The ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions were evaluated for formalin induced paw oedema and membrane-stabilizing property. The result obtained indicates that the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fraction (200 mg/kg, p.o.) significantly decreased the formalin induced paw oedema. The extract and its fractions also exhibit membrane-stabilizing property, as significantly (P<0.001) reduced the levels of haemolysis of erythrocytes exposed to hypotonic solution.

**Keywords:** *Ipomoea staphylina*, formalin, paw oedema, membrane-stabilizing property, haemolysis.

**MATERIALS AND METHODS**

**Plant Material**

Leaves of *Ipomoea staphylina* were collected from forest area of Karnataka near to Bangalore. The *Ipomoea staphylina* plant taxonomically identified and authenticated by Dr. K. Karthigeyan at Central National Herbarium, Botanic Garden, Howrah, where the voucher specimen is conserved under the reference number SMF-01. The leaves of *Ipomoea staphylina* were cleaned and dried under shade at room temperature for several days and powdered. The powder was defatted with petroleum ether (60-80 GR) for 72 h and then the dried powder was extracted with ethyl alcohol to get a yield of 10.2 % w/w. The ethanolic extract was dispersed in distilled water and partitioned with ethyl acetate in a separating funnel till the colourless ethyl acetate fraction is obtained.

Then the aqueous part is then partitioned with n-butanol to get the butanol fraction. Ethyl acetate and butanol fraction so obtained was concentrated by keeping in boiling water bath to get the solid residue. The dried extracts were stored in airtight container and placed in refrigerator.

**Phytochemical screening**

Preliminary phytochemical screening of ethanolic extract of *Sechium edule* fruits and its ethyl acetate and n-butanol fractions were performed for the presence of alkaloids, phenolics, flavonoids, saponins, proteins, carbohydrates and glycosides.

**Drugs and chemicals**

Diclofenac sodium and aspirin were obtained from Micro Labs, Bangalore, India. All other chemicals used in this study were obtained commercially and were of analytical grade.

**Experimental animals**

In-breed wistar rats (150-200 g) of either sex weighing 20-25g maintained under controlled conditions of temperature (23±2°C) and humidity (50 ± 5%) and a 12-hour light-dark cycle, were used for the experiment. They were housed in sanitised polypropylene cages containing sterile paddy husk as bedding. They had free access to standard rat pellet diet and water ad libitum. The animals were given a week's time to get acclimatized with the laboratory conditions. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals (CPCSEA), ministry of social justice and empowerment Government of India, norms and approved by the Institutional Animal Ethics Committee (IAEC).

**Acute toxicity studies**

Mice were kept overnight fasting prior to drug administration. Animals were received a single oral dose (2000 mg/kg, b.w.) of ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions. After the administration of *Ipomoea staphylina* leaves extract and its different fractions food was withheld for further 3–4 h. Animals were observed individually at least once during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (pistizes, drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks.

**Formalin induced inflammation**

In this method rats (n=6) were pre-treated orally with the ethanolic extract of leaves of *Ipomoea staphylina* its ethyl acetate and n-butanol fractions (200 mg/kg, p.o and 100 mg/kg, p.o), Diclofenac (10 mg/kg, p.o) and saline (10 ml/kg, p.o) respectively. Thirty minutes after administration of different substances, 0.1ml of 1% of

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**ABSTRACT**

The treatment of inflammatory diseases is mainly dependent on nonsteroidal anti-inflammatory drugs (NSAID). Nonsteroidal anti-inflammatory drugs (NSAID) act by blocking cyclooxygenase enzyme (COX) thus inhibit the conversion of arachidonic acid to prostaglandin. But long-term administration of NSAID induces gastro-intestinal ulcers and bleeding due to their non-selective inhibition of both isoforms of the COX enzyme (COX-1 and COX-2 isozyme). Selective COX-2 inhibitors are associated with adverse cardiovascular effects. On the other hand, steroid drugs as anti-inflammatory agents are not safe due to their multiple side effects. Therefore, developing new agents with more powerful anti-inflammatory activity with lesser side effects is, at present, of great interest. The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the lysosomal membrane. Therefore, as membrane stabilizes that interfere in the release and or action of inflammatory mediators like histamine, serotonin, prostaglandins, leukotrienes etc.

