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Evaluation of cardioprotective activity of *Chonemorpha fragrans* alston root extract

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Abstract

Objective: The objective of the present study was to investigate the cardioprotective effects of ethanolic extract of root of *Chonemorpha fragrans* Alston against Isoproterenol-induced myocardial infarction (MI) in rats.

Method: The rats were divided into five experimental groups viz., Normal control, ISO-treated (Disease control), Propranolol (10 mg/kg + ISO), *Chonemorpha fragrans* (100 mg/kg +ISO) and *Chonemorpha fragrans* (200 mg/kg + ISO). Myocardial infarction in rats was induced by the administration of isoproterenol at a dose of 85mg/kg i.p., the rats in group IV and group V were pretreated with ethanolic extract of *Chonemorpha fragrans* in the dose of 100mg/kg b.w. and 200mg/kg b.w. through oral route. Cardiac marker enzymes, lipid profile and antioxidant enzymes as biomarker of cardiotoxicity were determined in experimental animals.

Result: Animals treated with root extract of *Chonemorpha fragrans* showed significant decrease in Triglycerides, AST, ALP, antioxidant enzymes viz., superoxide dismutase, LPO and increase in HDL-Cholesterol.

Conclusion: The results of the study demonstrate that *Chonemorpha fragrans* strongly protected the myocardium against isoproterenol-induced infarction and suggest that the cardioprotective effects could be related to antioxidant activities.

Keywords: cardioprotection, isoproterenol, antioxidant, *Chonemorpha fragrans*, myocardial infarction

Introduction

Cardioprotection includes" all mechanism and means that contribute to the preservation of the heart by reducing or even preventing myocardial damage [1]. Cardiovascular disease (CVD) remains the principle cause of death in both developed and developing countries. It may present as a typical heart attack, a sudden death or it may be detected at an advanced stage and be described as a silent infarct. CVD includes high blood pressure, coronary heart disease, congestive heart failure, stroke and accounts for 17,000,000 deaths per annum worldwide. The contributing factor for growing burden of CVDs are increase in prevalence of cardiovascular risk factor specially hypertension, dyslipidemia, diabetes, overweight or obesity, physical inactivity and use of tobacco. It is an area where death gains can be made through the implementation of primary care intervention and basic public health measures targeting diet, lifestyles and environment.

According to World health organization data 16.7 million people die each year owing heart attacks. The figure is one-third of number of deaths worldwide. By 2020-30 more deaths will be caused by heart attacks and India will lead in such number of deaths in worldwide. Myocardial Infarction, commonly known as heart attack is a disease that occurs when blood to a part of heart is interrupted, causing death of heart tissue. It means necrosis of region of myocardium caused by an interruption in the blood supply to the heart usually as a result of occlusion of coronary artery also called as cardiac infarction. Acute myocardial infarction is characterized by varying degree of chest pain, sweating, weakness, vomiting, arrhythmia and cause loss of consciousness and even sudden death. Several factors increasing the risk of heart attack include elevated level of low density lipoprotein, triglycerides, reduces high density lipoproteins level, blood cholesterol and blood pressure. An increased risk of coronary heart disease (CHD) is associated with high levels of serum total cholesterol and low density lipoprotein (LDL) and decreased levels of high density lipoprotein (HDL).

Materials and Methods

Collection and authentication of *Chonemorpha fragrans alston*

The dried root of the *Chonemorpha fragrans alston* were collected from Thengamam, Pathanamthitta, Kerala and authenticated by Dr. Kavitha R, Assistant Prof. of Botany, Government College Nattakom, Kottayam. The roots were cleaned and shade dried and milled into coarse powder by a mechanical grinder.

Preparation of plant extract

The powdered root are extracted using ethanol by soxhlet extractor. In this process the powdered drug are placed into the soxhlet extractor with ethanol as solvent. After extraction the extract is concentrated by evapoaration then it is kept in a refrigerator for further use [12, 13].

Animals

In-house laboratory bred healthy male albino rats of Wistar strain weighing 150-220gm were included for the study. Animals were housed in polypropylene cages.

Animals were maintained under controlled temperature at $25^0C \pm 2^0C$ with 12hr light/dark cycle having access to food and water ad libitum.The experiments were carried out as per the guideline of CPCSEA, New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC).

