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Effect of *Commiphora mukul* extract on cardiac dysfunction and ventricular function in isoproterenol-induced myocardial infarction

Shreesh K Ojha [a], Mukesh Nandave[a], Sachin Arora [a], Raj D Mehra [b], Sujata Joshi[a], Rajiv Narang [c] & D S Arya* [a]

[a]Cardiovascular Laboratory, Department of Pharmacology, All India Institute of Medical Sciences, New Delhi 110 029, India

[b]Department of Anatomy, All India Institute of Medical Sciences, New Delhi 110 029, India

[c]Department of Cardiology, All India Institute of Medical Sciences, New Delhi 110 029, India

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In present study, hydroalcoholic extract of *C. mukul* significantly improved the cardiac function and prevented myocardial ischemic impairment manifested in the form of increased heart rate, decreased arterial pressure, increased left ventricular end diastolic pressure, and altered myocardial contractility indices. *C. mukul* treatment additionally also produced a significant increase in lactate dehydrogenase levels and prevented decline of protein content in heart. *C. mukul* preserved the structural integrity of myocardium. Reduced leakage of myocyte enzyme lactate dehydrogenase and maintenance of structural integrity of myocardium along with favorable modulation of cardiac function and improved cardiac performance indicate the salvage of myocardium with *C. mukul* treatment. Guggulsterones which are considered to be responsible for most of the therapeutic properties of *C. mukul* may underlie the observed cardioprotective effect of *C. mukul* against cardiac dysfunction in isoproterenol-induced ischemic rats.

Keywords: Cardiac function, *Commiphora mukul*, Guggul, Isoproterenol, Ventricular function

Epidemiological studies indicate that heart diseases will constitute the major disease-burden worldwide by the year 2020[1]. In heart diseases, myocardial ischemia is a state of myocardial impairment which results from inadequate coronary perfusion of oxygenated blood relative to the metabolic demands of myocardium. Depressed myocardial contractile function, arrhythmias and myocardial necrosis or infarction are the major consequences of myocardial ischemia[2]. Reduction of mortality rate and prevention of myocardial infarction are of utmost importance. Synthetic agents such as angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, angiotensin II receptor antagonists, etc. have been proven to have cardioprotective effects in both preclinical and clinical studies. However, in the last few decades, much interest has been focused on herbs or herb based supplements as possible alternative medicine for long-term prevention of heart attack in high risk patients. A search for novel pharmacotherapy from medicinal plants for ischemic heart diseases is in progress for last decade. This is reflected by a large number of herbal preparations for which cardioprotective potential has been reported in a variety of animal models[3,4].

Catecholamines which participate in cardiac function rapidly undergo auto-oxidation and it has been suggested that oxidative products of catecholamines are responsible for changes in myocardium.[5] Administration of isoproterenol (ISP), a β adrenergic agonist, and synthetic catecholamine, depletes the energy reserve of cardiac muscle cells, and cause complex biochemical and structural changes leading to cell damage and necrosis comparable to those taking place in human myocardial infarction[4-7]. Certain natural substances have been recognized to have potential to reduce the detrimental effect of a number of cardiovascular risk factors[8-10].

Commiphora mukul Engl. (*C. mukul*), a small bushy tree, is used for thyroid, rheumatic, gastrointestinal and cardiac disorders[11]. Its standardized extract is one of the components of

*Correspondent author - Phone: +91-11-26588500 extn-4266;
Fax: +91-11-26588641
E-mail: dsarya16@hotmail.com

