IJPSR (2011), Vol. 2, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 May, 2011; received in revised form 01 August, 2011; accepted 28 September, 2011

EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY EFFECT OF *TERMINALIA ARJUNA* ETHANOL EXTRACT

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Keywords:

Terminalia arjuna,
Ethanol extract,
Anti-inflammatory,
Analgesic,
Pain

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ABSTRACT

The present study was conducted to assess the anti-inflammatory, analgesic and cytotoxic properties of 50% ethanol extract of the stem bark of *Terminalia arjuna* plant on laboratory animal. *In vitro* cytotoxicity test was studied by Brine Shrimp Lethality Bioassay and results illustrated significant ($p<0.05$) cytotoxicity against *A. salina,* that were expressed as LC_{50}. *Terminalia arjuna* ethanol extract showed brine shrimp cytotoxicity with lethal concentration 50 (LC_{50}) value of 50.11µg/ml. Carrageenan-induced paw edema method was done to study the anti-inflammatory effect and it was found that *T. arjuna* can be effective in acute inflammatory disorders and in that case it showed significant result ($p<0.001$) with both of the 250mg/kg and 500mg/kg dose level. The extract was also used to evaluate the centrally acting analgesic potential using formalin, hot plate and peripheral pharmacological actions using acetic acid induced writhing test in mice. The extract of the plant were found to have significant ($p<0.01$; $p<0.001$) analgesic activity at the oral dose of 250 & 500mg/kg body weight, in the tested models. In hot plate test, at both dose levels (250mg/kg and 500mg/kg), *T. arjuna* extract showed significant ($p<0.001$) increased latency period than the control group. In acetic acid induced writhing test and formalin test *T. arjuna* also showed reduced number of writhes than the control group at two dose levels which are significant ($p<0.05$; $p<0.001$) compared to control. The results obtained support the use of stem bark of *T. arjuna* in painful conditions acting both centrally and peripherally.

INTRODUCTION: Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses [1].

Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases [2]. Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary.

There is a frequent association of pain with inflammation and it is a feature of many cardiovascular diseases like myocardial infarction and angina. Very often, drugs with anti-inflammatory effect possess analgesic properties. The experience of pain is the final product of a complex information processing network involving the central and peripheral pathways. Various clinical trials, have also demonstrated the beneficial effects of *T. arjuna* in stable angina and in patient with ischaemic cardiomyopathy [3, 4].

Terminalia arjuna (family: Combretaceae), a large tree, is found throughout the South Asian region. It is one of the most versatile medicinal plants having a wide spectrum of biological activity [5, 6]. There are very few reports regarding to its anti-inflammatory and analgesic effects using various parts of this plant. Some scientists used leaves [7] and some other scientists used crude powder [8] to screen its anti-inflammatory and analgesic activity.

The aim of the present work was to evaluate the cytotoxic, anti-inflammatory, analgesic assays to support the pharmacological effects and phytochemical investigation of this plant as well. Although numerous studies have shown the medicinal values of this plant, there still remains ample scope for further in depth research.

So far, for the first time an attempt was taken to investigate the anti-inflammatory and analgesic effect of *Terminalia arjuna* by using 50% ethanol extract of the bark. So far, there is no published report of the anti-inflammatory and analgesic activity of *T. arjuna* bark extract with polar solvent. Accordingly, we disclose herein the cytotoxic, anti-inflammatory and analgesic effects of the bark of *Terminalia arjuna* to further establish the scientific basis of the traditional uses of this plant.

MATERIALS AND METHODS:

Plant materials and preparation of test sample: The barks of *Terminalia arjuna* were collected from Khamarpara, a village of Magura, Bangladesh. The plant was identified by the Bangladesh National Herbarium, Dhaka and the specimens were stored in there for the further reference (Voucher Specimen No.- DACB-35235).

The stem barks of the *T. arjuna* were cut into small pieces and then water washed carefully. After washing, the fresh barks were air dried and then oven dried at 40°C temperature. The dried barks are then grinded to make powder, which were then screened to get fine powder. 1500g of barks were dried in oven and finally 500 g of fine powder was obtained. 500 g of dried bark powder were soaked in 50% ethanol. These suspensions were filtered with thin and clean cloth and then filtered by filter paper.

