Research Article

# Ameliorative Effect of Galangin on Isoproterenol Induced Myocardial Infarction in Rats

**Naveen Elangovan, Jeevitha Palanisamy,**

**Diviya barathi Mathialagan and Murugesan Madhesh***

Department of Biochemistry, Sivaraj Siddha Medical College, Salem, Tamil Nadu, 636 307, India.

**ABSTRACT**

This study evaluates whether the galangin, a dietary flavanoids have many vital role in the treatment of cardiovascular disease. Pretreatment with galangin from *alpinia officinarum* prevents the elevation of serum marker enzymes and their oxidative stress markers like lipid peroxidase, glutathione, catalase and superoxide dismutase are get altered by ISO induced myocardial infarction in rats. The rats were divided into four groups (n=6). Group 1 received 0.5% CMC treated as normal control group. Group 2 received isoproterenol (85mg/kg body weight) intraperitoneal (i.p) for two consecutive days ($14^{th}$ and $15^{th}$ days). Group 3 received Galangin (20 mg/kg b.wt) intragastric intubation for 15 days. Group 4 rats received Galangin as in Group 3 and additionally isoproterenol was given for two consecutive days ($14^{th}$ and $15^{th}$ days). The isoproterenol-induced rats indicated increase in the level of TBARS and decreased in the activities of serum and tissue antioxidants in MI rats. On treatments with Galangin at a daily dose of (20mg/kg b.wt) showed significantly decrease in the levels of serum and tissue lipid per-oxidation, raise in the antioxidant levels and also diminish in the marker enzymes. The present study exposed that Galangin ameliorates lipid peroxidation metabolism due to its free radical Scavenging, antioxidants.

**Key words:** Galangin, Isoproterenol and antioxidants.

## INTRODUCTION

Cardiovascular diseases are a heterogeneous group of disorders that affect the heart and blood vessels. It is still one of the main causes of death in spite of advances in diagnostic and therapeutic procedures.[1] Myocardial infarction (MI) is a common presentation of ischemic heart disease (IHD). World Health Organisation (WHO) predicts that Myocardial Infarction is the leading causes of death worldwide by the year 2020.[2] Whilst this prediction, on the other side of the world, developing countries like India is getting more vulnerable to non-communicable diseases owing to changing lifestyle such as increased junk food consumption, reduced physical activities and lack of proper exercises. It is a clinical syndrome arising from sudden and persistent curtailment of myocardial blood supply resulting in necrosis of the myocardium. Myocardial infarction usually results from abrupt reduction in coronary blood flow to a segment of a myocardium, which initiates a continuum of progressively more severs cellular changes that unless interrupted by culminate in cell death and tissue necrosis.[3] Isoproterenol (ISO), a synthetic catecholamine and beta-adrenergic agonist that cause severe stress in myocardium and infarct like necrosis of heart muscle.[4] It induces myocardial necrosis by a multiple step mechanism and has been reported to show metabolic and morphologic aberrations in the heart tissue of experimental animals similar to those observed in humans.[5] Higher levels of catecholamines deplete the energy reserve of cardiac muscle cells, leading to complex biochemical and structural changes that cause irreversible cellular damage that ultimately necrosis.[6] It is a acute condition of myocardial necrosis which causes cardiac dysfunction, increased lipid peroxidation, altered activity of cardiac enzymes and antioxidant.[7]

Free radicals are produced in cells by cellular metabolism and by exogenous agents. This species react with biomolecule in cells, including DNA. The resulting damage to DNA, which is also called as oxidative damage to

DNA, is implicated in myocardial necrosis, mutagenesis, carcinogenesis and aging.[8] Oxidative stress produced by free radicals are reactive oxygen species (ROS) as evidenced by marked increase in production of lipid peroxidative products and transient inhibition of endogenous antioxidant defense such as superoxide dismutase (SOD), catalase (CAT) and reduced glutathione(GSH) has been shown to underlie myocardial damage during myocardial infarction.

