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Research Article

THE POTENTIAL OF INTERACTION OF HYDROALCOHOLIC EXTRACT OF *SEMECARPUS ANACARDIUM* NUT EXTRACT WITH PROPRANOLOL IN ISCHEMIA-REPERFUSION INDUCED MYOCARDIAL DAMAGE USING ISOLATED RAT HEART

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ABSTRACT
The present study was carried out to determine the effect of hydroalcoholic extract of *Semecarpus anacardium* nut (SANE) and the possible interaction with propranolol (PRO) on experimentally induced myocardial infarction (MI) in rats. Isolated rat hearts were infused with propranolol 10 µg/ml (PRO), SANE 1 µg/ml (SANE 1), SANE 10 µg/ml (SANE 10), SANE 25 µg/ml (SANE 25), SANE 50 µg/ml (SANE 50) and SANE 10 + PRO dissolve in KH solution using modified Langendorff's apparatus through the aorta and subjected to ischemic reperfusion injury (IRI) by stopping the KH flow. The influence of different treatment was analysed by quantification of % recovery in terms of heart rate and force of contraction, biomarkers and antioxidants in post ischemic condition and histopathological studies. The superoxide dismutase and catalase activities were reduced in the heart tissue of isolated heart perfused with PRO, SANE 1, SANE 10, SANE 25, SANE 10 +PRO compared with IRI control. The biomarker LDH activity decreases in perfusate with PRO, SANE 1, SANE 10, SANE 25, SANE 10+PRO treatment and rises in heart tissue homogenate (HTH) of SANE 10 and SANE 10+PRO group compared with IRI control. CKMB activity decreases significantly in perfusate of SANE 10 and SANE 10+PRO groups and elevated in HTH in the groups PRO, SANE 10, SANE 25, SANE 10+PRO as compared with IRI control. Significant level of % recovery in terms of heart rate and developed tension were observed in groups infused with SANE 10 and SANE 10+PRO compared with IRI control. Histopathological studies reported SANE 10+PRO as a best protective group. To conclude, perfusion of SANE 10 with PRO in combination demonstrated most significant cardioprotective efficacy in isolated rat heart subjected to ischemia reperfusion injury.

KEYWORDS: Cardioprotection, Propranolol, *Semecarpus anacardium*, Ischemia-reperfusion injury, isolated rat heart.

INTRODUCTION

Ischemic heart disease (IHD) leading cause of morbidity and mortality in developed countries but it is a worldwide health problem[1, 2]. Ischemia which is an acute or chronic form of cardiac complication occurs due to the imbalance between the myocardial supply and demand for oxygenated blood.[2] Herbs and the herbal therapy nowadays become very much popular due to potency and less toxicity profile. When synthetic drug combine with herbal therapy it may potentiate, mimic or oppose the pharmacological activity[1, 3]. The possibility of herb–drug interactions is theoretically higher than drug–drug interactions, could be because synthetic drugs usually contain single chemical entities[3].

Semecarpus anacardium belongs to Anacardiaceae family, commonly known as bhallataka or marking nut[4]. The principle chemical constituents of fruit are flavonoids, saponins, and tannins. The nut of Semecarpus shell contains biflavonoids, biflavone A, C, A_1, A_2, tetrahydrorobustaflavone, jeediflavone, semecarpuflavone and gulluflavone. Oil from nuts contains Bhilavinol and the leaves contain amentoflavone as a sole biflavonoid[5]. *Semecarpus anacardium* nut is already reported for various diseases such as insanity, fever, asthma, dysentery, neurological disorders, cardiac complication, enlargement of spleen, alopecia, ulcers, corns, leprosy, leukoderma, hemorrhoids, rheumatism and cancer[6]. 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 too [7]. Further, studies showed that nut extract significantly lowered blood glucose level in alloxan induced diabetic ratand also in normal rats[8], antifungal property[9] and antioxidant property[10]. The present study was designed to demonstrate the potential interaction of SANE with PRO during IRI damage to myocardium using isolated perfused rat heart preparation.

MATERIALD AND METHODS

Chemicals

All chemicals used were of analytical grade and purchased from standard companies. Biochemical kits like LDH and CK-MB were procured from Crest Biosystems (Goa, India).

Plant extract

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. The nuts were mechanically grinded and detoxified with the solvent n-butanol for five days with the daily change of the solvent[11]. 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

Hydroaclcoholic 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[12,13].

