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Analgesic and antipyretic effects of aqueous extract from *Clerodendrum inerme* (L.) Gaertn. leaves in animal models

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ABSTRACT

In this study, the analgesic, and antipyretic effects of aqueous extract obtained from Clerodendrum inerme leaves, the so-called AECI was investigated. Analgesic effect of AECI was evaluated by Hot plate, Tail Flick and Tail immersion methods in albino rats. Antipyretic activity of AECI was evaluated by milk-induced hyperpyrexia in rabbits. The AECI produced significant (P<0.001) analgesic activity in all models. Further, the AECI potentiated the Diclofenac sodium-induced analgesic effect in albino rats. Treatment with AECI showed a significant (P<0.001) dose-dependent reduction of pyrexia in rabbits. The results suggest that AECI possess potent analgesic and antipyretic activity.

Keywords: Pain, Fever, *Clerodendrum inerme*, Analgesic activity, Antipyretic activity.

INTRODUCTION

Pain has been defined by International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [1]. Pain is an unpleasant sensation no doubt, but on the whole it is usually beneficial to man (or animal). It is mainly a protective mechanism for the body, occurs whenever any tissues are being damaged, and it causes the individual to react to remove the pain stimulus [2].Typically, it is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause (e.g. trigeminal neuralgia), or persistent long after the precipitating injury has healed (e.g. phantom limb pain). [3].

Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics. All of these drugs known to possess side and toxic effects. Moreover synthetic drugs are very expensive to develop since, for the successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested where the cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs [4]. As NSAIDs causes adverse side effects the use of these drugs as analgesic agents have not gain importance hence, drugs with no such effects been searched all over the world. During this process, plant-based drugs used in the traditional medicine is concern since they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs [5].

Pyrexia is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defence to create an environment where infectious agent or damaged tissue cannot survive [6]. Normally the infected or damaged tissue initiates the enhanced formation of pro inflammatory 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 [7]. As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high, it dilate the blood

vessels and increase sweating to reduce the temperature; but when the body temperature become very low, hypothalamus protect the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration and existing complaints, as found in HIV [8]. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE-2 biosynthesis. Moreover, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, golmeruli, cortex of brain and heart muscles, whereas natural COX-2 inhibitors have lower selectivity with fewer side effects [9].

Clerodendrum inerme (L.) Gaertn., (Family: Verbenaceae), is a common shrub that grows in India, both in the wild and as a garden hedge. Its leaves are used as alterative, febrifuge and as a substitute for *Swertia chirayita*. Its leaves have been shown to possess antimicrobial activity [10] and are reported to be cardiovascular system active. They also stimulate uterine motility in rats and inhibit intestinal motility [11]. In Indian tribal medicine, leaves of *C. inerme* are used for treating fever, cough, skin rashes, chronic pyrexia and boils, and are used in conjunction with other plant leaves. They are also used to treat umbilical cord infection and for cleaning the uterus in local medicine [12, 13]. Aerial parts of *C. inerme* showed potent anti-viral activity against Hepatitis B virus [14]. Whole plant parts of *C. inerme* are used as to treat coughs, scrofulous infection, venereal infection, skin diseases and Beriberi diseases [15]. It is also used as febrifuge, vermifuge and antioxidant. The plant contains mainly iridoids, flavonoids, diterpenes, sterols, triterpenes and neolignans [16-20].

Rationale in selection of this plant for the current study is based on its traditional use to treat fever [12, 13].

MATERIALS AND METHODS

Collection of the plant sample

Fresh leaves of *Clerodendrum inerme* (L.) Gaertn. (Verbanaceae) were collected from the region of Sriperambathur, Chennai, Tamilnadu. The plant was identified, and authenticated by comparing with an authentic specimen by botanist Dr. P. Jayaraman, Plant Anatomical Research Centre (PARC), Tambaram, Chennai, T.N, India., bearing a voucher no **PARC/2011/1010** dated 18.11.2011

Preparation of plant extract

Fresh entire selected plant leaves were washed with water to remove the adhering matter. The finely cut plant material (50 g) was taken in a Clevenger apparatus and graduated receiver with water to avoid any air bubbles. The distillation solvent used i.e. water and glycerol (200: 25) ensured a higher boiling point than water. The distillation unit was run for 5 hours. The distillate was collected in the graduated receiver in which the H_2O portion of distillate automatically separated and returned to the distillation flask [21].

