ORIGINAL ARTICLE

Anti-inflammatory and analgesic activities of leaf extract of *Wattakaka volubilis* (Dreagea volubilis)

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Wattakaka volubilis (Family: Asclepiadaceae) has been reported to possess medicinal effects. In the present study, the dried leaf extract [methanol–water (1:1)] of *W. volubilis* designated as 'the extract' was evaluated for pharmacological activity in rats and mice. The anti-inflammatory activity was evaluated using acute, sub-chronic and chronic models of inflammation in rodents. The antipyretic and analgesic activities were evaluated in mice models. In the acute toxicity study, it was found that the extract was non-toxic up to 1 g/kg, i.p. The extract (50, 100 and 200 mg/kg, i.p.) was found to possess, anti-inflammatory, analgesic and antipyretic activities in a dose-dependent manner and the effect was comparable with that produced by the standard drug, ibuprofen. The extract significantly inhibited the arachidonic acid-induced paw oedema in rats, indicating that the extract inhibited both the cyclo-oxygenase and lipo-oxygenase pathways of arachidonic acid metabolism. The extract also significantly enhanced the macrophage count in mice in a dose- and time-dependent manner. It is possible that the saponins present in the extract may be responsible for these activities.

Key words: *Wattakaka volubilis*, saponins, anti-inflammatory activity, leaf extract

INTRODUCTION

Wattakaka volubilis (family: Asclepiadaceae) is used in the treatment of various ailments since ancient times.[1] The literature survey revealed that among the various saponins obtained from the stem and flower of *W. volubilis*, two compounds are active against Ehrlich's ascites carcinoma.[2,3] Since *W. volubilis* has been reported to possess medicinal effects, the present study attempts to evaluate the anti-inflammatory, analgesic and anti-pyretic activities exhibited by the constituents present in the dried leaf extract [methanol–water (1:1)], named 'the extract', of this plant.

MATERIALS AND METHODS

Plant Materials

The leaves of *W. volubilis* (family: Asclepiadaceae) were collected from Tiruchirappalli district of Tamil Nadu, India, during the month of December 2004. The Botanical Survey of India, Coimbatore, Tamil Nadu, India, identified the plant and a voucher specimen was submitted (Ref No: BSI/SC/5/21/04-05/Tech-1704 dated 23.12.2004).

Extraction and Preparation of the Sample

The leaves of *W. volubilis* were dried under shade, ground to half dust (1 kg) and soaked in 3 l of aqueous methanol for 48 hours at room temperature (28–32°C) with occasional shaking. The solvent was filtered and to the residue same amount of solvent was added and the same procedure was carried out thrice. A rotary evaporator at 40°C was used to evaporate methanol, and water was evaporated using a high vacuum lyophilizer. The crude brownish coloured solid mass (130 g) was collected and stored at 4°C in a closed container. The aqueous methanol extract was completely dried (free of solvent methanol) and the residue was dissolved in water in which it was totally soluble. A 100 mg/ml solution of the crude extract was prepared in distilled water to make a stock solution which was stored at 4°C for a maximum period of 4 days. Suitable dilutions were made from the stock solution before experiment.

Animals

Experiments were carried out on albino rats (Sprague-Dawley strain) weighing 225–250 g and Balb-C mice weighing 20–25 g bred in the Institute's Animal House. The animals were housed under conditions of 22 ± 1°C, 50 ± 10% humidity and 12-hour light and 12-hour dark cycle. During maintenance, the animals received a diet of food pellets (fortified with minerals and vitamins) prepared in the animal house and water ad libitum. All animal studies were carried out in accordance with the internationally accepted

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principles for laboratory animal use and care after getting clearance from the Institute Animal Ethics Committee.

Chemicals

Arachidonic acid, Brewer's yeast, carrageenan, croton oil, Freund's complete adjuvant, ibuprofen and indomethacin were purchased from Sigma, phenidone from Biomol Co., acetic acid and silicotungstic acid from M/S E.Merck and all other chemicals were of analytical grade purchased locally.

