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# Methanolic Extract Induced Analgesic and Anti-pyretic Activity of *Hemigraphis hirta*

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***Authors' contributions***

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Objective:** The objective of this research is to investigate the analgesic and antipyretic activity of methanolic extract of *Hemigraphis hirta.*
**Methods:** Whole dried plant of *Hemigraphis hirta* was extracted with pure methanol. Analgesic activity was tested by both acetic acid induced writhing test and formalin induced paw licking test. And anti-pyretic activity was tested by Brewer's yeast-induced pyrexia test.
**Results:** In paw licking analgesic activity test, after 0- 5 minutes, $p < 0.05$ (44.79% inhibition) and after 30 minutes, the $p < 0.001$ (90.07% inhibition) in case of 200 mg/kg bodyweight which was more significant than 400 mg/kg body weight. But in both cases, we saw significant reduction of paw licking compared to standard group (Dioclofenac Sodium). In the contrary, in acetic acid induced writhing test the *p* value for reduction of writhing for dose of 200 mg/kg body weight was < 0.05 (72.45% inhibition) which was more significant than 400 mg/kg body weight. In this test, we

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also observed very significant reduction of writhing compared to standard group. In Brewer's yeast-induced pyrexia test the methanolic extract at the dose of 400 mg/kg and 200 mg/kg body weight, reduced hyperthermia in mice in 1 hour observation and also lowering of temperature from 2 hours to 4 hours observation period in comparison to control (Paracetamol).
**Conclusion:** These finding suggests that *Hemigraphis hirta* can be a potential source of analgesic and anti-pyretic medication.

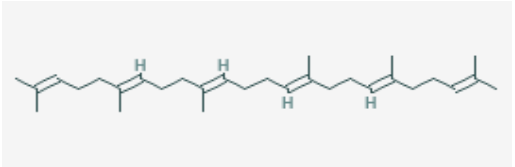
*Keywords: Hemigraphis hirta; analgesic; anti-pyretic; folk medicine.*

## 1. INTRODUCTION

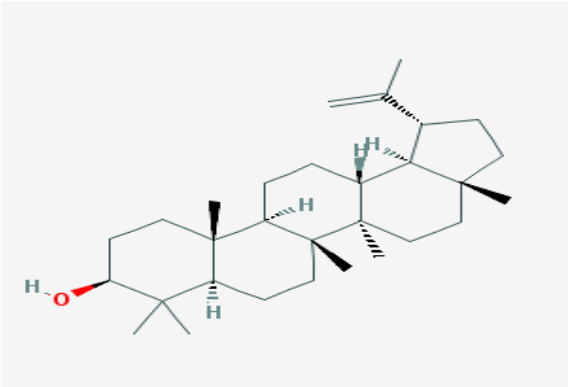
Inflammation is the body's attempt at self-protection; its aim is to remove harmful stimuli, including damaged cells, irritants, and pathogens and begin the healing process. Analgesia and pyrexia is a common symptoms of inflammatory process. Analgesia (pain) is an ill-defined, unpleasant sensation, usually evoked by an external or internal noxious stimulus. Typically, it is a direct response to an untoward event associated with tissue damage, such as injury and inflammation, but severe pain can arise independently of any obvious redisposing cause, or precipitate healing after injury for a relatively long time. It can also occur as a consequence of brain or nerve injury. Pain signalling to the central nervous system is initiated when harmful excitement and primary afferent nociceptive C and A fibres are frequently caused by activation of several types of ionotropic channels and etabotropic receptors [1,2]. 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 and inability to concentrate. This increase in set point triggers increased muscle tone and shivering [4].

