Journal of Pharmaceutical Research Vol. 9, No. 2, April 2010 : 90-93.

PHYLLANTHUS RETICULATES INHIBITS ISOPRENALINE INDUCED OXIDATIVE STRESS IN RAT'S HEART

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Received on : 01.02.2010 Revised : 20.04.10 Accepted : 30.04.10

ABSTRACT

The objective of the present study was to carryout cardioprotective effect of *Phyllanthus reticulatus* leaves extract (PRLE) against isoprenaline (ISP) induced myocardial dysfunction in rats. Rats were administered low (100 mg/kg) and high (500 mg/kg) doses of PRLE orally for three weeks, while propranolol (PRO) was given for one week. At the end of treatment, isoprenaline (150 mg/kg, s.c) was administered for two consecutive days. LDH and CK-MB were estimated in serum as well as in heart tissue homogenate (HTH) apart from SOD, Catalase and TBARS determinations in HTH. Both high and low doses of PRLE as well as PRO were found to decrease the LDH and CK-MB activities in serum and increase in HTH. SOD and Catalase activities were also found to rise significantly in all treated groups. The estimation of TBARS demonstrates an inclination in free radical formation in ISO group that was ameliorated by prophylactic treatment of both high and low dose of PRLE and PRO. The best results were found in group subjected to high dose of PRLE. The results of the study revealed efficacies of both high and low doses of PRLE and PRO on isoprenaline induced myocardial damage in rats.

Keywords: *Phyllanthus reticulatus; Antioxidants; Cardioprotection; Isoprenaline.*

INTRODUCTION

In recent times, advances in modern scientific research have brought about rapid developments in medicinal treatment. Many potentially fatal diseases can be completely cured by modern treatments but some particular chronic disease cases are increasing due to ineffectiveness of conventional drug treatment as well as potential side effects[1]. Herbal medicine is being widely used in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The herbal medicines are derived from rich traditions of ancient civilizations and scientific heritage. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicine is available[2]. Some herbs used as immune therapies include ashwagandha, astragalus, atractylodes, ginseng, shatvari and shiitake. Herbs used as antimicrobials include garlic, neem, propolis and sanguinaria. Ginger, ginkgo, milk thistle and turmeric are used as antioxidant herbs and Aloe vera for skin problems [2,3]. Several herbs have been used to treat cardiovascular ailments including venous insufficiency. Epidemiologic studies support the potential of dietary antioxidants and flavonoids present in several herbs to improve cardiovascular health intermittent claudication, hyperlipidemia, hypertension and congestive heart failure. Garlic believed to thin the blood, reduce cholesterol, decrease blood pressure, inhibit atherosclerosis and improve circulation. Herbs like *Digitalis purpurea, Digitalis lanata, Apocynum cannabinum* (black Indian hemp), *Plumeria rubra* (frangipani), *Thevatia peruviana* (yellow oleander) are rich sources of cardiac glycosides [4].

Phyllanthus reticulatus Poir (Euphrobiaceae) is an important medicinal plant and popularly known as 'potato-bush'. It is reported to be useful in vitiated condition of *pitta*, strangury, gastropathy, ophthalmodynia, diarrhoea, skin eruption and obesity. Chemical studies reported that it contains phytosterol (sitosterol), descendants of friedelin, olean and lupan type (butelin and glochidonol), polyphenols, flavonoid glycosides, tannic acid, taraxerone, betasitosterol, octacosanol, taraxeryl acetate, and 21-alpha-hydroxyfriedelan-3-one and exhibits the significant antioxidant activity through the scavenging of free radicals which have been reported to participate in pathophysiology of various diseases including myocardial infarction[5-8]. However, till now there is no scientific confirmation of cardioprotective behaviour of *phyllanthus reticulates* against myocardial injury in rats. Hence the present study was designed with an aim to evaluate to cardioprotective potential of *phyllanthus reticulates* leaves extract in isoprenaline induced myocardial infarction using rats

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EXPERIMENTAL

Plant material

The leaves of *Phyllanthus reticulatus* were collected during June 2009 from Tumkur, and were taxonomically identified with the help of Regional Research Institute, Bangalore.

Plant extract

Successive hot extraction of the air-dried ground powder (500 mg) with petroleumether (60°-80°), ethyl acetate and ethanol (95%) were performed for 24 Hours. The extract, in all these cases were filtered off and evaporated to dryness in *vacuo*, to get a concentrated gummy mass [9] and practical yield was 7%.

