Research Article

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CARDIO PROTECTIVE ACTIVITY OF TRINETRA RASA IN EXPERIMENTAL ANIMAL MODEL

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ABSTRACT
Cardiovascular diseases are described under Hridroga in Ayurvedic classics. Trinetra Rasa is a herbo-mineral formulation explained in the context of Hridroga. The present study was aimed to evaluate Cardio protective activity of Trinetra Rasa against Isoprenaline induced cardiac damage in rats and attempt to understand mechanism of its therapeutic effect with respect to biochemical markers, ECG and Histopathological changes. Albino rats of Wistar strains of either sex between 150 to 250 g, randomly divided into 6 groups, 8 rats in each group. Group 1- Control group given tap water 10 ml/kg, Group 2- Isoprenaline control group -100 mg/kg, Group 3- Standard group given Proprenalol-10 mg/kg, Group 4- Honey (Vehicle control/Anupana)- 10 ml/kg (Test 1), Group 5-Trinetra Rasa in therapeutic dose along with Honey-750 mg/kg (Test 2) and Group 6- Trinetra Rasa in 2 times the therapeutic dose along with Honey- 1500 mg/kg (Test 3) were administered according to the body weight of animals by oral route to respective groups at morning hours and continued for 20 days. On 19th day, ECG was recorded to assess Myocardial infarction and 1st dose of Isoprenaline injection (s/c) was given, on 20th day after 24 hours of 1st dose, 2nd dose of Isoprenaline injection was given (s/c) and ECG was recorded. Biochemical and histopathological assessment was done. In biochemical parameters better effect was observed with Test 2 group but histopathological profile showed good effect at Test 3 group higher dose level. The study provided evidence for presence cardio protective activity of Trinetra Rasa against Isoprenaline induced cardiac damage in rats.
Keywords: Trinetra Rasa, Hridroga, Isoprenaline, Cardio protective activity

INTRODUCTION

Cardiovascular disease is the most frequent cause of adult death in industrialized societies, and is increasingly important in developing countries[1]. By 2030, almost 23.6 million people will die from cardiovascular disease[2]. Coronary heart disease in Indians has been shown to occur prematurely, that is, at least a decade or two earlier than their counterparts in developed countries[3]. Myocardial infarction (MI) is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand[4]. Isoprenaline, a β-adrenoceptor agonist has been reported to produce MI in large doses; upon auto-oxidation, Isoprenaline generates highly cytotoxic free radicals known to stimulate the peroxidation of membrane phospholipids causing severe damage to the myocardial membrane. Hence, it is widely used as a model to produce MI in rats[5,6].

Ayurveda emphasizes on the preventive aspect of health as well as curing of diseases. Cardiovascular diseases are described under Hridroga in Ayurvedic classics[7,8] where various herbo-mineral formulations are being described in Rasashastra texts. Ayurvedic treatment and dietary guidelines are very important for prevention and treatment of Hridroga in today's stressful life and Trinetra Rasa is one such potent herbo-mineral formulation explained in the context of Hridroga. The formulation of Trinetra Rasa is selected for the present study from Bhaishajya Ratnavali - Hridroga Chikitsa. It consists of Shudha Parada, Shudha Gandhaka and Abhraka Bhasma, where Bhavana Dravya is Arjuna Twak Kwatha which is administered along with Anupana Honey[9]. No earlier experimental studies have been carried out on this yoga. Myocardial cell protection and prevention of cell ischemia or necrosis have been therapeutic targets for a long time. Hence an effort was made to study the cardio protective effect of Trinetra rasa. Here an attempt is made to establish pharmacological basis for the use of Trinetra Rasa in Hridroga, i.e. evaluation of Cardio protective activity in Isoprenaline induced cardiac damage in rats and to understand mechanism of its therapeutic effect with respect to biochemical markers, ECG, Cardiac cyto-architecture (Histopathology).

