Acmella calva (DC.) R.K. Jansen Flower: A Comprehensive In vivo Study of its Central and Peripheral Analgesic, Hypoglycemic, Antidepressant, and Anti-Diarrheal Properties

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(Received: February 5, 2023 Accepted: May 3, 2023; Published (web): June 21, 2023)

ABSTRACT. Acmella calva (DC.) R.K. Jansen (Local name “Surzo konna”) is a Southeast Asian perennial herb mostly used in rural areas as an ailment of dental disorders. This study involved the evaluation of peripheral and central analgesic activities, hypoglycemic, anti-diarrheal and anti-depressant activities of the methanolic extract of A. calva flower at different doses (200 mg/kg, 400 mg/kg and 600 mg/kg of body weight) on Swiss Albino mice models. The sample exhibited moderate central analgesic activity at all doses whereas the acetic acid-induced writhing test in mice revealed 67.69% reduction in peripheral pain response for both 400 and 600 mg/kg doses (69.23% for standard diclofenac sodium). Reduction in diarrheal faeces was found to be 41.03% and 46.46% at dose of 200 mg/kg and 400 mg/kg respectively compared to the standard loperamide 53.85%. Hypoglycemic activity in diabetic mice showed statistically significant data depending on increasing doses and thiopental induced sleeping test revealed the sedative activity (P<0.05) compared to diazepam. The in-vivo bioactivities suggest that this plant part can be a potential source of active lead compounds.

KEY words: Acmella calva, in-vivo activity, analgesic, antidepressant, anti-diarrheal, hypoglycemic.

INTRODUCTION

Plants have been exploited to cure certain diseases due to the potential active secondary metabolites present in them. Preliminary screening of lead compounds from natural sources involves a number of in vitro and in vivo assays. Whilst the in vitro assays detect the efficacy of the sample in test cells, in vivo assays carried out on animal models define the sensitivity and safety limit to be used in further human trials. Inflammatory and pain associated disorders, diabetes, neuro-degenerative diseases are some of the leading topics of interest in health and medicine of the current world. Though synthetic drugs are available to combat but the adverse effects related with these pharmaceuticals challenge the researchers to find out alternative sources of treatment. Ethnopharmacological use of plants, animals and microbes by people in several regions of the world and the large variations of these ingredients in nature have established the search of future drugs from these sources. Bangladesh, being a country rich in flora with a large number of native species with therapeutic benefits, has promising aspects for future drug development.

Acmella calva (DC.) R.K. Jansen is indigenous to tropical and subtropical parts of the world; found in damp and swamp conditions and as a roadside weed. There are about 28 species of Acmella genus found in India and 9 of that in Bangladesh. Besides being used as an ornamental plant or as an insecticide and as a food item, the extracts of the plant part have been actively used in the treatment of pain associated with tooth and gums, as anti-inflammatory agent, as local anaesthetics. A. calva flowers and leaves have pungent taste and is accompanied by tingling
sensation and numbness when in contact with skin. Chemical analysis of *A. calva* reported its major pungent compounds, spilanthol and butylated hydroxytoluene. It is also found to contain a number of active N-alkylamides and several essential oils such as limonene, β-caryophyllene, Z-β-ocimene, γ-cadinene, thymol, germacrene D and myrcene. Different species of *Acmella* genus have shown antioxidant, anti-inflammatory, antibacterial, antifungal, insecticidal and local anaesthetic properties.

Despite being commonly used in folk medicine and revealing the potential to be an active source to important pharmaceuticals, there were no reported studies on the bioactivities of this plant part in in-vivo assays. So, the present work involves the evaluation of central and peripheral analgesic, hypoglycemic, anti-diarrheal and anti-depressant activities of *A. calva* flower on Swiss-Albino mice models.

**Materials and Methods**

**Collection and preparation of the flower sample.** The fresh flower sample of *A. calva* was collected from Nandail, Mymensingh, Bangladesh in March, 2022 and was taxonomically identified by the Dhaka University Salar Khan Herbarium (Voucher specimen number: DUSH-10813). Freshly plucked flowers were cleaned, dried at room temperature for several days and then in an oven (40 °C). The dried sample was then grounded to powder and kept in airtight container. The powdered sample was used for further extractions.

