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Research Article

Investigation of *in vivo* Analgesic, Anti-Inflammatory, *in vitro* Membrane Stabilizing and Thrombolytic Activities of *Atylosia scarabaeoides* and *Crotalaria spectabilis* Leaves

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Abstract

Background and Objective: Medicinal plants are stockroom of phytochemical constituents used for the treatment of various ailments from the ancient era. The search for new therapeutic remedies from natural sources is an ongoing process. Therefore, the aim of this study was to examine the analgesic, anti-inflammatory, membrane stabilizing and thrombolytic activity of *Atylosia scarabaeoides* and *Crotalaria spectabilis* leaves those are widely used as folkloric medicine in Bangladesh. **Materials and Methods:** The analgesic and anti-inflammatory effects were assessed by using acetic acid induced writhing test and carrageenan induced paw edema test in mice respectively using aspirin and ketorolac as standards. Membrane stabilizing activity was examined by using hypotonic solution induced human erythrocyte lysis model considering aspirin as standard. *In vitro* thrombolytic model was used to evaluate the clot lysis effect using streptokinase as standard. One way ANOVA with Dunnett's *post hoc* test was used for the statistical analysis using SPSS 20 (Chicago, IL, USA). **Results:** In the analgesic assay the n-hexane soluble fraction of *A. scarabaeoides* at 450 mg kg^{-1} b.wt., displayed height pain inhibitory activity, 63.72% ($p<0.001$). In carrageenan induced anti-inflammatory assay the highest percentage of inhibition of inflammation, 38.38% ($p<0.001$) was exhibited by the n- hexane soluble fraction at 450 mg mL^{-1} at 4th h. All the plant extracts treated for membrane stabilizing showed significant activity ($p<0.001$) at 1 mg mL^{-1}. Among the two plant extracts, the n-hexane soluble fraction of *C. spectabilis* exhibited highest thrombolytic activity, 31.553±0.928% ($p<0.001$) at 10 mg mL^{-1}. However, at 10 mg mL^{-1} significant ($p<0.001$) thrombolytic activity was also demonstrated by the ethanolic and its n-hexane soluble fraction of *A. scarabaeoides* and ethanolic extract of *C. spectabilis*. **Conclusion:** In this study both the plant extracts possessed effective analgesic, anti-inflammatory, membrane stabilizing and thrombolytic activities. Therefore, further research may be recommended to find the promising compound(s) of the plant parts.

Key words: *Atylosia scarabaeoides*, *Crotalaria spectabilis*, analgesic activity, anti-inflammatory activity, membrane stabilizing activity, thrombolytic activity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pain and inflammation are two most considerable areas of global scientific interest because they are the most prevalent manifestations of many ailments affecting millions of people[1,2,3]. Inflammation is the initial physiological response to detrimental stimuli to diminishing their expandability. Which primary manifestations are pain and edema at the site of injury due to release of some pro-inflammatory mediators such as interleukin 6 (IL-6), IL-12, interferon (INF-γ), Tumor Necrosis Factor (TNF), cyclooxygenase-2 (COX-2) and nitric oxide along with leakage of fluid from the vascular tissues that is related to an increase in vessel permeability, migration of inflammatory cells, tissue damage and healing[4,5]. Where pain is an unflattering sensory and pathological experience associated with actual or potential tissue damage. Inflammation is the initiating response of all types of pain, whether it is acute or chronic, peripheral or central[6]. The COX enzymes specially COX-2 helps in the synthesis of prostaglandins (PGs) precisely PGE2 and PGF2 from the membrane phospholipids of cell membrane which is found highly at the inflammation site, thus pain and inflammation can be linked by COX[7]. As many of the presently existing analgesic and anti-inflammatory drugs have numerous detrimental effects such as gastrointestinal ulcers, bleeding and renal disorders etc., discovery of alternative therapies for treatment of inflammation and pain is continuing throughout the universe[8]. Membrane stabilizing activity of red blood cell membrane is an effective *in vitro* method for measuring the anti-inflammatory activity of various compounds[9]. Membrane stabilization is a process, which act by retaining the integrity of biological membranes such as erythrocyte and lysosomal membranes against hypotonic solution induces lysis by interfering with the release either the action of inflammatory mediator like histamine, serotonin, prostaglandins, leukotrienesetc[10,11]. As the erythrocyte membrane resembles to lysosomal membrane, stabilization of erythrocyte membrane resembles to stabilization of lysosomal membrane[12].