Experimental Design: *In vivo* methods [15]

Isoproterenol induced acute myocardial infarction method

The rats are divided into 5 groups of 6 animals each as follows:

Group I (Normal control): Rats were administered vehicle once daily for 30 days.

Group II (ISO Control): Rats were administered vehicle orally for 30 days and on day on29th and 30th day injected with ISO (85 mg /kg i.p.)

Group III: Rats were administered standard drug Propranolol(10mg/kg) orally for 30 days and on 29th and 30th day injected with ISO (85 mg /kg i.p.)

Group IV (*Chonemorpha fragrans* 100 mg/kg) - Rats were administered ethanolic extract of *Chonemorpha fragrans* orally for 30 days and on 29th and 30th day injected with ISO (85 mg /kg i.p)

Group V (*Chonemorpha fragrans* 200 mg/kg) - Rats were administered ethanolic extract of *Chonemorpha fragrans* orally for 30 days and on29th and 30th day injected with ISO (85 mg /kg i.p.)

Twelve hours after the second injection of ISO all the rats were sacrified by cervical dislocation. Blood was collected and serum separated after centrifugation. Serum wass used for various biochemical estimations. The heart wass dissected out, wash immediately in ice-chilled saline, blotted and weighed. A known weight (200 mg) of the heart tissue was homogenized in 5 ml of 0.1 M Tris–HCl (pH-7.4) buffer solution. The homogenate wass centrifuged at 3000 rpm for 5 min. The supernatant is used for the estimation of various biochemical parameters.

Serum biochemical estimation

The activities of aspartate aminotransferase (AST),alkaline phosphatase (ALP) are assayed using standard kits. The results were expressed as units/liter (IU/L). The levels of plasma HDL and TG were estimated in the serum using standard commercial kits.

Superoxide dismutase estimation

SOD activity was determined by the method of Marklundand Marklund(1974). To the supernatant, 2.85ml of 0.1M phosphate buffer(pH8.4) and 50 ml of 7.5mM pyrogallol are added and absorbance is measured at 420nm for 3min at 30s intervals. SOD levels are expressed as U/mg protein

Estimation of Lipid Peroxidation

The extent of lipid peroxidation in tissues is assessed by measuring the level of malondialdehyde (MDA) as described by Wilbur. Briefly 1 ml of trichloroacetic acid (TCA) 20% and 2 ml of thiobarbituric acid (TBA) 0.67% are added to 2 ml of homogenate supernatant. The absorbance of the mixture is recorded at 530 nm and the values are expressed as ηM of MDA formed /mg of protein.

Haemodynamic measurements

Animals were anaesthetized by intraperitoneal injection of ketamine 48 h after the first dose of isoproterenol. When the rats no longer responded to external stimuli,ECG was recorded using student physiograph.

Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's Multiple Range Test. Results are expressed as mean ± S.E.M. from six rats in each group.

Results

***In vivo* Cardioprotective activity**

In the present study, ISO-treated rats showed significant (P<0.001) increase in the activities of AST, TG, ALP but significant (P<0.001) decrease in the activity of HDL ISO-treated rats when compared to the control group.

ISO and pretreatment with flavonoid of leaves of *Chonemorpha fragrans* group (100mg/kg, b.w. p.o.) showed significant reduction (P<0.001) in AST, TG, ALP but a significant increase in HDL-Cholesterol (P<0.001) activities when compared to ISO-treated group.

ISO and pretreatment with flavonoid of leaves of *Chonemorpha fragrans* group (200mg/kg, b.w. p.o.) showed significant reduction (P<0.001) in AST, TG, ALP but a significant increase in HDL-Cholesterol (P<0.001) activities when compared to ISO-treated group.

Similarly, ISO-treated rats showed significant (P<0.001) increase in the SOD and LPO activitieswhen compared to the control group (Table 1).

ISO and pretreatment with flavonoid of leaves of *Chonemorpha fragrans* group (100mg/kg, b.w. p.o.) showed significant reduction (P<0.001) in SOD and LPO activities when compared to ISO-treated group.

ISO and pretreatment with flavonoid of leaves of *Chonemorpha fragrans* group (200mg/kg,

b.w. p.o.) showed significant reduction (P<0.001) in SOD and LPO activities when compared to ISO-treated group.

Table 1: Effect of flavonoid of leaves of *Chonemorpha fragrans* on plasma TG, HDL, AST, ALP, CK-MB, SOD, LIPID Peroxidation levels in ISO induced myocardial necrosis in rats.

