Anti-nociceptive, Anti-inflammatory and Antipyretic Activities of Ethanolic Extract of Atylosia scarabaeoides (L.) Benth (Family: Fabaceae) Leaves in Experimental Animal

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Authors’ contributions

This work was carried out in collaboration between all authors. Author MM carried out the collection of plants extraction process, conducting the experiments. Authors SMRD and AD wrote the manuscript and done the statistical analysis. Author MSS carried out conception and design of the study, analysis and interpretation of data. All authors read and approved the final manuscript.

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ABSTRACT

Background: Atylosia scarabaeoides (L.) Benth, belonging to the family Fabaceae and locally known as Banurkali or Thitkalai, has been widely used in folkloric medicine. Hence, the objective of the present study was to evaluate the anti-nociceptive, anti-inflammatory and antipyretic activities of ethanolic extract of A. scarabaeoides leaves using Swiss-albino mice as test animal.

Methods: The anti-nociceptive activity was assessed using both chemical-induced (acetic acid writhing response test, formalin test) and heat-induced (tail-flick test, hot-plate test) nociception

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models in mice at different doses (150, 300 and 450 mg/kg, p.o.) of the extract. The anti-inflammatory activity was investigated using in vivo carrageenan-induced paw oedema and in vitro membrane stabilising tests. Antipyretic activity was evaluated using Brewer’s yeast-induced pyrexia model.

**Results:** Oral administration of the ethanolic extract exhibited a significant ($P<0.05 - <0.001$) dose-dependent analgesia in various pain models. In acetic acid-induced writhing test, the writhing response inhibitory percent by the extract was 29.82% and 31.58% at 300 and 450 mg/kg doses respectively. The extract also significantly inhibited the licking response in both the early phase (61.84%, $P<0.01$) and the late period (89.36%, $P<0.001$) in the formalin-induced writhing test at a dose of 450 mg/kg b.w. Furthermore, in tail flick and hot plate test, the plant extract at varying concentrations increased the reaction time to the thermal stimuli up to 120 min. The extract also significantly ($P<0.05$, $P<0.01$ and $P<0.001$) inhibited carrageenan-induced inflammatory response in mice in a dose-related manner. In the in-vitro anti-inflammatory test, the extract (1 mg/ml) demonstrated significant ($P<0.01$) protection against RBC hemolysis with 77.13 ± 6.61% inhibition which was closer to that of the standard drug acetylsalicylic acid (84.25 ± 3.73%). The extract also reduced hyperthermia induced by Brewer’s yeast depending on the dose, and the maximum activity was found at the dose of 450 mg/kg b.w.

**Conclusion:** Since the study revealed that the ethanolic extract of *A. scarabaeoides* leaves possesses anti-inflammatory, antinociceptive and antipyretic activities. Therefore, the findings supported the use of the plant extract in the traditional medicine as an oral remedy for pains, inflammation, and fever.

**Keywords:** Atylosia scarabaeoides; anti-nociceptive; anti-inflammatory; antipyretic.

1. **BACKGROUND**

Inflammation and pain have become the principal areas of global scientific research [1], as they are the most common manifestations of various diseases affecting millions of people worldwide [2,3]. Inflammation is a complex biological response of vascular tissues to harmful stimuli [4] and is triggered by mechanical injuries, microbial infections, burns, allergens, and other noxious stimuli [5]. It is also known to be a critical adaptive response for humans, and its mechanism includes a complex interaction between immune cells and molecular mediators like prostaglandins, leukotrienes, histamine, bradykinin, platelet-activating factor and interleukin-1 [6]. While the pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It is always a warning signal and primarily protective but often causes a lot of discomforts and lead to many adverse effects [7]. Harmful stimuli lead to activation of nociceptors through the release of variety of chemical mediators, such as excitatory amino acids, vasoactive amines (histamine, serotonin), proteins, peptides, nitric oxide (NO), arachidonic acids (prostaglandins E2, leukotrienes), and cytokines [TNF-α and interleukin-1], which act on specific receptors and ion channels contributing to the induction of pain and inflammation [5]. Moreover, prostaglandins (PGs) induce hyperalgesia by affecting the transducing property of free nerve endings, bradykinins, TNFα, interleukins (ILs) and induce pain. Again pyrexia or fever is caused by a secondary impact of inflammation [8]. Fever occurs due to infection produced by a generation of pyrogens including, ILs, TNF-α, interferes in which induces PGE2 production in the hypothalamus and its temperature set point. Therefore, it may be said that inflammation, pain and fever are all associated with enhanced production of prostaglandins [2].

