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EVALUATION OF CARDIOPROTECTIVE EFFECT OF *ACHYRANTHES ASPERA* ON ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS

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ABSTRACT

The present study was designed to investigate the cardio protective effect of hydro alcoholic extract of *A. aspera* whole plant (AAPE) against isoproterenol induced myocardial infarction in rats. Male wistar albino rats (200-250gm) were pre-treated with AAPE (250 and 500 mg/kg) for 28 days. After the pre-treatment period, myocardial infarction in rats was induced by isoproterenol (ISO) administration (85 mg/kg, S.C.) at an interval of 24 h for two consecutive days. Isoproterenol administration showed significant elevation in the serum levels of cardiac injury markers (creatine kinase-MB, lactate dehydrogenase, alkaline phosphatase, aspartate transaminase and alanine transaminase, Total Proteins), lowered antioxidant defence status (Catalase, SOD, GSH, and LPO) in the heart. AAPE (low and high dose) pre-treatment restored ISO induced altered levels of marker enzymes and disturbed antioxidant status in rats. It was further confirmed by the Histopathological studies. In the AAPE alone treated group no significant alterations were observed. Results of the present study suggest that AAPE has a significant dose dependent protective effect on heart against isoproterenol induced myocardial infarction probably by improving endogenous antioxidant enzyme activities.

KEYWORDS: ***Achyranthes aspera*, Myocardial infarction, Isoproterenol, Antioxidant.**

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INTRODUCTION

Myocardial infarction (MI) is the main cause of death from cardiovascular disease CVD[1]. Cardiovascular disease (CVD) is one of the cause of death worldwide and is the world's largest killer, claiming 17.1 million lives a year[2]. Myocardial infarction is the acute condition of myocardial necrosis consistent with ischemia that occurs as a result of critical imbalance between coronary blood supply and myocardial oxygen demand[3]. It is the most dreaded sequel among ischemic heart disease and is invariably followed by several biochemical alterations such as lipid peroxidation, free radical damage, hyperglycaemia and hyper lipidaemia, leading to qualitative and quantitative alterations of myocardium[4]. Risk factors for myocardial infarction include smoking, hypercholesterolemia, hyper lipoproteinemia, high blood levels of triglycerides, low density lipoproteins, low blood levels of high density lipoproteins, diabetes, high blood pressure. Cardiovascular disease (CVD), remain the principal cause of death in both developed and developing countries which includes high blood pressure, coronary heart disease, congestive heart failure and stroke. It is predicted that CVD will be the most important cause of mortality in India by 2020[6]. Myocardial infarction (MI) occurs when the blood supply to a part of the heart is interrupted, causing death of heart tissue[5]. It is the most important consequence of coronary artery disease. Many patients may die within the first few hours of the onset, while remainder suffers from effects of impaired cardiac function.

Objectives

- Collection and authentication of *Achyranthes aspera L.* plant
- Extraction of the *Achyranthes aspera L.* plant
- Carrying out preliminary phytochemical screening
- Acute toxicity study
- In *vivo* evaluation of cardio protective activity using ISO induced myocardial infraction

MATERIALS AND METHODS

Collection and Authentication of the Plant

Plant of *Achyranthes aspera L.* was collected from a forest of Talakona, Chittoor district, Andhra Pradesh, India and the plant was identified on 02-FEB-2015 and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India. In the present study Achyranthes *aspera* whole plant was selected for evaluating cardio protective activity against isoproterenol induced myocardial infarction in male wistar rats.

Figure 1
Achyranthes aspera L

Preparation of the Extract

The whole plant was shade dried and powdered by mechanical grinder. About 500 gm of powder was extracted with hydro alcoholic mixture (ethanol: water: 70:30) at 40-60^{0}C by soxhlet apparatus for 3-4 days. The obtained extract was concentrated under reduced pressure using a rotary flash evaporator and stored in an airtight container in a refrigerator.

Phytochemical Investigation[9, 10]

The hydro alcoholic extract obtained was subjected to different preliminary qualitative phytochemical tests to determine the chemical constituents present in the extract. Various tests to determine the presence of proteins, steroids, flavonoids, alkaloids and carbohydrates were performed as per the following process.

Tests for Carbohydrates

Molisch's test

The test solution was treated with few drops of alcoholic α-napthol and 0.2ml of concentrated sulphuric acid was added slowly along the sides of test tube. Formation of purple to violet colouring at the junction indicates the presence of carbohydrates.

