INTRODUCTION

Inflammation in the body response to noxious or injurious stimuli, characterized by warmth, redness of the skin, pain, swelling and loss of function. Inflammation is a part of host defense mechanism. There are several tissue factors that are known to be involved in the inflammatory reactions such as release of histamines, bradykinin and prostaglandins. [1, 2]

Asthma is a chronic inflammatory disease of the respiratory tract that is characterized by increased airway hyper-responsiveness and mucus production that leads to episodes of wheezing, coughing and shortness of breath. Asthma is common among the individuals (up to 10% in adults and 35% in children), and as a result, large quantities of asthma medications are needed to stop the asthma prevalence increase [3].

Since generations, in India people are using the extracts and leachates of different herbs in order to stimulate and promote the growth of specific herbs. The example of Alangium lamarkii is one of them. Traditionally, the leaves are useful in treatment of inflammations, blood disorders, burning sensation, spermatorrhoea, gleet, acute fever and lumbago [4, 5]. In case of intense pain due to gout, the patients are advised by the healers to apply the Ankol leaves in affected parts. The leaves are also used in treatment of asthma. The leaves are dried and put on fire. The patients are advised to inhale the fumes [6, 7]. However this plant has not been studied for anti-inflammatory and anti-asthmatic activity. Based on this an attempt has been made to evaluate the inflammatory and anti-asthmatic activity of Alangium lamarkii with their phytoconstituents.

MATERIALS AND METHODS

Plant material

The leaves of Alangium lamarkii are collected form Asansol, West Bengal, India. A herbarium sheet was prepared and it was identified and authenticated (CNH/35/2011/TECH II/446) by the Botanical Survey Research.
of India, Howrah, West Bengal, India. The leaves were dried in shade to avoid too many chemical changes occurring and made into a coarse powder. Methanol was used as solvent for extraction and extraction was performed in Soxhlet Apparatus.

**Fig.1:** Picture showing the *Alangium lamarkii* leaves

**Preparation of extract**
The air dried crushed leaves (1000g) were soaked for 12 hr in Methanol (3L) at room temperature. The residue was extracted with hot Methanol under reflux 3 times (each 1500 ml) after vacuum filtration. All solvent was evaporated under vacuum and extract was then lyophilized, to yield approximately 12% w/w of the residue, which was stored at 20°C until use.

**Experimental animals**
Healthy male and female rats (Wistar albino) and Guinea pigs of 4-8 weeks old were selected after physical and behavioral veterinary examination from Institutional Animal House of Gupta College of Technological Sciences. The weight range was fall within ± 20% of the mean body for each sex at the time of initiation of treatment. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal ethics committee (955/A/06/CPCSEA).

Sixty young adult male Wistar rats (120–150 g) and Guinea pigs (350 to 400 g), were obtained from the Institutional Animal House of Gupta College of Technological Sciences. The rats were housed in polyethylene cages in the Animal House. The rats were housed in polyethylene cages, allowed one week of acclimatization, and maintained on standard rat chow and standard laboratory conditions throughout the experiment.

**Phytochemical Screening**
The concentrated extracts were used for preliminary screening of various phytoconstituents viz. carbohydrate, amino acid, alkaloids, tannins and flavonoids were detected by usual methods prescribed in standard tests. The various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value and alcohol soluble extractive value were determined by the method reported by Sailor et al. 10 with slight modification. [8]

**Acute toxicity test**
Acute toxicity study was performed as per OECD guidelines 423. (Acute toxicity class method). [9]

**Anti-inflammatory activity study**

**Carrageenan – induced rat paw edema**
This is the simplest and most widely used model for studying the anti inflammatory activity of new compounds. The development of edema after a sub plantar injection of carrageenan in the animal is attributed to the release of histamine, serotonin, kinins and prostaglandins. [10]

The animals were weighed and numbered. The right hind paw just beyond tibio tarsal junction was marked, so that every time the paw was dipped into water column upto the fixed mark to ensure constant paw volume. The initial paw volume of each rat was measured by water displacement method. The animals were divided into five groups each comprising of six rats.

Group I: Control animals were received normal saline solution at the dose of 10ml/kg.

Group II: Animals received standard Diclofenac sodium at the dose of 10mg/kg body weight.

Group III: Animals received methanolic extract at the dose of 100mg/kg body weight.

Group IV: Animals received methanolic extract at the dose of 200mg/kg body weight.
Group V: Animals received methanolic extract at the dose of 400mg/kg body weight. All groups received intra peritoneal injection. After 30 minutes 0.1 ml of 1% (w/v) caregeenan was injected in the plantar region of the right paw of each rat. The paw volume was measured after 30, 60, 120 and 180 minutes of administration of carrageenan. Compare the mean percentage change in paw volume in control, extract, and diclofenac treated animals and expressed as percent oedema inhibition.

