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Original Article

Cytotoxic, anti-inflammatory, analgesic, CNS depressant, antidiarrhoeal activities of the methanolic extract of the *Artocarpus Lakoocha* leaves

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ABSTRACT

The study was carried out to assess the cytotoxic, anti-inflammatory, analgesic, CNS depressant and antidiarrhoeal activities of the *Artocarpus lakoocha* methanolic leaves extract. In brine shrimp lethality bioassay, the LC_{50} value of the *Artocarpus lakoocha* leaves was 2.83±0.11 (µg/ml). The anti-inflammatory effect of the extract was observed significant effect at 1st, 2nd and 3rd hour and 200mg/kg showed 64.90% inhibition which is closed to standard (69.86%). The extract (200 mg/kg) exhibited higher analgesic activity against acetic acid induced pain in mice than 100mg/kg dose extract. At both test doses produced a higher percent of protection that is about similar to reference Indomethacin (10 mg/kg) in formalin test. The extract also expressed significant dose dependent CNS depressant activity at 60min, 90min and 120min in both hole cross and open field test. The extract (100 and 200 mg/kg) reduced the frequency and severity of diarrhea in test animals. The results demonstrated that the *Artocarpus lakoocha* methanolic leaves extract had the potential phytomedicine value for its significant cytotoxic, anti-inflammatory, analgesic, CNS depressant and antidiarrhoeal activity.

KEYWORDS: Cytotoxic, Anti-inflammatory, Analgesic, CNS depressant, antidiarrhoeal, *Artocarpus lakoocha*.

INTRODUCTION

Artocarpus lakoocha (A. lakoocha) belongs to the family of Moraceae, popularly regarded as a medicinal plant in the South East Asia region. It is commonly called as Monkey jack. Experimentally, *Artocarpus lakoocha* are shown to possess anti-inflammatory, antiviral, anticancer, antiHIV acftivity [1] and antiaging activity [2]. The fruit pericarp extract has shown dose dependent antibacterial, antioxidant, anthelmintic and insecticidal activity. Bark when applied externally, draws out purulent matter; heals boils, cracked skin and pimples. Seeds are purgative, haemagglutinating [3].

The seed and bark of the plant are reported to be effective in the treatment of stomach and liver disease [4]. The various compounds have been isolated from the plant such as cycloartenone, cycloartenol α- amyrin and leupeol acetate 8 [3]. Stem bark contains a stilbene, lupeol acetate, ß-amyrin acetate, cycloartenol and related compounds. Root barks contain glycoflavanol, lupeol and ß-sitosterol. Wood contains a polyhydric phenolic compound, tetrahydorxystilbene. Two isolectins have been isolated from the seeds [5]. After administration of *Artocarpus lakoocha* (Ma-Haad or Puag Hadd), no pathological change of the viscera was occured. Moreover, the rest of the animals unsanctified were still alive healthily at the end of the study. So *A. lakoocha* would probably be a drug of choice for the treatment of taeniasis due to its previously approved efficacy. With cool water this plant may be wide margin of safety [6].

Literature reviews indicated that no studies combining the anti-inflammatory, analgesic, CNS depressant, cytotoxic as well as antidiarrhoeal activities of the *A. lakoocha leaves*. Taking this in view, the present study aimed to evaluate the cytotoxic, analgesic, anti-inflammatory, CNS activities, along with their antidiarrhoeal activities.

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MATERIALS AND METHODS

Plant materials: *Artocarpus lakoocha* were collected from the adjoining area Tangail, Bangladesh during February 2012. The plant material was taxonomically identified by the National Herbarium of Bangladesh whose voucher specimen maintained in our laboratory for future reference.

Chemicals: Indomethacin, and Diazepam were obtained from Square Pharmaceuticals Company Ltd., Bangladesh; Acetic acid, was purchased from Merck, Germany. Normal saline water (0.9% NaCl), a product of Beximco Infusion Company Ltd., Bangladesh was purchased from local market. BDH Chemicals Ltd kindly provided tween-80, formalin, castor oil, caeageenan and all other chemicals were of analytical grade.

Phytochemical screening of the extract: The extract of *Artocarpus lakoocha* was subjected to qualitative analysis of various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins and flavonoids described elsewhere [7].

Preparation of plant extract: The plant material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The dried powder material (1.2 kg) was refluxed with Methanol for three hours. The total filtrate was concentrated to dryness, in vacuum at 40^0C to render the Methanol extract (310 g).