MATERIALS AND METHODS

Preparation of Trinetra Rasa

Trinetra Rasa -Shudha Parada, Shudha Gandhaka and Abhraka Bhasma in equal proportion along with Bhavana Dravya-Arjuna Twak Kwatha were taken and Bhavana was given for 21 times as per Bhaishajya Ratnavali[9]. Necessary processing of raw materials and preparation of yoga was done in the laboratory of P.G Department of Rasashastra Alva's Ayurveda Medical College, Moodbidri, Karnataka, India as per textual references.

Chemicals

Chemicals used in the study were of analytical grade.

Screening and Maintenance of animals

Albino rats of Wistar strains of either sex between 150 to 250 g were obtained from animal house attached to department of Pharmacology, SDM Research Centre Udupi, Karnataka, India. The experimental protocol was

approved from the institutional ethical committee under the reference no. SDMCAU, IAEC, 2012-13 MDB 01. The animals were fed with normal rat diet and water *ad libitum* throughout the study. They were acclimatized in the laboratory condition for two weeks prior to the experimentation. The housing provided has the following conditions: controlled lighting of 12:12 h light and dark cycle, temperature of 25ºC and relative humidity of approximately 50 %.

Study Design

Wistar albino rats of either sex weighing 150 g to 250 g, 48 rats were divided into six different groups, eight in each group.

Group 1- Control group was given tap water 10 ml/kg,
Group 2- Isoprenaline control group -100 mg/kg,
Group 3- Standard group was given Proprenalol-10 mg/kg,
Group 4- Honey (Vehicle control/Anupana) – 10 ml/kg (Test 1),
Group 5-Trinetra Rasa in therapeutic dose + Honey-750 mg/kg (Test 2),
Group 6- Trinetra Rasa in 2 times the therapeutic dose + Honey-1500 mg/kg (Test 3)

The initial weight of all animals was recorded prior to administration of drug and dose of medicine calculated accordingly. The dose selection was done on the basis of body surface area ratio using the table of Paget and Barne's (1969)- Human dose X 0.018 for rat weighing 200 g X 5(converting to mg/kg by multiplying with suitable factor 5)- 750 mg x 0.018 x 5 - 67.5 mg/kg body weight of the rat was the therapeutic dose. Test drugs, vehicles, standard drug were administered according to the body weight of animals by oral route with help of suitable gastric catheter sleeved onto a syringe to respective groups at morning hours and continued for 20 days. On 19th day, body weight was again recorded. Accordingly Isoprenaline dose was calculated and ECG was recorded and considered as initial reading to assess Myocardial infarction. After the reading, 1st dose of Isoprenaline injection (s/c) was given. On 20th day after 24 hours of 1st dose, 2nd dose of Isoprenaline injection was given (s/c). After 2nd dose, ECG was recorded. Blood samples were collected through supra orbital puncture by capillary and sent to laboratory for estimation of serum biochemical parameters. The animals were sacrificed by overdose of diethyl ether anaesthesia. The abdomen was opened by midline incision and then heart were dissected out along with aorta and transferred to normal saline for anti-oxidant study and specimen meant for histopathology were transferred to 10 % formalin solution.

Electrocardiography

The ECG recording was done on 19th day, 20th day and 24 h after 2nd dose of Isoprenaline injection. ECG was recorded after mildly anesthetizing the animals with diethyl ether. Anesthetized rats were placed in the supine position on a board and ECG was recorded continuously by using a portable electrocardiogram machine (IWORX), 3 standard limb leads were attached. The speed of the ECG machine was set to 25 mm/sec. The ECG was measured to determine duration and amplitude of the P wave, QRS complex, and ST segment alterations

Procedure for biochemical parameters

For this purpose a requisite quantity of serum was fed to the auto analyser (Erba FM 200 of Transasia) which was automatically drawn in to the instrument for estimating different parameters.

Statistical Analysis

One way ANOVA followed by Dunnett's multiple comparison T test with post HOC test using graph pad in stat software. Where $P < 0.05$ considered mild significant, $P < 0.01$ considered moderately significant, $P < 0.001$ considered highly significant.