Anti-asthmatic activity study

Histamine induced Bronchoconstriction
Twenty four guinea pigs of either sex (150-350) were divided into four groups containing six in each. To screen the sensitivity of guinea pigs were placed in a Plexiglas’s chamber (Histamine chamber) and sprayed with 0.1% histamine under the average pressure of 180 ± 30mmHg. The time to onset of respiratory distress (preconvulsive time) during the challenge with these agent was measured. The guinea pigs were randomly allotted to different groups with 6 per each group. The negative control received distilled water orally, and the positive control animals received Pheniramine Maleate by intra peritoneal, the other two groups were treated with methanol extract of Alangium lamarkii (200, 400 mg/kg BW) doses respectively. All groups treated with a single dose daily for five days prior to the challenge, the last dose given 2 hrs before the challenge. The methods of challenge were the same as those of screening the sensitive guinea pigs. The delitescence of convulsion for each guinea pig and tumble numbers for each group during challenge within exposure period were recorded. Protection from anoxic convulsion was calculated as (1 – T₁/T₂) X 100 where T₁ is the mean of pre-convulsion time before treatment and T₂ is the mean of pre-convulsion time 5 days after treatment and % protection was expressed relative to control.[11]

Guinea pig Tracheal chain preparation
Guinea pigs of either sex (200-250g) were divided into 3 groups. Each group contains 6 animals and is allowed to starve overnight and free access to water. The animals were killed by blow on the head and exsanguinations. The isolated trachea was mounted in a 30ml organ bath containing Tyrode solution, maintained at 37± 1°C and gassed with air. The tissue was equilibrated for 45min during which the bath solution is replaced every 10min. At the end of the equilibrium period, histamine (0.5μg/ml) induced contraction as well as effect of extract (upto 800μg/ml) was recorded. A drug tissue contact time of 1min was maintained. The percent response of each group was calculated from the height of the peak obtained.

Statistical analysis
All the values were statistically analyzed by one-way analysis of variance (ANOVA) followed by multiple comparison test. Comparison between control and drug treated groups were considered to be significant p<0.01, p<0.001. All values are expressed as mean ± SEM.

RESULTS AND DISCUSSION

Phytochemical studies
From the Phytochemical study, it has evaluated the presence of alkaloid, amino acid and steroid in leaves.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Compound</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>2.</td>
<td>Tannin</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Amino acids</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Flavonoids</td>
<td>-</td>
</tr>
</tbody>
</table>

++ means strongly present, + means present and – means absent

The physicochemical characters are presented in Table 2. The total ash content value and water soluble ash value of powdered Alangium lamarkii leaves are found to be more in crude drug. Ash value is a measure of the quality and purity of the crude drug. Alcohol and water soluble extractive values were determined to find out the amount of water and alcohol soluble compounds. The leaves showed more
amounts of water soluble compounds than alcohol soluble compounds.

Table 2: Physico-chemical characters of the leaf powder of *Alangium lamarkii*.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameter</th>
<th>Values (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total Ash</td>
<td>20%</td>
</tr>
<tr>
<td>2.</td>
<td>Acid insoluble ash</td>
<td>30%</td>
</tr>
<tr>
<td>3.</td>
<td>Water Soluble Ash</td>
<td>10%</td>
</tr>
<tr>
<td>4.</td>
<td>Determination of Water Soluble Extractives</td>
<td>1.6%</td>
</tr>
<tr>
<td>5.</td>
<td>Determination of Alcohol Soluble Extractives</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

Acute toxicity studies

The extracts of *Alangium lamarkii* did not show any sign of toxicity up to 2000 mg/kg body weight and hence it was considered to be safe.

Anti-inflammatory study

Methanolic Extract of *Alangium lamarkii* was evaluated for Anti-inflammatory activity. In Carrageenan induced paw oedema, the intraperitoneally administration of leaves extract produced a significant anti-inflammatory activity in a dose-dependent manner respectively (100, 200 and 400mg/kg body wt) in the rats. In 400mg/kg dose was showed highest anti-inflammatory potential comparing with the standard anti-inflammatory agent.

Table 3: Effect of Methanolic extract of *Alangium lamarkii* on carrageenan induced inflammation

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Dose</th>
<th>Initial volume</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>0.6±0.02</td>
<td>0.8±0.01</td>
<td>0.94±0.02</td>
<td>1.2±0.05</td>
<td>1.32±0.07</td>
</tr>
<tr>
<td>2.</td>
<td>Standard Diclofenac Sodium (10mg/kg b.w)</td>
<td>0.48±0.04</td>
<td>0.56±0.02* (30%)</td>
<td>0.65±0.01* (30.85%)</td>
<td>0.58±0.02* (51.6%)</td>
<td>0.56±0.03* (57.5%)</td>
</tr>
<tr>
<td>3.</td>
<td>Extract (100mg/kg b.w)</td>
<td>0.50±0.03</td>
<td>0.58±0.02* (27.5%)</td>
<td>0.64±0.05* (31.9%)</td>
<td>0.83±0.03* (30.8%)</td>
<td>0.97±0.08* (26.51)</td>
</tr>
<tr>
<td>4.</td>
<td>Extract (200mg/kg b.w)</td>
<td>0.40±0.05</td>
<td>0.52±0.03* (35%)</td>
<td>0.60±0.02* (36%)</td>
<td>0.80±0.04* (33%)</td>
<td>0.67±0.03* (49%)</td>
</tr>
<tr>
<td>5.</td>
<td>Extract (400mg/kg b.w)</td>
<td>0.45±0.03</td>
<td>0.50±0.05* (37.5%)</td>
<td>0.63±0.03* (32.9%)</td>
<td>0.75±0.02* (37.5%)</td>
<td>0.60±0.03* (54%)</td>
</tr>
</tbody>
</table>

