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# Cardioprotective effects of *Commiphora mukul* against isoprenaline-induced cardiotoxicity: A biochemical and histopathological evaluation

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**Abstract**

*Commiphora mukul* commonly known as Guggul is one of the oldest and commonly consumed herb for promoting heart and vascular health. Present study was undertaken to evaluate cardioprotective potential of *Commiphora mukul* against isoprenaline-induced myocardial necrosis in rats. *Wistar albino* rats were divided into three main groups: sham (saline only), isoprenaline control (saline and isoprenaline) and *Commiphora mukul* treated (*Commiphora mukul* and isoprenaline) groups. *Commiphora mukul* was administered in three doses 100, 200 and 400 mg $kg^{-1}$ p.o. for 30 days. On 29$^{th}$ and 30$^{th}$ day, the animals of isoprenaline control and *Commiphora mukul* pretreatment groups were administered isoprenaline (85 mg $kg^{-1}$; s.c.), consecutively at an interval of 24 hr. Isoprenaline administration produced a significant ($p<0.05$) decrease in myocardial antioxidants; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), reduced glutathione (GSH), and myocyte injury marker enzymes creatine- phosphokinase - MB (CK-MB) and lactate dehydrogenase (LDH) along with enhanced lipid peroxidation; malondialdehyde (MDA) in heart. *Commiphora mukul* pretreatment reversed the isoprenaline-induced oxidative changes in rat myocardium by significant ($p<0.05$) increase in SOD, CAT, GSHPx, GSH and reduction of MDA. In addition to improving myocardial antioxidant status, *Commiphora mukul* also prevented the leakage of LDH and CK-MB from heart. Further, histopathological examination showed the reduction of necrosis, edema and inflammation following *Commiphora mukul* pretreatment. Based on present findings, it is concluded that *Commiphora mukul* may be a potential preventive and therapeutic agent against the oxidative stress associated ischemic heart disease owing to antioxidant and antiperoxidative activity.

**Key words**

Cardiotoxicity, Guggul, Histopathology, Antioxidant enzymes, Necrosis

## Introduction

Myocardial infarction (MI) is a clinical problem defined as acute necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand (Dhalla *et al.,* 2000). Ischemia caused due to reduced blood supply to heart causes several biochemical alterations which may lead to cardiac dysfunction and ultimately cell death (Ferrari *et al.,* 1990). It is well recognized that free radicals generated in ischemic tissues causes metabolic stresses which results in degradation of tissue defense system, leading to myocardial damage and necrosis (Hearse, 1991). The development of myocardial ischemia and infarction is a dynamic process with the widespread occurrence of coronary atherosclerosis and involvement of oxidative stress in the humans. Among several pharmacological interventions to protect heart against oxidative stress, the use of antioxidants is most promising.

Epidemiological, clinical and experimental studies have provided compelling evidence that MI is largely preventable by antioxidant intervention via suppression of free radical generation

and/or augment endogenous antioxidant (Cooper *et al.,* 2000; Hertog *et al.,* 1993). Over the years, there has been a concerted effort to develop non toxic and relatively safe antioxidants from natural products to minimize the damage to the heart during ischemia and/or to develop preventive agents. Medicinal plants, plants based foods and their constituents have received great attention for their salutary effects and potential to treat many aspects of ischemic heart disease or MI (Goyal *et al.,* 2010; Ojha *et al.,* 2008; Mohanty *et al.,* 2004). In parallel, the use of herbs in pharmacotherapy is also rising along with a realization that herbal products can influence the course of heart diseases and may provide an integrated approach of nutritional substances, which helps in restoring and maintaining, the balanced body systems (Hertog *et al.,* 1993).

The present investigation was prompted in the purview of the claims of the most reputed medicinal plant of ayurveda and contemporary modern medicine, *Commiphora mukul* (family; Burseraceae) popularly known as Guggul. It is used extensively for the treatment of different ailments including dysmenorrhea, endometritis, hypercholesteremia, hypertension, bronchitis, inflammation, arthritis, cancer and cardiovascular disorders (Shishodia *et al.,* 2008). Its therapeutic potential as antiatherogenic (Tripathi *et al.,* 2004), anti-inflammatory (Francis *et al.,* 2004), antithyroid (Panda and Kar, 1999), hypolipidemic (Ulbricht *et al.,* 2005) and hepatoprotective (Gowrishankar *et al.,* 2008) has been reported. *Commiphora mukul* reported to contain a number of steroidal alkaloid and flavonoids (Zhu *et al.,* 2001; Singh *et al.,* 1990). The chemical constituents Z- and E- isomers of guggulsterone and its related guggulsterols I-IV have been reported to show a wide number of pharmacological activities, including antioxidant, free radical scavenger, anti-inflammatory, hypolipidemic and antihypertensive. The cardioprotective effects of *Commiphora mukul* are believed partly from the steroidal alkaloid and flavonoid constituents.