RESULTS

In the present study, as shown in Table 1; the observed SGPT elevated extremely significantly in Isoprenaline control and standard groups but not significantly increased in Test 2 and Test 3 groups. Significant elevation of SGPT was found in Test 3 group. Serum lipid lowering effect was seen in Standard group. The cholesterol lowering effect was not affected by vehicle and test drug treatment. The triglyceride lowering was moderately reversed in vehicle and test drug administered groups. Significant decrease in total protein content of the serum especially the globulin level was observed. This decrease was found to be moderately reversed in all the treated groups in comparison to Isoprenaline control groups. Serum Creatinine significant decrease was observed in Test 2 and Test 3 groups. Elevation of Alkaline Phosphatase in Isoprenaline group was found to be significantly reversed in standard, Test 2 and Test 3 treated group. Glucose levels did not increase in Isoprenaline group but standard group and Test 1 group significant increase was observed where as surprisingly even though honey was administered in Test 2 and Test 3 no increase was seen in blood glucose was observed. CRP level in the serum was not modified in to significant extent by Isoprenaline. However, significant elevation was observed in this marker in reference standard and vehicle treated group. Significant elevation of LDH activity was observed after Isoprenaline injection this was reversed to moderate extent by reference standard and test drug but not in vehicle treated group. Serum uric acid level was not affected to significant extent in Isoprenaline treated group in comparison to the values of normal control. However, interestingly in all the treatment groups a significant decrease was observed in comparison to the Isoprenaline control group. Significant elevation in CK-MB was observed in Isoprenaline control group indicating marked damage to the myocardium. This elevation was found to be significantly reversed in reference standard, vehicle and test drug administered group. Catalase was found to be moderately decreased in all the treated groups in comparison to Isoprenaline control group. The lipid peroxidation was found to be moderately increased in non-significant manner in standard, vehicle and Test 3 groups where significant increase was observed in Test 2 group. Glutathione peroxidise activity was found to be

significantly decreased in standard group, no effect was observed in vehicle group and in the other two groups a moderate but non-significant decrease was observed. Isoprenaline administration in rats showed treatment-related adverse effects in electrocardiogram. There was a significant decrease in duration of P, PR interval, TP duration, QT interval, time at R and significant increase in heart rate (Figure 5) in comparison to Normal control group (Figure 6). None of these parameters were found to be reversed by different types of treatment modalities. Histopathological examination of the heart sections from Isoprenaline control group revealed drastic pathological changes in the form of necrosis, oedema, myocarditis and fibrosis in some cases (Figure 1). Ventricular dilatation was observed in some rats indicating a failing heart. These changes were attenuated to moderate extent in all the three test drug administered groups as well as reference standard treated group. The best effect was observed in Test 3 dose given group (Figure 2). Changes were observed in the aorta in the form of decrease in the thickness of tunica adventia, loss of lamellar arrangement in the tunica media and disturbance of the endothelial lining in few rats in Isoprenaline control group (Figure 3). These changes were found to be reversed in the entire three test drugs administered groups with the effect being better at higher dose level (Figure 4). In biochemical parameters better effect was observed with Test 2 group but histopathological profile showed good effect at Test 3 group higher dose level.

