

MYOCARDIAL POTENCY OF *SEMECARPUS ANACARDIUM* NUT EXTRACT AGAINST ISOPROTERENOL INDUCED MYOCARDIAL DAMAGE IN RATS

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ABSTRACT

The present study was undertaken to evaluate the cardioprotective effect of hydroalcoholic extract of *Semecarpus anacardium* nuts (SANE) against isoproterenol (ISO) induced myocardial damage in rats. Rats of either sex were administered SANE (100 and 500 mg/kg) for three weeks and propranolol (PRO, 10 mg/kg) for one week orally in their respective groups. Myocardial damage was induced by subcutaneous administration of isoproterenol (150 mg/kg) for two consecutive days. A change in biomarkers and antioxidants levels reflects the influence of prophylactic treatment. The CK-MB activities were fallen in serum and elevated in heart tissue of animals treated with low and high doses of SANE as well as PRO compared to ISO control. The LDH activity were significantly reduced in serum with both and high doses of SANE while no change was noted in heart tissue with both doses compared to ISO control. Further, high and low doses of SANE caused significant elevation in SOD and CAT activities compared to ISO control. Hence it is concluded that *semecarpus anacardium* possess potential to ameliorate the myocardial damage induced by isoproterenol in rats.

Keywords: *Semecarpus anacardium*; Antioxidants; Cardioprotection; Isoproterenol. Myocardial infraction

INTRODUCTION

Myocardial infarction (MI) is an acute or chronic form of cardiac disability arising due to the imbalance between the myocardial supply and demand for oxygenated blood¹. MI causes irreversible necrosis of heart tissue that is responsible for the principle cause of death in developed and developing countries². MI is making increasingly important contribution to mortality statistics due to advanced life style in developing countries, such as India, particularly in metropolitan cities of such countries³. Recently the use of herbs and herbal medicines in chronic diseases like MI is burgeoning in many parts of the world⁴.

Semecarpus anacardium belongs to family Anacardiaceae that usually found in outer Himalayas from Sulej to Sikkim, and throughout the hotter parts of India, as far east as Assam. In Ayurvedic, Unani and Siddha system of medicine, it is called as Bhallataka, Bhilavaa, and Sorankottai respectively. The parts generally used are detoxified nut and oil⁵. The nut of *Semecarpus* shell contains biflavonoids, biflavone A, C, A₁, A₂, tetrahydrorobustaflavone, jeediflavone, semecarpuflavone and gulluflavone. Oil from nuts contains Bhilavinol and the leaves contain amentoflavone as a sole biflavonoid⁶. The fruit of this plant is traditionally used as a folk remedy in certain regions of India for the treatment of piles in non – bleeding conditions. It is an effective adjuvant in the treatment of ascites and tumours. It reduces the bronchospasms and their frequency too⁷. Nuts of *Semecarpus anacardium* Linn. (Anacardiaceae) are known in folklore for treating various diseases such as anti-fungal⁷, anti-carcinogenic and anti-arthritis⁸. The nut of the plant proved to have protective effect in aflatoxin B1 mediated hepatocellular carcinoma through induction of *in vivo* antioxidant defense system⁹. The fruits are - acrid, bitter, astringent, weat themogenic, emollient digestive, carminative, anathematic, purgative, liver tonic, expectorant, alternate aphrodisiac, antiarthritic, depurative,

anticarcinomic, stimulant, urinary astringent, antiseptic, anti-inflammatory, cardiogenic, uterine stimulant, sudorific, febrifuge, rejuvenating and tonic⁸. Further, studies showed that nut extract significantly lowered blood glucose level in alloxan induced diabetic rat and also in normal rats¹⁰. Furthermore, the nut extract demonstrated antioxidant¹¹ and anti atherosclerotic properties¹².

Till now there is no scientific evidence of cardioprotective activity of *Semecarpus anacardium*. Therefore the current research was designed to evaluate cardioprotective role of semecarpus anacardium in an experimental model of isoproterenol (ISO) induced myocardial damage in rat heart.

MATERIALS AND METHODS

Chemicals

Isoproterenol was purchased from Sigma-aldrich, U.S.A. LDH & CKMB Kits for enzyme estimation were purchased from Crest Biosystems, Coral clinical systems, Goa, India. Other chemicals used were obtained from SD fine chemicals Ltd. (Mumbai, India). All chemicals used in the present study were of analytical grade.