various traditional formulations to treat inflammation, obesity, and lipid disorders.[11] It is an important herb used in the treatment of several degenerative disorders in modern medicine too and established as a hypolipidemic drug[12]. It also increased fibrinolytic activity and decreased platelet adhesiveness and scavenges free radicals in patients with atherosclerotic ischemic diseases, thus reducing cellular damage from ischemia[8,13,14]. Clinical trials have also indicated *C. mukul* as an effective medicine for angina pectoris, atherosclerosis and myocardial infarction.[13-17] Till date, several chemical components such as diterpenes, sterols, steroids, esters and higher alcohols have been identified in *C. mukul*[18,19]. The active ingredient responsible for the use of the plant in maintenance of healthy cholesterol levels is guggulsterones [4, 17 (20)-pregnadiene-3, 16-dione], specifically guggulsterone E and guggulsterone Z[19]. Purified *C. mukul* used in the current study is a hydroalcoholic extract and standardized to contain a minimum of 2.5% guggulsterones E and Z. The present study was undertaken to study the effect of *C. mukul* extract on cardiac function parameters in ISP-induced myocardial ischemia in rats.

Materials and Methods

Plant material and composition of extract—Standardized hydro-alcoholic lyophilized extract of whole plant of *C. mukul* was supplied by Sanat Products Ltd., New Delhi, India. The identity of *C. mukul* was authenticated by Dr. Santosh Kumari, Division of Plant Physiology, Indian Agriculture Research Institute, New Delhi, India. The voucher specimen of lyophilized extract of *C. mukul* (No. CM 0407) was deposited in Cardiovascular Laboratory, Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India. Phytochemical analysis of extract revealed the total andrographolides content not less than 10% w/w.

Experimental animals—With the prior permission of the Institutional Animal Ethics Committee of All India Institute of Medical Sciences, New Delhi, the present study was conducted in accordance with the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals (227/05). Male albino Wistar rats (10-12 week old; weighing 150-200 g) were obtained from the Central Animals House facility of All India Institute of Medical Sciences, New Delhi, India. The animals were kept in polypropylene cages containing a maximum of four animals in a cage. The animals were housed under standard laboratory conditions (25±2ºC, relative humidity 50±10%, 12 hr dark/light photoperiod) in departmental Animal House. They were fed with commercial pellet diet (Ashirwad Industries Ltd, Chandigarh, India) and tap water *ad libitum*. The commercial pellet diet contained (w/w): carbohydrate (55%), fat (5%), protein (24%), fiber (4%), calcium (0.6%), phosphorous (0.3%), moisture (10%) and ash (0.9%).

Chemicals used—All chemicals used in the study were of analytical grade and distilled water was used for all biochemical estimations. Isoproterenol hemisulphate was obtained from Sigma Chemicals Company (St. Louis, MO), USA. Isoproterenol solution was prepared under sterile condition with saline (0.9%) and used within 10 min of preparation of the solution.

Experimental design—The animals were randomly divided into five experimental groups with 10 animals in each group. After a week of acclimatization under laboratory conditions, rats were administered saline or extract through oral route using oral gavage. Animals of group I, designated as sham group were administered 0.9% of normal saline orally once daily for 31 days; and on day 29 and 30, 0.5 ml of normal saline was injected subcutaneously at an interval of 24 hr. Animals of group II, designated as ISP control group were administered 0.9% of normal saline orally once daily for 31 days; and on days 29 and 30, ISP (85 mg/kg) was injected subcutaneously at an interval of 24 hr. Animals of group III, IV and V were designated as *C. mukul* extract treated group and orally received alcoholic extract at dose of 100, 200 and 400 mg/kg per day, respectively for 31 days. In addition, on days 29 and 30 rats of *C. mukul* treated group were administered subcutaneous injection of ISP (85 mg/kg) at an interval of 24 hr. On days 31, rats of each group were subjected to hemodyanmic and left ventricular function measurement.