Table 1: Results of all Parameters

Parameter	Group 1 (Normal control)	Group 2 (Isoprenaline control)	Group 3 (standard control)	Group 4 (Test 1)	Group 5 (Test 2)	Group 6 (Test 3)
SGOT (IU/L)	139 ± 6.80	237 ± 7.99**	313 ± 17.51*	344 ± 28.122**	293 ± 21.44	286 ± 17.08
SGPT (IU/L)	49.6 ± 4.402	107 ± 8.664	164.8 ± 18.540	112.33 ± 20.790	148.5 ± 11.890	225.83 ± 29.248**
Serum Total cholesterol (mg/dl)	75.66 ± 4.047	54 ± 3.367**	67.6 ± 0.400*	56.33 ± 2.348	51.33 ± 3.062	54.33 ± 1.874
Serum triglyceride (mg/dl)	171 ± 21.575	69.5 ± 1.232**	140.8 ± 9.820**	85.66 ± 3.630	91 ± 3.521	81.5 ± 1.408
Serum albumin (g/dl)	3.2 ± 0.1581	3.066 ± 0.04216	2.3 ± 0.0949**	3.0166 ± 0.03073	2.733 ± 0.021*	2.516 ± 0.1014**
Serum globulin (g/dl)	3.90 ± 0.3225	2.27 ± 0.076**	3.18 ± 0.14**	2.43 ± 0.14	2.70 ± 0.058	3.00 ± 0.09**
Total Protein (g/dl)	7.05 ± 0.16	5.33 ± 0.08**	5.64 ± 0.04	5.45 ± 0.14	5.48 ± 0.06	5.63 ± 0.14
Serum Creatinine (mg/dl)	0.65 ± 0.034	0.692 ± 0.0083	0.92 ± 0.037**	0.733 ± 0.021	0.535 ± 0.029**	0.367 ± 0.021**
Serum Alkaline Phosphatase (IU/L)	254.66 ± 36.218	483.17 ± 43.374**	140.4 ± 5.862**	271.66 ± 15.251**	431.66 ± 21.512	94.66 ± 7.424**
Serum glucose (mg/dl)	118.33 ± 6.422	132.66 ± 9.294	185 ± 12.693**	195.66 ± 15.692**	131.33 ± 4.112	115.33 ± 6.48
C-Reactive Protein (mg/L)	0.746 ± 0.044	1.079 ± 0.172	0.852 ± 0.005354	0.907 ± 0.1164	0.874 ± 0.06126	0.907 ± 0.05725
LDH activity (IU/L)	982.8 ± 190.88	1877.3 ± 337.51*	1380 ± 151	2038.3 ± 197.72	1318.3 ± 85.129	1500.3 ± 150.75
Serum uric acid (mg/dl)	6.533 ± 0.5239	6.216 ± 0.2880	1.86 ± 0.0872**	3.792 ± 0.5367**	3.35 ± 0.1711**	4.163 ± 0.4644**
CK-MB (IU/L)	91.946 ± 2.452	380.3 ± 44.507**	84.134 ± 11.405**	40.983 ± 4.334**	21.775 ± 2.206**	96.276 ± 11.081**
Biochemical parameters in the Heart Homogenate						
Catalase (µmoles/min/mg protein)	-	518.76 ± 143.90	440.09 ± 59.437	427.76 ± 87.308	420.62 ± 48.82	392.075 ± 25.33
Lipid Peroxidation (µmoles of MDA formed/g wet tissue)	-	26820.5 ± 4161.4	13014.8 ± 1595.6*	25667.9 ± 5308.8	14684.9 ± 2433.6	15621.27 ± 1362.3
Glutathione Peroxidase activity (µmoles of glutathione/mg protein/min)	-	1252.3 ± 240.97	2025.11 ± 151.2	1761.55 ± 236.44	3108.74 ± 262.57**	1783.57 ± 368.91
ECG parameters						
Time at R	85.815 ± 14.357	42.5 ± 10.804**	24.18 ± 5.370	23.45 ± 3.53	38.4 ± 6.87	51.49 ± 4.96
TP duration	192 ± 5.612	117 ± 4.06**	122 ± 2	136 ± 12.19	113.33 ± 3.076	106.66 ± 2.108
P duration	65 ± 7.906	25 ± 4.183**	18 ± 2	33 ± 7.176	23.33 ± 3.33	24.166 ± 1.537
PR- Interval	63.00 ± 16.956	24.00 ± 1.87**	27.00 ± 2.00	33.00 ± 9.87	15.83 ± 2.50	22.50 ± 2.81
QT Interval	137 ± 5.148	93 ± 2.55**	93 ± 4.637	103 ± 9.434	97.5 ± 2.386	84.16 ± 2.386
T duration	75 ± 16.956	37 ± 3.391*	43 ± 2.55	43 ± 9.950	50 ± 3.416	31.66 ± 4.595
Heart rate	320 ± 16.583	558.38 ± 10.26*	898 ± 176.36**	462.29 ± 29.513	542.8 ± 8.754	564.25 ± 13.389

