



AN *IN-VIVO* STUDY ON ANALGESIC AND ANTIPYRETIC ACTIVITY OF BARK EXTRACTS OF *GMEILINA ARBOREA*

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Accepted on: 30-06-2011; Finalized on: 25-09-2011.

ABSTRACT

The objective of the present study is to evaluate the analgesic and antipyretic activity of benzene, chloroform, ethanol, and aqueous extracts of the heart wood bark of the plant *Gmelina arborea*. Healthy albino mice weighing about 30-40gm were used to evaluate the analgesic activity. The peripheral analgesic activity of different extracts of *Gmelina arborea* was investigated by the acetic acid induced writhing test and the central analgesic action was assessed by tail-immersion test in mice. Wister strain albino rats with Yeast induced Pyrexia (n = 6) were administered with benzene, chloroform, ethanolic and aqueous bark extracts of the plant *Gmelina arborea* (420 mg/kg) to evaluate the antipyretic activity. The aqueous and ethanolic extracts of bark of the plant *Gmelina arborea* exhibited significant antipyretic activity as compare to the standard drug paracetamol (50 mg/kg body weight). However the analgesic activity of test compounds was found to be more significant on acetic acid-induced test than tail flick test and thus it appears that the test compounds inhibit predominantly the peripheral pain mechanism. It is concluded that barks of the plant *Gmelina arborea* has significant analgesic and antipyretic activity as compared to the standard drug.

Keywords: Analgesic activity, Antipyretic activity, yeast induced pyrexia, *Gmelina arborea*.

INTRODUCTION

Gmelina arborea is an unarmed deciduous medium size to large plant about 12-30 m, height 60–100 cm diameter with white spikes¹. This plant is commonly known as Bhadrarni or Gambhari in Orissa. The roots, fruits and the leaves of gambhari have great medicinal value. Almost all parts of this tree are used in folk medicine for treating various stomach disorders, fevers and skin problems². Externally, the paste of the leaves is applied on the forehead to alleviate the headache, especially in fever. To mitigate the burning sensation of the body, fresh juice of leaves is massaged, with great benefit. Internally, the roots and fruits of gambhari are used in vast range of diseases. The fruits are recommended in raktapitta, excessive thirst, dysuria, sexual debility in males and habitual abortion. The roots alleviate flatulence, augment the appetite and are salutary in piles, being mild laxative.

Roots of *Gmelina* are used in commercial Ayurvedic preparations³. The plant extracts are reported to exhibit anti-inflammatory^{4,5} and wound healing properties⁶ and are also known to inhibit platelet aggregation⁷. The ethanolic extract of the dried root reported to possess anti-diarrhoea activity⁸. In Vitro studies on bark and fruit extracts showed antioxidant activity⁹ and protected liver slice culture cells by alleviating oxidative stress-induced damage to liver cell. However no systematic study on analgesic and antipyretic activity of the heart wood bark has been reported. In the present investigation we have screened the Benzene, chloroform ethanolic, and aqueous extracts of the heart wood bark of the plant *Gmelina arborea* (family Vervencaceae) for analgesic and anti-pyretic activity.

MATERIALS AND METHODS

Collection of plant materials: The heart wood bark of *Gmelina arborea* was collected in the month of Sept – October 2007 from the tribal area of Bhadrak and Balasore district of Orissa and prepared herbaria of some plant was authenticated at Botanical Survey of India, at Central National Herbarium, PO – Botanical garden, Howrah – 711103. A voucher specimen no CNW/I-I/(207)2007/TECH-II has been deposited in the same department. Heart wood bark of the *Gmelina arborea* was shade dried and coarsely powdered for preparation of extracts.

The powdered plant materials was subjected to continuous hot extraction (soxhlation) with benzene, chloroform, ethanol and the aqueous extracts was prepared by simple maceration process at room temperature. These extracts were stored in desiccators and used for the screening of antipyretic activity.

Animals: Inbred male albino rats (Wister strain) weighing between 120-150 gm and healthy albino mice weighing about 30-40gm used in the study were selected from animal house, Gayatri College of pharmacy, Sambalpur, Odisha. They were housed under standard laboratory conditions for week before the experiments. The housing conditions were maintained at controlled temperature (23°C) and humidity (50%). They received standard diet and water. The animals were transferred to the laboratory 1 hr before starting the experiment. The experiment was carried out according to committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines and Institutional Animal Ethical Committee approved all the procedures.



Experiments were conducted between 9:00 to 14 hr. Each animal was used only once.

Determination of LD₅₀: LD₅₀ was found from previously published report⁵.

Analgesic Activity: The peripheral analgesic activity of different extracts of *Gmelina arborea* was investigated by the acetic acid induced writhing test and the central analgesic action was assessed by tail-immersion test in mice.