Experimental animals

Laboratory bred female 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. 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).

Experimental Procedure

Modified langendorff apparatus was used to perfuse the isolated heart [14]. Animals of all the groups were heparanised (Heparin 100 I/U, Intraperitoneal route). After half an hour the animals were anaesthetized with ketamine (70 mg/kg, i.p) and xylazine (10 mg/kg, i.p). The heart was isolated and infused with Kreb-Henseleit (K-H) solution gassed with carbogen (95% O_2 and 5% CO_2) at 37 ^oC at a constant flow rate of 5 ml/min. The composition of K-H solution was (mM) NaCl 118, KCl 4.7, $NaHCO_3$ 25, $NaHPO_4$ 1.0, $MgSO_4.7H_2O$ 0.57, $CaCl_2$ 2.5 and glucose 11). The pH of K-H solution was adjusted to 7.4 to avoid K-H buffer acidosis that may occur after prolonged gassing with carbogen. The heart was allowed to equilibrate for 10 min and then regular recordings were taken for a perfusion period of 15 min. Measurement of contractile force was done using force displacement transducer and recorded on a Polygraph. Apart from IRI control group in all the other groups the KH solution is replaced with drug with KH solution. Drug with KH solution was prepared according to treatment protocol Group II (PRO 10µg/ml in KH), Group III (SANE 10 µg/ml in KH), Group IV (SANE 25 µg/ml in KH), Group V (SANE 50µg/ml in KH), Group VI (SANE 50 µg/ml in KH), Group VII (SANE 10 µg/ml in KH)+ PRO 10(PRO 10 µg/ml in KH). Treatment was given to isolated heart of respective group for 10 min and the measurement of contractile force was done. 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/K-H with drug solution & carbogen supply followed by 15 min of reperfusion[15]. The heart rate and developed tension were measured during pre-ischemic and post-ischemic period and recovery (%) was calculated. Lactate dehydogenase (LDH) and creatine kinase-MB (CK-MB) activity were measured in post-ischemic perfusate. The heart was then homogenized to prepare heart tissue homogenate (HTH) using sucrose (0.25 M) [16] and the activity of LDH, CK-MB, superoxide dismutase (SOD) [17] and catalase[18-20] was determined. Microscopic slides of myocardium were prepared for histopathological studies after post-ischemia.

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.

RESULT

Effect on LDH and CK-MB activities in perfusate (Figure 1)

SANE 1, SANE 10, SANE 25, SANE 10+PRO treated group shows an extremely significant ($p<0.001$) decrease in the LDH activity in perfusate compared to IRI control group and the treated group SANE 10+PRO shows just significant ($p>0.05$) decrease in LDH activity compared with the SANE 10 treated group. The CK-MB activity in perfusate shows an extremely significant ($p<0.001$) decrease in SANE 10+PRO treated group and a very significant ($p<0.01$) decrease in SANE 10 treated group compared with the IRI control group. SANE 10+PRO treated group shows a very significant ($p<0.01$) decrease in CK-MB activity compared with the PRO treated group.

Effect on LDH and CK-MB activities in heart tissue homogenate (Figure2)

There was an extremely significant increase ($p<0.001$) in LDH activity in perfusate of SANE 10 and SANE 10+PRO treated group when compared to the IRI control group. LDH activity of HTH in SANE 10+PRO shows an extremely significant increase ($p<0.001$) compared with the PRO and a very significant ($p<0.01$) increase compared with the PRO-10 treated group.

Effect on SOD and catalase activity (Table 1)

SANE 1, SANE 10, SANE 25, SANE 10+PRO treated group shows an extremely significant ($p<0.001$) increase in SOD activity compared with IRI control group. SANE 10+PR0 treated group shows an extremely significant ($p<0.001$) increase in SOD activity when compared with PRO and an extremely significant ($p<0.001$) increase in SOD activity when compared with SANE 10 treated group.PRO, SANE 1, SANE 10, SANE 25, SANE 10+PRO treated group shows an extremely significant ($p<0.001$) increase in catalase activity and the SANE 50 treated group shows a just significant ($p>0.05$) increase in catalase activity when compared with IRI control group. SANE 10+PR0 treated group shows an extremely significant ($p<0.001$) increase in catalase activity when compared with PRO and a very significant ($p<0.01$) increase in catalase activity when compared with SANE 10 treated group. SANE 10+PR0 treated group shows an extremely significant ($p<0.001$) increase in catalase activity when compared with PRO and an extremely significant ($p<0.001$) increase in catalase activity when compared with SANE 10 treated group.