Though, the beneficial properties of *Commiphora mukul* have been demonstrated in atherosclerosis and myocardial infarction (Shishodia *et al.,* 2008). But, the effect of *Commiphora mukul* on myocardial antioxidant defense system and histopathology in isoprenaline-induced myocardial necrosis in an *in vivo* animal model resembling MI in humans have not yet been studied. Isoprenaline is a synthetic catecholamine and β adrenergic agonist known to cause oxidative damage and cardiac dysfunction in rats. It is considered as the most authenticated model for the evaluation of drugs in MI (Mohanty *et al.,* 2004).

In present study, we aimed to investigate the effects of hydroalcoholic extract of *Commiphora mukul* on endogenous antioxidant status *e.g.* reduced glutathione (GSH) and antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx), and lipid peroxidation product *e.g.* malonaldialdehyde (MDA) as well as myocyte injury marker enzymes *e.g.* creatine phosphokinase-MB isoenzyme (CK-MB) and lactate dehydrogenase (LDH). The biochemical effects were further corroborated with the histopathological changes in light microscopic observations.

## Materials and Methods

**Guggul *(Commiphora mukul)* extract:** The identity of *Commiphora mukul* was authenticated on the basis of routine pharmacognistical studies including organoleptic tests, macroscopic and microscopic observations. The voucher specimen of *Commiphora mukul* extract has been deposited in Cardiovascular Laboratory, Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India for further reference. Phytochemical analysis of the extract was performed using a high-performance liquid chromatography (HPLC, Waters, Milford, MA) for the determination of guggulsterone, the active chemical constituent of *Commiphora mukul*. The extract was standardized to contain a minimum of 2.5% guggulsterones E and Z. The doses of *Commiphora mukul* (100, 200 and 400 mg $kg^{-1}$) were selected on the basis of previously published literature assessed pharmacological activities. The lyophilized hydroalcoholic extract was dissolved in normal saline and freshly prepared before administration.

**Experimental animals:** Laboratory bred male 10 to 12 week old, *Wistar albino* rats weighing 150-200 g were obtained from the Central Animal House Facility of All India Institute of Medical Sciences, New Delhi, India. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee in accordance with the Indian National Science Academy Guidelines for the use and care of experimental animals in research. The animals were acclimatized to departmental animal house and housed under standard laboratory conditions of temperature at 25 ± 2°C, relative humidity of 55 ± 10% and light: dark cycle of 12 hr photoperiod. All experiments were performed between 9.00 and 16.00 hr. They were group housed in polypropylene cages with not more than four animals per cage and had free access to food pellets and tap water.

**Treatment protocol:** A total of 120 rats were used and randomly divided into eight main experimental groups, each containing fifteen animals. After one week of acclimatization, they were pre- and co-treated orally with saline/*Commiphora mukul* along with subcutaneous isoprenaline/saline injection on the scheduled days as per the groups described in the Table 1. On day 31, 48 hr after the first injection of isoprenaline or normal saline administration, rats were euthanized. The heart was excised and washed in cold saline phosphate buffer saline and snap frozen in liquid nitrogen until biochemical analysis. Left ventricle from the heart was isolated and stored in formalin solution for histopathological assessment.

**Biochemical studies:** Hearts stored in liquid nitrogen were brought to room temperature and weighed. After mincing the heart, a 10% homogenate was prepared in phosphate buffer (50 mM, pH 7.4) and the aliquot was used for biochemical estimations.

**Estimation of SOD activity:** Activity of SOD in heart was measured by the method described by Misra and Fridovich (1972). To a cuvette containing 0.75 ml of carbonate buffer (100 mM, pH 10.2) and 10 µl of epinephrine (3 mM), 50 ml of supernatant was added. The change in absorbance of each sample was then recorded spectrophotometrically on 480 nm for 120 sec at an interval of 15

sec. One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto-oxidation.

**Estimation of CAT activity:** Myocardial CAT activity was determined by the method of Aebi (1974). Briefly, supernatant (50 ml) was added to a 3.0 ml cuvette containing 1.95 ml phosphate buffer (pH 7.0, 50 mM) and 1.0 ml of 30 mM hydrogen peroxide. Changes in absorbance were recorded spectrophotometrically for 30 sec on every 5 sec at 240 nm. Catalase activity is expressed as units $mg^{-1}$ protein as compared to the standard. One unit of CAT activity represents 1 mmol of $H_2O_2$ decomposed per min at 25°C.

