



**EVALUATION OF CARDIOPROTECTIVE ACTIVITY OF SAPONINS (*SAPINDUS
MUKOROSI*) ON WISTAR RATS**

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ABSTRACT

Sapindus mukorossi Linn belongs to the family Sapindaceae. The fruit is valued for the saponins (10.1%) present in the pericarp and constitutes up to 56.5% of the drupe known for inhibiting tumour cell growth and traditionally fruit of *Sapindus mukorossi* is used for epilepsy, chlorosis (Iron deficiency anaemia) and excessive salivation. In the present study an attempt was made to isolate saponins from *Sapindus mukorossi* and to evaluate the cardioprotective potential of isolated saponins against isoproterenol induced cardio toxicity in wistar rats. The pericarp of *Sapindus mukorossi* is powdered, extracted with water and was subjected for phytochemical screening. The saponins (200mg/kg) exhibited significant cardio protective effect against Isoproterenol (2 mg/Kg, i.p.) induced myocardial damage by

showing the reduction in cardiac biomarkers like CK, CK-MB, and troponin (I). It is also evidenced by histopathological studies isoproterenol exhibited pathological changes in cardiac muscle including several congestions, subendocardial necrosis and abundant hyperplasia along with increased edematous intramuscular space while administration of the isolated saponins (200 mg/Kg) exhibited normal appearance with mild changes in congestions and necrosis as like with standard drug propranolol (10 mg/Kg). This research proved that the presence of saponins in *Sapindus mukorossi* fruits can protect the heart from the myocardial infarction condition.

Keywords: *Sapindus mukorossi*, saponins, cardiotoxicity, cardioprotective, biomarkers, Sub-endocardial necrosis

INTRODUCTION

Myocardial infarction (MI) is a complex phenomenon affecting the mechanical, electrical and structural and biochemical properties of the heart [1]. Myocardial infarction (MI) is the world's leading cause of morbidity and mortality, and will be the leading cause of death in the world by 2020, according to the world health organization [2]. Simultaneously with the increasing strain on society and health systems caused by non-communicable disease such as myocardial infarction, developing countries such as India are struggling to manage the effects of infectious diseases [3]. MI is the acute myocardial necrosis syndrome arising from an imbalance between the supply of coronary blood and myocardial demand [4]. Myocardial cell damage is caused by the production of toxic reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radical

[5]. Myocardial infarction (MI) results from prolonged myocardial ischemia with necrosis of myocytes due to interruption of blood supply to an area of heart. Free radicals and reactive oxygen species have an impact in various disorders like cardiac diseases and cancer which result due to exposure to chemicals and environmental agents, experimental and clinical studies have shown that there is increased generation of reactive oxygen species such as super oxide anion (O_2^-) and hydroxyl radicals (OH) in heart failure, which involved in the formation of lipid peroxides, damage of cell membrane, and destruction of antioxidative defence system [6]. Isoproterenol (ISP), a synthetic catecholamine and β -adrenergic agonist that causes severe myocardial pressure and heart muscle necrosis as an infarction [7]. ISP mediated myocardial injury entails changes in membrane permeability

resulting in the loss of myocardial membrane functions and integrity [8]. ISP is a well-known standard model for studying myocardial necrosis [10]. It should be appreciated that over the years, while more specific biomarkers of myocardial necrosis became available, the accuracy of detecting myocardial infarction has changed. Such changes occurred when creatinine kinase (CK) and the MB fraction of CK, i.e. CK-MB activity and CKMB mass. Current more specific and sensitive biomarkers and imaging methods to detect myocardial infarction are further refinements in this evolution [6].

Herbal medicines play a significant role in health care for a large part of the world's population and have been regarded as a component of the cultural heritage of different tribes. Polyphenols exercise cardio protective function by inhibiting low-density lipoprotein oxidation [10]. Most of the effective pharmacological drugs come from plants. Plant derivatives as medicines play an important role for animals and humans in health care systems around the globe [11]. The use of herbal medicines has been steadily increasing over the past decade. Some medicinal plants have been shown to have beneficial effects in conditions such as cancer, liver disease, cataract and myocardial ischemia. A significant number of these plants and plant-based products have been commonly

used [12, 13]. Therefore, herbal medicines with antioxidant properties can be protective for cardiovascular diseases [14]. *S. mukorossi* Linn (Sapindaceae), commonly known as Ritha or Aritha is found throughout India. The major constituents of its fruit are saponins (10%-11.5%), sugars (10%) and mucilage [15]. The fruit of the plant is reported to have expectorant, emetic, alexipharmic, and abortifacient effects. It is also used in excessive salivation, epilepsy and chlorosis [16, 17]. *S. mukorossi* also possess Insecticidal [18], Spermicidal [19], Anti-protozoal [20], Anti-inflammatory [21], Piscicidal [22], Cytotoxic [23], Hepatoprotective [24], Anti-platelet aggregation [25], Anti-trichomonas [26] and Anti-fungal [27] activities. Based on the wide intensity of usage we made an attempt to isolate saponins from *S. mukorossi* and to evaluate the cardioprotective potential of isolated saponins against isoproterenol induced cardiotoxicity.