Acute Toxicity Study

Mice were divided into 10 groups and the extract was injected i.p. in doses from 100 mg to 1 g/kg, i.p. The LD_{50} (24 hour) was calculated according to Ghosh.[4]

Anti-inflammatory Studies

Carrageenan-induced paw oedema

The rats were divided into five groups (n = 6) and the first group served as negative control and received normal saline (0.1 ml/100g, i.p.). The second group was administered ibuprofen (100 mg/kg, i.p.) as the standard drug and rats of groups III, IV and V were administered 50,100 and 200 mg/kg, i.p. of the extract, respectively. Oedema was produced by the method described by Winter *et al*.[5] Carrageenan (0.1 ml/100 g from a 10 mg/ml solution) was injected into the planter aponeurosis of right hind paw of the rats of all the groups 30 minutes later. The left hind paw served as the control. The paw volume was measured after 4 hours using a plethysmometer (UGO BASILE). After measuring the paw volume, the rats were killed by cervical dislocation.

Cotton pellet-induced granuloma

Two autoclaved cotton pellets weighing 10 ± 1 mg were implanted subcutaneously into both sides of the groin region of each rat.[6] The animals were divided into five groups containing six animals in each group. Group I served as negative control and received normal saline daily at a dose of 0.1 ml/100 g body weight i.p., group II received 100 mg/kg, i.p. ibuprofen (standard drug) and groups III, IV and V received the extract at doses of 50, 100 and 200 mg/kg, i.p., respectively, daily for seven consecutive days. On the 8^{th} day, the animals were sacrificed by cervical dislocation and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60°C, weighed and compared with control.[6]

Croton oil-induced ear inflammation

Croton oil irritant solution (0.1 ml) prepared according to Brooks *et al*.[7] was applied externally to the outer surface of the right ear of mice. The mice were divided into five groups of six animals in each group. While mice of group I (negative control) received 0.1 ml/10 g of normal saline i.p., those of group II received 100 mg/kg ibuprofen (standard drug) i.p. and mice of groups III, IV and V received 50,100 and 200 mg/kg, i.p. of the extract, respectively, 30 minutes before croton oil application. The mice were sacrificed by cervical dislocation after 4 hours, and 7-mm punches were made in the ear by a cork borer. Each ear disc was weighed and compared with control.

Freund's adjuvant-induced polyarthritis

The method of Newbould[8] was followed. A total of 30 male albino rats were divided into five groups of six animals in each group. On day 1, 0.1 ml of Freund's complete adjuvant was injected into the planter pad of right hind paw of each rat. While rats of group I (negative control) received 0.1 ml/100 g of normal saline i.p., those of group II received 100 mg/kg ibuprofen (standard drug) i.p. and rats of groups III, IV and V received 50, 100 and 200 mg/kg, i.p. of the extract, respectively, for consecutive 21 days. The paw volume of each group was measured using a plethysmometer (UGO BASILE) on day 0 before administration of adjuvant and on day 21 after treatment. Number of ear nodes and tail nodes were also noted on the same day.

Arachidonic acid-induced paw oedema

A total of 36 male albino rats were divided into six groups of 6 animals in each group. Rats of group-I (negative control) received 0.1 ml/10 g of normal saline i.p., those of group-II received 100 mg/kg of phenidone (dual blocker) i.p., rats of group III received indomethacin (10 mg/kg, i.p.) and rats of groups IV, V and VI received 50, 100 and 200 mg/kg, i.p. of the extract, respectively. Paw oedema was induced by a single injection of 0.1 ml 0.5% arachidonic acid in 0.2 (M) carbonate buffer (pH 8.4) into right hand paw (subplantar) of rats 30 minutes after drug treatment. Hind paw volume was measured 1 hour after arachidonic acid injection.[9]

Analgesic Activity

Writhing in mice

Balb-C mice were randomly divided into five groups (six in each). Mice of group I received normal saline (0.1 ml/10 g, i.p.) the animals of the second group received ibuprofen (100 mg/kg, i.p.) and groups III, IV and V received 50, 100 and 200 mg/kg, i.p. of the extract, respectively. Thirty minutes later, each mouse was given 0.1 ml/10 g, i.p. of 1% acetic acid. Writhing response was observed by the method of Turner.[10] The time of onset of writhing and the number of writhing within 15 minutes were noted.

