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Anti-Oxidant Activity of *Piper longum* Linn.

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Abstract: Myocardial ischemia is a serious cardiac problem which should be diagnosed and treated effectively to prevent its complications. Many plant based medicine have been utilized for the effective treatment in cardiac problems. Myocardial ischemia was induced in rats by administration of isoproterenol. Petroleum ether extract of root and piperine from roots of *Piper longum* Linn. were subjected for evaluation of their anti-oxidant activity by DPPH scavenging method. Lipid peroxide and Glutathione values in myocardial ischemic rats have also been estimated by inducing myocardial ischemia by using isoproterenol. It has been found that at 50 mg mL⁻¹ concentration pet ether extract and piperine exerts 74.12 and 72.13% of inhibition. Pet ether extract and piperine pretreatment decreases lipid peroxide level and maintain glutathione content to near normal in treated rats. The present study shows that the extract of the root of the plant and piperine exert anti-oxidant activity and are protective in the myocardial ischemic condition.

Key words: Myocardial ischemia, cardio protective, piperine, antioxidant

INTRODUCTION

Pharmacological activities of plants and plant derived drugs necessitates for the search of new and useful drugs globally. Fifty percent of the estimated 250,000 plant species found on earth come from tropical forests. The number of higher plant species (angiosperms and gymnosperms) on planet is estimated at 250,000, with a lower level at 215,000 and an upper level as high as 500,000 of these, only about 6% have been screened for biologic activity and a reported 15% have been evaluated phytochemically. India is the largest producer of medicinal herbs. India has 15,000-18,000 species of flowering plants, 2500 algae, 23000 fungi and 1600 types of microorganisms. These values has shown a vast and tremendous biodiversity potential in India, which can be utilized in drug industry (Chaudhari, 1996).

Indian long pepper is indigenous to North-Eastern and southern parts of India and Srilanka. In the Western part of India aqueous extract of the roots of *Piper longum* Linn. are used as food material (Kirtikar and Basu, 1984). Different species of piper like *Piper nigrum*, *Piper longum* Linn. and *Piper retrofractum* Vahl. have been widely used as spice all over the world.

Diverse pharmacological activities of fruits of *Piper longum* Linn. like stomachic, aphrodisiac, thermogenic, carminative, expectorant, digestive, emollient, anti-girdiasis, anti-amoebic and antiseptic activity has been mentioned in literature (Warrier *et al.*, 1995). Dry

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fruits of *Piper longum* Linn. have been used in the prevention of recurrence of asthma as well as *Piper longum* Linn. has also found to be indicated in cardiac related disorders (Dahanukar and Karandikar, 1984). *Piper longum* Linn. is effective in gastric ulceration when given along with the extracts of *Zingiber officinalis* and *Ferula* species (Agarwal *et al.*, 2000).

Anti-inflammatory activity of *Piper longum* Linn. was observed in Brahmi Rasayana (Jain *et al.*, 1994). The benzene extract of fruits of *Piper longum* Linn. along with the methanolic extract of the *Embelia ribes* berries showed 80% of inhibition of pregnancy when administered to female rats (Kholkute *et al.*, 1979). *Piper longum* Linn. has been found to possess immunomodulatory activity (Devasagayam and Sainis, 2002). Extract of the plant also shows hepatoprotective action against carbon tetrachloride induced liver damage (Rege *et al.*, 1994). Extract of the plant has also found to increase beta glucuronidase activity of brunners glands in duodenal ulcers of rats (Thiraviraj and Pillai, 1989). *Piper longum* Linn. (Piperaceae) is also widely used as a folk medicine to cure diseases such as leprosy and tuberculosis. Alkaloidal amide of *Piper longum* Linn. shows increase in the bioavailability of various therapeutically diverse drugs (Shankaracharya *et al.*, 1997).

Phytochemical investigation of the plant showed presence of Piperlongumine, Piperlonguminine, Piperine, Sesamin, 3, 4, 5-trimethoxycinnamate, beta-Sitosterol, Aristolactum, Piperyline, Piplartine and Hexacosanoic acid isobutyl amide (Dutta *et al.*, 1977).

Literature survey reveals that the *Piper longum* Linn. roots have been indicated in many diseases and found to be useful in heart diseases. Hence, in order to evaluate cardio protective role of plant in myocardial ischemia its anti-oxidant activity and lipid peroxide and glutathione levels have been estimated in the present study. Petroleum ether has been used as solvent for isolation of piperine. Petroleum ether extract as well as piperine were selected for the study to evaluate their potential as anti-oxidant agent and their usefulness in myocardial ischemia.

MATERIALS 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 411038.

