

Interaction of *Semecarpus anacardium* L. with propranolol against isoproterenol induced myocardial damage in rats

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With a view to evaluate the cardioprotective effect of ethanolic extract of *S. anacardium* nut and the possible interaction with propranolol against isoproterenol induced myocardial damage in rats, female Sprague-Dawley rats were pre-treated with propranolol (10 mg/kg for 7 days), low and high doses of *S. anacardium* (100 and 500 mg/kg for 21 days) and their combination orally and subsequently subjected to isoproterenol administration (150 mg/kg, sc) for two consecutive days. The influence of prophylactic treatment was analysed by quantification of biomarkers and antioxidants, electrocardiographic parameters and histopathological observations. The activities of lactate dehydrogenase and creatinine phosphokinase-MB were reduced in serum and raised in heart tissue with concurrent elevation in superoxide dismutase and catalase activities as well as reduction in thiobarbituric acid reactive species levels significantly in all treated groups compared to isoproterenol group. Similarly, electrocardiographic changes were restored to normalcy in all treated groups. To conclude, combination of high dose of *S. anacardium* with propranolol was found to be most effective in alleviating the abnormal conditions induced by isoproterenol.

Keywords: Cardioprotection, Electrocardiographic parameters, Isoproterenol, Propranolol, *Semecarpus anacardium*

In spite of tremendous therapeutic innovations for cardiovascular manifestations, there is dearth of medicinal solution with enhanced safety and efficacy carrying minimal toxicity^{1,2}. Certain chronically ill patients with concurrent administration of conventional drugs and herbs have achieved the goal of this therapeutic objective without procuring scientific evidence in its support. Herbs and drugs may cover the side effect and low efficacy of each other when they are combined together. However, they raise the alarm of dangerous adverse herb drug interaction and counteraction. Hence it is inevitable to explore scientific proof for commonly available herb and their possible interaction with conventional drug for validating their combined use.

Semecarpus anacardium L. belongs to Anacardiaceae family, commonly known as bhallataka³. The principle chemical constituents of fruit are flavonoids, saponins, and tannins. 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 biflavonoid⁴. 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 too³. Nuts of *S. anacardium* are known in folklore as anti-fungal³, anti-carcinogenic⁵, anti-arthritis⁶, antioxidant⁷ and immunomodulator⁸. Earlier studies showed that nut extract lowered blood glucose level in normal and alloxan induced diabetic rat⁹. However, the nuts of *S. anacardium* have not been explored for cardioprotective properties during myocardial stress. As it is one of the herb which possess large number of therapeutic efficacies, it was felt worthwhile to evaluate its role during myocardial damage either alone or in presence of conventional cardioprotective drug. The present research has been designed to determine the cardioprotective activity of *S. anacardium* and its interaction with propranolol in isoproterenol induced myocardial damage in rats.

Materials and Methods

Chemicals—Isoproterenol was purchased from Sigma-aldrich, U.S.A, lactate dehydrogenase (LDH) and creatinine phosphokinase-MB (CK-MB) Kits

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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—Healthy adult female Sprague-Dawley (SD) rats weighing 175-250 g, housed in polypropylene cages, maintained under standardized condition (12 h L:D cycles, $28^{\circ}\pm 2^{\circ}\text{C}$) with paddy husk bedding at the Central Animal House, Krupanidhi College of Pharmacy, Bangalore were provided with standard pellet food and had free access to purified drinking water. The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India were followed and prior permission was sought from the Institutional Animal Ethics Committee for conducting the study (KCP/IAEC-27/2008-09).

Plant material—The shade dried nuts of *S. 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 (500 g) were mechanically grinded and detoxified with the solvent n-butanol for 5 days with the daily change of the solvent¹⁰. 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 desiccator until further use. The yield was around 24% (w/w). Prior to the treatment, dried extract was suspended in water using 0.5% carboxy methyl cellulose.