Effect on developed tension and heart rate (Figure 3)

The isolated heart infused with PRO 10 ($p>0.05$), SANE 10 ($p<0.001$) and SANE 10+PRO ($p<0.001$) shows significant % recovery to ischemic heart in terms of heart rate after global ischemia. SANE 10+PRO shows significant level of ($p>0.05$) % recovery compared with PRO. % recovery in developed tension shows moderately significant ($p<0.01$) in SANE 10 and extremely significant ($p<0.001$) in SANE 10+PRO treated group compared with the IRI control. SANE 10 +PRO shows a significant ($p<0.01$) compared with PRO and moderately significant ($p>0.05$) compared with SANE 10 in % recovery of developed tension.

Effect on histological score (Figure 5)

DISCUSSION

The research was proposed to determine the effect of infusion of different doses of SANE and its comparison with PRO-10 dissolve in KH solution during IRI induced myocardial infarction (MI) in isolated rat heart preparation. In this study we induced the myocardial injury by stopping the flow of carbogenated KH which produces ischemia followed by reperfusion which increases the extent of damage. Sudden occlusion of physiological salt solution (PSS) results in immediate biochemical alterations[21,22]. The increase in intracellular Na^+ serves to drive Ca^{2+} intracellularly via Na^+/Ca^{2+} exchange that results in irreversible damage to myocardium at the end of 15 min global ischemia[23].

In this study we infused the heart with two doses of PRO (10 and 100µg/ml) and found that the high dose of PRO causes the immediate cessation of heart response but with low dose the isolated hearts gave the normal drug response with significant recovery from ischemic damage in developed tension and heart rate. Hence low dose of PRO (10µg/ml) was selected as a safe dose of propranolol. Isolated rat heart were also infused with different concentration of SANE (1, 10, 25, 50 µg/ml) and the recording were taken on polygraph. In the post ischemic perfusate antioxidant and biomarker levels was measured and subjected for histopathological study. The results showed SANE 10 (10 µg/ml) as the most effective dose. Going a step ahead we combine the doses SANE 10 and PRO 10 and measured the %recovery in terms of developed tension, heart rate, activity of marker enzymes, antioxidant level, histopathological study.

Earlier studies reported that different marker enzymes are organ specific and released from the damaged organ during damage[24]. Damage to myocardial tissue causes the release of cardiac biomarkers such as LDH and CK-MB into the perfusate with resultant decrease in their activities in HTH and increase in perfusate[25]. In this study we found that the SANE 10 and SANE 10+PRO is the most protective doses by decrease the biomarker levels in the perfusate and increase in HTH. SANE 10+PRO 10 was found to best effective combination as it demonstrated superior protection compared with the alone infusion of both SANE 10 and PRO 10.

Different studies already demonstrated that generation of oxygen free radicals and the hydrogen per oxide during the MI elevates the extent of damage resulting in decrease level of antioxidants such as SOD and catalase in HTH[26]. Infusion of SANE 1, SANE 10, SANE 25 and SANE 10+PRO causes significant level of elevation in antioxidant level but in SANE 50 the antioxidant levels does not show the similar type of activity. Among this group SANE 10+PRO found to be most effective dose as it causes the significant level of elevation compared with PRO and SANE 10 alone. Percentage recovery in post ischemic condition is calculated considering preischemic condition as maximum. % recovery in terms of developed tension and heart rate was found most effective in SANE 10 and SANE 10+PRO but the combination with PRO concluded as most effective as it was giving better recovery compared with both SANE 10 and PRO infused heart. Histopathological studies also suggested SANE 10+PRO as a most effective dose.

CONCLUSION

The present study showed that the infusion of isolated heart with the groups SANE 10 and SANE 10+PRO was found to be protective in myocardial damage induced by ischemic reperfusion injury (IRI). However the combination of SANE 10+PRO was found to be most effective group compared with the all the other group.