Natural products are known to possess wide range of biological activity. Flavonoids and polyphenolic compounds are the active antioxidant principles found in large number of natural products. Consumption of these plant products certainly prevents the free radical mediated damage in the cell and therefore protects the body from several health problems.[9] Several epidemiological studies have supported the hypothesis that the antioxidant actions of flavanoids may reduce the risk of developing cardio vascular disease.[10]

Galangin is naturally occurring flavanoid found in alpinia officinarum (lesser galanga). Flavanoids are well known antioxidant, which can protect cells from being damaged from free radicals.[11,12] Galangin has antioxidant[13,] anti-obesity[14,] anti-cancer activity and antiviral[15] properties. Galangin has also shown anti-inflammatory properties. [16] Thus, an attempt was made to investigate its cardioprotective effect on myocardial necrosis induced by isoproterenol with reference to cardiac markers, lipid peroxidation, antioxidant enzymes, non-enzymatic antioxidants, lipid profile.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

Isoproterenol hydrochloride was purchased from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used were of analytical grade.

### 2.2. Formulation and administration of galangin

Galangin powder was suspended in 0.5% Carboxymethyl Cellulose (CMC) and each animal belonging to three different groups received 1.0 ml of galangin suspension at a dose of 20 mg/kg body weight everyday respectively by intragastric intubation.[17]

### 2.3. Induction of Myocardial Infarction

Myocardial Infarction was induced by intraperitoneal (i.p.) injection of isoproterenol hydrochloride (85 mg/kg body weight) on 14$^{th}$ and 15$^{th}$ days.[18]

### 2.4. Animal Housing and Diets

Male Wistar albino rats aged 6 weeks and weighing about 150g were obtained from Sri Venkateshwara Enterprises Bangalore, India. After one week of acclimatization all animals were housed six per polypropylene plastic cage covered with metal grids and a hygenic bed of husk in a specific-pathogen free animal room under controlled conditions of a 12h light/12 hour dark cycle, and provided with standard food pellets (diet composition, wheat broken-moisture 9.0%, crude protein, 11.5% crude fat, 1.9% crude fibre 4% ash 0.2%, nitrogen-free extract 73.4%) supplied by Hindustan Lever Ltd, Mumbai, India) and tap water *ad libitum*. The study was carried on after getting a clearance from the Institutional Animal Ethical Committee (IAEC) (Reg .no P.Col/52/2010/IAEC/VMCP) of Vinayaka Mission College of Pharmacy, Salem, Tamil Nadu.

#### 2.4.1. Experimental Design

The rats in group I obtained 1.0 ml of 0.5% CMC daily via intragastric intubation and served as the untreated control. The rats in group II received galangin via intragastric intubation at a daily dose of (20 mg/kg body weight) respectively for a period of 15 days. Group III rats received isoproterenol (85 mg/kg body weight) intraperitoneally twice at an interval of 24h on the 14$^{th}$ and 15$^{th}$ days

At the end of the experimental period, rats were sacrificed by cervical decapitation. The blood was collected and serum obtained after centrifugation were used for various biochemical estimations. Hearts were removed, cleared of blood and immediately transferred to ice cold containers containing 0.9% sodium chloride. Samples of tissues were homogenized in appropriate buffer and used for the determination of the following parameters.