The suspensions were evaporated by BUCHI Rota vapor R-114 [BUCHI, Germany], connected with BUCHI water bath B-480 at 50^{o}C. In this case, 175mbar (to remove ethanol), 72mbar (to remove water) pressure and 160rpm rotation speed were maintained constantly. Finally, small amount of liquid were evaporated from the semi-solid extracts by using a freeze-drier (HETOSICC, Heto Lab Equipment, Denmark) and 75 g of ethanol extracts were obtained.

Animals: Young male Swiss-albino mice, 4-5 weeks old (weighing 25-30 gm) & male Long-Evans rats (weighing 180-200 gm) were used to conduct the *in vivo* experiment. They were kept in the animal house of the Department of Pharmacy, North South University, Bangladesh and maintained at a constant room temperature of 22±5^{o}C, 40-70% humidity conditions and the natural day-night cycle with an *ad libitum* access to food. The mice and rats had no access to food during the whole day of experiment. The influence of circadian rhythms was avoided by starting all experiments at 8.30 a.m.

Phytochemical Screening: The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Molisch's reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride solutions and saponins with ability to

produce stable foam and steroids with Liebermann-Burchard reagent.

Gum was tested using Molisch's reagent and concentrated sulphuric acid; reducing sugars with Benedict's reagent; terpenoids with chloroform and conc. sulphuric acid. These were identified by characteristic color changes using standard procedures [9].

Cytotoxicity Screening: Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds by the method Meyer [10]. Here, simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp were collected and hatched in artificial seawater (3.8% NaCl solution) for 48 hr to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method. The test samples (extract) were prepared by dissolving them in distilled water (20mg/ml). 2.5, 5, 10, 20, 40, and 80µl of solutions for each test sample were taken in 6 vials and 4ml of sea water was added to each vial containing 30-35 brine shrimp nauplii.

So, the concentrations of the test sample in the vials were 12.5, 25, 50, 100, 200, and 400µg/ml respectively. A vial containing 50µl DMSO diluted to 5ml was used as a control. Standard Colcichine was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial were counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

Acute Toxicity Studies: The acute oral toxicity studies were performed to study the acute toxic effects and to determine minimum lethal dose of the drug extracts. Swiss albino mice and Long-Evans rat of male sex weighing 25-30 gm and 180-200 gm were used for the study. The ethanol extracts were administered orally to different groups of over night fasted mice at the doses of 50, 100, 250, and 500 mg/kg body weight. After administration of the extracts, animals were observed continuously for the first three hours for any toxic manifestation. Thereafter, observations were made at regular intervals for 24 hrs. Further the animals were under investigation up to a period of one week [11].

Analgesic Activity:

Hot Plate Test Method: The hot-plate test method was employed to assess the analgesic activity which was previously described by Lanhers *et al*., [12] and modified by Mahomed and Ojewole [13]. The experimental mice were divided into four groups designated as group-I, group-II, group-III and group-IV consisting of five mice in each group for control, positive control and test sample group respectively. Each group received a particular treatment i.e. control (distilled water, 10ml/kg), positive control (Diclofenac sodium 10mg/kg) and the test sample (50% ethanol extract of 250 mg/kg & 500 mg/kg respectively). The animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5 ^{0}C. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60, 120,180 and 240 min after oral administration of the samples. Percent analgesic score was calculated as;

$$(PAS) = (Tb-Ta/Tb) \times 100$$

Where, Tb= Reaction time (in second) before drug administration, Ta = Reaction time (in seconds) after drug administration.

Acetic acid induced Writhing Method: To evaluate the analgesic effects of the plant extract, the method described by Dharmasiri *et al.,* [14] was used with slight modifications. Different groups of five mice each received orally normal saline solution (10 ml/kg) (i.e. control), diclofenac (10mg/kg), or plant extract (250 and 500mg/kg). Thirty minutes later, 0.6% acetic acid (10ml/kg) solution was injected intraperitoneally to all the animals in the different groups. The number of writhes (abdominal constrictions) occurring between 5 to 15 min after acetic acid injection was counted. A significant reduction of writhes in tested animals compared to those in the control group was considered as an antinociceptic response.

Formalin assay: The formalin test was carried out as described by previous workers Hunskaar S. *et al*., [15]. Four groups of mice (n = 5) were treated orally with the ethanol extract of *T. arjuna* (250 and 500 mg/kg), diclofenac (10 mg/kg) and normal saline (10 ml/kg bw).

Formalin solution (0.5% v/v) was injected into the sub-plantar region of the right hind paw of the animals 30 min post treatment.

