Int. J. Chem. Sci.: 8(3), 2010, 1545-1552

CARDIO PROTECTIVE ACTIVITY OF *INULA RACEMOSA*

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ABSTRACT

Myocardial ischemia is one of the serious cardiac related disorders. Myocardial ischemia was induced in the rats by isoproterenol administration (20 mg/100 g subcutaneously twice at an interval of 24 hrs.). *Inula racemosa* has been indicated for its use in cardiac disorders. Hence, extract of roots of the plant *Inula racemosa* and alantolactone, which has been isolated from the roots of *Inula racemosa* were subjected for evaluation of their cardio protective activity in myocardial ischemia (100 mg/kg body weight). Lipid peroxides and glutathione contents were estimated. It has been found that the alantolactone effectively reduces the lipid peroxide levels in the ischemic rats and bring the glutathione content to near normal level as compared to the petroleum ether extract.

Key words: Cardio protective, Isoproterenol, Myocardial ischemia, Lipid peroxides, Glutathione, *Inula racemosa*

INTRODUCTION

Medicinal plants and plant derived products are used as medicines in a large group of world population. Plants exert useful pharmacological activity in the therapeutic treatment of various disorders. Since ancient times, plant and their products have been used for the therapeutic treatment of different diseases. The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicines[1]. The literature evidences for the therapeutic actions of herbal drugs are indicated and well documented in Ayurvedic, Chinese, European and African systems of medicine[2]. Medicine, in several developing countries, using local traditions and beliefs, is still the major source of health care. The practice of traditional medicine is widespread in China, India, Japan,

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Pakistan, Sri Lanka and Thailand. Many of drug molecules have been derived and designed from the herbal drugs. Plants serve as a major source of active lead molecules for the treatment of different diseases. Plants are used in traditional medicines and serve as a source of novel therapeutic agents.

The increasing attention of western countries has emerged in the development of many drug molecules[3]. Plant based remedy is the important precursor of many drug molecules. Resistance, toxicity and economic burden of the modern drugs other than the herbal drugs necessitate the discovery of new drug molecules, which will be useful for the society[4].

The genus Inula is widely occurring perennial herb in Europe, East Asia and Himalayas[5]. *Inula racemosa* is a traditionally used herb. It has been indicated in Ayurvedic system of medicine for its therapeutic actions[6] in heart diseases, infectious diseases. Inula grows in the hilly regions in the northwestern Himalayas.

Cardio protective effect of plant extract *I. racemosa* in rabbits was demonstrated[7]. β adrenergic activity of *Inula racemosa* in ischaemic heart disease was also studied[8]. Pushkarbrahmigugglu is an Indian system of Ayurvedic formulations consisting of *Inula racemosa* (Pushkarmoola), *Bacopa monnieri* (Brahmi) and *Commiphora mukul* (Gugglu), which was found to be useful in the management of ischaemic heart disease[9]. Extract of roots of the plant *Inula racemosa* was found to show anti-anginal activity[10]. Studies also show the use of *Inula racemosa* in formulations for cardiac disorders. Lipistat, a formulation comprising of equal proportions of extracts of *Terminalia arjuna, Inula racemosa* Hook and latex of *Commiphora mukul* was found to be effective in the treatment and management of ischemic heart disease[11].

Globally millions of people are affected by cardiac diseases. Sudden arrest of cardiac system has become a major reason of mortality amongst a large group of people. In spite of the availability of the drugs for the treatment of cardiac diseases, they have posed a big challenge for their treatment. *Inula racemosa* has been found to exhibit significant pharmacological activity in cardiac problems. Hence, the roots of the plant have been selected to isolate alantolactone, which is one of the major constituent of the plant. Petroleum ether extract of the roots of *Inula racemosa* and alantolactone were administered in myocardial ischemic rats to evaluate its cardio protective activity.

EXPERIMENTAL

Material and methods

Identification and authentification of material

Identification and authentification of the plant material was done on the basis of organoleptic characters, exomorphology and pharmacognostic study at Department of Pharmacognosy, MAEER's Maharashtra Institute of Pharmacy, Pune and Botanical Survey of India (BSI/2005)

Lipid peroxide and glutathione activity

Chemicals, drugs and equipment

Petroleum ether and alcohol was purchased from Yash Traders, Pune. Auto analyzer BIOTRON BTR 830 was used for enzymatic estimations. Isoproterenol injection and water for injection was obtained from a local medical supplier.