Test for Starch

To the test solution, weak aqueous iodine solution was added. Appearance of blue color indicates the presence of starch, which disappears on heating and reappears on cooling.

Tests for Proteins

Biuret Test

To 2ml of test solution, 2ml of biuret reagent was added. Appearance of violet color indicates the presence of proteins.

Warming Test

Test solution was boiled in a boiling water bath. Appearance of coagulation indicates the presence of proteins.

Test with TCA

To the test solution, 5% Trichloro acetic acid was added. Appearance of precipitate indicates the presence of proteins.

Tests for Sterols and Tri terpenoids

Libermann - Burchard Test

5ml of test solution was boiled with few drops of acetic anhydride boiled and cooled then concentrated sulphuric acid was added along the side of test tube. Appearance of brown ring at the junction of two layers is taken as inference. If the upper layer turns green, sterols are present whereas formation of deep red color indicates the presence of tri terpenoids.

Salkowski's Test

Test solution was treated with few drops of concentrated sulphuricacid and shaken well and the solution was allowed to stand for some time. Appearance of red color in the lower layer indicates the presence of sterols whereas formation of yellow color in the lower layer indicates the presence of tri terpenoids.

Tests for Flavonoids

Shinoda Test (Magnesium hydrochloride reduction test)

To the test solution, few fragment of magnesium ribbon were added then concentrated hydrochloric acid was added drop wise. Appearance of pink scarlet, crimson red or occasionally green to blue color after few minutes indicates the presence of flavonoids.

Alkaline Reagent Test

To the test solution, few drops of sodium hydroxide solution was added ,formation of an intense yellow color which turns to colorless by the addition of few drops of dilute acetic acid indicates the presence of flavonoids.

Ferric chloride Test

To 2ml test solution, few drops of ferric chloride solution were added. Formation of intense green color indicates the presence of flavonoids.

Tests for Alkaloids

Hager's Test

Alkaloids give yellow color precipitate with Hager's reagent (Saturated solution of Picric acid).

Mayer's Test

Alkaloids give cream color precipitate with Mayer's reagent (Potassium mercuric iodide solution).

Dragendorff's Test

Alkaloids give reddish brown precipitate with Dragendorff's reagent (Potassium bismuth iodide solution).

Wagner's Test

Alkaloids give reddish brown precipitate with Wagner's reagent (Solution of Iodine in Potassium Iodide).

Tests for Tannins

Lead acetate Test

To 5ml of test solution, few drops of 10% lead acetate were added. Appearance of precipitate indicates the presence of tannins.

Bromine water Test

0.5g of the extract was dissolved in distilled water and 10ml of bromine water was added. De colorization of bromine water indicates the presence of tannins.

Ferric chloride Test

To 5ml of test solution, few drops of ferric chloride test reagent were added. Appearance of intense green or blue color indicates the presence of tannins. Blue color indicates hydrolysable tannins whereas green color indicates condensed tannins.

Experimental Animals

Healthy male wistar albino rats weighing 200-250 g were used in this study. They were housed in polypropylene cages and maintained at room temperature under 12h dark/light cycles. They were fed with standard pelleted diet and water was provided *ad libitum*. The animals were acclimatized for one week under laboratory conditions before experiments on the animals. The experiment was carried out according to the prescribed guidelines by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India

Acute Toxicity Test

The acute oral toxicity was carried out using female wistar rats (200-250g) for hydro alcoholic extract of *Achyranthes aspera* whole plant *(*AAPE) in accordance with the Organization for Economic Co-operation and Development (OECD) Guideline no. 420-fixed dose procedure which was adopted for toxicity studies.

Dose Selection of Isoproterenol (ISO)

In the present study the dose of Isoproterenol 85mg/kg was selected as per the earlier studies.

Dose Selection of A. aspera Plant Extract (AAPE)

The extract was found to be devoid of mortality even at 2500mg/kg. Hence 1/10 (250mg/kg, p. o.) and 1/5 (500mg/kg, p.o.) of this dose were selected for the main study.

Table 1
Needle Selection for the induction process in rats.

S. No.	Purpose	Needle size
1.	Oral dosing for Rat	18 G, 3 inch length, curved, 2.25 ball diameter
2.	I. P injection	26 G, 1/2"
3.	S. C injection	26 G, 1/2"

Induction of Myocardial Infarction in rats
Isoproterenol (85mg/kg) was dissolved in normal saline and injected subcutaneously to rats at the end of extract treatment period for 2 consecutive days (*i.e.,* on 29th and 30th day) at an interval of 24 hrs to induce experimental myocardial infarction in rats[11].

