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Antioxidant Activity of A*ndrographis paniculata* in Ischemic Myocardium of Rats

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Abstract: Hydroalcoholic extract of *Andrographis paniculata* prevented isoproterenol induced increase in lipid peroxidation and increased the activities of antioxidant enzymes viz. super oxide dismutase, catalase, glutathione peroxidase and the levels of reduced glutathione in hearts. In addition, the extract also prevented the leakage of lactate dehydrogenase from heart and salvages the heart from isoproterenol induced myocardial ischemic injury. The results indicate the antioxidant, antilipid peroxidative and antiischemic activity of *A. paniculata* and justify its use in ischemic heart diseases.

Key words: *Andrographis paniculata* · Antioxidant · Heart · Oxidative stress

INTRODUCTION

Andrograph paniculata (Burm f.) Nees (Acanthaceae) is a perennial herb widely cultivated in tropical and subtropical areas, south-east Asia and India. In India it is known as 'Kalmegh' and in China it is called 'Chuan Xin Lian' and traditionally used as febrifuge, tonic, stomachic and anthelmintic [1]. Andrographis forms the principal ingredient of several pharmaceutical preparations and household medicines too. Modern pharmacological studies have demonstrated its hepatoprotective [2], antithrombotic [3], antiinflammatory [4], immunostimulant [5], antimalarial [6], antihyperglycemic [7] and cardioprotective properties [8, 9].

The role of free radicals generated oxidative stress in isoproterenol-induced myocardial ischemic injury is well established. Several herbal drugs possessing antioxidant activity have been demonstrated protective in the isoproterenol-induced ischemic injury of the myocardium. Andrographis is one of the plants used as antioxidant and acclaimed to provide benefit in cardiovascular diseases in traditional literature. Therefore, this study has been undertaken to evaluate the effect of hydroalcoholic extract of *A. paniculata* (APHE) on oxidative stress markers in isoproterenol-induced myocardial ischemic injury in rats.

MATERIALS AND METHODS

Plant Material: The identity of locally collected plant *A. paniculata* was authenticated by Botanist, Division of Plant Physiology, Indian Agriculture Research Institute, New Delhi, on the basis of routine pharmacognistical studies including organoleptic tests and macroscopic and microscopic observations. The voucher specimen of lyophilized hydroalcoholic extracts of *A. paniculata* has been deposited for further reference.

Extraction: The whole plant was macerated with hydro-alcohol mixture (methanol: water; 50: 50) and evaporated to dryness to obtain an extract. The total andrographolides content determined was not less than 10% w/w.

Phytochemical Screening: The hydroalcoholic extract of *A. paniculata* was screened for various constituents (alkaloids, saponins, tannins, glycosides, fixed oils, phlobatannins and simple sugars) using routine chemical identification methods.

Animals: Laboratory bred, 10 to 12 week old, Wistar male albino rats (150-200 g) were maintained under standard laboratory conditions. The animals were to acclimatize for one week before the experiments and had free access to commercial food pellets and tap water. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee and conformed to the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals in Research.

Isoproterenol-Induced Myocardial Stress: Five groups each containing of 6 rats was allotted to different treatment groups. Group I (control) was treated with normal saline (10 ml/kg) as vehicle only and 29th and

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30th day, at an interval of 24 h isoproterenol (ISP; 85 mg/kg; subcutaneous) was administered [10]. Rats of groups II to IV were administered orally *A. paniculata* extract 100, 200 and 400 mg/kg respectively and the group V was given 100 mg/kg of vitamin E as a reference drug.

Measurement of Markers of Oxidative Stress: All the animals were sacrificed under the overdose of anesthesia and heart were excised for the estimation of markers of oxidative stress and lipid peroxidation. A 10% homogenate of whole heart was prepared in phosphate buffer and an aliquot was used for the assay of malonaldehyde; MDA [11] and reduced glutathione [12]. Rest of the homogenate was centrifuged and the supernatant was used for the estimation of protein [13], superoxide dismutase; SOD [14], catalase; CAT [15], glutathione peroxidase; GSHPx [16] and lactate dehydrogenase; LDH [17].

Statistical Analysis: All values were expressed as mean±S.E.M. A p value less than 0.05 was considered statistically significant.

Drugs and Chemicals: Vitamin E was obtained from Merck India and isoproterenol hydrochloride was obtained from Sigma Chemicals, USA.

RESULTS AND DISCUSSION

APHE on antioxidant enzymes APHE produced a significant increase in the activity of SOD, CAT and GSHPx enzyme [Table 1]. The extract significantly reduced the levels of MDA [Table 2] and increased the levels of GSH [Table 3] in heart homogenates. APHE treatment also produced a significant restoration of LDH activity in heart tissue by preventing leakage of enzyme from cardiac cells [Table 4]. In addition, Vitamin E was also found to significantly improve the endogenous myocardial antioxidant enzymes and inhibit lipid peroxidation and prevented the leakage of LDH from heart.

