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Research Article

ANTIPYRETIC ACTIVITY OF ETHANOL AND AQUEOUS EXTRACT OF ROOT OF *Asparagus racemosus* IN YEAST INDUCED PYREXIA

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ABSTRACT

Objective: To evaluate and compare the *in vivo* antipyretic activity of the aqueous and the ethanol extract of root of *Asparagus racemosus*.
Methods: The presence of phytochemicals like flavonoids and saponins were identified by TLC and the *invivo* antipyretic activity was determined by brewer's yeast induced pyrexia method.
Results: The results showed that the ethanol extract of root of *Asparagus racemosus* possessed significant antipyretic effect compared to the aqueous extract which may be attributed to the presence of flavonoids and saponins in the extracts whose presence were observed in TLC.
Conclusion: This study provides evidences for the antipyretic activity of *Asparagus racemosus* which could partly contribute to its ethno medical use.

Keywords: *Asparagus racemosus, Antipyretic effect, flavonoids ,saponins, TLC*

INTRODUCTION

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders. Herbal products are often perceived as safe because they are "natural". In recent years herbal medicine is a major component in all traditional medicine systems, and a common element in Siddha, Ayurvedic, Homeopathic, Naturopathic, Traditional Chinese medicine, and Native American medicine. Considerable efforts have been directed towards the development of natural products from various plant sources [1].

Today a substantial number of drugs are developed from plants which are active against a number of diseases. The majority of these involve the isolation of the active ingredient (chemical compound) found in a particular medicinal plant and its subsequent modification. In the developed countries 25 percent of the medical drugs are based on plants and their derivatives and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries [2].

Pyrexia or Fever is defined as an elevation of body temperature. It is a response due to tissue damage, inflammation, malignancy or graft rejection. Cytokines, interleukin, interferon and Tumor Necrosis Factor α (TNF- α) are formed in large amount under this condition, which increase PGE2 which in turn triggers hypothalamus to elevate body temperature [3].Fever is associated with symptoms of sickness behavior which consist of lethargy, depression, anorexia, sleepiness, & inability to concentrate. This increase in set point triggers increased muscle tone & shivering. However antipyretic medication can be effective at lowering the temperature which may include the affected persons comfort [4].

According to Ayurveda, pyrexia originates from a combination of indigestion, seasonal variations and significant alterations in daily routine [5].Due to poor hygiene practices and malnutrition, children in developing countries frequently suffer from various forms of infections which present as fevers. These fevers are often accompanied by aches and pains which all lead to morbidity and mortality [6].

Antipyretics are drugs which can reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus which regulate the set point of body temperature. Drugs like paracetamol do not influence body temperature when elevated by factors such as exercise or increase in ambient temperature [7].

Asparagus racemosus Willd. commonly known as 'Shatavari', is a much-branched, spinous under shrub found growing wild in tropical and sub-tropical parts of India. The leaves are like pine-needles, small and uniform. The inflorescence has tiny white flowers, in small spikes and the roots are finger-like and clustered [8].

The plant has been used in India for thousands of years for its therapeutic and tonic properties. It is an all-round tonic and rejuvenates which can be given to a person with any type of constitution, males or females, youngsters or elders [9]. It is mainly known for its phytoestrogenic properties. It has also been shown to have many other properties like antistress, anti-diarrhea, ant dyspepsia, adaptogenic action, antiulcerogenic action, and antioxidant & cardio protection [10].

The root of *Asparagus racemosus* is the preferred part of the plant used in Folklore traditional medicine for treating fever. Extract of 250g fresh tuber of Asparagus racemosus boiled with 100ml water is consumed lukewarm[11].The preliminary phytochemical study of the Asparagus racemosus revealed the presence of alkaloids, carbohydrates, phytosterols, tannins, flavonoids [8].The antipyretic activity of many plants has been attributed to their saponins, terpenoids, flavonoids and steroids contents[12].

