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Original Research Article

Assessment on antinociceptive actions of soluble fractions derived from edible mollusc (*Bellamya bengalensis* Lam.)

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ABSTRACT

Background: *Bellamya bengalensis*, an edible bivalve mollusc is traditionally used in the treatment of joint pain, bone fracture, jaundice and eye infections. Present study was designed to find out the most potent analgesic fractions derived from the body mass of *Bellamya bengalensis*.
Methods: The test specimen was collected, identified and fractionated with solvent medium like, phosphate buffer saline (PB), ethyl acetate (EB), methanol (MB) and chloroform (CB). Protein concentration of each fraction was determined. The antinociceptive activities were measured either by thermal models like, hot plate and tail immersion (central analgesic action) or by chemical model like acetic acid induced writhing (peripheral analgesic action) in mice. Diclofenac sodium was used as analgesic standard.
Results: Significant peripheral and central analgesic activity showed by phosphate buffer saline fraction at 100mg/kg, even better than diclofenac standard at 10mg/kg. In hot plate and tail immersion tests, phosphate buffer saline showed the highest activity followed by methanol, chloroform and ethyl acetate fraction respectively. However, in case of peripheral analgesic experiment, phosphate buffer fraction exhibited maximum writhing inhibitory properties and that was followed by chloroform, methanol and ethyl acetate fraction respectively.
Conclusions: Phosphate buffer saline fraction of *Bellamya bengalensis* showed maximum potential central and peripheral analgesic activity than any other fractions.

Keywords: Analgesic, *Bellamya bengalensis*, Eddy's hot plate, Mice, Mollusc

INTRODUCTION

Pain is a noxious stimuli which involves sensory, emotional and cognitive experience and leads to from potential tissue damage.[1] Severe pain needs the use of strong analgesics which can act peripherally by blocking the generation of impulses at chemoreceptor site of pain or can act centrally by raising the threshold and altering the physiological response to pain.[2] Non Steroidal anti-inflammatory drugs (NSAIDs) is still the backbone therapy in the treatment of pain by inhibiting cyclooxygenase, the enzyme which catalyzes the rate-limiting step in the metabolic conversion of arachidonic acid to prostaglandins and related eicosanoids.[3,4] Unfortunately, chronic use of NSAIDs may cause of health hazards, range from dyspepsia to gastric irritation or bleeding and acute renal failure.[4,5]

The ethnopharmacological knowledge provides enough information on the folklore practices and traditional aspects of therapeutically important natural products.[6] Plant based drugs are used as the source of traditional medicine since ancient times in India. But use of animal products, as an important component of traditional medicine has been much less studied in the country.[7] Molluscs are a diverse group of organisms produces many medicinally significant metabolites. The conotoxin from the cone-shell snail inhibits pain as well as accelerates the recovery of injured nerves.[8] *Bellamya bengalensis*, a freshwater mollusc, (class: Gastropoda) are also used in the treatment of various diseases such as arthritis, asthma, and conjunctivitis.[9] It has been documented in earlier works, that the extrapallial fluid of *Bellamya bengalensis* possess anti arthritic and analgesic activity.[10]

Mollusc usually consists of hard shell, mantle and a foot. The whole soft body lies within an enlarged mantle cavity. Elutions with different polar and non polar solvents convey diverse chemical molecules. Phosphate buffer saline is a polar solution that mimics the physiological body fluid, whereas the chloroform, methanol and ethyl acetate have non polar characteristics.[11,12] Hence, the present study was aim to explore the maximum antinociceptive properties of different fractionations eluted with polar to non-polar solvents from soft body mass of *Bellamya bengalensis*.

METHODS

Collection and identification of the mollusc

Bellamya bengalensis was collected from Kolkata, West Bengal, India. The bivalve mollusc was authenticated from Zoological Survey of India, Kolkata (No:1242/lot No-63), as *Bellamya bengalensis* Lam. of the Viviparidae family.