Animal: Swiss albino mice (25-30g) were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water *ad libitum.* The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of five animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accor-dance with guidelines of the Institutional Animal Ethics Committee [8].

Screening of Cytotoxic Activity: The cytotoxic activity of the plant extract was evaluated using Brine Shrimp lethality bioassay method [9]. Here simple zoological organism (*Artemia salina*) was used as an expedient monitor for the screening. The eggs of the brine shrimp, *Artemia salina*, were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 hrs to mature shrimp called nauplii. The test samples (extract) were prepared by dissolving them in DMSO (not more than 50 µl DMSO in 5 ml solution to avoid toxicity of itself) plus sea water (3.8% NaCl in water) to attain concentrations - 10, 20, 40, 60, 80, 160µg/ml. A vial containing 50 µl DMSO diluted to 5 ml of what was used as a control. Then matured shrimps were applied to each of all experimental vials and control vial. The number of the nauplii that died after 24 hrs was counted. The median lethal concentration LC of the test 50 sample after 24 hrs was obtained by a plot of percentage of the dead shrimps against the logarithm of the sample concentration. Vincristine sulphate was used as a reference standard in this case.

Anti-inflammatory activity

Carrageenan induced paw edema test in mice: Swiss albino mice (25-30g) were divided into five groups of four animals each. The test groups received 100 and 200 mg/kg body weight, p.o. of the extracts of *A. lakoocha* . The reference group received Indomethacin (10 mg/kg body weight, p.o.) while the control group received 1 ml/kg body weight normal saline. After 30 min, 0.1 ml, 1% carrageenan suspension in normal saline was injected into the sub plantar tissue of the right hind paw. The paw volume was measured at 1, 2, and 3 h after carrageenan injection using a vernier caliper. The percentage inhibition of the inflammation was calculated from the formula:

% inhibition = $(1-D_t/D_o) \times 100$

Where, D_o was the average inflammation (hind paw edema) of the control group of mice at a given time, D_t was the average inflammation of the drug treated (i.e., extract or reference indomethacin) mice at the same time [10]

In vivo analgesic activity

***Acetic acid induced writhing test*:** The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice [11]. The animals were divided into six groups with five mice in each group. Group I animals received vehicle (1% Tween 80 in water, 10 ml/kg body weight), animals of Group II received Indomethacin (10 mg/kg body weight) while animals of Group III, IV were treated with 100 and 200 mg/kg body weight (p.o.) of the *Artocarpus lakoocha* leaves extract. Test samples and vehicle and Indomethacin were administered orally 30 min before intraperitoneal administration of 0.7% v/v acetic acid. The mice were observed for specific contraction of body referred to as 'writhing' for the next 20 min [10].

The percent inhibition (% analgesic activity) was calculated by the equation $\{(A-B)/A\} \times 100$

Where, A= Average number of writhing of the control group; B= Average number of writhing of the test group.

Formalin induced licking and biting test: The antinociceptive activity of the drugs was determined using the formalin test described by [10].Control group received 5% formalin. 20 µl of 5% formalin was injected into the dorsal surface of the right hind paw at 30 min after administration of methanolic extract of *A. lakoocha* (100 and 200 mg/kg, p.o.) leaves and Indomethacin (10 mg/kg, p.o.). The mice were observed for 30 min after the injection of formalin, and the amount of time spent licking and biting of the injected hind paw was recorded. The first 5 min of the formalin injection is referred to as the early phase and the period between 15 and 30 min as the late phase. The total time spent licking and biting of the injured paw (pain behavior) was measured with a stop watch.

CNS depressant activity

Hole Cross Test: The method used was done as described by Takagi et al. [12]. A steel partition was fixed at the middle of a cage having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The animals were divided into different group and each group contain 5 animals. The control group received vehicle (1% Tween 80 in water at the dose of 10 ml/kg p.o.) whereas the test group received *A. lakoocha* extracts (100 and 200 mg/kg p.o.) and standard group received diazepam (1mg/kg body weight p.o.). Each animal was then placed on one side of the chamber and the number of passages of each animal through the hole from one chamber to the other was recorded for 3 min on 0, 30, 60, 90 and 120 min during the study period.