Thrombosis is the process which is accountable for life denunciatory coronary disorders such as pulmonary emboli, deep vein thrombosis, strokes and heart attacks. It is characterized by the development of a blood clot in the circulatory system due to deposition of blood cell on the endothelial cell surface. This type of complications may lead to fatal consequences, such as myocardial or cerebral infarction vascular blockade, as well as death[13]. Anticoagulation therapy is the first choice of treatment in such complications; those are done by using some thrombolytic drugs such as tissue plasminogen activator (t-PA), streptokinase (SK), urokinase[14]. In order to restrain several adverse effects like bleeding and embolism related to streptokinase use researchers has conducted a number of studies to discover new thrombolytic drug from natural sources with minimal adverse effect.

From the beginning of the civilization, people were used to utilize medicinal plants for treating various diseases and ailments[15]. In addition, medicinal plants are becoming very popular as an alternative to synthetic drugs for their abundant number of scientific evidences addressing the benefits of using medicinal plants[16]. Therapies with drugs obtained from synthetic sources have many side effects and cannot be afforded by the people due to higher cost of the drug[17]. But at the same time drugs obtained from plant sources have lesser chance of side effects and besides these treatments with natural drug is economic. Now a days people were migrated towards the medicinal plant for the treatment of many complicated diseases, after discovering some lifesaving drugs from plant source like atropine, codeine, digoxin, morphine, quinidine, quinine, vincristine, vinblastine, etc[18,19].

Atylosia scarabaeoides (*A. scarabaeoides*) a perennial climber with densely grey-dowry stems from the family Fabaceae locally known as Banurkali or Thitkalai is widely distributed throughout Bangladesh, India, Malaysia, China, Mauritius and Madagascar[20, 21]. This plant has a wide range of traditional use in night fever, dropsy, burns, wounds, small-pox and cholera. During pregnancy, it is used to reduce swelling and pain in leg and also for vitality to the mother after childbirth[22,23]. It has profound antimicrobial activity. Three terpenes such as caryophyllene-4, 5-oxide, α-amyrin and β-amyrin were obtained from this plant[24].

Crotalaria spectabilis (*C. spectabilis*) locally named as Pipuli, Jhanjhani is a woody shrub from the genus *Crotalaria* belongs to the family Fabaceae is native to Asia and largely distributed throughout Bangladesh in fallow lands[25]. These plants are used in scabies and impetigo[25]. Ethanolic extract of the plant exhibit antioxidant activity and alcoholic extract of the plant can possess little antibacterial activities as well[26,27].

Searching new drug for different disease is very important. *Atylosia scarabaeoides* and *C. spectabilis* are two medicinal plants that were not previously inversigated for their thrombolytic, membrane stabilizing, analgesic and anti-inflamatory activities. Both the plants are very effective in the folk medicine for the treatment of pain and inflammation. Therefore the purpose of this study was to inspect the analgesic, anti-inflammatory, membrane stabilizing and thrombolytic activities of *Atylosia scarabaeoides* and *Crotalaria spectabilis* leaves.

MATERIALS AND METHODS

Chemicals and drugs: All the chemicals used in this study were of analytical grade. Tween 80, aspirin, ketorolac, ethylenediaminetetraacetic acid (EDTA), sodium chloride (NaCl), phosphate were purchased from Sigma Chemical Co., (St. Louis, MO, USA) and streptokinase (Lyophilized Altepase) vial of 15,00,000 IU was collected from Beacon Pharmaceutical Ltd., Bangladesh.

Plant collection and identification: The leaves and stems of *A. scarabaeoides* and *C. spectabilis* were collected (about 4 kg each) from Dhaka, Bangladesh during the month of October, 2015. The plants were identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (Accession No. DACB-40293 and DACB-40294 respectively) and two voucher specimen were also submitted at the Herbarium for future reference.