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Table 1: Phytochemical investigation for various compound

SL NO	TEST	INFERENCE	RESULT
1.	**Test for alkaloids**		
1.a	Hager's test	Yellow colour -	+
1.b	Mayer's test	cream precipitate -	+
1.c	Dragendroff's test	orange pricipitate	+
1.d	Wagner's test	red-brown pricipitate	+
2.	**Test for carbohydrates**		
2a	Molish test	Violet colour	+
2b	Fehling's test	Break red colour	+
2c	Borfoed's test	Red colour	+
2d	Benedict's test	Red colour	+
3.	**Test for steroids, triterpenoids and glycosides**		
3a	Liebermann-buchard test	Redish- violet colour	+++
3b	Salkowski test	Red colour	+
3c	Baljet test	Orange colour	+++
3d	Keller killani test	Red colour	+
4.	**Test for saponins**		
4.a	Froth test	1 cm foam	+
4.b	Haemolytic test	No pricipitate	+
5	Test for tannins		
5.a	Ferric chloride test	Blue colour	+
5.b	Lead acetate test	Yellow colour	+
6	**Test for proteins and Amino acids**		
6.a	Millon's test	Red colour	+
6.b	Biuret test	Violet colour	+
6.c	Ninhydrin test	Violet colour	+
7	**Test for flavanoids**		
7.a	Ferric chloride test	Blackish red colour	+
7.b	Lead acetate test	Yellow pricipitate	+
8	**Test for specific flavanoids**		
8.a	Test for carotenoids	Deep blue colour	+
9	Test for phenolic compound	Radish brown colour	+

Table 2: Effect on SOD and catalase in heart tissue homogenate

Groups	Heart tissue homogenate	
	SOD (unit/mg protein)	**CAT (unit/mg protein)**
IRI control	1.70±0.09	2.07±0.08
PRO-10	2.07±0.04	3.46±0.10***
SANE 1	3.07±0.08***	3.96±0.08***
SANE 10	6.38±0.17***	6.76±0.23***
SANE 25	2.83±0.07***	3.27±0.11***
SANE 50	2.06±0.07	2.86±0.15*
SANE 10+PRO-10	7.53±0.15$^{***\bullet\bullet\bullet\blacklozenge\blacklozenge\blacklozenge}$	7.58±0.17$^{***\bullet\bullet\bullet\blacklozenge}$

All values are mean ± SEM, n=6; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P< 0.001$ when compared to IRI control; $^{\bullet}P<0.05$, $^{\bullet\bullet}P<0.01$, $^{\bullet\bullet\bullet}P< 0.001$ when compared to PRO ; $^{\blacklozenge}P<0.05$, $^{\blacklozenge\blacklozenge}P<0.01$, $^{\blacklozenge\blacklozenge\blacklozenge}P< 0.001$ when compared to SANE 10 with SANE 10+PRO ; IRI, ischemia reperfusion induced injury; PRO-10, propranolol 10 µg/ml; SANE 1, *Semecarpus anacardium* nut extract 1µg/ml; SANE 10, *Semecarpus anacardium* nut extract 10 µg/ml; SANE 25, *Semecarpus anacardium* nut extract 25µg/ml; SANE 50, *Semecarpus anacardium* nut extract 50 µg/ml.

