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Evaluation of cardioprotective activity of Panchanana rasa in Isoprenaline Induced Myocardial Infracted Experimental rats

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ABSTRACT
Rasasastra is an important branch of Ayurveda which deals with the use of metallic and mineral drugs which are pharmaceutically processed and rendered fit for internal administration.The Rasa medicines are known for their faster action in smaller doses,quicker assimilation,excellent therapeutic values,longer shelf life and easy preparation.Among the many health predictions of the new millennium,the most alarming is that of cardio vascular disorders,heart diseases and stroke topping the list for death and disability.The present study is concerned with the formulation called Panchanana rasa mentioned in Brihat rasa raja sundara which has indication in Hridroga.Till now,no scientific studies has been carried out on this formulation. In the experimental study,the drug was proved to be non-toxic in acute oral toxicity study.Finally myocardial infarction adequately reproduced in laboratory was used to check its cardioprotectiverole.Statistical analysis on the basis of biochemical parameters was done and the results combined with histopathological changes observed in the heart,concluded in the novel idea of cardioprotective action of Panchanana rasa.Based on the empirical evidences and baseline classical principles,logical reasoning was done to end up in an accurate report of the present study.
KEY WORDS-Panchanana rasa,hridroga,isoprenaline,cardioprotective activity

I. INTRODUCTION

Ayurveda is a traditional system of medicine with historical roots in the Indian subcontinent.In Ayurveda, materials from natural sources are being used for the preparation of Ayurvedic formulation. This includes plants, minerals/metals and animals. Rasashastra is a branch of Ayurvedic medicine which deals with formulations containing minerals/metals and significantly Parada. From the fact that the name of this branch has been given after Parada (Rasa in Rasashastra) indicates the emphasis laid by the then health care professionals on the use of Mercury or Rasa or Parada in the use of therapeutics.According to Ayurvedic Formulary of India, mercury is reported to be the widely used heavy metal. But nowadays, modern scientists are concerned with the use of heavy metals in Ayurvedic preparation. According to Ayurveda, before these metals are used for the treatment,purification process should be carried out so that the possibility of adverse effects gets eliminated.

Rasashastra is existing since vedic period. It has also been reported that Buddhist sages were the first to use mercury and believed to be one of the creators of treatment by using metals and minerals in their appropriate form. Many drugs used for rasayana chikitsa (rejuvenation therapy) contains Parada(rasa) along with other metals. These are classified as rasaousadhies. Since rasaoushadies are effective in small doses and gives quick relief as compared to pure herbal formulations,they have gained significance tremendously.Great caution should be taken while using metals. In the text it is suggested that they should be reduced to micro-fine powder.Before these metals are being used in the formulation, they should be treated in an appropriate manner by different processing techniques like shodhana, marana, amritikarana, etc. This is responsible for the elimination of the possible adverse effects.In ancient rasa literature it is reported that ancient rasaacharyas have recognized dosas in almost all drugs including metals, minerals, etc. To remove these dosas, certain techniques and procedures were prescribed like shodhana and marana. For purification, samanya shodhana and vishesha shodhana methods are carried out. But if not performed properly then it may lead to many complications like loss of vision, skin diseases, vomiting and even death. In Ayurveda, heavy metals are converted into non-metallic form.These compounds should be initially detoxified.. From

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the ancient times, garlic has been useful for purification of Parada. Thousands of such purification procedures are mentioned for all the minerals and metals. It is now agreed that manufacturing of Ayurvedic preparation as per ancient scripts and using modern techniques in combination will lead to non-toxic formulations.

Heart diseases are mostly seen with the advancement of age.But in the present scenario,heart diseases cause one in every four deaths.Heart disease is a term covering any disorder of the heart.Coronary heart disease,arrhythmia and myocardial infarctions are some examples of heart disease.Heart disease is often used interchangeably with the term cardio vascular disease.Among the many health predictions of the new millennium,the most alarming is that of cardio vascular disorders,heart diseases and stroke topping the list for death and disability.Earlier studies suggest that by the year 2020 India will have the largest CVD burden in the world.One fifth of the deaths in India are from coronary heart disease,studies show that it will account for one third of all deaths.Sadly many of these Indians will be dying young.The risk factors and higher mortality from CVD has been proved from the data obtained in developing countries including India.Heart disease in India occurs 10-15 years earlier than in the West.Innovative research across the translational section is essential for equitable CVD prevention and control in India.Contemporary treatment attempts to restore blood flow to the heart by percutaneous coronary intervention where arteries are pushed open and may be stented or coronary artery bypass surgery followed by life style modification.Angioplasty is the gold standard for the treatment of acute myocardial infarction.This dissertation work aims to evaluate a noninvasive intervention in CAD resulting in MI using a medicated mineral drug processed as per classics of Ayurveda. Direct reference to CVD in ancient ayurvedic classics is Hridroga.