#### Biochemical estimations

Lipid peroxidation was estimated by measuring the level of thiobarbituric acid reactive substances (TBARS) in tissues via the method of Niehaus and Samuelson[19] and plasma or erythrocyte via the method of Yagi.[20] The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation, was measured at 532 nm. The values are expressed as nmoles/ 100 g tissue or nmoles/mg Hb. Reduced glutathione (GSH) content was determined via the method of Moron et al.[21] GSH determination is based on the development of yellow colour when 5, 5´dithio 2-nitro benzoic acid (DTNB) is added

to compounds containing sulfhydryl groups. The values are expressed as nmoles $g^{-1}$ wet tissue. Glutathione peroxidase (GPx EC.1.11.1.9) activity was assayed via the method of Rotruck [22] with a modification: a known amount of enzyme preparation was incubated with $H_2O_2$ in the presence of GSH for a specified time period. The amount of $H_2O_2$ utilized was determined via the method of Habig et al.[23] The values are expressed as mmoles of GSH utilized/min/mg Hb or protein. Superoxide dismutase (SOD EC.1.15.1.1) was assayed using the method of Marklund and Marklund[24] based on the 50% inhibition of the formation of NADH- phenazine methosulfate-nitroblue tetrazolium formazan at 520 nm. One unit of the enzymes is taken as the amount of enzyme for 50% inhibition of NBT reduction/min/mg protein. The activity of catalase (CAT EC. 1.11.16) was determined via the method of Sinha[25]: dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide ($H_2O_2$), with the formation of perchromic acid as an unstable CAT intermediate. The chromic acetate formed was measured at 590 nm. Catalase was allowed to split $H_2O_2$ for different periods of time. The reaction was stopped at different time intervals via the addition of a dichromate acetic acid mixture, and heating the reaction mixture and measuring chromic acetate colorimetrically and determined the remaining $H_2O_2$.

The levels of cTnT and cTnI in serum were estimated using standard Kit by chemiluminenscence immunoassay (Roche Diagnostics, Switserland). Activities of AST and ALT were assayed by the method of Reitman.[26] The liberated oxaloacetate and pyruvate reacts with 2, 4-dinitrophenyl hydrazine to form 2, 4-dinitrophenyl hydrazone, which was read at 540 nm. Activities of aspartate and alanine transaminase were expressed as IU/L. The activity of lactate dehydrogenase (LDH) was assayed in serum using a commercial kit (Product No. 72351) purchased from Qualigens Diagnostics, Mumbai, India. The enzyme activity was expressed as IU/L for serum and nmol of pyruvate liberated/min/mg protein for tissue. Creatine kinase activity was assayed by the method of Okinada.[27] The enzyme activity was expressed as IU/L for serum and µmoles of phosphorus liberated/min/mg protein for tissue. Creatine kinase- MB activity was assayed in serum using a commercial kit (Product No 11207001) obtained from Agappe Diagnostics, Kerala, India. The enzyme activity in serum was expressed as IU/L.

### 2.7. Preparation of hemolysate

Blood was collected in heparinized tubes and plasma was separated by centrifugation at 2000×g for 10 min. After the separation of plasma, the buffy coat was removed and packed cells (RBCs) were washed thrice with cold physiological saline. To determine the activity of RBC antioxidant enzymes, RBC lysate was prepared by lysing a known volume of RBCs with hypotonic phosphate buffer, pH 7.4 and centrifuged at 3000×g for 10 min at 2°C to separate the hemolysate.

### 2.8. Preparation of tissue homogenate

Heart tissue was removed immediately and washed with ice-cold saline and homogenized in the appropriate buffer in a tissue homogenizer.

### 2.9. Statistical analysis

The results presented here are the means ± SD of 6 rats in each group. The results were analyzed using one-way analysis of variance [ANOVA] and the group means were compared using Duncan's multiple range test [DMRT] using SPSS version 12 for Windows. The findings were considered as statistically significant if $P < 0.05$.[28]

## 3. RESULTS

### 3.1. Body Weight Changes in experimental rats

Table 2. Shows the levels of changes in body weight and heart weight of control and experimental group of rats. There was a change of increase in heart weight to body weight in the ISO induced (group 2) as compared with the control rats (group 1). Galangin administration of ISO induced rats (group 4) prevent heart weight changes.