The number of times paw was licked/bitten within the time frames of 0-5 min (neurogenic phase) and 15-30 min (inflammatory phase) after formalin administration was counted.

Anti-inflammatory Activity:

Carrageenan induced Paw Edema Method: The ethanol extract *on* carrageenan induced inflammation in rat paw was investigated by following the method of Winter *et al.,* [16] with minor modifications. The rats were divided into four groups containing five rats in each group. 0.1 ml of 1.0% carrageenan in normal saline (0.9% w/v NaCl) was injected to the sub plantar region of right hind paw. The ethanol extract of *T. arjuna* was administered to the rats half an hour before carrageenan injection and the basement value was taken at 0 hour. Different groups were treated as follows:

- Group I (Control): Carrageenan (0.1 ml of 1.0% carrageenan/rat to the sub plantar region) and saline water (10 ml/kg bw)
- Group II (Positive control): Carrageenan + Diclofenac (10 mg/kg bw)
- Group III and IV: Carrageenan + ethanol extract of *T. arjuna* (250 mg/kg and 500 mg/kg bw respectively)

The paw value was measured at ½, 1, 2, 3, 4 and 8 hour after carrageenan injection, using Plethysmometer. The left hind paw served as a reference non-inflamed paw for comparison. The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula-

$$\% \text{ Inhibition of paw edema} = (V_c - V_t / V_c) \times 100$$

Where V_c and V_t represent average paw volume of control and treated animal respectively.

Statistical analysis: Data from the experiments were analyzed using the Statistical Package for Social Science (SPSS) software for windows version 17 (SPSS Inc., Chicago, Illinois, USA). All the triplicate data were expressed as Mean ± SD as appropriate. Statistical analysis of the results was performed by using the One way ANOVA (analysis of variance) followed by Bonferroni *post hoc* and Dunnett test. The limit of significance was set at $p<0.05$. The LC_{50} values were calculated from linear regression analysis.

RESULTS:

Photochemical screening: Phytochemical screening of the crude extract revealed the presence of tannins, flavonoids, saponins, gums, steroids, alkaloids, reducing sugar and terpenoids. The intensity of the component content was high in all of the tested groups except saponins and terpenoids (**Table 1**).

TABLE 1: RESULT OF CHEMICAL GROUP TEST OF THE ETHANOL EXTRACT OF *T. ARJUNA*.

Plant Extract	Tannins	Flavonoids	Saponins	Gum & Carbohydrate	Steroids	Alkaloids	Reducing sugar	Terpenoids
50% ethanol	+++	+++	++	+++	+++	+++	+++	++

High = +++; Moderate = ++

Brine Shrimp Lethality Assay: Following the procedure of Mayer, the lethality of the extracts of *T. arjuna* to brine shrimp was determined on *A. salina* after 24 hours of exposure of the samples and the positive control, colcichine. The results of the different extracts of *T. arjuna* (% mortality at different concentrations and LC_{50} values) were shown in **Table 2** and **Fig. 1-2**. The percent mortality increased with an increase in concentration. The 50% ethanol extract of *T. arjuna* and colcichine showed almost 100% mortality to brine shrimp at 400 µg/ml. The LC_{50} obtained from the best-fit line slope were found to be 50.11µg/ml and 12.59µg/ml for ethanol extract and standard respectively.

TABLE 2: RESULTS OF BRINE SHRIMP LETHALITY ASSAY FOR *T. ARJUNA* ETHANOL (50%) EXTRACT.

		% mortality at different concentrations						
	Sample	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	LC_{50} µg/ml
T. arjuna	Control	6.18±0.57	8.98±0.57	10.65±0.57	11.76±0.57	11.76±0.57	20.00±0.57	-
	Colcichine	44.4±0.57*	52.94±0.57*	58.94±0.57*	76.47±0.57*	87.50±0.57*	100±0.57*	12.59
	Ethanol Extract	13.33±0.57*	23.54±0.57*	23.41±0.57*	55.61±0.57*	78.67±0.57*	94.78±0.57*	50.11

Data were expressed as mean±SD and analyzed by one way ANOVA, Post hoc and Dunnett test. All of the results were compared with the standard (colchicine); P<0.05