Plant extract preparation: After collection and cleaning by clean water the plants were first air dried for 3 days and then again sun dried for 4 days. By the use of a mechanical grinder those leaves and stems were then powdered into coarse powder. Those powdered plant parts were taken in clean sterile glass bottle (each of about 500 g) and were soaked into 2.5 L each of 96% ethanol solution for 14 days at room temperature in addition with occasional shaking. At first those socked extracts were filtered through a pitch of cotton plague and then using Whatman No. 1 filter paper. After then those filtrated extracts were subjected to evaporation at a temperature of 54°C using rotary evaporator until they were came into a consolidated mass. The yields of each extracts were then measured. After that these concentrated ethanolic extracts were fractionated with n-hexane and consolidated following the same process. These ethanolic and n-hexane extracts were then preserved at +4°C for further biological assay.

Experimental animal: For the assessment of *in vivo* analgesic and anti-inflammatory activity 100 Swiss Albino male mice (average weight of 95 g) were brought from the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh. Throughout the experiments they were kept under standard experimental conditions 25°C temperature, 55-65% relative humidity and fed the ICDDR,B formulated food and water. The study protocol was approved by the ethics committee of the Department of Pharmacy, Southeast University, Dhaka, Bangladesh. The use and care of animals was performed as per the guide for National Institutes of Health.

Analgesic activity test

Acetic acid induced writhing test: For the assessment of analgesic activity acetic acid induced writhing model in mice was utilized by following the model Bhowmick *et al.*[6], with minor modification. The rats were randomly divided into five groups, each consisting of three animals including group I (control group), group II (standard) and three test groups from group III-V. The mice of group III, IV and V were received 150, 300 and 450 mg kg^{-1} b.wt., plant extracts respectively. Where standard group received aspirin as standard drug at the dose of 300 mg kg^{-1} b.wt., and control group was treated with 1% tween 80 in distilled water at a dose of 2 mL kg^{-1} b.wt., respectively. After that, the mice were been subjected to take intraperitoneal injection of 0.7% acetic acid as a substance to initiate pain. In addition, after 30 min of intraperitoneal acetic acid injection test samples, standard drugs and control were administered orally to the mice. Immediately after 15 min, the mean abdominal writhing (constriction of abdomen, turning of trunk and extension of hind legs) were recorded on each mice for 5 min. The percentage inhibition of writhing was calculated using the following formula[11]:

$$\text{Inhibition of writhing } (\%) = \frac{W_C - W_T}{W_C} \times 100\%$$

where, W_C is average writhing of control group and W_T is average writhing of treated group.

Anti-inflammatory activity test

Carrageenan-induced rat paw edema test: Carrageenan induced mice hind paw edema was used as the animal model of acute inflammation according to the method of Ferreira *et al.*[28], with minor modification. In this experiment, the mice were divided into five groups of three animals each and were fasted for a period of 24 h. Group I was treated as control and received 2% tween 80 in normal saline at a dose of 2 mL kg^{-1} b.wt. Group II was treated as standard and received 10 mg kg^{-1} b.wt., of ketorolac orally. Group III, IV and V were treated as test group and received 150, 300 and 450 mg kg^{-1} b.wt., dose of extract to each mice 30 min after the oral administration of the tested materials 0.05 mL carrageenan suspension (1% carrageenan suspended in 0.9% NaCl) along with 2% tween 80 was given through subplantar injection of the right hind paw of the rats in order to produce acute inflammation. The paw volume was measured with micrometer screw gauze at 1, 2, 3 and 4 h after the

administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the following expression[28]:

$$\text{Inhibition of inflammation } (\%) = \frac{V_c - V_t}{V_c} \times 100$$

where, V_c is average degree of inflammation by the control group and V_t is average degree of inflammation by the treated group.

Membrane stabilizing activity test: The hypotonic solution induced human erythrocyte lysis model is used for membrane stabilizing activity test following the design designed by Sikder *et al.*[29], with minor modification. Using syringes containing anticoagulant (EDTA) 7 mL of venous blood was collected from each healthy male human volunteer. Then the blood samples were centrifuged for 10 min at 3000 rpm in a centrifugal machine. After centrifugation blood cells were washed 3 times with 154 mM NaCl solution in 10 mM sodium phosphate buffer (pH 7.4). The test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the aqueous solution extracts (1.0 mg mL^{-1}) or acetyl salicylic acid (0.1 mg mL^{-1}). The control sample consisted of 0.5 mL of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10 min at room temperature and then centrifuged the tubes for 10 min at 3000 rpm and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation[29]:

$$\text{Inhibition of haemolysis } (\%) = \frac{OD_1 - OD_2}{OD_1} \times 100$$

where, OD_1 is optical density of hypotonic-buffered saline solution alone (control) and OD_2 is optical density of treated group in hypotonic solution.

