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Antipyretic activity of Root of *Marsdenia tenacissima* in Rats

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Abstract

Objective : To evaluate ethanol & aqueous extracts of root of *Marsdenia tenacissima* Wight & Arn. at different doses (100 mg/kg, and 200 mg/kg p.o.) for antipyretic activity in yeast induced pyrexia in rats. Materials and Methods : The ethanol extract of root of *Marsdenia tenacissima* was obtained by continuous soxhlet extraction and aqueous extract by cold maceration process**.** Both the extracts were subjected to phytochemical investigation to identify the phytoconstituents and further assessed for antipyretic activity by yeast induced pyrexia in experimental rats and compared with standard drug, Paracetamol. Results : Upon evaluation of antipyretic activity on experimental animals, the ethanol and aqueous extracts at the dose (100 mg/kg, and 200 mg/kg p.o.) showed significant ($P < 0.01$) antipyretic activity as observed from evaluation parameter. The ethanol and aqueous extracts revealed the presence of steroid glycosides, alkaloids, saponins on qualitative chemical tests. Conclusion : From the results, it can be concluded that the active ethanol and aqueous extracts (100 mg/kg and 200 mg/kg p.o.) of root of *Marsdenia tenacissima* is worthwhile to develop the bioactive principles for antipyretic activity.

Key words : Antipyretic, *Marsdenia tenacissima* , Root, Ethanol extract, Aqueous extract.

1. Introduction

Pyrexia or fever is caused as a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator's (cytokines like interleukin 1β, α, β and TNF-α), which increase the synthesis of prostaglandin E_2 (PGE_2) near preoptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature [1]. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE_2 biosynthesis. Moreover, these synthetic agents

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irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cortex of brain and heart muscles, whereas natural COX-2 inhibitors have lower selectivity with fewer side effects [2]. A natural antipyretic agent with reduced or no toxicity is therefore, essential.

Marsdenia tenacissima Wight & Arn (Asclepidaceae), commonly known as *Marua bel* (in Hindi), a large twining shrub found in the Himalayas from Kumaon to Assam up to an altitude of 1500 m. and extending southwards to the Deccan Peninsula [3]. As per Ayurvedic texts, Murva should possess characters such as a creeper; exudes milky juice or latex; possesses strong fibers & useful in abdominal pain due to worms, vata conditions, in fever, heart diseases, skin diseases etc... According to Dr.B.Vaidya *Marsdenia tenacissima* possesses all these characters and hence it is the true Murva [4]. Shushruta used the plant as an appetizer, in vomiting, indigestion, colic pain, fever etc.. The roots and seeds are rich in pregnane glycosides of 3-oxy sugars. The ethanol extract of root showed mild CNS depressant effect on mice and anthelmintic activity against earth worms [5] The root is traditionally used in the treatment of fever by local practitioners (personal communication). A literature survey revealed that no scientific investigation regarding antipyretic activity of the root of plant. In the present investigation, an attempt was made to test the root of the plant for antipyretic activity in rats and to establish phytopharmacological profile to justify the traditional and folklore claim.

2. Material and Methods

The root of *Marsdenia tenacissima* was collected from surrounding area of Gadag city in the month of November 2008 and was authenticated by Dr.R.C.Mathad, Professor, Department of Dravya Guna, K.L.E.S's Shri.B.M.Kankanawadi Ayurvedic Medical College, Shahpur, Belgaum. A voucher specimen (CG – 10) has been preserved in the department of the Pharmacognosy and Phytochemistry of the institute.

2.1 Preparation of extracts

The shade dried root of the plant approximately (500 g) was powdered to coarse particle size no. (#) 40 and subjected to continuous hot extraction with 90 % ethanol in a soxhlet extractor for 48 h. The total ethanol extract was filtered and concentrated to dryness at 40°C under reduced pressure in a rota evaporator. The yield of ethanol extract was found to be 100 gm (20% w/w). The extract was kept in a dessicator till the experiment.