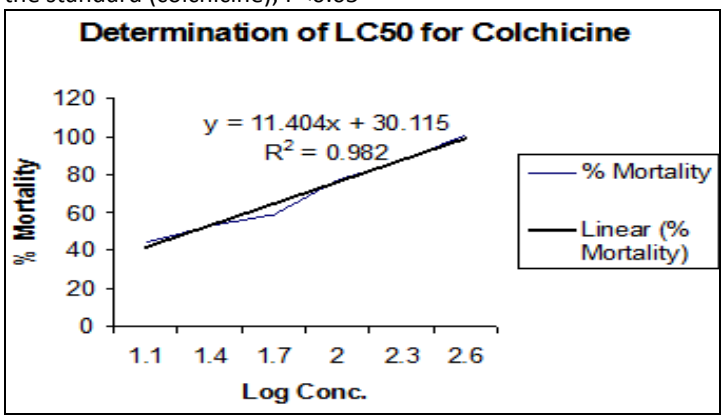

FIG. 1: DETERMINATION OF LC_{50} OF CHOLCHICINE AGAINST BRINE SHRIMP NAUPLII

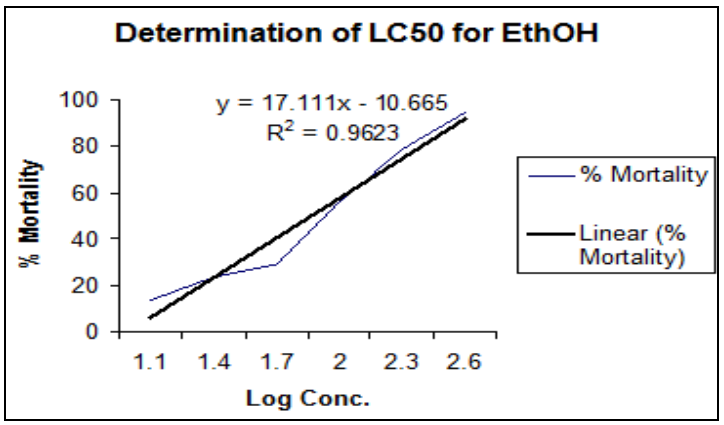

FIG. 2: DETERMINATION OF LC_{50} OF ETHANOL EXTRACT OF *T. ARJUNA* AGAINST BRINE SHRIMP NAUPLII

Acute Toxicity: Acute toxicity studies show that drug is safe up to the dose of 500 mg/kg with the 50% ethanol extract of *T. arjuna* to rats and mice. No mortality was recorded in any group after 72h of administering the extract to the animals.

Hot-plate Test: Results of hotplate test are presented in **table 3** for the ethanol extract of *T. arjuna*. The extract of the plant was found to exhibit a dose dependent increase in latency time when compared with control. At 180 and 240 minutes, the percent inhibition of two different doses (250 and 500 mg/kg body weight) were 45.74% & 6.34% and 76.37% & 34.32% for *T. arjuna* respectively. The results were found to be statistically significant (p<0.001).

Acetic acid induced writhing in mice: The ethanol extract of *T. arjuna* and diclofenac induced significant decrease in the number of writhes when compared to the control (**Table 4**). The extract at both concentrations showed significant result but the dose 500 mg/kg showed more significant result than the 250 mg/kg dose level.

TABLE 3: HOT-PLATE TEST FOR *T. ARJUNA* ETHANOL EXTRACT

	Experimental Groups			
Treatment	**Control (n=5)**	**Positive control (n=5)**	***T. arjuna* (250 mg/kg) (n=5)**	***T. arjuna* (500 mg/kg) (n=5)**
0 Min	12.36±1.23	9.46 ± 0.65	11.98 ±1.52	8.04 ±1.41
30 Min	10.74±0.89	12.20±0.44	13.76±1.64	10.84±1.25
60 Min	9.22 ± 0.66	13.70±0.47**	15.36±1.26*	12.46±0.95**
120 Min	8.14±0.60	15.28±0.50***	16.54±0.77***	13.32±1.02***
180 Min	6.66±0.55	6.66±0.55	17.46±0.59***	14.18±0.97***
240 Min	6.02±0.54	14.40±0.29***	11.22±0.87*	10.80±0.67***
		% Inhibition		
30 Min		28.96	14.84	34.82
60 Min		44.82	28.21	54.97
120 Min		61.52	38.06	65.07
180 Min		79.70	45.74	76.37
240 Min		52.21	6.34	34.32

Values were expressed as mean ± SEM (n=5 animals per group). *P<0.05, **P< 0.01, ***P< 0.001 vs. control

TABLE 4: EFFECT OF *T. ARJUNA* EXTRACT ON ACETIC ACID-INDUCED WRITHING IN MICE.