**Extraction.** About 500 g of the sample powder was extracted in methanol by cold extraction method which involved keeping the methanol-soaked sample in a flat-bottomed container with frequent stirring. The methanolic solution of the sample was then cotton filtered followed by additional filtering with Whatman filter paper. The crude extract was found by evaporating the solution in a rotary vacuum evaporator below 40 °C. Crude methanol extract at three different doses- 200 mg/kg, 400 mg/kg and 600 mg/kg body weight doses were used for in-vivo bioactivity evaluation.

**Reagents and drugs.** All the chemicals and reagents used in the experimental procedures were of analytical grade. Standard drugs for bioactivity assays such as glibenclamide and loperamide were collected from Square Pharmaceuticals Ltd.; morphine, diclofenac-sodium and thiopental-sodium were procured from Gonoshasthya Pharmaceuticals, Essential Drugs Company and Incepta Pharmaceuticals Ltd. respectively. Glucose sample and castor oil were obtained from local market.

**Experimental animal models.** Swiss Albino mice (28-30 g) of either sex (4-5 weeks old) was collected from the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR,B), Dhaka. They were kept in polypropylene cages and fed ICDDR,B prepared rodent chow and water in a 12-hour light dark cycle at a regulated ambient temperature (24 ± 2 °C; relative humidity 60-70 percent) (ad-libitum). Ethical guidelines regarding the use of animal models for bioactivity tests were strictly maintained and duly followed.

**Central analgesic activity.** The central analgesic activity of *A. calva* flower was evaluated by tail flicking induced by heat method. Methanolic crude extract at three different doses- 200 mg/kg, 400 mg/kg and 600 mg/kg of body weight indicated by ME-200, ME-400 and ME-600, respectively were used as test samples for the assay. A total of twenty Swiss-Albino mice were taken as test animals; divided into five groups: four mice in each group. The control group (CTL) and test groups (ME-200, ME-400, ME-600) were fed normal saline and test sample, respectively. Morphine (2 mg/kg of body weight) was used as standard and was administered subcutaneously to the standard group (STD). The tail of each test animal was immersed in hot water of 55 °C and the time taken for the withdrawal of the tail from the water was recorded at an interval of 30, 60 and 90 minutes after the administration of standard and sample doses. Following formula was used to calculate the elongation in tail flicking responses with time:

\[
\text{% elongation} = \left( \frac{T_c - T_s}{T_c} \right) \times 100
\]

Where, \( T_c \) = Average tail flicking time of the sample group and \( T_s \) = Average tail flicking time of control group.
Peripheral analgesic activity. Peripheral analgesic activity or the ability of the test sample in creating analgesia in pain stimulation through peripheral nervous system was carried out by writhing test induced by acetic acid. Mice models divided into five different groups with four in each were used for the evaluation. Test samples were given orally in three different doses- 200 mg/kg, 400 mg/kg and 600 mg/kg of body weight. 1% Tween-80 in normal saline was used as control and fed orally at a dose of 0.1 ml/ 10 g of body weight. 50 mg/kg dose of diclofenac-Na was injected intra-peritoneally as standard. After 30 minutes, acetic acid (1%) was administered in intra-peritoneal route to each of the test animals. The number of abdominal constrictions for each mouse was counted from five minutes after injection for a period of ten minutes. Inhibition in writhing (in percentages) was calculated using the formula as:

\[
\% \text{ Inhibition of writhing} = \left( \frac{W_c - W_s}{W_c} \right) \times 100
\]

Where, \( W_c \) = Average writhing of control, \( W_s \) = Average writhing of sample.

Hypoglycemic activity. The effect of the test sample in lowering the blood glucose level in diabetic mice was tested by moderated version of oral glucose tolerance test in mice. The experimental models were divided into five groups and the blood glucose level of each mouse in each group was recorded. All groups were then treated with 10% glucose solution at a dose of 2 g/kg of body weight. After 30 minutes, the increase in blood glucose level were measured; control (1% Tween-80 solution in saline), glibenclamide (as standard at a dose of 10 mg/kg of body weight) and test samples (200, 400 and 600 mg/kg of body weight doses) were administered orally to separate groups. After 60, 90 and 180 minutes of glucose loading, the glucose level in each mouse was measured by glucometer by taking blood from tail vein. Reduction percentage in glucose level due to crude methanol extract of the sample was calculated.

