ELAEOCARPUS SERRATUS L. EXHIBITS POTENTIAL ANALGESIC AND ANTIDIARRHEAL ACTIVITIES IN MICE MODEL

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Background. Elaeocarpus serratus L. (Family: Elaeocarpaceae) is a tropical fruit tree, traditionally used in the treatments of poisoning, diarrhea, arthritis, and other diseases.

Objectives. The current study was performed to conduct the analgesic, antidiarrheal, and hypoglycemic activity of E. serratus in mice model using methanolic bark crude extract.

Methods. To assess the peripheral and central analgesic activity, the acetic acid-induced writhing and tail immersion methods were respectively used. The castor-oil mediated antidiarrheal method was used to assess the antidiarrheal activity whereas, the tail tipping technique was conducted to determine the hypoglycemic activity of the plant extract.

Results. In the peripheral analgesic assay, the methanolic bark crude extract of E. serratus significantly inhibits the number of writing 69.77% (200 mg/kg) and 73.26% (400 mg/kg) respectively (p<0.05) which was strongly comparable with standard NSAID drug diclofenac sodium 75.58% (p<0.05). Similarly, it shown a significant tail flicking response for 30 minutes, 60 minutes and 90 minutes of central analgesic activity assay. In antidiarrheal activity assay, the E. serratus substantially reduced the number of diarrheal feces 64.26% (200 mg/kg, p<0.05) and 78.57% (400 mg/kg, p<0.05) which was also comparable with the positive control loperamide. The hypoglycemic activity of E. serratus extract was not convincing.

Conclusions. Our investigation demonstrated the significant analgesic and antidiarrheal activities of methanolic bark extract of E. serratus (200 and 400 mg/kg) in mice model.

KEYWORDS: Elaeocarpus serratus; analgesic activity; antidiarrheal activity; hypoglycemic activity.
addition, the leaf of *E. Serratus* contains alkaloids, flavonoids, and glycosides (eg. anthraquinone) [7]. Moreover, a list of bioactive compounds also contained in *E. serratus* such as myricitrin, mearnsit 3-O-β-D-glucoside, mearnsit, and tamarixetin 3-O-α-L-rhamnopyranoside where, myricitrin is an established potential antioxidant [8]. Historically, leaves of *E. serratus* extracts are used for the treatments of arthritis and various poisoning [9]. Equally, appetite, diarrhea, dysentery and other neuro-motors related diseases are commonly treated with fruits or fruit extracts [6,10]. Moreover, the previous studies also reported that the leaf, bark and fruit of *E. serratus* have antimicrobial and antifungal activities [11,12]. For all we know, there is no scientific report conducted on analgesic, hypoglycemic, and anti-diarrheal properties of *E. serratus* yet. Therefore, our main objective was to assess the analgesic, hypoglycemic, and antidiarrheal activity of methanolic bark crude extract of *E. serratus* in mice model.

**Methods**

**Collection and extraction of plant**

In February 2018, the bark of *E. Serratus* was acquired from Chandpur, Bangladesh. The collection of bark samples was verified by Bangladesh National Herbarium (BDNH), Dhaka, Bangladesh. An herbarium specimen number (DACB-31155) was provided and preserved for their further reference. The barks were cleaned and cut into small pieces to accelerate the drying process. Then the sundried fragments were crushed to a fine powder. About 400 g of powder was put in a flat bottom amber sterile glass container and socked with 1.5L methanol for two weeks. Continuous shaking and stirring were maintained over time. Afterwards, the entire mixture was filtered with Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate was then kept for a week to monitor any suffering or distress and fasted overnight prior to the experiments. The animal experiments were conducted according to the Ethics Committee of State University of Bangladesh (SUB), Dhaka, Bangladesh.

**Drug treatments and chemical reagents**

Diclofenac sodium, glibenclamide, and loperamide hydrochloride were purchased from Beximco Pharmaceuticals Ltd (Bangladesh). Phenobarbitone sodium and morphine sulphate were supplied by Incepta Pharmaceuticals Ltd (Bangladesh), and Beacon Pharmaceuticals Ltd (Bangladesh). Tween 80, normal saline (0.9% NaCl) and castor oil were kindly given by BDH Chemicals Ltd (United Kingdom). The remaining chemicals and reagents were purchased from Sigma-Aldrich (Munich, Germany).