### 3.2. Effect of galangin on cardiac markers in the myocardial infarction rats

Table 1 depicts the effect of galangin on cardiac markers (Troponin I and T, CK, CK-MB, LDH, AST and ALT) in the control and experimental rats. The level of cardiac markers were significantly ($P < 0.05$) increased in the ISO-induced rats (group 2) as compared to the control rats (group 1). However, pretreatment with galangin reduced cardiac markers (Troponin I and T, CK, CK-MB, LDH, AST and ALT) levels near to normal levels. Galangin administration to isoproterenol treated rats (group 4) significantly ($P < 0.05$) decreased the levels of cardiac markers (Troponin I and T, CK, CK-MB, LDH, AST and ALT) as compared to the unsupplemented ISO-induced rats (group 2).

### 3.3. Effect of galangin on lipid peroxidation in the myocardial infarction rats

Table 1 depicts the effect of galangin on circulatory and tissue levels of TBARS in the control and experimental rats. The levels of plasma, erythrocyte and tissue TBARS was significantly ($P < 0.05$) increased in the ISO-induced rats (group 2) as compared to the control rats (group 1). However, pre-treatment with galangin reduced TBARS levels to normal levels. Galangin administration to isoproterenol treated rats (group 4) significantly ($P < 0.05$) decreased in the levels of TBARS tissue erythrocyte and serum as compared with ISO-induced rats (group 2).

### 3.4. Effect of galangin on circulatory antioxidants in the myocardial infarction rats

Table 2 illustrates the effect of galangin on circulatory antioxidants (SOD, CAT, GSH and GPx) in the control and experimental rats. The circulating levels of (SOD, CAT, GSH and GPx) were significantly ($P < 0.05$) decreased in the ISO-induced rats (group 2) as compared to the control rats (group 1). However, pre-treatment with galangin (group 3) increased the circulatory antioxidants near to normal levels. galangin administration to the ISO-induced rats (group 4) was significantly ($P < 0.05$) increased the levels of antioxidants (SOD, CAT, GSH and GPx) as compared to the unsupplemented ISO-induced rats (group 2).

### 3.5. Effect of galangin on tissue antioxidants in the myocardial infarction rats

Table 3 depicts the effect of galangin on heart tissue antioxidants (SOD, CAT, GSH and GPx) of control and experimental rats. The tissue antioxidant levels (SOD, CAT, GSH and GPx) were significantly ($P < 0.05$) decreased in the ISO-induced rats (group 2) as compared to the control rats (group 1). However, pre-treatment with galangin (group 3) increased the circulatory antioxidants near to normal levels. Galangin administration in the ISO-induced rats (group 4) were significantly ($P < 0.05$) increased the levels of tissue antioxidants (SOD, CAT, GSH and GPx) as compared to the unsupplemented ISO-induced rats (group 2).

## DISCUSSION

Heart failure is a serious clinical syndrome with progressive myocardial dysfunction and a poor clinical outcome. Several studies demonstrated that oxidative and inflammation plays an important role in the progression of heart failure.[29] Our study is about the protective effects of galangin against MI induced by ISO in rats. In the present of study, the heart weights increased significant with relatively unchanged body weights following ISO administration; this contributed to the increased heart weight to the body weight ratio. The increased heart weights might be attributed to increased water content and edematous intramuscular space[30] and also for the increased free radical generation.[31,32] Increased organ wet weight is the indication of damaged and inflamed cardiac cells. Initial loss of the myocardium due to MI can induce progressive ventricular enlargement and deposition of interstitial collagen and fibrosis. However, pretreatment with galangin maintained near normal heart weights.