Experimental Study Design
The adult male wistar rats weighing 150-200g rats were divided into seven groups of six animals in each and received the treatment as per the design (Table 2).

Table 2
Experimental Design of Induction of Myocardial Infarction in rats

Groups	Treatment
Group I (Control)	Received normal saline p. o.
Group II (ISO)	Received Isoproterenol (85mg/kg/s.c.) for two consecutive days.
Group III (ISO+AAPE)	Received hydro alcoholic extract of *A. aspera* low dose (250mg/kg) orally for 28days + Isoproterenol (85mg/kg/s. c.) for two consecutive days.
Group IV (ISO+AAPE)	Received hydro alcoholic extract of *A.aspera* high dose (500mg/kg) orally for 28days + Isoproterenol (85mg/kg/s. c.) for two consecutive days.
Group V (AAPE Low dose alone)	Received hydro alcoholic extract of *A. aspera* Low dose (250 mg/kg) orally for 28 days.
Group VI (AAPE High dose alone)	Received hydro alcoholic extract of *A.aspera* high dose (500mg/kg) orally for 28days

At the end of experimental period (after 24 hrs of second Isoproterenol injection), all the rats were anaesthetized. Then blood was collected from retro orbital plexus. The serum was separated and used for the estimation of diagnostic marker enzymes like AST (aspartate aminotransferase), ALT (alanine amino transferase), LDH (lactate dehydrogenase), CK-MB (Creatine phosphokinase-MB), TG (triglycerides), TC (total cholesterol), LDL (low density lipoproteins) and HDL (high density lipoproteins), TP (total proteins). The animals were sacrificed and heart was dissected out. The samples of heart tissue were analysed for tissue Catalase (CAT), Glutathione (GSH), Superoxide dismutase (SOD) and lipid peroxidation for measurement of thiobarbituric acid reactive substances.

RESULTS

Yield of extract
The yield of hydro alcoholic extraction of *A. aspera* whole plant was found to be 12.6%w/w.

Phytochemical investigation
Hydro alcoholic extract of *A. aspera whole plant* was subjected to qualitative chemical tests to determine the chemical constituents present in it (Table 3).

Table 3
Results of Phytochemical investigation

S. No.	Chemical test	Inference
1	Test for Flavonoids	+
2	Test for Alkaloids	+
3	Test for Tannins	+
4	Test for Glycosides	-
5	Test for Saponins	+
6	Test for Carbohydrates	+
7	Test for Proteins	+
8	Test for Amino acids	+
9	Test for Steroids	+
10	Test for Triterpenoids	-

Acute toxicity test
The extract was found to be devoid of mortality even at 2500mg/kg, thus 1/10th (250 mg/kg, p.o.) and 1/5th (500 mg/kg, p.o.) of this dose were selected for the main study.

Effect on Body weight, Heart wt. and Heart to Body weight ratio

Isoproterenol treated rats showed slight reduction in percentage change in body weight which was not significant when compared to control rats. Significant difference in percentage change in body weight was not observed between the groups (table 4). Significant ($p<0.05$) increase in heart weight was observed in ISO administered rats when compared to control rats. Significant ($p<0.05$) decrease in heart weight was observed in rats pre-treated with low dose and high dose AAPE followed by ISO administration when compared with ISO alone administered rats. There was no significant difference in heart weight between high dose AAPE alone and control treated rats.

Table 4

Effect of hydro alcoholic extract of A. aspera plant extract (AAPE) on body weight in control and experimental rats

Group	Initial body weight (gm)	Final body weight (gm)	Percentage change in body weight
Control	201.0±11.94	224.2±12.53	11.75±1.703
ISO	204.0±9.48	225.2±9.583	10.84±1.454*
AAPE (250mg/kg) + ISO	207.0±8.51	232.7±8.468	11.71±1.928#
AAPE (500mg/kg) + ISO	213.5±3.78	236.5±3.948	11.31±1.162#
AAPE (250mg/kg)	201.7±9.524	228.7±10.80	11.37±1.558
AAPE (500mg/kg)	213.0±3.191	236.3±3.062	11.91±1.822

All the values were expressed as Mean ± SEM using one way ANOVA followed by Tukey's multiple comparison test, where n=6; *--P<0.05; Group I vs Group II.