Tail clip method

It was done in mice by applying a metal artery clip at the base of tail with its jaw sheathed with thin rubber tubing.[11] Those mice which made efforts to dislodge the clip within 15 seconds were selected and were randomly divided into five groups containing six in each group. Group I received normal saline (0.1 ml/10 g, i.p.), group II received ibuprofen

(100 mg/kg, i.p.) and groups III, IV and V received 50, 100 and 200 mg/kg, i.p. of the extract, respectively. The clip was applied 30, 60 and 120 minutes after drug administration. It was considered as a response if there was no attempt by the mouse to dislodge the clip within 15 seconds.

Tail flick method

The antinociceptive effect of the test substances was determined by the hot tail flick method described by Sewell and Spencer.[12] One to two cm of the tail of mice was immersed in warm water bath (Swan scientific instruments) kept constant at 55 ± 1°C. The reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as a mean of the next two readings. Balb-C mice were randomly divided into five groups (six in each). Mice of group I received normal saline (0.1 ml/10 g, i.p.) group II received ibuprofen (100 mg/kg, i.p.) and groups III, IV and V received 50, 100 and 200 mg/kg, i.p. of the extract, respectively. Thirty minutes later, the tail was immersed in the water bath and the tail flick response was recorded. The same experiments were repeated after 60 minutes and 120 minutes again.

Antipyretic Studies

Brewer's yeast-induced pyrexia

All experiments were conducted at a room temperature of 28 ± 1°C. Rats (six in a group) were randomly divided into four groups. Pyrexia was induced in rats by injecting Brewer's yeast (2 mg/kg, i.p.) using the method of Bruguerolle and Roucoules.[13] After 18 hours, those rats were selected whose rectal temperature was minimum 101.3°F and divided into four groups (n = 6). Group I received normal saline (0.1 ml/100 g, i.p.) and groups II, III and IV received 50, 100 and 200 mg/kg, i.p. of the extract, respectively. Rectal temperature was recorded first after 30 minutes and then after 60 minutes for next 4 hours.

Effect on normal peritoneal cell

Ninety-six mice were divided into four groups of 24 mice in each group. While group I received 0.1 ml/10 g, i.p. normal saline, groups II, III and IV received 50, 100 and 200 mg/kg, i.p. of the extract, respectively. The number of macrophages was determined by staining with 1% neutral red solution using a haemocytometer[14] from each group (six mice at a time) at 6, 12, 24 and 48 hours of treatment and compared.

Statistics

Data are presented as arithmetic mean ± S.E.M of at least six experiments. Statistical analysis was performed by one-way analysis of variance (ANVOA) followed by Dunnett's test or by Student's paired *t*-test. '*P*' value of <0.05 was considered as statistically significant.

RESULTS

Acute Toxicity Studies

It was found that the extract was non-toxic up to 1 g/kg, i.p. body weight up to 24 hours. Thus one-tenth of it, i.e, 100 mg/kg, i.p. was taken as the initial starting dose and the other two selected doses were 50 mg/kg, i.p. and 200 mg/kg, i.p, respectively.

Anti-inflammatory Studies

Carrageenan-induce oedema

The extract inhibited carrageenan-induced paw oedema by 60% at a dose of 50 mg/kg, 73% at the dose of 100 mg/kg and 65% at the dose of 200 mg/kg i.p. The effect of 100 mg/kg of the extract was comparable to the effect produced by ibuprofen [Table 1].

Cotton pellet-induced granuloma

The increase in the dry weight of cotton pellet granuloma was compared with the control and it was found that the extract inhibited the increase in dry weight by 35% at a dose of 50 mg/kg, 45% at a dose of 100 mg/kg and 58% at a dose of 200 mg/kg as compared to saline control [Table 1].

Freund's adjuvant-induced poly-arthritis

The extract inhibited polyarthritis in rat and this effect was slightly more as compared to that produced by the standard drug ibuprofen used in the experiment [Table 1]. It also reduced the number of ear and tail nodes significantly at all doses as compared to control [Table 1].

Croton oil-induced ear inflammation

The extract inhibited croton oil-induced ear inflammation in mice in a dose-dependent manner which is comparable to that produced by the standard drug ibuprofen [Table 1].