Cardiac markers in blood are highly useful in detecting myocardial infarction. AST, ALT, LDH and CK-MB are present in the myocardium, and these are the diagnostic markers of myocardial infarction. The elevation of these two cardiac markers in the blood is an indication of myocardial infarction. Cardiac troponin I (cTn I) is a low-molecular-weight regulatory protein of the heart muscle that controls calcium ion ($Ca^{2+}$)-mediated interactions between actin and myosin.[33] Normally, this protein is highly preserved in cardiac cells but is released upon myocardial injury, thus making this contractile protein a highly specific and sensitive diagnostic marker for MI.[34] As expected, an elevated level of cTn I was observed in rats treated with ISO alone compared with level measured for the normal controls. The results are consistent with those of previous studies.[35] Animals pretreated with galangin followed by ISO challenge, however, showed significantly lower cTn I levels compared with those measured for rats treated with ISO alone. This phenomenon may be attributed to the galangin which may contribute to the preserved structural and functional integrity of the contractile apparatus in the rat's myocardium indicated by the prevention of oxidative injury of cardiac muscles.

CK-MB isoenzyme activity is a useful early diagnostic index for MI or any type of myocardial injury. Cytosolic enzymes including CK-MB, LDH, AST, and ALT, which serve as diagnostic markers, leak out from the damaged tissue into the blood stream when the cell membrane becomes more permeable or ruptures. The amount of these cellular enzymes in the serum reflects alterations in the plasma membrane integrity and/or permeability.[36] ISO administration causes an imbalance between the supply of oxygen and myocardial hyperactivity resulting in a coronary

hypotension. As a result of a cascade of these events, the cell membrane becomes fragile and ruptured, resulting in the release of cellular components into the bloodstream.[37] In the present study, the activities of these diagnostic markers enzymes were altered in ISO-induced rats, whereas, in galangin pre-treated rats their activities were restored to normalcy in serum and heart tissues. This could be due to the protective effect of galangin on the myocardium by preserving the integrity of cell membrane and increasing the oxygen supply. The observed results are in concordance with previously reported studies.[38, 39]

In the present study decreased activities of antioxidant enzymes in MI induced rats results agree well with the previous reports.[40] Superoxide radicals generated at the site of damage modulate SOD and catalase, resulting in decreased activities of these enzymes.[41] This can lead to the accumulation of superoxide anion which further damages the myocardium.[42] Decreased activities of catalase and SOD bring out the loss of function and integrity of the myocardial membrane, which is usually observed during myocardial necrosis.[40] The decreased concentration of GSH leads to decreased activities of glutathione dependent enzymes such as GPx and GST in the heart of MI induced rats. Inactivation of GR leads to the oxidation of GSH and shifts the formation of GSSG, which in turn inactivates the enzymes with SH groups.[43] Since the redox cycle of glutathione is important for the efficiency of glutathione utilizing detoxification, the inhibition of glutathione recycling enzyme would aggravate MI induced oxidative stress in myocardium.

## CONCLUSION

Since ancient times, plants contribute quite extensively for the survival and wellness of human health by the supply of primary nutrients and secondary metabolites. In the current study, galangin treatment protected myocardium from isoproterenol induced myocardial functional and structural injury via favorably improved biochemical parameters, suggesting its cardioprotective action. These findings enlighten the protective role of galangin in ISO induced MI rats by preserving the structure and functions of cardiac cells. Assuming that these animal studies can be extended to humans, galangin may be useful to treat cardiovascular diseases in humans caused by oxidative stress associated cardiomyocyte apoptosis. Thus, further research is warranted to evaluate therapeutic efficacies in a clinical setup.

**Table 1: Effect of galangin on circulatory and plasma lipid peroxidation in control and experimental rats**

| Groups | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| Plasma TBARS(nmoles ml) | $1.99\pm0.04^{a}$ | $3.44\pm0.12^{d}$ | $1.86\pm0.06^{b}$ | $2.66\pm0.06^{c}$ |
| Tissue TBARS(nmoles/mg of protein) | $2.39\pm0.40^{b}$ | $3.66\pm0.69^{c}$ | $1.92\pm0.05^{a}$ | $2.52\pm0.07^{b}$ |

Data are presented as means ± SD of 6 rats in each group. $^{a-d}P<0.05$ the values not sharing a common superscript letter differ significantly, (Analysis of variance followed by DMRT).