**Peripheral analgesic activity**

The acetic acid-mediated writhing method by Kaushik, D., et al. 2012 was copied to assess the peripheral analgesic activity of the *E. serratus* crude extract [13]. The intraperitoneal acetic acid injection was given to all mice with a view to exhort the abdominal pain followed by writhing in mice. The potentialities of the test samples were measured by calculating their competency in the reduction of writhing numbers. Test group (ES-I and ES-II) were orally administered, containing the doses of 200- and 400 mg/kg of body weight (b.w.), respectively. Whereas, the NC group orally received 1% tween 80 in saline and the PC group orally received diclofenac sodium at 50 mg/kg dose [14]. To induce writhing in mice, 1% v/v acetic acid was given intraperitoneally to all mice (10 ml/kg b.w.) followed by a resting period of 40 minutes after test samples administration. The writhing cases were carefully observed and documented for 10 minutes after giving 10 minutes resting period. The acetic acid-induced pain reduction was calculated by using the following equation:
Central analgesic activity

Pizziketti et al., 1985 described the tail-flick method was implemented to assess the central analgesic activity of *E. serratus* crude extract in mice [15]. In this method, the mice were orally given a different dose of drugs and *E. serratus*, and the tips of the mice tails were submerged in a constant radiant heat source (hot water bath at 55±0.5°C). The reaction time (mice tail deflects from the heating source) of each mouse was recorded using a stopwatch. To prevent the damage of tail, we maintained a cut off period of 15 seconds. Similar to the peripheral analgesic study, the NC group orally received 1% tween-80 in saline, and the PC group was subcutaneously injected with morphine (2 mg/kg b.w.) [16]. The ES-I and ES-II were prescribed orally to the test groups of mice. The tail-flick reaction was counted and recorded in 0 minutes, 30 minutes, 60 minutes, and 90 minutes after administration of the test samples. The following equation was used to measure the pain inhibition percentage (PIP).

\[
\text{PIP} = \left( \frac{\text{Mean latency of treatment} - \text{Mean latency of control}}{\text{Mean latency of control}} \right) \times 100\%
\]

Hypoglycemic activity

The tail tipping technique according to the method described by Durschlag et al., 1996 was repeated to assess the hypoglycemic activity of test samples in mice model [17]. Here, the NC group was treated with 1% tween-80 (0.1 ml/10 mg b.w.), and PC group treated with glibenclamide (5 mg/kg b.w.) whereas, Group-III and Group-IV were treated with ES-I and ES-II respectively. All treatments were applied orally [14]. To accelerate the blood sugar level of mice, a 10% glucose solution was orally given to all mice after an hour resting period at dose 2 g/kg b.w. Blood is withdrawn from the tail tip and blood sugar was measured and recorded by using a glucometer (Biland G-423 S) in 0 minutes, 30 minutes, 60 minutes, 120 minutes, and 180 minutes respectively.

Antidiarrheal activity

The antidiarrheal activity of *E. serratus* crude extract was determined by the method described by Shaoba and Thomas [18], where forceful diarrhea is induced by orally administrating 1.0 ml of castor oil to all mice. Similar to other studies, various oral treatments were applied to the mice such as NC group received 1% tween-80 in saline, PC group received loperamide hydrochloride (50 mg/kg b.w.) and the remaining two groups were given ES-I and ES-II respectively [19]. All groups of mice were housed in individual cages with a blotting paper placed beforehand. The number of diarrheal feces were recorded for each mice over four hours of the experiment. The percentage of diarrheal prohibition was accounted for using the following formula:

\[
\text{Percentage inhibition} = \left( \frac{\text{Mean defecation of control} - \text{Mean defecation of test sample or standard}}{\text{Mean defecation of control}} \right) \times 100\%
\]

Statistical analysis

The values are represented here are set of mean ± standard error of mean (M±SEM). All the calculation was performed using student t-test or one way ANOVA followed by Dunnett’s test to determine the statistically significant differences between the groups. A p-value < 0.05 was considered statistically significant.