Measurement of hemodynamic and left ventricular dynamics—On day 31, animals of all the experimental groups were anesthetized intraperitoneally with pentobarbitone sodium (60 mg/kg). Atropine (4 mg/kg) was administered along with the anesthesia to maintain the heart rate especially during surgery and to reduce tracheo-bronchial secretions. The body temperature was monitored and maintained at 37^{o}C with the help of a thermal lamp at dissection table

during surgery. The neck was opened with a ventral midline incision to perform tracheostomy. The rats were ventilated with room air from a positive pressure ventilator (Inco, Ambala, India) using compressed air at a rate of 90 strokes/min and a tidal volume of 10 ml/kg. Ventilator setting and PO_2 were adjusted as needed to maintain the arterial blood gas parameters within the physiological range. The left jugular vein was cannulated with polyethylene tube for continuous infusion of saline solution (0.9%). The right carotid artery was cannulated with a heparinized saline filled cannula. The cannula was connected with Cardiosys CO-101 (Experimentria, Hungary) using a pressure transducer and the signal was amplified by means of an amplifier for measurement of systolic, diastolic and mean arterial pressure (SAP, DAP and MAP) and heart rate (HR). The left thoracotomy was preformed at the fourth-fifth intercostal space on left side and the heart was exposed. After incising pericardium, the heart was exteriorized by gentle pressure on ribs. A sterile metal cannula (1.5 mm bore) was introduced into cavity of left ventricle from posterior apical region of heart for measuring left ventricular dynamics such as (+) LV dP/dt, a marker of contractility, (-) LV dP/dt, a marker of relaxation, and LVEDP, a surrogate marker of preload. The cannula was connected to a pressure transducer (Gould Statham P23ID, USA) through a pressure-recording catheter on Polygraph (Grass 7D, USA). After the stabilization time of 10 min, tracings were recorded on polygraph paper following baseline measurements at different standardized sensitivity and speed. Thoracic cavity was covered with saline-soaked gauze after surgery to prevent heart from drying. Rate-pressure-product, the marker of energy expenditure, was calculated by multiplying systolic arterial pressure and heart rate and dividing the product by 100 (ref. 20).

Evaluation of myocardial injury—After recording cardiac function, the rats were euthanized with an overdose of anesthesia using sodium pentobarbitone (100 mg/kg, iv). Hearts of experimental animals were excised and rinsed with 0.9% chilled phosphate buffer saline (*p*H 7.4, 50 m*M*). A 10% of homogenate of whole heart was prepared in phosphate buffer saline and centrifuged at 7000 rpm for 15 min. The supernatant was used for estimation of myocardial injury specific enzyme LDH[21] and protein[22] by spectrophotometrically. Protein content was calculated by using Bovine Serum Albumin (BSA) as a standard reference point. One unit of LDH has been defined as the amount of enzyme required to reduce 1 µmole of pyruvate to D-lactate per min at *p*H 7, 25°C.

Statistical analysis—Data was subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by Bonferroni Multiple Range Test (SPSS software). P value of less than 0.05 was considered to indicate statistical significance.

Results

Effect of C. mukul on arterial pressure—Isoproterenol produced a significant fall in systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP) in comparison with sham group (Table 1). *C. mukul* extract at 200 and 400 mg/kg produced a significant improvement in SAP, DAP and MAP.

Effect of C. mukul on heart rate—ISP administration produces a significant increase in heart rate as compared to sham control (Table 2). Administration of *C. mukul* 100, 200 and 400 mg/kg significantly decreased heart rate in comparison to ISP control.

Effect of C. mukul on left ventricular end diastolic pressure—Subsequent to ISP challenge a significant increase in LVEDP was observed in comparison to sham control (Table 2). However, a significant reduction was observed in LVEDP at different doses of *C. mukul*.

Effect of C. mukul on left ventricle performance—A significant fall was observed in left ventricular (+) dP/dt and (-) dP/dt in ISP group in comparison with sham group (Table 3). Treatment with *C. mukul* at

Table 1—Effect of *Commiphora mukul* (CM) on systolic, diastolic and mean arterial pressure (mmHg) in different experimental groups
[Values are mean ± SD of 8 rats in each group]

Experimental groups	SAP	DAP	MAP
Group I (Sham; saline)	139 ± 19	123 ± 22	130 ± 20
Group II (ISP control; Saline+ISP)	123 ± 8*	97 ± 15*	105 ± 7*
Group III (CM 100 mg/kg+ISP)	124 ± 10	104 ± 13#	110 ± 11#
Group IV (CM 200 mg/kg+ISP)	129 ± 11#	107 ± 10#	115 ± 9#
Group V (CM 400 mg/kg+ISP)	132 ± 9#	109 ± 12#	116 ± 11#

*P<0.05, when compared to sham, #P<0.05, when compared to ISP control

200 and 400 mg/kg increased the left ventricular (+) dP/dt. However, a subtle but insignificant improvement in left ventricular (-) dP/dt was observed.

