

Research Article

Anti-inflammatory and Analgesic Activities of *Amorphophallus bulbifer* (Roxb) Kunth Whole Plant

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Abstract

Purpose: To investigate the anti-inflammatory and analgesic activities of the *Amorphophallus Bulbifer* in Wistar rats and mice.

Methods: The anti-inflammatory activity of the hydroalcohol extract of *A. bulbifer* whole plant at dose levels of 100 and 200 mg/kg p.o. in rats was determined with a plethysmograph paw volume difference of the animals pre- and post-treatment. Ibuprofen (10 mg/kg) was employed as reference standard. Analgesic activity was evaluated using tail flick and tail immersion techniques, by measuring the reaction time of the animals treated with either standard or extract. Pentazocin (30 mg/kg) was used as reference standard.

Results: The extract showed significant anti-inflammatory and analgesic activities at the two test dose levels at the 4th hour ($p < 0.001$). The extract exhibited anti inflammatory activity of 56.5 ($p < 0.001$) and 57.1 % ($p < 0.001$) inhibition compared to the control group in the carrageenan and histamine-induced inflammation model at a dose of 200 mg/kg. For analgesic activity, the extract showed reaction times of 7.33 ($p < 0.001$) and 7.83 ($p < 0.001$) min in the tail flick and tail immersion models, respectively, at a dose of 200 mg/kg while the normal and reference groups exhibited reaction times of 2.16, 2.66 and 8.16 ($p < 0.001$) and 8.5 ($p < 0.001$) in the tail flick and tail immersion methods, respectively.

Conclusion: Based on the findings, it can be concluded that *Amorphophallus bulbifer* possesses anti-inflammatory and analgesic properties and this lends some support for its use in traditional medical practice.

Keywords: *Amorphophallus bulbifer*, Anti-inflammatory activity, Analgesic activity

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INTRODUCTION

Amorphophallus bulbifer (Roxb) Blume, a member of *Araceae* family is a cormous herb; it is commonly known as Devil's Tongue in Western countries. The plant is widely distributed in Burma and Himalayan regions of Asia [1]. The plant is highly valuable in traditional Indian medicine for the treatment of piles, and in this regard, fresh rhizome pieces are taken twice or thrice daily for one month for curing piles and gonorrhoea. It is also used to treat hemorrhoids and diarrhea [2]. Small pieces of fresh petiole and bulbils of this plant are cooked as vegetable with dry fish and taken with rice once a day for 10 - 12 days for rheumatic muscular and joint pain while crushed tubers are used as antidote to poisoning from animal bites [3,4]. Water soluble amino acids [5], alkaloids from the dried entire plant [6] and sulfur compounds from the flowers [7] have been reported from the plant.

Studies substantiating its use in inflammation and pain are lacking although the plant is an important remedy for various ailments in traditional medicine, including pain and inflammation [1]. Therefore, the present study was aimed to evaluate the anti-inflammatory and analgesic activities of the whole plant in order to determine if there is any scientific basis for its use in traditional medicine.

EXPERIMENTAL

Collection of plant material

The fresh whole plant of *A. bulbifer* (3 kg) was procured from the rural belt of Annaram forest of Nizamabad district, Andhra Pradesh, India in October 2008 and authenticated by Professor VS Raju, a taxonomist in the Department of Botany, Kakatiya University, Warangal, Andhra Pradesh, India. A voucher specimen (AU/UCPSc/PCG/KSR/04/2008) was deposited in the herbarium of the Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India for future reference. The material was air-dried,

pulverised, and passed through a sieve with aperture size of 0.355 μm and used for further studies.

Preparation of extract

The dried and powdered material (500 g) was extracted by cold maceration with 3 L of ethanol: water (3:2) at room temperature for 72 h. It was filtered and the solvent was removed under reduced pressure thereafter the extract was used for the toxicity studies as well as for anti-inflammatory and analgesic studies. The yield of the extract was 8.49 %w/w. The extract was first suspended in 0.5 %w/v aqueous solution of sodium carboxymethyl cellulose prior to use.

Experimental animals

Adult Wistar rats (150 – 200 g) and Swiss albino mice of either sex were used in the studies. The animals were kept in standard polypropylene cages at room temperature ($30 \pm 2^\circ\text{C}$) and 60 - 65 % relative humidity [8]. All the experimental protocols were approved by the institutional animal ethical committee of Vaagdevi College of Pharmacy, Hanamkonda, Andhra Pradesh, India, vide approval no. 1047/AC/09/CPCSEA. The animals used in the studies were handled according to international guidelines [8].

Gross behavioral and acute toxicity studies

The extract was screened for the gross behavioral and toxicity studies in Swiss albino mice. Groups of mice comprising six animals each were treated with 100, 200, 400, 800, 1000, 2000 and 3000 mg/kg of the extract suspended in 0.5 % w/v sodium carboxymethyl cellulose (Na CMC) orally via a gastric catheter. The animals were then observed continuously for the first 4 h for any behavioral changes and also for mortality at the end of 72 h [9]. The control group was given the vehicle (0.5 % w/v sodium carboxymethyl cellulose). Based on the acute

toxicity study, 100 and 200 mg/kg doses were selected for subsequent studies.