MATERIALS AND METHODS

1. Materials:

The study was conducted on wistar albino rats of weight 200 - 250 gm and maintained under standard conditions (room temperature: 24-25°C, humidity: 60-65% with 12 hrs light and dark periods). Food in the form of dry pellets

(Amrut lab. Pune) and water were available ad libitum.

2. Isolation of Saponins from *Sapindus Mukorossi*:

The powdered pericarps of *S.mukorossi* Great (500 gm) were heated on a water bath for 6 hrs with distilled water (1 Lit) at 100°C. The supernatant liquid was decanted. Reddish clear extract was concentrated to one-third its volume on a thermostatic water bath at 50°C. The concentrated extract was treated with saturated solution of ammonium sulphate with vigorous shaking until a pale brown slimy mass floated on the surface. The liquid left out was again treated with saturated solution of ammonium sulphate to get maximum yield of the brown mass. The brown mass collected from each set was dissolved in saturated solution of ammonium sulphate and extracted with ethylacetate (50x3). The combined ethylacetate extract was evaporated to dryness. The white solid (75 gm) M.P. (140-143°C) was collected. This was confirmed as saponins of *S.mukorossi* [28].

3. Identification Test for Saponins:

Saponins were identified by using HPLC method. The normal- and reverse-phase HPLCs are commonly used for separation, identification, and

purification of saponins. But for the best separation of saponins, RP-HPLC is normally used. HPLC is increasingly used in the separation of various compounds including saponins. This technique is rapid, selective, and highly sensitive. Separation of saponins can be affected by HPLC using a variety of stationary and mobile phases. The hydrolyzed saponins of *sapindus mukorossi* were separated and quantified by HPLC method with UV detection. The total concentrations of saponins in aqueous extract were found to be 352µg/ml [29].

4. Experimental Design:

The experimental rats were divided into 4 groups, each consisting of 6 animals.

→ **Group I:** The control group, served as negative control, and received an intra-peritoneal (i.p.) injection of normal saline (2.5 ml/kg) daily for 30 days.

→ **Group II:** Served as a positive control and were treated with normal saline (i.p. 2.5 ml/kg) daily for 30 days and ISP (i.p. 2 mg/kg) on 31st and 32nd day.

→ **Group III:** The rats were administered with isolated saponins of 200 mg/kg, given orally once daily up to 30 days, followed by ISP administration on 31st and 32nd day.

→ **Group IV:** Animals were pre-treated with propranolol as standard drug (10 mg/kg) and the subcutaneous injection with ISP (2 mg/kg) serve as standard treatment control.

The duration of the treatment was 32 days. At the end of the treatment, blood was collected from all the groups by puncturing the retro-orbital plexus and was allowed to clot at room temperature and serum was separated by centrifugation at 2500 RPM for 10 min. The animals were sacrificed and hearts were excised and sent for histopathological studies [30, 31].

RESULTS AND DISCUSSION

Myocardial infarction remains a leading cause for death worldwide and prompt treatment for a heart attack is indispensable to save the life. In the traditional Indian medicinal system, a major role has been played by the plants, especially, in the aspect of cardioprotection. Several herbs and herbal products have been recommended for prophylactic and therapeutic effects in reducing cardiovascular diseases (CVDs) and that have been reviewed. In this context, there is a need to reveal the cardioprotective activity of isolated saponins from the plant *Sapindus mukorossi*. Myocardial ischemia results from the reduction of coronary flow to such an extent that supply of oxygen to the

myocardium does not meet the oxygen demand of myocardial tissue. When this ischemia is prolonged and irreversible then myocardial cell death and necrosis occurs which is defined as myocardial infarction. Recent studies suggest that increased free radical formation and subsequent oxidative stress associated with the occurrence of a relative deficit in the endogenous antioxidants, may be one of the mechanisms for the development of heart failure after myocardial infarction. In our study, ISP was used to induce myocardial damage and it has been found to cause a severe stress in the myocardium resulting in necrosis of the heart muscle. It is also well known to generate free radicals and stimulate lipid peroxidation, which may be a causative factor in irreversible damage to the myocardial membrane in experimental myocardial infarction.

The animals pre-treated with saponins in group IV shows a significant reduction in the cardiac markers like CK, CK-MB and Troponin-I levels when compared with the ISP treated rats (Group II). The decrement of cardiac marker is almost similar to the standard treatment with propranolol 10 mg/kg i.e. group II animals (**Table 1**). The histopathological examination of myocardial tissue of normal animals shows a clear integrity of myocardial cell membrane. Endocardium and pericardium were seen with in the

normal limits. No inflammatory cells infiltration was observed in the normal heart. In isoproterenol treated animals showed that a focal myonecrosis with myophagocytosis and lymphocytic

infiltration (myocarditis) was observed. The animals pre-treated with isolated saponins (*sapindus mukorossi*) at 200 mg/kg were found less damaged when compared with control group.