Preparation of streptokinase suspension: Streptokinase (SK) was collected as solid powder for thrombolytic activity test. Mixing that powder with 5 mL of sterile distilled water SK suspension was prepared. From which 100 µL (30,000 IU) suspensions was used as a stock solution for *in vitro* thrombolytic assay.

Thrombolytic activity test: By considering SK as standard the thrombolytic activity was evaluated according to the method developed by Prasad *et al.*[30], with minor modification. After managing volunteers (n = 20), 5 mL venous blood was taken from each volunteer by an expert pathologist, after then bloods were transferred into five different pre-weighed sterile microcentrifuge tube and subjected to incubation for 45 min at 37°C. After clot formation, serum was carefully removed from the clot so that the clot formed was not brooked. The remaining clots in each tube then weighed again to determine the clot weight which was calculated by subtracting the empty tube weight from the weight of clot containing tube. As a standard, 100 µL of SK and as a control, 100 µL of distilled water along with 100 µL of each samples were separately added to the microcentrifuge tubes. All the tubes were then incubated for 90 min at a temperature of 37°C and were observed for clot lyses. When the incubation was completed, the released fluids were removed from the tubes and then again weighed as a motive to measure the percentage of clot lyses. Finally percentage of clot lyses was determined using the following equation[30]:

$$\text{Clot lysis } (\%) = \frac{\text{Weight of released clot}}{\text{Clot weight}} \times 100$$

The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Statistical analysis: The results were expressed as Mean ± SD (n = 3). One way ANOVA with Dunnett's *post hoc* test was used for this experiment. SPSS 20 (Chicago, IL, USA) and Microsoft Excel 2010 (Roselle, IL, USA) was used for the statistical and graphical evaluations. The $p<0.05$ were considered to be statistically significant[31].

RESULTS

Determination of analgesic activity: In this study acetic acid induced writhing test model was used to evaluate the analgesic activity of ethanolic and n-hexane extracts of *A. scarabaeoides* and *C. spectabilis* leaf. Both of the solvent extracts of the plants showed potent peripheral analgesic activity at three different doses (Table 1, 2).

Determination of anti-inflammatory activity: Table 3 and 4 shows that administration of ethanolic and n-hexane extract of *A. scaraboides* and *C. spectabilis* shows inhibition of carrageenan induced paw edema in rats. In most cases the highest anti-inflammatory effect were observed at the 4th h.

Determination of membrane stabilizing activity: In hypotonic solution induced conditions, the samples were found (Table 5) to inhibit the lysis of erythrocyte membrane.

Among all the test samples, the n-hexane extract of *A. scaraboides* leaf displayed the maximum percentage of inhibition of RBC lysis as compared to aspirin.

Determination of thrombolytic activity: As a part of discovery of cardio protective drugs from natural sources ethanolic and n-heaxne extract of *A. scarabaeoides* and *C. spectabilis* leaf were assessed for thrombolytic activity and the results are presented in following Table 6 and 7.

DISCUSSION

In the analgesic assay some plant extracts were exhibited potent analgesic activity compared to the aspirin. Among those n-hexane extract of *A. scarabaeoides* leaf showed the maximum ($p<0.001$) activity at a dose of 450 mg kg^{-1} b.wt., while the lowest activity ($p<0.01$) was examined for the case of n-hexane extract of *C. spectabilis* leaves. For both plant, they showed almost similar activity for both ethanolic and n-hexane fraction with respect to their dose. These findings

Table 1: Analgesic activity of different solvent extract/fraction of *A. scarabaeoides* using acetic acid induced writhing test

Group	Dose (mg kg^{-1} b.wt.,)	Total writhing	Inhibition (%)
Control	2	37.67±2.52	-
Standard (aspirin)	300	6.67±2.52***	82.30
CEEAS	150	21.67±1.15***	42.48
	300	18.33±2.08***	51.33
	450	14.67±3.21***	61.06
NFAS	150	23.00±3***	38.94
	300	17.33±1.53***	53.98
	450	13.67±2.08***	63.72

Values are expressed as Mean±SD (n = 3). ***Indicates $p<0.001$; one-way ANOVA followed by Dunnett's test as compared to control, CEEAS: Crude ethanolic extract of *Atylosia scarabaeoides*, NFAS: n-hexane soluble fraction of *Atylosia scarabaeoides*