Anti-diarrheal activity. Anti-diarrheal activity of the crude extract was determined by previously reported castor-oil induced diarrhoea method with moderate adaptations. In brief, loperamide hydrochloride (50 mg/kg) was used as positive control during the assay. Methanolic crude extract of the sample at three different doses- 200 mg/kg, 400 mg/kg and 600 mg/kg of body weight were administered as suspension in saline water to three different groups of mice. Normal saline solution was used as control and was fed to another group of test mice. Each mouse was taken in separate boxes with blotting paper. After 30 minutes, 1 ml of highly pure analytical grade castor oil was given to each mouse to induce diarrhoea. The numbers of fecal stools were recorded for each mouse over a period of 4 hours. Reduction percentage in diarrhoeal feces compared to the control was calculated by the following equation:

\[
% \text{ Inhibition of diarrhoea} = \left( \frac{T_c - T_i}{T_c} \right) \times 100
\]

Where, \( T_c \) = Total number of diarrheal feces in the control group and \( T_i \) = Total number of diarrheal feces in the sample treated groups.

Anti-depressant activity. Thiopental sodium induced sleeping time test was carried out for the evaluation of antidepressant activity of A. calva flower extract. The test sample at three different doses were given orally to three groups of mice; normal saline was given to another group as control. Diazepam at a dose of 25 mg/kg was administered intra-peritoneally as positive control. Thiopental sodium was injected to each after 30 minutes to induce hypnosis or sleeping behaviour. The onset of sleep and total sleeping time were recorded for all groups.

Statistical analysis. All measured and calculated data were presented as mean ± SEM. Data were statistically analysed by Student’s t-test using GraphPad Software, USA. The results with \( P \) value less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

Central analgesic activity. The ability of the crude extract at different doses to create analgesia in central nervous system which stimulated nociceptive pain sensation was tested by tail withdrawal test in mice and the elongation percentage in tail flicking time after 30, 60 and 90 minutes of observations are shown in figure 1.
Figure 1. % Elongation in tail flicking responses in mice with time by the effect of A. calva flower crude methanol extract at different doses.

The analysis and statistical evaluation of the data indicates that the methanolic extract of A. calva showed moderate central analgesic activity at doses of 200 mg/kg, 400 mg/kg and 600 mg/kg of body weight after 60 and 90 minutes after administration with the highest activity for 600 mg/kg after 90 minutes as (305.32 ± 0.2056) % elongation of tail immersion time.

The methanolic extract of the sample increased the pain response time in mice models in a dose dependent manner comparable to that of the standard morphine. Morphine-like analgesics cure both acute and chronic pain by increasing the response time.\textsuperscript{12} The results from the present study indicates the potential of the flower to be a source of active analgesic compounds.

**Peripheral analgesic activity.** Three different doses (200 mg, 400 mg and 600 mg/kg of body weight) of methanolic crude extract were used to evaluate peripheral analgesic activity of A. calva flower and the results obtained are shown in table 1.

Peripherally induced inflammation or pain responses are usually generated by the synthesis of prostaglandin as a mediator to the stimuli. Responses in case of acetic acid injection are generally writhing of abdominal areas and licking. Agents that reduce the number of writhing responses will produce analgesia in test mice by inhibition of prostaglandin synthesis.\textsuperscript{13} In the present study, all three doses of methanolic extract of the sample showed significant analgesic activity as the standard diclofenac sodium. Observed peripheral analgesic activity of the flower may be attributed to the presence of several N-alkylamides or alkamides such as spilanthol in the plant extract.\textsuperscript{14}

**Hypoglycemic activity.** Blood glucose lowering ability of the sample extract at different doses was evaluated by glucose tolerance test. Anti-diabetic drug glibenclamide was taken as the standard in this assay. Plasma glucose level of the control, standard and sample treated mice were recorded after 60, 120 and 180 minutes of administration (Table 2). The crude extract at 600 mg/kg of body weight dose
reduced the plasma glucose level in mice from 11.15 ± 1.69 mmol/l to 7.18 ± 0.24 mmol/l after 30 minutes of glucose administration. For 400 mg/kg of body weight dose, the reduction occurred from 10.43 ± 1.20 mmol/l to 7.23 ± 0.56 mmol/l.

The percentage of reduction in the blood glucose level of mice in standard and sample groups compared to that of control groups are shown in figure 2.

Table 1. Peripheral analgesic activity of the methanolic extract of A. calva flower.