**Estimation of GSHPx activity:** The GSHPx activity in heart was measured by the method described by Paglia and Valentine (1967). To a 1 ml cuvette containing 400 µl of potassium phosphate buffer (pH 7.0, 0.25 M), 100 ml of 10 mM GSH, 100 ml of 2.5 mM NADPH and 100 ml of glutathione reductase (6 U $ml^{-1}$), 200 ml of supernatant was added. Hydrogen peroxide (100 ml of 12 mM) was then added and change in absorbance was recorded spectrophotometrically on 340 nm for 120 sec at an interval of 15 sec. GSHPx activity is expressed as units/mg protein as compared to the standard. One unit of GSHPx enzyme activity is defined as one nmol of NADPH utilized per min on 37°C.

**Estimation of GSH:** Myocardial GSH was estimated by the method of Maron *et al.* (1979). To 0.5 ml of the tissue homogenate, 4 ml of 0.3M $Na_2HPO_4$ (pH 8) and 0.5 ml of dithiobis-2-nitrobenzoic acid (DTNB, 0.6mM) prepared in 1% tri-sodium citrate were added and vortexed. Absorbance of the resultant yellow color was recorded spectrophotometrically at 412 nm.

**Estimation of MDA:** In heart tissues, MDA, a stable product of lipid peroxidation was estimated by method of Ohkawa *et al.* (1979). Briefly, to 0.2 ml of heart tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% thiobarbituric acid (TBA) were added in succession. Volume was made up to 4 ml with double distilled water. The mixture was incubated at 95°C in a temperature controlled water bath for 60 min and then allowed to cool. A mixture of 5 ml of n-butanol: pyridine (15:1) was added to it and centrifuged after vortex. The organic layer was separated and absorbance was read spectrophotometrically at 515 nm.

**Estimation of CK-MB isoenzyme activity:** Myocyte specific injury markers of early necrosis, CK-MB isoenzyme was estimated spectrophotometrically using a kit from Randox Laboratories, USA, according to the method of Lamprecht *et al* (1974). The sample (50 µl) was added to cuvette containing 1 ml of imidazole buffer consisting of adenosine-mono-phosphate (5.2 mM), adenosine-di-phosphate (2.1 mM), nicotinamide adenine dinucleotide phosphate (2.1 mM), glucose-6-phosphate dehydrogenase (1.6 U/l), creatine phosphate (31.2 mM) and N-acetyl cysteine (21 mM). The cuvette consisting of sample and imidazole buffer was incubated for 2 min at room temperature. Absorbance was recorded on 340 nm for 180 sec at every 60 sec. One unit of CK-MB isoenzyme is defined as the amount of enzyme that will transfer one µmol of phosphate from phosphocreatine to ADP per min at pH 7.4 on 30°C.

**Estimation of LDH activity:** Activity of the myocardial injury marker enzyme, LDH was measured by the method of Cabaud and Wroblewski (1958). To 0.1 ml of sample, 0.9 ml of double distilled water was added and placed at 95°C for 15 min in a water bath along with a separate tube containing pyruvic acid-buffered substrate (0.02% in 0.05M phosphate buffer). Pre-incubated pyruvic acid-buffered substrate (1 ml) was then added to the tube containing the sample mixture. Further, 1 ml of dinitrophenyl hydrazine (0.02% in 1N HCl) was added 30 min after adding the substrate and the tube was taken out from water bath. The contents were mixed by swirling and allowed to stand for 20 min at 37°C. It was vortexed after adding 10 ml of 0.4 N NaOH. Percent transmittance was read spectrophotometrically on 340 nm for 180 sec at an interval of 60 sec. One unit of LDH is defined as the amount of enzyme required to reduce one µmol of pyruvate to D-lactate per min on pH 7 at 25°C.

**Estimation of protein:** Protein was estimated by the method of Lowry *et al.* (1951) in hearts. To appropriately diluted samples with phosphate buffer, 5 ml of copper sulphate reagent consisting of $Na_2CO_3$ (1%), sodium potassium tartrate (2%) and $CuSO_4$ (1%) was added. The solution was vortexed and kept for 10 min. To this solution, 0.5 ml of Folin-Ciocalteau phenol reagent was added. The solution was then vortexed and kept at room temperature for 30 min before absorbance reading. Absorbance was read at 620 nm on Beckman's spectrophotometer. The protein content was calculated using Bovine Serum Albumin (BSA) as a standard.

**Histopathological studies of myocardium:** At the end of the experiment, the myocardial tissue was immediately fixed in 10% buffered formalin solution. Cross sections of 5 µm thickness of myocardial tissues were cut and stained with hematoxylin and eosin and was examined under microscope (Nikon. Tokyo, Japan) to examine the myocardial histoarchitecture. Representative area images were captured in an image analysis system. The slides were evaluated under for myonecrosis, inflammatory cell infiltration and edema. A minimum of 10 fields for each slide were examined and graded for severity of changes.