Table 2: Analgesic activity of different solvent extract/fraction of *C. spectabilis* using acetic acid induced writhing test

Group	Dose (mg kg^{-1} b.wt.,)	Total writhing	Inhibition (%)
Control	2	37.67±2.52	-
Standard (aspirin)	300	6.67±2.52***	82.3
CEECS	150	28.67±2.52**	23.89
	300	24.00±2***	36.28
	450	17.67±2.89***	53.1
NFAS	150	30.67±2.08**	18.58
	300	25.33±1.53***	32.47
	450	20.67±2.08***	45.13

Values are expressed as Mean±SD (n = 3). ***Indicates $p<0.001$ and **Indicates $p<0.01$; one-way ANOVA followed by Dunnett's test as compared to control, CEECS: Crude ethanolic extract of *Crotalaria spectabilis*, NFAS: n-hexane soluble fraction of *Atylosia scarabaeoides*

Table 3: Anti-inflammatory activity of different solvent extract/fraction of *A. scarabaeoides* using carrageenan-induced paw edema test

Group	Dose (mg kg^{-1} b.wt.,)	Edema diameter (mm)				Inhibition (%)			
		1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
Control	2	6.11±0.21	5.90±0.09	5.47±0.40	5.18±0.44	-	-	-	-
Standard (Ketorolac)	10	3.22±0.34	2.93±0.37	2.21±0.19	1.52±0.15	47.32	50.33	59.57	70.70
CEEAS	150	5.50±0.60*	4.59±0.44**	4.15±0.31***	3.79±0.52**	17.39	22.20	24.15	26.85
	300	5.36±0.53	4.98±0.29*	4.45±0.32**	3.58±0.39*	12.27	15.59	18.54	30.91
	450	4.75±0.26**	4.35±0.47**	3.96±0.20*	3.45±0.58**	22.25	26.33	27.62	33.29
NFAS	150	5.25±0.52*	4.84±0.36*	4.38±0.44**	4.31±0.38*	14.18	17.97	19.88	16.74
	300	4.94±0.40*	4.60±0.27**	4.16±0.25**	3.78±0.19***	19.19	22.09	23.90	26.98
	450	4.78±0.15*	4.36±0.40**	3.73±0.31***	3.19±0.22***	21.75	26.04	31.77	38.38

Values are expressed as Mean±SD (n = 3).**Indicates $p<0.01$ and *Indicates $p<0.05$, one-way ANOVA followed by Dunnett's test as compared to control, CEEAS: Crude ethanolic extract of *Atylosia scarabaeoides*, NFAS: n-hexane soluble fraction of *Atylosia scarabaeoides*

Table 4: Anti-inflammatory activity of different solvent extract/fraction of *C. spectabilis* using carrageenan-induced paw edema test

Group	Dose (mg kg^{-1} b.wt.,)	Edema diameter (mm)				Inhibition (%)			
		1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
Control	2	6.11±0.21	5.64±0.05	5.47±0.40	5.18±0.44	-	-	-	-
Standard (Ketorolac)	10	3.22±0.34***	2.93±0.37***	2.21±0.19***	1.52±0.15***	47.32	48.08	59.57	70.70
CEECS	150	5.60±0.19	5.28±0.12	4.53±0.6	4.22±0.81	8.40	10.56	17.07	18.48
	300	5.64±0.22	5.01±0.58*	4.41±0.56*	3.96±0.81	7.80	15.03	19.39	23.50
	450	5.13±0.29**	4.73±0.35**	4.58±0.23	3.71±0.48	16.03	19.83	16.58	28.27
NFCS	150	5.26±0.49	4.84±0.37**	4.36±0.22**	3.89±0.16**	13.85	14.00	20.12	24.55
	300	5.10±0.26**	4.41±0.38***	4.33±0.48**	3.68±0.56**	16.63	21.85	20.73	28.98
	450	5.14±0.11	4.66±0.12**	4.32±0.07**	3.87±0.43**	15.87	17.42	20.91	25.11

Values are expressed as Mean±SD (n = 3).**Indicates $p<0.01$ and *Indicates $p<0.05$, one-way ANOVA followed by Dunnett's test as compared to control, CEECS: Crude ethanolic extract of *Crotalaria spectabilis*, NFCS: n-hexane soluble fraction of *Crotalaria spectabilis*