Symptoms of inflammation process can be alleviated by non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit mainly the cyclooxygenase enzyme and reduce the synthesis of prostanooids. Corticosteroids can also prevent the formation of prostaglandins by causing the release of lipocortin, which by inhibition of phospholipase A2 decreases arachidonic acid release [6]. Thus, most anti-inflammatory agents are also expected to possess analgesic and antipyretic activities [9]. Although they are useful in alleviating these manifestations but unfortunately, they may have adverse reactions such as respiratory depletion, cardiovascular instability, gastric damage, tachycardia, hypotension, hepatotoxicity, nephrotoxicity, Reye’s syndrome, and blood dyscrasias [10]. As a result, there is a high demand for the search of new drugs with lesser or no side effects [1].
Many researchers have focused in recent years on medicinal plants derived natural products such as flavonoids, steroids, polyphenols, coumarins, terpenes and alkaloids due to their full range of pharmacological significance including anti-inflammatory, analgesic and antipyretic activities with lesser side effects [11].

Drugs of natural origin are an essential source for the treatment of many diseases worldwide [12]. Medicinal plants used in the folkloric treatment of pain related ailments have a long and popular usage especially in developing countries [13]. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use of the 252 drugs considered as necessary by the World Health Organization (WHO). 11% are exclusive of plant origin, and a significant number are synthetic drugs obtained from natural precursors. Interestingly, the World Health Organization (WHO) has recommended the integration of traditional medicines proved to be useful into national health care programs, and developed a strategy to address issues of policy, safety, efficacy, quality, access and rational use (WHO Traditional Medicine Strategy 2014–2023) [4]. In the context, the current trend of research has shifted towards medicinal plants because of their affordability and accessibility with lesser side effects [1].

Atylosia scarabaeoides (L.) Benth (Fabaceae), locally known as Banurkali or Thitkalai, is a slender, twining herb with densely grey-dowry stems that is distributed throughout Bangladesh, India, Malaysia, China, Mauritius and Madagascar [13]. It has been used in folk medicine for its various medicinal properties like antimicrobial [13], and dysenteric, anticholera, febrifuge [14]. The plant is also used in the treatment of anaemia, anasarca and hemiplegia [14]. The leaf of the plant is given with honey to women after childbirth. The plant is also used for the prevention of hair loss and even to cure diarrhoea in cattle [15]. But no data is reported regarding the anti-nociceptive, anti-inflammatory and antipyretic activities to date.

In review of the evidences from the existing literatures showing that A. scarabaeoides has multipurpose traditional uses against different sorts of diseases, the present study aimed to investigate the anti-nociceptive, anti-inflammatory and antipyretic activities of ethanolic extract of A. scarabaeoides leaf.

2. MATERIALS AND METHODS

2.1 Plant Material

A. scarabaeoides leaves were collected from Kustia district of Bangladesh. The plant was identified and authenticated by an expert botanist of Bangladesh National Herbarium (DACB), Mirpur, Dhaka (Accession No. 40293) and a voucher specimen was submitted at the herbarium for future reference.

2.2 Extract Preparation

After washing and cutting the leaves into small parts, the leaves were air-dried in a shade and finally in a mechanical drier (Ecocell, MMM Group, Germany) at 55-60°C. The dried samples were ground to coarse powder with a mechanical grinder (NOWAKE, Japan). Weighed (630 g of the dried and powdered) sample was soaked in 2200 ml of 80% ethanol in clean, sterilized and flat-bottomed glass container. Afterwards, it was sealed and maintained for 15 days accompanying occasional stirring and agitation. The complete mixture was then subjected to coarse filtration on a piece of clean, white sterilized cotton material and Whatman® filter paper. The extract was obtained by evaporation using rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 4 rpm and 65°C temperature. It rendered a gummy concentrate of greenish color. The gummy concentrate was designated as crude extract or ethanolic extract. Then the crude ethanolic extract was dried by freeze drier and preserved at +4°C (yield 1.33%).

2.3 Test Animals

For the screening of in vivo analgesic, anti-inflammatory and antipyretic potential of ethanolic extracts of A. scarabaeoides leaves, young Swiss-albino mice (aged 20–25 days) of either sex, average weight 20–25 g were used. They were collected from the Jahangirnagar University, Dhaka, Bangladesh. After collection, they were kept in favorable condition for one week for adaptation and fed rodent food and water ad libitum formulated by ICDDR, B (International Centre for Diarrheal Disease and Research, Bangladesh). Throughout the experiments, all animals received human care according to the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’, 8th edition, prepared by the National Academy of Sciences and published by the National Institute of Health (US).
2.4 Anti-nociceptive Activity Test

In the current investigation, four different methods were employed for testing the possible peripheral and central analgesic effects of *A. scarabaeoides* leaves; namely acetic acid-induced writhing, formalin-induced licking response, hot plate and tail-flick test methods in mice respectively.