Effect of C. mukul on LDH levels in heart homogenates—Isoproterenol induced myocardial necrosis produced a significant decrease in LDH in heart homogenates as compared to sham control (Fig. 1a). *C. mukul* at all doses significantly increased the level of LDH in heart homogenates.

Effect of C. mukul on rate pressure product—ISP administration induced a significant decrease in RPP in comparison with sham control (Fig. 1b). However, treatment with *C. mukul* produced a subtle, but insignificant restoration of RPP at all the doses.

Effect of C. mukul on protein in heart homogenates—Isoproterenol induced myocardial necrosis produced a significant rise in protein contents as compared to sham control (Fig. 1c). All the doses of *C. mukul* produced a significant decrease in protein contents as compared to ISP control.

Table 2—Effect of *Commiphora mukul* (CM) on heart rate and left ventricular end diastolic pressure in the different experimental groups
[Values are mean ± SD of 8 rats in each group]

Experimental groups	HR (Beats/min)	LVEDP (mmHg)
Group I (Sham; saline)	356 ± 40	4.80 ± 0.55
Group II (ISP control; Saline+ISP)	227 ± 51*	8.63 ± 1.32*
Group III (CM 100 mg/kg+ISP)	299 ± 35	6.46 ± 1.43#
Group IV (CM 200 mg/kg+ISP)	341 ± 35#	6.57 ± 1.32#
Group V (CM 400 mg/kg+ISP)	347 ± 46#	6.60 ± 1.41#

*P<0.05, when compared to sham, #P<0.05, when compared to ISP control

Table 3—Effect of *Commiphora mukul* (CM) on left ventricular function in different experimental groups
[Values are mean ± SD of 8 rats in each group]

Experimental groups	LV (+) dP/dt (mmHg/sec)	LV (-) dP/dt (mmHg/sec)
Group I (Sham; saline)	3354 ± 111	3192 ± 162
Group II (ISP control; Saline+ISP)	2875 ± 105*	2375 ± 92*
Group III (CM 100 mg/kg+ISP)	2945 ± 125	2785 ± 135#
Group IV (CM 200 mg/kg+ISP)	3133 ± 115#	3035 ± 125#
Group V (CM 400 mg/kg+ISP)	3112 ± 122#	2995 ± 145#

*P<0.05, when compared to sham, #P<0.05, when compared to ISP control

Discussion

The present study showed that oral administration of *C. mukul* extract ameliorated the cardiac necrosis and improved cardiac functioning by significant

