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Research Article

ANTIPYRETIC AND ANALGESIC EFFECT OF METHANOLIC EXTRACT OF DIFFERENT PARTS OF *ABROMA AUGUSTA* LINN

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ABSTRACT

Abroma augusta Linn (Family-Malvaceae) commonly known as Ulatkambal traditionally used as dysmenorrhoea, ammenorrhoea, wound healing, sterlilty and other menstrual disorder. The purpose of this study was to evaluate the methanolic extract *Abroma augusta* Linn for antipyretic and analgesic activity, and determine its probable mechanism of action. Pyrexia was induced in rats by intravenous injection of 15% suspension of Brewer's yeast. Rectal temperature was monitored at 30, 60, 120 and 180 min post-administration of 250 mg/kg of leaves, barks and roots extract. The analgesic effect of the extract was evaluated using Eddy's hot plate, acetic acid-induced mouse writhing test and tail immersion test using Swiss albino mice. It revealed the significant antipyretic activity of the extract (250 mg/kg) comparing with paracetamol standard. The extract also showed significant analgesic effect in different model where bark has showed the higher effect than leaf and root comparing with the different standard analgesics. The extract possesses antipyretic and analgesic activities which validate its use in the treatment of pains and fevers.

Keywords: *Abroma augusta* Linn (Family-Malvaceae), Methanolic extract, Antipyretic, analgesic.

INTRODUCTION

Over the years, plants have generally proven to be veritable sources of drugs used in orthodox medicine. This has in recent times encouraged the search for newer more efficacious and better tolerated drugs from plants. A criterion that has been used over the years for the selection of plants for pharmacological investigations is reported use in traditional medicine. Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states[1]. It is the body's natural function to create an environment where infectious agents or damaged tissues cannot survive. Normally, the infected or damaged tissue initiates the enhanced formation of proinflammatory mediators (cytokines, such as interleukin 1β, α, β, and TNF- α), which increase the synthesis of prostaglandin E2 (PgE2) near hypothalamic area and thereby trigger the hypothalamus to elevate the body temperature[2]. When body temperature becomes high, the temperature regulatory system, which is governed by a nervous feedback mechanism, dilates the blood vessels and increases sweating to reduce the temperature. When the body temperature becomes low, hypothalamus protects the internal temperature by vasoconstriction.

High fever often increases faster disease progression by increasing tissue catabolism, dehydration, and existing complaints, as found in HIV[3]. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE2 biosynthesis[4]. These synthetic agents irreversibly inhibit COX-2 with a high selectivity and are toxic to the hepatic cells, glomeruli, cortex of brain, and heart muscles.

Abroma augusta Linn (Family-Malvaceae) commonly known as Ulatkambal in Hindi and Devil's cotton in English. A genus of evergreen plant large, spreading, quick-growing hairy shrub or a small tree with velvety branches, found in tropical Asia, South and eastern Africa, and Australia. It is mainly used for dysmenorrhoea, ammenorrhoea, wound healing, sterlilty and other menstrual disorder. Powdered root act as anabortifacient and anti-fertility agent. Leaves are useful in treating uterine disorders, diabetes, rheumatic pain of joints, and headache with sinusitis. Leaves and stem are demulcent and an infusion of fresh leaves and stem in cold water is very efficacious in gonorrhea. The root-bark is used as an emmenagogue and uterine tonic. [5, 6, 7]

However this plant has not been studied for antipyretic and analgesic activity. The aim of this study is to investigate the analgesic and antipyretic activities of the methanolic different parts of plant extract of *Abroma augusta* in rodents using different pharmacological models.

MATERIALS AND METHODS

Collection of plant materials

The leaves, bark, root of *Abroma augusta* was collected from Siliguri, Raigang, West Bengal, India. A herbarium sheet was prepared & it was sent to A.J.C.B INDIAN BOTANIC GARDEN, Shibpur, Howrah and West Bengal, India for authentication. The authentication no. of the study plant is" CNH/111/2011/Tech.II/627". The leaves, bark, root of *Abroma augusta* was collected and dried under shade. These dried materials were mechanically powdered, sheaved using 80 meshes and stored in an airtight container. These powdered materials were used for further physiochemical, phytochemical study (Figure 1).

Fig 1: Picture showing the *Abroma augusta* plant

Preparation of extract

The air dried crushed leaves, bark and roots (1000g) were soaked for 12 hr in Methanol (3L) at room temperature. The residue was extracted with hot Methanol under reflux 3 times (each 1500 ml) after vacuum filtration. All solvent was evaporated under vacuum and extract was then lyophilized, to yield approximately 12% w/w/) of the residue, which was stored at 20ºC until use. The concentrate was suspended in 5% w/v Tween 80 and given at dose 1ml/100gm body weight.