Table 1: Effect of *Wattakaka volubilis* leaf extract on four different models of inflammation

Drug	Dose (i.p.)	Carrageenan-induced paw oedema (vol. in ml.)	Croton oil-induced ear inflammation (mg)	Increase in cotton pellet wt. (mg)	Freund's adjuvant-induced arthritis (vol. in ml.)
N. Saline	0.1 ml/100g	0.57 ± 0.06	11 ± 5	62 ± 4	0.52 ± 0.04
Ibuprofen	100 mg/kg	0.14 ± 0.02*	8 ± 2*	40 ± 2*	0.33 ± 0.04*
Extract	50 mg/kg	0.23 ± 0.05*	10 ± 5*	40 ± 2*	0.30 ± 0.28*
Extract	100 mg/kg	0.15 ± 0.05*	8 ± 4*	34 ± 1*	0.21 ± 0.04*
Extract	200 mg/kg	0.20 ± 0.05*	9 ± 4*	26 ± 1*	0.26 ± 0.02*

Data are mean ± SEM of six experiments. Data were analysed by ANOVA and Dunnett's test. *Significant inhibition as compared to control (*P* <0.05).

Arachidonic acid-induced paw oedema

Arachidonic acid injection (subplantar) in right hand paw produced significant oedema after 1 hour. Indomethacin, the cyclo-oxygenase blocker, inhibited it by 16% whereas phenidone, a dual blocker, inhibited it by 80%. The extract at doses of 50, 100 and 200 mg/kg inhibited the oedema by 67%, 73% and 87%, respectively, which suggested that the extract behaved like phenidone [Table 2].

Analgesic Studies

Writhing in mice

Intraperitoneally injected acetic acid produced abdominal constrictions, which is characterized by stretching response. The extract produced a dose-dependent reduction of acetic acid-induced writhing in mice and also significantly increased the onset of writhing [Table 3].

Tail clip method

Though all the doses of the extract used in the study produced significant analgesic activity in this model, the effect produced by the lowest dose, i.e., 50 mg/kg, i.p. was much more pronounced than that produced by the higher doses (100 and 200 mg/kg, i.p.) as well as by the standard agent ibuprofen [Table 4].

Tail- flick method

The extract produced a dose-dependent analgesic activity in this model and the effect produced by 200 mg/kg, i.p. of the extract was comparable to that produced by ibuprofen, the standard agent [Table 5].

Brewer's yeast induced pyrexia

The extract produced a dose-dependent decrease of rectal temperature of rats that lasted up to 4 hours of its administration with the maximum decrease occurring at 2 hours [Table 6].

Effect on normal peritoneal cell

It was observed that the average number of macrophages was increased after the extract treatment in a time- and dose-depedent manner as compared to the control [Figure 1]. The linear increase was effective up to 24 hours and then on the 48th hour the count came down [Figure 1].

Table 2: Effect of *Wattakaka volubilis* leaf extract on arachidonic acid-induced paw oedema of rat

Drug	Dose (i.p.)	Difference in paw volume in ml (mean± S.E.M)	% Inhibition as compared to control
Saline control	0.1/100 g	3.8 ± 0.20	–
Indomethacin	10 mg/kg	2.0 ± 0.37*	47
Phenidone	100 mg/kg	0.75 ± 0.12*	80
Extract	50 mg/kg	1.25 ± 0.12*	67
Extract	100 mg/kg	1.0 ± 0.23*	73
Extract	200 mg/kg	0.5 ± 0.11*	87

Data are mean ± SEM of six experiments. Data were analysed by ANOVA and Dunnett's test. *Significant inhibition as compared to control (P <0.05).

Table 3: Effect of *Wattakaka volubilis* leaf extract on acetic acid-induced writhing in mice

Drug	Dose (i.p.)	Onset of writhing (min) (mean ± S.E.M)	No. of writhing in 15 minutes (mean ± S.E.M)
Saline control	0.1 ml/100 g	3.42 ± 0.68	38.4 ± 6.06
Extract	50 mg/kg	9.46 ± 2.17*	5.0 ± 1.14*
Extract	100 mg/kg	9.29 ± 2.17*	3.6 ± 1.08**
Extract	200 mg/kg	9.27 ± 2.31*	3.6 ± 0.86**

Data were analysed by Student's *t*- test. *Significant delay in onset as compared to control (P <0.05). **Significant reduction in number of writhing as compared to control (P <0.05).