### 1.1 Plant Details

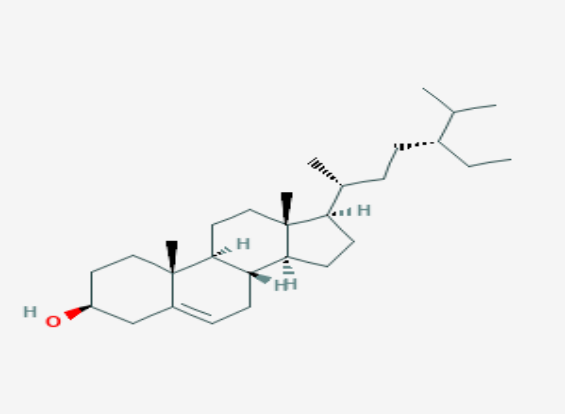
*Hemigraphis hirta* is a soft herb; stem 15-45 cm long, creeping in grass. It has small leaves, 1.25-2.5 cm long, ovate crenate. Heads 2-6-flowered, axillary. Bracts 1.25 cm long, elliptic; sepals linear, or in fruit subspathulate; corolla 1.25 cm, subequal, pale lavender blue. Capsule is 1.25 cm long, 12-seeded. *Hemigraphis hirta* [Family: Acanthaceae; English Name: *Hairy Hemigraphis;* Botanical name: *Hemigraphis hirta;* Local name: *Buripana, Borati gas;* Tribal Name: *Mrawnna (Marma)*] is a regular folklore medicine in some regions of Bangladesh as an antidiarrheal, antishigellotic, analgesic, antipyretic etc [5]. Major chemical compounds of this plant are-squalene, lupeol and B-sitosterol [6] which are shown in Fig. 1. Phytochemical investigation of *Hemigraphis hirta* has led to the isolation of stigmasterol and n-hentriacontanol from the petroleum ether extract [7].



**Squalene (PubChem CID: 638072)**



**Lupeol (PubChem CID: 259846)**



**Beta-sitosterol (PubChem CID: 222284)**

**Fig. 1. Chemical structure of Squalene, Lupeol and Beta-sitosterol**

Generally it is considered that compounds produced naturally rather than synthetically and will be biodegraded more easily and therefore being more environmentally acceptable. It is a renowned medicinal plant used extensively in rural area of Bangladesh for treatment of abdominal pain, glossitis, stomatitis, acute wounds and anthelmintic. But no scientific data is currently available as its proof of action. However, this research is conducted to justify the local myth of its analgesic and antipyretic activities and establish its regular usage scientifically.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Materials

The plant of *Hemigraphis hirta* was collected from Jessore University of Science & Technology Campus, Jessore, Bangladesh; and the areas of Navaron, Sharsha, Jessore, in August, 2016. The collected plants were identified and confirmed by National Herbarium, Bangladesh. The accession number for the plant is DACB-45076.

### 2.2 Preparation and Extraction of Plant Material

The whole plants of *Hemigraphis hirta* were thoroughly washed with fresh water to remove all contaminants and dried in shade at room temperature (25±2℃) for one week. The materials were grinded into coarse powder and cold extraction method was used to extract the active components. The grinded powders were soaked in Methanol for 7 days and stirred periodically. The whole mixture was primarily filtered through cotton and then through Whatman No.1 filter papers and was concentrated with a rotary evaporator under reduced pressure at 40℃ temperature to afford crude extract.

### 2.3 Experimental Animals

The experiment of analgesic activity was conducted on Swiss albino mice of either sex, aged 4-5 weeks, weighing about 25-35 gm. Before initiating the experiment, the mice were kept in standard environmental conditions (12 h light/ 12 h dark cycle) and had free access to feed and water ad libitum. All protocols for animal experiment were approved by the institutional animal ethical committee of Jessore University of science & Technology, Jessore, Bangladesh. Five mice were used for each treatment group. The animals were housed in a well-ventilated hygienic experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment.

### 2.4 Evaluation of Analgesic & Antipyretic Activity of the Methanolic Extract of *Hemigraphis hirta*

#### 2.4.1 Drugs and chemicals

- Tween 80 (1%)
- Sterile normal saline's solution (0.9% NaCl)
- Diclofenac sodium

- 0.7% acetic acid solution
- 3% formalin solution
- Paracetamol 500 mg
- Brewer's yeast

### 2.4.2 Route of administration

For analgesic test, the extracts were administered orally [per oral (p.o.) route]. Diclofenac sodium, 0.7% acetic acid solution and 3% formalin solution were administered intra-peritoneally (i.p.). For antipyretic test, the extracts, standard drug paracetamol (100 mg/kg) were administered orally [per oral (p.o.) route]. Brewer's yeast 15% (w/v) was injected subcutaneously (s. c.).