Treatment of animals

Healthy male and female rats (Wistar albino) and Mice (Swiss Albino) (25-30g) of 4-8 weeks old were selected after physical and behavioral veterinary examination from Institutional Animal House of Gupta College of Technological Sciences. The weight range was fall within ± 20% of the mean body for each sex at the time of initiation

of treatment. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal ethics committee (955/A/06/CPCSEA).

Sixty young adult male Wistar rats (120–150 g) and Swiss Albino Mice (25-30g), were obtained from the Institutional Animal House of Gupta College of Technological Sciences. The rats and mice were housed in polyethylene cages in the Animal House. They were housed in polyethylene cages, allowed one week of acclimatization, and maintained on standard rat chow and standard laboratory conditions throughout the experiment.

In-vivo antipyretic study

Brewer's yeast induced pyrexia

The subcutaneous injection of Brewer's yeast suspension is known to produce fever in rats. A decrease in temperature can be achieved by administration of compounds with antipyretic activity. Male or female rats with a body weight between 120 -180 g were used. The animals were starved overnight. The animals were fasted for 18 hours prior to the experiment. Animals were divided into five groups of six animals each and marked. By insertion of a thermometer to a depth of 2 cm into the rectum the initial rectal temperatures are recorded. 15% suspension of Brewer's yeast in 0.9% saline was prepared. Groups of 6 male or female Wistar rats with a body weight of 150 g were used. The animals were fevered by injection of 10 ml/kg of Brewer's yeast suspension subcutaneously in the back below the nape of the neck. The site of injection was massaged in order to spread the suspension beneath the skin. Immediately after yeast administration, food is withdrawn. 18 h post challenge, the rise in rectal temperature was recorded. Only animals with a body temperature of at least 38 °C were taken into the test. The room temperature is kept at 22–24 °C. The measurement was repeated after 30 min. drug. Group I received Tween-80 (1%, i.p.) and served as control, GroupII received Paracetamol (100mg/kg) and served as standard. Group III, group IV, group V received the leave, bark, root (250mg/kg i.p.) respectively[8]. Rectal temperatures were recorded again 30, 60, 120 and 180 min post dosing. The results were tabulated by % inhibition by using formula

Temperature reduced in control= Temperature reduced in test / Temperature reduced in control ×100

In-vivo Analgesic Activity

A) Eddy's hot plate method

In this method heat is used as source of pain. Animals are individually placed on a hot plate maintained at constant temperature (55 ºC) and the reaction of the animal such as paw licking or jump response is taken as end point. The method was first described by Eddy and Leimbach. Male or female Swiss albino with a body weight between 120 -180 g are used. The animals were starved overnight. The animals were fasted for 18 hours prior to the experiment. Animals are divided into five groups of six animals each and marked. Group I received Tween-80 (1%, i.p.) and served as control. Group II received Diclofenac sodium 5mg/kg and served as standard. Group III, group IV, group V received the leave, bark, root (250mg/kg i.p.) respectively. After administration of test and standard drug, the test for analgesia was carried out by placing the mice on electrically heated plate at 55ºC +/- 0.5 º C and noting the signs of discomfort, i.e., it may lick its fore paws or jump out of the plate. The time was noted in seconds. Test was carried out similarly for animals of control group. The observations were made at 30' and 60'. The results are tabulated graphically[9].

B) Acetic acid induced writhing reflex

Pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhing. The test is suitable to detect analgesic activity. An irritating agent such as phenyl quinone or acetic acid is injected intraperitoneally to mice and the stretching reaction is evaluated. Male or female Swiss albino mice with a body weight between 20-30 g were used. The animals were starved overnight. Animals were divided into five groups of six animals each and marked. Group I received Tween-80 (1%, i.p.) and served as control. After 15 min, acetic acid solution1%v/v (inject 1ml/100g of body weight of the animal) was inject to the Group I. Number of Wriths were noted during a period of 10min. Group II received Tramadol 5mg/kg and served as standard. Group III, group IV, group V received the leave, bark, root (250mg/kg i.p.) respectively. After 15 min, acetic acid solution1%v/v (inject 1ml/100g of body weight of the animal) was inject to the Group I,II,III,IV,V& number of Wriths were noted during a period of 10min,respectively. The results are tabulated by % of inhibition