Phytochemical estimations of the extract^{11,12}—Hydroalcoholic extract of *S. anacardium* nuts (SANE) was subjected to phytochemical analysis to investigate the presence of various constituents such as alkaloids, carbohydrates, glycosides, phytosterols, proteins, saponins, tannins and flavonoids

Acute toxicity study—The acute oral toxicity study was performed according to the Office of Prevention, Pesticide and Toxic Substance (OPPTS) guidelines following the limit test procedure¹³. The animals were fasted over night prior to the experiment. Test dose of 2 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. Two different concentrations of SANE,

0.2 and 0.04 g/ml, corresponding to 500 and 100 mg/kg body weight of animal were prepared.

Experimental protocol—The animals were divided into 7 groups of 8 animals each. Group I and Group II received saline for three weeks and termed as normal control and ISO control respectively; Group III, IV and V were administered standard propranolol (PRO, 10 mg/kg, for 7 days), *S. anacardium* nut extract 100 mg/kg (SANE-100, 21 days) and 500 mg/kg (SANE-500, 21 days) respectively by oral route. Group VI and Group VII were treated with SANE-100 and SANE-500 respectively along with PRO. All treatments were done by oral route.

*Isoproterenol (ISO) induced myocardial necrosis in rats*¹⁴—After treatment of animals from group II to VII according to the protocol, isoproterenol (ISO; 150 mg/kg, sc) was administered for two consecutive days. After 48 h from first dose of ISO, electrocardiographic parameters were recorded, blood samples were collected and hearts were excised under humane conditions. Serum was separated and biochemical markers LDH and CK-MB were estimated. Four hearts from each group were homogenized with sucrose solution (0.25 M)¹⁵ for estimations of LDH, CK-MB, superoxide dismutase (SOD)¹⁶, catalase¹⁷ and thiobarbituric acid reactive species (TBARS)^{18,19}.

*Electrocardiographic studies*²⁰—Under anesthetic conditions induced by combination of ketamine hydrochloride (75 mg/kg, ip) and xylazine (8.0 mg/kg, ip), leads were attached to the dermal layer of both the front paws and hind legs and recording were made with the help of computerized ambulatory ECG system.

Histological analysis—Heart sections were prepared from the remaining four hearts in each group, stained with Hematoxylin and Eosin (H&E) and change in histology were observed. The myocardial damage was determined by scoring method depending on the severity as follows²⁰, no change=0 score; mild=1 score (focal myocytes damage or small multifocal degeneration with slight degree of inflammation); moderate=2 score (extensive myofibrillar degeneration) and marked=3 score (necrosis with diffuse inflammation).

Statistical analysis—Results are expressed as mean \pm SE. 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 and CK-MB activities—The LDH and CK-MB activities were significantly increased in serum and decreased in heart tissue homogenate (HTH) in groups treated with isoproterenol compared to normal control (Table 1). Prophylactic administration of SANE-100, SANE-500, SANE-100+PRO and SANE-500+PRO showed significant fall in LDH and CK-MB activities in serum and elevation in HTH compared to ISO control. The decline in LDH activity in serum of animals pretreated with SANE-500+PRO

was significantly different compared to PRO alone. Similarly, CK-MB activity was raised significantly in animals treated with SANE-500+PRO compared to PRO alone.

Effect on SOD and catalase (Table 2)—The SOD and catalase activities were reduced in ISO control compared to normal control. Administration of PRO, SANE-100/500 and their combinations resulted in significant rise in SOD and catalase activities compared to ISO control. Concurrent administration of SANE-100/500 with PRO resulted in significant elevation in SOD and catalase activities compared to PRO alone.

Effect on TBARS (Table 2)—A significant elevation in TBARS levels were found in ISO control compared to normal control. Treatment of animals with SANE, PRO and their combination demonstrated significant fall in TBARS levels compared to ISO control. Incorporation of PRO during the last seven days of SANE treatment resulted in synergistic fall in TBARS levels compared to PRO alone.