Column Chromatography

Chemicals

Petroleum ether, ethanol, ethyl acetate (Emerk) purchased from Yash Traders, Pune. Column grade silica (#100-200) was obtained from S.D. Fine Chem. Limited, Mumbai and TLC grade silica (S. D. Fine Chem) was obtained from Yash Traders, Pune.

Roots of the plant *Piper longum* Linn. have been selected for the isolation of piperine from it. The dried roots of the plant *Piper longum* Linn. were powdered and pass through appropriate mesh size so as to get uniform coarse powder.

Slurry of column grade silica (#100-200) was prepared and column was packed with slurry. One hundred grams of dried root powder of the plant *Piper longum* Linn. was dissolved in 500 mL of the petroleum ether with intermittent shaking and extracted at room temperature for about 48 h. This extract was then filtered and the filtrate was then evaporated under reduced pressure in rotary evaporator 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. Then the cotton having a diameter of column size was placed on the top

surface of mass so that it does not get disturb by the addition of mobile phase during elution process. Column was eluted by gravity at a flow rate of 1 mL min⁻¹ by using mobile phase of the solvents petroleum ether and ethyl acetate (8:2).

CHARACTERIZATION OF THE ISOLATED CONSTITUENTS

General Experimental Procedure

Infra Red (IR) spectra were recorded on Shimadzu 8400 FT Infrared spectrometer. ¹H Nuclear Magnetic Resonance (NMR) spectrum was recorded on Varian-Mercury (300 MHz) instrument with chemical shift data reported in ppm. Gas Chromatograph-Mass Spectrums (GCMS) were recorded on GC-MS Shimadzu (QP 5050) instrument.

ANTIOXIDANT ACTIVITY

Plant Material

Emulsions of petroleum ether extract as well as that of piperine of *Piper longum* Linn. were prepared individually by triturating with Tween 80 (2.5%) in glass mortar with gradual addition of water for injection to make volume.

Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH), was purchased from Sigma (Sigma-Aldrich, Germany). All other chemicals used were of analytical grade and were obtained from Merck and Sigma (Sigma-Aldrich, Germany).

The DPPH free radical scavenging activity of extracts was measured according to DPPH radical scavenging method (Shimada *et al.*, 1992). Briefly, 0.1 mM solution of DPPH/in ethanol was prepared and 1 mL of this solution was added to 3 mL of extract solution (1, 10, 50 mg mL⁻¹ concentration) in water. The mixture was shaken vigorously and incubated at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer (Shimadzu UV-1700 PharmaSpec) against a blank. The free radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\text{DPPH scavenging effects (\%)} = 100 - ((A_0 - A_1/A_0) \times 100)$$

where, A₀ was the absorbance of the control reaction and A₁ was the absorbance in the presence of the sample of pet ether extract and isolated piperine.

Lipid Peroxide and Glutathione Activity

Chemicals, Drugs and Equipment

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

Preparation of Dosage Forms

Dosage forms of extract and piperine were prepared as per the following method. Emulsion of petroleum ether extract of *Piper longum* Linn. was prepared by triturating with Tween 80 (2.5%) in glass mortar with gradual addition of water for injection to make volume. Similarly, piperine 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. Isopreterenol was administered by subcutaneous route.

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°C. 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.

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 h). Group III rats were administered with 100 mg kg⁻¹ of pet ether extract for a period of 7 days orally. Group IV rats were pretreated with pet ether extract for 7 days and were given isoproterenol (20 mg/100 g subcutaneously twice at an interval of 24 h) at the end of treatment period. Group V rats were pretreated with piperine for 7 days and were given isoproterenol (20 mg/100 g subcutaneously twice at an interval of 24 h) at the end of treatment period.

After the experimental period, the rats were scarified by cervical decapitation. The heart was dissected out, immediately washed in ice-cold saline and a homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4). Homogenate was centrifuged and supernatant was used for the assay of glutathione and lipid peroxides in serum and heart homogenate (Karthick and Stanely, 2006).

RESULTS

Column Chromatographic isolation of pet ether extract of the roots of the plant *Piper longum* Linn. resulted in isolation of piperine. Fraction PL33 was the pale yellow needle shaped crystalline constituent which was identified as piperine. IR, NMR, ¹³C NMR and GC-MS spectral analysis indicates that it has structural similarities with that of piperine (Fig. 1).

Structural Data of Piperine

¹H NMR (CDCl₃) δ : 3.90 (9H), δ : 4.1 (2H), δ : 1.6 (6H), δ : 1.4 (10H), δ : 5.9 (2H), δ : 6.8 (3H).

¹³C NMR

C-1 166, C-2 120, C-3 142, C-4 125, C-5 138

C-1' 130, C-2' 105, C-3' 148, C-4' 147.2, C-5' 108, C-6' 122, C-7' 100.2.