Isoproterenol, a synthetic catecholamine has been shown to produce myocardial injury in animals similar to humans [11, 18]. The involvement of oxidative stress exhibited due to generation of free radicals and oxidative products of ISP are major determinant of myocardial injury in a large number of studies [18-20].

Free radicals generation leading to oxidative stress enhances the process of peroxidation of membrane lipids, thus altering the permeability of cellular and sub-cellular membrane and further causes excessive influx and reduced efflux of calcium resulting in calcium overload and loss of energy phosphates. This free radical induced lipid peroxidation also causes leakage of LDH from myocardial cells [18-20].

Table 1: Effect of *A. paniculata* hydroalcoholic extract (APHE) on antioxidant enzymes in hearts

		Improvement in enzyme activities		
Treatment	Dose (mg/kg)	SOD (U/mg protein)	CAT (U/mg protein)	GSHPx (U/mg protein)
Saline	-	4.40±1.70	12.90±2.30	0.30±0.08
APHE	100	5.31±1.12	13.63±3.24	0.43±0.05
	200	8.83±1.61*	15.86±2.64*	0.71±0.06*
	400	9.94±1.46*	18.75±3.84	0.98±0.04*
Vitamin E	100	8.06±1.21*	19.15±2.36*	0.45±0.06

Values are expressed as mean±SEM of 8 rats. *P< 0.05 vs. control

Table 2: Effect of *A. paniculata* hydroalcoholic extract (APHE) on lipid peroxidation in hearts

Treatment	Dose (mg/kg)	MDA (nmol/g tissue)	% Inhibition
Saline	-	263.90±8.47	-
APHE	100	188.50±13.42*	28.57
	200	176.24±23.52*	33.12
	400	137.64±11.07*	47.90
Vitamin E	100	165.54±10.18*	37.27

Values are expressed as mean±SEM of 8 rats. *P< 0.05 vs. control

Table 3: Effect of *A. paniculata* hydroalcoholic extract (APHE) on glutathione level in hearts

Treatment	Dose (mg/kg)	GSH (µmol/g tissue)	% Improvement
Saline	-	1.22±0.52	-
APHE	100	1.58±0.41*	22.78
	200	1.62±0.48*	24.69
	400	1.80±0.44*	32.22
Vitamin E	100	1.65±0.38*	26.06

Values are mean±SEM of 8 rats. *P< 0.05 vs. control

Table 4: Effect of *A. paniculata* hydroalcoholic extract (APHE) on lactate dehydrogenase in hearts

Treatment	Dose (mg/kg)	LDH (IU/mg protein)	% Improvement
Saline	-	104.72±16.91	-
APHE	100	160.62±19.25*	34.80
	200	176.33±18.65*	40.61
	400	188.25±19.22*	44.37
Vitamin E	100	178.33±18.25*	41.27

Values are mean±SEM of 8 rats. *P< 0.05 vs. control

SOD catalyzes the dismutation of superoxide radicals [14] and CAT catalyzes the reduction of hydrogen peroxides and protects the tissue from highly reactive hydroxyl radicals [15]. GSH functions as free radical scavenger and in the repair of radical caused biological damage [12]. However, GSHPx catalyzes the reduction of hydrogen peroxides and hydroperoxides to nontoxic products [15]. Following isoproterenol administration myocardial ischemic injury was clearly evident by a significant fall in antioxidant enzymes, SOD, CAT, GSHPx and reduced GSH. In addition to an increase in lipid peroxidation, a concomitant decrease in LDH was suggestive of cardiomyocyte injury. Treatment with APHE increased the activities of endogenous antioxidant enzymes SOD, CAT, GSHPx and levels of reduced GSH. In addition, extract also attenuated increase in lipid peroxidation product, MDA with a concomitant rise in myocyte enzyme, LDH.

The chemical constituents of *A. paniculata* are the diterpene lactones, andrographolides, neoandrographolide and kalmeghin, several flavones and flavonoids [21]. The two main diterpenoids isolated from *A. paniculata* are 14-deoxyandrographolide and 14-deoxy-11,12-didehydroandrographolide [22]. Presence of these constituents could attribute to cardioprotective activity of *A. paniculata* by antioxidant, free radical scavenging and antilipid peroxidation property.

The present study results clearly indicate that hydroalcoholic extract of *Andrographis paniculata* possesses antioxidant activity against oxidative alterations in myocardium and confer significant cardioprotective activity by helping in retaining the cardiac function in a normal manner.

In conclusion, the study provides a scientific basis for its use in the treatment of ischemic heart disease. Considering the safety, efficacy and acceptability further researches are needed to establish its therapeutic and preventive role in myocardial ischemic injury.

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