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ABSTRACT

Objective: To investigate the anxiolytic activity of newly isolated compound by our lab called ursolic acid stearoyl glucoside (UASG) from the leaves of Lantana camara (L. camara). Methods: Column chromatography was used to isolate USAG. Anxiolytic potential was experimentally proved and demonstrated through Elevated plus-maze, Open field and light and dark test. Results: The UASG showed marked increased in time spent (%) and number of frequent movements made by animals in open arm of elevated plus-maze apparatus. In light and dark model, UASG produced marked increase in time spent by animal, number of crossing and reduced duration of immobility in light box. Conclusions: UASG showed significant increase in number of rearing, assisted rearing and number of square crossed in open field established test model. UASG showed its anxiolytic effect in dose dependent manner.

1. Introduction

Anxiety disorders are the most common psychiatric disorders[1]. They accounts for heavy burden on public health. Present epidemiological data gives a different glimpse of this disorder, as traditionally thought, almost involving half of the population[2]. Every eighth human of total population is linked with anxiety, making this field an important area of research, for psychopharmacologists[3], hence it became need of an hour to develop new anxiolytics. Many secondary plant metabolites are reported in the treatment of psychological disorders. The anti anxiety activities plant metabolites are used in traditional medicine practice, either directly or indirectly affecting the central nervous system there by altering gamma–amino butyric acid, serotonin noradrenaline, benzodiazepine neurotransmitters activities. It is already proved that ursolic acid and its derivative is used in the treatment of central nerve system (CNS) disorders[4]. Ursolic acid obtained from Nepeta sibthorpii reported for CNS depressing, anticonvulsant and analgesic activity[5]. Awad et al in 2009 also reported in-vitro assays of ursolic acid inhibited GABA–T by 20% at a dose of 100 μ g/mL[6].

Lantana camara (L. camara) L. is well known traditional and tropical folk medicine[7-11]. Ursolic acid a stearoyl glucoside (USAG), pentacyclic triterpenoid was isolated from L. camara L. (family: verbanaceae)[12]. The traditional uses of L. camara L. mainly refer for the treatment of asthma, ulcers, measles, chickenpox, eczema, tumors, cancers, high blood pressure, bilious fevers, catarhal infections, tetanus, rheumatism, malaria, ataxy of abdominal viscera[13,14], epilepsy[15], memory weakness, enhance intellect and cognition[16]. The plant
is reported to possess anticonvulsant[17], anticancer[18,19], antiulcer[20], antioxidant[19], anti-diabetic[21,22], antifungal, antibacterial[23–25], anti-feedant, larval mortality/repellency[2], antimotility[29], analgesic and anti-inflammatory activities[30].

In Indian traditional herbal medicine, it is reported for its use in treatment of mental disorders and found to be effective as a brain tonic. Hence, our this work was an effort to use UASG isolated from L. camara L. for its anxiolytic effect against elevated plus-maze test (EPM), open field test and light and dark test induced anxiety in experimental animals.

2. Materials and methods

2.1. Animals

The experimental mice were obtained from the animal house of Siddhartha Institute of Pharmacy, Dehradun, India, (1435/PO/a/11/CPCSEA). The animals were housed as per the guide lines of CPCSEA. All the animals were provided with standard pellet diet (Lipton rat feed, Ltd., Pune) and water ad libitum throughout the experimental protocol. All the animal studies were approved by the Institutional Animal Ethical Committee of Siddhartha Institute of Pharmacy, Dehradun, India. The animals were randomly segregated into four groups, having six mice in each group. Group 1, solvent control (received 0.9% (w/v) of saline); Group 2, a positive control (diazepam 1 mg/kg); Group 3 and 4 were received UASG 25 and 50 mg/kg suspended in 1% Tween 80 (v/v). All the animals were administered with above drugs/solvent etc intraperitoneally, 30 min prior to start the experiment.

2.2. Plant material

L. camara L. leaves were collected from Dehradun and identified by Dr. S. B. Singh, Scientist, NISCAIR, New Delhi. A voucher specimen (NISCAIR/RHMD/consult/20–09–10/1322/125) was deposited in the herbarium of NISCAIR, India.

2.3. Extraction and isolation of UASG

Dried powder of Lantana camara leaves (4 kg) was extracted with methanol (12 L) at 50 °C for 1 d. Extract was concentrated to dryness under reduced pressure to obtain slurry (605 g).

The slurry was dissolved in minimum amount of methanol and was adsorbed on silica gel (60–120 mesh). The slurry was subjected to a silica gel column using CHCl3/MeOH gradient system (49:1; 2.0 L for gradient system). It leads to elution of colorless crystals of UASG (yield 11.2 g, 0.28%). It was found to be 100% pure by HPTLC by using solvent system CHCl3/MeOH (99:1). Structure of compound was identified by comparison of their spectroscopic data from the reported literature[12]. The structure of USAG is depicted in Figure 1.

2.4. Chemicals

Diazepam (Ranbaxy, New Delhi, India) was purchased through institution. All the chemicals were of analytical grade. Tween 80 (1% v/v) in saline was used to suspend UASG and was kept for its use.

2.5. Behavioral parameters used to test anxiolytic activity

2.5.1. Elevated plus-maze test (EPM)

The EPM an apparatus designed by institute with following dimensions four arms elevated 25 cm from the floor. Each arm is at 90° relative to the adjacent arms. Two arms enclosed with high walls (35 cmx5 cmx20 cm), and the other two linked via a central area (5 cmx5 cm) giving an appearance of plus sign. The maze floor and the walls of enclosed arms were black. An illumination with a 40 W lamp was made in the room. The animals were administered with vehicle, diazepam (1 mg/kg)[31] and USAG (25 and 50 mg/kg) intraperitoneally, 30 min prior to the test. Initially the mice were accustomed to lab condition half an hour prior to behavioral testing.

