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## Research Article

### EVALUATION OF ANTIPIRETTIC ACTIVITY OF ETHANOLIC EXTRACT OF *WEDELIA TRILOBATA*

M.Madhu Babu\*, A.Lakshmana Rao, V.Harshitha, V.Madhavi, V.N.S.Lavanya, V.Vineetha, M.Sai Sailaja

Department of Pharmacology, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, Andhra Pradesh.

**Keywords:** *Wedelia trilobata*, Brewer's yeast, Paracetamol, Digital clinical thermometer.

#### ABSTRACT

The aim of present study was to investigate antipyretic activity of ethanolic extract of leaves of *Wedelia trilobata* in yeast induced pyrexia in wistar albino rats. In which pyrexia was induced by an intraperitoneal injection of 20% brewer's yeast (10 ml/kg b.wt.). The body temperature of rats were measured before the injection of yeast and injected ethanolic extract of leaves of *Wedelia trilobata* (100 mg/kg b.wt.) and (200 mg/kg b.wt.) and followed by treatment with paracetamol (150 mg/kg b.wt.). The body temperature of experimental animals were recorded in the time interval of 0 hr, 1 hr, 2 hr and 3 hr with help of digital clinical thermometer which is placed in rectum in the depth of 2 cm and recorded body temperature values shown that the leaves extract of *Wedelia trilobata* possess antipyretic activity.

#### INTRODUCTION

*Wedelia trilobata* is a mat forming perennial herb with rounded stems. Leaves are fleshy, usually 2 to 4 inches long and 1 to 5 inches wide, with irregularly toothed margins. Flowers are solitary, one inch in diameter and yellow-orange in color. The major components were germacrene D,  $\alpha$ -phellandrene,  $\alpha$ -pinene, E-caryophyllene, bicyclic-germacrene, limonene and  $\alpha$ -humulene. The percentage of most of the individual constituents present in *W. trilobata* essential oil changed significantly during the months. The plant has reported various pharmacological activities i.e., antimicrobial, antiproliferation, wound healing, antioxidant, antiinflammatory, *in-vitro* thrombolytic, antiproteinase, antifungal, antitumour and leishmanicidal activities<sup>[1]</sup>. Aerial parts of this plant used in traditional medicine against bronchitis, colds, abdominal pains, dysmenorrhoea, fertility enhancer. Antipyretic compounds available in the market still present a wide range of undesired effects, leaving an open door for new and better compounds. Therefore, the present study was made on antipyretic effects on *Wedelia trilobata*.

#### EXPERIMENTAL METHODOLOGY

##### Identification & collection of plant material

The whole plant of *Wedelia trilobata* was collected from Gudlavalleru. These plants were identified and authenticated by Department of Botany, Hindu College, Machilipatnam. The plants were sorted, cleaned and air dried at room temperature for one week. Then it was ground to powder. Powdered sample was collected and stored in air and water proof containers protected from direct sunlight and heat until used for extraction.

##### Preparation of plant material

The powdered material of *Wedelia trilobata* was extracted with maceration for 3 days with distilled water followed by simple distillation. The extracts were concentrated to dryness till free from the solvents.

##### Qualitative Phytochemical screening

The following tests were carried out on standardized herbal extract to detect the presence of various phytoconstituents like saponins, tannins, flavonoids, alkaloids, steroids, carbohydrates, proteins and phenols by different methods.

### Experimental animals

Rats weighing 180-200 gm are kept in polypropylene cages, 3 in each cage, at an ambient temperature of  $25\pm 2^{\circ}\text{C}$  and relative humidity of 55-65%. A 12 hrs light and dark schedule was carefully maintained in the air conditioned animal house. All the rats are fed with common diet for 1 week after arrival and then divided into groups with free access to food and water [2].

All experiments were carried out according to the guidelines for care and use of experimental animals and approved by the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). The study was approved by Institutional Animal Ethics Committee (IAEC) and the approval number is -1847/PO/Re/S/16/CPCSEA.

### Acute toxicity studies

According to most essential OECD 423 (Organization for Economic Co-operation and Development) apprenticeship and recommendations we have conducted the acute toxicity studies. Animals are divided in groups (n=5). For about 4-5 hrs prior to experiments, all the animals are fasted with free access to only distilled water. The suitable extract of *Wedelia trilobata* are administered to different groups of rats in doses of 5, 50, 300 and 1000 mg/kg by gavage and observed for mortality and consideration physical and behavioral changes for over 14 days<sup>[3-4]</sup>.