**Statistical analysis:** Data were presented as mean±SD. One way analysis of variance (ANOVA) was applied for statistical analysis following post-hoc analysis (Bonferroni Multiple Range Test). The criterion of statistical significance was set at $p<0.05$.

## Results and Discussion

The administration of isoprenaline in rats resulted significant reduction in the activities of myocardial SOD, CAT and GSHPx enzymes (Table 2). However, pretreatment with *Commiphora mukul* prevented the reduction of these enzymes to near normal levels (Table 2). SOD, CAT and GSHPx enzymes constitute a mutually supportive enzyme system of the first line cellular defense in myocardium against oxidative injury, decomposing $O_2$ and $H_2O_2$

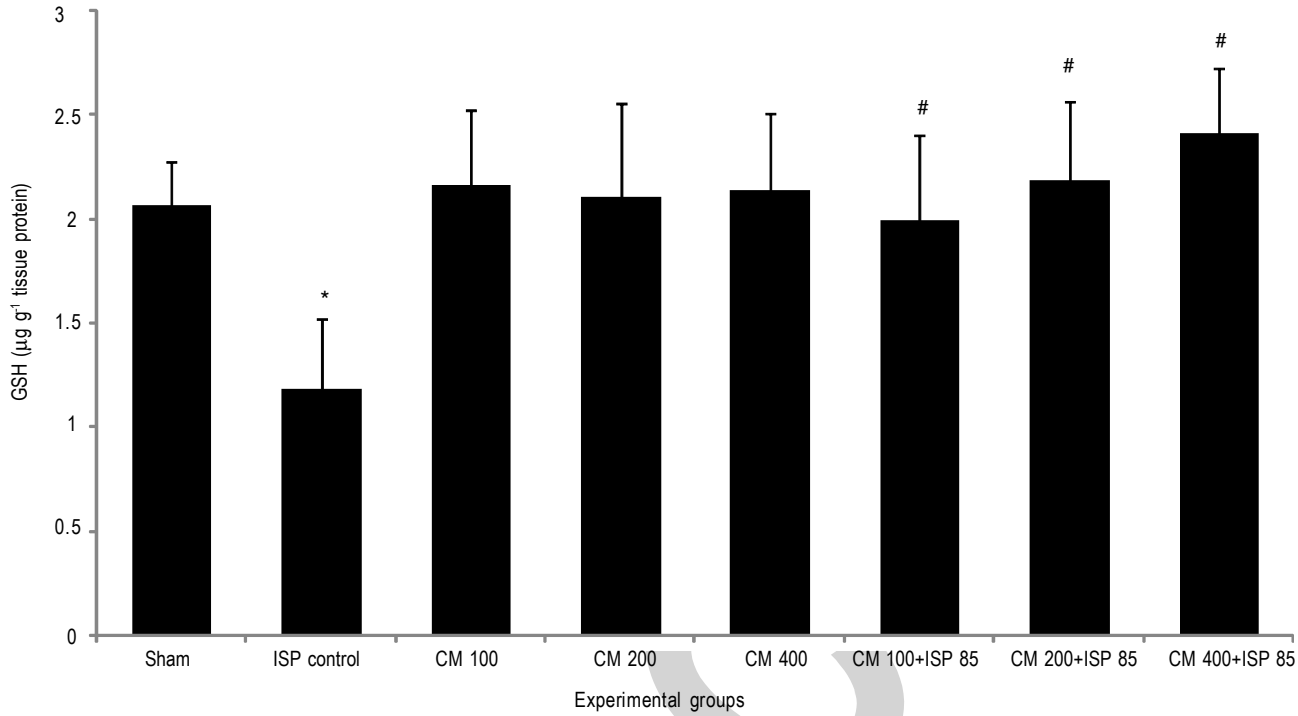


**Fig. 1:** Myocardial glutathione activity in different experimental groups ISP 85 = Isoprenaline 85 mg $kg^{-1}$; CM 100 = *Commiphora mukul* 100 mg $kg^{-1}$; CM 200 = *Commiphora mukul* 200 mg $kg^{-1}$; CM 400 = *Commiphora mukul* 400 mg $kg^{-1}$; GSH = Reduced glutathione. Values are mean ± SD of eight animals. *p<0.05 vs sham, #p<0.05 vs ISP control.

before their interaction to form the more harmful hydroxy radical. In present study, decreased SOD activity in isoprenaline control animals may be due to excessive formation of superoxide anions or the decreased removal of superoxide anions, which can be harmful to the myocardium. The activities of $H_2O_2$ scavenging enzymes, CAT and GSHPx also decreased significantly after isoprenaline challenge. Reduction in the activity of these enzymes may be explained by the fact that excessive superoxide anions may inactivate SOD, thus resulting in an inactivation of the $H_2O_2$ scavenging enzymes.