2.4.1 Acetic acid induced writhing test

The analgesic activity of the crude ethanolic extract was studied using acetic acid-induced writhing model in mice following the procedure described by Bhowmic et al. [16], with minor modification. The animals (Swiss Albino male mice) were divided into five groups (Each group consisting 5 mice) including control (Group I), positive control (Group II) and three test groups (Group III-V). The animals of group III, IV and V were administered test substance at the dose of 150, 300 and 450 mg/kg body weight respectively. Positive control group received aspirin (standard drug) at the dose of 300 mg/kg body weight and vehicle control group was treated with 1% Tween 80 in distilled water at the dose of 10 ml/kg body weight. Test samples, standard drugs and control vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After 15 min of time interval, the writing (constriction of abdomen, turning of trunk and extension of hind legs) was observed on mice for 5 min.

2.4.2 Formalin-induced writhing test

The method used was similar to that described previously [4]. The mice were divided into five groups each containing 5 mice and were administered with either distilled water (1 ml/kg, i.p.), ethanolic extract of *A. scarabaeoides* leaves (150, 300 and 450 mg/kg, i.p) or aspirin (50 mg/kg, s.c). Thirty minutes after this treatment; 50 μL of a freshly prepared 0.6% solution of formalin was injected subcutaneously under the plantar surface of the left hind paw of each mice. The mice were placed individually in an observation chamber and monitored for one hour. The time (in second) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Anti-nociceptive effect was determined in two phases. The early phase (phase 1) was recorded during the first 5 minutes, while the late phase (phase 2) was recorded during the last 15–20 minutes after formalin injection.

2.4.3 Tail-flick test

The procedure used was similar to that used by Abdala et al. [6], with slight modification. The apparatus used consisted of a circulating immersion water heater. The thermostat was adjusted so that a constant temperature of 52 ± 2°C was maintained in the water bath. Before treatment, the terminal 3 cm of each mouse’s tail was immersed in the water bath and the time in seconds taken to flick the tail was recorded. Each mice group served as its own control. Only mice showing a pretreatment reaction time less or equal to 4s were selected for the study. Immediately after basal latency assessment, the three doses ethanolic extract (150, 300 and 450 mg/kg p.o.), the positive control morphine (10 mg/kg i.p.) or 1% tween 80 p.o. (negative control) were administered to groups of mice (5 to 15 animals per group) and there action time was again measured 1 and 2 h after the treatment, except for morphine where measurement began 30 min after administration. Cut-off time was 6s for tail flick measurements in order to minimize tissue injury. The percentage of pain reduction was calculated using the following ratio: (treated mean – control mean) × 100/ control mean.

2.4.4 Hotplate test

The antinociceptive activity of the extracts was also measured by hot-plate method [17]. Mice were divided into five groups of five animals each. Group I treated as control (saline water 10 mg/kg), group II received standard drug (aspirin 300 mg/kg p.o.), and groups III-V received crude ethanolic extract of *A. scarabaeoides* leaves (150, 300 and 450 mg/kg bw p.o, respectively). The temperature of the hot-plate was maintained at 55 ± 1°C. The mice were placed in a 24 cm diameter glass cylinder on the heated surface and the time between placement and licking of the paws or jumping was recorded as the latency. A cut off time of 20s was followed to avoid any thermal injury to the paws and was defined as complete analgesia. The reaction time were recorded before (0 min) and after 30, 60, 90 and 120 min following administration of test samples or standard drug.

2.5 Anti-inflammatory Activity Test

2.5.1 Carrageenan-induced rat paw edema

Carrageenan-induced mice hind paw edema was used as the animal model of acute inflammation according to the method of Ferreira et al. [18]. In
this experiment, the mice were divided into five groups of five animals each. Group I (control) received 2% Tween 80 in normal saline (2 ml/kg). Group II (Positive control) received 10 mg/kg body wt. of ketorolac orally. Group III, IV and V received 200, 300 and 400 mg/kg body wt. of the extract orally respectively. Acute inflammation was induced in all the five groups by subplantar injection of 0.05 ml of its suspension of Carrageenan with 2% Tween 80 in normal saline in the right Paw of the mice 30 minutes after the oral administration of the tested materials. The paw volume was measured with micrometer screw gauze at 0, 1, 2, 3, 4 and 5h after the administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the following expression [18].

\[
\text{Percentage inhibition of inflammation} = \frac{(V_c - V_t)}{V_c} \times 100
\]

Where, \( V_c \) is the average degree of inflammation by the control group and \( V_t \) is the average degree of inflammation by the test group.

