazolium chloride (TTC) was purchased from BDH chemicals Ltd (England). Thiopentone sodium was supplied by Abott Lab Ltd (Ankleshwar, India). Nitroblue tetrazolium, NADH, reduced glutathione, oxidized glutathione, 1-chloro-2, 4-dinitrobenzene, Folin's phenol reagent, Ellman's reagent and TritonX-100 RS were purchased from Sisco Research Laboratories Pvt Ltd (Mumbai, India). Phenazine methosulphate was purchased from National Chemicals (Vadodara, India). All other chemicals and reagents were used of analytical grade.

## ANIMALS

Albino Wistar rats (National Institute of Nutrition, Hyderabad, India), of either sex, weighing 200–250 g, were selected. Animals were maintained under standard laboratory conditions at 25 ± 2∞C, relative humidity 50 ± 15% and normalphotoperiod (12 h dark/12 h light). Commercial pellet diet (Rayon's Biotechnology Pvt Ltd, India) and water were provided ad libitum. The experimental protocol has been approved by the Institutional Animal Ethics Committee and by the Animal Regulatory Body of the Government **(Regd. No. P6/VCP/IAEC/2012/4/AE13).**

**Experimental design**

The rats were randomly divided into 14 groups, of which seven were normal groups and seven diabetic groups. The usage pattern of number of rats for the determination of biochemical parameters was as follows. n = 6 for percentage left ventricle necrosis and malondialdehyde(MDA) levels, n = 5 for heart rate, superoxide dismutase (SOD) and catalase levels. Group 1, sham control group; group 2, control I/R group treated with saline (0.2 ml); group 3, vehicle control I/R group treated with 0.2 ml of 50% dimethyl sulfoxide (DMSO); groups 4 and 5 were treated with myricetin 5 and 10 mg/kg, respectively; groups 6 and 7 were treated with kaempferol 5 and 10 mg/kg, respectively; group 8, diabetic sham control group; group 9, diabetic control I/R group treated with saline; group 10, diabetic vehicle control group treated with DMSO; groups 11 and 12, diabetic control I/R treated with myricetin at doses of 5 and 10 mg/kg, respectively; groups 13 and 14, diabetic control I/R treated with kaempferol at doses of 5 and 10 mg/ kg, respectively. Myricetin and Kaempferol were dissolved in 50% DMSO and administered intraperitoneally (i.p.) 10 min before reperfusion.

**Induction of diabetes**

Diabetes was induced by a single i.v. injection of STZ, 45 mg/kg of body weight, dissolved in citrate buffer (pH 4.5), into the tail vein of animals lightly anaesthetized with ether. Age-matched rats were injected with citrate buffer only. Diabetes was confirmed after the third day of STZ injection by estimation for serum glucose by an autoanalyzer (Dade Behring, USA). Following 2 weeks of diabetes induction, rats were subjected to the surgical procedure. About 20% mortality was observed in diabetic rats even before being subjected to I/R injury. Diabetic rats showing more than 350 mg/dl of serum glucose concentration were used for the experiment.

**Surgical procedure**

Rats were anaesthetized with thiopentone sodium (30 mg/kg, i.p.), tracheotomized and ventilated with room air by a Techno positive pressure mechanical respirator (animal respirator, Crompton Parkinson Ltd, UK). The right jugular vein was cannulated in order to inject drugs. A left thoracotomy and pericardiotomy were performed, and the left coronary artery was dissected free above the first diagonal branch and ligated just below the origin of left circumflex artery with the help of a silk thread (6–0). The artery was occluded for 30 min

by a knot. The silk thread was removed after 30 min with the help of two knot releasers to allow reperfusion of the heart for the next 4 h.

**Electrocardiograph**

After surgical preparation, rats were allowed 10 min for stabilization and then control measurements of ECG were taken. The rats were then subjected to a 30 min occlusion. At 15 min of occlusion the ECG was taken again. Measurements of ECG were repeated immediately after release of the occlusion and at 1, 2, 3 and 4 h intervals for all the groups of animals. A lead II electrocardiogram was monitored using Cardiart 408 (BPL) with 20 mm/mV sensitivity at a paper speed of 50 mm/s. Heart rates were expressed as beats per minute.