#--P<0.05; Group II vs Group III; Group III vs Group IV.

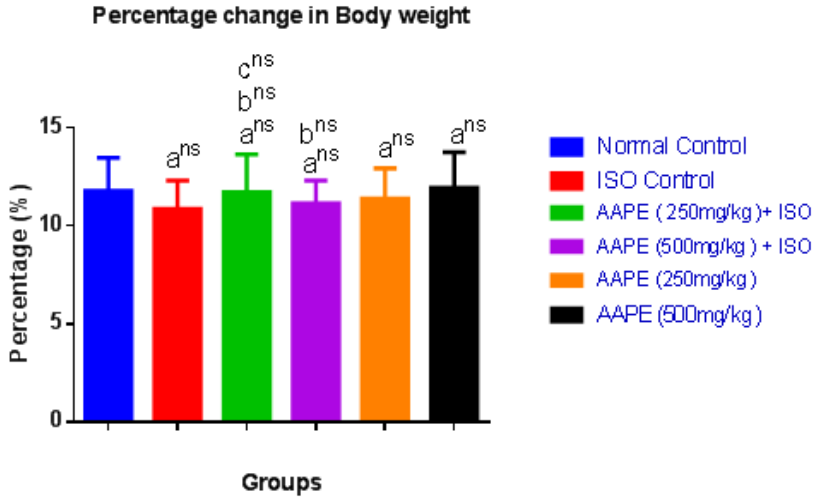
Table 5

Effect of hydro alcoholic extract of A. aspera plant extract (AAPE) on heart and heart to body weight ratio of control and experimental rats

Group	Heart weight (gm.)	Heart/body weight ratio
Control	0.6422±0.045	0.2861±0.014
ISO	1.3940±0.157	0.5982±0.1314*
AAPE (250mg/kg) + ISO	0.9299±0.0577	0.3918±0.010#
AAPE (500mg/kg) + ISO	0.8542±0.034	0.3651±0.012#
AAPE (250mg/kg)	0.6383±0.041	0.2888±0.013
AAPE (500mg/kg)	0.6308±0.043	0.2868±0.013

All the values were expressed as Mean ± SEM using one way ANOVA followed by Tukey's multiple comparison test, where n=6; *--P<0.05; Group I vs Group II.

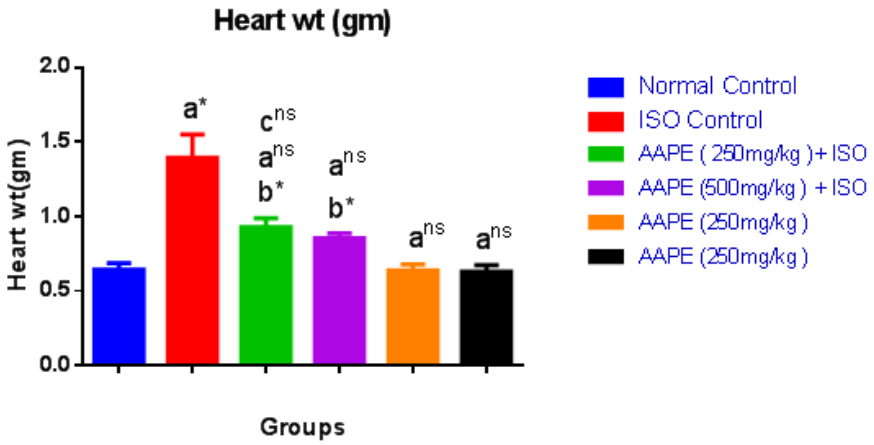
#--P<0.05; Group II vs Group III; Group III vs Group IV



All values are expressed as MEAN ± SEM using one way ANOVA followed by Tukey's multiple comparison test, where n=6; a-when compared with Control; b-when compared with ISO (85mg/kg); C-when compared with AAPE (500 mg/kg) + ISO; *--P<0.05; ns-no significance.

