

# Anti-inflammatory, Analgesic and Antipyretic activities of root of *Wattakaka volubilis*

Amit Kumar Shukla<sup>1\*</sup>, S. P. Mishra<sup>2</sup>, Ravi Varma<sup>3</sup>

<sup>1</sup>Department of Pharmacognosy, <sup>2</sup> Department of Pharmaceutics  
Institute of Pharmacy, Sitapur 261001, Uttar Pradesh, India,

<sup>3</sup>Department of Pharmacology, Acharya Narendra Dev College of Pharmacy, Babhnan  
Gonda Uttar Pradesh, India.

\*Corres.author: amitmedical66@yahoo.com  
Tel: (+91) 5862-250035; (+91) 9453276266

**Abstract:** *Wattakaka volubilis* (Family: Asclepiadaceae) has been reported to possess medicinal effects. In the present study, the dried root ethanol extract of *W. volubilis* designated as 'the extract' was evaluated for pharmacological activity in rats and mice. The anti-inflammatory and antipyretic activities were evaluated in rodents. The analgesic activity was evaluated in mice models. In the acute toxicity study, it was found that the extract was non-toxic up to 1 g/kg, p. o. The extract (100, 200 and 300 mg/kg, p. o.) was found to possess, anti-inflammatory, analgesic and antipyretic activities in a dose-dependent manner and the effect was comparable with that produced by the standard drug, ibuprofen. The extract significantly inhibited the arachidonic acid-induced paw oedema in rats, indicating that the extract inhibited both the cyclo-oxygenase and lipo-oxygenase pathways of arachidonic acid metabolism.

**Keywords:** *Wattakaka volubilis*, arachidonic acid-induced paw oedema, anti-inflammatory activity, root extract.

## Introduction

*Wattakaka volubilis* (family: Asclepiadaceae) is used in the treatment of various ailments since ancient times. <sup>1</sup> The literature survey revealed that among the various saponins obtained from the stem and flower of *W. volubilis*, two compounds are active against Ehrlich's ascites carcinoma. <sup>2, 3</sup> Since *W. volubilis* has been reported to possess medicinal effects, the present study attempts to evaluate the anti-inflammatory, analgesic and anti-pyretic activities exhibited by the constituents present in the dried root ethanol extract.. Pharmacological activity studies reported earlier on these two plants are:

*W. volubilis*: Antifungal, antibacterial <sup>4</sup>, hypoglycemic <sup>5</sup>, anti-inflammatory, analgesic and anti-lipid peroxidative <sup>6</sup>, protection against selenite induced cataract in rat lens <sup>7</sup>, *in vitro* anti-leishmanial and anti-tumour <sup>8</sup>, hepatotoxicity <sup>9</sup>, prevention of proteolysis in rat lens <sup>10</sup>, apoptosis inducing potential. <sup>11</sup>

## Material and methods

### Plant materials

Roots of *W. volubilis*, (Amit Kumar Shukla June 2010), roots were collected from Tirunelveli District, Tamil Nadu (India). The plants were authenticated by Taxonomist. The respective voucher

specimens have been deposited in the herbarium and museum of Institute of Pharmacy, Sitapur, India.

### Animals

Albino Wistar rats weighing 170-200 g and Balb-C mice weighing 20-25 g of either sex were used for the study. Animals were acclimatized to the experimental conditions in the animal house of Acharya Narendra Dev College of Pharmacy, for two weeks prior to the study. Animal house was well maintained under standard hygienic conditions, at a temperature ( $22 \pm 2$  °C), room humidity ( $60 \% \pm 10 \%$ ) with 12 h day and night cycle, with food and water *ad libitum*. Pharmacological studies were carried out at Acharya Narendra Dev College of Pharmacy.

### Acute Toxicity Study

Mice were divided into 10 groups and the extract was injected p. o. in doses from 100 mg to 1 g/kg, p. o. The LD<sub>50</sub> (24 hour) was calculated according to OECD.<sup>12</sup>

### Anti-inflammatory Studies Carrageenan- induced paw oedema

The rats were divided into five groups (n = 6) and the first group served as negative control and received normal saline (0.1 ml/100g, p. o.). The second group was administered ibuprofen (100 mg/kg, p. o.) as the standard drug and rats of groups III, IV and V were administered 100, 200 and 300 mg/kg, p. o. of the extract, respectively. Oedema was produced by the method described by Winter *et al.*<sup>13</sup> Carrageenan (0.1 ml/100 g from a 10 mg/ml solution) was injected into the planter aponeurosis of right hind paw of the rats of all the groups 30 minutes later. The left hind paw served as the control. The paw volume was measured after 4 hours using a plethysmometer.