(The data are expressed as mean ± S.E.M. Significant differences in each group versus the control were as follows: *P < 0.05. **P < 0.01).
The isolated guinea pig tracheal chain preparation showed dose dependent significant (P<0.001) inhibition of the contraction of the tracheal muscles induced by histamine as compared to control group.

**Table 4:** Effect of methanol extract of *Alangium lamarkii* on guinea pigs bronchoconstriction induced by Histamine

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Preconvulsive at 0 day (sec)</th>
<th>Preconvulsive time at 5th day (secs)</th>
<th>% protection (1- T1/T2)X100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control as Distilled water</td>
<td>5ml/kg</td>
<td>93.0±0.5</td>
<td>90.0±0.5</td>
<td>-</td>
</tr>
<tr>
<td>Standard (Pheniramine Maleate)</td>
<td>2.5</td>
<td>92.5±0.3</td>
<td>137.0±0.61**</td>
<td>33</td>
</tr>
<tr>
<td>Extract</td>
<td>200</td>
<td>78.5±0.4*</td>
<td>104±0.58**</td>
<td>24.51</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>90.5±0.5**</td>
<td>133.0±0.44**</td>
<td>32.71</td>
</tr>
</tbody>
</table>

(Values are presented as means ± S.E.M, n = 6, **P < 0.01 and * P < 0.05statistical significance against control. statistical test done by t-test)

The isolated guinea pig tracheal chain preparation conducted with crude *Alangium lamarkii* leaf extracts found to exhibit significant anti inflammatory activity whose effect is comparable to that of standard drug-Diclofenac reported in this study. Extract caused significant (P < 0.001) reduction in paw edema from the second hour at the 200mg/kg and 400mg/kg dose level, whereas significant (P< 0.001) reduction in paw edema was not observed from the second hour at the 100mg/kg dose level.

**DISCUSSION**

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. An inflammatory response has been associated with various manifestations such as elevated body temperature and pain [12, 13, 14 and 15]. Preliminary pharmacological screening experiments were conducted with crude *Alangium lamarkii* leaf extracts found to exhibit significant anti inflammatory activity whose effect is comparable to that of standard drug-Diclofenac reported in this study. Extract caused significant (P < 0.001) reduction in paw edema from the second hour at the 200mg/kg and 400mg/kg dose level, whereas significant (P< 0.001) reduction in paw edema was not observed from the second hour at the 100mg/kg dose level.

Bronchial asthma is commonly characterized by increased airway reactivity to spasmogens. An initial event in asthma appears to be the release of inflammatory mediators like histamine, triggered by exposure to allergens that directly cause acute bronchoconstriction [16, 17]. In the present study, histamine was used as spasmogens in the form of aerosol to cause immediate bronchoconstriction in the form of PCD in guinea pigs. Bronchodilating effect of MEAL was evaluated by observing its effects at the time of PCD. In our study, we found that the time of occurrence of PCD was significantly increased, suggestive of bronchodilating activity following treatment with *A. lamarkii* against spasmogens. The preconvulsive time increased significantly (P<0.01) after administration of the extract at doses of 200 and 400 mg/kg b.w. p.o. However the extract was found to be less potent compared to the standard drug, Pheniramine used in the study, as shown by the percentage protection values. Increasing evidence suggests that the frequently observed association between activated T lymphocytes and eosinophils...
plays a major role in the development of airway inflammation and in the accompanying bronchial hyper reactivity [18, 19]. Neutrophils and monocytes play a pivotal role in the disease process as they are a source of variety of inflammatory mediators which are responsible for bronchial hyper responsiveness and airway inflammation [20, 21, 22 and 23].

The relaxant effects of MEAL on tracheal chains of guinea pigs might be produced by different chain mechanisms including stimulation of β-adrenergic receptors, inhibition of histamine H1 receptors or an anticholinergic property of this plant. The relaxant effect of all concentrations of the extract of MEAL obtained where significantly lower than control group. These finding suggest probable β-adrenergic stimulatory, muscurinic or histaminic H1 blocking properties of the plant extract.

Phytochemical screening showed presence of alkaloid, amino acid and steroid etc. Anti-inflammatory and anti-asthmatic activity may be due to presence of above constituents.

Our data suggest that the methanolic extract of the leaves of A. lamarkii possesses significant anti-inflammatory and anti-asthmatic activity. Further studies are needed to establish molecular mechanism and to isolate and characterise the active principles which are responsible for anti-inflammatory and anti-asthmatic property.

Conflict of interest:
There is no conflict of interest associated with the authors of this paper.

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