Table 1: Effect of saponins (*sapindus mukorossi*) on cardiac marker enzymes

GROUP	TREATMENT	CK-MB	CK	TROPONIN-I
Group I	Negative Control	54±2.43	166±0.55	Negative
Group II	Positive Control (ISP-2 mg/Kg)	96±1.61	935± 1.43	Positive
Group III	S.M (200 mg/Kg) + ISP	32±0.79	146±2.01	Negative
Group IV	ISP + Propranolol (10 mg/Kg)	49±1.07	164± 1.87	Negative

ISP: Isoproterenol; CK: creatinine kinase; CK-MB: Creatinine kinase myocardial band. Data represented as Mean ± SEM (n=5)

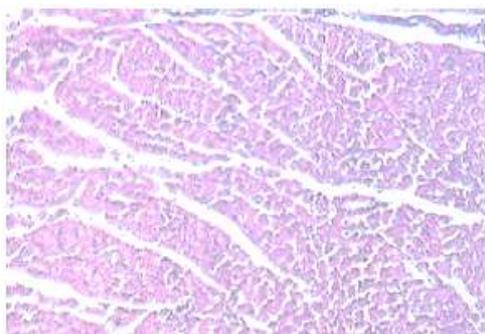


Figure No- 1 Negative control (saline solution)

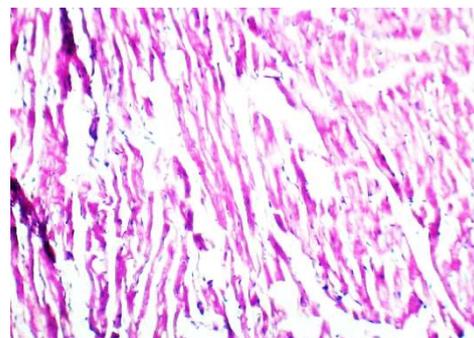


Figure No-2: Positive control (isoproterenol)

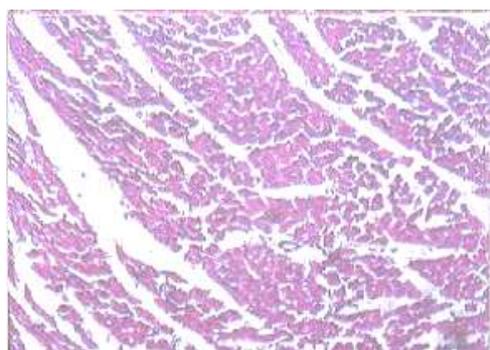


Figure No- 3: ISP + Propranolol

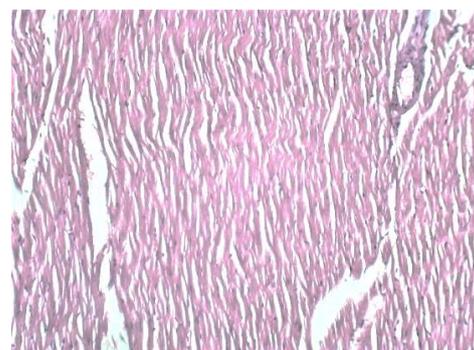


Figure No-4: SM (saponins) + ISP

The enzyme levels of CK, CK-MB and Troponin-I were decreased compared with positive control (ISP administered) rats. Thus it shows the cardioprotective activity of test saponins (*sapindus mukorossi*). The histopathological studies are as follows.

From the histopathological studies of heart the negative control rats showed the regular arrangement with clear striations of myocardial fibres without any histological alterations because of degeneration or necrosis. ISP induced rats

(Group II- positive control) showed pathological changes in heart including several congestions, subendocardial necrosis and abundant hyperplasia along with increased edematous intramuscular space. The heart was having near normal appearance with mild changes in congestions and necrosis in rats treated with standard drug propranolol. Same pattern was obtained in rats treated with isolated saponins (*sapindus mukorossi*) 200mg/kg. So this research proved that the isolated saponins (*sapindus mukorossi*) fruits can protect the heart from the myocardial infarction condition.

CONCLUSION

The saponins (200mg/kg) exhibited significant cardioprotective effect against Isoproterenol (2 mg/Kg, i.p.) induced myocardial damage by showing the reduction in cardiac biomarkers like CK, CK-MB, and troponin (I). It is also evidenced by histopathological studies isoproterenol exhibited pathological changes in cardiac muscle including several congestions, subendocardial necrosis and abundant hyperplasia along with increased edematous intramuscular space while administration of the isolated saponins (200 mg/Kg) exhibited normal appearance with mild changes in congestions and necrosis as like with standard drug propranolol (10 mg/Kg). This research proved that the presence of saponins in *Sapindus*

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Conflict of Interest:

The authors of this manuscript declare no conflict of interest.

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