Open field test: Mice were treated as discussed above. The experiment was carried out following the methods described by Takagi [12]. The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had 40 cm height a wall. The number of squares visited by mice was counted for 3 min on 0, 30, 60, 90 and 120 min after oral administration of test samples.

Antidiarrhoeal Activity:

Castor oil induced diarrhea: This study was conducted by the method described by Shoba and Thomas [13]. The animals were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhea were selected for the final experiment. The animals were divided into following four groups containing five mice in each group. Group I was treated with vehicle (Saline 10 ml/ kg p.o.). Group II and Group III were treated with 100 and 200 mg kg-1 body weight (p.o.) of *Artocarpus lakoocha* leaves extract respectively. Group IV received loperamide (3mg/kg body weight p.o. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. Diarrhoea was induced by oral administration of 0.5 ml castor oil to each mice, 30 min after the above treatments. During an observation period of 4 h, the total number of fecal output and the number of diarrheic feces excreted by the animals were recorded.

Statistical analysis: All values were expressed as the mean ± SEM of three replicate experiments. The analysis was performed by using SPSS statistical package for WINDOWS (version 16.0; SPSS Inc, Chicago). All *in vivo* data are subjected to ANOVA followed by Dunnett's test and $p<0.05$ were considered to be statistically significant.

RESULTS

Brine Shrimp lethality bioassay: The result of Brine Shrimp lethality bioassay is given in Table (1). *Artocarpus lakoocha* leaves extract displayed strong toxic potentiality. LC_{50} value of the extract was very potent 2.83±0.11µg/ml).

Carrageenan induced paw edema test: Table (2) shows the results of the anti-edematous effects of orally administered of *Artocarpus lakoocha leaves* extract, on carrageenan induced paw edema in mice. The *A. lakoocha* leaves extract showed dose dependent anti-inflammatory activity and statistically significant ($P<0.05$). *Artocarpus lakoocha* exhibited anti-inflammatory effects at 200 mg/kg dose (64.90% inhibition) compared to indomethacin (69.86 %).

Acetic acid-induced writhing test: Table (3) shows the effects of both extract of on acetic acid-induced writhing in mice. The oral administration of both doses of *Artocarpus lakoocha* leaves extract significantly ($p<0.05$) inhibited (29.63% and 57.41%) writhing response respectively.

Formalin induced licking and biting test: *Artocarpus lakoocha* (100 and 200 mg/kg, p.o.) significantly ($P<0.05$) suppressed the licking and biting activity in either phase of the formalin-induced pain in mice in a dose dependant manner (Table 4). *Artocarpus lakoocha* leaves extract (200 mg/kg body weigh) showed the almost similar with the standard drug Indomethacin of inhibitory activity against licking and biting of the pain at both early and late phase.

Hole-cross test: In the Hole- cross test, *Artocarpus lakoocha* extracts produced a decrease in the

movements of the test animals at all dose levels. They were statistically significant (P<.05) for all dose and followed a dose-dependent response. The depressing effect was most intense during the second (60 min) and third (90 min) observation periods (Table 5).

Open field test: In the Open field test, *Artocarpus lakoocha* extracts expressed a decrease in the movements of the test animals at both dose levels tested. They were also statistically significant (P<.05) for both 100 and 200 mg/kg dose and followed a dose-dependent response (Table 6).

Antidiarrhoeal Activity: Table (7) shows the castor oil induced diarrhea. *Artocarpus lakoocha* leaves extract decreased castor oil induced diarrhea of the test animals at both (100 and 200 mg/kg) dose and they were statistically significant (P<.05).

DISCUSSION

Brine shrimp lethality is a general bioassay, which is indicative of cytotoxicity, pesticidal effects and various pharmacologic actions [14]. Several studies have been approved on brine shrimp lethality of extracts from natural sources. In the study of Raghavendra *et al*. [15], the extract of *Artocarpus lakoocha* was found to cause mortality of brine shrimps in a dose dependent manner. In our cytotoxic test, the LC_{50} of the *Artocarpus lakoocha* leaves was less toxic compared to standard. The presence of saponins, alkaloids and cardiac glycosides may be responsible for the observed brine shrimps lethality activities of the extracts. Moreover, the reason of the lethal nature of the extract may be the presence of secondary metabolites present in the extract.

Acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesic and represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipid [16]. The response is thought to be mediated by peritoneal mast cells [17], acid sensing ion channels [18] and the prostaglandin pathways [19]. The organic acid has also been postulated to act indirectly by inducing the release of endogenous mediators, which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics [20]. It is well known that non-steroidal anti-inflammatory and analgesic drugs mitigate the inflammatory pain by inhibiting the formation of pain mediators at the peripheral target sites where prostaglandins and bradykinin are proposed to play a significant role in the pain process [21]. Therefore, it is likely that *Artocarpus lakoocha* might have exerted its peripheral antinociceptive action by interfering with the local reaction caused by the irritant or by inhibiting the synthesis, release or antagonizing the action of pain mediators at the target sites. In acetic acid induced test *Artocarpus lakoocha* exhibited 57.41% analgesic activity at the dose 200mg/kg which is about to similar with the standard Indomethacin. The above findings clearly demonstrated the peripheral mechanisms are involved in the antinociceptive action of *Artocarpus lakoocha*. Interestingly, compounds like flavonoids and steroids, triterpenes in part, have been shown to possess anti-inflammatory, analgesic activity and the claim made by Pritam *et al*. [22].

The formalin model normally postulates the site and the mechanism of action of the analgesic. This biphasic model is represented by neurogenic (0-5 min) and inflammatory pain (15-30 min), respectively [23]. Drugs that act primarily on the central nervous system such as narcotics inhibit both as steroids and NSAIDs suppress mainly the late phase [20]. The suppression of neurogenic and inflammatory pains by the extract might imply that it contains active analgesic principle that may be acting both centrally and peripherally. This is an indication that the extract can be used to manage acute as well as chronic pain. The mechanism by which formalin triggers C-fibers activation remained unknown for a relatively long time. Recently, McNamara *et al*. [24] demonstrated that formalin activates primary afferent neurons through a specific and direct on TRPA1, a member of the transient receptor potential family of cation channels, expressed by a subset of C-fiber nociceptors, and this effect is accompanied by increased influx of Ca^{2+} ions. TRPA1 cation channels at primary sensory terminals were also reported to mediate noxious mechanical stimuli [25]. These experiments suggest that Ca^{2+} mobilization through TRPA1cation channels is concomitant with noxious chemicals and mechanical stimuli as they produce their analgesic action. It is likely that the inhibitory effect of *Artocarpus lakoocha* to pain response is due to inhibit the increase of the intracellular Ca^{2+} through TRPA1, presumably evoked by formalin. The *Artocarpus lakoocha* leaves extract (200mg/kg) showed significant analgesic action at both early (51.13%) and late phase(82.14%) compared to standard (84.28%). In accordance of analgesic action, the compound of *Artocarpus lakoocha* leaves may affect the metabolism of Ca^{2+}.

Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2h) of the carrageenan model is

mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [26, 27]. Since the extract significantly inhibited paw edema induced by carrageenan in the second phase and this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme. Flavonoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation. In previous study, we found that *A. lakoocha ethanolic* extract contained polyphenolic compounds [28] especially tannins and flavonoids. So, these compounds may be involved for its anti-inflammatory activity.

Locomotor activity considered as an increase in alertness and decrease in locomotor activity indicated sedative effect [29]. The 100mg/kg decreased movements similar with the standard but 200mg/kg extract showed higher depressing effect than standard. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidated their action through GABA, therefore it is possible that extracts of *Artocarpus lakoocha* may acts by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation [30] on of GABA receptor by the extracts (Many research showed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders [31]. Earlier investigation on phytoconstituents and plants suggests that many flavonoids and neuroactive steroids were found to be ligands for the $GABA_A$ receptors in the central nervous system; which may act as benzodiazepine like molecules [29].

Several mechanisms have been previously proposed to explain the diarrheal effect induced by the castor oil including inhibition of intestinal Na^+, K^+-ATPase activity to reduce normal fluid absorption [32], activation of adenylate cyclase or mucosal cAMP mediated active secretion [33], stimulation of prostaglandin formation [34], platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil [35]. However, it is well evident that castor oil produces diarrhea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [36]. According to the test, the methanolic extract of the *Artocarpus lakoocha* (100mg/kg) leaves showed moderate effect where as at 200mg/kg dose successfully inhibited (68.11%) which is closed to the standard drug loperamide (71.01%). Hence, the extract might have exerted its antidiarrheal action via antisecretory mechanism which was also evident from the reduction of total number of wet feces in the test groups of the experiment.