### Arachidonic acid-induced paw oedema

A total of 36 male albino rats were divided into six groups of 6 animals in each group. Rats of group-I (negative control) received 0.1 ml/10 g of normal saline p. o., those of group-II received 100 mg/kg of phenidone (dual blocker) i. p., rats of group III received indomethacin (10 mg/kg, i. p.) and rats of groups IV, V and VI received 100, 200 and 300 mg/kg, p. o. of the extract, respectively. Paw oedema was induced by a single injection of 0.1 ml 0.5% arachidonic acid in 0.2 (M) carbonate buffer (pH 8.4) into right hand paw (subplantar) of rats 30 minutes

after drug treatment. Hind paw volume was measured 1 hour after arachidonic acid injection.<sup>14</sup>

### Analgesic Activity

#### Tail- flick method

The antinociceptive effect of the test substances was determined by the hot tail flick method described by Sewell and Spencer.<sup>15</sup> One to two cm of the tail of mice was immersed in warm water bath (Swan scientific instruments) kept constant at  $55 \pm 1$  °C. The reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as a mean of the next two readings. Balb-C mice were randomly divided into five groups (six in each). Mice of group I received normal saline (0.1 ml/10 g, p. o.) group II received ibuprofen (100 mg/kg, p. o.) and groups III, IV and V received 100, 200 and 300 mg/kg, p. o. of the extract, respectively. Thirty minutes later, the tail was immersed in the water bath and the tail flick response was recorded. The same experiments were repeated after 60 minutes and 120 minutes again.

### Anti-pyretic activity studies

Albino rats of Wistar strain of either sex weighing between 170-200 g were used. Pyrexia was induced by subcutaneous injection of 20 % w/v of brewer's yeast (10 ml/kg) in distilled water, under the skin, in between the shoulder blades. 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. The febrile rats were divided into different experimental groups each consisting of 6 animals. Test doses of the extracts were fixed based on acute toxicity results. Paracetamol 150 mg/kg body weight was used as the standard antipyretic drug. Rectal temperature of animals was noted at regular intervals following the respective treatments.<sup>16</sup>

### Statistics

Data are presented as arithmetic mean  $\pm$  S.E.M of at least six experiments. Statistical analysis was performed by one-way analysis of variance (ANVOA) followed by Dunnett's test or by Student's paired t-test. 'P' value of  $<0.05$  was considered as statistically significant.

**Table1. Effect of *Wattakaka volubilis* root extract on inflammation**

Drug	Dose	Carrageenan-induced paw oedema (vol. In ml.)
N. Saline	0.1 ml/100g	0.60± 0.06
Ibuprofen	100 mg/kg	0.14±0.02
Extract	100 mg/kg	0.23±0.05**
Extract	200 mg/kg	0.21±0.05**
Extract	300 mg/kg	0.20±0.05**

The values are expressed as Mean ± SEM; *n* = 6 animals in each group.  
Data were analysed by Tukey-Kramer multiple comparison test.

**Table2. Effect of *Wattakaka Volubilis* root extract on arachidonic acid- induced paw oedema of rat**

Drug	Dose	Difference in paw volume in ml (mean±S.E.M)	% inhibition as compared to control
Saline control	0.1/100g	3.1±0.20	--
Indomethacine	10 mg/kg	2.1±0.34*	32
Phenidone	100 mg/kg	0.69±0.10*	77
Extract	100 mg/kg	1.75±0.10*	43
Extract	200 mg/kg	1.08±0.20*	65
Extract	300 mg/kg	0.9±0.10*	71

The values are expressed as Mean ± SEM; *n* = 6 animals in each group.  
Data were analysed by Tukey-Kramer multiple comparison test.

**Table 3. Effect of *Wattakaka volubilis* root extract on thermal nociception in mice (Tail flick method)**

Drug	Dose	Min. after treatment		
		30	60	120
		Response in s (mean± S. E. M)		
Saline control	0.1 ml/100g	2.2± 0.20	2.2± 0.51	2.0±0.21
Ibuprofen	100 mg/kg	2.2± 0.20	3.0± 0.15*	3.0± 0.40*
Extract	100 mg/kg	2.5± 0.30*	2.7± 0.31*	3.0± 0.52*
Extract	200 mg/kg	2.6± 0.31*	3.0± 0.22*	3.0± 0.21*
Extract	300 mg/kg	3.1± 0.50*	3.3± 0.60*	3.3± 0.56*

The values are expressed as Mean ± SEM; *n* = 6 animals in each group.  
Data were analysed by Tukey-Kramer multiple comparison test.

## Results

### Acute Toxicity Studies

It was found that the extract was non-toxic up to 1 g/kg, p. o. body weight up to 24 hours. Thus one-tenth of it, i.e, 100 mg/kg, p. o. was taken as the initial starting dose and the other two selected doses were 100 mg/kg, p. o. and 200 mg/kg, p. o., respectively.