Table 5: Membrane stabilizing activity of different solvent extract/fraction of *A. scarabaeoides* and *C. spectabilis*

Group	Concentration (mg mL^{-1})	Absorbance of hypotonic solution	Membrane stabilization (%)
Control	1	0.893±0.035	-
Standard (aspirin)	0.10	0.143±0.012	83.980±1.920***
CEEAS	1	0.236±0.041	73.674±3.503***
NFAS	1	0.23±0.022	74.228±2.003***
CEECS	1	0.317±0.022	64.395±3.617***
NFCS	1	0.245±0.023	72.503±2.313***

Values are expressed as Mean±SD (n = 3). ***Indicates p<0.001, one-way ANOVA followed by Dunnett's test as compared to control. CEEAS: Crude ethanolic extract of *Atylosia scarabaeoides*, NFAS: n-hexane soluble fraction of *Atylosia scarabaeoides*, CEECS: Crude ethanolic extract of *Crotalaria spectabilis*, NFCS: n-hexane soluble fraction of *Crotalaria spectabilis*

Table 6: Thrombolytic activity of different solvent extract/fraction of *A. scarabaeoides*

Doses of standared/ *A. scarabaeoides*	Clot lysis of crude ethanolic extract (%)	Clot lysis of n-hexane fraction (%)
Control	4.418±1.405	-
Standard (streptokinase)	58.120±1.001***	-
2 mg mL^{-1}	6.465±1.114	10.102±0.489***
4 mg mL^{-1}	8.459±0.779**	13.602±0.908***
6 mg mL^{-1}	10.367±1.287***	18.242±1.084***
8 mg mL^{-1}	13.603±0.908***	22.072±1.414***
10 mg mL^{-1}	16.224±0.567***	26.207±0.489***

Values are expressed as Mean±SD (n = 3). ***Indicates p<0.001 and **Indicates p<0.01, one-way ANOVA followed by Dunnett's test as compared to control

Table 7: Result of thrombolytic activity of different solvent extract/fraction of *C. spectabilis*

Doses of standared/ *A. scarabaeoides*	Clot lysis of crude ethanolic extract (%)	Clot lysis of n-hexane fraction (%)
Control	4.418±1.405	-
STD (streptokinase)	58.120±1.001***	-
2 mg mL^{-1}	7.33±0.763**	18.266±0.763***
4 mg mL^{-1}	9.594±1.101***	23.3±0.673***
6 mg mL^{-1}	12.4±0.824***	26.203±1.423***
8 mg mL^{-1}	15.808±1.703***	28.502±1.17***
10 mg mL^{-1}	22.675±0.598***	31.553±0.928***

Values are expressed as Mean±SD (n = 3). ***Indicates p<0.001 and **Indicates p<0.01, one-way ANOVA followed by Dunnett's test as compared to control

may be the possible representation of peripheral analgesic activity through the peritoneal receptors mediated cyclooxygenase inhibition. An earlier study by Hossain *et al*.[31] on analgesic activity of extract of *Xanthosoma sagittifolium* leaves also reported promising activity[31]. Pain is associated with the pathophysiology of various clinical consequences such as arthritis, muscular pain, cancer and vascular diseases[32]. Acetic acid induced writhing response is a suitable method for assessing peripheral analgesic effects as it is sensitive for various classes of analgesic drugs. Pain perception in acetic acid induced writhing assay is revealed by producing localized inflammatory response due to release of free arachidonic acid from tissue phospholipids by the help of COX, which intrans produce prostaglandin specifically PGE2 and PGF2α, the level of lipoxygenase products may also increases in peritoneal fluids and cause inflammation and pain by increasing capillary permeability. Thus the substance inhibiting the writhing will have analgesic effect preferably by inhibition of prostaglandin synthesis[33,34].