As the roots of *Asparagus racemosus* is a Folklore traditional medicament used in ailments that caused fever, it will be a cost effective alternative approach to study the root extract of this plant for the development of an effective antipyretic agent. So the present study has been carried out to evaluate and compare the in vivo antipyretic activity of the aqueous and the ethanol extract by yeast induced pyrexia method.

MATERIALS AND METHODS

Collection of the Plant Material

The roots of *Asparagus racemosus* were collected from Thrissur, Kerala. They were identified and authenticated by Dr.V.V.Radhakrishnan, Department of Medicinal Plants, Kerala Agricultural University – Kerala, India.

Preparation of the Plant Extracts

Fresh roots of *Asparagus racemosus* were washed, shade dried, powdered, passed through a #60 mesh sieve and were extracted

with alcohol (95% v/v) in a soxhlet apparatus by continuous heat extraction for about 48 h. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50ºC. For experimental purpose the alcohol extract was prepared in distilled water containing 2% v/v Tween 80 (as a suspending agent).

The aqueous extract was prepared by maceration in chloroform water (72hrs).The macerate was filtered through Whatmann No.1 filter paper and concentrated in a rotary flash evaporator at a temperature not exceeding 50ºC[8].

Experimental Animals

Wistar albino rats of either sex weighing about 150-200g were employed for this study. They were procured from KMCH Institute of Pharmacy, Coimbatore and maintained on the suitable nutritional and environmental condition throughout the experiment. They were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment. This study was approved by institutional ethics committee for animal studies (KMCRET/PhD/7/2009).The animals had access to laboratory chow and water ad libitum [13].

Experimental Design

The LD50 of *Asparagus racemosus* is 1g/kg. No toxic effects or mortality were observed with doses ranging from 50mg/kg to 1g/kg for four weeks. Acute and sub acute (15 – 30 days administration) toxicity studies did not detect any changes in vital organ function tests [14]. Hence antipyretic activity of the aqueous and ethanol root extracts of *Asparagus racemosus* was studied as per the following.

Experimental design

Body weights of the animals were recorded and they were randomly divided into 5 groups of 6 animals each as follows:

Group I : animals served as control

Group II : animals were treated with yeast via subcutaneous injection (10ml/kg).

Group III : animals were administered with yeast (10 ml/kg) and the standard drug paracetamol (150mg/kg b.w.), orally

Group IV : animals were administered with yeast (10ml/kg,) and with aqueous root extract of Asparagus racemosus (500mg/kg b.w.), orally.

Group V : animals were administered with yeast (10ml/kg,) and with ethanol root extract of Asparagus racemosus (500mg/kg b.w.), orally.

Yeast induced pyrxia

Pyrexia was induced by subcutaneous injection of 20 % w/v of brewer's yeast (10ml/kg) in distilled water. Basal rectal temperature was measured before the injection of yeast, by inserting digital clinical thermometer to a depth of 2 cm into the rectum. The rise in rectal temperature was recorded 19 h after yeast injection. Paracetamol 150mg/kg body weight was used as the standard antipyretic drug. Rectal temperature of animals was noted at regular intervals following the respective treatments.The temperature was measured at 1st, 2nd, and 3rd hour after drug administration [15].

Statistical Significance

The statistical analysis was done by ANOVA followed by Dunnet's test for multiple comparisons. $P < 0.01$ was considered significant in the experiment [11].

Thin Layer Chromatographic (TLC) Technique

The selection of a solvent for application of the sample can be a critical factor in achieving reproducible chromatography with distortion free zones. In general, the application solvent should be a good for the sample and should be as volatile as possible and more non-polar [16]. Flavonoids and Saponins from the roots of Asparagus racemosus have been analysed by TLC.