DISCUSSION

Leaves of medicinal plants are common ingredients of many folk and herbal medicines,[15,16] and leaf extracts of a number of medicinal plants have been reported to possess pharmacological activity, including anti-inflammatory activity.[15,17,18] The present study reveals that the dried leaf extract of *W. volubilis* possesses significant anti-inflammatory, analgesic and anti-pyretic activities in experimental animals. Different parts of *W. volubilis* plant enjoy considerable reputation for their various medicinal uses. The leaf paste is used to clear boils. Plant paste is mixed with hot milk and taken for urinary troubles. Leaf juice is inhaled to stop sneezing. The alcoholic extract of the plant is widely used in India as a traditional medicine for boils and abscesses.[19] The alcoholic extract of the plant is also reported to show activity on the central nervous system, as well as anticancer activity against sarcoma 180 in mice.[3] Two dregosides isolated from the methanolic extract of stem

Table 4: Effect of *Wattakaka volubilis* leaf extract on physical nociception in mice (tail clip test)

Drug	Dose(i.p.)	Min after treatment		
		30	60	120
		Response in s (mean ± S.E.M)		
Saline control	0.1 ml/100g	5.2 ± 1.0	5.2 ± 1.0	5.0 ± 1.3
Ibuprofen	100 mg/kg	8.5 ± 3.12*	12.3 ± 3.7*	5.0 ± 2.0**
Extract	50 mg/kg	17.6 ± 4.3*	18.0 ± 6.2*	16.0 ± 4.9**
Extract	100 mg/kg	9.6 ± 1.83*	10.6 ±1.06*	5.0 ± 1.4**
Extract	200 mg/kg	11.6 ± 3.3*	12.0 ± 1.8 *	8.0 ± 1.6**

Data were analysed by Student's *t*test. *Significant delay of time as compared to control (P <0.05). **Significant reduction in response as compared to 60th minute (P <0.05).

Table 5: Effect of *Wattakaka volubilis* leaf extract on thermal nociception in mice (tail flick test)

Drug	Dose (i.p.)	Min. after treatment		
		30	60	120
		Response in s (mean± S.E.M)		
Saline control	0.1 ml/100 g	2.5 ±0.22	2.5 ± 0.56	2.0 ± 0.25
Ibuprofen	100 mg/kg	2.5 ±0.22	3.2 ± 0.17*	3.2 ± 0.48*
Extract	50 mg/kg	2.6 ± 0.33*	2.8 ± 0.30*	3.0 ± 0.57*
Extract	100 mg/kg	2.8 ± 0.37*	3.0 ± 0.20*	3.0 ± 0.24*
Extract	200 mg/kg	3.3 ± 0.55*	3.5 ± 0.67*	3.3 ± 0.54*

Data were analysed by Student's t-test. *Denotes significant delay of time as compared to control (P <0.05).

Table 6: Effect of *Wattakaka volubilis* leaf extract on brewer's yeast induced pyrexia in rat

Drug	Dose (mg/kg, i.p.)	Temp.(°F) 18 hours after brewer's yeast injection	Temp. (°F) after treatment			
			1 hour	2 hours	3 hours	4 hours
N.Saline	0.1 ml/100 g	101.8±0.4	99.2±0.7	99.5±0.6	99.9±0.7	99.8±0.8
Extract	50 mg/kg	100.8±0.5	99.2±0.5*	98.9±0.5*	100.1±0.2*	98.1±1.3*
Extract	100 mg/kg	101.5±0.3	98.5±0.5*	98.9±0.5*	99.0±0.5*	99.2±0.3*
Extract	200 mg/kg	102.3±0.6	97.3±0.7*	97.8±0.7*	98.3±0.7*	97.7±0.6*

Data were analysed by Student's t-test. *Significant decrease in rectal temperature (P<0.05).

