

ANALGESIC, ANTI-PYRETIC AND ANTI-INFLAMMATORY ACTIVITY OF THE LYMPH EXTRACTED FROM BELLAMYA BENGALENSIS F. ANNANDALEI IN RODENT MODELS

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ABSTRACT

Innumerable scientific reports have identified the therapeutic roles of animal toxin and secretions. Animal secretions exhibit physiological responses like hemolysis, platelet aggregation, and modulation of membrane bound enzymes etc. *Bellamya bengalensis*, a freshwater edible snail, has been used as medicine since ancient time for the cure of ailments like asthma, conjunctivitis etc. The present study aims to evaluate the anti-inflammatory, analgesic and antipyretic activity of the secretion of *Bellamya bengalensis* f. *annandalei* designated as LBB in rodent models. Acute toxicity, acute anti-inflammatory study with carrageenan induced rat paw oedema, croton oil induced ear inflammation in mice, subacute study with cotton-pellet induced granuloma and chronic study with Freund's adjuvant induced poly-arthritis. Mechanism of action, using arachidonic acid induced rat paw-

oedema. Analgesic studies include writhing, hot plate, tail clip method. Anti-pyretic activity with Brewer's yeast induced pyrexia in rat. Pretreatment with LBB inhibits arachidonic acid-induced paw inflammation by both the COX-LOX pathway of arachidonic acid metabolism. This study emphasizes on the exploration of selective and less toxic lead therapeutic with better therapeutic indices.

KEYWORDS: *Bellamya bengalensis*, Secretion, Inflammation, Pain, Pyrexia.

INTRODUCTION

Chronic inflammatory diseases remain one of the world's major health problems.^[1] Inflammation has become the focus of global scientific research because of its implication in human and animal diseases. Ant-inflammatory drugs have attributed to various adverse effects such as gastric lesions caused by (NSAIDs), tolerance and dependence induced by opiates. Therefore, new anti-inflammatory and analgesic drugs lacking these side effects are being researched as alternatives to NSAIDs and opiates.^[2,3] Ethnic folk medicine still makes use of animals and products derived from animal organs; examples of current uses of animal-derived remedies can be found in many urban, semi urban and more remote localities in different parts of the world. *Bellamya bengalensis* f. *annandalei*, a freshwater edible snail species has been used for the cure of a number of ailments like asthma, conjunctivitis etc.^[4] Gomes et al. 2011 reported hepatoprotective activity of the edible snail (*Bellamya bengalensis*) flesh extract against carbon tetrachloride induced hepatotoxicity in rats.^[5] A potent anti-bacterial peptide was also isolated from the secretion extract of *Bellamya bengalensis*.^[6] The snail flesh is known to increase bone strength and prevents joint disorders. The snail shell is used in acupuncture treatment in Chinese folk medicine. The santhal tribe uses the water of snail in treating ophthalmia, hazy vision and night blindness. Snail extract cream helps in maintaining skin tone and wrinkle free skin. Cone snail is also used in diabetic neuropathy and in different CNS disorders. However, studies with the isolated lymph of *Bellamya bengalensis* f. *annandalei* have not been performed so far. The present study attempts to evaluate the anti-inflammatory, analgesic and antipyretic activities of the isolated lymph of *Bellamya bengalensis* f. *annandalei* named "LBB".

MATERIALS AND METHOD

Reagents

Carrageenan, Croton oil, Arachidonic acid, Acetyl Salicylic acid, Indomethacin, Paracetamol, Brewer's yeast, Freund's complete adjuvant, Pentazocin was purchased from Sigma, Phenidone from Biomol. Co., Acetic acid, Pyridine, sodium chloride, diethyl ether (Merck), and all other chemicals of analytical grade were procured locally.

Collection and preparation of Test Sample

Freshly collected live adult *Bellamya bengalensis* f. *annandalei* (Kobelt,1908), family Viviparidae were collected commercially from the Howrah market and authenticated by Dr. R. Venkitesan Scientist- 'C' O/C Mollusca Section, Zoological Survey of India Kolkata- 53.

Authentication No: F.No.229-10/98-Mal (1) dated 24th May 2010. Live snails were washed twice with tap water and Mili-Q water. The lymph of adult snail was isolated without any injury by sterile disposable syringe at the anterior end where the operculum is attached. 100 ml of lymph was collected, kept in ice and filtered by Whatman filter paper to avoid the mud and debris. The filtered lymph was passed through 3 kDa Biomax polyethersulfone (PES) cut-off membrane using Amicon Stirred Ultra Filtration Cells (MA, USA). The partially purified filtrate was lyophilized to get the powder form which was stored at -20°C. Stock solution of 10mg/ml was prepared in Mili-Q water for experimentation. The lymph extract of *Bellamya bengalensis* f. *annandalei* is designated as LBB.