Experimental animals

Laboratory bred Sprague-Dawley (SD) rats weighing 175-250 g were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol (KCP/IAEC-27/2008-09). The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Plant material

The shade-dried nuts of *Semecarpus anacardium* were purchased from the local market of Bangalore (India) and Regional Research Institute (Ay), Bangalore authenticated the nuts (RRI/BNG/SMP/Drug Authentication/2009-10/535). The nuts were mechanically grinded and detoxified with the solvent n-butanol for five days with the daily change of the solvent¹³. The detoxified nuts were subjected to exhaustive extraction in a soxhlet apparatus-using ethanol. The extract was concentrated in water bath and stored in a desicator until further use.

Phytochemical estimations of the extract^{14, 15}

Hydroalcoholic extract of *Semecarpus anacardium* nuts (SANE) was subjected to qualitative analysis to investigate the presence of various phytochemical constituents like alkaloids, carbohydrates, glycosides, phytosterols, proteins, saponins, tannins and flavonoids.

Acute toxicity study

The acute oral toxicity study was performed according to the OPPTS guidelines (Office of Prevention, Pesticide and Toxic Substance) following the limit test procedure¹⁶. The animals were fasted over night prior to the experiment. Test dose of 2 g/kg and 5 g/kg were given orally to mice. Both doses were found to be safe. Hence 1/10th and 1/50th of the maximum safe dose corresponding to 500 and 100 mg/kg orally were selected as high and low doses respectively.

Experimental protocol

The animals were divided into five groups of eight each. Group I and Group II received saline for three weeks and termed as normal control and ISO control respectively; Group III was treated with standard Propranolol (PRO) 10 mg/kg, *p.o* for one week after two week of saline treatment; Group IV and Group V were administered SANE 100 and 500 mg/kg orally respectively for three weeks.

Isoproterenol (ISO) induced myocardial necrosis in rats⁴

After the treatment of animals for three weeks from group II to V according to the protocol, isoproterenol (ISO) 150 mg/kg, *s.c* was administered for two consecutive days. Forty eight hour after the first dose of ISO administration the rats were sacrificed. Blood samples were collected by the retro orbital puncture method. Serum was separated and biochemical markers LDH and CKMB were estimated. Heart tissue homogenate (HTH) was prepared in sucrose solution (0.25 M) and used for estimation of endogenous marker enzymes and biological antioxidants viz., superoxide dismutase (SOD)¹⁷ catalase¹⁸ and TBRS^{19,20} activities.

Statistical analysis

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

RESULTS

Preliminary phytochemical investigation

The preliminary phytochemical investigation of the SANE extract showed the presence of alkaloids, carbohydrates, flavonoids, cardiac glycosides, proteins, saponins, tannins and terpenoids. The percentage yield of SANE was found to be 24%.

Effect on LDH activity (Table 1)

The LDH activity of SANE-100 and SANE-500 were compared with normal and ISO control. No significant change in the serum LDH activity was observed with SANE-100, SANE-500 and PRO compared with normal control, whereas, significant fall in serum LDH activity was seen with PRO, SANE-100 and SANE-500 compared with ISO control. Two doses of ISO caused significant fall in LDH activity in heart tissue homogenate compared to normal control. However, no significant change occurred in groups treated with SANE-100, SANE-500 and PRO compared to ISO control.

Table 1: Effects on LDH and CKMB level in serum and heart tissue homogenate against isoproterenol induced myocardial damage

Treatment	CK-MB ACTIVITY		LDH ACTIVITY	
	Serum (unit/lit)	HTH (unit/gm)	Serum (unit/lit)	HTH (unit/gm)
Normal control	11 \pm 1.4	210 \pm 20	324 \pm 16	16.2 \pm 2.1
ISO CONTROL	92 \pm 2.2 ***	34 \pm 10 ***	643 \pm 3.1 **	3.4 \pm 0.7 ***
PRO	19 \pm 5.2 ***	96 \pm 1.6 ****	396 \pm 40 **	7.2 \pm 0.6 **
SANE-100	20 \pm 3.6 ***	137 \pm 2.1 ****	416 \pm 18 **	5.8 \pm 1.2 **
SANE-500	16 \pm 0.5 ***	167 \pm 4.8 ***	456 \pm 43*	7.2 \pm 1.5 ***

All values are mean \pm SEM, n=8, * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$ when compared to normal control; * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$ compared to ISO control. SANE -100 (*Semecarpus anacardium* nut extract 100 mg/kg), SANE -500 (*Semecarpus anacardium* nut extract 500 mg/kg). All treatments were done for 21 days by oral route.