Effect on histological score—Myocardial integrity was disturbed by administration of isoproterenol for two consecutive days that was evident with significant rise in histological score compared to normal control (Fig. 1, Table 2). Isoproterenol injections caused necrosis of cells with degeneration of myofibril and increased interstitial space (Fig. 2). Prior treatment of animals with PRO (Fig. 3), SANE-100 (Fig. 4) and their combination (PRO plus SANE-100, Fig. 6) before subjecting to isoproterenol induced myocardial damage showed significant fall in histological scores compared to ISO control. High dose of SANE found to cause mild multifocal degeneration with slight inflammation (Fig. 5). Concurrent administration of PRO with SANE (500 mg/kg) resulted in significant

Table 1—Effects of *S. anacardium* and propranolol on LDH and CKMB level in serum and heart tissue homogenate against isoproterenol induced myocardial infarction

[Values are mean \pm SE from 8 animals in serum groups and 4 animals in HTH groups]

| Treatment | CK-MB activity | | LDH activity | |
|----------------|---------------------------|-----------------------------|------------------------------|--------------------------|
| | Serum (unit/lit) | HTH(unit/ g wet wt) | Serum (unit/lit) | HTH(unit/ g wet wt) |
| Normal control | 11 \pm 1 | 210 \pm 20 | 324 \pm 16 | 16 \pm 2 |
| ISO CONTROL | 92 \pm 2 ^{***} | 34 \pm 10 ^{***} | 643 \pm 31 ^{**} | 3 \pm 0 ^{***} |
| PRO | 19 \pm 5 ^{***} | 96 \pm 1 ^{***} | 396 \pm 40 ^{**} | 7 \pm 0 ^{**} |
| SANE-100 | 20 \pm 3 ^{***} | 137 \pm 2 ^{***} | 416 \pm 18 ^{**} | 5 \pm 1 ^{**} |
| SANE-500 | 16 \pm 0 ^{***} | 167 \pm 4 ^{***b} | 456 \pm 43 [*] | 7 \pm 1 ^{***} |
| SANE-100+ PRO | 16 \pm 1 ^{***} | 134 \pm 12 ^{***} | 495 \pm 59 [*] | 8 \pm 2 [*] |
| SANE-500 + PRO | 13 \pm 3 ^{***} | 163 \pm 7 ^{***b} | 198 \pm 14 ^{***a} | 10 \pm 1 |

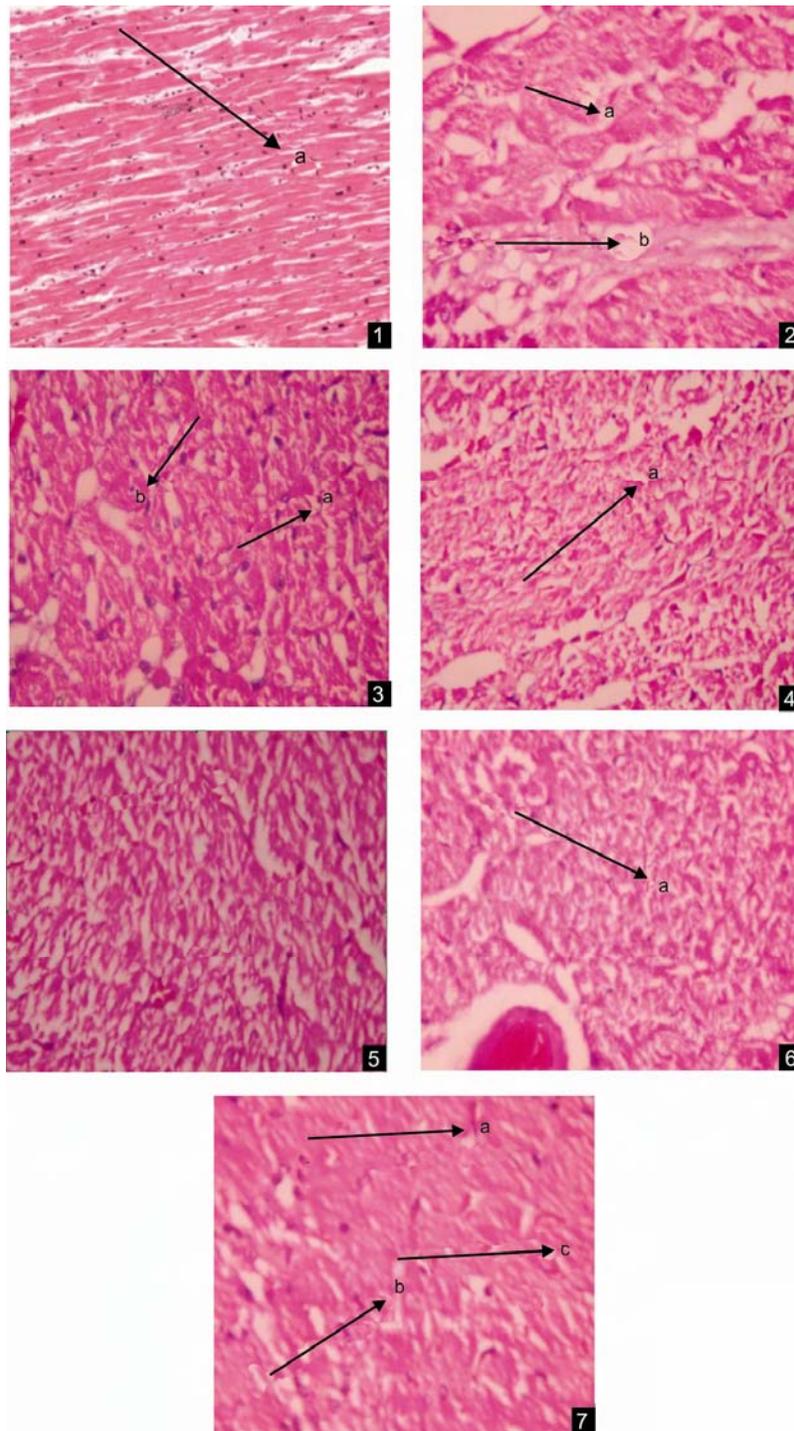
* $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$ when compared to normal control; ^a $P < 0.1$, ^b $P < 0.01$, ^c $P < 0.001$ compared to ISO control and ^a $P < 0.1$, ^b $P < 0.01$, ^c $P < 0.001$ compared to propranolol. SANE-100 (*S. anacardium* nut extract 100 mg/kg), SANE-500 (*S. anacardium* nut extract 500 mg/kg) and PRO (propranolol 10 mg/kg).