Pretreatment of isoprenaline challenged rats with *Commiphora mukul* prevented decrease in SOD, CAT and GSHPx activities, which may be correlated directly to the scavenging of free radicals by *Commiphora mukul* resulting in protection of these enzymes from reactive oxygen species. *Commiphora mukul* has been demonstrated to be effective in oxidative modification of LDL and counteracted the generation of reactive oxygen species such as superoxide anions and hydroxyl radicals. Several studies have reported the modulation of endogenous antioxidants by herbal formulations or plants extract in cardiovascular pathologies (Goyal *et al.,* 2010; Ojha *et al.,* 2008; Mohanty *et al.,* 2004; Tripathi *et al.,* 2004; Mary *et al.,* 2003).

Glutathione antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. It protects the myocardial cellular membrane against oxidative damage by regulating the redox status of proteins in the cell-surface membrane (Farvin *et al.,* 2004). The cellular tripeptide, GSH (glutamyl cysteinyl glycine) thwarts peroxidative damage by neutralizing the free radicals. A significant decrease in GSH was observed following isoprenaline administration (Fig. 1). However, rats treated with *Commiphora mukul* showed lesser depletion of GSH from myocardial tissues. The significant reduction in GSH along with concomitant reduction of activities of glutathione-dependent antioxidant enzyme, GSHPx demonstrates enhanced oxidative stress following isoprenaline administration in consonance to the previous reports (Shiny *et al.,* 2005; Mohanty *et al.,* 2004). Reduction observed in the activities of GSHPx in isoprenaline-induced myocardial infarction might be due to decreased availability of their substrate, reduced glutathione. This in turn may lead to increased accumulation of oxidative free radicals and also enhance the susceptibility of myocardial cell membrane to peroxidative damage.

Glutathione depletion further increases the susceptibility of myocardial membrane to reactive oxygen metabolites and lipoperoxidative necrotic damage and explains the myocardial damage and increased lipid peroxidation (Anandan *et al.,* 2003). Depletion of reduced glutathione is known to result in enhanced lipid peroxidation evidenced by increased MDA levels following isoprenaline administration (Table 3).

Subsequent to isoprenaline administration an increased formation of lipid peroxidation product, MDA was also observed in rats (Table 2). Lipid peroxidation is an important pathogenic event in myocardial necrosis and the accumulation of lipid hydroperoxides reflects cardiac damage (Sevenian and Hochstein, 1985). The

**Table - 1:** Treatment protocol in different experimental groups

| Group | Experimental groups | Treatment days 1-30 | Day 29 & 30 |
|---|---|---|---|
| I | Sham | Saline p.o. | Saline p.o. + saline s.c. |
| II | ISP control | Saline p.o. | Saline p.o. + ISP 85 mg $kg^{-1}$ s.c. |
| III | CM 100 | Commiphora mukul 100 mg $kg^{-1}$ p.o. | Commiphora mukul 100 mg $kg^{-1}$ p.o. + saline s.c. |
| IV | CM 200 | Commiphora mukul 200 mg $kg^{-1}$ p.o. | Commiphora mukul 200 mg $kg^{-1}$ p.o. + saline s.c. |
| V | CM 400 | Commiphora mukul 400 mg $kg^{-1}$ p.o. | Commiphora mukul 400 mg $kg^{-1}$ p.o. + saline s.c. |
| VI | CM 100 + ISP 85 | Commiphora mukul 100 mg $kg^{-1}$ p.o. | Commiphora mukul 100 mg $kg^{-1}$ p.o. + ISP 85 mg $kg^{-1}$ s.c. |
| VII | CM 200 + ISP 85 | Commiphora mukul 200 mg $kg^{-1}$ p.o. | Commiphora mukul 200 mg $kg^{-1}$ p.o. + ISP 85 mg $kg^{-1}$ s.c. |
| VIII | CM 400 + ISP 85 | Commiphora mukul 400 mg $kg^{-1}$ p.o. | Commiphora mukul 400 mg $kg^{-1}$ p.o. + ISP 85 mg $kg^{-1}$ s.c. |