### 2.4.3 Preparation of the test materials and standard for the test

Crude methanol extract were triturated by the addition of small amount of suspending agent (Tween-80). Normal saline (0.9% NaCl) was slowly added to make the concentration of the solution 400 mg/kg and 200 mg/kg. For analgesic test, for the preparation of standard, Diclofenac sodium (10 mg) was dissolved into 0.9% Normal saline to make the concentration 10 mg/kg. For the preparation of 0.7% acetic acid solution, 0.7 ml glacial acetic acid was mixed with distilled water to 100 ml. For antipyretic test, for the preparation of standard, (Paracetamol 500 mg) was dissolved into 0.9% Normal saline to make the dose 100 mg/kg. Control sample containing tween- 80 (1%) in 0.9% Normal saline.

### 2.4.4 Designing of the experiment

The experimental animals were randomly divided into four groups consisting of five mice in each group. The groups were denoted from group-I to group- IV. Group I and II used as control and standard and group III and IV used as treatment groups. Each group of mice received a specific treatment. Prior administering the drugs, each mice was weighed properly and the doses were adjusted accordingly.

### 2.4.5 Acetic acid-induced writhing test in mice

The test was conducted by modified method of Koster et al [8]. Swiss albino mice were divided into four groups containing five mice each. The first group served as control and was given 10 ml/kg intra peritoneal normal saline to act as negative control. Groups III and IV received 200 and 400 mg/kg body weight in intra-peritoneal route respectively, while the II group was given 10 mg Diclofenac sodium per kg body weight i. p to act as positive control. Thirty minutes later, each mouse was injected with 0.7% acetic acid of 1 ml per 100 g (i. p). The number of abdominal constriction for each mouse was counted after injection of acetic acid for a period of twenty minutes.

Percentage inhibition of writhing was calculated using the formula.

$$\text{Inhibition \%} = \frac{\text{Mean no of writhes (control)} - \text{mean no of writhes (test)}}{\text{Mean no of writhes (control)}} \times 100$$

### 2.4.6 Formalin-induced paw licking in mice

The formalin-induced nociception test was performed by slight modification of Nakamoto et al. [9]. Mice were divided into four groups of five mice each and were injected with 20 µl of 3% formalin into the subplantar space of the left hind paw 30 min after extract (200 and 400 mg/kg) or vehicle (1% tween 80) or standard drug (Diclofenac sodium 10 mg/kg, i.p.) administration. Mice were observed and the time spent in licking, biting, and shaking behaviors was measured in seconds during the early phase (0–5 min) and late phase (15–30 min).

$$\text{\% Inhibition} = \frac{\text{Mean paw licking time (control)} - \text{mean pawlicking time (test)}}{\text{Mean paw licking time (control)}} \times 100$$

### 2.4.7 Brewer's yeast-induced pyrexia test

This test was performed with slight modification of Turner et al. [10]. Mice were fasted overnight with water *ad libitum* before inducing pyrexia. Their rectal temperatures were recorded before inducing pyrexia by using an electric thermometer, which was connected with a probe and inserted 2 cm into the rectum. Subcutaneous injection of a 15% (w/v) suspension of brewer's yeast at a dose of 10 mL/kg in the back below the nape of the neck induced pyrexia. The suspension was spread under the skin by massaging the injection site. The increase in rectal temperature was recorded 18 h after injection, and the mice that showed an increase in temperature of at least 0.6℃ were considered pyretic mice and used for brewer's yeast-induced pyrexia test. The tested samples including paracetamol (100 mg/kg) as standard, normal saline (0.9% NaCl) as control and plant extracts as described before, which were given orally to

the pyretic mice, were investigated for their antipyretic activity. All group's temperatures were recorded at 1, 2, 3 and 4 hours.