In the anti-inflammatory assay both solvent extracts of *A. scarabaeoides* and *C. spectabilis* leaves were displayed significant (p<0.05, p<0.01 and p<0.001) inhibition of inflammation compared to the ketorolac. *A. scarabaeoides* showed relatively higher anti-inflammatory effect compared to *C. spectabilis* which suggested that the phytoconstituents present in the plants may inhibit the inflammation via their suppressive action on prostaglandin. In the study of anti-inflammatory potentials of extracts of *Buddleja crispa* and its fractions, Bukhar *et al*.[35] reported notable activity. Inflammation arrive when the immune system try to remove something that may turn out to be harmful[36]. The feasible mechanism of carrageenan mediated inflammation is bi-phasic. The first phase is characterized by the release of histamine, serotonin and kinins in the 1 h; followed by the release of prostaglandins and lysosome enzymes in 2-4 h in second phase[37].

In this study the sample were found very significant (p<0.001) to inhibit lysis of erythrocytes compared to aspirin. Where, the n-hexane extract of *A. scarabaeoides* leaf displayed the height inhibition of hemolysis of RBC. Therefore it may be assumed that the plant extracts may conserve certain phytoconstituent that may be responsible for its membrane stabilization activity. Hossain *et al*.[31] reported similar outcome in the study of membrane stabilization activity of different extracts from *Spilanthes paniculata* leaves[38]. Membrane stabilization is the process through which the analgesic compounds work and is the possible assay to study the possible anti-inflammatory effect of any compound[39]. The hypotonic solution induced human erythrocytes lysis test is the effective model to examine. During inflammation, lysosomal enzymes and hydrolytic components are released from the phagocytes to the extracellular space results in damages of the surrounding organelles and tissues and also guide a variety of disorders[40]. Nonsteroidal anti-inflammatory drugs act either by inhibiting these lysosomal enzymes or through stabilization of lysosomal membranes. Furthermore, exposure of RBC to noxious substances such as hypotonic medium, heat, etc, results in the lysis of the membranes due to oxidation and the lysis of hemoglobin[41]. So retaining the RBC membrane integrity by suppressing hypotonicity induced membrane lysis was taken as a possible mechanism of anti-inflammatory activity[42].

As a part of that continuous research work we also examined the thrombolytic potentiality of the ethanolic and n-hexane extract of *A. scarabaeoides* and *C. spectabilis* leaves. By treating the clots with the extractives from *A. scarabaeoides* and *C. spectabilis* leaves and comparing with SK, a significant ($p<0.01$ and $p<0.001$) thrombolytic activity was observed. In which n-hexane soluble fraction of *C. spectabilis* displayed the most promising clot lysis potential with relatives to their dose. Ali *et al.*[43] in the study of thrombolytic potential of *Averrhoa bilimbi*, *Clerodendrum viscosum* and *Drynaria quercifolia* revealed remarkable thrombolytic activity[43]. Thrombosis is process of blood clot formation in the blood vessels due to accumulation of tissue factor and fibrin on to the vascular endothelial cell surface. In the formation process the major role is played by activated platelets through forming platelets to platelets bonds. These activated platelets further bind to the leucocytes and results them into a complex process of plaque formation and growth[44]. Plasmin is a natural antithrombic agent that has the potentiality to lyses clot[45]. The SK has a remarkable effect on clot lysis by converting the plasminogen to plasmin. But this first generation thrombolytic agent had no fibrin binding capabilities and caused systemic plasminogen activation with concomitant destruction of haemostatic proteins that may increase the risk of hemorrhage, thromboembolism, anaphylaxis, hypotension etc[46,47]. To overcome these complications a number of studies have been conducted and also in-process by various researchers in order to discover better thrombolytic drug from the natural source with minimal adverse effect.

CONCLUSION AND FUTURE RECOMMENDATION

From the results of this study it can be summarized that the plant extracts have notable analgesic, anti-inflammatory, membrane stabilizing and thrombolytic activities. Further studies can be undertaken to substantiate definite structure of the ingredients in the extracts and to explore the precise mechanism of action.

SIGNIFICANCE STATEMENT

This study discovers the *in vitro* thrombolytic, membrane stabilizing and *in vivo* analgesic, anti-inflammatory potentials of the leaf extracts of *A. scarabaeoides* and *C. spectabilis* that can be beneficial to explore new drug(s) from natural source. This study will help the researcher to uncover the aforementioned activities of the medicinal plants that many researchers were not able to explore. This work will be creating a new insight on the potent activities of the phytoconstituents of the plant extracts.

ETHICAL APPROVAL

The study protocol was approved by the ethics committee of the Department of Pharmacy, Southeast University, Dhaka, Bangladesh. The use and care of animals was performed as per the guide for National Institutes of Health. The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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