*P < 0.05, **P < 0.01; Where, Group 1- control group given tap water 10 ml/kg, Group 2- Isoprenaline control group -100 mg/kg, Group 3- Standard group given Proprenalol-10 mg/kg, Group 4- Honey (Vehicle control/Anupana)- 10 ml/kg (Test 1), Group 5-Trinetra Rasa in therapeutic dose + Honey-750 mg/kg (Test 2), Group 6- Trinetra Rasa in 2 times the therapeutic dose + Honey-1500 mg/kg (Test 3)

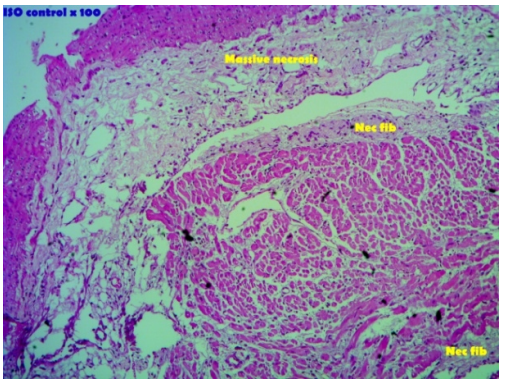

Figure 1(photomicrograph of section of Heart -Isoprenaline treated group)

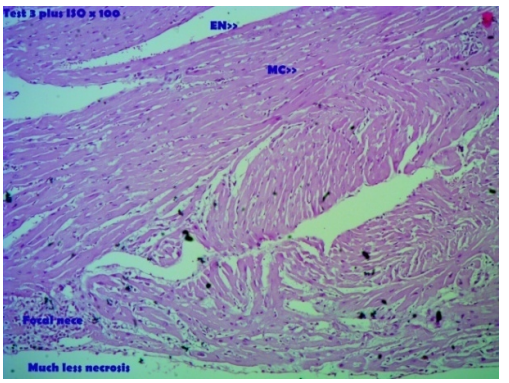

Figure 2(photomicrograph of section of Heart-Test 3 group)

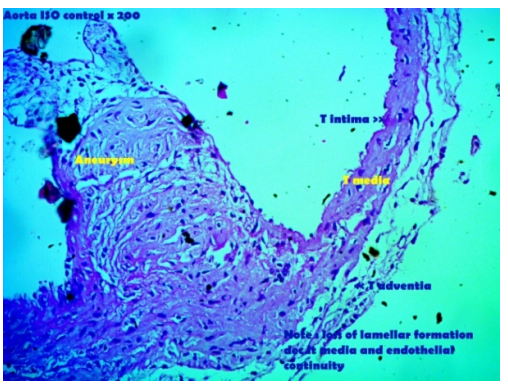

Figure 3 (photomicrograph of section of Aorta -Isoprenaline treated group)

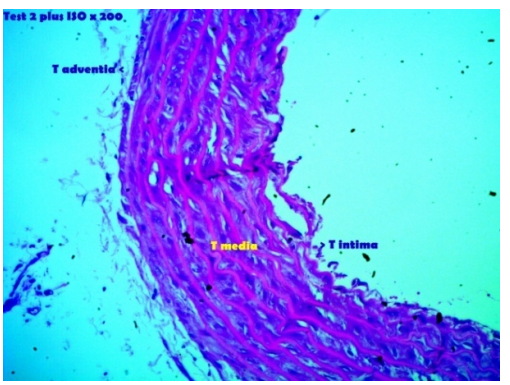

Figure 4(photomicrograph of section Aorta-Test 3 group)