Table 2: Effects on SOD, Catalase and TBARS in Heart tissue homogenate against isoproterenol induced myocardial damage

Treatment	Heart tissue homogenate		
	SOD	Ca Catalase	TBARS
Normal control	14.4±0.15	6.56±0.04	72.5±5.0
ISO CONTROL	4.1±0.07***	0.04±0.01***	24.1±2.2***
PRO	6.9±0.26***	2.73±0.28***	31.7±2.2***
SANE-100	5.1±0.37***	0.62±0.02***	34.8±2.2***
SANE-500	6.6±0.31***	2.28±0.04***	57.66±0.3***

All values are mean ± SEM, n=8, * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$ when compared to normal control; * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$ compared to ISO control. SANE -100 (*Semecarpus anacardium* nut extract 100 mg/kg), SANE -500 (*Semecarpus anacardium* nut extract 500 mg/kg). All treatments were done for 21 days by oral route.

Effect on CK-MB activity (Table 1)

Two doses of ISO resulted in extremely significant elevation in CK-MB activities in serum and fall in heart tissue homogenate. Comparing the treatment groups viz. SANE-100, SANE-500, PRO with the ISO group, an extremely significant decrease ($p < 0.001$) in CK-MB activity in serum was observed. In heart tissue homogenate, there was an extremely significant ($p < 0.001$) decrease in CK-MB activity were seen in ISO group compared to normal control. On the contrary, pre-treatment of animals with SANE-100, SANE-500 and PRO caused significant ($p < 0.001$) increase in the level of CK-MB compared to ISO control.

Effect on SOD and Catalase (Table 2)

The SOD and Catalase activity were estimated in the heart tissue homogenate. Subcutaneous administration of ISO caused significant fall in SOD and CAT activities in heart tissue homogenate compared to normal control. Prophylactic administration of PRO and SANE-500 resulted in significant ($p < 0.001$) rise in SOD and CAT activities as compared to ISO control where as SANE-100 causes no significant changes compare with ISO group.

Effect on TBARS (Table 2)

TBARS levels increased significantly ($p < 0.001$) upon ISO administration and remained high in PRO and SANE-100 pretreated groups compared to normal control. In SANE-500 group an extremely significant decrease ($p < 0.001$) was observed as compared to ISO group.

DISCUSSION

Isoproterenol, which is known as a synthetic catecholamine and β -adrenoceptor agonist, is reported to produce myocardial infarction in large doses²¹. Isoproterenol causes the cardio toxicity by several mechanisms including relative hypoxia, coronary microcirculatory disturbances however intracellular calcium load is now the most accepted cause of catecholamine cardio toxicity²². Two consecutive doses of isoproterenol by subcutaneous leads to an increase in calcium uptake and energy consumption leading to cell death²³. Elevation in the level of marker enzymes in serum

is due to the leakage of enzymes from the heart as a result of isoproterenol-induced necrosis²⁴. Isoproterenol also causes the production of cytotoxic free radicals through its auto-oxidation. It has been suggested that the oxidative products of catecholamines produce changes in the myocardium by stimulating lipid peroxidation and cause irreversible damage to the myocardial membrane. This alters membrane permeability, leading to the loss of function and integrity of myocardial membranes²⁵.

As discussed above ISO induces free radical formation (TBARS) and reduction in antioxidant activities such as superoxide dismutase (SOD) and Catalase and also reduction in marker enzyme such as LDH and CKMB in HTH. Pretreatment of animals with low dose of SANE (100 mg/kg, *p.o*) produces no significant changes in antioxidant activities compare with the ISO group. High dose of SANE (500 mg/kg, *p.o*) causes significant level of elevation in SOD activities with simultaneous increase in CAT and TBARS activity, increased SOD activity may lead to intracellular accumulation of H_2O_2 with detrimental effects²⁶. Hence simultaneous rise in SOD and CAT scavenge free radicals more effectively.

It has been known that biochemical markers are tissue specific and leak from the damaged tissue. Damage to the membrane induced by the ISO causes release of enzymes in the serum and deficiency of enzymes in HTH reflects the damage to the myocardium. Low and high doses of SANE (100 & 500 mg/kg, *p.o*) decreases serum CKMB and both the doses causes increase in CKMB activities in HTH. In LDH activity both the high and low dose of SANE causes rise in serum but no significant change is observed in LDH activity in heart tissue homogenate.

CONCLUSION

From our study it may be concluded that the both high dose of SANE (500 mg/kg) and low dose of SANE (100 mg/kg) possess good cardio protective activity against isoproterenol (ISO) induced myocardial necrosis in rats. Further studies should be carried out to elucidate the active constituents responsible for the said effect with extensive evaluations of histological and ultra structural changes.

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