Preparation of dosage forms

Emulsion of petroleum ether extract of *Inula racemosa* was prepared by triturating with tween 80 (2.5%) in glass mortar with gradual addition of water for injection to make desired volume. Similarly, alantolactone dosage form was prepared. All the dosage form of extracts and drug solutions were prepared freshly on the day of experiment and stored in tight amber colored vials. Extracts were administered by oral route. Isopreoterenol was administered by subcutaneous route.

Isolation of alantolactone

100 g of dried root powder of *Inula racemosa* was dissolved in 500 mL of the petroleum ether with intermittent shaking and extracted at room temperature for about 72 hrs. This extract was filtered and the filtrate was then evaporated under reduced pressure to obtain a viscous mass (8 g) by mixing silica gel (2 g). The dried mass was then poured in column slowly and the mass was completely settled down and then the cotton having a diameter of column size was placed on the top surface of mass so that sample does not get disturbed by the addition of mobile phase during elution process. Column was eluted by gravity at a flow rate of 1 mL/min. Solvents like petroleum ether and ethyl acetate were first distilled and used in a ratio 8 : 2 as mobile phase[12].

Alantolactone was isolated and characterized by the spectral studies like IR, NMR and GCMS.

Animals

Adult male albino rats of Wistar strain weighing 120-150 g were used for the study. They were fed with commercial pelleted rat chow and given water *ad libitum* and maintained in a clean polypropylene cages at 25^0C. The rats were divided into five groups, each consisting of 6 animals.

Approval of protocol

All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of National Institute of Virology, Pune, constituted under committee for Purpose of Control and supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Ethical guidelines were strictly followed during all the experiments.

Acute toxicity studies

Petroleum ether extract and alantolactone were administered in doses of 20, 100, 200, 1000, 2000 and 5000 mg/kg or vehicle (5 mL/Kg) intra peritoneally. The rats were observed for mortality or any sign of toxicity for 24 hrs. Study was carried out as per OECD (Guideline 425)

Procedure

Group I served as control with normal diet and saline, Group II rats were administered with isoproterenol (20 mg/100 g subcutaneously twice at an interval of 24 hrs.) at the 8^{th} and 9^{th} day of experiment. Group III rats were administered with 100 mg/kg of petroleum ether extract once daily (10 am) for a period of 7 days orally. Group IV rats were pretreated with petroleum ether extract for 7 days and were given isoproterenol (20 mg/100 g subcutaneously twice at an interval of 24 hrs.) at the end of treatment period.

After the experimental period, the rats were scarified by cervical decapitation. Lipid peroxide values and glutathione levels in control and experimental animals were evaluated. Similar protocol was followed for the alantolactone[13]. (Software used: GraphPad Instat)

RESULTS AND DISCUSSION

Plant derived drugs offer many drug molecules for the treatment of serious diseases like cardiac disorders. In the present investigation, petroleum ether extract and alantolactone

were subjected for evaluation of lipid peroxide values and glutathione content in ischemic rats.

Plant was authentified and acute toxicity studies were carried out (Table 1). Petroleum ether extract of the roots and alantolactone were found to safe upto 1000 mg/kg of body weight. Isolation of the major constituent alantolactone was done by column chromatography techniques. Its structure was confirmed by IR, NMR and GCMS.

Table 1: Acute toxicity study

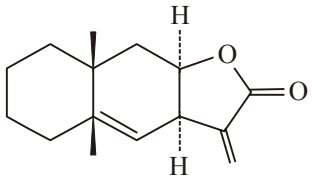
Extract	Dose in mg/kg body weight					
	20	**100**	**300**	**1000**	**2000**	**5000**
Petroleum ether	0	0	0	0	X	X
Alantolactone	0	0	0	0	X	X
0 = Alive, X = Dead						

Acute toxicity studies shows that the extract as well as alantolactone are safe up to dose of 1000 mg/kg body weight.

^{1}H NMR ($CDCl_3$) δ : 5.3 (1H, m) , 3.95 (1H m), 5.50, 6.20 (1H each, s), 1.45 (3H, s) and 1.8 (3H, s).