About 500 g of shade dried coarsely powdered root was subjected to cold maceration with chloroform water I.P. in a two litre conical flask, for about 14 days at room temperature. The flask was securely plugged with absorbent cotton and was shaken periodically till complete maceration. After maceration, the marc was pressed in a muslin cloth and the filtrate was concentrated to a dry residue at room temperature. The yield of aqueous extract was found to be 60 gm (12% w/w). Some part of the total extract was reserved for phytochemical investigation and rest of the extract was used for biological activity.

2.2 Preliminary Phytochemical studies

The preliminary Phytochemical investigations of ethanol extract of the root revealed presence of sterols, triterpenes, alkaloids, saponins, lactones etc.. while aqueous extract revealed the presence of steroid glycosides, saponins, carbohydrates [6-8]

2.3 Acute toxicity studies (LD_{50})

The acute toxicity of both the extracts was evaluated on Albinomice, 5 groups of 6 animals

each. The ethical clearance was obtained by the Institutional Animal Ethics committee (Registration number 221/CPCSEA) before the experiment. Animals were kept in polypropylene cages & fasted for 24 h with water *ad libitum*, maintained at an ambient temperature of 25 ± 2 ^{0}C. Animals were then administered by oral route with ethanol & aqueous extracts (50 - 2000 mg / kg), suspended in 2% w / v gum acacia solution (vehicle). Control group received only vehicle. Animals were observed for clinical signs and mortality continuously for the initial 4 h and intermittently for next 6 h and then again 24 h and 48 h after dosing. The parameters observed and recorded were sedation, hyperactivity, grooming, loss of righting reflex , respiratory rate and convulsion. 1/10 of lethal dose was taken as the screening dose [9].

2.4 Evaluation of antipyretic activity

Antipyretic activity was assessed using brewer's yeast (*Saccharomyces cerevisiae*) induced pyrexia as per the method described [10]. The ethical clearance was obtained by the Institutional Animal Ethics committee (Registration number 221/CPCSEA) before the experiment. The adult Wistar rats, weighing between 100 - 150 g, were procured from the animal house of J. N. Medical College, Belgaum. Rats were kept in polypropylene cages and fed on standard laboratory diet (Lipton India Ltd) with water *ad libitum*, maintained at an ambient temperature of 25 ± 2 ^{0}C. The animals were exposed to 12 h of darkness and light each. Rats of either sex were divided into 6 groups of 6 animals each. The normal body temperature of each rat was measured rectally at 1 h interval on a thermometer. After measuring the basal rectal temperature, animals were given a subcutaneous injection of 2 ml/kg of 15% w/v brewer's yeast suspended in 0.5% w/v methyl cellulose solution. Rats were then returned to respective cages. After 19 h of yeast administration, Group-1 served as control received vehicle (5 ml/kg, p.o.), Group-2 received Paracetamol dissolved in distilled water (150 mg/kg, p.o.) ; Groups 3 and 4 received ethanol extract (100 mg/kg and 200 mg/kg, p.o, respectively) suspended in 2% w / v gum acacia solution; Groups 5 and 6 received aqueous extract (100 mg/kg and 200 mg/kg, p.o, respectively) suspended in distilled water. The rats were allowed to remain quiet in the cage for sometime. A flexible thermister probe coated with lubricant was inserted 3-4 cm deep into the rectum and fastened to the tail by adhesive tape. The temperature was measured on a thermometer in ^{0} C at 1 h interval up to 23 h after yeast injection.

2.5 Statistical analysis

All the results are expressed as Mean ± SE. The statistical significance was analyzed by performing one-way ANOVA followed by Dunnet's "t" test [11]. Level of significance was set at $P < 0.01$.

3. Results and Discussion

The effect of different doses of ethanol and aqueous extracts of roots of *Marsdenia tenacissima* on yeast induced hyperpyrexia are expressed in Table 1. The ethanol extract at the dose (100 mg/kg and 200 mg/kg, p.o,) exhibited significant antipyretic activity ($P < 0.01$) at 20 , 21, 22 & 23 h of yeast administration as compared to control. The significant reduction in elevated body temperature was observed in the group treated with ethanol extracts, which was comparable with reference drug, at all hours of testing. However, aqueous extract at the dose (100 mg/kg and 200 mg/kg, p.o,) exhibited significant antipyretic activity ($P < 0.01$) at 21, 22 & 23 h of yeast administration .