Acetic acid induced writhing in mice: The extracts and standard compound were suspended in 10% tween-20 and administered subcutaneously. The control group animals, however received the same volume of vehicle. In this study, the animals were administered a 420mg/kg (body weight) dose of the extracts and 50 mg/kg (body weight) dose of standard drug Paracetamol. The writhing¹⁰ was induced by injecting 0.1 ml of a 0.6% acetic acid intraperitoneally after 30 minutes of drug administration and writhes were recorded 10min after administering acetic acid for next 10min. The nociceptive effect was expressed as the percentage of protection compared to the control group and reported in Table 1.

Table 1: Analgesic Activity of *Gmelina arborea* extracts on writhing responses in mice.

Compounds	Writhing times (Mean ± SEM)	Protection (%)
Control	50 ± 0.68	-
Benzene Extract	30 ± 0.31**	40
Chloroform Extract	26 ± 0.06**	48
Ethanol Extract	14 ± 0.40**	72
Aqueous Extract	19 ± 0.25**	62
Paracetamol (50 mg/kg)	17 ± 0.25**	66

n= 6 in each group. **P < 0.0001, *P < 0.001

Tail Immersion Test: Healthy albino mice weighing about 30-40gm were taken. They were then placed into individual restraining cages leaving the tail¹¹ hanging out freely. The animals are then allowed to adapt in the cages for 30 minutes before testing. The lower 3.5 cm portion of the tail was immersed in a cup of freshly filled water of exactly 55±5°C. Prior to analgesic experiment, the animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55±5°C. The animals withdrawing the tail from hot water within 5 seconds were selected for the study. The selected mice

were then divided into 6 groups having 6 each and numbered. The animals marked group I received orally 1ml/100gm of body weight of 0.5 %w/v solution of sodium lauryl sulphate and served as control. The animals marked group II received orally 25mg/kg body weight of diclofenac sodium in 0.5%w/v suspension of sodium lauryl sulphate and served as standard. The animals marked test group III to VI received 420 mg/kg body weight of Benzene, Chloroform, Ethanol and Aqueous extracts of the barks of *Gmelina arborea* were screened for analgesic activity. After the drug was administered the reaction time was recorded at an interval of 0, 30, 60, 90 and 120 minutes (Table 2). The latency to withdraw the tail was recorded in sec with a stopwatch and a cut-off maximum latency of 7 second was established in order to prevent tissue damage.

Induction of pyrexia in Wister strain albino rats: Prior to the experiment, the animals were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature for 7 days were selected for the study. They were divided up to 6 groups having 6 animals each and fasted for 24 hours before inducing pyrexia. Pyrexia was induced¹² by subcutaneous injection of 20 ml/kg (S. C.) of 20 % brewer's yeast suspension in saline solution below the nape of the neck and rectal temperature was recorded by clinical thermometer by introducing one inch into rectum and keeping it inside for one minute immediately before (-18hr) and 18hr after (0 hr) brewer's injections.

Antipyretic study: The pyrexia rats marked group I received 1ml/100 gm of body weight of 0.5% w/v solution of carboxy methyl cellulose orally and served as control. The group II animals received 50mg/kg of body weight of Paracetamol and served as standard. The test group III to VI received 420 mg/kg body weight of various extracts orally as a suspension.

The rectal temperature were recorded using a clinical thermometer by introducing one inch into rectum at 1, 2, 3, and 4 hour after zero hour reading and the effects of extracts were observed and compared with the effect of standard paracetamol drug.

Statistical Analysis: The results have been impressed mean + SEM and significance of the result were analyzed by student 't'-test-compared with control¹³.

Table 2. Analgesic activity of *Gmelina arborea* extracts on tail immersion method in mice.

Compounds	Predrug Reaction time in sec (Mean±SEM)	Post Drug reaction time in sec. (Mean ± SEM)			
		30 Min.	60 Min.	90 Min.	120 Min.
Control	3.28±0.24	3.31±0.17	3.38±0.18	3.43±0.12	3.38±0.24
Benzene Extract	3.57 ± 0.22	3.65 ± 0.13	3.84 ± 0.24*	4.23 ± 0.14**	4.37 ± 0.09**
Chloroform Extract	4.05 ± 0.15	4.21 ± 0.11	4.50 ± 0.14	4.82 ± 0.12*	5.06 ± 0.01**
Ethanol Extract	4.16 ± 0.09	4.28 ± 0.02	4.56 ± 0.11*	4.83 ± 0.11*	5.20 ± 0.12**
Aqueous Extract	4.21 ± 0.10	4.38 ± 0.09	4.56 ± 0.12	4.89 ± 0.15**	5.30 ± 0.10**
Diclofenac sodium (25 mg/kg)	3.15±0.08	4.62±0.31***	4.85±0.27***	5.16±0.25***	5.61±0.45***

n= 6 in each group. **P < 0.0001, *P < 0.001



Table 3: Antipyretic effect of bark extract of *Gmelina arborea*.