CONCLUSION

The results of the experiments suggest that *Artocarpus lakoocha* leaves may be used as an alternative or supplementary herbal remedy for the treatment of analgesic, inflammatory, hyperactive and diarrheal disease. Because of its analgesic and anti-inflammatory effects, *Artocarpus lakoocha* may show beneficial effects together with drugs known for a strong analgesic as well as anti-inflammatory effects. Thus the present study warrants further investigation involving components of *Artocarpus lakoocha* for possible development of new class of drugs.

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Conflict of interest: There is not any conflict of interest in this study.

Table 1: Brine Shrimp lethality bioassay of the ***Artocarpus lakoocha*** methanolic leaves extracts

Test sample	Concentration (µg/ml	Log C	No. of dead shrimps(out of 10)	% of Mortality	LC_{50} (µg/ml)	
A.lakoocha	10	1	7	70	*A.lakoocha*	Vincristine sulphate
	20	1.301	7.33	73.3	2.83±0.11	0.21±0.19
	40	1.602	7.7	77		
	80	1.903	8	80		
	160	2.204	8.34	83.4		

Table 2**:** Effects of the *Artocarpus lakoocha* methanolic leaves extract on carrageenan induced paw edema test.

Group		Oedema diameter (mm)			Inhibition (%)		
Group	Dose (mg/kg)	1h	2h	3h	1h	2h	3h
Group I	Vehicle	10.8±1.03	11.40±0.89	11.4±1.94			
Group II	10	6.40±0.89*	5.0±0.55*	3.80±0.70*	43.86	56.14	69.86
Group III	100	7.60±1.14*	6.40±1.92*	5.0±1.30*	29.62	43.84	56.14
Group IV	200	6.20±1.10*	5.4±1.09*	4.0±1.94*	41.72	52.62	64.90

Values are mean ± SEM, (n = 5), * P<0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (1% Tween 80 in water), Group II received Indomethacin 10 mg/kg body weight, Group III, IV, were treated with 100 and 200 mg/kg body weight (p.o.) of ***Artocarpus lakoocha*** extract respectively

Table 3**:** Effects of the *Artocarpus lakoocha* methanolic leaves extract on acetic acid Induced writhing in mice

Groups	**Dose (mg/kg)**	**No. of writhing**	**% inhibition**
Group I	Vehicle	18±1.34	-
Group II	10	7.5±0.91*	58.33
Group III	100	12.67±1.32*	29.63
Group IV	200	7.66±1.5*	57.41

Values are mean ± SEM, (n = 5), * P<0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (1% Tween 80 in water), Group II received Indomethacin 10 mg/kg body weight, Group III, IV, were treated with 100 and 200 mg/kg body weight (p.o.) of ***Artocarpus lakoocha*** respectively.

Table 4**:** Effects of methanolic extract of ***Artocarpus lakoocha*** on licking and biting of hind paw in the formalin test.

Groups	Dose (mg/kg)	Early phase (Sec)	% protection	Late phase (Sec)	% protection
Group-I	Vehicle	22.16 ± 1.82	-	23.33± 2.03	-
Group-II	10	9.83 ± 1.21*	55.63	3.67 ± 1.10*	84.28
Group-III	100	13.5 ±1.47*	39.09	5 ± 1.18 *	78.57
Group-IV	200	10.83 ± 1.31*	51.13	4.17 ± 1.21*	82.14

Values are mean ± SEM, (n = 5); *p<0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (1% Tween 80 in water), Group II received Indomethacin 10 mg/kg body weight, Group III, IV were treated with 100 and 200 mg/kg body weight (p.o.) of ***Artocarpus lakoocha*** leaves extract respectively.

Table 5: Effect of the ***Artocarpus lakoocha*** methanolic leaves extract on hole cross test in mice.

Group	Dose	Number of Movements				
		0 min	30 min	60 min	90 min	120 min
Group-I	Vehicle	9.00 ± 125	7.20 ± 1.28	7.60± 1.34	9.00± 1.25	1.00± 1.27
Group-II	1mg/kg	6.60 ± 1.06*	5.40± 1.06*	3.8o ±0.91*	3.60± 1.34*	2.20 ± 0.91*
Group-III	100 mg/kg	7.20 ± 1.14*	3.20±0.91*	5.20±1.14*	2.40± 0.74*	0.80 ± 0.91*
Group-IV	200 mg/kg,	6.60 ± 1.34*	8.00± 1.25*	3.80± 1.14*	1.80±0.91*	1.00± 0.84*

Values are mean ± SEM, (n = 5); * p<0.05, as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (1% Tween 80 in water), Group II received diazepam 1 mg/kg body weight, Group III , Group IV were treated with 100 and 200 mg/kg body weight (p.o.) of the ***Artocarpus lakoocha*** leaves extract.