Mean of control group= Mean of treated group / Mean of Control group ×100

C) Tail immersion test

The procedure is based on the observation of drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55 °C. Male or female Swiss albino with a body weight between 20-30 g was used. The animals were starved overnight. Animals were divided into five groups of six animals each and marked. They were placed into individual restraining cages leaving the tail hanging out freely. The lower 5 cm portion of the tail was marked. This part of the tail was immersed in a cup of freshly filled water of exactly 55 °C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time was recorded by a stopwatch. After each determination the tail was carefully dried. Group I received Tween-80 (1%, i.p.) and served as control. Group II received Tramadol 5mg/kg and served as standard. Group III, group IV,group V received the leave, bark, root (250mg/kg i.p.) respectively. After 15 min, tail withdrawing reaction was recorded. The results are tabulated graphically[10, 11].

Statistical analysis

Results are expressed as the mean value ± standard error of mean (S.E.M.). Within group comparisons were performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups was assessed by student's t-test. A probability level of less than 5 %($P < 0.05$) was considered significant.

RESULTS

In-vivo antipyretic activity

Methanolic Extracts were evaluated for Antipyretic activity. In Brewer's induced pyrexia, the intraperitonially administration of leaves, bark and root induced a significant antipyretic activity in a dose-dependent manner in the rats. Bark extract has shown significant analgesic effect than root and leaves (Table 1) (Figure 2).

Table 1: Effect of Methanolic extract of *Abroma augusta* on Brewer's yeast induced pyrexia

GROUP	DOSE Mg/kg	Rectal temperature							
		30min		60min		120min		180min	
		RT(ºC)	TI%	RT(ºC)	TI %	RT(ºC)	TI%	RT(ºC)	TI %
Control	----	37.9±0.13		37.9±0.08		38.5± 0.09		38.9±0.05	
Standard	100	37.2±0.14	1.88	36.66±0.8*	3.27	36.36±0.04**	5.55	35.9±0.12**	7.71
Bark	250	37±0.12	1	36.75± 0.79*	3.03	36.71± 0.09*	4.67	36.63± 0.07*	5.8
Root	250	36.76±0.12	1.87	36.71±0.09*	3.26	36.7± 0.08*	4.80	36.7± 0.11*	5.65
Leaves	250	37.06±0.12	0.9	36.65±0.08*	3.27	36.65± 0.09*	4.67	36.7± 0.09*	5.01

The data are expressed as mean ± S.E.M. Significant differences in each group versus the control were as follows: * P < 0.05. ** P < 0.01.

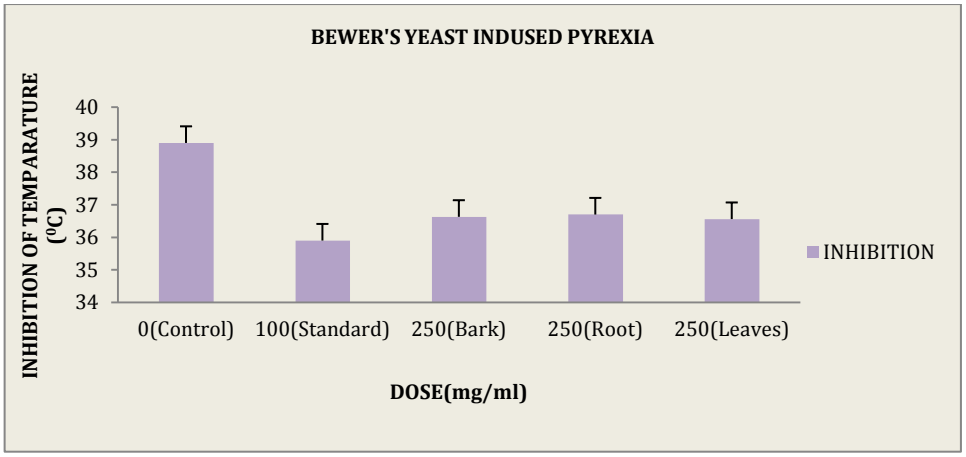

Fig 2: This histrogram showing the inhibition of Brewer's Yeast induced pyrexia in rats.