PHARMACOLOGICAL ACTIVITIES

ACUTE TOXICITY STUDIES

Determination of LD50 value of *Clerodendrum inerme*

The procedure was followed by using OECD guidelines 423 (Acute toxic class method). The acute toxic class method is a step wise procedure with 3 animal of single sex per step. Depending on the mortality and / or moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion. The method uses defined doses (2000mg/kg body weight) and the results allow a substance to be ranked classified according to the globally harmonized system (GHS) for the classification of chemicals which cause acute toxicity.

Procedure

Three animals male Wister albino rats (20-25gm) were kept in an environment with temperature (22^{o}c±3^{o}c)] are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels 5, 50,300 and 2000mg/kg body weight. The starting level should be that which is most likely to produce mortality in some of the dosed animals. The starting dose level of extracts of *Clerodendrum inerme* was 300mg/kg body weight. The time interval between treatment groups is determined by the onset, duration and severity of toxic signs. Most of the crude extracts possess LD50 value more than 2000mg/kg of the between of animal used. Dose volume was administered 0.1ml/100mg body weight to the animal p.o. after giving the dose the toxic signs were observed within 3-4hrs. Body weight of the animal before and after administration, onset of toxicity and signs of toxicity like changes in skin, fur, eyes, mucous membrane and also respiratory, circulatory, autonomic, central nervous system and behaviour pattern, signs of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma was also to be noted if any.

ANTINOCICEPTIVE ACTIVITY

Drugs and chemicals

Acetic acid was obtained from Merck. Tween-80 was obtained from Hindustan Chemicals. Normal saline solution was purchased from Beximco Infusion Ltd. and Diclofenac sodium was obtained from Chitra Pharmaceuticals Ltd.

Animals

Healthy male Wistar albino rats weighing 120–180 g and from the Central Animal House of Darshan Institute of Pharmacological Studies, Puliyur, Karur TK, Tamilnadu **(1084/ac/07/CPCSEA)** were used throughout the study. They were kept under standard environmental conditions at 25 °C with 12:12 h light–dark cycle in ventilated plastic cages. The rats were fed with a standard rat feed and water ad libitum .The experiment was performed in accordance with the guidelines was approved by IAEC.

ANTINOCICEPTIVE ACTIVITY

Analgesic activity was determined viz three standard methods, which are as follows:

1. Hot plate method

Experimental animals of either sex were randomly selected and divided into four groups designated as group-I, group-II, group-III and group-IV consisting of five albino rats in each group for control, positive control and test sample group respectively. Each group received a particular treatment i.e. control (1% Tween-80 solution in water), positive control (Diclofenac sodium 10 mg/kg, p.o.) and the test sample (aqueous extract of 100 mg/kg, p.o. & 200 mg/kg, p.o. respectively). The animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5 ^{0}C. A cut off period of 15 s [22] was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples [23-25].The Intraperitoneal administration of samples to albino rats as shown in Fig.1

Fig: 1 Intraperitoneal administration of samples to albino rat

2. Tail flick method

The pre screened animals (reaction time: 3-4 sec) were divided into groups as shown in Table 2. Diclofenac sodium 10 mg/kg acted as the standard drug. The drugs were administered intraperitoneally. The tail flick latency was assessed by the analgesiometer (Inco, India). The strength of the current passing through the naked nicrome wire was kept constant at 6 Amps. The distance between the heat source and the tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut-off reaction time was fixed at 10 sec to avoid tissue damage [26].

3. Tail immersion method

Albino rats in groups of five each were treated with vehicle, Diclofenac sodium 10 mg/kg and the test sample (aqueous extract of 100 mg/kg, p.o. & 200 mg/kg, p.o. respectively).The distal 2 - 3 cm portion of mouse-tail was immersed in hot water maintained at 55 ± 0.5ºC [27]. The time taken by the mouse to withdraw the tail from hot water was noted as reaction time.