**Quantification of infarct size**

In all the groups, after sacrificing the animal by giving excess anaesthesia, the heart was rapidly excised from the thorax and the greater vessels were removed. The left ventricle was separated from the heart and weighed. It was sliced parallel to the atrioventricular groove into 2–3 mm thick sections and the slices were incubated in 1% TTC solution, prepared in phosphate buffer pH 7.4, for 30 min at 37∞C.[21] In the viable myocardium, TTC is converted by dehydrogenases to a red formazan pigment that stains tissue dark red.[22] The infracted myocardium, which does not take TTC stain where the dehydrogenases are drained off, remains pale in colour. The pale necrotic tissue was separated from the stained portions and weighed on an electronic balance (Dhona 200D, Dhona instruments Ltd, India). Infarct size was calculated as a percentage of necrotic tissue of the left ventricle.

**Determination of malondialdehyde levels in serum**

In all the groups, before sacrificing the animal at the end of 4 h of reperfusion, 2 ml of blood sample was collected from the left ventricle for the estimation of malondialdehyde (MDA) levels in blood serum. Serum MDA levels were estimated by the method developed by Yagi.[23] Tetraethoxypropane (0.5, 1, 2, 4, 6, 8 and 10 nmol) served as the external standard. MDA levels in serum were expressed as nanomoles per millilitre of serum.

**Determination of malondialdehyde levels in myocardium**

MDA levels in the myocardium were measured by the method developed by Ohkawa et al.[24] Briefly, the infracted left ventricular tissues were homogenized with 1.15% KCl (10% w/v). The assay mixture consisted of 0.1 ml of tissue homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of

20% acetic acid (adjusted to pH 3.5 with NaOH) and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid (TBA). This was heated for 60 min at 95∞C. Thereafter, the mixture was cooled and extracted with 5 ml of a mixture of n-butanol and pyridine (15:1, v/v). After centrifugation at 4000 rpm for 10 min, the organic phase was assayed spectrophotometrically at 532 nm. Tetraethoxypropane (2, 4, 6 and 8 nmol) served as an external standard. MDA levels in myocardium were expressed as nanomoles per gram of tissue.

**Determination of superoxide dismutase in myocardium**

The SOD level was estimated by the method described by Kakkar et al.[25] The assay mixture contained 0.1 ml of sample, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of phenazine methosulphate (186 mM), 0.3 ml of 300 mM nitro blue tetrazolium and 0.2 ml of NADH (750 mM). Reaction was started by addition of NADH. After incubation at 30∞C for 90 s, the reaction was stopped by the addition of 0.1 ml of glacial acetic acid. The reaction mixture was stirred vigorously with 4.0 ml of n-butanol. The mixture was allowed to stand for 10 min, centrifuged and the butanol layer was separated. The colour intensity of the chromogen in the butanol was measured spectrophotometrically at 560 nm and the concentration of SOD was expressed as units per milligram of protein.

**Determination of catalase in the myocardium**

The catalase (CAT) level was measured by the method of Aebi.[26] Supernatant (0.1 ml) was added to a cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM $H_2O_2$. The rate of decomposition of $H_2O_2$ was measured spectrophotometrically from changes in absorbance at 240 nm. The activity of CAT was expressed as micromoles of $H_2O_2$ metabolized per milligram of protein per minute.

**Statistical analysis**

The results are expressed as mean ± SD. Differences in infarct size, serum and tissue lipid peroxide levels, SOD and CAT were determined by factorial one-way analysis of variance. Individual groups were compared using Tukey's test. Heart rate data were tested for differences between groups by using a two-way ANOVA for repeated measurements followed by a Bonferroni post test when the differences were significant. Differences with $P < 0.05$ were considered statistically significant.