In-vitro analgesic activity

Analgesic activity of *Abroma augusta* was evaluated using both models to detect central and peripheral analgesics respectively. Acetic acid induced writhing test is used for detecting both central and peripheral analgesics, where as Hot plate model and Tail immersion test is more sensitive to centrally active analgesics

Methanolic Extracts were evaluated for analgesic activity. In Eddy's hot plate method Acetic acid induced writhing response and Tail Immersion method; the intraperitonially administration of leaves, bark, root induced a significant($p<0.01$,$p<0.001$) analgesic activity in a dose-dependent manner respectively in the mice and rats. Root extract has shown significant analgesic effect than bark and leaves, but less compared to Diclofenac sodium as a standard drug. But in Tail immersion method bark extract has shown significant analgesic effect than root and leaves with compare of Tramadol HCl as a standard drug [4] **(Table 2, 3 and 4) (Figure 3, 4 and 5)**

Table 2: Effect of Methanolic extract of *Abroma augusta* on Eddy's Hot Plate Apparatus

Treatment	Reaction time (mean ± s.e.m.) (sec)				
	0 min	30min	60min	90min	120min
Control	9.39 ± 0.21	9.54 ± 0.13	9.63 ± 0.26	9.47 ± 0.19	9.48 ± 0.41
Standard	10.01± 0.28	19.55 ± 0.39	21.98 ± 0.77*	23.00 ±0.69**	26.01 ± 0.51**
Bark	9.85 ± 0.36	10.64 ± 0.39	11.78 ± 0.45	10.92 ± 0.31*	9.58 ± 0.26*
Root	9.87 ± 0.40	14.51 ± 0.40	18.56 ± 0.24*	21.44 ± 0.25**	23.05 ±0.12**
Leaves	9.60 ± 1.4	10.96 ± 1.5	12.57 ± 1.6	13.50 ± 1.81	14.21 ± 1.71*

The data are expressed as mean ± S.E.M. Significant differences in each group versus the control were as follows: * P < 0.05. ** P < 0.01.

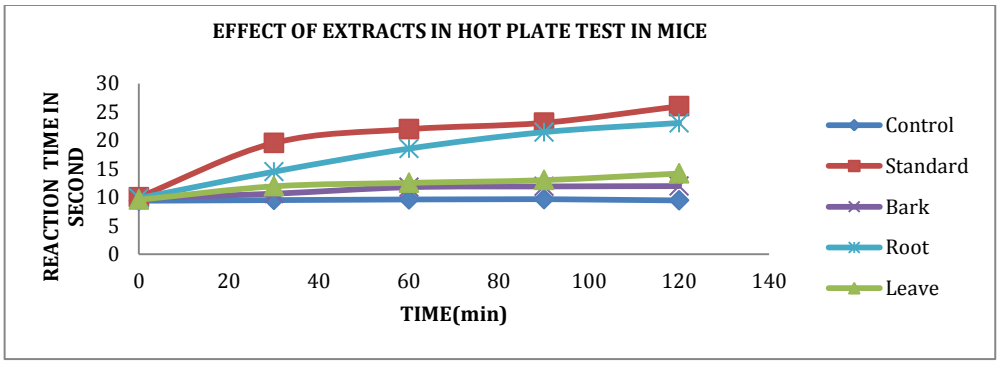

Fig 3 : This graphical representation shows the various response of mice in Eddy's hot plate having constant temperature 55±1°C in 30 min, 60min, 90min, 120min after drug administration.

Table 3: Effect of Methanolic extract of *Abroma augusta* on Writhing Reflex in mice

Group	Dose	Mean number of writhing(10)	%of inhibition
Control	----------	57.66 ± 2.92	----------
Standard	5	16 ± 0.33	72.25**
Bark	250	25.66 ± 0.45	55.49*
Root	250	22.33 ± 0.085	61.27**
Leaves	250	28 ± 0.54	51.24*

The data are expressed as mean ± S.E.M. Significant differences in each group versus the control were as follows: * P < 0.05. ** P < 0.01.