Animals

Experiments were carried out on Wistar albino rats weighing 120-150gm and Swiss albino mice weighing 22-25gm. The animals were housed under conditions of 22 ± 1 ° C, $50 \pm 10\%$ humidity and 12 hours light and 12 hours dark cycle. The animals received a diet of food pellets (fortified with minerals and vitamins) prepared in the animal house and water *ad libitum*. The animals were bred in the animal house of Gupta College of Technological Sciences. The animal experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care (CPCSEA) after getting clearance from Institutional Animal Ethics Committee (GCTS/IAEC/2012-June/06 dated 06.06.2012).

Acute toxicity studies

The acute toxicity studies were performed in accordance with the OECD guidelines 423.^[7] For acute toxicity study, different doses of LBB were injected intra-peritoneally (i.p.) to the group of mice and 24hrs mortality was recorded.^[8]

Anti-inflammatory study

Carrageenan-induced rat paw oedema

Wistar albino rats were divided into four groups. Group 1 served as negative control and received normal saline (0.1ml/100gm i.p). Group 2 was administered the standard drug, Acetyl Salicylic acid (100mg/kg i.p). Group 3 and 4 received LBB at 50 & 100mg/kg i.p respectively. Post-administration of Carrageenan (0.1ml in 1% saline) into the plantar aponeurosis of right hind paw of all the rats' oedema was observed.^[9] The left hind paw served as control. The paw volume of each rat was measured at intervals of 1, 2, 3 and 4hrs of treatment using digital plethysmometer (Orchid scientific). The initial and final volume of the right hind paw of each rat was calculated, and the % inhibition was calculated for each group.

Croton oil induced ear inflammation in mice

Swiss albino mice were divided into four groups. Group 1 served as the negative control and was treated with normal saline i.p, Group 2 received 100mg/kg i.p Acetyl Salicylic acid, the standard drug, Group 3 and 4 were treated with 50mg/kg and 100mg/kg i.p LBB respectively, 30 mins prior to croton oil application. 0.1ml Croton oil irritant solution was applied to the inner surface of the right ear of mice ^[10]. The mice were sacrificed by cervical dislocation after 4 hrs and 7mm punches using a cork borer were made in the right ear. Each ear disk was weighed and compared with control.

Cotton-pellet induced granuloma

Autoclaved cotton pellets weighing 10 ± 1 mg were implanted subcutaneously in both sides of the groin region of the rat. ^[12] Animals were divided into four groups (n=3). Group 1 was treated with PBS (1 ml /kg i.p) Group 2 received Acetyl Salicylic acid (standard drug, 100mg/kg i.p) and Groups 3 & 4 were treated with 50mg/kg and 100 mg/kg i.p LBB respectively for seven consecutive days. On the eight-day the animals were sacrificed by cervical dislocation and the pellets along with the granuloma tissues were removed, dried in an oven at 60°C, weighed and compared with control. ^[11]

Freund's adjuvant induced poly-arthritis

Rats were divided into four groups (n=3). 0.1 ml of Freund's complete adjuvant was injected into the plantar region of each rat on day 1. All test drugs were administered for 21 consecutive days. Group 1: 1 ml/kg normal saline i.p, Group 2: Acetyl Salicylic acid 100 mg/kg i.p and Groups 3 & 4: 50mg/kg and 100mg/kg i.p LBB. The paw volume for each group was measured using plethysmometer (Orchid scientific) on day-0 before administration of adjuvant and on day-21 post treatment. Number of ear nodes and tail nodes were also noted on the same day and percentage inhibition was thereby calculated. ^[12]

Arachidonic acid induced paw-oedema

The rats were divided into four groups (n=3). Group 1(negative control) was treated with 1ml/kg normal saline, Group 2 was treated with 100mg/kg Phenidone i.p, Group 3 received 50mg/kg LBB i.p. and Group 4 was given 10mg/kg Indomethacin i.p. Paw oedema was induced by single injection of 0.1 ml (0.5%) arachidonic acid in 0.2M carbonate buffer (pH 8.4) into the right hind paw (sub plantar) of rats 30 min after drug treatment. Hind paw volume was measured 1 hour after arachidonic acid injection. ^[13]

Analgesic study in mice

Writhing in mice

One-hour post administration of control, LBB and standard the mice were injected with 1% v/v (10ml/kg i.p) acetic acid. Group 1 received vehicle while Group 2 received 100 mg/kg i.p of the standard drug Acetyl Salicylic acid, the other two groups received 50 and 100mg/kg i.p. LBB respectively. Writhing response characterized by abdominal constrictions and the stretching of hind limbs was observed by the method of Turner. ^[14] Number of writhing movements was counted for 15 min and percentage inhibition of writhing movement was calculated.