Results

The peripheral analgesic activity of *E. serratus* crude extract is demonstrated in Table 1. A significant reductions of abdominal muscle contractions caused by the administration of 0.1 ml acetic acid were exhibited in both experimental groups where ES-II showed higher writhing inhibition and was close to the PC group. Our results indicated that the *E. serratus* bark crude extracts significantly inhibit the number of writhing 69.77% and 73.26% at dose 200 and 400 mg/kg b.w. gradually whilst diclofenac sodium displayed 75.58% writhing inhibition.

Values are represented here as mean of ±SEM. NC=1% tween 80 in water, PC= diclofenac sodium, ES-I: *E. serratus* crude extract-I, ES-II: *E. serratus* crude extract-II. M1-4=mice 1 to 4 respectively. (n=4, *p<0.01)

The result of the tail-flick method to assess the central analgesic activity of *E. serratus* are...
shown in table 2. Both experimental groups ES-I and ES-II increased the response time by 37.32% and 53.72% respectively in the initial 30 minutes of the experiment, whereas PC morphine increased by 85.24%. In addition, 82.76% (200 mg/kg b.w.), 98.94% (400 mg/kg b.w.) tail flicking response were recorded in 60 minute, and 149.27% (200 mg/kg b.w.) and 179.46% (400 mg/kg b.w.) were recorded in 90 minutes of the experiments. The whole experiment followed a dose-dependent tail flicking response over the time.

Values are represented here as mean of ±SEM. NC=1% tween 80 in water, PC= morphine sulfate, ES-I: *E. serratus* crude extract-I, and ES-II: *E. serratus* crude extract-II. (n=4, *p<0.01)

The bark crude extract of *E. serratus* not displayed any significant blood glucose-lowering activity at doses 200 and 400-mg/kg b.w. However, the percent of blood sugar reducing activity was found to be followed in a dose-dependent manner. The results shown in table 3 indicated that the highest glucose lowering activity was displayed at dose 400 mg/kg b.w. relative to ES-I groups.

Values are represented here as mean of ±SEM. NC=1% tween 80 in water, PC=glibenclamide, ES-I: *E. serratus* crude extract-I, and ES-II: *E. serratus* crude extract-II. (n=4, *p<0.01)

The remarkable antidiarrheal activities were displayed by ES-I and ES-II in mice. The potential antidiarrheal activity of the *E. serratus* crude extract is shown in table 4. The ES-I and ES-II substantially reduced the number of castor oil-incited diarrheal feces by 64.29% and 78.57% compared to the NC. The highest diarrheal reduction was shown by PC group.

Values are represented here as mean of ±SEM. NC=1% tween 80 in water, PC=loperamide hydrochloride, ES-I: *E. serratus* crude extract-I,

<table>
<thead>
<tr>
<th>Mice group</th>
<th>Writhing count (sec)</th>
<th>Number of writhing (Mean±SEM)</th>
<th>% Inhibition of writhing</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>M-1</td>
<td>M-2</td>
<td>M-3</td>
</tr>
<tr>
<td>NC</td>
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</tr>
<tr>
<td>PC</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>ES-I</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>ES-II</td>
<td>6</td>
<td>6</td>
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<th>60 minutes of assay</th>
<th>90 minutes of assay</th>
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<tr>
<td></td>
<td>% of elongation</td>
<td>% of elongation</td>
<td>% of elongation</td>
</tr>
<tr>
<td>NC</td>
<td>3.49±0.32*</td>
<td>3.55±0.08*</td>
<td>3.60±0.20*</td>
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<td>PC</td>
<td>6.47±0.11*</td>
<td>9.57±0.25*</td>
<td>169.39</td>
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<tr>
<td>ES-I</td>
<td>4.79±0.34*</td>
<td>6.49±0.35*</td>
<td>82.76</td>
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<tr>
<td>ES-II</td>
<td>5.37±0.32*</td>
<td>7.07±0.39*</td>
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<th>180 minutes of assay</th>
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<tr>
<td></td>
<td>% Reduction</td>
<td>% Reduction</td>
<td>% Reduction</td>
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<tr>
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<td>8.55±0.31</td>
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<tr>
<td>ES-I</td>
<td>10.18±0.61</td>
<td>19.25</td>
<td>7.05±0.39</td>
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<tr>
<td>ES-II</td>
<td>10.08±0.93</td>
<td>20.04</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mice group</th>
<th>Dose</th>
<th>Number of diarrheal feces (Mean±SEM)</th>
<th>% Reduction of diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>10 ml/kg b.w.</td>
<td>3.5±0.58*</td>
<td>–</td>
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<tr>
<td>PC</td>
<td>50 mg/kg b.w.</td>
<td>0.5±0.48*</td>
<td>85.71</td>
</tr>
<tr>
<td>ES-I</td>
<td>200 mg/kg b.w.</td>
<td>1.25±0.85*</td>
<td>64.29</td>
</tr>
<tr>
<td>ES-II</td>
<td>400 mg/kg b.w.</td>
<td>0.75±0.71*</td>
<td>78.57</td>
</tr>
</tbody>
</table>
and ES-II: *E. serratus* crude extract-II. (*n*=4, *p*<0.01)

