

Protective effects of *Semecarpus anacardium* fruit extract against myocardial ischemia-reperfusion injury in rats

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Citation

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Abstract

The purpose of the present study was to identify bioactivity in ethanolic extract of *Semecarpus anacardium* (EESA) fruits on experimentally induced myocardial damage in rats. The excised heart from previously treated rats was mounted and subjected to ischemia-reperfusion injury (IRI). The high dose of EESA significantly decreases endogenous biomarker enzymes such as LDH and CK-MB activities in perfusate and increases in heart tissue homogenate (HTH). Moreover, biological antioxidants viz., SOD and catalase activities were significantly increased in HTH. High dose of EESA were also found to provide significant recovery in developed tension and heart rate from ischemia. The biochemical findings were confirmed by histological investigations.

INTRODUCTION

The use of complementary and alternative medicines is burgeoning globally for ischemic heart diseases (IHDs), especially in developed countries including US [12]. However, majority of the population in developing countries depend on traditional healing modalities, including herbal remedies, for health maintenance and therapeutic management disease [3]. Although many studies identified the increasing prevalence of herbal use throughout the world, only a few reported on how patients perceived the efficacy of this healthcare modality in specific diseases including IHDs [45].

Nuts of *Semecarpus anacardium* Linn. (Anacardiaceae) (SA nuts) are known in folklore for treating various diseases such as anti-helminthic, anti-fungal, anti-carcinogenic and anti-arthritis [67]. Moreover, they were also exploited for managing variety of cardiac complications such as angina, hypertension, myocardial dysfunctions and cardiac disabilities [8]. However, there is no scientific confirmation for the above said traditional claims. The present study is design to ascertain the effect of ethanolic extract of fruits of *semecarpus anacardium* during myocardial damage induced by ischemia-reperfusion.

MATERIALS AND METHODS

CHEMICALS

All chemicals used were of analytical grade and purchased from standard companies. Biochemical kits such as LDH

and CK-MB were procured from Crest Biosystems (Goa, India).

PLANT EXTRACT

Authentic sample of *S. anacardium* nuts was purchased from the medicinal garden Danvantri vana, Bangalore University, India. They were washed and only good quality nuts, which did not float in water, were selected for further study. They were dried, weighed and subjected to extraction with endotoxin free ethanol (95%) (Sigma, St. Louis, MO, USA) in soxhlet for about 72 hrs. The obtained syrupy mass was concentrated and dried in hot air oven and the percentage yield was calculated. The obtained mass was collected in aliquots and stored at -20°C till used.

ACUTE TOXICITY STUDIES

The acute toxicity study was carried out according to the limit test described by the OPPTS guidelines. Test dose of 2 g/kg and 5 g/kg were given to mice. Hence, 1/25th and 1/50th of the safe dose corresponding to 200 mg/kg and 100 mg/kg orally were selected as high and low dose respectively [9].

EXPERIMENTAL ANIMALS

Laboratory bred female Wistar albino rats weighing between 200-250 g were housed at $25^{\circ} \pm 5^{\circ}\text{C}$ in a well-ventilated animal house under 12:12 hour light and dark cycle. The rats had free access to standard rat chow (Amrut Laboratory Animal feed, Maharashtra, India) containing protein 22.10%, oil 4.13%, fibre 3.15%, ash 5.15%, sand (silica) 1.12% w/w

and water ad libitum. There was no significant difference in the body weight of the treated rats when compared with control, either at the beginning or at the end of the study period. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

EXPERIMENTAL PROTOCOL

The animals were divided into different treatment groups. The first group served as IRI control. The animals of group II were treated orally for 30 days with propranolol orally at a dose of 10 mg/kg [10]. The animals of group III and group IV were treated with ethanolic extract of fruits of *semecarpus anacardium* (EESA) 100 and 200 mg/kg (30 days, p.o), respectively.