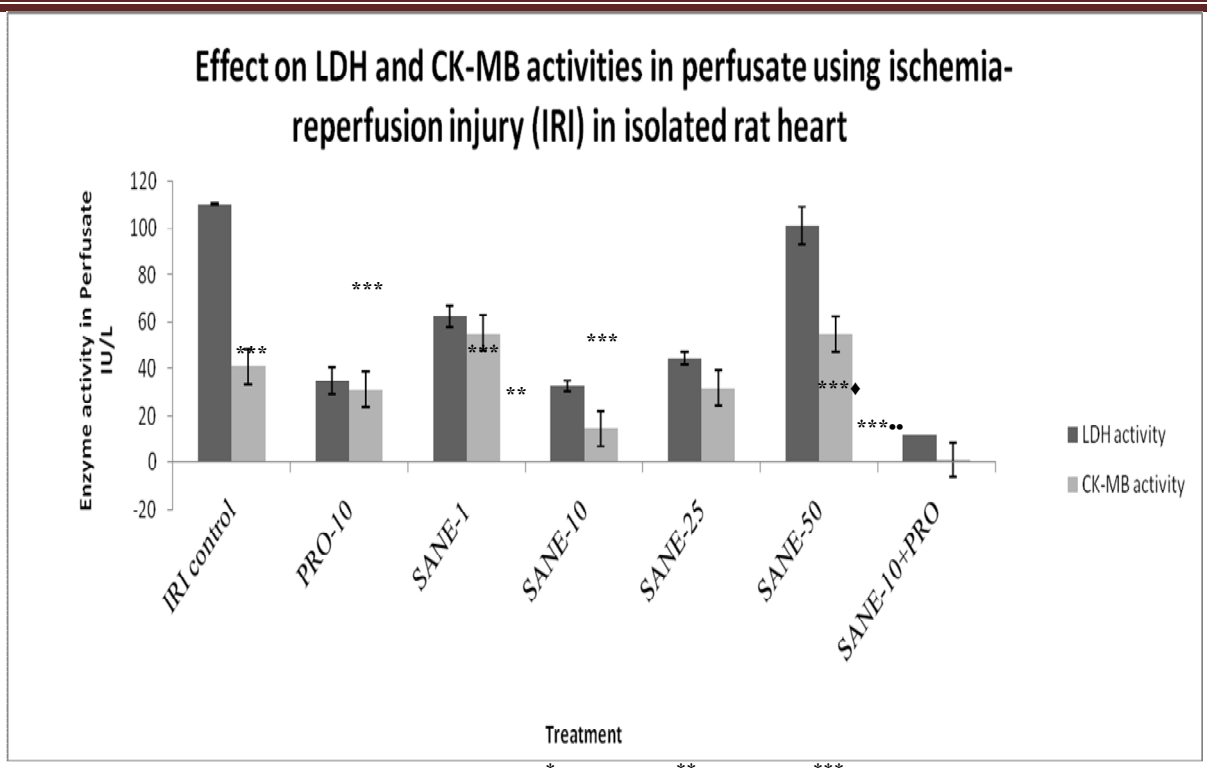


Figure 1: All values are mean ± SEM, n=6; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P< 0.001$ when compared to IRI control; $^{\bullet}P<0.05$, $^{\bullet\bullet}P<0.01$, $^{\bullet\bullet\bullet}P< 0.001$ when compared to PRO ; $^{\blacklozenge}P<0.05$, $^{\blacklozenge\blacklozenge}P<0.01$, $^{\blacklozenge\blacklozenge\blacklozenge}P< 0.001$ when compared to SANE 10 with SANE 10+PRO ; IRI, ischemia reperfusion induced injury; PRO-10, propranolol 10 µg/ml; SANE 1, *Semecarpus anacardium* nut extract 1µg/ml; SANE 10, *Semecarpus anacardium* nut extract 10 µg/ml; SANE 25, *Semecarpus anacardium* nut extract 25µg/ml; SANE 50, *Semecarpus anacardium* nut extract 50 µg/ml.

