

## Research Article

# Aerial parts of *Enicostemma littorale* Blume serve as antipyretic and antacid: *in vivo* and *in vitro* evaluations

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**ABSTRACT:** **Introduction:** *Enicostemma littorale* Blume (Gentianaceae) is traditionally used as medicine for the treatment of diabetes, fever, rheumatism, stomachache, dyspepsia, hernia, itching, insect poisoning and malaria. In the present study, we investigated the antipyretic activity using Swiss Albino mice and *in vitro* antacid activity of the aqueous extract of the aerial parts of this plant. **Materials and Methods:** The extract was screened for various essential phytoconstituents by qualitative phytochemical screening. The antipyretic activity was evaluated by lipopolysaccharide (LPS) induced pyrexia using mice as the animal model. The *in vitro* acid neutralising capacity was determined to evaluate the antacid potency of the extract as compared to standard antacid drugs. **Results:** Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, steroids, glycosides, saponins and flavonoids in the aerial parts of *E. littorale*. The extract significantly reduced the LPS elevated body temperature in mice at 100, 200, 300, and 400 mg/kg doses throughout the observation period of 5 hours. The extract showed optimum antipyretic activity at 200 mg/kg dose. The extract also showed the antacid potency comparable with standard antacid drugs. **Conclusion:** We suggest that the aqueous extract of the aerial parts of *E. littorale* might play an important role in the antipyretic and antacid like activities.

**KEY WORDS:** *Enicostemma littorale* Blume, Gentianaceae, lipopolysaccharide, antipyretic activity, *in vitro* antacid activity

## INTRODUCTION

*Enicostemma littorale* Blume (family Gentianaceae) is a perennial herb and found throughout India, most commonly in coastal areas. Ancient literature suggests the aqueous extract of the plant was used in the treatment of diabetes, fever, rheumatism, stomachache, dyspepsia, hernia, itching, insect poisoning and malaria.<sup>[1-3]</sup> Several studies also suggested the role of the plant with antitumor,<sup>[3,4]</sup> antiarthritic,<sup>[3,5]</sup> hypoglycemic<sup>[3,6]</sup> and antimalarial<sup>[3,7]</sup> properties.

Pyrexia or fever results as a secondary effect of many medical conditions including viral diseases, malaria, malignancy, tissue damage, inflammation, graft rejection and other disease states.<sup>[8]</sup> The infected or damaged tissues

are developed due to the diseased states to enhance the production of proinflammatory mediators such as cytokines. It is reported that these cytokines [interleukin  $\alpha$ ,  $\beta$ ,  $1\beta$  and tumour necrosis factor (TNF)- $\alpha$ ] increase the synthesis of prostaglandin (PG)  $E_2$  near the hypothalamus and thereby elevate the body temperature.<sup>[8]</sup> Hypothalamus regulates body temperature (hyperthermia or hypothermia) by modulating the size of blood vessels. Most antipyretic drugs inhibit the enzyme cyclo-oxygenase (COX)-2 expression to reduce the elevated body temperature.<sup>[8]</sup> Synthetic agents irreversibly inhibit COX-2 with a high selectivity and develop toxicity to hepatic cells, glomeruli, brain cortex and heart muscles.<sup>[9]</sup> However, plant derived antipyretics, from turmeric, ginger and hops<sup>[10]</sup> show lower COX-2 selectivity and thus have fewer side effects.<sup>[9]</sup> Therefore, selection of a natural antipyretic agent with reduced or no toxicity would be beneficial in the treatment of pyrexia. Since *E. littorale* is traditionally reported to have uses in fever,<sup>[2]</sup> it is assumed that the plant may serve as a cost effective and alternative natural antipyretic agent.

The stomach normally secretes acid which has an essential role in the digestion of food, although excess production of this may result in acidity. Heartburn, dyspepsia and eructation are common symptoms of acidity. Antacids

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provide a symptomatic relief from these symptoms by neutralizing the excess gastric acid upon oral administration. Acid neutralizing capacity (ANC) is the most commonly used measure to express potency of an antacid. Acid neutralizing capacity can be defined as the number of milli equivalents (mEq) of 1N hydrochloric acid that is brought to a pH of 3.5 in 15 min by a unit dose of an antacid preparation.<sup>[11,12]</sup> Various known artificial antacids are commonly used to treat hyperacidity. Despite this, drugs obtained from the plant kingdom may serve as useful sources in the development of new natural antacids. Hence, the present study was aimed at the evaluation of *in vivo* antipyretic activity using mice and *in vitro* antacid potency of aqueous extract of aerial parts of *E. littorale*, since the drug is traditionally used in the treatment of fever, stomachache and dyspepsia.

