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Evaluation of antipyretic activity of *Azima tetracantha* Lam. in rats

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ABSTRACT

Azima tetracantha Lam. belongs to Salvodoraceae and known as Kundali in Ayurvedic medicine. The chloroform and alcoholic extracts of *Azima tetracantha* Lam leaves at doses of 250 and 500mg/kg, p. o. were tested against brewers's yeast induced pyrexia in rats to assess antipyretic activity. The pyrexia in rats was reduced significantly compared to that of respective control. The effects of these extracts are compared to standard drug Paracetamol. The results obtained from the present investigation indicate that plant extracts possess antipyretic activity. The phytochemical screening of the extracts revealed the presence of secondary metabolites like alkaloids, flavonoids, tannins, triterpenoids, steroids, saponins etc.,

KEYWORDS

Azima tetracantha;
Lam;
Antipyretic;
Brewers's yeast;
Pyrexia.

INTRODUCTION

Fever (also known as pyrexia), is a frequent medical sign that describes an increase in internal body temperature to levels above normal. Fever is most accurately characterized as a temporary elevation in the thermoregulatory set-point, causing typical body temperature to rise, and effector mechanisms are enacted as a result. A feverish individual has a general feeling of cold despite an increased body temperature, and increases in heart rate, muscle tone and shivering, all of which are caused by the body's attempts to counteract the newly perceived hypothermia and reach the new thermoregulatory set-point. A fever is considered one of the body's immune mechanisms to attempt a neutralization of a perceived threat inside the body, be it bacterial or viral.

Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator's (cytokines, such as interleukin 1β, α, β, and TNF-α), which increase the synthesis of prostaglandin E2 (PGE2) near peptic hypothalamus area and there by triggering the hypothalamus to elevate the body temperature[1]. As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high, it dilate the blood vessels and increase sweating to reduce the temperature; but when the body temperature become very low hypothalamus protect the internal temperature by vasoconstriction.

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High fever often increases faster disease progression by increasing tissue catabolism, dehydration and existing complaints, as found in HIV[2]. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE-2 biosynthesis[3]. Moreover, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, golmeruli, cortex of brain and heart muscles, whereas natural COX-2 inhibitors have lower selectivity with fewer side effects[3]. A natural antipyretic agent with reduced or no toxicity is therefore, essential. Though several synthetic chemical entities are screened for the purpose, folklore plants still serve as major group to be tapped.

Azima tetracantha Lam. belongs to Salvodoraceae and known as Kundali in Ayurvedic medicine. The plant is reported to possess antidiarrhael[4], antimicrobial activity in fruits[5], diuretic and rinderpest in leaf[6,7]. Leaf powder has shown anti-inflammatory activity[8], the juice of the leaves is used in tooth and earache[9], diuretic[10], antiulcer[11] and analgesic[12] activity have been reported. There are few reports on phytochemical composition of the plant like presence of dimeric piperdine alkaloids azimine, azacarpaine, carpaine[13], triterpenoids[14], isorhamnetin 3-rutinoside[15], and novel fatty acids[16]. Presence of neoascorbinogen and glucosinolates[17], has also been reported. The present study evaluates the antipyretic effects of chloroform and ethanolic extracts of dried leaves of *Azima tetracantha* in rats.

TABLE 1 : Results of qualitative chemical tests for phytoconstituents in *Azima tetracantha* Lam.

Tests	Pet. ether	Chloroform	Alcoholic	Aqueous
Alkaloids	-	-	+	-
Carbohydrates	-	-	+	+
Flavonoids	-	-	+	-
Triterpenoids	+	+	+	-
Protiens	-	-	-	-
Resins	-	-	-	-
Saponins	-	-	+	+
Steroids	+	+	+	-
Tannins	+	-	+	-
Starch	-	-	-	-

Plant material

The leaves of *Azima tetracantha* was collected from the regions of Chitradurga District, Karnataka, India and authenticated by Prof.B.B.Nandyal, Department of Botany and Biotechnology, SJVP Science College, Harihar, Karnataka.

Preparation of extracts

The leaves were collected and dried under shade. Dried leaves were powdered and extracted successively with petroleum ether 60-80, chloroform and alcohol in a Soxhlet apparatus, finally with chloroform water by maceration. All the extracts were distilled and dried. Chloroform and alcohol extracts were used for the present study.

Phytochemical investigation

Phytochemical tests were carried out to find out the presence of phytoconstituents viz., alkaloids, flavonoids, tannins, triterpenoids, saponins etc.[18] and the results are shown in TABLE 1.