ANTIPYRETIC ACTIVITY

Drugs and Chemicals

Paracetamol tablet was obtained from the market and used as an antipyretic agent. The standard solution was prepared by dissolving the tablet in the solvent to obtain 7.5mg paracetamol per ml solution. The dose of paracetamol was maintained at 10 mg/kg body weight.

Experimental Group

It contains 2 groups for checking the various concentration of the *C. inerme*. One group receiving dose of 100mg/kg and another group receiving 200mg/kg of AECI of the sample.

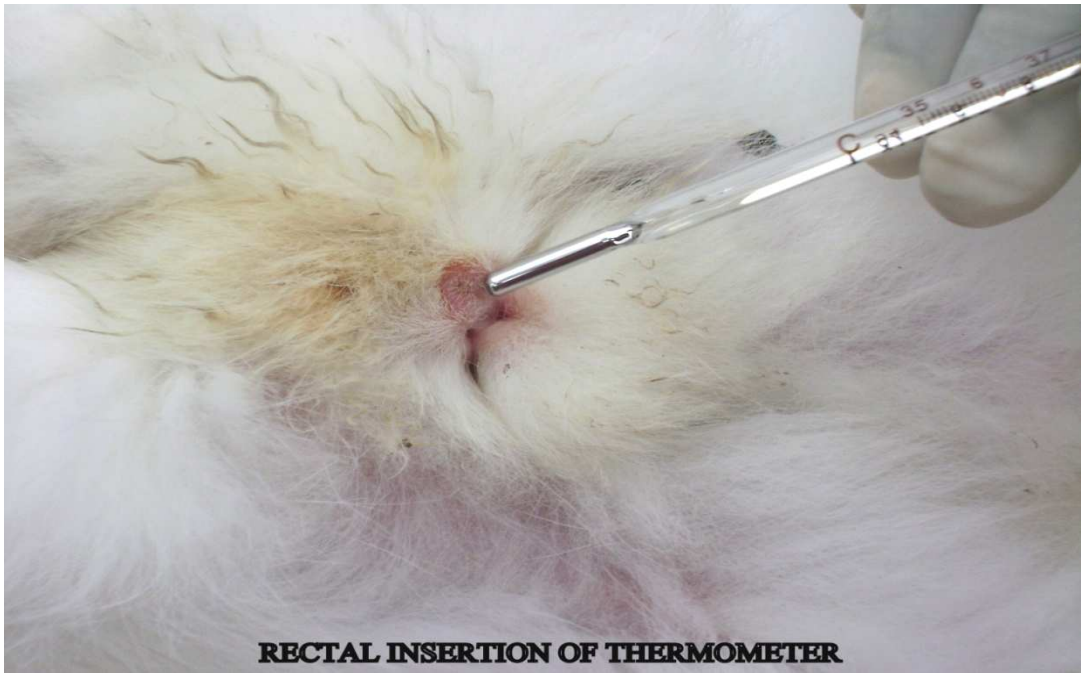
Control Group

It consists of Positive control group –receives standard drug to check anti pyretic effect. Control group- receives the solvent used. Number of rabbits in each group is four.