## RESULTS

### CONSOLIDATED TABLE – 1.

| GROUP | PLVN (%) | Serum MDA (nmol/ml of serum) | Tissue MDA (nmol/gwet tissue) | SOD (U/mg protein) | Catalase Decomposed ( µ moles) |
|---|---|---|---|---|---|
| Normal Sham Control | 4.30±0.30 | 2.16±0.10 | 4.16±0.14 | 25.19±0.47 | 26.64±0.50 |
| Normal Control I/R | 47.42±0.75 | 26.25±0.39 | 119.03±0.68 | 13.07±0.38 | 12.22±0.14 |
| Normal Veh Control I/R | 46.86±0.59* | 25.98±0.30* | 100.88±0.49 | 13.36±0.37* | 12.50±0.15* |
| Normal Myrc 5mg | 21.59±0.62 | 18.89±0.25 | 61.25±0.53 | 16.38±0.34 | 16.57±0.31 |
| Normal Myrc 10mg | 8.88±0.30 | 11.94±0.44 | 25.85±0.23 | 22.22±0.75 | 22.08±0.28 |
| Normal Kaemp 5mg | 20.03±0.49 | 17.87±0.25 | 53.43±0.23 | 17.13±0.50 | 16.65±0.34 |
| Normal Kaemp 10mg | 3.09±0.20*** | 9.90±0.36 | 22.50±0.40 | 22.17±0.55 | 23.45±0.38 |
| Diab Sham Control | 8.88±0.35 | 5.98±0.19 | 13.56±0.79 | 22.22±0.31 | 21.24±0.49 |
| Diab Control I/R | 57.36±0.35 | 53.31±0.46 | 152.41±0.38 | 11.47±0.24 | 10.67±0.22* |
| Diab Veh control I/R | 53.12±0.75 | 48.93±0.30 | 140.17±0.70 | 11.23±0.36* | 12.54±0.42+ |
| Diab Myrc 5mg | 31.27±0.69 | 32.07±0.76 | 93.18±0.55 | 13.00±0.28 | 15.21 ±0.06 |
| Diab Myrc 10mg | 11.95±0.42 | 17.24±0.28 | 40.26±0.91 | 18.66±0.30 | 18.51±0.30 |
| Diab Kaemp 5mg | 26.98±0.28 | 30.94±0.39 | 90.96±0.63 | 14.23±0.34 | 15.78±0.37 |
| Diab Kaemp 10mg | 7.04±0.22+++ | 16.47±0.35 | 30.95±0.49 | 19.34±0.39 | 19.56±0.28 |

Units for catalase are expressed as micromoles of $H_2O_2$ decomposed per milligram of protein per minute. All the values are expressed as mean±S.D (n=6 for PLVN and MDA levels, n=5 for SOD and CAT). PLVN-Percent Left Ventricle Necrosis; MDA-Malanoldehyde; SOD-Superoxide dismutase; CAT-Catalase; Norm-Normal; Diab-Diabetic; Myrc-Myricetin; Kaemp-Kaempferol; Veh-Vehicle. *P<0.05, Not significant vs Normal Control I/R, **P<0.05, Not significant vs Normal vehicle control I/R, ***P<0.05, Not significant vs Normal sham control. $^{+}$P<0.05, Not significant vs diabetic control I/R, $^{++}$P<0.05, Not significant vs Vehicle diabetic control I/R, $^{+++}$P<0.05, Not significant vs diabetic sham control.