EXPERIMENTAL PROCEDURE

A modified langendorff apparatus for the isolated perfused heart was set up as mentioned elsewhere [11]. The heart was isolated from each animal 2 hrs after the last dose of the drug(s) under ketamine (70 mg/kg, i.p) and xylazine (10 mg/kg, i.p) anesthesia. The isolated heart was perfused with Krebs-Henseleit (K-H) solution gassed with carbogen (95% O₂ and 5% CO₂) at 37 ° C at a constant flow rate of 5 ml/min. Measurement of contractile force was done using force displacement transducer and recorded on a Student Physiograph (INCO, Mumbai, India). After the initial pre ischemic perfusion, heart was subjected to 15 min of global no-flow ischemia by blocking the flow of K-H solution & carbogen supply followed by 15 min of reperfusion [12]. The heart rate and developed tension were measured during pre-ischemic and post-ischemic period and recovery (%) was calculated. Lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) activity were measured in pre-ischemic and post-ischemic perfusates. The heart was then homogenized to prepare heart tissue homogenate (HTH) using sucrose (0.25 M) [13] and the activity of LDH, CK-MB, superoxide dismutase (SOD) [14] and catalase [15] was determined. Microscopic slides of myocardium were prepared for histopathological studies after post-ischemia.

The myocardial damage was determined by giving scores depending on the intensity as follows [16]; no changes – score 00; mild – score 01 (focal myocytes damage or small multifocal degeneration with slight degree of inflammatory process); moderate – score 02 (extensive myofibrillar

degeneration and/or diffuse inflammatory process); marked – score 03 (necrosis with diffuse inflammatory process).

STATISTICAL ANALYSIS

Results are expressed as mean ± SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P<0.05 was considered significant.

RESULTS

PHYTOCHEMICAL ESTIMATIONS OF THE EXTRACTS

The percentage yield of EESA was found to be 13%. Chemical and phytochemical analysis of EESA nut reveals the presence of biflavonoids, biflavones, phenolic compounds, bharilawanols, minerals, vitamins and amino acids.

EFFECT ON LDH AND CK-MB ACTIVITIES

The biological activities of endogenous enzymes such as LDH and CK-MB were evaluated in coronary effluent (perfusate) during pre-ischemic and post-ischemic period as well as in heart tissue homogenate (HTH). In perfusate, high dose of EESA-200 and PRO-10 showed a significant (p<0.001) decline in activation of both LDH and CK-MB when compared with IRI control. However, low dose of EESA -100 showed no significant alteration in LDH and CK-MB activity when compared with IRI group (Figure 1 & 2). In heart tissue homogenate, high dose of EESA-200 and PRO-10 was found to significantly (p<0.001) elevate the enzyme activities compared to IRI. However, EESA-100 fails to show similar significant change in enzyme activities when compared to IRI control. (Figure 1 & 2).

Figure 1

Figure 1

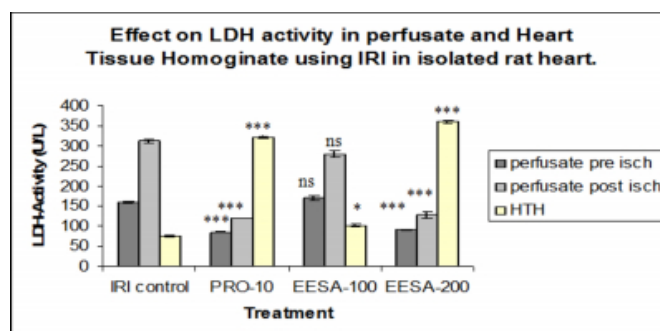
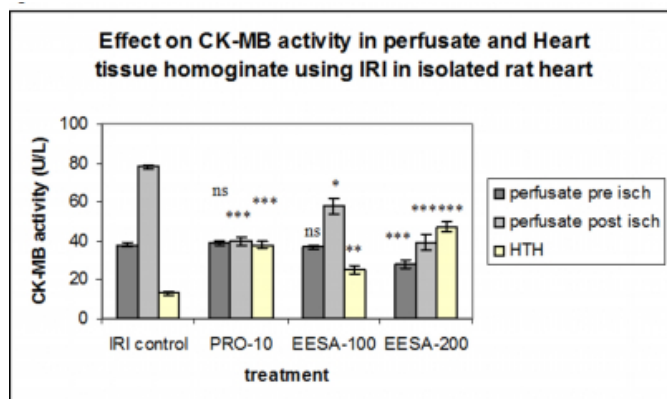


Figure 2

Figure 2



Values are expressed as mean \pm SEM for eight rats in each group.