Experiment Protocol

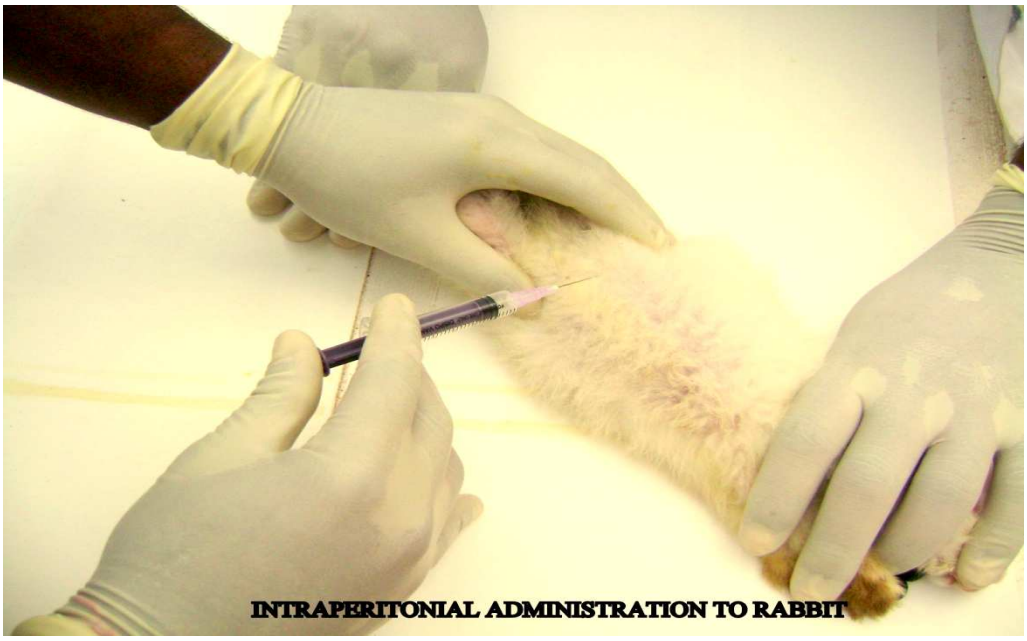
The rectal temperatures of the rabbits were noted by using well lubricated thermometer inserting it into rectum (Fig.2). Care was taken to insert it to the same depth each time (about 3 cm). Milk was collected from local cow had been boiled. When temperature of the boiled milk equilibrates to room temperature then rabbits were injected boiled milk at the dose of 0.5 ml/kg body weight, to induce pyrexia. Induction of fever was taken about one to two hour [28].

Fig: 2 Rectal insertion of lubricated Thermometer in to Albino rabbit anus



The aqueous extract of the leaves were given to experimental group, standard drug was given to positive control and solvent were given to control group. Intra peritoneal administration was used (Fig.3). Temperature was recorded 1 hour interval till 3 hours.

Fig: 3 Intraperitoneal administration of samples to Albino rabbits



Statistical analysis

The results are presented as mean ± standard error of mean (SEM). The one-way ANOVA test with Tukey-Kramer Multiple Comparisons Test was used to analyze and compared with control. Statistical significance is expressed as $p < 0.001$.

RESULTS

Antinociceptive activity

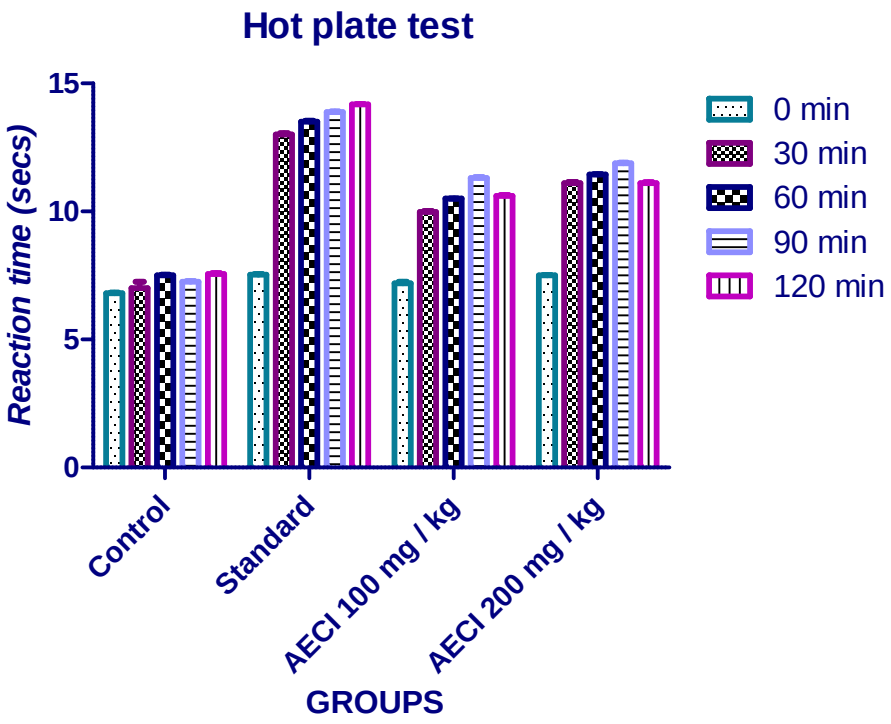
Hot-plate test was assayed to characterize the analgesic activity of the AECI (Aqueous extract of *Clerodendrum inerme*). The results presented in Table 1 and graph 1 show that the i.p administration of the AECI at doses 100mg/kg, p.o and 200mg/kg, p.o. significantly **(P<0.001)** raised the pain threshold at different time of observation (0 - 120 min) in comparison with control. Diclofenac sodium (10 mg/kg, p.o), used as standard drug, also produced a significant analgesic effect during all the observation times when compared with control values **(P<0.001).**