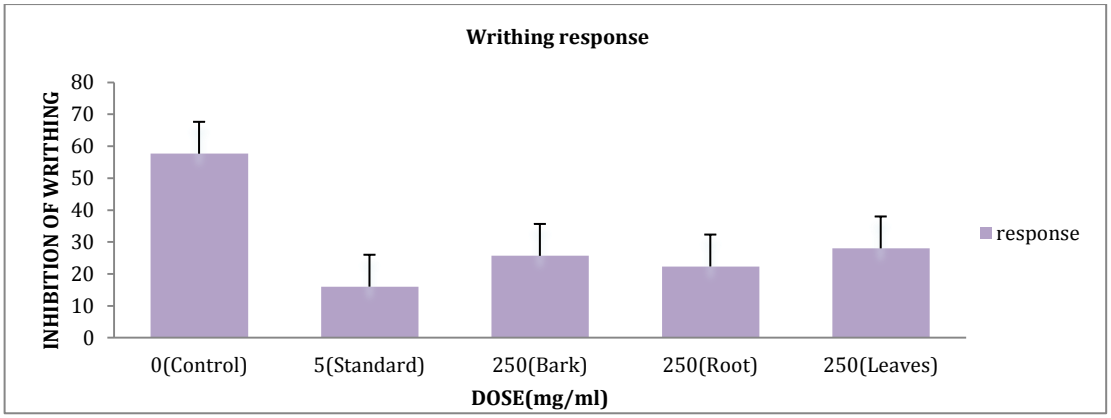

Fig 4: This histrogram showing the inhibition of writhing response after induced acetic acid in mice.

Table 4: Effect of Methanolic extract of *Abroma augusta* on Tail Immersion in rats

Treatment	Reaction time (mean ± s.e.m.) (sec)			
	0 min	30min	60min	90min
Control	3.11± 0.20	3.22± 0.20	3.69± 0.22	3.88± 0.22
Standard	3.77± 0.12	6.48 ± 0.12*	8.58 ± 0.16**	11.09±0.12**
Bark	3.89 ± 0.07	5.81 ± 0.12	7.44± 0.12**	9.51± 0.12**
Root	3.65 ± 0.04	4.87 ± 0.13	6.32± 0.06*	6.59± 0.9*
Leaves	3.82± 0.14	4.04± 0.12	4.30± 0.07	4.66±0.26*

The data are expressed as mean ± S.E.M. Significant differences in each group versus the control were as follows: * P < 0.05. ** P < 0.01.

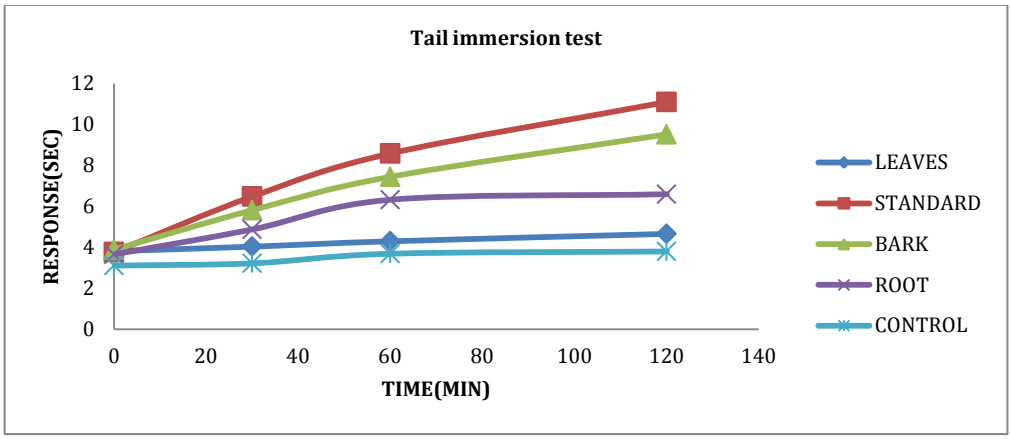

Fig 5: This graphical representation shows the various responses of mice in Tail immersion test having constant temperature of water 55^{0}C in 30 min, 60min, and 120min after drug administration

DISCUSSION

The data presented here suggests that the *Abroma augusta* possesses analgesic and antipyretic activities. The extract at the doses tested was shown to possess analgesic activity evident in all the nociceptive models, signifying it possesses both central and peripherally mediated activities. The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. In general, acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response. The method has also been associated with prostanoids in general, that is, increased levels of PGE2 and PGF2α in peritoneal fluids, as well as lipoxygenase products[12, 13]. The significant reduction in acetic acid-induced writhes by *Abroma augusta* suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances. The hot-plate and tail-immersion tests are useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level[12]. The significant increase in pain threshold produced by *Abroma augusta* in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems[13-16]. The analgesic effect produced by the extract may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leucotrienes, and other endogenous substances that are key players in inflammation and pain. Antipyretic are the agents, which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature. Yeast induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature[17]. The present results show that *Abroma augusta* possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats, and its effect is comparable to that of paracetamol (standard drug). So inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol[18]. Also, there are several mediators or multiprocesses underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis[19-21].

CONCLUSION

The results obtained in this study indicate that different parts of *Abroma augusta* possesses potent analgesic and antipyretic properties, which are mediated via peripheral and central inhibitory mechanisms. This could provide a rationale for the use of this plant in fever, pain and inflammatory disorders in folk medicine.

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