Eddy's Hot plate method

Mice were kept on Eddy's hot plate (Orchid Scientifics) having constant temperature of $55 \pm 0.1^\circ\text{C}$. Group 1 received normal saline. Group 2 served as standard and were injected Pentazocin (10mg/kg). Group 3 and 4 were administered 50 and 100mg/kg i.p LBB. Various responses (paw licking and jumping) were recorded before and after 30, 60, and 90 min. ^[15]

Anti-pyretic study

Brewer's Yeast induced pyrexia

Initially a digital thermo- meter was inserted 3-4cm deep into the rectum, after fastened the tail to record the basal rectal temperature. Rats (n=3) received 1 ml subcutaneous of 20% Brewer's yeast at the nape of neck. After 18 hour of yeast injection, rats which showed a rise in temperature of at least 1°C were taken for the study. Animals in the various groups were treated as follows: Control group received 0.1ml/100gm i.p. normal saline and treated groups received LBB (50 and 100 mg/kg i.p). Group 4 received Paracetamol the standard drug at a dose of 100mg/kg. Rectal temperature was recorded every hour for four hours after administration of drugs.

Statistical Analysis

Values reported are mean \pm S.E.M. Student's t test was used for statistical analysis and a probability level of $p < 0.05$ was considered significant & $p < 0.001$ as highly significant. Percent inhibition was calculated by the formula hereby: % Inhibition = (mean of control – mean of treated group/ mean of control) x 100.

RESULTS

Acute Toxicity Studies

The isolated lymph of *Bellamyia bengalensis* f. *annandalei* was found to be safe up to 1.6gm/kg dose intraperitoneal.

Anti-inflammatory activity

Carrageenan induced rat paw oedema

The LBB inhibited Carrageenan induced paw oedema in a dose dependant manner. The effect of 50mg/kg of LBB was comparable to the effect produced by Acetyl Salicylic acid, standard anti-inflammatory drug. [Table 1].

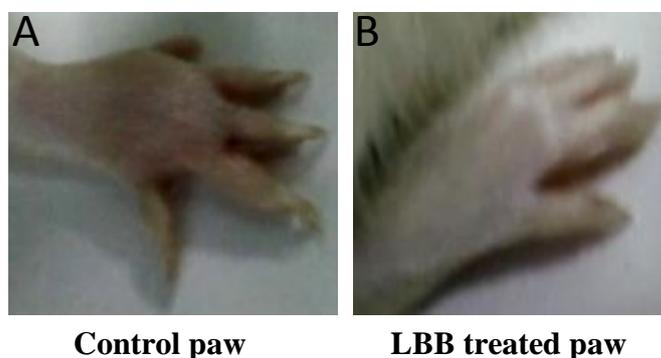


Table 1: Effect of LBB on two acute models of inflammation.

Drug	Dose (mg/kg)	Carrageenan-induced paw oedema (volume in ml) in rat	Croton oil-induced ear inflammation in mice (mg)
Saline Control	1ml/kg i.p	4.3 ± 0.02	45 ± 15.0
Acetyl Salicylic acid	100mg/kg i.p	2.8 ± 0.42*	11 ± 2.4 *
LBB	50mg/kg i.p	2.9 ± 0.20*	21 ± 2.3 *
LBB	100mg/kg i.p	2.3 ± 0.10*	10 ± 1.8*

Values are mean ± S.E.M, Number of rat/mice in each group (n=3), *Denotes significant reduction from control ($P < 0.05$).

Croton oil induced ear inflammation in mice

There was a dose-dependent inhibition in the croton oil induced ear inflammation in mice, comparable to that produced by the standard Acetyl Salicylic acid. [Table 1] The reduction in weight of ear disc produced by LBB was highly significant as compared to control.

