Toxicological Profile of Ethanolic Extract of Leaves and Barks of Buddleja asiatica Lour

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Abstract: In the present study, the plant was evaluated for cytotoxic and phytotoxic activities. Toxicological studies showed that plant are safe for human consumption. BLE showed significant cytotoxic result with LD50 value 469.63 µg/ml and BBE showed non-significant cytotoxic effect with LD50 value 30487.44 µg/ml. Lemna minor phytotoxicity assay of BLE and BBE was carried out, showed non-significant phytotoxic effect with Fl50 values 27997.37 and 15152.54 µg/ml respectively. Analgesic effects of the ethanolic extract of B. asiatica leaf and bark was tested in mice. The dose dependent analgesia was noticed. The BLE and BBE both have the ability to inhibit the pain sensitivity and showed significant results. The present study revealed that the B. asiatica plant is pharmacologically active for various types of ailment and is recommended that this plant may be further explored.

Key words: Buddleja asiatica Lour. • Toxicological studies • Cytotoxic • Phytotoxic

INTRODUCTION

Buddleja plants are widely distributed throughout the world and only four species are found in Pakistan, i.e., B. asiatica, B. crispa, B. davidii and B. lindleyana [1]. They have been used in treatment of cancer and as a cure of articular rheumatism in the Chinese traditional medicine. The whole plant B. asiatica has been used medicinally as an abortifacient and in skin complaints [2]. It is planted in gardens as an ornamental shrub and the wood may be used for making walking sticks [3]. Toxicology is the aspect of pharmacology that deals with the adverse effect of bioactive substance on living organisms. In order to establish the safety and efficiency of a new drug, toxicological studies are very essential in animals like mice, rat, guinea pig, dog, rabbit, monkey etc under various conditions of drug. Toxicological studies help to make decision whether a new drug should be adopted for clinical use or not. No drug is used clinically without its clinical trial as well as toxicity studies [4]. The current study deals with the cytotoxic and phytotoxic profile of Buddleja asiatica.

MATERIALS AND METHODS

Plant Material: The leaves and bark of Buddleja asiatica were collected from Department of Botany University of Peshawar, Peshawar during the month of January 2013. The plant was identified with the help of available literature in the herbarium, Department of Botany, University of Peshawar and preserved for ready reference in future having voucher No. 20035 (PUP).

Preparation of Plant Extract: The Plant materials were rinsed, cleaned and dried in shade for 15 days. After drying, these were grinded into fine powder. It was stored in a well closed container free from environmental climatic changes till usage. The powdered materials were soaked in ethanol for successive seven days. The resulted ethanolic extract was evaporated through rotary evaporator and then used for various biological activities [5-7].

Animals: BALB/c mice (20 to 25 g) of either sex were used in all experiments. Animals were purchased from the Pharmacology Section of the Department of Pharmacy,
University of Peshawar, Peshawar. The animals were
maintained in standard laboratory conditions (25 °C and
light/dark cycles i.e. 12/12h and were fed with standard
food and water ad libitum.

**Acute Toxicity Studies:** The acute toxicity study was
carried out for the ethanolic extract of leaves (BLE) and
bark (BBE) of the selected plant according to standard
protocol [8]. The study was carried out using albino mice
weighing 20-25 g of either sex. The animals were randomly
distributed into seven groups each with four animals. The
animals were acclimatized to the Laboratory
conditions before the commencement of experiment. All
the animals were deprived from food overnight, the
control group received normal saline and the
remaining II-IV groups were treated with 500, 1000 and
2000 mg/kg body weight, respectively with crude
ethanolic extract of leaves, while group V-VII groups were
treated with 500, 1000 and 2000 mg/kg, respectively with
ethanolic extract of bark. The animals were observed
continuously for the first 4 h and then for next 24 h for
any toxic symptom.

**Phytotoxicity:** Lemma plants are miniature aquatic
monocot consists of a central oval frond or mother frond
with two attached daughter fronds and a filamentous root.
Under normal conditions, the plants reproduce
exponentially with budding of daughter fronds from
pouches on the sides of the mother frond. Using the
Lemma assay, it is observed that natural anti tumour
compounds can inhibit Lemma growth. In addition, it was
also discovered that some substances stimulate frond
proliferation and the assay may be useful to detect new
plant growth stimulants [12].

The phytotoxic activity of Buddleja asiatica
(leaf and bark) was evaluated using Lemma minor as test
species following the standard protocol [11].

**Cytotoxicity:** Brine shrimp assay is a rapid and
inexpensive assay used routinely for detection of
cytotoxic effects of any compound [9]. It is an excellent
and simple preliminary method to determine the
cytotoxicity of crude plant extract, pure natural
compounds and development of anti-cancer drugs [10].
The preliminary cytotoxic potential of the ethanolic and
n-hexane extract from the leaf and bark of Buddleja asiatica were carried out using brine shrimp assay by following the method of [11].