### 2.5.2 Hypotonic solution-induced hemolysis of RBC

The membrane stabilising the activity of the extract was evaluated by using hypotonic solution induced human erythrocyte hemolysis, designed by Debnath et al. [19]. To prepare the erythrocyte suspension 7 ml blood was obtained using syringes (containing anticoagulant EDTA) from healthy male volunteers (aged 20-23 years and without a history of oral contraceptive or anticoagulant therapy and free from diseases) through puncture of the antecubital vein. The blood was centrifuged, using the centrifugal machine, for 10 min at 3000 g and blood cells were washed three times with solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4). The test sample, consisted of stock erythrocyte (RBC) suspension (0.50 mL), was mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the extract (1.0 mg/mL) or acetylsalicylic acid (0.1 mg/mL). The control sample, consisted of 0.5 mL of RBCs, was mixed with hypotonic-buffered saline alone. The mixture was incubated for 10 min at room temperature, centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm using UV spectrometer. The percentage inhibition of either hemolysis or membrane stabilization was calculated using the following equation:

\[
\text{% Inhibition of hemolysis} = 100 \times \left(\frac{\text{OD}_1 - \text{OD}_2}{\text{OD}_1}\right)
\]

Where, 

- \( \text{OD}_1 \) = Optical density of hypotonic-buffered saline solution alone (control) and
- \( \text{OD}_2 \) = Optical density of test sample in hypotonic solution

### 2.6 Antipyretic Activity Test

#### 2.6.1 Brewer’s yeast-induced pyrexia

The antipyretic activity was evaluated by Brewer’s yeast induced pyrexia in experimental animal. Hyperpyrexia was induced by subcutaneous administration of 10 ml/kg body weight 20% aqueous suspension of brewer’s yeast. The selected animals were fasted overnight with water ad libitum before the experiments. Initial rectal temperature of animals was recorded using an Ellab thermometer (33.19 ± 0.40°C). After 18 h of subcutaneous administration, the animals that showed an increase of 0.3–0.5°C in rectal temperature were selected for the antipyretic activity. Crude ethanolic extract of plant was given orally (150, 300 and 450 mg/kg b.w p.o.). Paracetamol (150 mg/kg orally) was used as reference drug, whereas, control group received distilled water (10 ml/kg) only. The rectal temperature was recorded at 30 minutes interval for 2 h after treatment [16].

### 2.7 Statistical Analysis

The data are expressed as the mean ± SEM analysed by one-way analysis of variance (ANOVA), and Dunnett’s t-test was used as the test of significance. \( P \) value <0.05 was considered as the minimum level of significance. All statistical tests were carried out using SPSS (version 16 for Windows®) statistical software.

### 3. RESULTS

#### 3.1 Anti-nociceptive Activity Test

#### 3.1.1 Acetic acid induced writhing test

The effect of aqueous and ethanol extract of \( A. \) scarabaeoides leaves on acetic acid-induced writhing test in mice was dose-dependent (Table 1) and significantly \((P<0.05; \ P<0.001)\) decreased the number of writhing movements induced by the intraperitoneal administration of the acetic acid comparing with positive control. The highest inhibition of pain exhibited by higher dose 450
mg/kg of ethanolic extract was 31.58% while the standard drug, aspirin, was 80.70% (300 mg/kg).

### 3.1.2 Formalin-induced writhing test

The results of the anti-nociceptive effects of *A. scarabaeoides* on formalin-induced paw pain response in mice are presented in (Table 2). It can be seen that the highest doses assayed caused a significant inhibitory effect, once again in a dose-dependent manner, on both phases of formalin-induced pain as compared to control. The mean of inhibition was 48.68 and 76.59% at 150 mg/kg; 50.00 and 87.23% at 300 mg/kg and 61.84 and 89.36% at 450 mg/kg b.w. in the first and second phase respectively. This potency was comparable to that of aspirin, the non-steroidal anti-inflammatory drug used as a reference peripheral analgesic compound in this test, which produced a significant inhibition (55.26%; \( P<0.01 \)) during the first phase and 95.74% inhibition (\( P<0.001 \)) during the second phase of the formalin-induced pain in mice.

### 3.1.3 Tail-flick test

In tail flick method the ethanolic extract of *A. scarabaeoides* leaves at 150, 300 and 450 mg/kg b.w. showed significant increase in tail flicking response time to the nociceptive stimuli from 30–120 min after administration. Only the dose of 300 mg/kg assayed produced significant (\( P<0.001; \ P<0.01 \)) increase in the reaction time of 3.67 ± 1.80 and 2.33 ± 1.87 sec to thermal stimuli at the 30 and 60 min respectively after administration of extract in comparison to control.