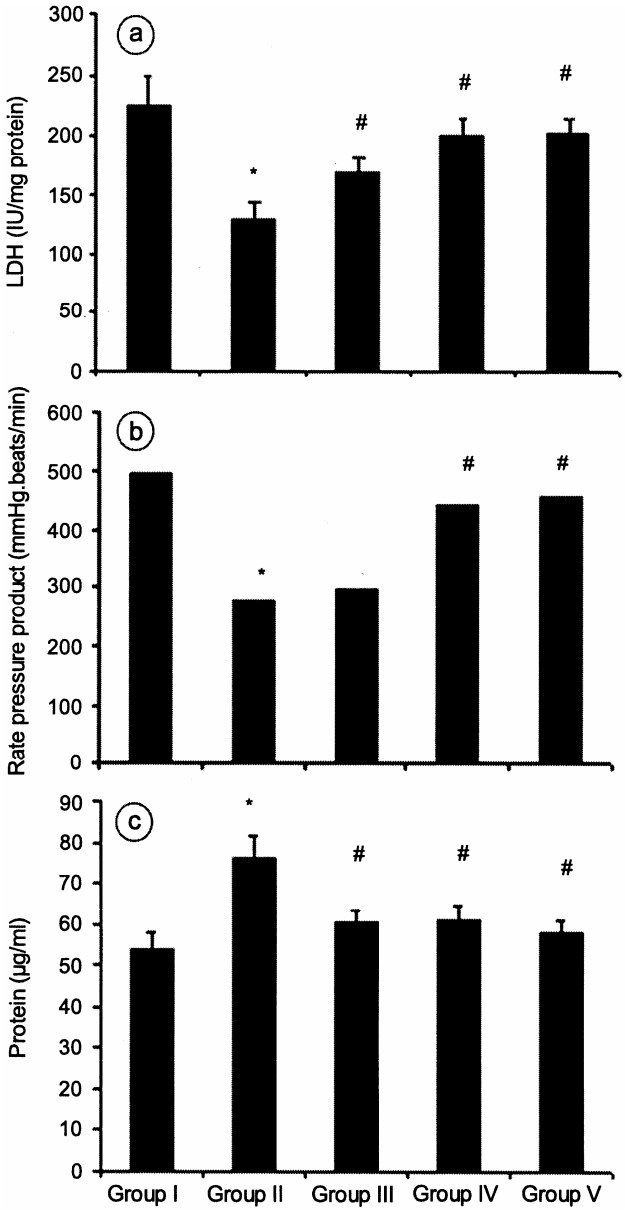

Fig. 1—Effect of *Commiphora mukul* (CM) on (a) myocyte injury marker enzyme, LDH; (b) Rate pressure product; and (c) protein in different experimental groups [The values are mean ± SD of 8 rats in each group. *P<0.05, when compared to sham, #P<0.05, when compared to ISP control]

improvement of hemodynamic and left ventricular function along with reduced myocardial injury in isoproterenol-induced myocardial infarction.

Myocardial injury commonly occurs in hypertension or following ischemia and reperfusion of the heart. Acute myocardial infarction causes severe ischemia by formation of an intra-arterial thrombus, which is treated through rapid restoring of blood flow using thrombolytic therapy or coronary angioplasty. Thus, traditional therapies for coronary artery disease or acute myocardial infarction are aimed at better matching coronary flow to the demand for myocardial mechanical power. Therefore hemodynamic and "plumbing'" approaches to the treatment of ischemic heart disease have proven relatively effective, especially for the treatment of acute myocardial infarction. However, an adjunctive treatment for ischemia is to manipulate energy metabolism, reduce oxidative stress in ischemic zone in a way that optimizes the metabolism of the ischemic cardiac muscle[23,24]. This approach, utilizing antioxidant intervention reduces the tissue injury and could potentially increase the rate of mechanical power.

The present study used a well established non invasive model for induction of myocardial infarction. As myocardial ischemia and infarction can be caused by increased inotropic activity they could be non invasively induced by administering a sympathetic activator such as isoproterenol, which has been described in several reports as causing myocardial necrosis[6], peroxide formation[25], alterations in coagulative parameters[26]. In supra-maximal dosages it produces acute myocardial necrosis and interstitial fibrosis. Some of the mechanisms have been proposed to explain isoproterenol-induced damage to cardiomyocyte including hypoxia due to myocardial hyperactivity and coronary hypotension, calcium overload, depletion of energy reserves and excessive production of free radicals resulting from oxidative metabolism of catecholamines.[6,25-29] It has been demonstrated that isoproterenol-induced myocardial necrosis is associated with dose-dependent increase in left ventricular end diastolic pressure, left ventricular volume and wall stress[27-29].