II. MATERIALS &METHODS

Preparation of Panchanana rasa

Sodhita parada &sodhita gandhaka is made into kajjali.Then it is given mardana in amalaki rasa,draksha rasa,yasti rasa and kharjura rasa each for 1 -2 days.Necessary processing of raw materials and preparation of yoga was done in the rasashala of P.G.Department of Rasasastra,MVR Ayurveda medical college,Parassinikkadavu,Kannur,Kerala,India as per textualreferences.

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.IEC/RSBK01/2020/05.

Six animals were housed in poly-propylene cage with stainless steel top grill. The dry wheat husk (post hulled) was used as bedding material and was changed every 3rd day. The animals were fed with normal rat diet- Laboratory Animal feed pellets supplied by VRK Nutritional Solutions and tap water ad libitum throughout the study. They were acclimatized in the laboratory condition for 14 days prior to the experiment in standard laboratory conditions: 12 ± 01 hour day and night rhythm, maintained at 25±3°C and relative humidity of approximately 50 %.

Study design

Wister albino rats of either sex weighing 150 g to 250g. 24 rats were divided into 4 different groups, 6 in each group.

Table showing Grouping and dosing in Cardioprotective study

Group no	Group name	Drug administered	Animal dose
Group 1	Control group	Tap water	-
Group 2	Isoproterenol control group	Isoproterenol	80 mg/kg (i.p)
Group 3	Standard group	Propranolol	20 mg/kg (i.p.)

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Group 4	Test drug group	Panchanana rasa with anupana	11.25mg/kg (oral)

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 Barn's (1969) and done as follows:

Therapeutic human dose x surface area ratio (convertibility factor) is the dose for rat.

Conversion of the dose obtained above to dose in mg / kg / day by multiplying with suitable conversion factor based on the average weight of the animal.

Dose for Rats = Human dose X 0 .018 for rat weighing 200g X 5 (converting to mg / kg by multiplying with suitable factor 5) i.e. 125mg x 0. 018 x 5;i.e. 11.25mg / kg body weight of the rat or 0.0108mg/g body weight of the rat was the therapeutic dose.

Stock solution of the drug was prepared in such a concentration that the requisite dose could be obtained by administration of stock solution in the volume of 1.125mg /100g body weight for 21days. i.e. 22.5 mg Panchanana rasa per 20ml was stock solution prepared which was administered to the rats according to their body weight.Anupana was prepared by mixing 5 gm dhatri churna and 5 gm sitha along with drug solution.. The test drug was administrated through oral route at different dose levels to respective animal through a suitable gastric catheter sleeved onto a syringe.Test drug and standard drug(intra peritoneally) were administered to respective groups at morning hours and continued for 21days. On 19th day, body weight was again recorded accordingly Isoproterenol dose was calculated and 1st dose of Isoproterenol injection(intra peritoneally) was given. On 20th day i.e. after 24 hours of 1st dose, 2nd dose of Isoproterenol injection was given (intra peritoneally). On 21st day, 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 anesthesia. The abdomen was opened by midline incision and then heart was dissected out along with aorta and transferred to normal saline and the specimen meant for histopathology to 10% formalin solution.

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.