## 3. RESULTS AND DISCUSSION

### 3.1 Evaluation of Analgesic activity

#### 3.1.1 Formalin-induced paw licking test

In early phase,

The standard drug (Diclofenac sodium, 10 mg/kg) exhibited a reduction of paw licking time in the early phase with a mean of (96.8 ± 13.56) sec but failed to produce significant result compared to that of control (105.6 ± 8.68). Percent of inhibition by the standard drug (Diclofenac sodium) was 56.56% compared to that of control. Methanol extracts at dose 200 mg/kg and 400 mg/kg reduced paw licking time in the early phase with a mean of (73.2 ± 6.93) sec and (73.8 ± 9.02) sec which were highly significant ($p < 0.05$) compared to both standard and control. Percent of inhibition by the two doses were 44.79% and 44.34% respectively, compared to that of control.

In late phase,

The standard drug (diclofenac sodium, 10 mg/kg), exhibited a reduction of paw licking time in with a mean of (4.2 ± 1.36) sec which was very highly significant ($p < 0.0001$) compared to that of control (44.6 ± 1.60). Percent of inhibition was 84.56 % compared to that of control. Methanol extracts at 200 mg/kg also reduced paw licking time in the late phase with a mean of (7.60 ± 6.1) sec which was highly significant ($p < 0.001$) and percent of inhibition was 90.07% which is comparable to standard drug (Diclofenac sodium). Methanol extracts at 400 mg/kg also exhibited a reduction of paw licking time in the late phase with a mean of (16.6±8.8) sec which was significant ($p < 0.05$) compared to that of control and percent of inhibition was 83.46%. The effect of the methanol extract of *Hemigraphis hirta* at 200 mg/kg was better than the standard drug both in the early and late phase and 400 mg/kg dose was better than the standard drug during the early phase, not in the late phase. The results are shown in Table 1.

During the formalin test, the response time of the animals spends in licking the injected paw were measured. Two distinct periods of high licking activity was identified, an early phase lasting the first 5 min and a late phase lasting from 20 to 30 min after the injection of formalin. The results demonstrate that two phases in the formalin test may have different nociceptive mechanisms. It is suggested that early phase is due to a direct effect on nociceptors and prostaglandins do not play an important role during this phase. The late phase seems to be an inflammatory response with inflammatory pain can be inhibited by anti-inflammatory drugs.

#### 3.1.2 Acetic acid-induced writhing test in mice

The analgesic effect of methanol extract of *Hemigraphis hirta* in acetic acid induced writhing test was shown in the Table 2.

The standard drug (Diclofenac sodium, 10 mg/kg) exhibited a reduction of number of acetic acid-induced writhes in mice with a mean of (28.20 ± 3.81) but failed to show significant result compared to that of control (39.20 ± 7.47). Percent of inhibition by the standard drug (Diclofenac sodium) was 38.27% compared to that of control. Methanol extracts at 200 mg/kg also reduced the number of acetic acid-induced writhing with a mean of (10.80 ± 4.25) which was

**Table 1. Effects of methanolic extracts of *Hemigraphis hirta* on formalin-induced paw licking test**

| Treatment groups | 0-5 min (early phase) | % inhibition | *p* value | 20-30 min (late phase) | % Inhibition | *p* value |
|---|---|---|---|---|---|---|
| Control (0.9% NaCl) | 105.6± 8.68 | 0.0 | - | 44.6± 1.60 | 0.0 | - |
| Diclofenac sodium (10 mg/kg) | 96.8±13.56 | 56.56 | 0.617 | 4.2±1.36*** | 84.56 | 0.0001 |
| Methanolic extract (200 mg/kg) | 73.2±6.93* | 44.79 | 0.004 | 7.60 ± 6.1** | 90.07 | 0.001 |
| Methanolic extract (400 mg/kg) | 73.8±9.02* | 44.34 | 0.005 | 16.6 ± 8.8* | 83.46 | 0.006 |

*Number of licking time inhibition are presented as (mean ± standard error of mean). *p <0.05, vs control; **p <0.001, vs control; ***p <0.0001, vs control. (Dennett's t test)*