**Hatching Techniques:** The hatching tray (a rectangular
dish (22×32 cm) was filled half with filtered brine solution.
The tray was then partitioned into two parts with
perforated partition. One part was sprinkled with 25 mg of
brine shrimp eggs powder and covered with black paper.
The other half was left open. The hatching tray was kept
at room temperature for hatching of eggs. A lamp was
suspected above the tray so as to illuminate the open
part of the tray. After hatching the nauplii were observed
to move through the perforated partition towards the
enlightened part.

**Sample Preparation:** 20 mg of an extract was dissolved in
2 ml of respective solvent and from the solution 5, 50 and
500 µl were transferred to vials (3 vials / concentration)
which was equivalent to 10, 100 and 1000 µg/ml
respectively. The solvent was allowed to evaporate
overnight and 5 ml seawater solution (38 gram/L) was
added to each vial. After 60 hours of hatching and
maturation as nauplii, 10 larvae were transferred to each
vial using a Pasteur pipette. The vials were then
placed at room temperature (25-27°C) under illumination.
For negative controls, vials filled with brine solution were
used.

**RESULTS AND DISCUSSION**

In the present study Buddleja asiatica was screened
out for various bioassays, due to which determine its
therapeutic value. The acute toxicity studies were
performed to study the acute toxic effects and to
determine lethal dose of the drug extracts. In the present
study ethanolic extract of Buddleja asiatica leaf and bark
at doses of 500, 1000 and 2000 mg/kg body weight was
evaluated for toxicological effects, using mice as test
animals. It did not produce any significant changes in
behavior, breathing, sensory nervous system responses
or gastrointestinal effects during the observation period.
No mortality was recorded in any group after 24 hr of
administering the extract to the animals. Acute toxicity
studies show that no death was observed at all
the doses showing that the plant is safe for human use
Table 1.
Table 1: Effect of acute toxicity test of ethanolic extract of leaves and barks of B. asiatica.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose</th>
<th>No. of animal died</th>
<th>% Mortality</th>
<th>Gross behavioral changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/Saline</td>
<td>10 ml/kg</td>
<td>0/6</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>BLE</td>
<td>500 mg/kg</td>
<td>0/6</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>1000 mg/kg</td>
<td>0/6</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2000 mg/kg</td>
<td>0/6</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>BBE</td>
<td>500 mg/kg</td>
<td>0/6</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>1000 mg/kg</td>
<td>0/6</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2000 mg/kg</td>
<td>0/6</td>
<td>0</td>
<td>None</td>
</tr>
</tbody>
</table>

Table 2: Cytotoxic activity of ethanolic extract of the Leaf and Bark of B. asiatica

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parts</th>
<th>Conc. (µl)</th>
<th>Total No. of Shrimps</th>
<th>No. of Shrimps survived</th>
<th>% age inhibition</th>
<th>LD₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BLE</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>20</td>
<td>469.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>10</td>
<td>7</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000</td>
<td>10</td>
<td>4</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>BBE</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>30487.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000</td>
<td>10</td>
<td>7</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Phytotoxic activity of ethanolic extract of the Leaf and Bark of B. asiatica

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parts</th>
<th>Conc. (µl)</th>
<th>No. of fronds in test</th>
<th>No. of fronds in ethanol (-ve control)</th>
<th>% age inhibition</th>
<th>F₁₀₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaf Extract</td>
<td>10</td>
<td>36</td>
<td>45</td>
<td>20.00</td>
<td>27997.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>31</td>
<td></td>
<td>31.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000</td>
<td>29</td>
<td></td>
<td>35.55</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Bark Extract</td>
<td>10</td>
<td>34</td>
<td>45</td>
<td>24.44</td>
<td>15152.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>31</td>
<td></td>
<td>31.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000</td>
<td>27</td>
<td></td>
<td>40.00</td>
<td></td>
</tr>
</tbody>
</table>

Cytotoxicity was evaluated using brine shrimp lethality assay. Brine Shrimp lethality test is a preliminary exploration for the detection and development of anti-cancer drugs. The BLE and BBE were examined for cytotoxicity while using Brine shrimps lethality assay. Shrimps larvae showed response towards different concentration of the test sample. The experimental findings confirm significant cytotoxic effect of BLE with percent inhibition of 20, 30 and 60% at 10, 100 and 1000 µg/ml respectively with LD₅₀ (469.63 µg/ml) and non-significant cytotoxic effect of BBE with percent inhibition of 10, 10 and 30% at 10, 100 and 1000 µg/ml respectively with LD₅₀ of (30487.44 µg/ml). The BBE was found to be relatively non-effective as it had an LD₅₀ value greater than BLE (Table 2).

**CONCLUSIONS**

It is concluded that BLE and BBE were found absolutely safe in acute toxicity test. BLE showed significant cytotoxic result and BBE showed non-significant cytotoxic effect. In phytotoxicity assay, BLE and BBE showed non-significant phytotoxic effect. However, further studies are necessary to find the active compound responsible for this pharmacological activity.

**REFERENCES**