Stastistical analysis

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

III. RESULTS

The ISO control group resulted in very significant elevation in **LDH** activity in comparison to the data from normal control group.In test group very significant decrease in LDH activity was noted in comparison to the ISO control group.In standard group also very significant decrease in LDH activity was noted in comparison to ISO group. The ISO control group showed very significant increase in **CKMB** activity in comparison to normal control group.In test group,statistically non significant decrease was noted in CKMB activity when compared to the ISO control group.In standard group,its non significant decrease was noted when compared to ISO group. In ISO control group **SGOT** activity was found to be non significantly decreased when compared with normal control.In test group,SGOT level showed non significant increase when compared to ISO group.In standard group it was non significantly decreased when compared to ISO group. In ISO control group **SGPT** activity was found to be non significantly decreased in comparison to normal control group.In test group SGPT level showed non significant increase when compared to ISO group.In standars group,it was found to be non significantly decreased when compared to ISO group. In ISO control group **cholesterol** activity was found to be very significantly increased in comparison to normal control.In test group cholesterol activity showed very significant decrease when compared to ISO group.In standard group it is non significantly decreased when compared to ISO group. In ISO control group **triglycerides** activity was found to be non significantly decreased when compared to normal control group.In test group triglycerides level was noted as non significant decrease when

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compared to ISO group.In standard group it was non significantly decreased when compared to ISO group. In ISO control group **HDL** activity was found to be very significantly decreased when compared to normal control.In test group HDL activity showed non significant increase when compared to ISO group.In standard group,it was found to be non significantly increased when compared to ISO group. In ISO control group **LDL** activity was found to be very significantly increased when compared to normal control group.In test group, LDL activity was found to be very significantly decreased when compared to ISO group.In standard group it was non significantly decreased when compared to ISO group. In ISO control group **VLDL** activity was found to be non significantly decreased when compared to normal control group.In test group,VLDL activity was found to be non significantly decreased when compared to ISO group.In standard group also it was non significantly decreased. In ISO control group **creatinine** activity was found to be very significantly increased when compared to normal control group.In test group,it is significantly decreased when compared to ISO group.In standard group it was very significantly decreased when compared to ISO group. In ISO control group **total protein** activity was found to be non significantly decreased when compared to normal control group.In test group it was non significantly decreased when compared to ISO group.In standard group it was non significantly increased when compared to ISO group. In ISO control group **weight of hearts** was found to be non significantly decreased when compared to normal control group.In test group it was non significantly increased when compared to ISO group.In standard group it was non significantly decreased when compared to the ISO group.

Results of All Parameters

Parameter	Group 1(normal control)	Group 2(positive control)	Group 3(standard drug)	Group 4 (test drug)
LDH	210.66±34.216	598.16±43.798	232.6±44.009	269±46.104
CKMB	8.133±0.8578	41.85±9.06	19.4±3.4	31.5±5.614
SGOT	104±4.715	102.71±6.44	95.2±5.093	102.833±10.512
SGPT	55.5±4.039	51.85±7.77	33.6±4.366	53.33±13.058
Cholesterol	53.166±2.88	103±2.8	100±9.274	47.8±11.83
Triglycerides	89.66±5.220	75.42±9.717	54.6±6.313	63.33±12.818
HDL	17.66±1.054	4.98±0.805	7.3±1.95	8.75±1.75
LDL	12.928±0.9798	79.63±1.75	76.22±12.96	22.08±9.182
VLDL	17.93±1.044	15.08±1.943	10.92±1.263	12.66±2.564
Creatinine	0.366±0.02108	0.735±0.062	0.486±0.0416	0.526±0.064
Total protein	6.433±0.182	5.142±0.573	6.3±0.557	4.216±0.635

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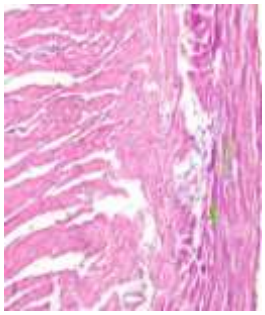
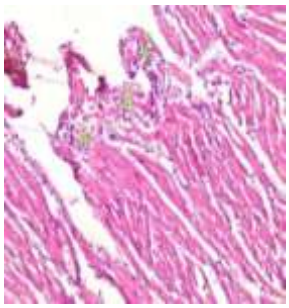
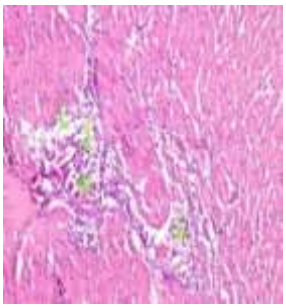
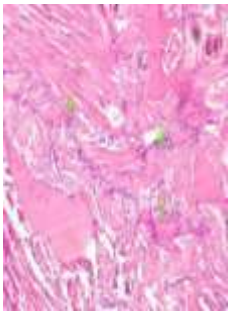
HISTOPATHOLOGY