**Table 2: Effect of galangin on circulatory antioxidants in control and experimental rats**

| Groups | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| SOD(Units/min/mg of Hb) | $11.27\pm0.17^{b}$ | $9.81\pm0.47^{a}$ | $13.23\pm0.41^{b}$ | $10.81\pm0.45^{c}$ |
| CAT (µmoles of $H_2O_2$ decomposed/min/mg of Hb) | $16.18\pm0.73^{b}$ | $13.24\pm0.77^{a}$ | $18.65\pm0.65^{c}$ | $15.16\pm0.59^{b}$ |
| GSH(µmoles/mg of Hb) | $1.77\pm0.04^{b}$ | $1.01\pm0.04^{a}$ | $2.45\pm0.09^{d}$ | $1.45\pm0.05^{c}$ |
| GPx(µmoles of GSH consumed /min/mg of Hb) | $15.41\pm0.63^{c}$ | $11.01\pm0.60^{a}$ | $18.08\pm0.60^{c}$ | $13.31\pm0.63^{b}$ |

SOD- Superoxide dismutase, CAT- Catalase, GSH- Glutathione and GPx- Glutathione Peroxidase
Data are presented as means ± SD of 6 rats in each group. $^{a-d}P<0.05$ the values not sharing a common superscript letter differ significantly, (Analysis of variance followed by DMRT).

**Table 3: Effect of galangin on tissue antioxidants in control and experimental rats**

| Groups | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| SOD(Units/mins/mg of protein) | 12.10±0.53[b] | 9.90±0.51[a] | 13.65±0.50[c] | 10.71±0.53[c] |
| CAT (µmoles of $H_2O_2$ decomposed/min/mg of protein) | 15.03±0.61[b] | 13.05±0.56[a] | 17.18±0.66[c] | 14.08±0.69[b] |
| GSH(mmoles/100g of tissue) | 1.71±0.04[c] | 1.09±0.04[a] | 2.55±0.88[d] | 1.49±0.56[b] |
| GPx(µmoles of GSH consumed/min/mg protein) | 14.95±0.62[c] | 11.65±0.59[a] | 16.85±0.74[d] | 13.21±0.67[b] |

SOD - Superoxide dismutase, CAT- Catalase, GSH- Glutathione and GPx - Glutathione Peroxidase
Data are presented as means ± SD of 6 rats in each group. [a-d]$P$<0.05 the values not sharing a common superscript letter differ significantly, (Analysis of variance followed by DMRT).

**Table 4: Effect of galanginon cardiac marker enzymes in control and experimental rats**

| Groups | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| Troponin I (ng/mL) | 0.24±0.06[a] | 0.79±0.40[c] | 0.19±0.11[a] | 0.42±0.13[b] |
| Troponin T (ng/mL) | 0.43±0.17[a] | 2.34±0.44[c] | 0.38±0.12[a] | 1.44±0.36[b] |
| CK(IU/L) | 162.45±1.23[a] | 215.8±1.07[c] | 145.67±1.74[b] | 161.45±1.35[d] |
| CK-MB(IU/L) | 103.11±1.98[a] | 173.88±1.88[c] | 97.68±1.39[b] | 127.87±1.24[d] |
| LDH(IU/L) | 284.30±1.26[a] | 452.63±1.25[c] | 237.18±1.29[b] | 327.97±1.22[d] |
| AST(IU/L) | 123.93±0.87[a] | 249.13±0.97[d] | 110.63±0.73[b] | 169.48±1.06[c] |
| ALT(IU/L) | 86.16±1.06[a] | 155.48±0.97[c] | 77.61±1.07[b] | 109.03±1.06[d] |

Data are presented as means ± SD of 6 rats in each group. [a-d]$P$<0.05 the values not sharing a common superscript letter differ significantly, (Analysis of variance followed by DMRT).

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