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### Table 1. Effect of ethanolic extract of *A. scarabaeoides* leaves on acetic acid induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Number of writhing</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Distilled water)</td>
<td>10</td>
<td>38.00 ± 2.64</td>
<td>-</td>
</tr>
<tr>
<td>Standard (Aspirin)</td>
<td>300</td>
<td>7.33 ± 2.52</td>
<td>80.70</td>
</tr>
<tr>
<td>EEAS 150</td>
<td>29.00 ± 2.64</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>EEAS 300</td>
<td>26.67 ± 9.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EEAS 450</td>
<td>26.00 ± 7.21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Standard error mean); (n = 5). ***indicates \( P<0.001 \), and *indicates \( P<0.05 \); one-way ANOVA followed by Dunnett’s t-test as compared to control. EEAS = Ethanolic extract of *A. scarabaeoides*

### Table 2. Effect of ethanolic extract of *A. scarabaeoides* leaves on formalin induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Licking number (Mean ± SEM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early phase (0-5 min)</td>
<td>Late phase (10-15 min)</td>
<td>Early phase (0-5 min)</td>
</tr>
<tr>
<td>Control (Distilled water)</td>
<td>10</td>
<td>25.33 ± 3.51</td>
<td>15.67 ± 1.15</td>
</tr>
<tr>
<td>Standard (Aspirin)</td>
<td>50</td>
<td>11.33 ± 1.15&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.67 ± 1.15&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>EEAS 150</td>
<td>13.00 ± 2.00&lt;sup&gt;**&lt;/sup&gt;</td>
<td>3.67 ± 1.15&lt;sup&gt;**&lt;/sup&gt;</td>
<td>48.68</td>
</tr>
<tr>
<td>EEAS 300</td>
<td>12.67 ± 1.15&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2.67 ± 1.15&lt;sup&gt;**&lt;/sup&gt;</td>
<td>50.00</td>
</tr>
<tr>
<td>EEAS 450</td>
<td>9.67 ± 3.78&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.67 ± 0.58&lt;sup&gt;**&lt;/sup&gt;</td>
<td>61.84</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Standard error mean); (n = 5). ***indicates \( P<0.001 \), and **indicates \( P<0.01 \); one-way ANOVA followed by Dunnett’s t-test as compared to control. EEAS = Ethanolic extract of *A. scarabaeoides*

### Table 3. Effect of ethanolic extract of *A. scarabaeoides* leaves on tail flicking reaction time of mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Tail flicking time (sec)</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Distilled water)</td>
<td>10</td>
<td>1.00 ± 0.00</td>
<td>1.20 ± 0.45</td>
<td>1.30 ± 0.45</td>
<td>1.50 ± 0.87</td>
<td></td>
</tr>
<tr>
<td>Standard (Morphine)</td>
<td>10</td>
<td>1.22 ± 0.44</td>
<td>2.11 ± 0.93&lt;sup&gt;**&lt;/sup&gt;</td>
<td>5.44 ± 2.70&lt;sup&gt;***&lt;/sup&gt;</td>
<td>5.89 ± 2.31&lt;sup&gt;***&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>EEAS 150</td>
<td>144 ± 0.53</td>
<td>1.44 ± 0.53</td>
<td>1.11 ± 0.33</td>
<td>1.00 ± 0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EEAS 300</td>
<td>3.67 ± 1.80&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2.33 ± 1.87&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.78 ± 0.97</td>
<td>3.00 ± 1.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EEAS 450</td>
<td>1.67 ± 1.00</td>
<td>1.22 ± 0.44</td>
<td>1.11 ± 0.33</td>
<td>1.44 ± 0.73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Standard error mean); (n = 5). ***indicates \( P<0.001 \), and **indicates \( P<0.01 \); one-way ANOVA followed by Dunnett’s t-test as compared to control. EEAS = Ethanolic extract of *A. scarabaeoides*
In contrast the reference opioid drug, morphine (10 mg/kg), produced an important significant inhibition in the 90 min (5.44 ± 2.70 sec; \( P<0.001 \)) and 120 min (5.89 ± 2.31 sec; \( P<0.001 \)) after its intraperitoneal administration (Table 3).