CM= *Commiphora mukul*; ISP = Isoprenaline; p.o. = Per oral; s.c. = Subcutaneous

**Table - 2:** Myocardial antioxidant enzymes in different experimental groups

| Experimental groups | SOD (U $mg^{-1}$ protein) | CAT (U $mg^{-1}$ protein) | GSHPx (U $mg^{-1}$ protein) |
|---|---|---|---|
| Sham (saline) | 7.94 ± 2.90 | 21.90 ± 3.10 | 0.33 ± 0.12 |
| ISP control (saline + ISP 85) | 3.40 ± 1.40* | 11.90 ± 2.00* | 0.19 ± 0.08* |
| CM 100 | 7.64 ± 1.92 | 22.68 ± 2.42 | 0.31 ± 0.05 |
| CM 200 | 8.01 ± 2.11 | 21.85 ± 3.04 | 0.37 ± 0.06 |
| CM 400 | 7.90 ± 2.41 | 22.32 ± 2.81 | 0.38 ± 0.12 |
| CM 100 + ISP 85 | 4.38 ± 1.70# | 15.68 ± 2.91# | 0.21 ± 0.06# |
| CM 200 + ISP 85 | 6.32 ± 2.82# | 20.73 ± 3.41# | 0.39 ± 0.07# |
| CM 400 + ISP 85 | 7.83 ± 2.14# | 21.56 ± 3.99# | 0.41 ± 0.05# |

ISP 85 = Isoprenaline 85 mg $kg^{-1}$; CM 100 = *Commiphora mukul* 100 mg $kg^{-1}$; CM 200 = *Commiphora mukul* 200 mg $kg^{-1}$; CM 400 = *Commiphora mukul* 400 mg $kg^{-1}$; SOD = Superoxide dismutase; CAT = Catalase; GSHPx = Glutathione peroxidase. Values are mean ± SD of eight animals. *$p<0.05$ vs sham, #$p<0.05$ vs ISP control

**Table - 3:** Lipid peroxidation and myocyte injury marker enzyme in different experimental groups

| Experimental groups | MDA (nmol $g^{-1}$ tissue protein) | LDH (IU $mg^{-1}$ protein) | CK-MB (IU $mg^{-1}$ protein) |
|---|---|---|---|
| Sham (saline) | 78.80 ± 10.45 | 223.80 ± 15.25 | 181.40 ± 22.30 |
| ISP control (saline + ISP 85) | 193.50 ± 12.32* | 117.45 ± 16.32* | 91.32 ± 16.67* |
| CM 100 | 78.75 ± 10.21 | 227.55 ± 15.35 | 188.65 ± 19.00 |
| CM 200 | 77.60 ± 12.25 | 220.15 ± 16.35 | 182.57 ± 12.36 |
| CM 400 | 78.10 ± 11.73 | 218.62 ± 15.12 | 191.64 ± 12.85 |
| CM 100 + ISP 85 | 122.15 ± 13.35 | 204.10 ± 16.30# | 148.55 ± 17.67# |
| CM 200 + ISP 85 | 105.25 ± 15.30# | 213.17 ± 13.72# | 156.52 ± 20.02# |
| CM 400 + ISP 85 | 92.40 ± 16.34# | 217.21 ± 16.06# | 170.10 ± 12.40# |

ISP 85 = Isoprenaline 85 mg $kg^{-1}$; CM 100 = *Commiphora mukul* 100 mg $kg^{-1}$; CM 200 = *Commiphora mukul* 200 mg $kg^{-1}$; CM 400 = *Commiphora mukul* 400 mg $kg^{-1}$; MDA = Malonaldialdehyde; LDH =Lactate dehydrogenase; CK-MB = Creatine-phosphokinase-MB isoenzyme. Values are mean ± SD of eight animals. *$p<0.05$ vs sham, #$p<0.05$ vs ISP control.

**Table - 4:** Histopathological changes of rat myocardium in different experimental group

| Experimental groups | Myonecrosis | Inflammation | Edema |
|---|---|---|---|
| Sham (saline) | - | - | - |
| ISP control (saline + ISP 85) | ++++ | ++++ | ++++ |
| CM 100 | - | - | - |
| CM 200 | - | - | - |
| CM 400 | - | - | - |
| CM 100 + ISP 85 | - | + | + |
| CM 200 + ISP 85 | - | - | - |
| CM 400 + ISP 85 | - | - | - |

ISP 85 = Isoprenaline 85 mg $kg^{-1}$; CM 100 = *Commiphora mukul* 100 mg $kg^{-1}$; CM 200 = *Commiphora mukul* 200 mg $kg^{-1}$; CM 400 = *Commiphora mukul* 400 mg $kg^{-1}$

increased lipid peroxides in isoprenaline-induced myocardial necrosis might be due to free radical mediated membrane damage. The decreased MDA level in heart following *Commiphora mukul* pretreatment might be due to augmentation of endogenous antioxidants. The results support the anti-lipidperoxidative property of *Commiphora mukul* in agreement with previous reports (Gowrishankar *et al.,* 2008; Ulbricht *et al.,* 2005; Singh *et al.,* 1990; Panda and Kar, 1999). *Commiphora mukul* extract is highly lipotropic and when administered exogenously, it can readily pass across the membrane lipid bilayer similar to the other lipotropic phytoconstituents substances. The ability of *Commiphora mukul* to diffuse into intracellular compartments aids the capabilities of this natural product as an antioxidant. Previous studies have indicated that lipotropes inhibit lipid peroxidation in cellular membranes as a result of distinct biophysical interactions with membrane lipid bilayer (Balkan *et al.,* 2004).