*** Significantly different from IRI control $P < 0.001$.

EESA-100 and EESA-200 = EESA - 100 mg/kg and 200 mg/kg

(30 days treatment, p.o)

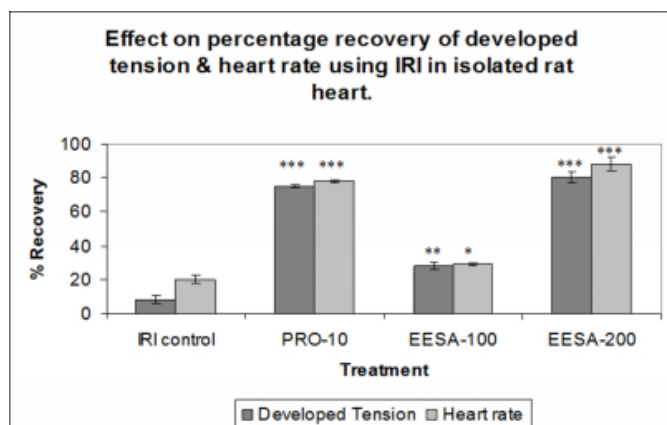
PRO-10 = Propranolol 10 mg/kg (30 days treatment, p.o)

EFFECT ON DEVELOPED TENSION AND HEART RATE

Pre treatment of animals with EESA-200 and PRO-10 showed a significant ($p < 0.001$) recovery (%) of ischemic heart in terms of both heart rate and developed tension when compared with IRI control. However, low dose of EESA (EESA-100) fails to recover the ischemic damage (Figure 3).

Figure 3

Figure 3



Values are expressed as mean \pm SEM for eight rats in each group.

*** Significantly different from IRI control $P < 0.001$.

EESA-100 and EESA-200 = EESA - 100 mg/kg and 200 mg/kg

(30 days treatment, p.o)

PRO-10 = Propranolol 10 mg/kg (30 days treatment, p.o)

EFFECT ON SOD AND CATALASE ACTIVITY

The SOD and catalase activity in the HTH were significantly ($p < 0.001$) increased after treatment with EESA-200 ($p < 0.001$), EESA-100 ($p < 0.01$) and PRO-10 ($p < 0.05$), when compared to IRI control (Table 1).

Figure 4

Table 1: Effect on SOD, catalase activities and histological scores in HTH using IRI induced MI

TREATMENT	SOD (Units/mg protein)	Catalase (Units/mg protein)	Histological scores
IRI-control	89 \pm 0.09	1.89 \pm 0.44	2.51 \pm 0.21
PRO-10	56 \pm 0.008***	3.54 \pm 0.38**	0.75 \pm 0.10***
EESA-100	21 \pm 1.05***	5.73 \pm 0.18***	2.25 \pm 0.81**
EESA-200	5.75 \pm 1.23***	8.39 \pm 0.08***	0.5 \pm 0.05***

Values are expressed as mean \pm SEM for eight rats in each group.

*** Significantly different from IRI control $P < 0.001$.

EESA-100 and EESA-200 = EESA - 100 mg/kg and 200 mg/kg

(30 days treatment, p.o)

PRO-10 = Propranolol 10 mg/kg (30 days treatment, p.o)

SOD Units: One enzymatic unit of SOD is the amount in the form of proteins present in 100 l of 10 % heart tissue required to inhibit the reduction of 24 mM NBT by 50%.

Catalase Units: One international unit of catalase is the amount, which catalyzes the decomposition of 1 mM hydrogen peroxide per minute at 37 $^{\circ}$ C.

EFFECT ON HISTOLOGICAL SCORE

Histological examination (Table-1) of myocardial tissue of the IRI control showed patchy areas of necrosis,

hyalinization of muscle fibers with focal cellular infiltrations. The muscle fibers showed vacuolar changes with fragmentation suggestive of necrosis. In animals pretreated with EESA-200 and PRO-10, damages due to IRI were significantly removed. However, EESA-100 fails to provide any protection during IRI damage.

DISCUSSION

The research envisaged was carried out to determine the effect of different doses of EESA and its comparison with PRO-10 during IRI induced myocardial damage in isolated rat heart preparation. The results show that high dose of EESA-200 and PRO-10 protects the myocardium against IRI damage. However, EESA-100 fails to provide the similar result.

Myocardial damage was induced using ischemia-reperfusion injury (IRI) model. The IRI was obtained following no-flow global ischemia, where sudden occlusion of physiological salt solution (PSS) results in immediate biochemical alterations [1718]. The increase in intracellular Na^+ serves to drive Ca^{2+} intracellularly via $\text{Na}^+/\text{Ca}^{2+}$ exchange that results in irreversible damage to myocardium at the end of 15 min global ischemia [19].