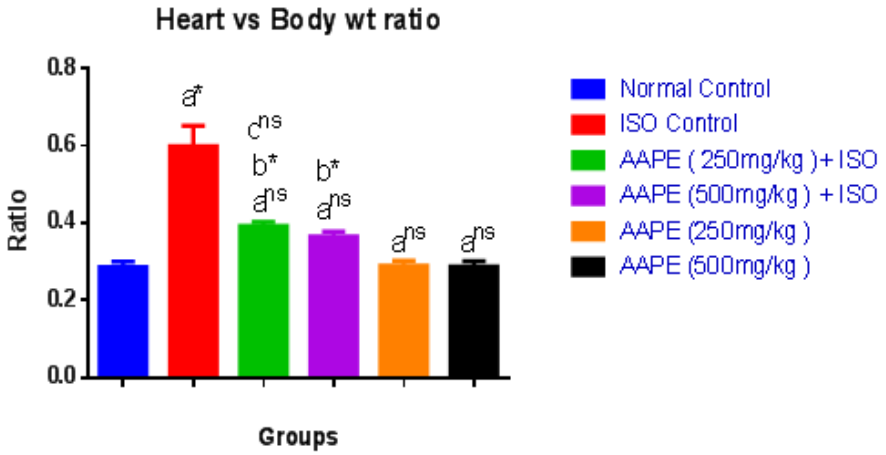
Figure 2

Effect of AAPE on percentage change in body weight



All values are expressed as MEAN ± SEM using one way ANOVA followed by Tukey's multiple comparison test, where n=6; a-when compared with Control; b-when compared with ISO (85mg/kg); C-when compared with AAPE (500 mg/kg) + ISO;*--P<0.05 ; ns-no significance

Figure 3
Effect of AAPE on Heart weight



All values are expressed as MEAN ± SEM using one way ANOVA followed by Tukey's multiple comparison test, where n=6; a-when compared with Control; b-when compared with ISO (85mg/kg); C-when compared with AAPE (500 mg/kg) + ISO; *--P<0.05; ns-no significance.

Figure 4
Effect of AAPE on Heart to Body weight ratio

Myocardial infarction remains a leading cause of morbidity and mortality worldwide. In the traditional Indian medicinal system, a major role has been played by the herbal plants in cardiac diseases. As a remedy for myocardial infarction, many synthetic drugs that exhibit considerable protection are being used, but they have been proved to have variable adverse effects. Consumption of natural antioxidants from food supplements, herbal drugs, and traditional medicine gives us an alternative solution to such problems[6] in this perspective, we have chosen *A. aspera* whole plant to test its protective effect on myocardium which has been reported for antioxidant activity in *vitro.* Experimental induction of myocardial infarction by isoproterenol in animals is a well-established and common model to study the protective role of different cardio protective agents[7]. Isoproterenol acts both on β_1 and $\beta_2$adrenoceptors, activation of which leads to positive inotropic and chronotropic effects. Thus, isoproterenol produces relative ischemia due to myocardial hyperactivity and coronary hypotension.

DISCUSSION

Myocardial infarction remains a leading cause of morbidity and mortality worldwide. In the traditional Indian medicinal system, a major role has been played by the herbal plants in cardiac diseases. Experimental induction of myocardial infarction by isoproterenol in animals is a well-established and common model to study the protective role of different cardio protective agents[8]. Isoproterenol acts both on β_1 and $\beta_2$adrenoceptors, activation of which leads to positive inotropic and chronotropic effects. Thus, isoproterenol produces relative ischemia due to myocardial hyperactivity and coronary hypotension[9]. Pre-treatment with high dose of AAPE followed by ISO showed mild congestion without severe alterations showed by ISO administered rats[10]. Low dose of AAPE pre-treatment showed mild oedema, inflammation, cardiac muscle separation without focal necrosis and haemorrhage[11]. Low and high dose of AAPE were found to be effective in protection against ISO induced myocardial infarction in male wistar rats. Dose dependent effect was

observed between two doses of AAPE. High dose AAPE has been shown activity nearer to normal control. AAPE alone treatment proved to be safe without any toxic effects at high dose.

CONCLUSION

Myocardial infarction (MI) occurs when the blood supply to a part of the heart is interrupted, causing death of heart tissue. It is the most important consequence of coronary artery disease. Many patients may die within the first few hours of the onset, while remainder suffers from effects of impaired cardiac function. In myocardial infarction free radical generation occurred which further leads to sever condition of MI. It can be conclude that, *A. aspera* whole plant extract has potential cardio protective effect on Isoproterenol induced myocardial infarction in male wistar rats. However, further investigations are needed to confirm its exact mechanism of action in cardio protection and to characterize the chemical.

CONFLICT OF INTEREST

Conflict of interest declared none.

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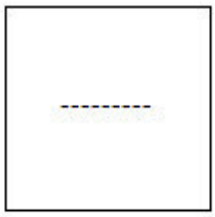

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