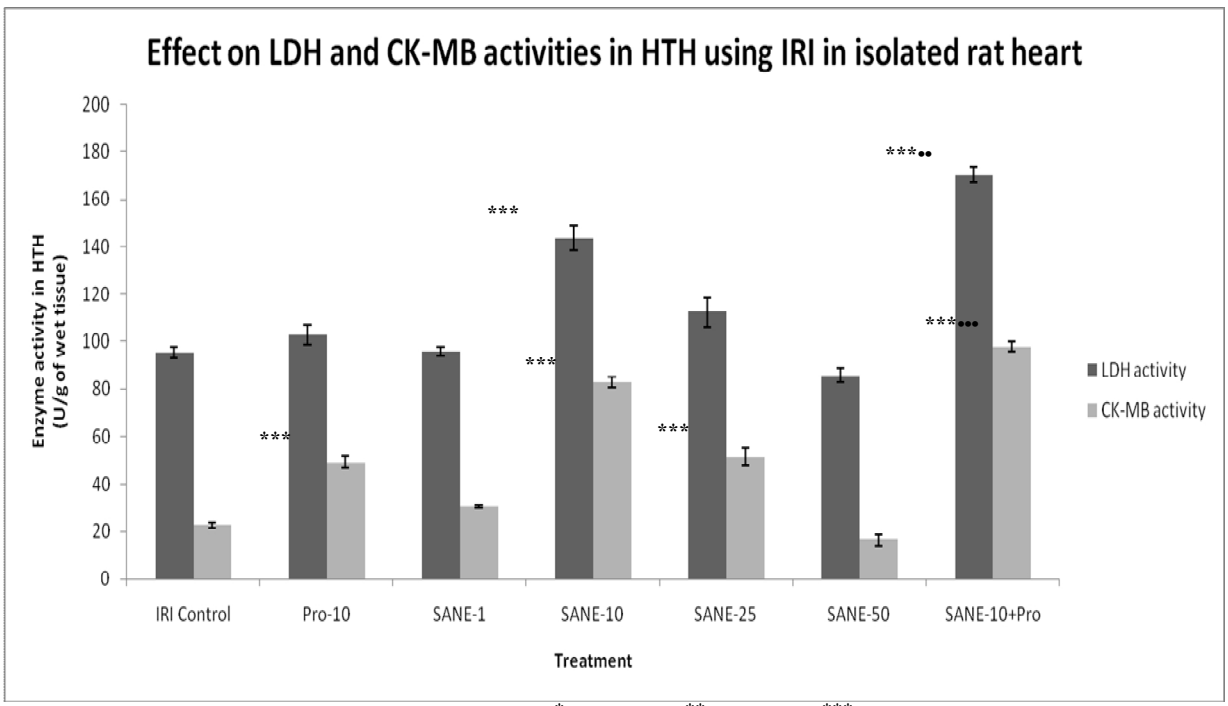


Figure 2: All values are mean ± SEM, n=6; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P< 0.001$ when compared to IRI control; $^{\bullet}P<0.05$, $^{\bullet\bullet}P<0.01$, $^{\bullet\bullet\bullet}P< 0.001$ when compared to PRO ; $^{\blacklozenge}P<0.05$, $^{\blacklozenge\blacklozenge}P<0.01$, $^{\blacklozenge\blacklozenge\blacklozenge}P< 0.001$ when compared to SANE 10 with SANE 10+PRO ; IRI, ischemia reperfusion induced injury; PRO-10, propranolol 10 µg/ml; SANE 1, *Semecarpus anacardium* nut extract 1µg/ml; SANE 10, *Semecarpus*

anacardium nut extract 10 µg/ml; SANE 25, *Semecarpus anacardium* nut extract 25µg/ml; SANE 50, *Semecarpus anacardium* nut extract 50 µg/ml.

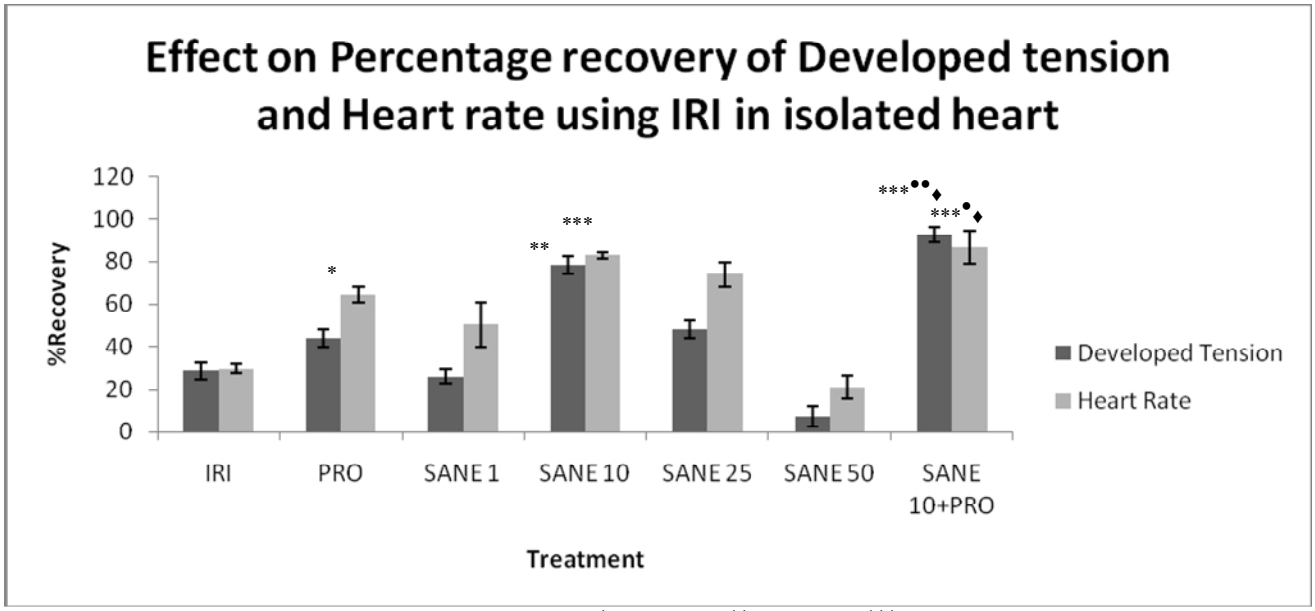
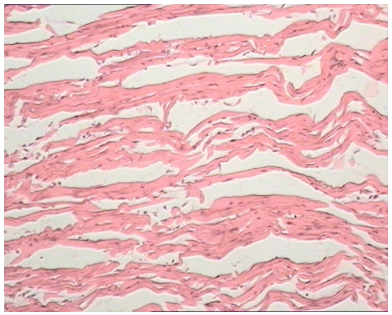
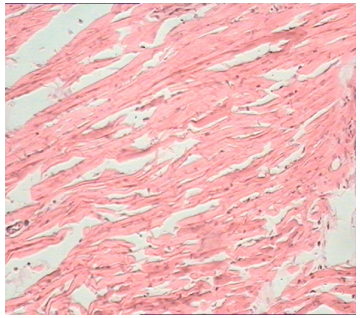