3.1.4 Hotplate test

The reaction time of three doses of ethanolic extract increased in dose-dependent manner to the thermal stimuli. The results are shown in (Table 4). The higher dose 450 mg/kg of ethanolic extract demonstrated the highest and significant \( (P<0.05) \) nociceptive inhibition of thermal stimulus which was comparable to the control. The maximum reaction time of ethanolic extract for the response against thermal stimuli at higher dose was 5.33 ± 0.58 and 4.67 ± 0.58 seconds at 180 and 120 minutes respectively whereas for the standard drug, diclofenac Na (10 mg/kg) was administered orally, it was 2.67 ± 2.08 and 2.00 ± 1.00 seconds at 180 and 120 minutes respectively.

3.2 Anti-inflammatory Activity Test

3.2.1 Carageenan-induced rat paw edema

The anti-inflammatory effect of the crude ethanolic extract and standard drug using carrageenan-induced edema tests is expressed in (Fig. 1). The intraplantar injection of 0.05 ml of carrageenan-induced significant and intense gradual increase in the volume of the paws of the mice treated an effect that reached a maximum level at 5 h after administration. The oral administration of different doses of the ethanolic leaf extract of \( A. scarabaeoides \) significantly \( (P<0.05, P<0.01 \) and \( P<0.001 \)) inhibited inflammatory response induced by carrageenan in rats in a dose-related manner. It can be observed from (Fig. 1) that \( A. scarabaeoides \) leaves, at the higher dose tested of 400 mg/kg, demonstrated prominent inhibition of edema formation (46.83%; \( P<0.01 \)) when compared to control group. Standard drug (ketorolac,10 mg/kg b.w.) was administered orally, also produced a significant \( (P<0.01) \) inhibition of the paw edema (54.43%) after 5 h when compared to the vehicle control.

3.2.2 Hypotonic solution-induced hemolysis of RBC

In hypotonic solution induced conditions, the test sample of \( A. scarabaeoides \) leaves was found to inhibit lysis of erythrocyte membrane by 77.13 ± 6.61% (Table 5). Besides, the standard drug acetylsalicylic acid (0.1 mg/ml) inhibited 84.25 ± 3.73% hemolysis of the RBC. The inhibitory capacity of RBC hemolysis of both the test sample and the standard were found to be significant \( (P<0.01) \).
Table 4. Effect of ethanol extract of *A. scarabaeoides* leaves in hot plate test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Reaction time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Control (Distilled water)</td>
<td>10</td>
<td>6.33 ± 0.58</td>
</tr>
<tr>
<td>Standard (Diclofenac Na)</td>
<td>10</td>
<td>0.33 ± 0.58</td>
</tr>
<tr>
<td>EEAS</td>
<td>150</td>
<td>1.33 ± 1.53</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>2.00 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>2.00 ± 1.00</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Standard error mean); (n = 5). ***indicates P<0.001, **indicates P<0.01 and *indicates P<0.05; one-way ANOVA followed by Dunnett’s *t*-test as compared to control. EEAS = Ethanolic extract of *A. scarabaeoides*.

Table 5. Effect of ethanol extract of *A. scarabaeoides* leaves on hypotonic solution induced hemolysis of erythrocyte membrane

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mg/ml)</th>
<th>Optical density of samples in hypotonic solution</th>
<th>Percentage inhibition of hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>0.90 ± 0.04</td>
<td>-</td>
</tr>
<tr>
<td>EEAS</td>
<td>1</td>
<td>0.21 ± 0.06</td>
<td>77.13 ± 6.61</td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td>0.1</td>
<td>0.14 ± 0.03</td>
<td>84.25 ± 3.73</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Standard error mean); (n = 5). **indicates P<0.01; One-way ANOVA followed by Dunnett’s *t*-test as compared to control. EEAS = Ethanolic extract of *A. scarabaeoides*.

Table 6. Effect of ethanol extract of *A. scarabaeoides* leaves on Brewer’s yeast-induced pyrexia in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Temperature in °C Initial</th>
<th>Pyretic</th>
<th>0.5 h</th>
<th>1 h</th>
<th>1.5 h</th>
<th>2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>35.78 ± 1.39</td>
<td>39.27 ± 0.43</td>
<td>38.24 ± 0.69</td>
<td>37.18 ± 0.65</td>
<td>37.00 ± 0.64</td>
<td>36.31 ± 0.34</td>
</tr>
<tr>
<td>Standard (Paracetamol)</td>
<td>150</td>
<td>35.46 ± 1.03</td>
<td>38.31 ± 0.92</td>
<td>36.09 ± 1.09</td>
<td>36.00 ± 1.01</td>
<td>35.79 ± 1.13</td>
<td>35.49 ± 0.99</td>
</tr>
<tr>
<td>EEAS</td>
<td>150</td>
<td>35.59 ± 0.99</td>
<td>38.11 ± 0.87</td>
<td>37.15 ± 0.16</td>
<td>37.05 ± 0.17</td>
<td>36.39 ± 0.48</td>
<td>35.83 ± 0.89</td>
</tr>
<tr>
<td>EEAS</td>
<td>300</td>
<td>35.46 ± 0.48</td>
<td>38.15 ± 0.44</td>
<td>36.54 ± 0.69</td>
<td>36.30 ± 0.82</td>
<td>35.89 ± 0.48</td>
<td>35.81 ± 0.36</td>
</tr>
<tr>
<td>EEAS</td>
<td>450</td>
<td>35.76 ± 0.61</td>
<td>37.92 ± 0.19</td>
<td>36.50 ± 0.72</td>
<td>36.29 ± 0.83</td>
<td>36.07 ± 0.56</td>
<td>35.79 ± 0.56</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Standard error mean); (n = 5). ***indicates P<0.001, **indicates P<0.01 and *indicates P<0.05; one-way ANOVA followed by Dunnett’s *t*-test as compared to control. EEAS = Ethanolic extract of *A. scarabaeoides*.
3.3 Antipyretic Activity Test