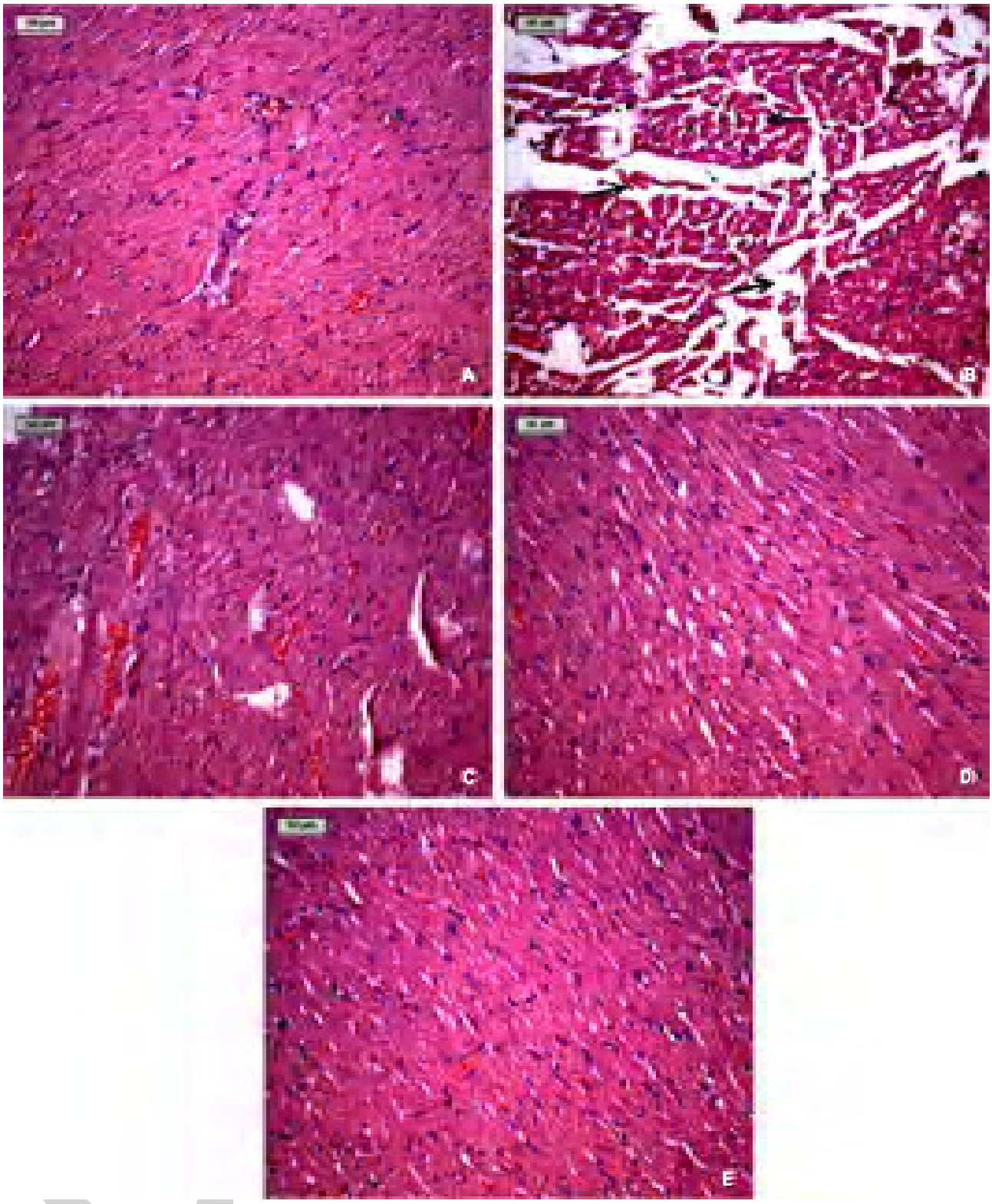


**Fig. 2:** Light micrograph (H&E 100x) of rat's myocardium of (A) sham group showing normal architecture of myocardium (B) ISP control group showing confluent focal necrosis of myofibrils, edema with infiltration of inflammatory cells and extravasations of red blood cells (C) *Commiphora mukul* 100 mg $kg^{-1}$ + ISP 85 mg $kg^{-1}$ treated rats showing lesser myocardial necrosis, vacuoles, edema with inflammatory cells (D) *Commiphora mukul* 200 mg $kg^{-1}$ + ISP 85 mg $kg^{-1}$ treated rats showing near normal myocardial histoarchitecture with lessened necrosis and edema (E) *Commiphora mukul* 400 mg $kg^{-1}$ + ISP 85 mg $kg^{-1}$ treated rats showing almost normal myocardial histoarchitecture