Table 1: Effect of AECI by hot plate test in albino rats

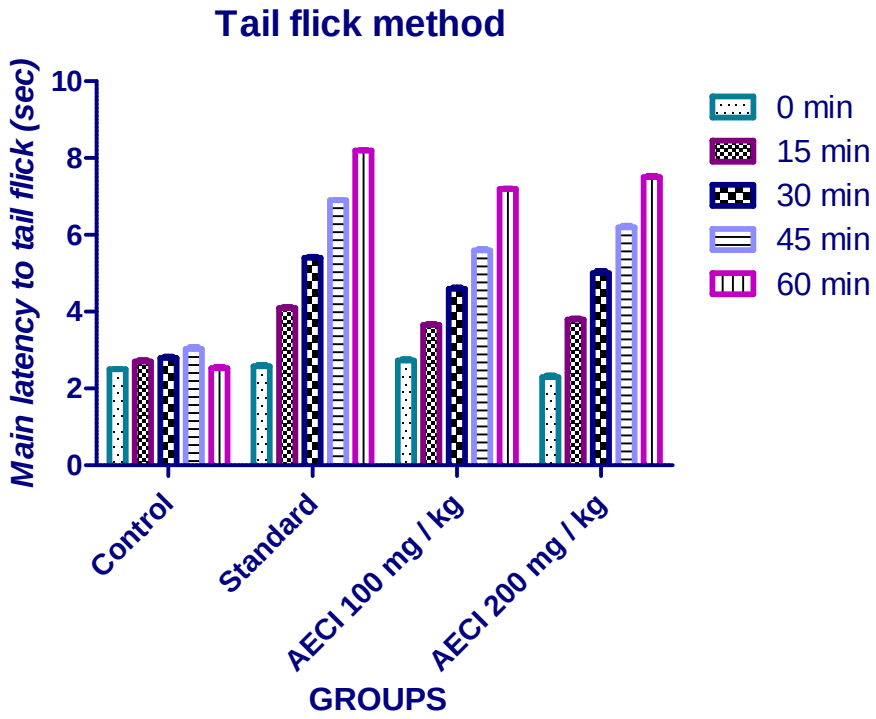
Group	Dose (mg/kg)	*Reaction time(Seconds)*				
		0 min	**30min**	**60min**	**90min**	**120min**
Group 1	Vehicle	6.8 ± 0.03	7 ± 0.25	7.5 ± 0.03	7.25 ± 0.03	7.56 ± 0.04
Group 2	Standard -10mg/kg	7.53 ± 0.03	13 ± 0.05*	13.51 ± 0.03*	13.88 ± 0.03*	14.18 ± 0.01*
Group 3	AECI-100mg/kg	7.2 ± 0.05	9.98 ± 0.04*	10.5 ± 0.004*	11.3 ± 0.04*	10.6 ± 0.04*
Group 4	AECI-200mg/kg	7.5 ± 0.02	11.1 ± 0.05*	11.45 ± 0.04*	11.88 ± 0.03*	11.11 ± 0.03*

*All values are expressed as mean ±SEM (n = 6), * P < 0.001 significant compared to control.*

Graph: 1 Effect by AECI to hot plate test in albino rats



Graph: 2 Effect of AECI by Tail Flick Method in albino rats



The effect of AECI on Tail Flick Method is shown as in Tables 2 and graph 2. The extract caused a significant (P<0.001) inhibition of pain at both the doses used (100mg/kg, p.o and 200mg/kg, p.o) Diclofenac sodium (10 mg/kg, p.o), used as standard drug, was highly effective (P<0.001).