Group	Number of writhings (5-15) Min	% Inhibition
Control	44.25±3.54	
Standard (Diclofenac)	18.75±0.47***	57.63
T. arjuna (250 mg/kg)	29.75±1.49**	32.76

T. arjuna (500mg/kg)	22.50±1.04***	49.15

Values were expressed as mean ± SEM (n=7 animals per group). ***P*< 0.01, ****P*< 0.001 vs. control

Formalin Assay: Treatment with the ethanol extract at 250, 500 and diclofenac at 10mg/kg caused significant decrease in licking time and frequency of licking of the formalin-injected paw of mice (**Table 5**). The 500mg/kg dose showed the highest effect.

TABLE 5: EFFECT OF *T. ARJUNA* ETHANOL EXTRACT ON FORMALIN-NDUCED PAIN IN MINCE

Group	Control	Standard (Diclofenac)	*T. arjuna* (250 mg/kg)	*T. arjuna* (500 mg/kg)
Early phase (0-5 Min)	25.25±0.47	12.75±0.75***	20.25±1.31*	14.50±1.32***
Late phase (15-30 Min)	14.50±1.65	4.00±0.40***	7.12±0.40**	5.50±0.54***
		% Inhibition		
Early phase (0-5 Min)		49.50	19.80	43.56
Late phase (15-30 Min)		72.41	50.89	62.08

Values were expressed as mean ± SEM (n=7 animals per group). *P< 0.05, **P< 0.01, ***P< 0.001 vs. control

Anti-inflammatory Activity: The effective values, calculated for each group are presented in **Table 6**. In control animals, the sub plantar injection of carrageenan produced a local edema that increased progressively to reach a maximal intensity three hour after the injection of the phlogistic agent. Ethanol extract of the of the *T. arjuna* showed a significant dose depended reduction both at 250mg/kg and 500mg/kg body weight. Maximum inhibition of *edema* was found to be 38.36 % at three hour and 42.28% at eight hour of study at a dose of 250 mg/kg and 500 mg/Kg body weight respectively.

TABLE 6: ANTI-INFLAMMATORY TEST FOR *T. ARJUNA* ETHANOL EXTRACT

Groups	Hr_0	Hr_30	Hr_1	Hr_2	Hr_3	Hr_4	Hr_8
Control	0.74±0.03	0.91±0.04	1.07±0.04	1.31±0.06	1.59±0.08	1.57±0.08	1.49±0.07
Standard	0.87±0.03	1.06±0.05	1.13±0.06	1.29±0.01*	1.41±0.13*	0.98±0.06***	0.90±0.07***
Ext_250	0.79±0.03	0.88±0.06	0.94±0.04	1.03±0.09*	0.98±0.13**	1.13±0.08**	0.95±0.05***
Ext_500	0.59±0.06	0.93±0.09	0.90±0.02	1.00±0.05*	1.12±0.04*	1.14±0.04**	0.86±0.03***
				% Inhibition			
Standard	17.56	16.48	5.60	1.52	11.32	37.57	39.59
Ext_250	6.75	3.29	12.14	21.37	38.36	28.02	36.24
Ext_500	20.27	2.19	15.88	23.66	29.55	27.38	42.28

One way ANOVA, Post hoc (Dunnet's test), *P<0.05, **P<0.01, ***P<0.001

DISCUSSION: The brine shrimp lethality assay (BSLA) has been used routinely in the primary screening of the extracts as well as isolated compounds to assess the toxicity towards brine shrimp, which could also provide an indication of possible cytotoxic properties of the test materials. Brine shrimp nauplii have been previously utilized in various bioassay systems. Among these applications have been the analyses of pesticidal residues, mycotoxins, stream pollutants, anesthetics, dinoflagelate toxins, morphine-like compounds, carcinogenicity of phorbol esters and toxicants in marine environment. A number of novel antitumor and pesticidal natural products have been isolated using this bioassay [17, 18, 19].