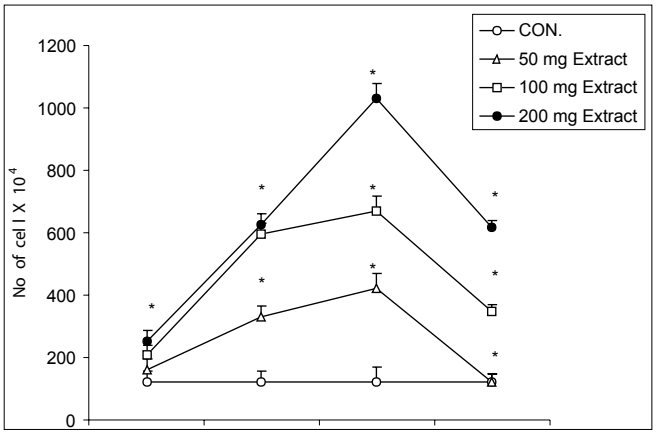

Figure 1: Graph showing the dose-dependent increase in macrophage count after extract administration at various time intervals. The points are mean SEM. Data were analysed by Student's t-test. *Significant increase in macrophage count as compared to control (P <0.05)

this plant showed anti-tumour activities against Ehrlich's carcinoma (solid type), and also showed activity against melanoma B-16.[2]

Acute toxicity study revealed that the extract is non-toxic up to 1 g/kg, i.p. The anti-inflammatory effect of the extract could be observed in acute (carrageenan and arachidonic acid induced paw oedema in rat and croton oil-induced ear inflammation in mice), sub-chronic (cotton pellet-induced granuloma in rat) and chronic (Freund's complete adjuvant-induced polyarthritis in rat) models of inflammation. In acute models, it was found that 100 mg/kg, i.p. of the extract was the most effective dose, whereas in subchronic and chronic models, the extract showed a dose-dependent inhibition of inflammation. Since the extract inhibited the oedema comparable to the dual-blocker phenidone in the arachidonic acid-induced paw oedema model in rat, it is possible that the extract produces its anti-inflammatory activity by inhibiting both the lipo-oxygenase and cyclo-oxygenase pathways of arachidonic acid metabolism. The finding that the extract significantly reduced inflammation in Freund's adjuvant-induced polyarthritis in rats shows that the extract has anti-arthritic activity as well.

In case of analgesic study, in writhing experiment, the extract reduced the number of paw stretching and increased the onset of writhing in a dose-dependent manner. In the tail clip method, the effective dose of the extract was 200 mg/kg, which was more effective as compared to the standard drug ibuprofen. In the case of tail flick method, it showed time- and dose-dependent analgesic activities. The extract also produced a significant antipyretic effect in the brewer's yeast-induced pyrexia model in rat.

In some of the experiments, such as in the acute and chronic models of inflammation and in acetic acid- induced writhing and tail clip models, the effect produced by the extract was not dose dependent. In fact, the effect produced by the lowest dose of the extract ie. 50 mg/kg i.p. was more than that produced by the higher doses (100 and 200 mg/kg) used in the study. This phenomenon is not unusual with plant extracts. It has been shown by many that lower doses of plant extracts produced more effect than the higher doses.[20, 21]

Extract treatment significantly enhanced the number of macrophages. Normally, it is found that 50% of the murine intraperitoneal cells are macrophages. A injection of certain materials such as casein, Freund's complete adjuvant, thioglycolate, starch, etc. leads to non-specific

accumulation of macrophages in the peritoneal cavity. Mature macrophages in the untreated peritoneal cavity are mostly residential. Intraperitoneal administration of different agents results in the exudation and intraperitoneal accumulation of new macrophages that differ from the mature macrophages. It has been reported that the exudate macrophages are more active than the residential mature ones in their ability to spread on the surface to which the cells are attached, receptor size of the cell coat, response to chemotactic stimuli, composition of cell wall, etc.[22] Though the actual role of the extract in the enhancement of macrophages can not be explained right now, it is possible that the pharmacological effects observed with the extract are mediated through changes in the immune system. However, experiments need to be performed to substantiate such a possibility.

It is well known that there is a close relationship between inflammation and cancer.[23-25] It has been reported that tumour promoters recruit inflammatory cells to the application site and cancer development may also act by aggravating inflammation in the tissue and vice versa and that inflammatory cells are capable of inducing genotoxic effects.[26] Since the present study indicates that the extract has significant anti-inflammatory activity, it is plausible that it may possess anti-cancer activity as well. In this context, it is worth mentioning that two saponins isolated from the stem and flower of *W. volubilis*, have been reported to be active against Ehrlich's ascites carcinoma.[2,3] It is possible that the saponins present in the extract may be responsible for the observed anti-inflammatory, analgesic and antipyretic activities in the present study.

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