Table 2: Effect of AECI by Tail Flick Method in albino rats

Group	Treatment	Mean latency to tail flick(sec)				
		0min	15min	30min	45min	60min
I	Control	2.5 ± 0.007	2.7 ± 0.04	2.8 ± 0.03	3.03 ± 0.05	2.53 ± 0.03
II	Standard 10mg/kg	2.58 ± 0.03	4.1 ± 0.02*	5.4 ± 0.03*	6.9 ± 0.004*	8.2 ± 0.005*
III	AECI-100mg/kg	2.73 ± 0.03	3.65 ± 0.03*	4.6 ± 0.03*	5.6 ± 0.03*	7.2 ± 0.006*
I V	AECI-200mg/kg	2.3 ± 0.04	3 .8 ± 0.02*	5.0 ± 0.06*	6.2 ± 0.04*	7.5 ± 0.04*

*All values are expressed as mean ±SEM (n = 6), * P < 0.001 significant compared to control*

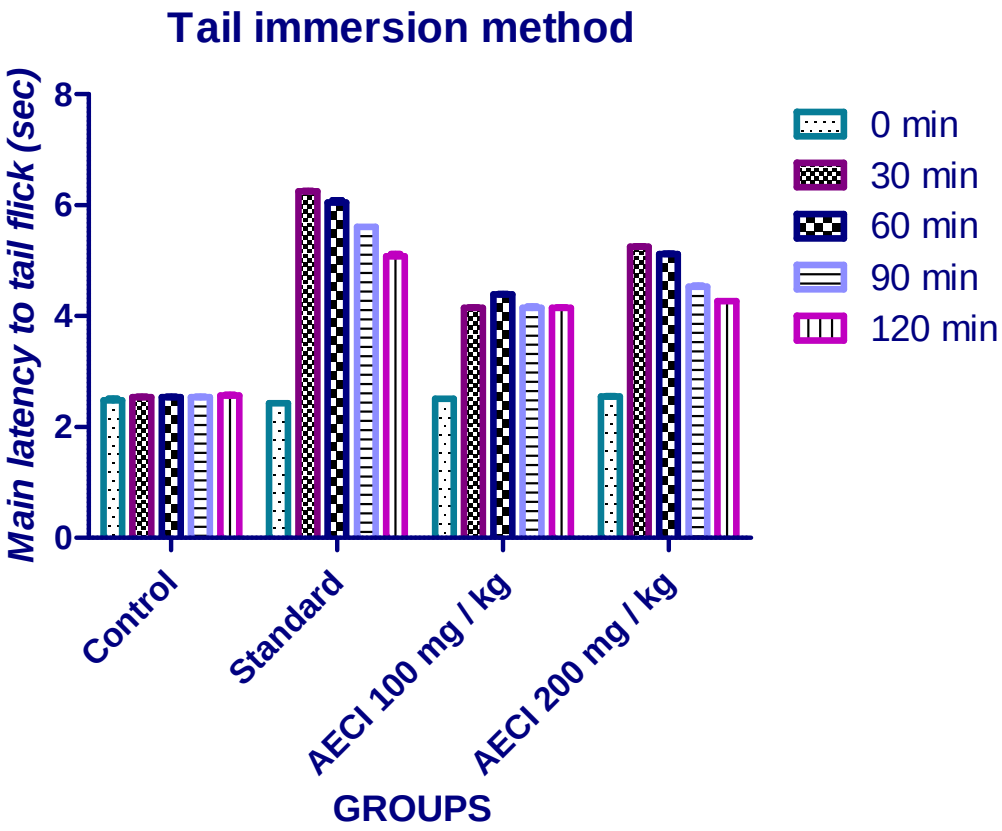
There was a significant dose-dependent inhibition of both phases of the Tail immersion method response in albino rats (Table 3 and graph 3), with a more potent effect on the second than the first phase. Diclofenac sodium (10 mg/kg, p.o), also inhibited both phases of the pain significantly (P<0.001) when compared to control group.