Furthermore, subsequent to isoprenaline injection we observed a decrease in myocytes injury marker CK-MB isoenzyme and LDH, an indicative of severity of isoprenaline-induced necrotic damage of myocardial membrane (Table 3). The leakage of CK-MB isoenzyme and LDH enzyme from heart is the diagnostic marker of myocardial infarction. Thus, the determination of CK-MB isoenzyme and LDH is a useful parameter for assessing myocardial damage. When myocardial cells, containing CK-MB isoenzyme and LDH are damaged or destroyed due to deficient oxygen supply or glucose, $Ca^{+2}$ overloading, production of free radicals and lipid peroxidation leads loss of the integrity of cell membranes and render the membrane more porous and permeable or may rupture those results in the leakage of this enzymes. This account for the decreased activities of CK-MB isoenzyme and LDH in heart tissue of isoprenaline challenged rats. This might be due to the damage caused to the sarcolemmal by the agonist, which has rendered it leaky (Mohanty *et al.,* 2004). However, pretreatment with *Commiphora mukul* prevented depletion of CK-MB isoenzyme and LDH enzyme from heart as compared to isoprenaline control group (Table 3), therefore, reduced the release of CK-MB isoenzyme and LDH from myocardium into the systemic circulation, a suggestive of cytoprotective action of *Commiphora mukul*.

Chemically, the heterocyclic ring structure in phytoconstituents of *Commiphora mukul* has been reported to quench singlet molecular oxygen and exert antioxidant action and protect membrane lipids (Zhu *et al.,* 2001). The unpaired electron present in the hydroxyl free radical, which is mainly responsible for the isoprenaline-induced necrotic damage to the myocardium (Saravanan and Prakash, 2004) might have been trapped and subsequently dismuted by constituents of *Commiphora mukul*. The protective effect of *Commiphora mukul* against isoprenaline-induced myocardial infarction observed in this study may also be associated with the conservation of energy phosphates and substrates, which contributes to an increase in the supply of substrate needed for the synthesis of GSH that protects the cell from reactive metabolites and reactive oxygen species.

Histopathological examination showing the extent and degree of necrosis was graded and scored as presented in Table 4. Sham group hearts showed normal myofibrillar architecture with striations, branched appearance and continuity with adjacent myofibrils (Fig. 2A). Rats challenged with isoprenaline showed severe myocardial necrosis with edema and inflammatory cells and separation of myofibers as compared to sham group (Fig. 2B). The histopathological perturbation in isoprenaline control rats are attributed to a decline in oxygen supply with paramount rise in wall-stress. *Commiphora mukul per se* treated rats showed normal cardiac myofibers without any significant pathological changes, therefore indicating that *Commiphora mukul* is relatively safe to cardiomyocytes. However, the isoprenaline challenged rats receiving *Commiphora mukul* pretreatment has exhibited near normal architecture of myofibrillar striations and continuity with adjacent myofibrils (Fig. 2C-E) compared to isoprenaline control group. The preserved morphology of cardiac myofibers indicates the cytoprotective action and membrane stabilizing action of *Commiphora mukul*.

Moreover, the protective actions of *Commiphora mukul* against the myocardial membrane damage can be explained by its lipophilic nature. It is presumed that *Commiphora mukul* similar to other lipophilic agents, such as vitamin E, antipyrine, nifedipine and β-blocker drugs intercalate into the lipid matrix and impart stabilization to myocardial cell membranes in relation to the degree of their lipophilicity (Cruickshank and Neil, 1985). Thus, it is possible that likewise *Commiphora mukul* may also render resistance as well as impart stabilization to myocardial cell membranes and thereby extend the viability of myocardial cell membranes from necrotic damage.

The present study concludes that protective effects of *Commiphora mukul* against isoprenaline-induced myocardial infarction in rats are related to its effects on counteraction of free radicals by its free radical scavenging and antioxidant and membrane stabilizing action. The observations highlight that *Commiphora mukul* may be one of the promising drug for improving defense mechanisms in the physiological systems against oxidative stress caused during myocardial infarction.

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