## MATERIALS AND METHODS

### Collection of plant material

Whole plants of *E. littorale* were collected fresh before the flowering stage in the month of August-September from Agalgaon, Solapur district (Maharashtra, India). The plant was identified and authenticated by the University Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur and a voucher specimen was deposited for further reference (Authentication Number 9247).

### Extraction and Phytochemical analysis

The aerial parts of the plants were separated from the roots and dried under shade. The dried material was ground into a uniform powder using a blender and stored in polythene bags at room temperature. The plant powder thus generated was further used for extraction using water by maceration method in the drug:solvent proportion of 1:5. The extracts were concentrated to dryness under controlled temperature in hot air circulated oven. All the extracts were subjected to qualitative phytochemical screening.<sup>[13-16]</sup>

### Animals

Adult male Swiss-Albino mice (25-30 g and 8 weeks old) were used for screening antipyretic activity. They were fed with standard laboratory diet *ad libitum*, allowed free access to drinking water and kept in 12 h light/dark cycle. Animals were further selected for the experiment after confirmation of approximate constant rectal temperature for consecutive 7 days.

### Antipyretic activity

Thirty six healthy mice were divided into six groups of six animals each. The rectal temperature was recorded using a digital telethermometer (Electrolab®, India) by introducing thermister probe 1 inch into the rectum and keeping it

inside for 1 min until constant rectal temperature was displayed. The initial rectal temperature of each animal was recorded, its hourly variation was noted up to 5 hours and the average was calculated. Pyrexia was induced by injecting a suspension of lipopolysaccharide (LPS) in normal saline by intraperitoneal (ip) route at the dose 100 µg/kg. After a half hour interval, animals were subjected to oral administration of the standard drug or herbal extracts using an oral syringe and behavioral study was carried out as follows: group I served as negative control which received vehicle (normal saline) orally. Group II was administered orally with reference standard paracetamol (150 mg/kg, Zim Laboratories, Nagpur, India) for comparison with the activity of *E. littorale* extract. Groups III-VI received aqueous herbal extracts at 100, 200, 300 and 400 mg/kg dose, respectively. The doses of the plant extracts were selected on the basis of previous literature,<sup>[3,17]</sup> which put forward the LD<sub>50</sub> value (> 2000 mg/kg) for the extract of *E. littorale*. A dose of 1/10<sup>th</sup> to 1/5<sup>th</sup> of the dose of the LD<sub>50</sub> was selected for the present study. Rectal temperature of animals was recorded at 30, 60, 120, 180, 240 and 300 min following a half hour interval after the last oral administration of vehicle, standard drug paracetamol as well as individual doses of herbal extract.

### *In vitro* antacid activity

The ANC value obtained for the aqueous extract (500, 1000 and 1500 mg/5 ml) was compared with the standard antacids mixture (containing magnesium hydroxide and aluminium hydroxide gel in 1:1 proportion, 250 mg each/5 ml; Zim Laboratories, Nagpur, India). To the 5 ml quantity of this mixture, water was added to make the total volume 70 ml and then mixed for 1 min. Thereafter, 30 ml of 1.0 N HCl was added into standard and test preparation (aqueous extracts 500, 1000 and 1500 mg/5 ml) and stirred for 15 min. The excess HCl was immediately titrated with 0.5 N sodium hydroxide solution to attain a stable pH of 3.5 (for 10 to 15 sec). The temperature of the mixture was maintained at 37 ± 0.5 °C throughout the entire experiment. Then, the number of mEq of acid consumed was calculated by the formula.

$$\text{Total mEq of acid consumed} = (30 \times \text{N HCl}) - (\text{V NaOH} \times \text{N NaOH})^{[11,12]}$$

Where N HCl and N NaOH are the normality of HCl and NaOH respectively, and V NaOH is the volume of NaOH used for titration. ANC values are reported as mEq of acid consumed by 5 ml of standard as well as test preparation.

### Statistical analysis

The data of antipyretic activity was analyzed by two-way analysis of variance (ANOVA) followed by post-hoc Bonferroni's multiple comparisons test. The data is presented as mean ± SEM. Differences obtained from the data were considered significant at P < 0.05, 0.01 and 0.001.