### 1. DETERMINATION OF PLVN

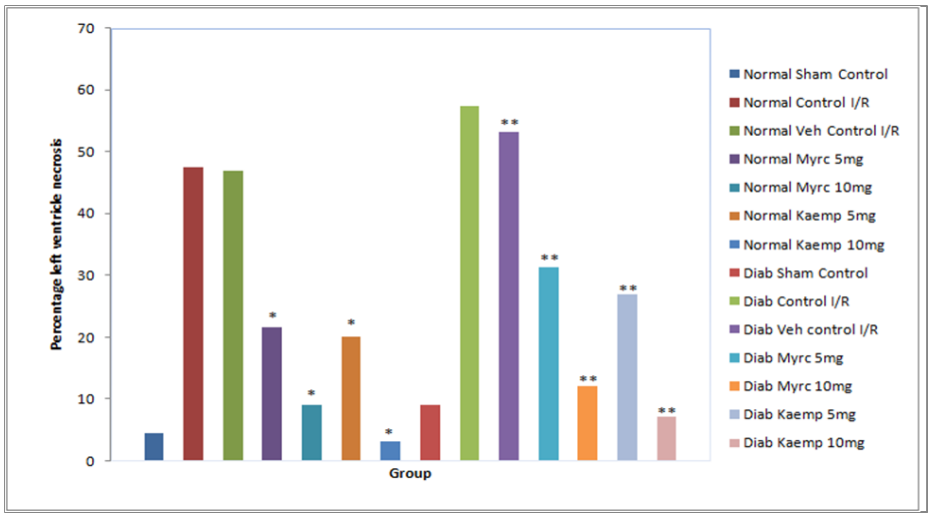

**Figure 1- Effect of myricetin and kaempferol on left ventricle necrosis of both normal and diabetic experimental group animals subjected to ischaemia reperfusion injury. All values are expressed as mean ± SD (n = 6). I/R, ischaemia-reperfusion; Norm vehi, normal vehicle-treated; Norm myrc,normal myricetin; Diab vehi, diabetic vehicle-treated; Diab myrc, diabetic myricetin. *P < 0.05 vs control I/R. **P < 0.05 vs diabetic control I/R.**

## DISCUSSION

In normal control animals (group 2) subjected to I/R injury the infarct size was found to be 47.42 ± 0.75% and statistically significant (P < 0.05) compared to sham control animals (group 1). In vehicle-treated animals, infarct size was found to be 46.86 ± 0.59% (group 3). There was no significant difference between the infarct size in the vehicle group (group 3) and the normal control group (group 2). Treatment with myricetin (5 and 10 mg/kg) (groups 4 and 5) significantly diminished the infarct size to 21.59 ± 0.62% and 8.88 ± 0.30%, respectively. Treatment with kaempferol (5 and10 mg/kg) (groups 6 and 7) significantly diminished the infarct size to 20.30 ± 0.49% and 3.09 ± 0.20%, respectively. It is noteworthy that kaempferol at a dose level of 10 mg/kg almost diminished the infarct size (3.09 ± 0.20%) to the level of the infarct size (4.30 ± 0.30%) of the sham control group. Furthermore, there was no significant difference between the infarct sizes of the group treated with kaempferol at 10 mg/kg (group 7) and the normal sham control group (group 1) (see Figure 1 and Table 1). In diabetic control animals subjected to I/R injury, the infarct size was significantly increased to 57.36 ± 0.35% (group 9) when compared to normal control animals (47.42 ± 0.75%) (group 2) and diabetic sham control animals (8.88 ± 0.35%) (group 8). In vehicle-treated diabetic animals, infarct size was significantly reduced to 53.12 ± 0.75% (group 10). Treatment with myricetin (5 and 10 mg/kg) (groups 11 and 12) significantly diminished the infarct size to 31.27 ± 0.69% and 11.95 ± 0.42%, respectively. Treatment with kaempferol (5 and 10 mg/kg) (groups 13 and 14) significantly diminished the infarct size to 26.98 ± 0.28% and 7.04 ± 0.22%, respectively. A similar pattern of complete cardioprotection was also observed in diabetic animals. This was clearly evident in that there was no significant difference between infarct sizes in the kaempferol 10 mg/kg treated group (7.04 ± 0.22%) (group 7) and the diabetic sham control group (8.88 ± 0.35%, group 8) (Figure 1). It is noteworthy that kaempferol at a dose level of 10 mg/kg diminished the infarct size almost to the level of the infarct size (4.30 ± 0.30%) of the sham control group.