### Assessment of anti-pyretic activity of Ethanolic extract of *Wedelia trilobata*

#### Study 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 (10 ml/kg b.wt.).

- Group III: animals were administered with yeast (10 ml/kg) and the standard drug paracetamol (150 mg/kg b.wt.).
- Group IV: animals were administered with yeast (10 ml/kg) and ethanolic extract of *Wedelia trilobata* (100 mg/kg b.wt.).
- Group V: animals were administered with yeast (10 ml/kg) and ethanolic extract of *Wedelia trilobata* (200 mg/kg b.wt.).

### Antipyretic activity test

Extracts were screened for *in-vitro* and *in-vivo* antipyretic activity by brewer's yeast induced pyrexia.

#### Yeast induced pyrexia

Brewer's yeast (15%) in saline (0.9%) was prepared as suspension. Six rats of either sex were taken in a group and five groups were formed [5].

The thermocouple was inserted for 2 cm into the rectum and the temperature was measured. An intraperitoneal injection of 20% w/v of brewer's yeast (10 ml/kg) in distilled water was injected to induce pyrexia. Also, the basal rectal temperature was measured before the injection of yeast. The sight of injection was massaged in order to spread the suspension beneath the skin. The experimental room temperature was set at  $22-24^{\circ}\text{C}$ , immediately after yeast administration, food was withdrawn and the increase in rectal temperature was recorded [6]. The dose of the test compound and standard drug was given subcutaneously. The rectal temperature was recorded again after 1, 2 and 3 hrs. Paracetamol (150 mg/kg) was chosen as a reference drug. The ethanol extracts were dissolved in saline.

#### Statistical significance

Data were analyzed by one-way ANOVA followed by Dennett's t-test using Instant® (Graph Pad software, U.S.A). At 95% confidence interval  $p < 0.05$  was considered statistically significant.

## RESULTS

### Phytochemical constituents present in ethanolic extract of leaves of *wedelia trilobata*

Table 1: Details of qualitative phytochemical tests

S. No.	Test	Methanolic Extract	Chloroform Extract	Ethanolic Extract
1	<b>Carbohydrates</b>			
	Mohlish's test	-	-	+
	Fehling's test	+	+	+
2	<b>Proteins and amino acids</b>			
	Ninhydrin test	-	-	-
	Biuret test	-	-	-

<b>3</b>	<b>Alkaloids</b>			
	Mayer's test	-	+	+
	Wagner's test	+	+	+
<b>4</b>	<b>Fixed oils and fats</b>			
	Spot test	-	-	-
<b>5</b>	<b>Glycosides</b>			
	Legal's test	+	+	+
	Keller killiani test	+	+	+
<b>6</b>	<b>Triterpenoids</b>			
	Tin + thionyl chloride	-	+	+
<b>7</b>	<b>Phenolics and tannins</b>			
	Ferric chloride test	+	+	+
	Gelatin test	-	+	+
	Lead acetate test	-	+	+
	Alkaline reagent test	-	-	+
<b>8</b>	<b>Saponins</b>			
	Foam test	-	+	+
	Haemolysis test	-	-	+
<b>9</b>	<b>Flavones and Flavonoids</b>			
	Shinoda test	+	+	+
	Lead acetate test	+	-	+

“+” – Present “-” – Absent

The strong presence of desired phytochemicals in ethanolic extracts when compared to methanolic and chloroform extracts. Hence, for the further studies ethanolic extract of *Wedelia trilobata* have been selected. Effect of ethanolic extract of *Wedelia trilobata* on brewer's yeast induced rectal temperature in rats is presented in table 2.

The intraperitoneal injection of brewer's yeast suspension markedly elevated the rectal body temperature after 18 hrs of administration.