**Table 1: Effect of aqueous extract of *E. littorale* on lipopolysaccharide (LPS) induced pyrexia in mice**

Treatment	Dose in mg/kg	Rectal Temperature in °C ± SEM						
		Initial	30 min	60 min	120 min	180 min	240 min	300 min
Naïve (Saline)		38.0 ± 0.2	37.9 ± 0.2	38.0 ± 0.2	37.8 ± 0.2	38.2 ± 0.2	37.9 ± 0.1	38.1 ± 0.3
Control (LPS + Saline)	0.1	38.0 ± 0.2	39.9 ± 0.3**	38.8 ± 0.1*	38.1 ± 0.2	38.2 ± 0.4	38.2 ± 0.2	38.8 ± 0.1*
Standard (LPS + Paracetamol)	150	38.6 ± 0.1	37.5 ± 0.2 <sup>#</sup>	36.6 ± 0.3 <sup>#</sup>	36.0 ± 0.3 <sup>#</sup>	36.2 ± 0.1 <sup>#</sup>	36.5 ± 0.2 <sup>#</sup>	37.1 ± 0.1 <sup>#</sup>
LPS + Herbal Extract	100	37.9 ± 0.3 <sup>a</sup>	37.9 ± 0.2	37.6 ± 0.1 <sup>b</sup>	37.4 ± 0.1 <sup>c</sup>	37.2 ± 0.1 <sup>c</sup>	37.6 ± 0.1 <sup>c</sup>	37.8 ± 0.2
LPS + Herbal Extract	200	38.2 ± 0.4	37.68 ± 0.3	36.9 ± 0.2	36.6 ± 0.1	37.2 ± 0.1 <sup>b</sup>	37.5 ± 0.1 <sup>c</sup>	37.8 ± 0.2
LPS + Herbal Extract	300	38.2 ± 0.1	37.1 ± 0.2	37.4 ± 0.1 <sup>a</sup>	36.9 ± 0.2 <sup>b</sup>	37.5 ± 0.2 <sup>c</sup>	37.6 ± 0.3 <sup>c</sup>	37.6 ± 0.1
LPS + Herbal Extract	400	38.3 ± 0.2	37.6 ± 0.1	36.8 ± 0.3	36.7 ± 0.2	37.5 ± 0.2 <sup>c</sup>	37.8 ± 0.1 <sup>c</sup>	37.9 ± 0.4 <sup>a</sup>

The effect of paracetamol and aqueous extract of aerial parts of *E. littorale* (100-400 mg/kg) in lipopolysaccharide (LPS) induced pyrexia was observed. The change in the rectal temperature was observed following the treatment by respective intraperitoneal (LPS) or oral (paracetamol and herbal extract) route. The data represent mean of °C ± SEM for each group (n = 6). The data were analyzed by two-way ANOVA followed by post hoc Bonferroni's multiple comparisons test. \*P < 0.05, \*\*P < 0.001 vs Naïve; <sup>#</sup>P < 0.001 vs LPS; <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.001 vs paracetamol.

## RESULTS

### Phytochemical screening

The aqueous extract of the aerial parts of *E. littorale* was subjected to the qualitative phytochemical screening. It revealed the presence of alkaloids, carbohydrates, steroids, glycosides, saponins and flavonoids in the aerial parts of the plant.

### Antipyretic activity

Animals were selected for the experiment after confirmation of approximate constant rectal temperature for consecutive 7 days. During the experiments the rectal temperature of animals were recorded at 30, 60, 120, 180, 240 and 300 min following the treatments. The initial constant rectal temperature of naïve mice was found to be in the range of 37.7-38.3 °C. Pyrexia was induced in mice by injecting a suspension of LPS in normal saline (ip, 100 µg/kg dose) and after half an hour interval saline, reference standard drug or herbal extract was administered orally and following half an hour interval, the body temperature was measured up to 5 hours. LPS treatment significantly elevated the body temperature of animals by 1.9 ± 0.1 °C as compared to the naïve untreated animals at 30 and 60 min following injections. Application of two-way ANOVA followed by post-hoc Bonferroni's multiple comparisons test showed a significant rise in temperature following the LPS treatment with respect to time. On the other hand, paracetamol treatment at the dose 150 mg/kg significantly decreased LPS-induced pyrexia at all the time points as compared to that of the control mice. Similarly, there was significant reduction in the LPS-induced pyrexia following oral treatment of the aqueous extract. It was observed that the oral treatment of aqueous extract of the aerial parts of the *E. littorale* (100, 200, 300 and 400 mg/kg) following LPS treatment significantly blocked the LPS-induced pyrexia. Moreover, the effects obtained at these doses were significant but the one obtained at 200 mg/kg dose was optimum as compared to that obtained by the reference standard paracetamol. However, the antipyretic effect obtained following paracetamol was more pronounced and significant but the application of