Cotton pellet induced granuloma

The increase in the dry weight of cotton pellet granuloma was compared to the control and it was found that LBB inhibited the increase in dry weight at the different doses. The effect of 50mg/kg of LBB was more effective than Acetyl Salicylic acid, standard drug. [Table 2]

Table 2: Effects of LBB on cotton pellet induced granuloma (Sub-Chronic) and Freund's adjuvant induced polyarthritis (Chronic) models of inflammation.

Drug	Dose (mg/kg)	Increase in cotton pellet weight in rat(mg)	Difference in paw volume between Day 0 and Day 21 in rat (ml)
Saline Control	1ml/kg(i.p.)	90 ± 3.62	1.42 ± 0.15
Acetyl Salicylic acid	100mg/kg (i.p.)	70 ± 8.75*	0.91 ± 0.01*
LBB	50mg/kg (i.p.)	65± 2.76*	0.82 ± 0.25 *
LBB	100mg/kg (i.p.)	55± 1.95*	0.54 ± 0.13 8*

Values are mean ± S.E.M, Number of rat in each group (n=3), *Denotes significant reduction from control (P < 0.05).

Freund's adjuvant induced polyarthritis

Prophylactic treatment with LBB at the doses of 50 and 100mg/kg i.p., inhibited development of arthritis as detected by reduction of paw volume and increased mobility of joints. LBB also showed 10% more inhibitory effect as compared to Acetyl Salicylic acid. [Table 2] The secondary lesions like tail and ear buds as well as joint movements were also inhibited after 21 days' consecutive treatment with LBB as compared to control.

Arachidonic acid-induced paw oedema

Arachidonic acid injection in sub plantar of aponeurosis of right hind paw produced significant oedema after 1 hour. Indomethacin, the cyclooxygenase blocker, inhibited it by 33% whereas Phenidone, a dual blocker, inhibited it by 70%. LBB at the dose of 50mg/kg inhibited the edema by 72%, which suggested that the secretion of *Bellamyia bengalensis* f. *annandalei* behaved like Phenidone. [Table 3].

Table 3: Comparisons of rat paw oedema in control and LBB treated animals after one hour of arachidonic acid injection.

Drug	Dose (mg/kg)	Difference in paw volume in ml (Mean \pm S.E.M.)	% inhibition (compared to saline control)
Saline Control	1ml/kg(i.p.)	50.75 \pm 3.62	-
Indomethacin	10 mg/kg (i.p.)	34.23 \pm 2.13	33%
Phenidone	100 mg/kg (i.p.)	15.12 \pm 1.65*	70%
LBB	50 mg/kg (i.p.)	14.01 \pm 2.76*	72%

Values are mean \pm S.E.M, Number of rat/mice in each group (n=3), *Denotes significant reduction from control (P < 0.05).

Analgesic study

Writhing in mice

Intraperitoneally injected acetic acid produced abdominal constriction, characterized by stretching response. LBB significantly reduced acetic acid induced writhing in mice in a dose-dependent manner and prolonged the onset of writhing [Table 4].

Table 4: Effect of LBB on acetic acid induced writhing in mice.

Drug	Dose (mg/kg)	Onset of writhing in min (Mean \pm S.E.M.)	No. of writhing in 15min (mean \pm S.E.M.)
Saline Control	1ml/kg(i.p)	6.18 \pm 0.40	139 \pm 2.13
Acetyl Salicylic acid	100mg/kg (i.p)	10.25 \pm 1.12	97 \pm 3.12
LBB	50 mg/kg (i.p)	9.26 \pm 2.14	54 \pm 2.14
LBB	100 mg/kg (i.p)	11.25 \pm 1.14	38 \pm 2.15*

Values are mean \pm S.E.M, Number of mice in each group (n=3), *Denotes significant reduction from control (P < 0.05).

Eddy's hot plate

LBB showed insignificant analgesic activity when tested using the hot plate method. [Table5] Since nonnarcotic analgesics can be differentiated from narcotic ones by their ineffectiveness in the hot plate method,^[14] it can be assumed that the analgesic activity of LBB is of the type produced by non-narcotic analgesics.

Table 5: Effect of LBB on thermal nociception in mice (Hot Plate).

Drug	Dose (mg/kg)	30 mins	Response after 60mins (Mean \pm S.E.M)	120mins
Saline Control	1ml/kg (i.p)	5.535 \pm 0.165	5.175 \pm 0.605	2.89 \pm 0.133
Pentazocin	10mg/kg (i.p)	8.45 \pm 0.122	6.25 \pm 0.21	5.15 \pm 0.156
LBB	50 mg/kg (i.p.)	4.97 \pm 0.356	4.63 \pm 0.265	4.21 \pm 0.236
LBB	100mg/kg (i.p.)	4.35 \pm 0.43	4.07 \pm 0.62	3.98 \pm 0.315

Values are mean \pm S.E.M, Number of mice in each group (n=3).