Mass spectrum : $C_{17}H_{19}NO_3$ Mol. Wt. 285, MS m/z (Rel. Int.): 232 (M^+), 217, 204, 187, 175, 158, 152, 131 and 106.

IR Spectrum : 3082 cm^{-1}: $C=CH_2$ stretching, 2853.3: $-CH_2-$ stretching, 1766.7: characteristic lactone peak, 1257.5 : CH_3 peak, 894.9: $-C=CH_2$ stretching and 815.8: trisubstituted alkene peak.



Alantolactone

Spectral studies show that the isolated compound is alantolactone. Rats administered with isoproterenol alone showed a significant increase in the levels of TBA reactive substances in serum and heart depletion of glutathione content in blood and heart. The alteration was minimum in rats pretreated with petroleum ether extract and alantolactone.

A significant increase in the levels of lipid peroxides in serum and heart on isoproterenol administration indicates enhanced lipid peroxidation by free radicals. Due to increased lipid peroxidation, glutathione levels were lowered significantly in blood and heart of Group II rats. Decrease in glutathione level may be due to its increased utilization in protecting SH groups containing proteins from the action of free radicals. Glutathione participates directly in the destruction of hydrogen peroxide and also promotes the formation of reduced form of ascorbate, which has high antioxidant activity. The isolated alantolactone treated rats showed significant levels of glutathione and lipid peroxides as indicated in Table 2. The petroleum ether extract and alantolactone were found to reduce the increased lipid peroxidation and even the levels of the glutathione were maintained at near normal values. Thus, the plant is useful in protecting the oxidative changes induced by the necrosis. In all the treated groups, the petroleum ether extract and alantolactone were found to reduce lipid peroxidation and maintains the levels of glutathione in serum as well as heart of control and experimental rats[14].

Table 2: Lipid peroxides and glutathione in serum and heart of control and experimental animals

Group	Lipid peroxides		Glutathione	
	Serum	Heart	Serum	Heart
Normal	2.8 ± 0.34	4.8 ± 0.41	1.40 ± 0.04	5.20 ± 0.23
Isoproterenol	3.5 ± 0.52*	5.5 ± 0.31*	1.11 ± 0.02*	4.15 ± 0.20*
Petroleum ether extract	2.4 ± 0.56	4.6 ± 0.24	1.56 ± 0.05	5.91 ± 0.22
Petroleum ether extract + Isoproterenol	3.2 ± 0.16#	5.1 ± 0.36#	1.70 ± 0.21#	5.86 ± 0.16
Alantolactone + Isoproterenol	3.1 ± 0.19#	4.9 ± 0.17#	1.56 ± 0.03#	5.47 ± 0.14#

Alantolactone has been found to show significant activity as compared to the petroleum ether extract. Plant exerts its effects by maintaining the normal levels of

glutathione in isoproterenol treated rats. Thus, it can be said that the extract and alantolactone are useful in reducing the lipid peroxidation and helps in maintaining the normal levels of glutathione. They exert protective actions and help to protect myocardium from the harmful effects of ROS and found to be helpful in protecting heart from the necrotic changes due to myocardial ischemia.

Values are expressed as mean ± SEM for 6 animals in each group. The level of lipid peroxides in serum is represented as nanomoles of TBA reactants/litre and in heart, it is expressed as nanomoles of TBA reactants/g protein. Glutathione content in blood is expressed as micromoles/litre and in heart as nanomoles of GSH/g tissue.*$p < 0.001$ significantly different from control group; #$p < 0.001$ significantly different from isoproterenol group. One way ANOVA with Dunnett's test.

SUMMARY AND CONCLUSION

Alantolactone and petroleum ether extract of the plant have been found to exert cardio protective activity in ischemic rats and prevents the myocardium of rat heart for the oxidative damages. Alantolactone particularly found to play significant role due to its chemical nature and these studies will be useful for evaluation of detail role of plant in preventing myocardial ischemia.

ACKNOWLEDGEMENT

Authors are thankful to the Management of Maharashtra Academy of Engineering and Educational Research, Pune and Director, National Institute of Virology, Pune for providing necessary facility for the present research work.

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Accepted : 02.02.2010