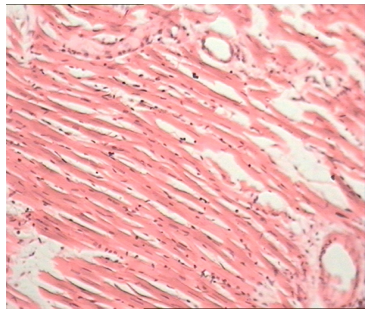
Figure 3: All values are mean ± SEM, n=6; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P< 0.001$ when compared to IRI control; $^{\bullet}P<0.05$, $^{\bullet\bullet}P<0.01$, $^{\bullet\bullet\bullet}P< 0.001$ when compared to PRO ; $^{\blacklozenge}P<0.05$, $^{\blacklozenge\blacklozenge}P<0.01$, $^{\blacklozenge\blacklozenge\blacklozenge}P< 0.001$ when compared to SANE 10 with SANE 10+PRO ; IRI, ischemia reperfusion induced injury; PRO-10, propranolol 10 µg/ml; SANE 1, *Semecarpus anacardium* nut extract 1µg/ml; SANE 10, *Semecarpus anacardium* nut extract 10 µg/ml; SANE 25, *Semecarpus anacardium* nut extract 25µg/ml; SANE 50, *Semecarpus anacardium* nut extract 50 µg/ml.



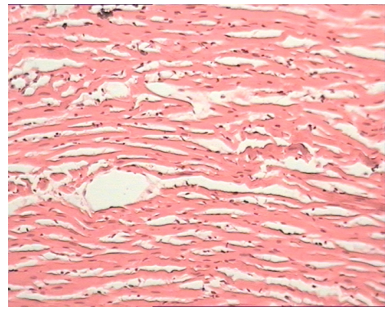
IRI CONTROL



PRO



SANE 1



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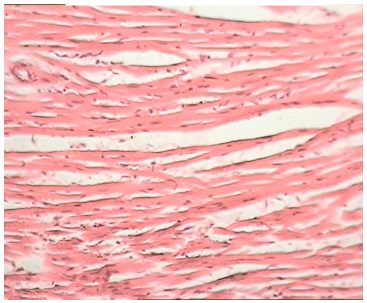

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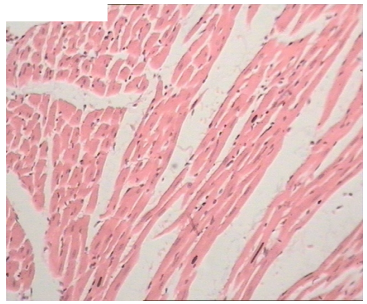
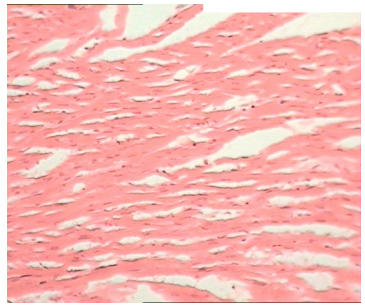


SANE 50



SANE 10 + PRO

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