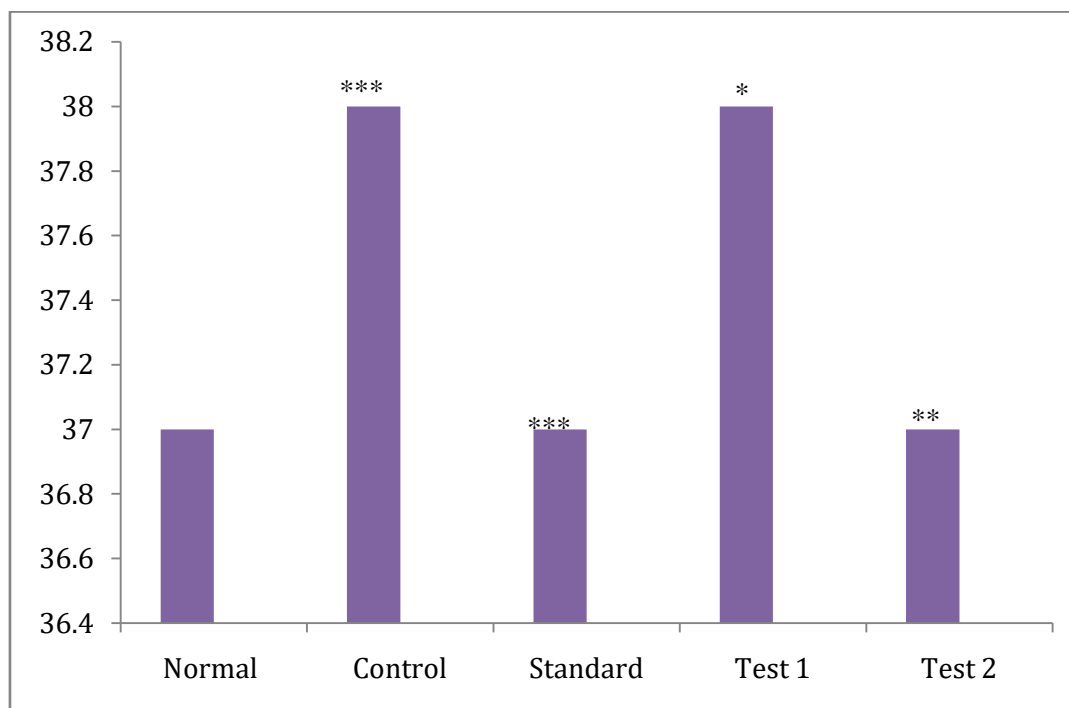
Treatment with *Wedelia trilobata* at doses of 100 and 200 mg/kg decreased rectal body temperature in dose dependent manner.

*Wedelia trilobata* higher dose 200 mg/kg significantly reduced yeast elevated rectal body temperature at 2 hrs and 3 hrs compared to control group.

**Table 2: Effect of ethanolic extract of leaves of *Wedelia trilobata* on body temperature in yeast induced pyrexia**

Groups	0 hr	1 hr	2 hr	3 hr
Normal control	37.18±0.6	37.46±0.33	37.46±0.33	37.45±0.33
Control group	39.34±0.163***	38.43±0.03**	38.47±0.16**	38.47±0.06***
Standard	39.31±0.18	38.7±0.2**	37.9±0.2**	37.2±0.08**
WTBT	39.3±0.2	38.57±0.18*	38.87±0.2*	38±0.2*
WTBT	39.28±0.17	38±0.35*	37.53±0.28**	37.3±0.49**

Values are mean ±SEM (n=6) one way ANOVA. Where, \* represents significant at p<0.05, \*\* represents highly significant at p<0.01, and \*\*\* represents very significant at p<0.001. All values are compared with toxicant.



**Fig 1: Effect of ethanolic extract of leaves of *Wedelia trilobata* on body temperature in yeast induced pyrexia**

## 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.

Fever is a complex physiologic response triggered by infections or aseptic stimuli. Elevation in body temperature occurs when the concentration of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) 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 PGE<sub>2</sub> within the hypothalamic region. A natural antipyretic agent with reduced or no toxicity is therefore, essential.<sup>[7]</sup>

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. Yeast induced pyrexia is called pathogenic fever which is due to the production of prostaglandins (PGE<sub>2</sub>) which set the thermoregulatory center at a higher temperature.<sup>[8]</sup>

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.

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 interleukin1 $\alpha$  production subsequent to interferon production. Flavanoids like baicalin have been shown to exert antipyretic effect by suppressing TNF- $\alpha$  and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing the fever and pain. This study also correlates with the study of *Zakaria et al*<sup>[9]</sup> 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 leaf extracts of *Wedelia trilobata* 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.<sup>[10]</sup>

In conclusion, this study provides evidences for the antipyretic activity of *Wedelia trilobata* 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. [11-13]

### CONCLUSION

This study indicates ethanolic extract of leaves of *Wedelia trilobata* significantly reduced the elevated levels of body temperature compared to control. Hence at this point it may be concluded that the ethanolic extract of leaves of *Wedelia trilobata* possess anti pyretic activity.

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#### \*Address for correspondence

**M.Madhu Babu**

Assistant Professor

Department of Pharmacology

V. V. Institute of Pharmaceutical

Sciences, Gudlavalleru, Andhra

Pradesh.

Email: [morthamadhu@gmail.com](mailto:morthamadhu@gmail.com)

Ph: +91- 9704285739