<table>
<thead>
<tr>
<th>Code no</th>
<th>Writhing count</th>
<th>Average of writhing</th>
<th>% Inhibition of writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-1</td>
<td>18</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>M-2</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>M-3</td>
<td>5</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>M-4</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± STD (n = 4). [M-1= mice no. 1, M-2= mice no. 2, M-3= mice no. 3, M-4= mice no. 4)

Figure 2. Hypoglycemic activity of the methanolic extract of the sample.

Table 2. Plasma glucose level in mice models treated with A. calva methanolic extract.

<table>
<thead>
<tr>
<th>Code no</th>
<th>0 min</th>
<th>30 mins</th>
<th>60 mins</th>
<th>120 mins</th>
<th>180 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>5.93 ± 0.38</td>
<td>12.83 ± 0.26</td>
<td>12.90 ± 2.74</td>
<td>7.88 ± 0.77</td>
<td>6.65 ± 0.59</td>
</tr>
<tr>
<td>STD</td>
<td>6.35 ± 0.36</td>
<td>10.70 ± 1.87</td>
<td>9.48 ± 1.37</td>
<td>7.23 ± 1.43</td>
<td>5.30 ± 0.48</td>
</tr>
<tr>
<td>ME-600</td>
<td>6.45 ± 0.28</td>
<td>11.15 ± 1.69</td>
<td>7.18 ± 0.24</td>
<td>7.28 ± 0.28</td>
<td>5.68 ± 0.59</td>
</tr>
<tr>
<td>ME-400</td>
<td>5.88 ± 0.11</td>
<td>10.43 ± 1.20</td>
<td>7.23 ± 0.56</td>
<td>7.23 ± 0.53</td>
<td>5.53 ± 0.43</td>
</tr>
<tr>
<td>ME-200</td>
<td>5.85 ± 0.69</td>
<td>8.15 ± 0.54</td>
<td>6.83 ± 0.37</td>
<td>6.65 ± 0.39</td>
<td>5.00 ± 0.13</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n = 4).

Several plant parts have been used for treating hyperglycemia in rural areas at a lower cost with less side-effects and as an alternative to available synthetic drugs. The present study reveals the prominent activity of the flower extract of A. calva in reducing blood glucose level which was not previously reported, thus showing the necessity of further extensive study into its phytochemicals.
Anti-diarrheal activity. The reduction in diarrheal feces in mice models by the sample crude extract of different doses compared to that of the standard loperamide is shown in figure 3.

The results indicate that the 200 mg/kg and 400 mg/kg of body weight doses of methanolic crude extract have very significant activity in reduction of diarrhoeal feces with highest activity at 400 mg/kg body weight compared to the standard loperamide. This is in line with the plant’s traditional use in GIT problems. Potential anti-diarrheal activity may be attributed to the presence of tannins, alkaloids, saponins, flavonoids, sterols and reducing sugars in the extracts.\textsuperscript{15}

![Figure 3. Anti-diabetic activity of the crude extract of the sample.](image)

Anti-depressant activity. In the thiopental induced hypnosis test, the sample extract at all doses showed significant lessening in the time of onset of sleep, increasing the duration of sleep in a dose dependent manner (Figure 4).

200 mg/kg, 400 mg/kg and 600 mg/kg body weight doses of the sample showed 56.53%, 65.51% and 53.10% increase in time of sleep respectively whereas that of the standard diazepam was found to be 87.10%. These results with statistical significance (P<0.05) reveal the potential sedative effect of the flower.

CONCLUSION

To date, the folkloric use of \textit{A. calva} flower as a medication to several anti-inflammatory disorders is remarkably valuable to the indigenous people of developing countries. The present study on the \textit{in-vivo} biological properties of this flower extract unveils its prospective central and peripheral
analgesic, anti-diabetic, anti-diarrheal and sedative activities. The results obtained are promising enough to carry out further extensive investigations for the active chemical compounds which are responsible for these therapeutic actions.

Conflict of interest

The authors declare that there’s no conflict of interest.

Funding

The research was partially funded by Bose Center for Advanced Study and Research in Natural Sciences, University of Dhaka, Dhaka, Bangladesh.

Data availability

The raw data used to support the findings of this study are available from the corresponding author upon request.

ACKNOWLEDGEMENTS

The authors are grateful to Bose Centre for Advanced Study and Research in Natural Sciences for providing financial aid to carry out the research work through Bose Fellowship program. Authors’ heartfelt gratitude goes to State University of Bangladesh for supporting the research work.

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