Table 2—Effects of *S. anacardium* and propranolol on SOD, catalase and TBARS in heart tissue homogenate against isoproterenol induced myocardial infarction

[Values are mean \pm SE from 8 animals in serum groups and 4 animals in HTH groups]

| Treatment | Heart tissue homogenate | | | |
|----------------|-------------------------------|--------------------------------|--------------------------------|---------------------------------|
| | SOD (unit/mg protein) | Catalase (unit/mg protein) | TBARS (μ mol/g wet wt) | Histopathological Score |
| Normal control | 14.4 \pm 0.1 | 6.56 \pm 0.04 | 24.1 \pm 2.2 | 0.50 \pm 0.28 |
| ISO control | 4.1 \pm 0.0 ^{***} | 0.04 \pm 0.01 ^{***} | 72.5 \pm 5.0 ^{***} | 2.83 \pm 0.16 ^{***} |
| PRO | 6.9 \pm 0.2 ^{***} | 2.73 \pm 0.28 ^{***} | 64.7 \pm 2.2 ^{**} | 2.66 \pm 0.16 ^{***} |
| SANE-100 | 5.1 \pm 0.3 ^{***} | 0.62 \pm 0.02 ^{***} | 59.6 \pm 0.3 ^{**} | 1.83 \pm 0.16 ^{**} |
| SANE-500 | 6.6 \pm 0.3 ^{***} | 2.28 \pm 0.04 ^{***} | 37.6 \pm 0.3 ^{***} | 1.53 \pm 0.03 ^{***} |
| SANE-100+ PRO | 6.7 \pm 0.0 ^{***} | 2.77 \pm 0.21 ^{***} | 38.3 \pm 1.4 ^{***b} | 1.66 \pm 0.16 ^{***a} |
| SANE-500 + PRO | 8.2 \pm 0.0 ^{***a} | 3.36 \pm 0.33 ^{***} | 28.8 \pm 2.2 ^{***c} | 1.46 \pm 0.03 ^{***b} |

$P < 0.1$, ** $P < 0.01$, *** $P < 0.001$ when compared to normal control; ^a $P < 0.1$, ^b $P < 0.01$, ^c $P < 0.001$ compared to ISO control and ^a $P < 0.1$, ^b $P < 0.01$, ^c $P < 0.001$ compared to propranolol. SANE -100 (*S. anacardium* nut extract 100 mg/kg), SANE -500 (*S. anacardium* nut extract 500 mg/kg) and PRO (propranolol 10 mg/kg).