3.3.1 Brewer’s yeast-induced pyrexia

From the results (Table 6), it was observed that, experimental mice showed a marked increase in rectal temperature, 18th h after Brewer’s Yeast injection. In the first 30 minute (0.5 h) the extract did not show anti-pyretic activity. The extract treatment, with 150, 300 and 450 mg/kg, significantly reduced the rectal temperature of the animals in the first and second hour after administration, reaching the peak of antipyretic effect with the highest dose (450 mg/kg) in the 2nd h (35.79 ± 0.56°C, \(P < 0.001\)), in relation to control (36.31 ± 0.34°C). The Paracetamol treatment (150 mg/kg) caused significant antipyretic effect at all time periods, reaching the peak in the 2nd h (35.49 ± 0.99°C, \(P < 0.001\)), in comparison to control.

4. DISCUSSION

The present study implemented different in vivo and in vitro test models with the aim of evaluating analgesic, anti-inflammatory and antipyretic properties of the leaves of *A. scarabaeoides* and the documented results showed that the ethanolic extract of the plant has marked effects with varying potency and a reasonable safety profile.

Several test models to induce nociception in animals are well-known and can be used to investigate the analgesic activity of different compounds. These models consist in providing noxious stimuli to the animals employing a thermal, chemical, electrical or mechanical pathway and further observation of the animal behaviour to the stimulus can be quickly done using those [6]. Acetic acid-induced writhing is a well-recommended protocol in evaluating the peripheral analgesic property of medicinal agents due to its sensitivity and response to the compounds at a dose which is not effective in other methods [20]. Here the pain induction is caused by liberating endogenous substances as well as some other pain mediators such as arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis (specifically lipoxygenase, PGE2 and PGF2α) [21]. These prostaglandin and lipoxygenase products cause inflammation and pain by increasing capillary permeability and affecting local pain receptor [21]. Thus the substance inhibiting the writing will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [21]. In our study, antinociceptive assessment using the acetic acid-induced writhing test showed that oral administration of *A. scarabaeoides* produced a statistically significant inhibition of writes compared to the control. This is an indication of the peripheral analgesic activity by the active principle(s) of the plant extract, since any agent that lowers the writhing number, demonstrates analgesia by inhibiting prostaglandin synthesis which is a peripheral mechanism of pain inhibition [22,23].

In order to obtain more specific evidence on the possible mechanism of anti-nociceptive activity of *A. scarabaeoides*, the effect of different doses of the ethanolic extract on the formalin test was examined. This test model is considered as a method of persistent pain produced by the intraplantar injection of formalin that induces a biphasic nociceptive behavior. The early phase (0–5 min) is characterised by neurogenic pain caused by C-fiber activation due to the inflammatory response elicited by formalin which is triggered by a combination of stimuli, including inflammation of the peripheral tissues and mechanisms of central sensitisation [6]. Again it is assumed that manifestation in the late phase is due to inflammation causing a release of serotonin, histamine, bradykinin and prostaglandins, which at least to some degree can cause the sensitisation of the central nociceptive neurons [24]. It is reported that substance P is involved in the first phase whereas histamine, serotonin, prostaglandins and bradykinin are responsible for the second phase of inflammation [6]. In our study, the extract demonstrated anti-nociceptive activity in blocking both phases of the formalin response although the effect of the extract was more pronounced in the late phase of test. As oral pretreatment with *A. scarabaeoides* inhibited the first (neurogenic pain) and second (inflammatory nociception) stages of formalin-induced licking in mice, therefore, it may be postulated that the plant extract may possess both peripheral and central effect.