Anti-inflammatory studies Carrageenan-induced paw edema model

Anti-inflammatory activity was evaluated in adult Wistar rats in treatment groups of six animals each. The test samples (100 and 200 mg/kg extract) or ibuprofen (reference, 10 mg/kg) or 0.5 %w/v Na CMC (control, 0.2 ml/100 g) were administered orally 1 h prior to subcutaneous injection of 0.1 ml of sterile saline carrageenan (1 %w/v) in the sub-plantar region of the left hind paw. The contra lateral paw was injected with an equal volume of saline. Paw volume was measured with a plethysmograph immediately after, i.e., at 0 h, and subsequently 1 h, 2 h and 4 h after administration. Anti-inflammatory activity was expressed as inhibition (%) of edema [10,11].

Histamine-induced paw edema model

Histamine (0.1 %w/v in normal saline) was injected into the right hind paw of each rat at a dose of 0.1 ml to induce edema. Paw volume was measured with a plethysmograph at 0, 1, 2 and 4 h. Anti-inflammatory activity was expressed as inhibition (%) of edema [12,13].

Analgesic studies

Tail flick method

Swiss Albino mice were screened for sensitivity test by placing the tip of the tail on the radiant heat source. Any animal that failed to withdraw its tail within 5 s was rejected from the study. The selected animals were then divided into four groups of six mice each. Each of the groups received one of the following: extract (100, 200 mg/kg), pentazocin (reference, 30 mg/kg) and 0.5% w/v sodium CMC (control, 0.1 ml/10 g) in normal saline intraperitoneally. Basal reaction time was measured initially (0 min) and at 15, 30, 45 and 60 min. A cut-off period of 10 s

was observed to avoid damage to the tail [14,15].

Tail immersion method

The animals were screened for sensitivity by immersing the tail of the mice gently in hot water maintained at 55 - 55.5 °C. The mice that lifted their tails from the hot water within 5 s were selected for the study. The selected mice were then divided into four groups (n = 6). Group-I animals received 0.5 % Na CMC (0.1 ml/10 gm) in normal saline intraperitoneally and served as control. Group-II received pentazocin (reference, 30 mg/kg) while Groups-III and IV animals received the extract in 0.5 %w/v Na CMC in normal saline at a dose of 100 and 200 mg/kg, respectively, in a similar manner. Following administration of the samples, reaction time was measured at 0, 15, 30, 45 and 60 min [15,16].

Statistical analysis

All the results are expressed as mean \pm standard error of mean (SEM). The data were analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by Dunnett's test using Graph Pad Prism, version 4.5, software (Graph Pad Software, Inc). Values of $p < 0.001$ were considered statistically significant.

RESULTS

Gross behavioral and acute toxicity

Gross behavioral and acute toxicity data revealed marked analgesia at all tested dose levels. No mortality was observed in any of the tested doses at the end of 72 h.

Anti-inflammatory activity

Anti-inflammatory data are presented in Table 1 for the carrageenan-induced paw edema model and Table 2 for histamine-induced inflammation model, The results reveal that the extract exhibited statistically

Table 1: Anti-inflammatory activity of the hydroalcohol extract of *A. bulbifer* in carrageenan-induced paw edema in Wistar rats

Group	Treatment	Dose	Paw volume (ml)				Inhibition of paw edema (%) at 4 h
			0 h	1 h	2 h	4 h	
I	Control	0.2ml/100g	0.75±0.038	0.997±0.044	1.21 ± 0.044	1.48±0.039	–
II	Ibuprofen	10mg/kg	0.706±0.020	1.06 ± 0.038	1.037±0.024	0.96±0.045*	64.0
III	Extract	100mg/kg	0.71±0.015	1.057±0.025	1.08 ± 0.023	1.04±0.027*	53.5
IV	Extract	200mg/kg	0.69±0.022	0.972±0.022	1.027±0.018	0.99±0.039*	56.5

All values are expressed as mean ± SEM, * $p < 0.001$, compared to control group

Table 2: Anti-inflammatory activity of hydroalcohol extract of *A. bulbifer* in histamine-induced paw edema in Wistar rats

Group	Treatment	Dose	Paw volume (ml)				% inhibition of paw edema at 4 h
			0 h	1 h	2 h	4 h	
I	NaCMC	0.2ml/100g	0.76 ± 0.013	0.94 ± 0.016	1.20 ± 0.043	1.30±0.029	–
II	Ibuprofen	10mg/kg	0.706±0.044	1.056±0.024	1.026±0.024	0.98±0.023*	61.18
III	Extract	100mg/kg	0.73 ± 0.028	1.14±0.196	1.10±0.021	1.08±0.02*	52.05
IV	Extract	200mg/kg	0.69 ± 0.013	1.063±0.031	1.02 ± 0.032	0.986±0.029*	57.101

All the values were expressed as mean ± SEM; * $p < 0.001$, compared to control group..