Mass Spectrum

C₁₇H₁₉NO₃ Mol. Wt. 285,

MS m/z (Rel. Int.): 238 (M⁺), 237, 146, 119, 92,

IR Spectrum

3012.6 cm^{-1} : Aromatic C-H stretching
 1587.3, 1629.7 : Symmetric and asymmetric stretching of C = C (diene)
 1492: Aromatic stretching of C = C (phenyl ring)
 1635: Stretching of -CO-NO<
 2893, 2937: CH_2 symmetric and asymmetric stretching, aliphatic C-H stretching
 1444: CH_2 bending
 1197, 1253: Asymmetric stretching of = C-O-C
 1029.9: Symmetric stretching of = C-O-C
 929.6: C-O stretching
 1130.2: In plane bending for phenyl CH
 842: CH bending for trans -CH = CH-

The scavenging effects of pet ether extract and piperine on the DPPH radical are shown in Table 1. Pet ether extract and piperine had significant scavenging effects on the DPPH radical. At the concentration of 50 mg mL^{-1} , pet ether extract and piperine showed DPPH-scavenging activities of 74.12 and 72.13% while that of Vitamin C was found to be 96.12%. Pet ether extract and piperine pretreatment decreases lipid peroxide level and maintain glutathione content to near normal as indicated in Table 2 (Statistical software used: GraphPad Instat).

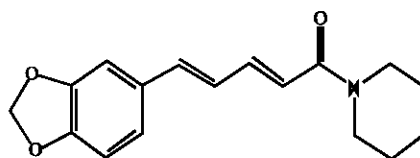


Fig. 1: Piperine

Table 1: DPPH free radical scavenging activity

Extracts	Concentration (mg mL^{-1})	DPPH radical-scavenging activity (%)
Pet ether	1	5.26 $\pm$ 2.48
	10	24.12 $\pm$ 2.86
	50	74.12 $\pm$ 3.10
Piperine	1	6.28 $\pm$ 1.09
	10	26.12 $\pm$ 2.42
	50	72.13 $\pm$ 2.09
Vitamin C	0.1	96.12 $\pm$ 3.12

Values are means of triplicate determinations $\pm$ SD

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

Groups	Lipid peroxides		Glutathione	
	Serum	Heart	Serum	Heart
Normal	2.9 $\pm$ 0.37	3.6 $\pm$ 0.51	1.61 $\pm$ 0.02	4.71 $\pm$ 0.18
Isoproterenol	4.5 $\pm$ 0.50*	5.4 $\pm$ 0.21	1.46 $\pm$ 0.01*	3.82 $\pm$ 0.21
Pet ether extract	3.3 $\pm$ 0.56	3.8 $\pm$ 0.21	1.74 $\pm$ 0.04	4.84 $\pm$ 0.21
Pet ether extract+Isoproterenol	3.4 $\pm$ 0.13#	4.1 $\pm$ 0.18	1.83 $\pm$ 0.24#	4.89 $\pm$ 0.14
piperine+Isoproterenol	3.1 $\pm$ 0.18#	3.9 $\pm$ 0.17	1.68 $\pm$ 0.02#	4.82 $\pm$ 0.17

Values are expressed as mean $\pm$ SEM for 6 animals in each group. * p <0.001 significantly different from control group; # p <0.001 significantly different from isoproterenol group

DISCUSSION

The DPPH radical is considered to be a model of lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid autoxidation. The petroleum ether extract and piperine were found to be useful as anti-oxidant agent. Piperine alone showed 72.13% inhibition which means piperine can also be used as anti-oxidant alone for the treatment of cardiac related problems to minimize stress levels in myocardial ischemia.

Sushmakumari (1989) has reported that 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. Decreases 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. Administration of petroleum ether extract of roots of *Piper longum* Linn. as well as piperine helps to minimize the lipid peroxide values in rats treated with isoproterenol and maintains glutathione levels to near normal in Group IV and V rats. The pretreatment of animals with extract and piperine helps to minimize the changes induced by the administration of isoproterenol suggesting protective action of plant in ischemic condition of heart in treated rats as reported by Sasikumar and Devi (2000). The present findings indicate the anti-oxidant activity of the plant and the protective nature of the plant against the isoproterenol induced myocardial ischemia in rats.

CONCLUSION

Piperine is one of the important constituent of *Piper longum* Linn. Piperine was isolated from the roots of the plant and by extracting with petroleum ether as solvent. Studies shows that the pet ether extract and piperine shows significant DPPH scavenging activity. The extract and piperine were also found to exert protective effect in the myocardial necrotic rats. They have protected myocardium from the harmful effects of lipid peroxidation and even maintained the glutathione levels to normal. Hence it can be concluded that the pet ether extract as well as piperine are useful in exerting protective activity in case of myocardial ischemia in treated animals.

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