**Discussion**

Acetic acid may trigger the writhing reflex in experimental animals where visceral pain is generated through activation of pain receptors on the visceral surface and extreme secretion of histamine, prostaglandins, bradykinin and serotonin [20]. In the experimental animals, acetic acid induces visceral pain which is commonly treated with NSAID drugs or chemicals, such as phenyl quine (prostaglandin E2 inhibitor). In addition, the level of analgesia is measured by calculating the percent reduction of abdominal contraction by drugs or crude extract after intraperitoneal administration of acetic acid to mice. In this study, *E. serratus* extracts significantly reduced the sum of abdominal contraction of 69.77% and 73.26% by ES-I and ES-II compared to NC. Importantly, the results of peripheral analgesic activity by ES-I and ES-II were almost equal to the diclofenac treatment. Therefore, by considering our results, we assumed that *E. serratus* extract may be inhibited the synthesis or release of endogenous substances in mice to act its potential peripheral analgesic activity. However, further research may need to explore the exact mechanisms.

In the central analgesic assay, the relative promotion of tail-flicking response (in percent) was obtained from *E. serratus* extract in a dose and time-dependent manner. Although, the responses from *E. serratus* crude extracts were a bit of lower than the PC-morphine however, higher dose might be shown an equals or higher potentiality like morphine. Pizziketti, et al., 1985 demonstrated that the tail flicking response is mostly generated from spinal reflex caused by radiant heat source however it may involve higher neuronal complex signals. In general, the pain is centrally originated via a number of complex signaling such as opiate, dopaminergic, noradrenergic and serotonergic nervous systems [15]. Our results described that *E. serratus* displayed a significantly higher level of pain threshold activity at 200 and 400 mg/kg b.w. respectively in mice model. The core mechanisms may be associated with the receptor-bind inhibition of pain-related nervous system or through peripheral mechanisms involved prohibited prostaglandins, leukotrienes, and other endogenous substances release and synthesis which are key mediators of pain [21]. Our results might be followed the same mechanisms to exhibit the potential analgesic activity in mice model.

Our bark crude extract of *E. serratus* showed lack of blood glucose lowering activity. Notwithstanding, a considerable number of studies have concluded that plant extracts exhibit potential anti-hyperglycemic activity by accelerating or regenerating β cells or promoting the secretion of insulin [22, 23]. The hypoglycemic activity by the natural product may also associated with excessive insulin secretion from β cells or trigger the peripheral glucose consumption, or promote insulin-mediated blood sugar absorbing mechanisms [22-24].

Apart from this, the statistical evaluation revealed that both doses of *E. serratus* showed a significant dose-dependent anti-diarrheal activity in mice. The ricinoleic fatty acid or 12-hydroxy-9-cis-octadecenoic acid is an active metabolite of castor oil. This metabolic fatty acid enhanced peristaltic activity in the small intestine to trigger the permeability of mucosal electrolytes thus resulting diarrhea [25, 26]. Furthermore, ricinoleic fatty acid enhanced mucosal irritation and inflammation which contribute to the excessive endogenous prostaglandin secretion. Moreover, in castor oil-induced diarrheal mechanisms it involved a cascade of signaling including, intestinal Na+/K+-ATPase inhibition, adenylate cyclase activation or promotion cAMP-mediated platelet-activating factor secretion [25, 27].