Table 6: Effect of methanolic extract of the ***Artocarpus lakoocha*** leaves extract on open field test in mice.

Group	Dose	Number of Movements				
		0 min	30 min	60 min	90 min	120 min
Group-I	Vehicle	239.40± 2.60	189.60±7.01	176.80± 2.4	159.0± 2.72	1.46.0±2.34
Group-II	1mg/kg	89.0 ± 2.60*	86.60± 1.34*	64.40 ±3.14*	50.00± 2.81*	37.60±2..36*
Group-III	100 mg/kg	177.80 ± 4.29*	127.0±4.38*	74.80±3.33*	58.20± 2.80*	38.20± 2.75*
Group-IV	200 mg/kg	1.33 ± 4.69*	87.00± 3.47*	70.20± 3.47*	47.80±3.85*	26.40± 2.47*

Values are mean ± SEM, (n = 5). * P<0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (1% Tween 80 in water), Group II received diazepam 1 mg/kg body weight, Group III and Group IV were treated with 100 and 200 mg/kg body weight (p.o.) of the the ***Artocarpus lakoocha*** methanolic leaves extract.

Table 7: Effect of ***Artocarpus lakoocha*** methanolic extract on castor oil induced diarrhea in mice.

Groups	**Dose**	**No. of faces in 4 hour**	**% inhibition of defecation**
Group I	Vehicle	27.6±1.44	
Group II	3mg/kg	8±1.25	71.01
Group III	100mg/kg	12±1.71	56.52
Group IV	200mg/kg	8.8±1.38	68.11

Values are mean ± SEM, (n = 5). * P<0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test). Group I animals received vehicle (1% Tween 80 in water), Group II received Loperamide 3 mg/kg body weight, Group III and Group IV were treated with 100 and 200 mg/kg body weight (p.o.) of the the ***Artocarpus lakoocha*** methanolic leaves extract.

REFERENCES

1. Vikas V et al. Estimation of two bioactive compounds from Artocarpus lakoocha roxb. Inter J of Pharma and Bio Sci 2011; 2(1): 860-866.
2. Prasit S et al. Anti-Aging Activity and Non-Toxic Dose of Phytooxyresveratrol from Artocarpus lakoochaRoxb. Trop J of Pharm res 2012; 11 (1): 69-74
3. Shailendra KMB et al. Screening of selected biological activitiesof *Artocarpus lakoocha* roxb (moraceae) fruit pericarp. J of Basic and Clin Pharm 2010; 249-255.
4. Pandey A, Bhatnagar SP. Preliminary Phytochemical screening and antimicrobial studies on Artocarpus lakoocha Roxb**.** Department of Pharmaceutical Sciences, BIT, Mesra, Ranchi 835215.
5. Ghani. Medicinal plants of Bangladesh 2003.
6. Nantaporn N et al. Toxicity test of Puag Haad (Artocarpus lakoocha). Bulletin of the depart of med Sci 1985; 27:1.
7. Khandelwal KR. Practical pharmacognosy: Technique and experiments. Nirali Prakashan, India 2006; 162-165.
8. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16:109
9. Meyer BN et al. Brine shrimp; a convenient general bioassay for active plant constituents. Planta Medica 1982; 45: 31-34.
10. Achinta S et al. The analgesic and anti-inflammatory activities of the extracts of *Phyllanthus reticulates* in mice model. Pharm Biol 2007; 45:355-359.
11. Winter CA et al. Carrageenan induced oedema in hind paw of the rats as an assay of anti-inflammatory drug. Proc Soc Exp Bio Med 1962; 111:544-547.
12. Takagi KM et al. Studies on the spontaneous movement of animals by the hole cross test: Effect of 2-dimethylaminoethane. Its acylates on the central nervous system. Japan J of Pharmacol 1971; 21:797.