Table 3: Effect of AECI on Tail immersion method in albino rats

Group	Treatment (mg/kg)	Mean latency to Tail immersion (sec)				
		0min	30min	60min	90min	120min
I	Control	2.48 ± 0.03	2.54 ± 0.004	2.54 ± 0.005	2.54 ± 0.004	2.57 ± 0.004
II	Standard	2.43 ± 0.003	6.25 ± 0.04*	6.05 ± 0.04*	5.61 ± 0.003*	5.08 ± 0.04*
III	AECI-100mg/kg	2.51 ± 0.003	4.15 ± 0.006*	4.39 ± 0.004*	4.15 ± 0.02*	4.15 ± 0.005*
IV	AECI-200mg/kg	2.55 ± 0.006	5.25 ± 0.004*	5.12 ± 0.004*	4.53 ± 0.02*	4.27 ± 0.003*

*All values are expressed as mean ±SEM (n = 6), * P < 0.001 significant compared to control.*

Graph: 3 Effect of AECI on Tail immersion method in albino rats



Antipyretic activity

Tested on Boiled Milk-induced pyrexia in rats, AECI significantly reversed hyperthermia at either dose (100mg/kg b.w and 200 mg/kg b.w.). Time of peak effect obtained were 1 to 3h after oral administration. The standard drug, Paracetamol (10mg/kg b.w.) also suppressed hyperthermia induced by Boiled Milk significantly (P<0.001) during all the observation times when compared with control values (Table 4).

Table 4: Effect of *Clerodendrum inerme* Aqueous leaves extract on Milk-induced pyrexia in Rabbits.

Groups	Dose	Rectal temperature (°C)		Rectal temperature after treatment (°C)		
		Normal	3 h after boiled milk administration	1 hr	2 hr	3 hr
Solvent	2ml	39.2 ± 0.02	41.14 ± 0.006	40.8 ± 0.004	40.2 ± 0.006	39.3 ± 0.004
Paracetamol	150mg/kg	39.4 ± 0.004	41.12 ± 0.005	39.93 ± 0.006*	39.7 ± 0.005*	39.51 ± 0.03*
AECI	100mg/kg	39.45 ± 0.04	41.3 ± 0.006	40.14 ± 0.004*	39.89 ± 0.007*	39.7 ± 0.004*
AECI	2oomg/kg	39.5 ± 0.02	41.13 ± 0.006	40.13 ± 0.05*	39.76 ± 0.005*	39.5 ± 0.03*

*All values are expressed as mean ±SEM (n = 6), * P < 0.001 significant compared to control.*

DISCUSSION

The data presented here suggests that the AECI possesses anti-nociceptive and antipyretic activities. The extract at the doses tested was shown to possess anti-nociceptive 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 [29]. 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 [30]. The method has also been associated with prostanoids in general, that is, increased levels of PGE2 and PGF2 bperitoneal fluids [31], as well as lipoxygenase products [32]. The significant reduction in acetic acid-induced writhes by AECI suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances.

Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems [33-36]. The analgesic effect produced by the extract may be due to the inhibition of prostaglandins, leucotrienes, and other endogenous substances that are key players in inflammation and pain. As it is known that Flavonoids inhibit prostaglandin synthetase [37] this effect of extract may be due to presence of Flavonoids as Phytochemical constituent.

The present results show that AECI 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. Also, there are several mediators or multiprocesses underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis [38].

CONCLUSION

The overall results demonstrate that aqueous extract of *Clerodendrum inerme* have analgesic, and antipyretic activities in laboratory animals and this may be mediated by the central and peripheral mechanisms. Nonetheless, the precise mechanism and the bioactive principles responsible for these actions remain to be elucidated.

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