The acute toxicity study of both extracts of root

of *Marsdenia tenacissima* revealed no mortality when administered orally up to a max dose of 2 g / kg body weight. At this dose there was no gross behavioral changes.

The phytochemical investigation of ethanol extract indicated presence of sterols, triterpenes, alkaloids, saponins while aqueous extract revealed the presence of steroid glycosides, saponin glycosides, carbohydrates. This study reports for the first time antipyretic activity of the root of *Marsdenia tenacissima*, supporting its traditional use.

The hypothalamus regulates the set point at which body temperature is maintained. In fever, this set point is elevated [12]. Fever may be a result of infection or one of the sequence of tissue damage, inflammation, graft rejection, malignancy or other disease states. A common feature of these conditions is the enhanced formation of cytokines such as IL-1B,IL-6, interferon alpha & beta and TNF. The cytokines increase the synthesis of PGE_2 circum ventricular organs in and near to the preoptic hypothalamic area , alpha PGE_2 , via increased cyclic AMP, triggers the hypothalamus to the elevate body temperature by promoting increase in heat generation and decrease in heat loss. NSAID's suppress this response by inhibiting the synthesis of PGE_2 [13]

With the observed significant antipyretic activity in experimental animals by aqueous & ethanol extracts of root of *Marsdenia tenacissima*, the mechanism of action could, probably, be due to suppression of the enhanced formation of different cytokines and there by inhibiting the synthesis of PGE_2 involved in pyrexia.

The antipyretic activity of many medicinal plants has been suggested to be due to steroid glycosides [14,15]. The presence of steroid glycosides in root of *Marsdenia tenacissima* could be attributed to prominent antipyretic

Table 1: Effect of the ethanol and aqueous extracts of root of *Marsdenia tenacissima* on Yeast induced Pyrexia in Rats.

Treatment	Initial Temp in (° C)	Temp. at 19 h after yeast adm.	Temp.(°C) at different hours after treatment Mean ± SEM			
	0 h	19 h	20 h	21 h	22 h	23 h
Control (Vehicle) 5 ml / kg , p.o,	37.64 ± 0.1	39.2 ± 0.31	39.3 ± 0.4	39.4 ± 0.15	39.5 ± 0.18	39.6 ± 0.2
Paracetamol 150 mg/kg, p.o,	37.2 ± 0.2	39.6 ± 0.21	37.3 ± 0.15*	37.2 ± 0.2*	37.1 ± 0.12*	37.2 ± 0.1*
Ethanol Extract 100 mg / kg, p.o,	37.32 ± 0.3	39.7 ± 0.22	37.3 ± 0.3*	37.0 ± 0.23*	37.0 ± 0.12*	37.3 ± 0.1*
Ethanol Extract 200 mg / kg, p.o,	37.32 ± 0.3	39.7 ± 0.22	37.1 ± 0.3*	36.8 ± 0.23*	36.7 ± 0.12*	37.0 ± 0.1*
Aqueous Extract 100 mg / kg, p.o,	37.71 ± 0.4	39.35 ± 0.2	38.8± 0.1	38.3 ± 0.17*	38.3 ± 0.19*	38.12 ± 0.18*
Aqueous Extract 200 mg / kg, p.o,	37.71 ± 0.4	39.35 ± 0.2	38.7± 0.1	38.2 ± 0.17*	38.0 ± 0.19*	37.8 ± 0.18*

* Indicates significant antipyretic activity at $P < 0.01$ vs. control.

Each value expressed as mean ± SE ; n = 6

activity. However, detailed Phytochemical investigation of root of the plant is worthwhile to pin point the activity and to elucidate the structure of bioactive principles responsible for antipyretic activity.

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