Thermal nociception models such as the tail flicking and the hot plate tests were used to evaluate central analgesic activity. These tests use a thermal stimulus that produces a predominantly spinal reflex which can be measured concerning the reaction times of mice.
These tests are employed to study the analgesic activity mediated by central mechanisms, like morphine, while peripheral compounds are inactive on this kind of painful stimulus [25]. Our study showed that *A. scarabaeoides* displayed significant antinociception response on both the tail flicking and hot plate test which indicates the potency of the extract to inhibit thermal stimulation associated with central neurotransmission. Therefore, the findings from chemically and thermally-induced nociceptive processes tested in this research work shown by ethanolic extract of *A. scarabaeoides* leaves suggest that the extract may contain bioactive compound(s) which are responsible for the central and peripheral anti-nociceptive actions.

We also researched *A. scarabaeoides* to assess its anti-inflammatory activity using both *in vivo* and *in vitro* test models. Carrageenan-induced paw oedema is a well-documented *in vivo* animal model which is used to evaluate the anti-inflammatory effect of natural products as well as synthetic chemical compounds [20]. This test is primarily used analysis for screening both steroidal and non-steroidal anti-inflammatory drugs since it involves several mediators [6]. The response of Carrageenan-induced oedema is said to be biphasic of which the initial phase occurs between 0 and 2.5 h after injection of the phlogistic agent. At this phase, the oedema volume reaches its maximum at least two h of post-treatment and then beginning to decline [6]. In the second phase (2–5) h the oedema formation is continued up to 5h [26]. The initial period has been induced due to the action of different inflammatory mediators such as histamine, serotonin and bradykinin on vascular permeability while the late phase or second phase edema has been shown to be the result of overproduction of prostaglandins in tissues, oxygen-derived free radicals and kinin-like substances [20]. In the oral study pretreatment with *A. scarabaeoides* produced a dose-dependent inhibition of paw oedema, showing the highest potency at the dose of 400 mg/kg to the maximum inflammatory response, causing an inhibition closer to that of the standard drug ketorolac. Thus the results suggest that the ethanolic extract may inhibit the release of prostaglandins proinflammatory mediators of acute inflammation and may act as a non-steroidal anti-inflammatory drug.

Stabilization of membrane by hypotonic solution induced hemolysis, one of the *in vitro* methods, is used for the evaluation of anti-inflammatory activity of different components. During inflammation, lysosomal enzymes and hydrolytic components are released from the phagocytes to the extracellular space, which causes damages of the surrounding organelles and tissues and also assists a variety of disorders. Again, exposure of red blood cells (RBCs) to poisonous substances such as hypotonic medium, heat etc. results in the lysis of the membranes, accompanied by oxidation and lysis of haemoglobin. It was found that the NSAIDs act either by inhibiting these lysosomal enzymes or by stabilising the lysosomal membranes [19]. In our study, we have also found that the extract of the plant showed potent RBC membrane stabilisation activity with good percentage protection against hypotonic solution induced lysis. Therefore it may be postulated that the plant extract may possess certain phytoconstituents that may be responsible for its anti-inflammatory activity.

Antipyretic activity is commonly mentioned as a characteristic of drugs/compounds which have an inhibitory effect on prostaglandin-biosynthesis [27]. Here Brewer’s yeast induced pyrexia method was used to assess the antipyretic activity of the plant extract as it is considered as a useful test for the screening of plants materials as well as synthetic drugs for their antipyretic effect [20,21]. Yeast is responsible for induction of hyperthermia which is caused by the activation of endogenous pyrogen and subsequent secretion of pro-inflammatory mediators that eventually act on the hypothalamus and stimulate PGE2 synthesis in the preoptic area of anterior hypothalamus thermoregulatory centres. Some NSAIDs, including paracetamol, function by inhibiting the synthesis of PGs within hypothalamus [28]. In the present investigation, we found that ethanolic leaf extract of *A. scarabaeoides* at different doses exhibit significant antipyretic activity after 1 to 2 h of treatment. The results seem to support the view that the plant has some influence on prostaglandin synthesis, i.e. it may possess antipyretic activity.

5. CONCLUSION

Based on the results of our present study, it can be concluded that the ethanolic extract of *A. scarabaeoides* has been proved to be a safe remedy for the treatment of analgesia, inflammation and pyrexia. Hence, our research contributes towards scientific rationale for the folk use of the plant as antipyretic, analgesic
and anti-inflammatory. Although the exact mechanism of action of the plant extract can't be ascertained by these tests nevertheless, the isolation of pure secondary metabolites and identification of lead compounds from the plant will help us further understand the mechanism of these activities.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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