*Ipomoea staphylina* is an extensive climber belonging to the family convolvulaceae. A literature review reveals antulcer property of *Ipomoea staphylina*. Other species of genus *Ipomoea* like *Ipomoea pes-caprae*, *Ipomoea imperati*, *Ipomoea involucrata* and *Ipomoea asarifolia* has been reported for anti-inflammatory activity. So, the present study was carried out to evaluate the anti-inflammatory activity of ethanolic extract and its ethyl acetate and n-butanol fractions of leaves of *Ipomoea staphylina*.

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**INTRODUCTION**

The prese...
formaline subcutaneously into the planter region was injected to all animals in the left hind paw. The paw volume, up to the tibiotarsal articulation, was measured using a plethysmometer (model 7150, Ugo Basile, Italy). The paw volume was determined at 0 h (before formalin injection) and 0.5, 1, 2, 3 and 4 h later \( ^{18, 19} \). Percentage inhibition of oedema = \( 100 \times \left[ \frac{V_1 - V_2}{V_1} \right] \) Where \( V_1 \) and \( V_2 \) are volume of formalin injected paws of drug treated group and control group respectively.

**Membrane stabilizing activity**

**Preparation of erythrocyte suspension**

Whole blood was obtained with heparinized syringes from rats through cardiac puncture. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4). The blood was centrifuged each time for 10 minutes at 3000 g.

**Hypotonic solution-induced rat erythrocyte haemolysis**

Membrane stabilizing activity of the extract was assessed using hypotonic solution-induced rat erythrocyte haemolysis. 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 the ethanolic extract of leaves of *Ipomoea staphylina* its ethyl acetate and n-butanol fractions (0.25-1.0 mg/ml) or aspirin (0.1 mg/ml). The test sample for each concentration was mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the ethanolic extract of leaves of *Ipomoea staphylina* its ethyl acetate and n-butanol fractions (0.25-1.0 mg/ml) or aspirin (0.1 mg/ml). The prepared solution was mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the ethanolic extract of leaves of *Ipomoea staphylina* its ethyl acetate and n-butanol fractions (0.25-1.0 mg/ml) or aspirin (0.1 mg/ml). The prepared solution was incubated for 10 minutes at room temperature and centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated \( ^{15} \) where:

% Inhibition of haemolysis = \( 100 \times \frac{OD1 - OD2}{OD1} \)

Where: $OD1 = \text{Optical density of hypotonic-buffered saline solution}$

$OD2 = \text{Optical density of test sample in hypotonic solution}$

### Table 1: Effect of *Ipomoea staphylina* (IS) leaves extract and its fractions on formalin induced paw oedema

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Mean increase in paw volume (ml)</th>
<th>% Inhibition at 4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.49±0.015</td>
<td>0.91±0.026</td>
</tr>
<tr>
<td>Diclofenac sodium (10)</td>
<td>0.50±0.022</td>
<td>0.92±0.026</td>
</tr>
<tr>
<td>IS Extract (200)</td>
<td>0.53±0.021</td>
<td>0.94±0.026</td>
</tr>
<tr>
<td>IS Extract (100)</td>
<td>0.51±0.022</td>
<td>0.95±0.026</td>
</tr>
<tr>
<td>Ethyl acetate fraction of IS (200)</td>
<td>0.48±0.022</td>
<td>0.96±0.026</td>
</tr>
<tr>
<td>Ethyl acetate fraction of IS (100)</td>
<td>0.47±0.022</td>
<td>0.97±0.026</td>
</tr>
<tr>
<td>n-Butanol fraction of IS (200)</td>
<td>0.48±0.022</td>
<td>0.98±0.026</td>
</tr>
<tr>
<td>n-Butanol fraction of IS (100)</td>
<td>0.47±0.022</td>
<td>0.99±0.026</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6; *** P<0.001, ** P<0.01, * P<0.05 considered statistically significant as compared to control group.

### Acute toxicity studies

In LD50 studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses of 100 and 200 mg/kg body weight.

### Effect on formaldehyde-induced paw oedema

The ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions were evaluated for formalin induced paw oedema. The result obtained indicates that the ethanolic extract of leaves of *Ipomoea staphylina* (200 mg/kg, p.o.) significantly decreased the formalin induced paw oedema at 0.5 h (P<0.001), 1, 2 and 4 h (P<0.001). The ethyl acetate fraction (200 mg/kg, p.o.) also significantly decreased the paw oedema at 0.5 h (P<0.001), 1, 2, 3 and 4 h (P<0.001). The n-butanol fraction of *Ipomoea staphylina* (200 mg/kg, p.o.) also significantly decreased the formalin induced paw oedema at 1, 2, 3 and 4 h (P<0.001). Maximum inhibition of paw oedema was observed with the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions (200 mg/kg, p.o.) at 4 h when compared to the control group (Table 1). Diclofenac sodium (10 mg/kg, p.o.) and the ethanolic extract of leaves of *Ipomoea staphylina* (200 mg/kg, p.o.) inhibited paw oedema by 31.87% and 30.82% respectively.