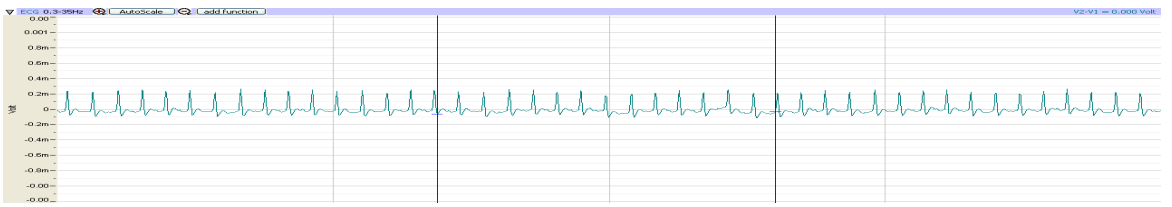

Figure 4: ECG of Isoprenaline treated group

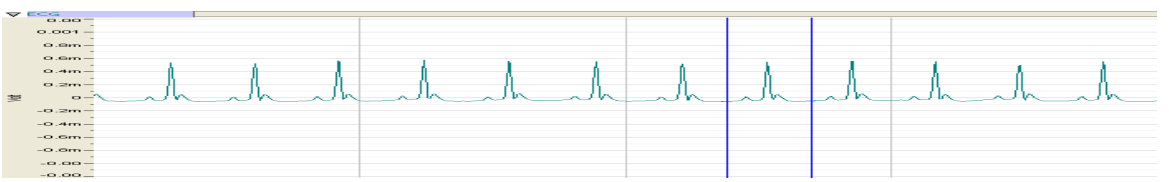

Figure 5: ECG of Normal control group

DISCUSSION

Cardio protectivity is classified as physiological approach of cardio protectivity and therapeutic approach of cardio protectivity. The same concept was explained in our texts thousands of years ago i.e. Hridya dravya and Hridrogahara dravya. Trinetra Rasa is one such unique combination of Hridya (physiological) and Hridrogahara (Therapeutic) dravya. Arjuna is having Hridya[10] property and Abhraka is having Hridrogahara[11] property, Kajjali as Yogavahi and Honey Hridya[12]. Holistic approach is the special feature of Ayurveda; hence the drug was given along with Anupana (Test 1). With a view to assess the action of drug it was given in normal therapeutic equivalent dose (Test 2) and doubles the normal therapeutic equivalent dose (Test 3). However in the present study even though the yoga is described in Hridroga context and indicated in all kinds of Hridroga, experimental evaluation was carried out on cardio protective activity on Isoprenaline induced MI in experimental animals. Myocardial infarction occurs due to sudden decrease in the coronary blood flow, most often it is due to occlusion of coronary artery already narrowed by atherosclerosis[13]. In the early stages of AMI (Acute Myocardial Infarction) there is generally leucocytosis,

elevation of serum enzymes like Serum Glutamic Oxaloacetic Transaminase (SGOT), Lactic dehydrogenase (LDH), and its isoenzymes, Creatinine Phosphokinase (CPK), and elevation in SGOT and CPK levels can be detected within 3 to 6 hours, which remain high up to 4 to 5 days after AMI. Hence, determination of these enzymes is useful for the early diagnosis of AMI. LDH levels rise from 12 to 48 hours after AMI and remain high for 10 to 12 days. If we analyze the result of experimental study in totality we can conclude that the injection of Isoprenaline leads to alterations in biochemical, ECG and histopathological parameters indicating features of marked myocardial perturbations- simulating changes found in myocardial infarction. These Isoprenaline induced changes are believed to be due to generation of highly cytotoxic free radicals which are responsible for the observed myocardial injury reflected in the form of changes in the biomarkers and infarct like necrosis in the cyto-architecture. The serum ALP, LDH and CPK enzyme activities are considered as important markers for both early and late phases of cardiac injury. The changes observed in the key bio-markers especially CK-MB activity, LDH activity results may be considered to indicate moderate myocardial protection in Test 2 and Test 3 groups and weak protection in Test 1 groups and histopathological changes indicate presence of at least moderate cardio protective activity in the test drug-Trinetra rasa. The results obtained by catalase, lipid peroxidation and glutathione peroxidise activity presented a complex picture. However, it may be suggestive that the reference standard and test drug promote glutathione conservation by modulating its metabolism. This may contribute to the lessening of the oxidative stress. However, dose dependent effect was not observed in all the parameters. In biochemical parameters better effect was observed with Test 2 group but histopathological profile showed good effect at Test 3 group higher dose level. Nevertheless the evidence generated can be considered as the basis for the observed effectiveness of this formulation in the clinical settings.

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