Sample preparation

The freshwater mollusc was collected live from the ponds and was washed well with distilled water for three times consecutively. The shell was carefully removed and the whole mass was eluted with four separate solvents-phosphate buffer saline, ethyl acetate, methanol and chloroform. In brief, the body mass of *Bellamya bengalensis* Lam. was left overnight at 4°C in the respective solvents. Thereafter, mass was grinded, followed by spinning down for 5000rpm for 10 min and filtered through Whatman filter paper. Four eluted fractionations were marked as PB for phosphate buffer saline, MB for methanol, EB for ethyl acetate and CB for chloroform.

Animals

Acute toxicity and analgesic studies were done with Swiss albino mice weighing 20-25g. The animal experiments were conducted in accordance with the accepted principles for laboratory animal use and care. The animals were kept in the animal house, maintaining standard condition and fed with proper diet and water *ad libitum*.

Acute toxicity study

Acute oral toxicity study of all the four fractions of mollusc namely PB, MB, EB, CB was performed according to Organization for Economic Cooperation and Development (OECD) guidelines 423.[13] Three animals in each group, albino female mice weighing 20-25g were used in each group of the study. All the four fractions were administered oral single dose in progressive manner up to 2g/kg of mass body weight of mollusc. The animals were observed for any sign of toxicity, morbidity or mortality for initially 24h and followed by next 72h.

In-vivo analgesic study

Thermal nociception by Eddy's hot plate

Ten groups of Swiss mice, where N=6 in each group, weighing 20-25g were selected for this study. The group divisions are shown in Table 1.

Table 1: Group division for in-vivo analgesic activity.

Groups	Treatment	Dose (Oral)
Control	Normal saline	0.5ml/kg b.w.
Standard	Diclofenac Sodium	10mg/kg b.w.
PB I	PBS fraction of *B. bengalensis*	100mg/kg b.w.
PB II	PBS fraction of *B. bengalensis*	200mg/kg b.w.
EB I	Ethyl acetate fraction of *B. bengalensis*	100mg/kg b.w.
EB II	Ethyl acetate fraction of *B. bengalensis*	200mg/kg b.w.
MB I	Methanol fraction of *B. bengalensis*	100mg/kg b.w.
MB II	Methanol fraction of *B. bengalensis*	200mg/kg b.w.
CB I	Chloroform fraction of *B. bengalensis*	100mg/kg b.w.
CB II	Chloroform fraction of *B. bengalensis*	200mg/kg b.w.

Eddy's hot plate (Orchid Scientifics, India) was used for this experimentation. The constant temperature was fixed at 55±1°C. All mice were placed individually on the hot plate after 30 min, 60 min and 90 min after drug administration. The time interval of pain responds either by paw licking or jumping was recorded. The cut of time for the response was 15 sec.[14,15]

Tail-immersion method

Ten groups, each group, consisting of six Swiss mice weighing 20-25g were randomly selected as above. After administration of the test drug, the lower end of the tail of

the animal was dipped in hot water up to 5cm. Temperature of the water was maintained at 55±1ºC. The sudden withdrawal of tail from the water was recorded as response. The cut off time was 10 sec. The reaction time was recorded just before the administration of the drug (0 min) and was compared with the response at 60 min and 90 min after drug administration.[15,16]

Acetic acid induced writhing

Swiss albino mice of either sex (20-25g) were divided in ten groups comprising of six animals each, as described above. Each mouse was injected with 0.6% acetic acid intraperitoneally, 30 minutes after the administration of test drugs at different doses in oral route. Number of writhes was counted for 10 min after acetic acid injection and the degree of analgesia was calculated as follows:[16]

$$\text{Degree of analgesia} = \frac{(\text{No. of writhing in control} - \text{no. of writhing in of test}) \times 100}{\text{No. of writhing in control}}$$

Statistical analysis

The results were presented as Mean±standard error of mean. The data were statistically analyzed by ANOVA and Dunnett's post-hoc test. The differences were considered statistically significant when $p \leq 0.05$ and $p \leq 0.01$.

RESULTS

Acute toxicity study

All the test fractions of *Bellamya bengalensis* L. namely, PB, MB, EB and CB were found to be safe up to oral dose of 2g/kg body weight in mice.