In summary, the plant *E. serratus* contained several flavonoids, anthraquinone glycosides, fatty acid, alcohol, aldehyde, hydrocarbons al-kaloids, terpenoids, and steroids. [7, 8, 28]. The presence of glycosides, steroids, and flavonoids which exhibited potential analgesic, hypoglycemic and anti-diarrheal activities in many plants [29-31]. In the present study, we concluded that *E. serratus* extract may contain a variety of bioactive phytochemicals. After successful isolation and characterization of phytochemicals, it might be used as an analgesic, and as an antidiarrheal agent.

**Conclusion**

The bark extract of *E. serratus* exhibited potential peripheral and central analgesic activity, very mild hypoglycemic activity but effective antidiarrhoeal activity in mice model. Therefore, further investigations are needed to isolate and characterization of bioactive molecules present in this plant. Further research may open a new therapeutic agents in the treatments of various diseases.
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Conflict of Interests
Authors declare no conflict of interest.

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Authors’ Contributions
Asma Aul Husna Pinkey, Mohammad Abdullah Taher – conceptualization, methodology; Asma Aul Husna Pinkey, investigation, data curation, formal analysis, writing – original draft; Zahirul Islam Khan – data curation, formal analysis, writing – reviewing and editing; Mahfuza Afroz Soma – writing – reviewing and editing.

ПРЕКЛІНІЧНІ ДОСЛІДЖЕННЯ ЗНЕБОЛЮЮЧОЇ ТА ПРОТИПРОНОСНОЇ ДІЇ ELAEOCARPUS SERRATUS L. НА МИШАХ

А.А.Н. Пінкі, *З.І. Кхан, М.А. Тахер, М.А. Сома

1 - ДЕПАРТАМЕНТ ФАРМАКІЇ, СТАТІСТУ ЕДАЛДЖ САНДІС ІНДІЗ, ДАКА, БАНГЛАДЕШ
2 - ДЕПАРТАМЕНТ ХЕЛТІНГ ТЕХНОЛОДЖІ ІНФОРМАТИКС, НЮ КОНГО ПОЛТЕКІНИК УНІВЕРСІТИТ, НЮ КОНГ О, КІНГДМ

Вступ. Elaeocarpus serratus L. (родина Elaeocarpaceae) - тропічне плодове дерево, фрукти, кора та інші частини якого традиційно використовуються при лікуванні отруєнь, діареї, артриту та інших захворювань.

Мета – дослідити фармакологічну активність (знеболювальну, протидіарейну та гіпоглікемічну дію) сухого метанольного екстракту кори E. serratus на мищах.

Методи. Для експериментальної оцінки центрального та периферічного компонентів у механізмі зневолювальної дії екстракту використовували метод оцінки больової реакції, що викликається хімічним подразненням – метод «цитовокислих карчів», та метод теплового подразнення, суть якого полягає в зануренні хвоста миші у гарячу воду (55±0.5°C). Для оцінки протипроносної активності використовували модель діареї, викликаної введенням рицинової олії, для визначення гіпоглікемічної активності екстракту використали метод Durschlag et al., 1996, забір крові проводили шляхом надрізів хвоста.

Результати. Встановлено, що застосування сухого метанольного екстракту кори E. serratus достовірно зменшує частоту розвитку карчів на 69,77% (200 мг/кг) та 73,26% (400 мг/кг) відповідно (p<0,05), що досягає рівня активності стандартного НПЗП диклофенаку натрію, який зменшує показник на 75,58% (p<0,05). Такі ж результати щодо частоти реакції хвостів піддослідних тварин протягом 30, 60 та 90 хвилин – показники центральної зневолюючої активності екстракту. Щодо протипроносної активності, то E. Serratus зменшував частоту діареї на 64,26% (200 мг/кг, p<0,05) та 78,57% (400 мг/кг, p<0,05), що також досягало також ж ефективності, як і у групі позитивного контролю з лоперамідом. Щодо гіпоглікемічної активності екстракту E. serratus – отримані нами результати були непереконливими.

Висновок. Наше дослідження продемонстровало значну зневолювальну та протидіарейну активність сухого метанольного екстракту кори E. serratus (200 та 400 мг/кг) на мищах.

КЛЮЧОВІ СЛОВА: Elaeocarpus serratus; зневолювальна активність; протипроносна активність; гіпоглікемічна активність

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