## 2. DETERMINATION OF MYOCARDIUM LEVELS IN SERUM AND MYOCARDIUM

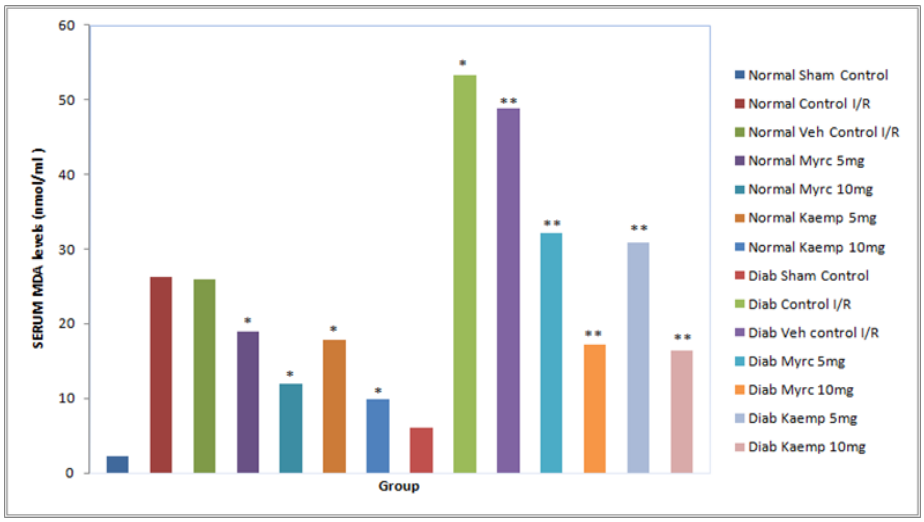


**Figure 2)A): Effect of myricetin and kaempferol on serum malondialdehyde levels of both normal and diabetic experimental group animals subjected to ischaemia-reperfusion injury. All values are expressed as mean ± SD (n = 6). MDA, malondialdehyde; I/R, ischaemia-reperfusion; Norm vehi,normal vehicle-treated; Norm myrc, normal myricetin; Diab vehi, diabetic vehicle-treated; Diab myrc, diabetic myricetin. *P < 0.05 vs control I/R. **P < 0.05 vs diabetic control I/R.**

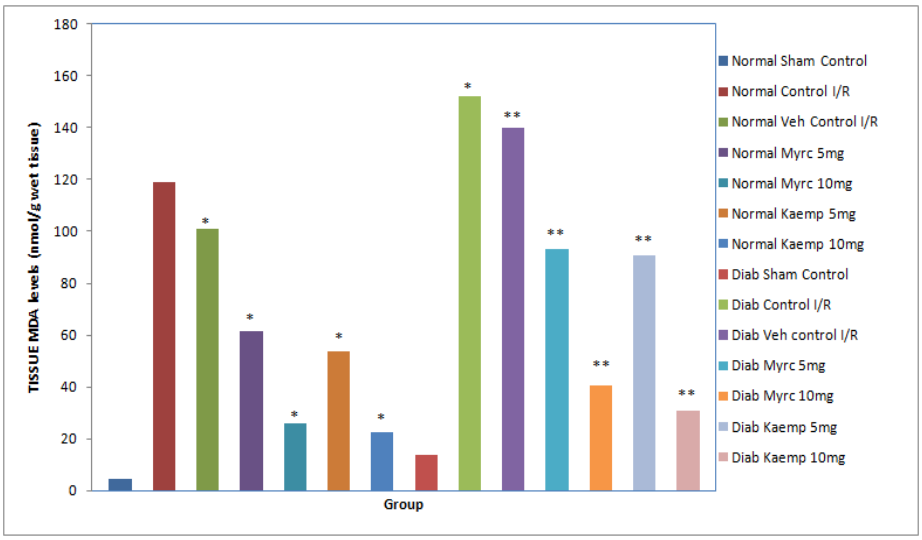


**Figure 2)B) Effect of myricetin and kaempferol on tissue malondialdehyde levels of both normal and diabetic experimental group animals subjected to ischaemia-reperfusion injury. All values are expressed as mean ± SD (n = 6). MDA, malondialdehyde; I/R, ischaemia-reperfusion; Norm vehi,normal vehicle-treated; Norm myrc, normal**

**myricetin; Diab vehi, diabetic vehicle-treated; Diab myrc, diabetic myricetin. *P < 0.05 vs control I/R.**P < 0.05 vs diabetic control I/R.**