Figs 1–7—Heart tissue from rats. 1—normal control (a=normal texture of cell); 2—isooproterenol (ISO) control (a=necrotic cells with degeneration of myofibril, b=increased interstitial space); 3—propranolol group (a=recovery from necrosis with mild inflammation, b=less interstitial space); 4—animals pretreated with SANE (100 mg/kg, po for 21 days) and isoproterenol (two doses of 150 mg/kg, sc) (a=extensive myofibrillar degeneration); 5—animals pretreated with SANE (500 mg/kg, po for 21 days) and isoproterenol (two doses of 150 mg/kg, sc) (a=small multifocal degeneration, b=slight inflammation); 6—animals pretreated with SANE (100 mg/kg, po) and propranolol (10 mg/kg, po) and isoproterenol (two doses of 150 mg/kg, sc) (a=marked diffuse inflammation and less interstitial space); 7—animals pretreated with SANE (500 mg/kg, po)+propranolol (10 mg/kg, po) and isoproterenol (two doses of 150 mg/kg, sc) (a=small multifocal degeneration, b=slight inflammation, c=fall in interstitial space). H&E (×400)

reduction in histological score compared to PRO alone. The protective effect of combined therapy of PRO with high dose of SANE was demonstrated with least multifocal degeneration, mild inflammation with reduction in interstitial space (Fig. 7).

Effect on electrocardiographic parameters—Electrocardiographic determination revealed a significant increase in heart rate of ISO control compared to normal control. Prior treatment of animals with PRO, SANE-100/500 and their combination resulted in significant fall in elevated chronotropic values compared to ISO control. A significant fall in heart rate was observed in SANE-500+PRO group compared to PRO alone (Table 3).

Subcutaneous administration of isoproterenol for two consecutive days showed enlargement of QRS duration and QT interval compared to normal control. Prior treatment of animals with PRO, SANE-100 and SANE-500 caused restoration of QRS duration and QT interval to normal conditions compared to ISO control. Treatment of animals with SANE-100/500 with PRO in conjunction caused further fall in QRS duration and QT interval compared to PRO alone (Table 3).

Increase in PR and RR intervals were noted in ISO control animals compared to normal control. Prophylactic administration of SANE-100/500, PRO and their combination showed recovery from abnormal PR and RR interval and best results were found in SANE-500+PRO group (Table 3).

Discussion

The aim of the present study was to elucidate the role of *S. anacardium* nut extract during myocardial dysfunction and metabolic derangement induced by isoproterenol in rat heart and also to explore its pharmacodynamic interaction with conventional cardioprotective drug, propranolol. The results

revealed the beneficial role of *S. anacardium* when treated concurrently with propranolol in conditions of anticipated cardiac injury.

Isoproterenol, a synthetic catecholamine and β -adrenoceptor stimulant, is known to cause myocardial damage at higher concentrations²². Disturbances in coronary microcirculation, anoxia and formation of catecholamine oxidative products apart from intracellular calcium load have been postulated to induce cardiac toxicity²³. Isoproterenol administration results in increase in calcium uptake and energy consumption leading to cell death²⁴. Elevation of different biomarker enzymes in serum is due to the leakage of enzymes from the heart as a result of isoproterenol-induced necrosis²⁵. Isoproterenol also increases the production of cytotoxic free radicals through its auto-oxidation. The oxidative products of catecholamines produce changes in the myocardium by stimulating lipid peroxidation and cause irreversible damage to the myocardial membrane^{26,27}. This alters membrane permeability, leading to the loss of function and integrity of myocardial membranes²⁸. Hence leakage of endogenous biological markers and free radical formation are attributed for myocardial distress in post isoproterenol administration²⁹. Ethanolic extract of *S. anacardium* nut extract dose dependently provides protection to myocardium by scavenging oxidative free radicals and thereby diminishing the permeability of these endogenous biomarkers to extra cardiac regions. Propranolol blocks beta-adrenergic receptors and prevents isoproterenol-mediated overstimulation of sympathetic system. Thus the combination of *S. anacardium* and propranolol caused synergistic cardioprotection to myocardium.