13. Shoba FG, Thomas M. Study of antidiarrheal activity of four medicinal plants in castor oil induced diarrhea. J Ethnopharmacol 2001; 76: 73-76.
14. MacLaughin JL et al. "Bench-Top" Bioassays for the discovery of Bioactive Natural Product: An update In: Studies in l natural product Chemistry, Ed. Ur-Rahman, A. Elsevier Sci Publi BV Amster 1991; 101-103.
15. Raghavendra HL et al. Screening for Cytotoxic activity of Methanol Extract of Putranjiva roxburghii Wall (Euphorbiaceae) Seeds. Pharmacog J 2010; 2(10): 335-337.
16. Ribeiro RA et al. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol 2000; 387:111-118
17. Voilley N. Acid-sensing ion channels (ASICs): New targets for the analgesic effects of Non-Steroid Anti-Inflammatory Drugs (NSAIDs). Curr Drug Targets-Inflamm Allergy 2004; 3:71-79.
18. Hossain MM et al. Antinociceptive activity of whole plant extracts of Paederia foetida. Dhaka Uni J Pharm Sci 2006; 5: 67-69.
19. Adzu B et al. Anti-inflammatory and anti-nociceptive effects of *Sphaeranthus senegalensis*. J Ethnopharmacol 2003; 84: 169-174.
20. Alam B et al. Antioxidant and analgesic activities of *Lannea coromandelica* Linn. bark extract. Int J Pharmacol 2012; 8: 224-233.
21. Kim HP et al. Anti-inflammatory plant flavonoids and cellular action mechanism. J Pharm Sci 2004; 96: 229-245.
22. Pritam, SJ et al. Analgesic activity of *Abelmoschus monihot* Extracts. Int J Pharmacol 2011; 7: 716-720.
23. Hunskaar S, Hole K. The formalin test in mice: Dissociation between inflammatory and non-inflammatory pain. Pain 1987; 30: 103-114.
24. McNamara CR et al. $TRPA_1$ mediates formalin-induced pain. Proc. Nat Aca Sci USA 2007; 104: 13525-13530.
25. Kerstein PC et al. Pharmacological blockade of TRPA1 inhibits mechanical firing in nociceptors. Mol Pain 2009; 5: 19-25.
26. Antonio AM, Brito ARMS. Oral anti-inflammatory and anti-ulcerogenic activities of a hydroalcoholic extract and partitioned fractions of Turnera ulmifolia (Turneraceae). J Ethnopharm 1998; 61: 215-228.
27. Gupta M et al. Anti-inflammatory evaluation of leaves of *Plumeria acuminate*. BMC Com Alt Med 2006; 6: 36-39.
28. Supawatchara S et al. Antioxidant and toxicity activities of *Artocarpus lakoocha* Roxb. heartwood extract. J of Med Plants Res 2010; 4(10): 947-953.
29. Verma A et al. Pharmacological Evaluation of *Saraca indica* Leaves for Central Nervous System Depressant Activity in Mice. J of Pharma Sci and Res 2010; 2: 338-343.
30. Koblyakov VA. Free radicals and inflammation (progress in inflammation research series, 1999). Biochem 2001; 66: 937-938.
31. Bhattacharya SK, Satyan KS. Experimental methods for evaluation of psychotropic agents in rodents: Anti-anxiety agents. Ind J of Experi Biol 1997; 35: 565-575.
32. Nell G, Rummel W. Action mechanism of secretagogue drugs. In: Csaky TZ, editor. Pharmacol of Intestinal Permeation. Berlin Springer-Verlag 1984; 464–474.
33. Capasso F et al. Dissociation of castor oil induced diarrhea and intestinal mucosal injury in rat: effect of NG-nitro-L-arginine methyl ester. Br J Pharmacol 1994; 113(4): 1127–1130.
34. Galvez A et al. Antidiarrhoeic activity of Euphorbia hirta extract and isolation of active flavonoid constituents. Planta Med 1993; 59(4): 333–336.
35. Mascolo N et al. Relationship between nitric oxide and platelet activating factor in castor oil induced mucosal injury in the rat duodenum. Naunyn Schmiedebergs Arch Pharmacol 1996; 353(6): 680–684.
36. Gaginella TS et al. Action of recinoleic acid and structurally related fatty acid on the gastrointestinal tract. II. Effect on water and electrolyte absorption in vitro. J Pharmacol Exp Ther 1975; 95(2): 355–356.