### Effect on erythrocyte membrane stability

The extract and its fractions at concentration range of 0.50-1.0 mg/ml significantly (P<0.001) protect the rat erythrocyte membrane against lysis induced by hypotonic solution. The standard drug aspirin (0.10 mg/ml) also offered a significant (P<0.001) protection of the rat erythrocytes membrane against the damaging effect of hypotonic solution. The ethanolic extract of *Ipomoea staphylina* and its ethyl acetate and n-butanol fraction shows 56%, 49% and 40% inhibition of haemolysis at a concentration of 1.0 mg/ml whereas the standard drug aspirin (0.10 mg/ml) produced 72% inhibition of RBC haemolysis (Table 2).
DISCUSSIONS
The results of the study suggest that the Ipomoea staphylina leaf extract and its ethyl acetate and n-butanol fractions possess anti-inflammatory activity, as it significantly inhibited paw edema induced by formalin in rats. The extract and its fractions also showed membrane stabilizing effect, as it offer significant protection of the erythrocyte against lysis induced by hypotonic solution.

Formalin, a potent oedematous agent, produced inflammation through the release of several inflammatory mediators including prostaglandins. Injection of 1% formalin subcutaneously into hind paw of rats produced localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic act and its later phase is mediated by chemical mediators such as substance P and bradykinins followed by a tissue mediated phase of swelling due to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Such injury to erythrocyte membrane will further make the cell more susceptible to free radical-induced lipid peroxidation.

Compounds with membrane-stabilizing properties are well known for their ability to interfere with the release of phospholipases that trigger the formation of inflammatory mediators such as histamine, serotonin, prostaglandins, leukotrienes etc. The extract and its fractions at concentration range of 0.50-1.0 mg/ml significantly (P<0.001) protect the rat erythrocyte membrane against lysis induced by hypotonic solution which suggests that its anti-inflammatory action observed in this study, may be related to the inhibition of the early phase of inflammatory events, the release of chemical mediators.

CONCLUSION
In conclusion, the results of the study suggest that Ipomoea staphylina leaf extract its ethyl acetate and n-butanol fractions possess anti-inflammatory and membrane stabilizing activity.

REFERENCES

Table 2: Effect of Ipomoea staphylina (IS) leaves extract and its fractions on rat erythrocyte haemolysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration</th>
<th>Optical density (OD)</th>
<th>% Inhibition of haemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypotonic medium</td>
<td>50 mM</td>
<td>0.83±0.018</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.25 mg/ml</td>
<td>0.64±0.010</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>0.50 mg/ml</td>
<td>0.55±0.010</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>0.75 mg/ml</td>
<td>0.42±0.010</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>1 mg/ml</td>
<td>0.36±0.011</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>0.25 mg/ml</td>
<td>0.68±0.011</td>
<td>18</td>
</tr>
<tr>
<td>IS Extract</td>
<td>0.50 mg/ml</td>
<td>0.58±0.018</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0.75 mg/ml</td>
<td>0.49±0.013</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>1 mg/ml</td>
<td>0.42±0.020</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>0.25 mg/ml</td>
<td>0.72±0.018</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>0.50 mg/ml</td>
<td>0.65±0.017</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>0.75 mg/ml</td>
<td>0.57±0.024</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>1 mg/ml</td>
<td>0.49±0.019</td>
<td>40</td>
</tr>
<tr>
<td>Acetate Fraction</td>
<td>0.10 mg/ml</td>
<td>0.23±0.017</td>
<td>72</td>
</tr>
<tr>
<td>n-Butanol fraction</td>
<td>0.25 mg/ml</td>
<td>0.65±0.017</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>0.50 mg/ml</td>
<td>0.57±0.024</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>1 mg/ml</td>
<td>0.49±0.019</td>
<td>40</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM of 6 experiments. ***P<0.001 considered statistically significant as compared to Hypotonic medium.


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