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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 LD_{50} value 469.63 µg/ml and BBE showed non-significant cytotoxic effect with LD_{50} value 30487.44 µg/ml. *Lemna minor* phytotoxicity assay of BLE and BBE was carried out, showed non-significant phytotoxic effect with FI_{50} 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,

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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.

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 suspended 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.

Phytotoxicity: *Lemna* 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 *Lemna* assay, it is observed that natural anti tumour compounds can inhibit *Lemna* 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 *Lemna minor* as test species following the standard protocol [11].

The E-medium was prepared using standard procedure [14] for *Lemna minor* assay. Different amount of salts dissolved in distillated water and the volume was mad up to 1000 ml. The pH was adjusted between 5.5- 6.00 by addition of KOH. After 24 h the number of dead and live nauplii was counted and the data was analyzed with Finney computer program to determine LD_{50} values with 95% confidence intervals [13, 14].

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.*

Treatments	Dose	No. of animal died	% Mortality	Gross behavioral changes
N/Saline	10 ml/kg	0/6	0	None
BLE	500 mg/kg	0/6	0	None
	1000 mg/kg	0/6	0	None
	2000 mg/kg	0/6	0	None
BBE	500 mg/kg	0/6	0	None
	1000 mg/kg	0/6	0	None
	2000 mg/kg	0/6	0	None

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

S. No.	Parts	Conc.(µl)	Total No. of Shrimps	No. of Shrimps survived	% age inhibition	LD_{50}
1	BLE	10	10	8	20	469.63
		100	10	7	30	
		1000	10	4	60	
2	BBE	10	10	9	10	30487.44
		100	10	9	10	
		1000	10	7	30	

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

S. No.	Parts	Conc. (µl)	No. of fronds in test	No. of fronds in ethanol (-ve control)	%age inhibition	Fl_{50}
1	Leaf Extract	10	36	45	20.00	27997.37
		100	31		31.11	
		1000	29		35.55	
2	Bark Extract	10	34	45	24.44	15152.54
		100	31		31.11	
		1000	27		40.00	

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 LD_{50} (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 LD_{50} of (30487.44 µg/ml). The BBE was found to be relatively non-effective as it had an LD_{50} value greater than BLE (Table 2).

Lemna plants are miniature aquatic monocotyledonous plants which are very sensitive to bioactive compounds. *Lemna* assay has been used to detect natural anti-tumor and phytotoxic compounds and may be useful to detect new plant growth stimulants. The effect of phytotoxic activity of the BLE and BBE is summarized in Table 3. The experimental findings confirm non-significant phytotoxic effect of BLE and BBE with percent inhibition of BLE was 20, 31.11 and 35.55 and BBE was 24.44, 31.11 and 40 at 10, 100 and 1000 µg/ml respectively. FI_{50} (Concentration which causing 50% fronds proliferation inhibition) was high (27997.37µg/ml) and FI_{50} (15152.54µg/ml) because of low toxic effect.

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.

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