Treatment group	ALP(IU/L)	AST(IU/L)	SOD (µg/ mg of protein)	TG (mg/dl)	HDL (mg/dl)	CK-MB (IU/L)	LPO(µg/mg of protein)
Normal control	92.3±0.106	16.55±0.13	0.014±0.004	75.7±0.15	50.72±0.15	56.35±0.07	0.625±0.007
Isoproterenol 85mg/kg	331±0.32	52.74±0.21	0.062±0.002	122.7±0.15	25.72±0.15	97.85±0.12	1.825±0.007
Propranolol 10mg/kg	115.7±0.21*	18.72±0.15**	0.022±0.002**	78.95±0.15**	45.38±0.15**	60.07±0.2**	0.825±0.007**
T1 100mg/kg + ISO	226.1±0.21*	42.83±0.16*	0.044±0.001*	95.7±0.16*	38.44±0.10*	74.36±0.07*	1.145±0.007*
T2 200mg/kg + ISO	166.1±0.18*	32.94±0.14*	0.028±0.001*	88.92±0.15**	40.45±0.09**	65.15±0.15**	0.975±0.007**

Data was analysed using one way ANOVA followed by Dunnett's t test

***P<0.001, **P<0.01, *P<0.05. n=6

Discussion

ISO induced myocardial infarction in rats

As *Chonemorpha fragrans* is rich in phytochemical constituents like flavonoids, polyphenols which are said to act as antioxidants. Based on these assumptions root of *Chonemorpha fragrans* was used to study the cardioprotective activity.

Isoproterenol induced myocardial infarction is widely used as a model for evaluating cardioprotective drugs. Radioactive oxygen species (ROS) are formed at an accelerated rate in ISO-treated myocardium. Cardiac myocytes, endothelial cells and infiltrating neutrophils contribute to this ROS production and can lead to cellular dysfunction and necrosis. 'Infarct-like' lesions are produced in the myocardium when injected with ISO. Myocardial necrosis induced by ISO is probably due to a primary action on the sarcolemmal membrane, followed by stimulation of adenylate cyclase, activation of Ca+ and Na+ channels, exaggerated calcium inflow and excess of excitation-contraction coupling mechanism leading to energy consumption cellular death. Free radicals generated by ISO initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of membrane structural and functional integrity. The metabolic damage of myocardium results in increase in the contraction of the marker enzymes like AST, ALP. The SOD were decreased while LPO increased in the myocardial homogenate of ISO administered rats indicating oxidative stress.

Chonemorpha fragrans (200mg/kg) prevented the alterations in marker enzymes of myocardial infarction and oxidative stress. Myofilamental alterations such as myocytosis and myofibrillar degeneration are reported in ISO treated rats. Cardiac sections of the ISO treated animals showed infiltration of inflammatory cells and continuity in the muscle fibre was lacking suggesting an irreversible cell injury. Rats pretreated with *Chonemorpha fragrans* (flavonoid) showed normal myofibrillar structure with striation and revealed a marked protection by the extract against myocardial necrotic damage. Administration of ISO raised LDL cholesterol and decreased HDL cholesterol level in the serum. An increase in concentration of total cholesterol and LDL cholesterol and a decrease in HDL cholesterol are associated with raised risk of myocardial infarction. High level of circulating cholesterol and its accumulation in heart tissue is accompanied with cardiovascular damage. *Chonemorpha fragrans* elevated HDL level and decreased LDL cholesterol level. Hypertriglyceridemia observed in ISO treated rats is clinically reported in ischemic heart disease. Pretreatment with *Chonemorpha fragrans* prevented the elevation of triglycerides cholesterol and LDL in serum signifying that the myocardial membrane is intact and not damaged.

Conclusion

From the experimental studies carried out, flavonoid of root of *Chonemorpha fragrans* at two different doses (100mg/kg and 200 mg/kg) showed dose dependent cardioprotective activity. The higher dose 200mg/kg showed significant protection compared to lower dose 100mg/kg.

The cardioprotective effect may be due to the presence of flavonoid. Flavonoid of root of *Chonemorpha fragrans* has shown free radical scavenging activity and the cardioprotective activity may be partially due to this activity.

Further studies need to be carried out to isolate the potential chemical constituents of flavonoid of root of *Chonemorpha fragrans* and to find out its mechanism of action in the treatment.

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