### Anti-inflammatory Studies Carrageenan-induce oedema

The extract inhibited carrageenan-induced paw oedema by 61% at a dose of 100 mg/kg, 65% at the dose of 200 mg/kg and 66% at the dose of 300 mg/kg p. o. [Table 1]

### Arachidonic acid-induced paw oedema

Arachidonic acid injection (subplantar) in right hand paw produced significant oedema after 1 hour. Indomethacin, the cyclo-oxygenase blocker, inhibited it by 32% where as phenidone, a dual blocker, inhibited it by 77%. The extract at doses of 100, 200 and 300 mg/kg inhibited the oedema by 43%, 65% and 71%, respectively. [Table 2].

### Analgesic studies Tail-flick method

The extract produced a dose-dependent analgesic activity in this model and the effect produced by 300 mg/kg, p. o. of the extract was comparable to that produced by ibuprofen, the standard drug [Table 3].

**Table 4 Effect of Wattakaka volubilis root extract on brewer's yeast induced pyrexia in rats.**

Drug	Dose	Temp.(° C) 19 hours after brewer's yeast injection	Temp. (° C) after treatment			
			60 min	120 min	180 min	300 min
N. Saline	0.1 ml/100g	38.7±0.4	37.3±0.7	37.5±0.6	37.7±0.7	37.6±0.8
Paracetamol	150 mg/kg	38.65±0.10	38.59±0.08***	38.19±0.14**	37.56±0.19**	37.16±0.24***
Extract	100 mg/kg	38.2±0.5	37.3±0.5**	37.1±0.5***	37.8±0.2**	37.2±1.3***
Extract	200 mg/kg	38.6±0.3	37.9±0.5**	37.1±0.5***	37.0±0.5***	37.3±0.3**
Extract	300 mg/kg	39.0±0.6	37.8±0.7***	37.2±0.7***	36.8±0.7***	36.0±0.6***

The values are expressed as Mean ± SEM;  $n = 6$  animals in each group. Data were analysed by Tukey-Kramer multiple comparison test. \*Significant decrease in rectal temperature ( $p < 0.05$ ).

### Antipyretic

The reduction in rectal temperature of febrile rats, treated with different doses of aqueous and alcohol extracts of the drugs, were recorded at 60, 120, 180 and 300 min post administration. The reduction in rectal temperature of treated animals at each interval was compared with that of untreated febrile rats.

### Discussion

Leaves of medicinal plants are common ingredients of many folk and herbal medicines,<sup>17, 18</sup> and leaf extracts of a number of medicinal plants have been reported to possess pharmacological activity, including anti-inflammatory activity.<sup>17, 19, 20</sup> The present study reveals that the dried root extract of *W. volubilis* possesses significant anti-inflammatory, analgesic and anti-pyretic activities in experimental animals. Different parts of *W. volubilis* plant enjoy considerable reputation for their various medicinal uses. The leaf paste is used to clear boils. Plant paste is mixed with hot milk and taken for urinary troubles. Leaf juice is inhaled to stop sneezing. The alcoholic extract of the plant is widely used in India as a traditional medicine for boils and abscesses.<sup>21</sup> The leaves extract of *W. volubilis* was reported as Anti-inflammatory and analgesic in rodent.<sup>22</sup> The alcoholic extract of the plant is also reported to show activity on the central nervous system, as well as anticancer activity against sarcoma 180 in mice.<sup>3</sup> Two dregosides isolated from the methanolic extract of stem this plant showed anti-tumour activities against Ehrlich's carcinoma (solid type), and also showed activity against melanoma B-16.<sup>2</sup> Acute toxicity study revealed

that the extract is non-toxic up to 1 g/kg, p. o. The anti-inflammatory effect of the extract could be observed in acute (carrageenan and arachidonic acid induced paw oedema in rat).

Since the extract inhibited the oedema comparable to the dual-blocker phenidone in the arachidonic acid-induced paw oedema model in rat, it is possible that the extract produces its anti-inflammatory activity by inhibiting both the lipo-oxygenase and cyclo-oxygenase pathways of arachidonic acid metabolism. In case of analgesic study, tail flick method, it showed time- and dose-dependent analgesic activities. The extract also produced a significant antipyretic effect in the brewer's yeast-induced pyrexia model in rat.

Since the present study indicates that the extract has significant anti-inflammatory activity, analgesic activity and antipyretic activity.

### Conclusion

It can concluded from present study that *W. volubilis* root extract can be used for development of standardized herbal therapeutic formulation for anti-inflammatory, analgesic and antipyretic conditions.

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