Each animal was individually placed on the central platform. The movement and duration of entries into the closed and open arms were observed for 5 min. Complete entry was considered and counted only when all four paws of the mouse entered an open or closed arm. The time spent (duration) in percentage for the open arms [100 × open/open
+ enclosed) and percentage of the number of open arm entries (frequency, 100 × open/total entries) were calculated for each mouse[32].

2.5.2. Open field test

The apparatus designed with modification and dimensions of a wooden box (60 cm×60 cm×60 cm), was used. The area of open field was divided into 16 equal squares (15 cm×15 cm). The 12 squares were at the periphery and 4 inner squares in the center. The experimental was performed in, dark room with sound proof facility. The open field area was illuminated with a 40 W lamp, focusing from the height of 75–100 cm. Treatment was started with vehicle, diazepam (1 mg/kg) and USAG (25 and 50 mg/kg), by placing individual animal in one of the corner squares and number of rearing, assisted rearing (forepaws touching the walls of the apparatus) and number of squares crossed were noticed for 5 min[33].

2.5.3. Light and dark test

The Light and dark test apparatus is top open wooden box, with two distinct chambers, a black (25 cm long ×35 cm wide ×35 cm deep), and white which is illuminated with 40 W light source as white, was placed approx. 25 cm above the open box. The two chambers were linked with a small opening, (7.5 cm long ×5.0 cm wide) located on the floor level at the center. The individual animal was placed in center of the light box and was observed for 5 min[34].

3. Result

As per our results animal treated with vehicle spent (53.29±1.52) s in open arm and it’s time for closed arm was recorded as (135.40±1.62) s with demonstrating the number of (4.86±0.28) entries in open arm and (33.85±0.74) in closed arm. The animal showed the increased stability in the open arm when treated with Diazepam (1 mg/kg) and USAG (25 and 50 mg/kg), significantly (P<0.05 and P<0.001, respectively), whereas significant (P<0.01 and P<0.001, respectively) reduction was observed in number of entries and time spent in closed arm. USAG (25 and 50 mg/kg) and diazepam significantly (P<0.001) increased the percentage of time spent and entries in open arm (Table 1).

84.85±1.58 squares were crossed by vehicle–treated animal and demonstrated (17.35±0.44) self rearing and 18.90±0.88 assisted rearing during the interval of 5 min, when the animals were treated with Diazepam and USAG (25 and 50 mg/kg), significant (P<0.05 and P<0.001, respectively) increase was noticed in the number of squares crossed by the animals. The self rearing and assisted rearing was altered significantly (P<0.05 and P<0.001, respectively) i.e. increased by USAG (25 and 50 mg/kg) and diazepam (Table 2). The animals treated with diazepam (1 mg/kg) and USAG (25 and 50 mg/kg) showed significant change (P<0.05, P<0.001 and P<0.001, respectively) in the number of crossing and duration of immobility, where as diazepam (1 mg/kg) and USAG (50 mg/kg) really showed significant (P<0.05 and P<0.01, respectively) increase in the number of crossing and decrease in the duration of immobility (Table 3).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of USAG on behavior of mice in elevated plus maze test.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (mg/kg, i.p.)</td>
<td>No. of entries</td>
</tr>
<tr>
<td></td>
<td>Open arm</td>
</tr>
<tr>
<td>Vehicle</td>
<td>4.86±0.28</td>
</tr>
<tr>
<td>Diazepam (1)</td>
<td>6.10±0.45</td>
</tr>
<tr>
<td>USAG (25)</td>
<td>8.36±0.52</td>
</tr>
<tr>
<td>USAG (50)</td>
<td>8.36±0.52</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM of 6 mice / treatment, Significant *P<0.05, **P<0.01 and ***P<0.001 compared with control.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effect of USAG on behavior of mice in open field test.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (mg/kg, i.p.)</td>
<td>No. of rearing</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>17.35±0.44</td>
</tr>
<tr>
<td>Diazepam (1)</td>
<td>31.67±1.46</td>
</tr>
<tr>
<td>USAG (25)</td>
<td>27.99±0.40</td>
</tr>
<tr>
<td>USAG (50)</td>
<td>28.92±1.64</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM of 6 mice / treatment, Significant *P<0.05, **P<0.01 and ***P<0.001 compared with control.
4. Discussion

Elevated plus maze, works on the fact that native mice has tendency to spend more time in closed arms as compared to open one. This may be due to fear generated in the open space. Present study on UASG, is a dose dependent, which induces significant increases both in the number of entries and time spent in the open arms, where as decreases the number of entries and time spent in the closed arm, proving its anxiolytic nature[35].

The open field model evaluates anxiety related activities characterized by the normal aversion of the animal to an open and bright area. Animals when subjected to change in their acclimatized location, expresses anxiety and fear[36]. UASG, in a dose dependent manner, significantly changes anxiety parameter that is the increase in the number of self rearing, number of assisted rearing and number of squares crossed, proving its anxiolytic effect by reducing such fearful behavior of animals in open field. The light and dark box method is a natural test for rodents which shows that they avoid bright places[37]. UASG may reduce the fear of animal which allows the animal to increase more the time in bright and open space. This effect may be due to the agonistic effect on GABA/benzodiazepine receptor complex. Ursolic acid and its derivatives crosses the blood brain barrier[38,39]. UASG is a derivative of ursolic acid. It is known to posses anxiolytic activity[6], and all the activities may be due to ursolic acid only. In conclusion, UASG exhibited significant activity in elevated plus maze, light and dark model and open field test induced anxiety models, proved its anxiolytic action. Exact mechanism of action needs a further detailed study.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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References