Experimental animals

Laboratory bred Wistar albino rats (200-250 g) of either sex were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 h light dark cycle. The rats had free access to standard rat chow (Amrut Laboratory Animal feed, Maharashtra, India) and water *ad libitum*. The animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA)

Chemicals

All chemicals used were of analytical grade and purchased from standard companies. Biochemical kits like lactate dehydrogenase (LDH) and creatinine kinase-MB (CK-MB) were procured from Crest Biosystems (Goa, India).

Acute oral toxicity study

The acute oral toxicity study was performed according to the OPPTS guidelines (Office of Prevention, Pesticide and Toxic Substance) following the limit test procedure[10] .The animals were fasted over night prior to the experiment. Test dose of 2 g/kg 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.

Experimental protocol

The animals were divided into five groups consisting of eight animals each. Group I and II were treated with normal saline for 21 days, Group III, IV and V were administered propranolol (PRO, 10mg/kg) [11], *phyllanthus reticulates* leaves extract (PRLE, 100mg/kg) and *phyllanthus reticulates* leaves extract (PRLE, 500 mg/kg) respectively, orally for twenty days.

Experimental procedure

At the end of treatment period, all the groups excluding normal control were administered isoprenaline (ISP, 150 mg/kg s.c.) for two consecutive days [5]. After 48 h of the first dose of ISP, the animal was anaesthetized with ketamine (70 mg/kg i.p) and xylazine (10 mg/kg i.p). Blood was withdrawn from retroorbital puncture and the serum was separated for estimation of biomarkers such as LDH and CK-MB. The heart was excised, weighed and homogenized to prepare heart tissue homogenate (HTH) using sucrose (0.25M). The activities of LDH, CK-MB, superoxide dismutase (SOD) [12], Catalase (CAT) [13] and thiobarbituric acid reactive substances (TBRS) [14] were estimated in HTH.

Statistical analysis

Results are expressed as mean ± SEM. 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

Effect on LDH and CK-MB activities

The serum LDH and CK-MB activity were significantly increased by ISP treatment when compared to normal. The serum CK-MB activities were significantly reduced in PRO, PRLE-100 and PRLE-500 treated groups compared to ISP control. The LDH and CK-MB activities were increased significantly (*P*<0.001) in heart tissue homogenate (HTH) compared to ISP control (Table 1).

***Table 1** : Effects on LDH and CKMB level in serum and heart tissue homogenate (HTH) against Isoprenaline induced myocardial infarction*

Groups	CKMB ACTIVITY		LDH ACTIVITY	
	Serum (unit/lit)	HTH (unit/gm)	Serum (unit/lit)	HTH (unit/gm)
Normal Control	11.9 ±1.4	210±20	324±16	16±2.1
ISP control	92.3±2.2***	34±10***	643±3.1***	3.4±0.7***
PRO	19.0 ±5.2***	96±1.7$^{***\#\#\#}$	396±40***	7.2±0.6$^{***\#\#\#}$
PRLE-100	17.5 ±3.1***	153±5.7$^{***\#\#\#}$	632±10***	7.9±1.5$^{***\#\#\#}$
PRLE-500	29.9±4.0$^{***\#\#\#}$	142±27$^{***\#\#\#}$	506±29$^{***\#\#\#}$	8.7±1.4$^{***\#\#\#}$

*All values are mean ± SEM, n=8; *P<0.05, **P<0.01, ***P< 0.001 when compared to Normal control; #P<0.05, ##P<0.01, ### P< 0.001 when compared to ISP control; LDH, lactate dehydrogenase; CKMB- creatinine phosphokinase-MB; ISP, isoprenaline; PRO-10, propranolol 10 mg/kg; PRLE-100, phyllanthus reticulates leaves extract 100 mg/kg; PRLE-500, phyllanthus reticulates leaves extract 100 mg/kg.*

Effect on SOD, CAT and TBRS activities

There was a very significant (*P*<0.001) decline in SOD and CAT activities and elevation in TBRS activity in HTH after ISP treatment when compared to normal control. Pretreatment with PRO, PRLE-100 and PRLE-500 significantly increased the activity of SOD and CAT in HTH compared to ISP. There was significant reduction in TBRS activity in PRLE-100 and PRLE-500 when compared to ISP group (Table 2).