Anti-pyretic activity

Brewer's Yeast induced pyrexia

The reductions in rectal temperature of febrile rats, treated with different doses of LBB, were recorded at 60, 120, 180 and 240 min post administration. The reduction in rectal temperature of treated animals at each interval was compared with that of untreated febrile rats. [Table 6].

Table 6: Effect of LBB on pyrexia in rat (Brewer's Yeast Method).

Drug	Dose (mg/kg) i.p	Temp.(°C) 18hrs after Brewer's yeast	Response after treatment at (Mean \pm S.E.M)			
			19hr	20hrs	21hrs	22hrs
Control	1ml/kg	38.04 \pm 0.47	38.09 \pm 0.24	38.09 \pm 0.24	38.09 \pm 0.24	38.09 \pm 0.24
PCM	100mg/kg	38.02 \pm 0.15	37.6 \pm 0.22	37.15 \pm 0.09	36.44 \pm 0.17	35.68 \pm 0.05**
LBB	50 mg/kg	37.80 \pm 0.03	37.70 \pm 0.07	37.02 \pm 0.57	36.24 \pm 0.95	36.10 \pm 0.20*
LBB	100 mg/kg	38.10 \pm 0.26	38.02 \pm 0.20	37.10 \pm 0.20	36.23 \pm 0.15*	36.01 \pm 0.20*

Values are mean \pm S.E.M, Number of rat in each group (n=3), *Denotes significant reduction from control (P < 0.05).

DISCUSSION

The results of the present investigation quite clearly indicate that lymph secretion of the *Bellamyia bengalensis* f. annandalei has significant anti-inflammatory, analgesic and antipyretic activity in Wister albino rat and Swiss mice in a dose-dependent manner on both acute models of inflammation. Oedema induced by phlogistic agents is a widely accepted model for the evaluation of anti-oedema effect of drugs.^[9] Carrageenan-induced paw oedema is a classical model of acute inflammation involving various types of chemical mediators of inflammation such as histamine, serotonin, bradykinin and finally prostaglandins.^[17] The anti-inflammatory effect of LBB could be observed in two types of rodent models of 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. LBB inhibited edema similar to that of the dual blocker Phenidone in the arachidonic acid induced paw oedema model in rat and since indomethacin failed to show any significant inhibitory effect in this model, it is plausible that LBB reduces inflammation by blocking both COX-LOX pathways of arachidonic acid metabolism. The observation that LBB significantly reduces inflammation in the Freund's adjuvant induced polyarthritis in rat reveals that LBB possesses anti-arthritic activity as well. All the animal models exhibit potent anti-inflammatory effect of LBB. We have further explored the analgesic activity of lymph extract of *Bellamyia bengalensis* f. *annandalei* on mice. LBB inhibits thermal induced pain as well as physical induced pain. The acetic acid-induced writhing is a visceral pain model and widely used for the evaluation of peripheral anti-nociceptive activity.^[18] The i. p administration of an agent that irritates the serous membranes causes a stereotypical behavior in mice, characterized by abdominal contractions, movements of the body as a whole, twisting of the dorsal abdominal muscles, and a reduction in the motor activity and coordination.^[19] LBB reveals that its analgesic activity is of the type produced by non-narcotic analgesics.^[14] Research suggests that LBB can henceforth be treated as NSAIDs from natural source. Lymph of *Bellamyia bengalensis* f. *annandalei* also plays pivotal role in Brewer's yeast induced pyrexia pathology by demonstrating significant anti-pyretic activity. For centuries, natural anti-inflammatory compounds have been used to mediate the inflammatory process and often with fewer side effects. We have experimentally found that the animal-derived natural compound, i.e. lymph of *Bellamyia bengalensis* f. *annandalei* exhibits similar effectiveness in treating the inflammatory reaction seen in both acute inflammation and pain syndromes encountered in a typical *in-vivo* practice. However, no lethal effects of LBB were found *in vivo* up to at a dose of 1.6 gm/kg body weight. Further study is aimed at isolating and elucidating the chemical structure of the bioactive principles responsible for the anti-inflammatory and analgesic properties.

Conflict of Interest

The authors declare they have no conflict of interest.

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