## DISCUSSION

Malondialdehyde is the end product of lipid peroxidation. There action of lipid peroxides with TBA has been widely adopted as a sensitive assay method for lipid peroxidation. MDA levels in serum and heart tissue in normal control animals were found to be 26.25 ± 0.39 nmol/ml (Figure 2-A) and 119.03 ± 0.68 nmol/g tissue (Figure 2-B), respectively, and statistically significant (P < 0.05) compared to the sham control group. In the normal vehicle control group, MDA levels in serum and tissue were found to be 25.98 ± 0.30 nmol/ ml and 100.88 ± 0.49 nmol/g tissue. In the vehicle-treated group there was a significant reduction in tissue MDA levels (P < 0.05) but the same was not observed for serum MDA levels. Myricetin treatment with 5 mg/kg significantly (P < 0.05) reduced the serum and tissue MDA levels to 18.89 ± 0.25 nmol/ml and 61.25 ± 0.53 nmol/g tissue, respectively. With myricetin at 10 mg/kg, there was a significant (P < 0.05) further reduction in the serum and tissue MDA levels to 11.94 ± 0.44 nmol/ml and 25.85 ± 0.23 nmol/g tissue, respectively. Kaempferol treatment at 5 mg/kg significantly (P < 0.05) reduced the serum and tissue MDA levels to 17.87 ± 0.25 nmol/ml and 53.43 ± 0.23 nmol/g tissue, respectively. With myricetin at 10 mg/kg, there was a significant (P < 0.05) further reduction in the serum and tissue MDAlevels to 9.90 ± 0.36 nmol/ml and 22.50 ± 0.40 nmol/g tissue, respectively (see Table 1). MDA levels in serum and heart tissue in diabetic sham control animals slightly increased to 5.98 ± 0.19 nmol/ml and 13.56 ± 0.79 nmol/g tissue when compared to the normal sham control group. MDA levels in serum and heart tissue in diabetic control animals were significantly raised to 53.31 ± 0.46 nmol/ml (Figure 2-A) and 152.41 ± 0.38 nmol/g tissue (Figure 2-B), respectively, when compared with normal control animals as well as the diabetic sham control group. In diabetic animals subjected to I/R injury, myricetin treatment at 5 mg/kg significantly (P < 0.05) reduced the serum and tissue MDA levels to 32.07 ± 0.76 nmol/ml and 93.18 ± 0.55 nmol/g tissue, respectively. With myricetin at 10 mg/kg, there was a a significant (P < 0.05) further reduction in the serum and tissue MDA levels to 17.24 ± 0.28 nmol/ml and 40.26 ± 0.91 nmol/g tissue, respectively. Kaempferol treatment at 5 mg/kg significantly (P < 0.05) reduced the serum and tissue MDA levels to 30.94 ± 0.39 nmol/ml and 90.96 ± 0.63 nmol/g tissue, respectively. With myricetin at 10 mg/kg, there was a significant (P < 0.05) further reduction in the serum and tissue MDA levels to 16.47 ± 0.35 nmol/ml and 30.95 ± 0.49 nmol/g tissue, respectively.