**Table 2: In vitro acid neutralizing capacity of aqueous extract of *E. littorale* (AEEL)**

Formulation	Vol. of NaOH consumed (ml)	mEq of acid consumed
AEEL batch 1	42.7	8.65
AEEL batch 2	41.8	9.1
AEEL batch 3	41.6	9.2
Standard antacids mixture	25.4	17.3

The acid neutralizing capacity of Standard antacids mixture and aqueous extract of aerial parts of *E. littorale* (500, 1000, 1500 mg/5 ml) was observed *in vitro*. The data represent mean of volume of NaOH consumed (ml) and mEq of acid consumed.

two-way ANOVA followed by post-hoc Bonferroni's multiple comparisons test suggested the effect of 200 mg/kg much closer to that of the paracetamol treated groups at 30, 60 and 120 min following treatment (Table 1).

### In vitro antacid activity

Initially, the ANC value of standard antacids mixture was determined for the comparison with ANC value of aqueous extract of *E. littorale* (AEEL). The three batches of AEEL were evaluated for their antacid potency by determining their acid neutralizing potency. The amounts of herbal extract taken in I, II and III batches were 500, 1000 and 1500 mg/5 ml, respectively. The ANC of AEEL was found to be in the range of 8.5-9.5. However, the ANC for standard antacids mixture was 17.3 (Table 2).

## DISCUSSION

The aqueous extract of aerial parts of *E. littorale* showed the presence of alkaloids, carbohydrates, steroids, glycosides, saponins and flavonoids following qualitative phytochemical screening, as reported earlier.<sup>[15,18]</sup> Therefore, it seems that the biological effects produced by the plant might be attributed to these constituents.

The aqueous extract of *E. littorale* produced significant antipyretic activity in the LPS-induced pyrexia, a well known animal model,<sup>[19]</sup> as observed by a decrease in the core body

temperature and was much closer to that of the reference standard paracetamol. It is understood that the LPS-induced pyrexia is a pathogenic fever and its etiology includes the role of PGs.<sup>[20,21]</sup> The antipyretic potentials of alkaloids, steroids and flavonoids have been previously reported.<sup>[22,23]</sup> Therefore, the antipyretic activity of the extract may be attributed to the alkaloids, steroids and flavonoids, which individually needs evaluation for *E. littorale*. Moreover, it is reported that paracetamol blocks the expression of PGE<sub>2</sub> and produces an antipyretic action.<sup>[8,24]</sup> Similarly, LPS-induced pyrexia was blocked by the herbal extract. Thus, we suggest that the antipyretic effect of *E. littorale* may involve the inhibition of PGs and help in the treatment of pathogenic fever.

The ANC value of standard antacid drugs was determined and compared to that obtained with the aqueous extract of *E. littorale*. Standard antacids mixture showed the ANC value of 17.3, which is also reported in the previous literature in the range of 12.49-19.90 depending on the concentration of the drugs in the mixture.<sup>[25]</sup> In the present study, the aqueous extract showed the ANC value in the range of 8.5-9.5 for three different batches containing 500, 1000 and 1500 mg/5 ml plant extract. The obtained range is far away from the values of standard suspension. This difference in the ANC value may be due to the presence of mixture of antacid drugs like magnesium hydroxide and aluminium hydroxide gel in the standard suspension. Therefore, it is not surprising that the ANC value obtained for the standard suspension was quite high compared to that of the plant extract. Moreover, compounds showing the ANC values near to 7 are even categorized as good antacids.<sup>[24]</sup> The extract contained phytochemical constituents including alkaloids, carbohydrates, steroids, glycosides, saponins and flavonoids. Therefore, the possibility of involvement of these phytoconstituents individually or in combination cannot be ruled out for the ANC of the herbal extract. Further investigations regarding the involvement of individual phytochemical constituent may be fruitful to formulate an effective herbal preparation that can be used to overcome gastrointestinal disorders like acidity in future.

## CONCLUSION

The present study demonstrates the antipyretic and antacid activities of aqueous extract of aerial parts of *E. littorale*. The antipyretic activity of the extract may be due to the presence of alkaloids, steroids and flavonoids and the possible mechanism involved may be the inhibition of PGE<sub>2</sub> synthesis. The antacid activity may also be due to alkaloids, carbohydrates, steroids, glycosides, saponins and flavonoids either of alone or in combination. Thus, this data provides a rationale for the use of active constituents of the plant in fever and gastrointestinal disorders with well established folk medicine.

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