Table 3: Analgesic activity of the hydroalcohol extract of *A. bulbifer*, evaluated by tail flick method

Group	Treatment	Dose	Basal Reaction time (s)	Reaction time (s)			
				15min	30min	45min	60min
I	Na CMC	0.1	2.0±0.333	2.33±0.192	2.5±0.204	2.66 ± 0.192	2.16±0.152
II	Pentazocin	ml/10g	2.16±0.28	4.0±0.408*	5.5±0.39**	6.83±0.723**	8.16 ± 0.597**
III	Extract	30mg/kg	2.33±0.192	3.5±0.204*	4.5±0.39*	5.83±0.549**	7.167±0.495**
IV	Extract	100mg/kg 200mg/kg	2.0±0.235	3.83±0.335*	5.0±0.235**	6.33±0.384**	7.33±0.561**

All values were expressed as mean ± SEM; * $p < 0.01$, ** $p < 0.001$, compared with control

significant ($p < 0.001$) anti-inflammatory activity at all the dose levels at the 4th hour of the study.

Analgesic activity

The results obtained from analgesic studies are presented in Tables .3 and 4 for tail flick and tail immersion methods, respectively. The extract showed statistically significant ($p < 0.001$) analgesic activity at all tested dose levels. The extract activity was statistically comparable to the reference drug employed in terms of analgesic activity, with reaction

time for the reference drug (pentazocin) and extract being 8.2 and 7.3 s, respective in the tail flick test, and 8.5 and 7.8 s, respectively, in the tail immersion test at 60 min interval. These results are statistically significant ($p < 0.001$), compared to control.

DISCUSSION

Inflammation is caused by release of chemicals from tissues and migrating cells. Most strongly implicated are the prostaglandins (PGs), leukotrienes (LT5), histamine, bradykinin, and, more recently, platelet-

Table 4: Analgesic activity of the hydroalcohol extract of *A. bulbifer*, evaluated by tail immersion method

Group	Treatment	Dose	Basal Reaction time (s)	Reaction time (s)			
				15min	30min	45min	60min
I	Na CMC	0.1ml/10g	2.5 ± 0.204	2.16±0.28	2.33±0.192	2.5±0.204	2.66±0.192
II	Pentazocin	30mg/kg	2.33±0.192	4.167±0.366*	5.5±0.311**	7.0±0.235**	8.5 ± 0.204**
III	Extract	100mg/kg	2.16±0.152	3.66±0.192*	4.5±0.204**	6.0±0.235**	7.0±0.577**
IV	Extract	200mg/kg	2.3 ± 0.192	3.83±0.28*	4.83±0.435**	6.5±0.456*	7.83±0.459**

activating factor (PAF) and interleukin-1[9].. The healing process of inflammation is biphasic, namely, primary and secondary phases. Histamine is involved in the primary phase whereas prostaglandins are associated with the secondary phase. Both are known as inflammatory mediators [9,10].

Inhibition of inflammation by the extract at the doses tested (100 and 200 mg/kg) in carrageenan in both inflammation models ranged from 52 - 57 %. Inflammation inhibition by the extract is less than that of the reference compound, ibuprofen due probably by interference by other compounds in the crude extract. However, the extract exhibited consistent anti-inflammatory activity irrespective of the model used, and this suggests that anti-inflammatory effect may be due to the inhibition of prostaglandin synthesis [9,10].

Tail-flick and tail immersion responses are believed to be spinally mediated reflex. The effectiveness of analgesic agents in the tail-flick pain model is highly correlated with relief of human pain [13]. In the two models used, though the data showed that the extract dose-dependently increased pain threshold, the increase in the pain threshold tail flick latency profiles of the extract were less than that of the standard drug, pentazocin. The μ receptor stimulation is generally associated with pain relief and has been shown to be potent in regulating thermal pain [13]. Non-analgesic effects via μ receptors include respiratory depression and, most importantly for therapeutic considerations, is its induction of physical dependence [14]. Activation of μ_2 opioid subtype leads to spinal analgesia and

commonly causes constipation as adverse effect. Therefore, taking all these data together we believe that the analgesic activity of the extract is most likely to be mediated by central action (spinally and supraspinally) [14] and indicates a codeine-like mechanism by binding with opioid receptors.

In tail flick method, the extract showed significant activity after 45 min but in the tail immersion method, the extract showed significant activity 30 min after administration.

CONCLUSION

On the basis of our findings, it can be inferred that the hydroalcohol extract of *A. bulbifer* has analgesic and anti-inflammatory activities. These activities are dose-related. The results corroborate the basis for the traditional use of the plant in folk medicine. However further investigations are required to identify the active components as well as undertake advanced studies on the efficacy of the identified compounds.

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