TLC fingerprint – to detect the presence of flavonoids and saponins

The aqueous and ethanol root extracts were characterized by thin layer chromatography (Silica gel coated, Merck) using mobile phase - chloroform: acetic acid: methanol: water in the ratio of 6.4: 3.2: 1.2: 0.8 for detection of flavonoids and chloroform: ethyl acetate: methanol in the ratio of 6: 4: 0.3 for detection of saponins. The Chromatograms were evaluated under UV light at 254nm[17].Silica gel was chosen as stationary phase, since it is an efficient adsorbent for the TLC separation of most of the plant extracts and plant drug extracts[18].

RESULTS

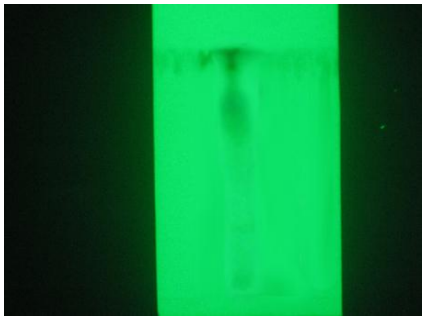
Natural herbs have been used for medicinal purposes in many countries and continue to be a medicament for various ailments even with the revolution in antibiotics and other synthetic medicine in modern scientific world. Non steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs due to their consistent effectiveness in the treatment of pain, fever, inflammation and rheumatic disorders. Since these drugs have toxic effect to the various organs of the body, search for safe herbal remedies with potent antipyretic activity received momentum recently. *Asparagus racemosus* Willd (Asparagaceae), which possess the active constituents like steroids, saponins, isoflavones, asparagamine, racemosol, polysaccharides, mucilage, vitamins A, B1, B2, C, E, Mg, P, Ca, Fe, and folic acid is an important medicinal plant indigenous to South Asian countries, whose medicinal properties are reported in traditional systems of medicine such as Ayurveda, Siddha and Unani. The present study has been carried out to evaluate and compare the in vivo antipyretic activity of the aqueous and the ethanol extract by yeast induced pyrexia method. Table 1 depicts the effect of the ethanol and aqueous root extract of *Asparagus racemosus* on yeast induced pyrexia. Plate 1 show the number of spots for flavonoids and saponins in both the extracts. The results indicated the highest antipyretic activity of ethanol extract when compared to the aqueous extract.

Table1: Effect of aqueous and ethanol extracts of *Asparagus racemosus* on body temperature in yeast induced pyrexia

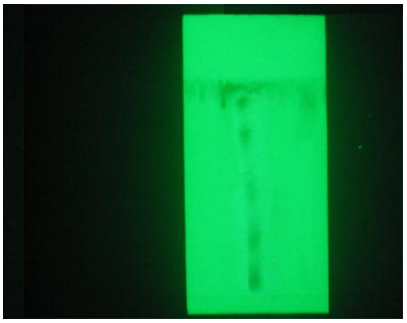
Groups	Rectal Temperature in ^{0}C after 18hrs of Yeast Injection			
	0 hr	1 hr	2 hr	3 hr
Group I	37.30±0.7	37.50±0.7	37.70±0.6	37.60±0.4
Group II	40.53±0.11	40.18±0.17	39.21±0.14	39.13±0.16
Group III	40.43±0.19	38.65±0.17	38.46±0.19*	37.66±0.18*
Group IV	40.61±00.14	39.63±0.19	39.13±0.24	38.68±0.12
Group V	40.58±0.11	39.23±0.12	38.01±0.14*	37.71±0.16*

Values are expressed as Mean ±SEM. n = 6 in each group, "*" indicate P < 0.01 compared to control

Plate 1Results of TLC performed on aqueous and ethanol root extract of *Asparagus racemosus* TLC plates for flavonoids