Excessive activation of sympathetic system by isoproterenol accompanied by vagal hypo activity produces severe myocardial damage³⁰. This is evident by disturbances in electrocardiography due to

Table 3—Effects of *S. anacardium* and propranolol on electrocardiographic parameters in isoproterenol induced myocardial infarction [Values are mean \pm SE from 8 animals in serum groups and 4 animals in HTH groups]

| Treatment | Heart rate | Hw/Bw | QRS duration (min) | QT segment (min) | RR interval (min) | PR interval (min) |
|----------------|-----------------|---------------------------------|----------------------------|--------------------|-------------------|--------------------------------|
| Normal control | 180 \pm 20 | 1.90 \pm 0.05 | 82 \pm 3 | 83.3 \pm 3.3 | 206.6 \pm 6.6 | 71.6 \pm 7.2 |
| ISO CONTROL | 340 \pm 20*** | 2.08 \pm 0.03 | 116 \pm 3*** | 133.3 \pm 6.6** | 290.0 \pm 5.7** | 190.0 \pm 10.0*** |
| PRO | 240 \pm 20** | 2.40 \pm 0.01 | 90 \pm 5* | 100.0 \pm 10.0** | 240.0 \pm 5.7 | 116.0 \pm 8.8** |
| SANE-100 | 200 \pm 20*** | 2.90 \pm 0.06*** ^b | 86 \pm 3* | 110.0 \pm 5.7 | 286.6 \pm 8.8** | 86.6 \pm 8.8*** |
| SANE-500 | 200 \pm 20*** | 2.90 \pm 0.02*** ^c | 93 \pm 8 | 80.0 \pm 5.7** | 253.3 \pm 13.0 | 105.3 \pm 5.7*** |
| SANE-100+ PRO | 240 \pm 10** | 2.50 \pm 0.08*** ^a | 66 \pm 3*** ^a | 93.3 \pm 3.3* | 253.3 \pm 3.3 | 72.3 \pm 5.7*** ^a |
| SANE-500 + PRO | 160 \pm 20*** | 2.20 \pm 0.12* | 61 \pm 1*** ^a | 86.6 \pm 3.3** | 220.0 \pm 20.0* | 70.0 \pm 5.2*** ^a |

*** $P < 0.1$, ** $P < 0.01$, * $P < 0.001$ when compared to normal control; ^a $P < 0.1$, ^b $P < 0.01$, ^c $P < 0.001$ compared to ISO control and ^a $P < 0.1$, ^b $P < 0.01$, ^c $P < 0.001$ compared to propranolol. SANE -100 (*S. anacardium* nut extract 100 mg/kg), SANE -500 (*S. anacardium* nut extract 500 mg/kg) and PRO (propranolol 10 mg/kg).

isoproterenol. The cholinergic blockage has a direct impact on ECG by extension of QT interval²². The prolongation QT interval at times of myocardial stress is an indication of arrhythmias³¹ and sudden cardiac collapse^{32,33}. Prophylactic therapy of SANE 100 and 500 mg/kg along with PRO or separately was potent enough to avoid the prolongation of QT interval indicating absence of arrhythmias and cardioprotective potential. Similarly, abnormally elevated QRS duration, PR and RR intervals are also predictors of myocardial damage. Prior treatment of animals with SANE-100/500 mg/kg, PRO and their combination showed substantial normalisation in electrocardiographic determinations. The best results were shown by high dose of SANE when given with PRO in conjunction before induction of myocardial damage by isoproterenol.

Conclusion

From the present results it may be concluded that the SANE both at low (100 mg/kg) and high doses (500 mg/kg) possess cardioprotective efficacy when given prophylactically in experimental animals. Moreover, combined therapy of SANE and PRO demonstrated synergistic cardioprotective potential than when they were used alone. The combination of high dose of SANE and PRO was found to have best effect. However, combined therapy of SANE and PRO must be further refined for adjustment of doses as higher doses of SANE and PRO may pose negative implication due to excessive pharmacological effects.

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