**Table 2. Effects of methanolic extracts of *Hemigraphis hirta* on Acetic acid-induced writhing test in mice**

| Treatment groups | Dose mg/kg | No of writhing | % of inhibition | *p* value |
|---|---|---|---|---|
| Control (0.9% NaCl) | 10 | 39.20±7.47 | 0.00 | - |
| Diclofenac sodium | 10 | 28.20±3.81 | 38.27 | 0.294 |
| Methanolic extract | 200 | 10.80±4.25* | 72.45 | 0.002 |
| Methanolic extract | 400 | 18.40±2.65* | 53.06 | 0.022 |

*Number reduction of writhing are presented as (mean ± standard error of mean). *p <0.05, vs control (Dennett's t test)*

**Table 3. Effects of methanolic extracts of *Hemigraphis hirta* in brewer's yeast induced pyrexia**

| Treatment group (Dose) | Temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | Initial | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr |
| Control (0.9% NaCl) | 33.74±.73 | 35.58±.65 | 35.28±.67 | 34.28±.53 | 33.10±.66 | 32.86±.56 |
| Paracetamol (100 mg/kg) | 32.90±.24 | 35.14±.14 | 31.74±.23 | 32.40±.31 | 32.56±.41 | 31.80±.25 |
| Methanolic extract (200 mg/kg) | 34.80±.35 | 35.32±.48 | 33.58±.39 | 33.54±.29 | 32.76±.26 | 33.32±.73 |
| Methanolic extract (400 mg/kg) | 35.62±19 | 36.34±.19 | 34.70±.32 | 33.36±.16 | 32.56±.12 | 32.68±.09 |

significant ($p < 0.05$) and percent of inhibition was 72.45% which is compared to that of control. Methanol extracts at 400 mg/kg also exhibited a reduction of number of acetic acid-induced writhing with a mean of (18.40 ± 2.65) which was significant ($p < 0.05$) and percent of inhibition was 53.06%, compared to that of control. The effect of methanol extract at both doses (200 mg/kg and 400 mg/kg) were better than the standard drug (Diclofenac sodium).

Acetic acid produced nociception by liberating endogenous substances including serotonin, bradykinin, histamine and prostaglandin, which may stimulate sensory nerve ending [11,12]. Therefore, *Hemigraphis hirta* extract might be inhibiting the synthesis and /or release of these endogenous substances. This may be concluded that, methanol extract of *Hemigraphis hirta* could be beneficial in the management of pain.

### 3.2 Evaluation of Antipyretic Activity Using Brewer's Yeast Induced Pyrexia Test

The antipyretic effects of *Hemigraphis hirta* in Brewer's yeast induced pyrexia test are shown in Table 3. The subcutaneous injection of yeast markedly increased the rectal temperature and the mean increment recorded was 1.24– 2℉ after 18 hours of administration. The extract and paracetamol treatment groups showed a reduction of temperature over period of time from 1 hour to 4 hours but failed to show any significant activity. The methanol extract at the dose of 400 mg/kg and 200 mg/kg body weight reduced hyperthermia in mice in 1 hour observation and also lowering of temperature from 2 hours to 4 hours observation period in comparison to control. Standard drug paracetamol also inhibited pyrexia in early and latter hours of observation time intervals.

Brewer's yeast used in this experiment is an exogenous pyrogen which initiates the synthesis and release of various endogenous cytokine factors like IL-1, IL-6, TNF-α [13,14]. These endogenous cytokines activates arachidonic pathway by easily crossing the blood brain barrier and acting on the preoptic/anterior hypothalamus which results in the synthesis and release of prostaglandins (PGE2) which set the thermoregulatory center at a higher temperature [15]. The methanolic extract of *Hemigraphis hirta* showed more pronounced effect in lowering the hyperthermia and found to have similar effect as the standard drug paracetamol in early and latter hours of observation time intervals. 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 [16].

## 4. CONCLUSION

The aim of this study was to identify the analgesic and antipyretic activity of *Hemigraphis hirta* and on the light of the conducted research

and data we can say that the plant extracts acts significantly in dose dependent manner in contrast to control medications to reduce analgesia and pyrexia which is a great promise for future experimentations. But it is yet to discover exactly which compounds and through which mechanism they are acting. We hope that this research would be helpful for the enthusiastic researchers in future who tend to identify the specific compounds and their mechanism of action.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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