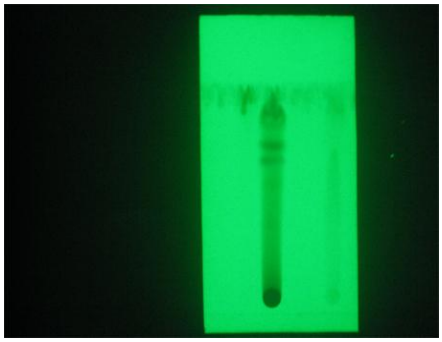


Aqueous extract

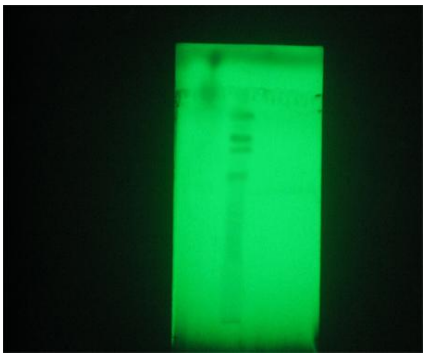


Ethanol extract

TLC plates for saponins



Aqueous extract



Ethanol extract

DISCUSSION

Herbal medicines derived from the plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available [19]. For the field of modern medical science, the herbal drugs are to be subjected for several processes such as identifications, isolation, purification, characterization, structural elucidation and therapeutic evaluation. The strategy of bioassay guided fractionation and isolation using chromatographic separation techniques revolutionized what could be achieved in medicinal plant research [20].

Fever is a complex physiologic response triggered by infections or aseptic stimuli. Elevation in body temperature occurs when the concentration of prostaglandin E2 (PGE2) increases within parts of the brain. Such an elevation contributes to a considerable alteration in the firing rate of neurons that control the thermoregulation process in the hypothalamus. It is now evident that most of the antipyretic drugs exert their action by inhibiting the enzymatic activity of cyclooxygenase and consequently reducing the levels of PGE2 within the hypothalamic region [3]. A natural antipyretic agent with reduced or no toxicity is therefore, essential [21].

Since antipyretic activity is commonly mentioned as a characteristic of drugs or compounds, which have an inhibitory activity on prostaglandins biosynthesis, the yeast induced hyperpyrexia in rat model was employed to investigate the antipyretic activity of the extract [6]. Yeast induced pyrexia is called pathogenic fever which is due to the production of prostaglandins (PGE2) which set the thermoregulatory center at a higher temperature [22].

The ethanol extract showed more pronounced effect in lowering the hyperthermia than the aqueous extract, but found to have similar effect as the standard drug Paracetamol at 3rd hour of administration. The extracts are likely to reduce pyrexia by reducing brain concentration of prostaglandin E2 especially in the hypothalamus through its action on COX-3 or by enhancement of the production of the body's own antipyretic substances like vasopressin and arginine [23].

Antipyretics have been shown to suppress fever by inhibiting prostaglandin synthetase, resulting in the blockade of the synthesis of prostaglandin in the brain or suppressing the rise of interleukin-1α production subsequent to interferon production Flavanoids like baicalin have been shown to exert antipyretic effect by suppressing TNF-α [24] and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing the fever and pain[25]. This study also correlates with the study of Zakaria *etal* [26] that the compounds like flavonoids and saponins are suggested to act synergistically to exert the observed pharmacological activity.The results of present study indicate that the ethanol root extracts of *Asparagus racemosus* possesses significant antipyretic effect compared to the effect of aqueous extract on yeast induced hyperthermia in rats. This may be attributed to the presence of flavonoids and saponins in the extracts which may be involved in inhibition of prostaglandin synthesis. Also, there are several mediators or multiprocessors underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis.

In conclusion, this study provides evidences for the antipyretic activity of *Asparagus racemosus* which could partly contribute to its ethno medical use. However, further investigation is required to isolate the active constituents responsible for these activities and to elucidate the exact mechanisms of action.

CONCLUSION

In conclusion, this study provides evidences for the antipyretic activity of *Asparagus racemosus* which could partly contribute to its ethno medical use. However, further investigation is required to isolate the active constituents responsible for these activities and to elucidate the exact mechanisms of action.

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