## III. DETERMINATION OF ANTI OXIDANT ENZYMES

### 3) A): DETERMINATION OF SUPEROXIDE DISMUTASE (SOD)

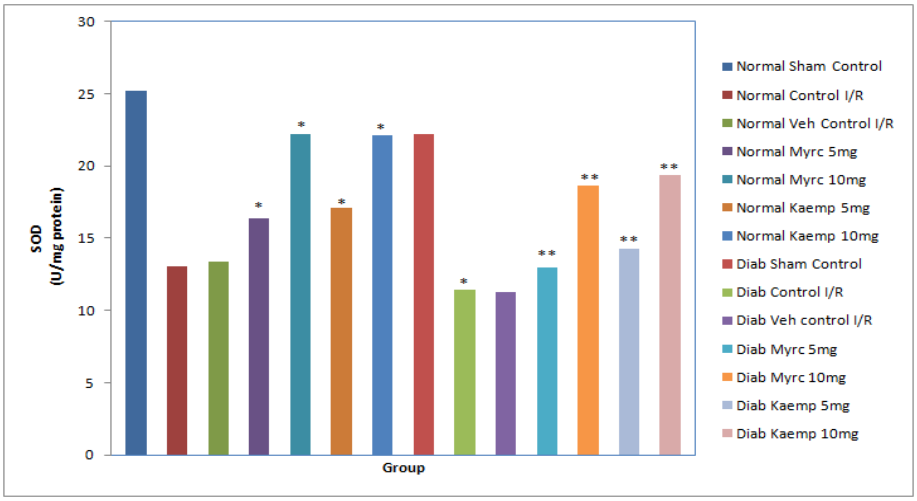


**Figure 3)A): Effect of myricetin and kaempferol on superoxide dismutase levels of both normal and diabetic experimental group animals subjected to ischaemia-reperfusion injury. All values are expressed as mean ± SD (n = 5). SOD, superoxide dismutase; I/R, ischaemia-reperfusion; Norm vehi,normal vehicle-treated; Norm myrc, normal myricetin; Diab vehi, diabetic vehicle-treated; Diab myrc, diabetic myricetin. *P < 0.05 vs control I/R.**P < 0.05 vs diabetic control I/R.**

### 3) B): DETERMINATION OF CATALASE

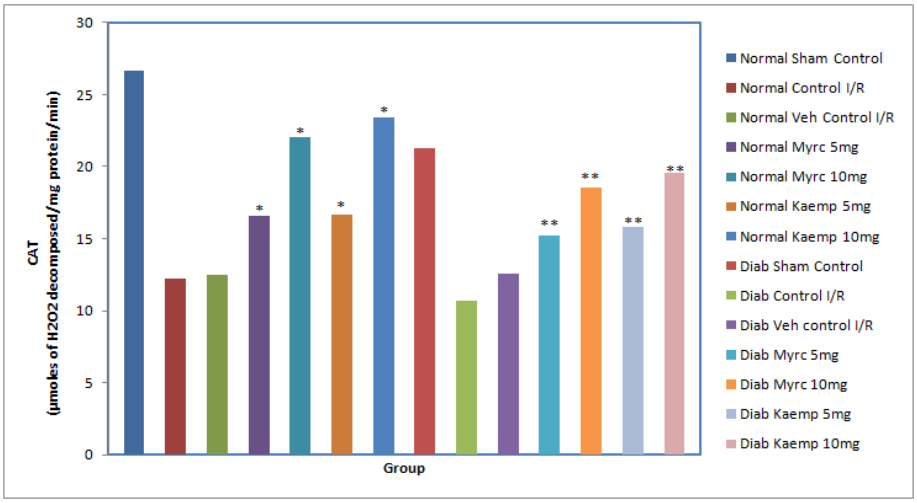


**Figure 3)B) Effect of myricetin and kaempferol on catalase levels of both normal and diabetic experimental group animals subjected to ischaemia-reperfusion injury. All values are expressed as mean ± SD (n = 5). CAT, catalase; I/R, ischaemia-reperfusion; Norm vehi, normal vehicle-treated; Norm myrc, normal myricetin; Diab vehi, diabetic vehicle-treated; Diab myrc, diabetic myricetin. *P < 0.05 vs control I/R. **P < 0.05 vs diabetic control I/R.**

## DISCUSSION

In control group animals, the levels of endogenous antioxidant enzymes such as SOD and CAT in both normal and diabetic heart tissue were reduced to 13.07 ± 0.38, 12.22 ± 0.14, 11.47 ± 0.24 and 10.67 ± 0.22, respectively (Figures 3-A, 3-B), and the differences were statistically significant ($P < 0.05$) compared to normal and diabetic sham control animals. In vehicle-treated animals, there was no significant increase in SOD and CAT in both normal and diabetic animals. Myricetin and kaempferol treatment significantly ($P < 0.05$) prevented the depletion of SOD and CAT in both normal and diabetic animals. The remaining data are shown in Figures 3-A and 3-B, and Table 1.