Treatment	Rectal temperature in °C at time (h)					
	-18 ^a	0 ^b	1 ^c	2 ^c	3 ^c	4 ^c
Control	36.84 ± 0.21	37.86 ± 0.35 (+1.02) ^c	37.96 ± 0.35	37.93 ± 0.21	38.73 ± 0.27	38.63 ± 0.27
Paracetamol (50 mg/kg)	36.66 ± 0.24*	37.83 ± 0.43* (+1.17) ^c	37.66 ± 0.23**	37.36 ± 0.31***	37.06 ± 0.29**	36.93 ± 0.27*
Benzene Extract	36.43 ± 0.17***	37.32 ± 0.15* (+0.89) ^c	37.7 ± 0.05*	37.83 ± 0.17**	37.3 ± 0.33*	37.16 ± 0.03**
Chloroform Extract	36.66 ± 0.26*	37.46 ± 0.38* (+0.80) ^c	38.03 ± 0.29*	37.9 ± 0.28*	37.23 ± 0.45*	37.05 ± 0.29*
Ethanollic Extract	36.7 ± 0.35*	37.63 ± 0.23* (+0.93) ^c	37.23 ± 0.39*	37.06 ± 0.45***	36.66 ± 0.13**	36.73 ± 0.24**
Aqueous Extract	36.73 ± 0.09*	37.82 ± 0.1* (+1.09) ^c	37.2 ± 0.17*	36.96 ± 0.21**	36.8 ± 0.34*	36.8 ± 0.17**

n: Six animals in each group, values are Mean ± SEM, * p<0.05, ** p<0.02, *** p<0.01; a: Temperature just before yeast injection; b: Temperature just before drug administration; c: Change in temperature following yeast injection

RESULTS AND DISCUSSION

Search for herbal remedies with potent antipyretic activity received momentum recently as the available antipyretics, such as paracetamol, nimesulide etc. have toxic effect to the various organs of the body. The body's ability to maintain a natural balance of COX 1 and 2 that regulate inflammatory response play a crucial role in supporting cardiovascular, immune, neurological, and joint and connective tissue systems. A number of plant extracts modulate enzymes of cyclooxygenase pathway, as reported with the rosmarinic acid of *Rosmarinus officinalis* that inhibit leukotriene and prostaglandins synthesis, while COX-1 and COX-2 was inhibited by cirsilineol, cirsimaritin, apigenin, rosmarinic acid and eugenol of *Ocimum sanctum* similar to ibuprofen, naproxen, and aspirin. The present work has demonstrated the antipyretic activity of the benzene, aqueous, chloroform and ethanolic extracts of the bark of the plant *Gmelina arborea* in yeast induced pyrexia in wister strain albino rats. The experimental rats showed a mean increase of about 0.86°C in rectal temperature, 18 h after Brewer's yeast injection. The ethanolic and aqueous extracts at a dose of 420 mg/kg body weight reduced the hyperthermia 1h after administration and its effect is comparable to that of the standard antipyretic drug paracetamol while chloroform and benzene extract reduced the temperature 3h after their administration but have mild effects.

However the analgesic activity of test compounds was found to be more significant on acetic acid-induced test than tail flick test and thus it appears that the test compounds inhibit predominantly the peripheral pain mechanism. In writhing tests, the abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. The results of the study indicate that the extracts of the bark of plant *Gmelina arborea* showed analgesic activity by reducing the abdominal constriction significantly and may supposed to have possible role in inhibition of cyclooxygenase in the prostaglandin pathways. Usually

most anti-inflammatory drugs possess analgesic and antipyretic activity. Previously published report suggests that Premnazole, an isoxazole alkaloid isolated from *Premna integrifolia* L. and *Gmelina arborea* L. (Verbenaceae) demonstrated significant anti-inflammatory activity in reducing cotton pellet-induced granuloma formation in rats and the anti-inflammatory activity was comparable to that of phenylbutazone⁵.

In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus. Therefore, the analgesic and antipyretic activity of chloroform and alcoholic extracts of *Gmelina arborea* is probably by inhibition of prostaglandin synthesis in hypothalamus.

CONCLUSION

It can be concluded that aqueous and alcoholic extracts have maximum antipyretic and analgesic activity as it reduce the rectal temperature significantly with compared to standard paracetamol and diclofenac sodium respectively in a regular interval of time. More extensive studies are required to isolate and characterize all the components and to confirm there mechanism of action so as to develop it as an antipyretic and analgesic drug.

Acknowledgement: The another sincerely thank the central national herbarium, PO Botanical garden, Howrah, for authentication of Herbarium and dept of pharmacognosy, Gayatri College of Pharmacy Sambalpur Orissa providing accompany facility for carrying out of this work.

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