***Table 2** : Effects on SOD, Catalase and TBARS in Heart tissue homogenate against Isoprenaline induced myocardial infarction*

Groups	HEART TISSUE HOMOGENATE		
	SOD (unit/mg protein)	CAT (unit/mg protein)	TBRS (unit/mg protein)
Normal Control	14±0.1	6.56±0.04	24.1±2.2
ISO control	4.1±0.7***	0.04±0.05***	72.5±5.0***
PRO	6.9±0.2$^{***\,\#\#\#}$	2.73±0.28$^{***\,\#\#\#}$	46.7±2.25$^{***\,\#\#}$
PRLE-100	5.7±0.3$^{***\,\#\#}$	1.12±0.09$^{***\,\#\#\#}$	59.0±2.4$^{***\,\#}$
PRLE-500	6.4±0.5$^{***\,\#\#\#}$	0.52±0.05$^{***\,\#\#\#}$	29.5±1.5$^{***\,\#\#\#}$

All values are mean ± SEM, n=8; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P< 0.001$ when compared to Normal control; $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}$ P< 0.001 when compared to ISP control; SOD, superoxide dismutase; CAT, Catalase; TBRS, thiobarbituric acid reactive substances; ISP, isoprenaline; PRO-10, propranolol 10 mg/kg; PRLE-100, phyllanthus reticulates leaves extract 100 mg/kg; PRLE-500, phyllanthus reticulates leaves extract 100 mg/kg.

DISCUSSION

The research envisaged was carried out to determine the effect of high and low dose of *phyllanthus reticulates* leaves extract (PRLE) against isoprenaline (ISP) induced myocardial damage in rat. The result of the present study demonstrates both 100 mg/kg and 500 mg/kg of PRLE protect the myocardium against ISP damage. An earlier study on the effect of PRLE on cardiovascular system suggests that PRLE-induced cardioprotection is due to its active Phytosterol (sitosterol), descendants of friedelin, olean and lupan type (butelin and glochidonol) and -alpha-hydroxyfriedelan-3-one[15-17] which have potent antioxidant activity[6].

Isoprenaline [1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanolhydrochloride] is a synthetic catecholamine and beta-adrenergic agonist that induces severe stress in the cardiac muscle leading to development of myocardial infarction (MI). The MI is produced due to its action on the cardiac â1-receptors. ISP-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membrane[18]. A number of studies are available that suggest the crucial role of free radicals in pathogenesis of ISP-induced myocardial damage. The pathophysiological changes following ISP administration are comparable to those taking place in human myocardial changes. Hence ISP-induced myocardial infarction model was used in this study.

The diagnostic marker enzymes of myocardial infarction (MI) are CK-MB and LDH. Presence of these biomarkers in heart tissue homogenate (HTH) is indicative of myocardial integrity and their release in serum signifies myocardial injury[5]. The release of cellular enzymes reflects a non-specific alteration in the plasma membrane integrity. In our study, there was substantial fall and rise in activities of marker enzymes in HTH and serum respectively upon ISP administration. Oral pre-treatment with PRLE-100 and PRLE-500 restored the activities of enzymes to near normal in both HTH and serum. Similar protective effect was also seen upon pre-treatment with PRO subjecting to myocardial damage. This indicates that both high and low doses of PRLE as well as PRO possesses protective effect individually. However, high dose of PRLE (PRLE-500) provide better protection to myocardium.

Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well being. Reactive oxygen and nitrogen species play key roles in normal physiological processes, including cellular life-death processes, protection from pathogens, various cellular signaling pathways, and regulation of vascular tone[19]. Superoxide dismutase is a free radical scavenging enzyme found in variety of cells, which converts superoxide anion to hydrogen peroxide[20]. Superoxide anion is formed via a large number of pathways, including normal cellular respiration, inflammatory cells, endothelial cells and in the metabolism of arachidonic acid. However, in necrotic tissues, the production of superoxide anion is increased at a rate that overwhelms the capacity of the endogenous SOD enzyme defence system to remove it. This imbalance results in superoxide anion mediated damage. Catalase is present in the peroxisomes and decomposes hydrogen peroxide to water and oxygen[20]. Pre-treatment of animals with PRLE (500 mg/kg), PRLE (100mg/kg) and PRO produced remarkable elevation in SOD and CAT activities when compared to control indicating cardioprotective effect. The result clearly demonstrates that PRLE reduces oxidative damage. In conclusion pretreatment of PRLE was found to offer dose dependent protection from myocardial injury in ISP-induced myocardial injury model at dose of 100 mg/kg and 500 mg/kg.

ACKNOWLEDGEMENT

Authors would like to extend their thanks to Prof. Dr. Suresh Nagpal Chairman, Prof. Sunil Dhamanigi, Secretary and Prof. Dr. Amit Kumar Das, Principal, Krupanidhi College of Pharmacy for providing essential requirement to complete this research study.

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