It is well established that the biological markers like endogenous enzyme are organ specific and leak from the damaged organ during necrosis [20]. Damage to the cardiac musculature due to IRI results in leakage of cardiac biomarkers such as LDH and CK-MB into the perfusate with resultant decrease in their activities in HTH and increase in perfusate [21]. Prophylactic administration of EESA-200 and PRO-10 were found to provide protection by preventing the damage to cardiac musculature during events of ischemia as shown by decrease activities of enzymes in perfusate and increase activities in HTH [22]. It can also be emphasized that the damage to myocardium cannot be reversed by low dose of EESA-100 as indicated by increase activity of LDH and CK-MB in perfusate and decrease activity in HTH.

There is substantial evidence that the associated contractile and rhythmic disturbances involve a contribution from oxygen free radicals (OFRs) [23]. During damage to myocardium, OFRs such as superoxide and hydrogen peroxide are produced in enormous amount that contribute to myocardial tissue injury [24]. IRI induced myocardial damage is associated with decreased endogenous antioxidants such as superoxide dismutase (SOD) and catalase in perfusate, which are structurally and functionally impaired by free

radicals resulting in damage to myocardium. IRI is highly dependent on the extent and duration of the ischemia and severity of coronary flow reduction. Inclination in endogenous antioxidant activities in HTH is indication for structural integrity and protection to the myocardium by prior administration of EESA. However, low dose of EESA does not show the similar rise in SOD and catalase when compared to high dose indicating the dose dependent effect of EESA. It is interesting to note the alteration in SOD is with concomitant fluctuation in catalase. Elevated activity of catalase in HTH is more beneficial than increase in SOD activity alone because without a simultaneous increase in catalase activity, increased SOD activity may lead to intracellular accumulation of H_2O_2 with detrimental effects [25]. However, EESA-100 failed to show the similar beneficial effect probably because low dose failed to reduce the oxidative stress mediated through superoxide and hydrogen peroxide.

The protection to myocardium during IRI can also be provided by scavenging/preventing the formation of OFRs. PRO causes decrease influx of calcium across the cell membrane leading to dephosphorylation of myosin light chain kinase. It deactivates voltage sensitive calcium channels in the heart via G_s mediated mechanism independent of cAMP concentration. Therefore pacemaker activity and conduction velocity are decreased with resultant increase in refractory period. As it is known that oxidative phosphorylation is a central site of reactive oxygen species production in the heart [26]. Therefore by dephosphorylation, generation of OFRs can be drastically reduced. On the basis of the present observation, it is speculated that PRO mediate cardioprotection without significantly elevating the SOD or catalase activities in HTH but by scavenging ability towards OFRs.

There was considerable recovery from ischemia-reperfusion induced MI in treated groups in functional parameters such as developed tension and heart rate. Increase in developed tension shows an improvement in cardiac contraction while a decrease in developed tension is an indication of injury to the heart. High dose of EESA are equipotent with PRO-10 in imparting recovery to myocardium as indicated by similar rise in developed tension after global 15 min no flow ischemia, whereas, EESA-100 does not show the same result.

Damage to cardiac musculature was also demonstrated and confirmed by histopathological parameter i.e. histological

scores. An increase in this parameter is indicative of myocardial damage [27]. In this study, it was found that EESA-200 and PRO-10 showed a remarkable reduction in the histological score when compared to ischemic heart. This effect of EESA-200 is may be due to augmentation of endogenous antioxidant synthesis. The recovery to myocardium could be attributed to NO scavenging property and generation of endogenous antioxidants due to prior administration of high dose of EESA. Hence it can be speculated that EESA-200 shows antioxidant activity by either inhibiting the release of OFR or enhancing the synthesis of endogenous antioxidants in IRI induced cardiotoxicity.

CONCLUSION

EESA in higher doses improves the myocardial recovery from injury induced by IRI. The observations made in the present study showed that prior administration of high dose of EESA prevents oxidative stress and associated structural changes induced by myocardial ischemic reperfusion injury (IRI). However, EESA in low dose failed to reduce the oxidative stress and thus unable to keep the myocardial integrity. In conclusion, further studies are required on ultrastructural changes in order to validate their use as cardioprotective and explore the possible mechanism of action.

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