Thermal nociception by Eddy's hot plate method

Results of thermal nociception activity by hot plate method are enumerated in Table 2. The responses were specified at 30 min, 60 min and 90 min after administration of the test fractions. The controls, which were treated with normal saline, did not show any significant change throughout the 90 min observation. The test sample, PB at the oral dose of 100 mg/kg body weight showed significant analgesic activity by increasing the pain latency from 3.1±0.27sec to 13.5±0.40**sec. MB and CB were also showed analgesic activity at 100 mg/kg dose which increased the latency period to 11.6±0.36** sec and 9.0±0.96** respectively when compared to control. Standard analgesic, Diclofenac sodium at the oral dose of 10 mg/kg was significantly increased the latency period from 2.9±0.15 sec to 12.0±0.44** sec (Table 2).

Table 2: *Bellamya bengalensis* L. fractions on reaction time in hot plate test.

Groups	Treatment/Dose	Reaction time in sec (Mean±SEM)			
		0 min	30 min	60 min	90 min
Control	0.5 ml/kg saline	3±0.25	3.6±0.42	3.8±0.30	3.1±0.64
Standard	10 mg/kg Diclofenac	2.9±0.15	8.1±0.40**	12.4±0.82**	12.0±0.44**
PB I	100 mg/kg PB	3.1±0.27	7.6±1.28**	12.0±1.71**	13.5±0.40**
PB II	200 mg/kg PB	2.9±0.80	6.3±0.33*	6.8±0.81*	6.3±0.22*
EB I	100 mg/kg EB	3.0±0.58	5.9±0.74	5.6±1.30	5.5±0.81
EB II	200 mg/kg EB	3.0±0.25	5.8±0.98	5.7±1.23	5.8±0.87
MB I	100 mg/kg MB	2.9±0.15	7.4±0.63**	11.6±0.97**	11.6±0.36**
MB II	200 mg/kg MB	3.0±0.25	6.8±0.85*	6.8±0.92*	6.7±0.64*
CB I	100 mg/kg CB	2.8±0.25	7.5±0.33**	8.0±0.49**	9.0±0.96**
CB II	200 mg/kg CB	3.0±0.25	7.5±0.61**	7.45±0.96**	7.55±0.81**

Results are expressed as Mean ± SEM (n=6); Statistical analysis was done by One way Anova followed by Dunnett test as post hoc analysis where *p<0.05 and **p<0.01

Tail-immersion method

The normal reaction time of tail flick response in mice to warm water was nearly 1.8-1.9 sec. In this study, test drug fraction, PB showed maximum analgesic activity at a dose of 100 mg/kg and it reduced reaction time of pain sensation from 1.8±0.27 sec to 6.0±0.82** sec within 90 min. Diclofenac sodium lowered the reaction time, the indicator of pain sensation was 1.8±0.28 to 5.0±.56**.

However, higher doses of PB did not showed any significant increase in response time. Moreover, MB and CB also showed significant enhancement in reaction time, but EB did not respond (Table 3).

Acetic acid induced writhing

The study showed that PB at 100 mg/kg dose significantly reduced the writhing response when compared to control.

PB at 100 mg/kg was showed 60.48% reduction in writhes, whereas, Diclofenac was showed 40.90% decrease in writhes compared to control. However, CB at 100mg/kg was showed 48.25% reduction and MB exhibited 38.11% reduction. EB was capable to reduced only 20.62% writhing (Table 4).

Table 3: *Bellamya bengalensis* L. fractions in tail immersion test.

Groups	Treatment/ dose	Reaction time in sec (Mean±SEM)		
		0 min	30 min	60 min
Control	0.5 ml/kg saline	1.8±0.11	1.8±0.21	1.9±0.30
Standard	10 mg/kg Diclofenac	1.8±0.28	5.9±0.33*	5.0±.56**
PB I	100 mg/kg PB	1.8±0.27	5.3±0.82*	6.0±0.82**
PB II	200 mg/kg PB	1.7±0.33	4.1±0.92*	4.9±0.90*
EB I	100 mg/kg EB	1.9±0.58	2.5±0.66	2.7±0.96
EB II	200 mg/kg EB	2.1±0.28	2.7±0.58	2.9±0.74
MB I	100 mg/kg MB	1.9±0.66	2.5±0.30	2.9±0.67
MB II	200 mg/kg MB	2.0±0.88	3.4±0.85*	3.9±0.96*
CB I	100 mg/kg CB	2.0±0.25	2.8±0.63	3.0±0.88
CB II	200 mg/kg CB	1.8±0.42	3.0±0.68*	3.7±0.93*

Results are expressed as Mean±SEM (n=6); Statistical analysis was done by One way Anova followed by Dunnett test;*p<0.05 and **p<0.01.