TABLE 2 : Effect of leaf extracts of *Azima tetracantha* Lam. chloroform (CE) and alcoholic (AE) extracts on yeast induced pyrexia in rats

	Rectal temperature (°C) after yeast injection					
Treatment	Before yeast	0 h	1 h	2 h	3 h	4 h
Normal control	37.4±0.09	37.33±0.29	37.89±0.16	37.80±0.07	37.93±0.04	37.78±0.12
Yeast [10ml/kg p.o]	37.2±0.05	39.31±0.34	38.77±0.27	38.98±0.50	38.93±0.30	39.75±0.55
Yeast+CE [250mg/kg p.o]	37.4±0.04	39.36±0.26	38.21±0.35	37.66±0.35	38.15±0.45	38.67±0.53
Yeast +CE [500mg/kg p.o]	37.3±0.04	39.49±0.27	36.58±0.11*	36.73±0.33*	37.47±0.21*	37.60±0.19*
Yeast +AE [250 mg/kg p.o]	37.4±0.06	39.27±0.18	38.12±0.22	38.23±0.43	38.70±0.26	38.74±0.25
Yeast +AE [500mg/kg p.o]	37.4±0.07	39.20±0.13	36.69±0.06*	36.07±0.04*	36.17±0.07*	37.59±0.24*
Yeast +PCM [100mg/kg p.o]	37.3±0.05	39.42±0.24	35.05±0.40*	35.62±0.28*	35.41±0.40	35.50±0.34*

Values of rectal temperature are expressed in °C. Data were analysed by ANOVA followed by Turkeys multiple comparison test ; Each value is the mean ± Standard Error of 6 rats weighing 125-150gm. * P < 0.05; Statistically significant from vehicle control

EXPERIMENTAL

Materials: Paracetamol was procured from Ranbaxy, India. Solvents and chemicals used for experimental work were of AR grade.

Animals used: Male albino rats (Wistar strain) of weighing between 125-150g were used in the experiments. The selected animals were maintained under standard laboratory conditions (temperature 27± 2°C relative humidity: 55±10% 12 hour light and dark cycles). The animals were fed with standard diet and water *ad libitum.* The animals were adapted to laboratory condition for 7 days prior to the experiments. The institutional Animal Ethical Committee (Sanction No SETCP/IAEC/2008-09/0544) approved all the pharmacological protocols, according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute toxicity studies

Rats weighing 125-150g were used for the study. These were divided into 6 groups of 6 animals each. The test extracts was administered orally as a suspension in Tween 80 (3ml of 1% solution) to the different groups in increasing dose levels of 10, 40, 100, 400, 1000 and 5000mg/kg body weight. The animals were then observed continuously for 3 h for general behavioral, neurological, autonomic profiles and then every 30 minutes for next 3 h and finally for death after 24 h[19]. From the studies it was observed that the animals were safe for maximum dose of 5000mg/kg-body weight. But there were few changes in the behavioral response like alertness, touch response and restlessness. Therefore 1/20th and 1/10th of maximum tolerated dose i.e.250 and 500mg /kg body weight was chosen for the study.

Statistical analysis

The data were expressed as Mean ± S.E.M for six rats in each group. Statistical comparisons were performed by one-way ANOVA followed by Turkey's post-test using GraphPad Prism version 4.0, USA. $P<0.05$ considered as statistically significant.

Preparation of yeast suspension: 15% W/v yeast suspended in normal saline solution.

Instrument: Elico Digital Thermometer.

Yeast induced pyrexia in rat

Rats were divided into six groups of six animals each. The normal body temperature of each rat was measured rectally at predetermined intervals and recorded. Fever was induced as per the method described by Smith and Hambourger, 1935[20]. The rats were acclimatized to remain quite in a restrained cage. A thermister probe was inserted 3-4cms deep into the rectum and fastened to the tail by adhesive tape. The temperature was measured on a thermometer. After measuring the basal rectal temperature, animals were given a subcutaneous injection of 10ml/kg BW of 15% W/v yeast suspended in saline solution. Rats were then returned to the housing cages. After 18th hour of yeast injection, the animals were again restrained in individual cages for the recording of their rectal temperatures as described previously, animals that showed increase in rectal temperature were selected. Nineteen hour after injection the vehicle, test extracts and paracetamol are given orally. Rats were restrained for recording the rectal temperature at 1hour intervals up to 23 hour after yeast injection.

RESULTS

The preliminary phytochemical screening of the chloroform and alcoholic fraction showed the presence of steroids, tannins, triterpenoids, alkaloids and flavonoids. The results of the effect of two extracts of leaf *Azima tetracantha* on yeast induced pyrexia in rats are depicted in TABLE 2. At doses of 250 and 500mg/kg body weight, even though both chloroform and alcoholic extract reduced the elevated rectal temperature in a dose dependent manner, significant antipyretic effect was noticed at 500mg/kg body weight in both the extracts. Further, the comparison among the extracts interms of antipyretic effect was revealed to be more effective in alcoholic extract than chloroform as evidenced from its lower mean values in rectal temperature.

DISCUSSION

Fever may be due to infection or one of the sequel

of tissue damage, inflammation, graft rejection, or other disease states. Antipyretic are agents, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature[21]. Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature[22].

It was of interest to note that the extracts of *Azima tetracantha* leaf parts produced a rather modest decrease in the body temperature in hyperthermic rats. The cause of this decrease may be central and/or peripheral in origin. Clinically available antipyretic drugs, such as paracetamol and the non-steroidal antiinflammatory drugs, are able to lower the body temperature only in feverish patients. Neuroleptic drugs and other central depressants can also reduce the normal body temperature[23]. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through inhibition of prostaglandin synthetase within the hypothalamus[24,25]. Therefore, it appears that the antipyretic action of the extract may also be related to the inhibition of prostaglandin synthesis.

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