The variation in BSLA results (Table 2) may be due to the difference in the amount and kind of cytotoxic substances (e.g. tannins, flavonoids or triterpenoids) present in the extracts. The cytotoxic effect of the *T. arjuna* was consistent with some other investigators though they investigated this activity by using the leaves of this plant [20]. Moreover, this significant lethality of the crude plant extracts (LC_{50} values less than 100 ppm or µg/mL) to brine shrimp is indicative of the presence of potent cytotoxic and probably insecticidal compounds which warrants further investigation. BSLA results may be used to guide the researchers on which part of the plant extracts/fractions to prioritize for further fractionation and isolation of these bioactive compounds. Other cytotoxicity tests and specific bioassays may be done on the isolated bioactive compounds later.

From acute toxicity studies, it was found that the drug is safe up to the dose of 500 mg/kg with 50% ethanol extract because it did not show any significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects

during the observation period. In future it will provide high margin of safety during formulation.

The extracts of the plants and diclofenac sodium (10 mg/kg) also presented a longer latency time than the control group in the hot plate test in a dose related manner. At 180 minutes, 250 mg/kg and 500mg/kg, p.o., administration of the *T. arjuna* plant extract, the percent inhibition was found 45.74% and 76.37% respectively which were statistically significant ($p<0.001$). Other scientists also found analgesic activity with the leaf extract by using this method [7]. The hot plat method is considered to be selective for the drugs acting centrally. The hot plat test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity [21]. It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally [22]. Therefore, the ethanol extract of the plant must have a central activity.

The acetic acid induced writhing test is normally used to evaluate the peripheral analgesic effect of drugs and chemicals. More significant response was found with 500 mg/kg dose level which is consistent with some other investigators thought in that case they used different parts and extracts of *T. arjuna* [7, 8]. The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway [23, 24]. Therefore, it may be inferred that the inhibitory effect of the compound could be due to the inhibition of prostaglandin pathway. The plant extract of *T. arjuna* exhibited both types of pain inhibition. The analgesic effect of the plants in both models suggests that they have been acting through central and peripheral mechanism [21].

Carrageenan-induced edema involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, especially the E series, histamine, bradykinins, leucotrienes and serotonin all of which also cause pain and fever [25]. Inhibitions of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorate the inflammation and other symptoms. In the present study it has shown that the extract of the *Terminalia arjuna* ethanol extract (50%) possessed a significant anti-edematogenic effect on paw oedema induced by carrageenan.

Development of edema induced by carrageenan commonly correlated with early exudative stage of inflammation [26]. Carrageenan edema is a multimediated phenomenon that liberates diversity of mediators. The first phase (1h) involves the release of serotonin and histamine while the second phase (over 1h) is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins [27].

Since, carrageenan-induced inflammation model is a significant predictive test for acute anti-inflammatory agents acting by the mediators of acute inflammation [28], the results of this study are an indication that *Terminalia arjuna* can be effective in acute inflammatory disorders and in that it showed significant result ($p<0.001$) with both of the 250mg/kg and 500mg/kg dose level. This result is consistent with some other scientists though they used different parts and extracts of *T. arjuna* [7, 8]. The extract also caused pronounced reduction in the edema produced by histamine.

Preliminary qualitative phytochemical screening reveals the presence of alkaloids, carbohydrates, tannins, gums, terpenoids & flavonoids in *T. arjuna*. Therefore, it is assumed that these compounds may be responsible for the observed analgesic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins [29, 30]. There are also reports on the role of tannins in anti-nociceptive activity [31]. Besides alkaloids are well known for their ability to inhibit pain perception [32], Tannins are important compounds known to be potent cyclooxygenase-1 inhibitors and with anti-phlogistic activity [33]. The mechanisms of anti-inflammatory activity may be related to the antiphlogestic action of the tannins. Flavonoids and other phenolics compounds of plant origin have been reported as antioxidants and as scavengers of free radicals [34, 35]. Antioxidants can also exert anti-inflammatory effects [36].

CONCLUSION: In conclusion, since the plant extract reduced significantly the formation of edema induced by carrageenan, as well as reduced the number of

writhes in acetic acid induced writhing models and hot plat test, the stem bark of *T. arjuna* exhibited anti-inflammatory and analgesic activities.

Beside these, it also possesses cytotoxic properties. Again, no mortality was recorded in the acute toxicity test; it showed that the plant is safe for use. The study has thus provided some justification for the folkloric use of the plant in several communities for conditions such as stomachache, pain and inflammations.

ACKNOWLEDGEMENT: We are thankful to all of the stuffs of the Department of Pharmacy, North South University, Dhaka, Bangladesh, for their cordial support.

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