Table 4: *Bellamya bengalensis* L. fractions in acetic acid induced writhing.

Groups	Treatment/Dose	Degree of analgesia (mean percentage±SEM)
Standard	10mg/kg Diclofenac sodium	40.90±1.01**
PB I	100mg/kg PB	60.48±1.06**
PB II	200mg/kg PB	58.32±1.22**
EB I	100mg/kg EB	20.62±1.10*
EB II	200mg/kg EB	17.13±2.64*
MB I	100mg/kg MB	38.11±2.07**
MB II	200mg/kg MB	32.86±1.29**
CB I	100mg/kg CB	48.25±1.37**
CB II	200mg/kg CB	40.55±1.54**

Results are expressed as Mean percentage±SEM (n=6); Statistical analysis was done by one way ANOVA followed by Dunnett test where*p<0.05 and **p<0.01.

DISCUSSION

Pain is classified into two types; one is fast pain which is mediated by A_δ fibers and another is slow pain which is mediated by C fibers whereas, nociception is the response of the sensory nervous system to the harmful noxious stimuli.[17,18] Present study compared the anti-nociceptive activity of the four different fractions of *Bellamya bengalensis* Lam. Assessment of analgesic actions of test fractions were done following thermal pain stimulus like, hot plate and tail immersion as well as chemical mediator of pain like acetic acid induced writhing. All the four fractions prepared from the body mass of mollusc namely PB, CB, MB and EB were found safe in mice even at oral dose of 2g/kg.

The phosphate buffer saline fraction of mollusc showed the most potent analgesic activity for both central and peripheral pathways. Hot plate and tail immersion method was used to assess the central analgesic activity. These models are based on the sensitization of the nociceptors of the sensory neurons with thermal stimuli. The contributions of endogenous substances like prostaglandins are limited in these cases.[19] Hot plate is also used to study non-inflammatory and acute analgesia. It is established that any agent that increases the latency period by hot plate method are acting centrally.[20] In the present study, PB fractions of *Bellamya bengalensis* Lam. showed maximum significant increase of latency period or analgesic properties in hot plate and tail immersion method followed by MB>CB>EB. Therefore, the phosphate buffer saline extract of body mass of the mollusc may have central analgesic activity.

Acetic acid writhing in mice is a standard model for evaluation of peripheral analgesic activity. Acetic acid is a chemical irritant that stimulates the local peritoneal receptors by releasing serotonin, bradykinin, histamine, prostaglandins (PGs).[21] Intraperitoneal administration of the chemical stimulants signalling to release of PGs especially, PGE_2, $PGF_{2\alpha}$ and lipooxygenase that mediated to elicit the visceral pain and abdominal constrictions.[22,23] There are very few studies exploring analgesic activity of *Bellamya bengalensis*. Previous report showed that the extrapallial fluid of *Bellamya bengalensis* has dose dependent analgesic action.[10] Another study reported foot muscle extract of *Bellamya bengalensis* has significant

central and peripheral analgesic activity.[20] In the present study, among the different fractions eluted from body mass of *Bellamya bengalensis* PB fraction showed maximum significant analgesic action at 100mg/kg followed by CB>MB>EB. From the above findings, therefore it may be suggested that the peripheral analgesic activity of PB fraction may be due to inhibition of prostaglandin mediated pathway for pain regulation.

In the present study, though phosphate buffer saline fraction showed maximum analgesic action but other two non-polar i.e., methanol and chloroform fractions have also mild analgesic properties.

Hence, it may be assumed that the components responsible for analgesic properties of body mass of *Bellamya bengalensis* have both polar and non-polar characteristics. Further, purification is required to characterization the compounds responsible for anti-nociceptive properties of the mollusc, which is presently continuing in our laboratory.

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