1 2 3 4

Fig.1 cells of myocardium: vacuole (Test group)
Fig.2 chronic inflammatory infiltrate (Positive control group)
Fig.3 Necrosis with inflammatory infiltration(Positive control group)Fig.4 Inflammatory infiltrate(Test group)

PC group shows degenerative changes in the form of pale vacuolated muscle fibers. Areas of necrosis with loss of cellular details and inflammatory infiltration seen. Fibrosed area seen in 1 slide. Chronic lymphocytic infiltration seen in all areas of the tissue.Test group shows mild to moderate degenerative changes, small areas of necrosis and mild chronic inflammatory infiltration. Compared with PC, reduction in inflammatory infiltration, degeneration and necrosisseen in few slides.

IV. DISCUSSION

Cardioprotectivity is classified as physiological approach and therapeutic approach of cardioprotectivity.The same concept was explained by our acharyas as hridya dravya(physiological) and hridrogahara (therapeutic)dravya.Panchanana rasa is a combination of the drugs having these properties.

The most superior among all the rasadravyas;parada is the major ingredient here which has yogavahi,rasayana and dehasidhikara properties.Along with parada when gandhaka which is vatakapha nasaka,rasayana,dipana and amapachana combines to form kajjali,the most basic preparation of most of the rasoushadhis which is having excellent rasayana properties along with antimicrobial action.It is having action at jataragni amd dhatwagni level thus bringing about srotosodhana;thus help in dhatuposhana and rasayana.When this kajjali undergoes continuous mardana with hridya and sita virya drava dravyas like amalaki swarasa,kharjura kvatha etc the viryavardhana occurs and is expected to have action in hridroga.It dosent hampers prana and ojas as it processes sita virya.It is a good rasayana drug in which all the ingredients having specific action in dhatus;Kharjura &draksa- in rasa dhatu,amalaki-in rakta dhatu&yasti-medhya rasayana(Charaka);The anupana is dhatri churna and sitha which is again having action in hridaya. Histopathological examination of the heart revealed that the test group shows mild to moderate degenerative changes, small areas of necrosis and mild chronic inflammatory infiltration. Compared with positive control, reduction in inflammatory infiltration, degeneration and necrosis seen in few slides. From, the change in value of biochemical parameters (due to the effect of different treatments) which are especially indicated in cardio-protective action like Serum Creatinine and cardiac markers like LDH and CK-MB, cardio-protective action of the testdrug can be inferred as follows:

The level of LDH significantly increased with ISO injection was significantly decreased in both test drug and STD drug. Similarly the level of CK-MB significantly increased with ISO injection was significantly decreased with both STD and Test drug. LDH and CK-MB enters the blood stream only when the organ Heart is damaged. The significant decrease in the level of these cardio-biomarkers strongly recommends the myocardial integrity protection through various mechanisms.The level of Serum creatinine showed a significant increase with ISO injection which was significantly decreased with Test drug. Increase in creatinine value indicates increased mortality due to MI. The test drug had shown a decrease in the

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level. This shows the test drug have more an action in maintaining Serum creatinine.The statistically significant reduction in the level of LDH, CK-MB and non-significant decrease in S. creatinine when read along with the histopathological findings of the heart in test drug causing mild-moderate toxic changes,we can conclude that the test drug " Panchanana rasa" can show cardioprotective action more or less equal to the Standard drug. This action of cardioprotection was noticed at an animal dose of 11.25mg/kg of test drug and 20mg/kg of STD drug. An equivalent human dose of test drug is 125mg.From the above experimental study, Panchanana rasa has an action in heart which is evident by the prevention of induced pathology of myocardial infarction. The reversal of pathology can be attributed to Ama pachana karma of the kajjali due to its Deepana Pachana nature resulting in Srotosodhana of Rasavaha and Pranavaha Srotas to produce a Rasayana Ojao vardhana effect. To be more subtle, kajjali due to processing with various dravadravyas might have Vayu- Agni predominant Panchabhautika constitution. These are expected to act against the Kapha-medo dushti responsible for Hridroga.Above all the Prabhava of the drug is ultimate power with which it does unimaginable action. Although each component of panchanana rasa has several pharmacological actions on different organs/systems of the body, they act through several mechanisms in management of derrangement of the health and in combined form they play a very important role in the management of Hridroga.

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