It is well documented that isoproterenol-induced ischemia produced a marked ventricular dysfunction[27,28]. Similar observations were also recorded in this study when subcutaneous isoproterenol was administered to rats. Following isoproterenol administration a significant decrease in systolic and mean arterial pressure was observed that indicated the activation of sympathetic nervous system. This decrease in SAP might be a compensatory mechanism of the myocardium to increase perfusion in order to meet the increased myocardial energy demand. *C. mukul* treatment was observed to increase the systolic and mean arterial pressure significantly with improved progression of cardiac function indicating the blunting of deteriorated metabolic and impaired state of ischemic myocardium. Decrease in heart rate depicted the injured state of myocardium. Following *C. mukul* treatment, a significant increase in heart rate was observed. Cardioprotection afforded by *C. mukul* may also be explained by the significant correction of MAP and HR that may increase blood flow through the sub-endocardial region of the ventricular muscle that bears the maximum brunt of ischemic insult. However, the current study, demonstrated that treatment with *C. mukul* prevented isoproterenol-induced impairment of inotropic (+LV dP/dt, marker of myocardial contraction) and lusitropic (-LV dP/dt, marker of myocardial relaxation) functions of heart. The extract also efficiently blunted isoproterenol-induced increase in left ventricular end diastolic pressure, a marker of preload that again reflected an improvement of left ventricular function. Myocardium gets perfused during diastolic phase of cardiac cycle through coronary arteries. These arteries are poor in collaterals therefore, under ischemic condition, the sub-endocardial region of heart is most vulnerable to ischemic necrosis because of disproportionate reduction in blood flow to subendocardial region, which is subjected to greatest extra-vascular compression during systole. In addition, increased left ventricular end diastolic pressure exerts an outward force on ventricular wall that reduces blood flow to the sub-endocardial region. By reducing left ventricular end diastolic pressure, *C. mukul* extract might have improved the perfusion to sub-endocardium thereby, reducing the myocardial injury. Along with improved ventricular function and contractility, a conservation of energy status was observed as evidenced by decrease in rate pressure product. In the present study, reduced after-load and decrease in heart rate account for decrease in rate-pressure-product, which is an approximation of myocardial oxygen consumption[20]. Decrease in arterial pressure and restored myocardial energy might translate into a beneficial effect of *C. mukul* as

a cardioprotective agent.

In previous studies isoproterenol causes increased left ventricular end diastolic volume, end diastolic pressure, left ventricular wall thickness and increased myocardial deposition of proteins[27,28]. In the present study a significant increase in myocardial proteins following isoproterenol challenge confirmed the presence of oxidative stress, a major mechanism of isoproterenol induced myocardial necrosis and protein degradation. Increased lipid peroxidation increases membrane permeability and thereby accumulation of lipid peroxidation products can directly produce membrane injury of cardiac myocyte. Decrease in protein content with *C. mukul* treatment reflects the salvage of myocardium.

Determination of LDH in heart is useful parameter for assessing myocardial damage[29]. Isoproterenol induced mediation of myocardial injury was evidenced by decrease in LDH activities in rat hearts. Release of LDH occurred after 24-48 hr of isoproterenol administration which is a delayed marker after the infarct has occurred. An increase of LDH content in heart of rats with *C. mukul* treatment indicated the prevention of leakage of LDH enzyme from myocardium. The equilibrium between cardiac function and biochemical parameters clearly emphasized the cardioprotective potential of *C. mukul*. The present results revealed mechanism of cardioprotective activity in addition to antihypertensive, antioxidant and antiatherogenic activity.

Guggulipid and guggulsterone have been demonstrated to reduce risk of cardiac events and improves cardiac function in experimental and clinical studies[12-16] . Guggulsterone obtained from gum resin of *C. mukul* contains isomers E and Z has been reports to inhibit platelet aggregation and provide protection from myocardial ischemia in rats[30-32]. The protective action of guggulsterone is due to antioxidant property because it inhibits the generation of oxygen free radicals[33].

Briefly, the present study results demonstrated that *C. mukul* treatment prevent myocardial impairment and ameliorated the depression of cardiac function by maintaining myocardial cell membrane integrity and antioxidant status. Further studies are warranted for time tested safety and efficacy in cardiovascular diseases.

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