**TABLE- 2: HEART RATE RECORDED AT VARIOUS STAGES OF MYOCARDIAL OCCLUSION AND REPERFUSION**

| Group | BO | MOP | IAR | 1h AR | 2h AR | 3h AR | 4h AR |
|---|---|---|---|---|---|---|---|
| Normal Sham Control | 418±12 | 417±25 | 418±23 | 422±23 | 421±20 | 421±26 | 418±25 |
| Normal Control I/R | 412±13 | 335±14 | 325±28 | 336±27 | 335±21 | 342±24 | 340±25 |
| Normal Vehi control I/R | 430±20* | 343±19 | 340±5 | 345±24 | 338±15 | 340±22 | 351±22 |
| Normal Myrc 5mg | 422±28* | 320±27 | 328±21 | 348±20 | 346±27 | 360±28* | 373±21* |
| Normal Myrc 10mg | 417±25* | 330±21 | 331±19 | 370±34 | 368±21 | 381±21 | 384±20* |
| Normal Kaemp 5mg | 405±20* | 323±19 | 318±20 | 338±25 | 350±24 | 363±28 | 398±21* |
| Normal Kaemp 10mg | 408±18* | 314±16 | 341±20 | 349±21 | 365±24 | 375±10 | 390±18 |
| Diab Sham Control | 361±41 | 360±20 | 360±22 | 358±26 | 357±32 | 360±32 | 358±25 |
| DiabControl I/R | 358±22** | 270±15 | 263±20 | 267±25 | 251±18 | 253±14 | 240±11 |
| Diab Vehi Control I/R | 364±19** | 280±14 | 281±18 | 272±22 | 262±24 | 250±14 | 245±18 |
| Diab Myrc 5mg | 382±21** | 292±15 | 291±18 | 295±24 | 298±18 | 308±18 | 312±20** |
| Diab Myrc 10mg | 393±15** | 283±20 | 287±28 | 293±32 | 285±21 | 322±18 | 324±28** |
| Diab Kaemp 5mg | 371±22** | 278±14 | 280±15 | 281±22 | 301±34 | 301±20 | 318±21** |
| Diab Kaemp 10mg | 388±31** | 252±15 | 250±16 | 266±21 | 315±24** | 340±16 | 314±20** |

Values are expressed as mean ± SD and expressed as beats per minute. BO, before occlusion; MOP, middle occlusion period; IAR, immediately afterreperfusion; AR, after reperfusion. Norm, normal; Diab, diabetic; quer, myricetin; veh, vehicle. *Not significant compared to normal sham control; **Not significant compared to diabetic sham control.

## DISCUSSION

In the normal control group, a continuous decrease in heart rate was observed during the 30 min of coronary artery ligation and throughout the reperfusion period compared to the sham control group (Table 2). In case of diabetic rats, there was a drastic drop in heart rate, particularly during the 30 min coronary artery ligation and throughout the reperfusion period. This result is in agreement with the previous study of Kolchin in which heart rate decreased during I/R. The groups treated with myricetin and kaempferol at doses of 5 and 10 mg/kg

showed a slight decrease in heart rate during the 30 min of coronary artery ligation, with a gradual increase throughout the reperfusion period. It was not possible to restore the heart rate to normal values at the end of 4 h in either diabetic or non diabetic rats (Table 2). In another experimentally induced myocardial infarction in dogs by 60-min occlusion of the coronary artery with subsequent 24-h reperfusion, the administration of myricetin solution was found to improve the contractile function of the left ventricular myocardium, decrease the incidence of heart rate and conductivity disorders, limit the ischaemic damage area, promote the preservation of the vessels' integrity, improve coronary circulation and prevent intravascular thrombus formation.

## CONCLUSION

The data strongly suggest that there is a possible cardioprotective action of myricetin and kaempferol in I/R-induced myocardial infarction in normal and diabetic rats. Protection is due in part to the attenuation of oxidative stress and a slight increment in antioxidant enzymes. Further studies are warranted to explore other mechanisms that contribute to the cardioprotective effects of myricetin and kaempferol.

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