TOXICITY TESTING AND ANTIBACTERIAL ACTIVITY OF NYCTANTHES ARBOR-TRISTIS CALYX EXTRACTS

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Accepted Date: 24/10/2013; Published Date: 27/12/2013

Abstract: Dried and powdered calyx of Nyctanthes arbor-tristis Linn. were extracted with various solvents and the extracts were evaluated for acute oral toxicity and Antibacterial activity against gram-positive and gram-negative microorganisms. All the extracts showed antibacterial activity at different concentrations with no symptoms of toxicity.

Keywords: Toxicity testing, Antibacterial activity, Nyctanthes arbor-tristis calyx.
INTRODUCTION

Nyctanthes arbor-tristis Linn. commonly known as Harsingar (Hindi), Parijatak (Marathi) is a hardy large shrub belonging to family Oleaceae (Nyctaginaceae). It blossoms in night & the flowers wither the next morning. The leaves of N. arbor-tristis Linn. are used extensively in Ayurvedic medicine for the treatment of various diseases such as sciatica, chronic fever, rheumatism, as a laxative, diaphoretic, diuretic.

The plant is a large shrub or small tree up to 10 metre in height with grey or greenish white rough bark and sharply quadrangular strigose young branches. Leaves are opposite 3.5-13cm long and 2-9cm wide, ovate, apex acuminate, base rounded or cuneate, margins entire or with a few large distant teeth, rough and scabrous above, densely pubescent beneath, petioles 0.5-2 cm long, pubescent. Flowers are very fragrant, small, white with an orange calyx, borne in 3-7 flowered fascicles; peduncles 4 angled, slender hairy, axillary and solitary and in terminal trichotomous cymes; corolla white, 4-8 lobed, about 1.3 cm long; tube bright orange, 0.6-0.8 cm long, obcordate, cuneate.1

MATERIAL AND METHOD

Preparation of Extracts

Flowers of Nyctanthes arbor-tristis L. were collected from the local areas of Saswad, Pune and authenticated at Agarkar Research Institute, Pune-411004. Then the calyx were separated from the flower, sun-dried, finely powdered and passed through sieve no #40 and then subjected to various extraction procedures like Maceration & Soxhlet Extraction for 18 hours using various solvents with increasing polarity like Petroleum Ether, Chloroform, Methanol, Ethanol & Water.2,3

Toxicity Testing4

According to the Organization for Economic and Cooperation Development (OECD) Guidelines for Testing Of Chemicals (No. 420) for ACUTE ORAL TOXICITY:

- Extract Used - Ethanolic
- Animal Used - Albino Mice (30 – 45 gm)

Total No. of animals used:

- a) for Sighting study - 5
- b) for Main study - 3
Sex of Animal - Either Of the Sex

Dose - 0.5 ml

Concentration

a) for Sighting study - 5, 50, 300, 2000, 5000 mg/kg of b.w.

b) for Main study - 5000 mg/kg of b.w.

Observation Time - 30 mins to 4 weeks.

At the end of 4 weeks, the animals were sacrificed using Ether anesthesia and the gross necropsy changes were recorded. The animals were observed for signs of convulsions, tremors, circling, depression, excitement and mortality.

Antibacterial Activity

The alcohol, water and methanolic extracts were screened against a total of two bacterial strains namely E. coli (NCTC 10418) and S. aureus (NCTC 6571).

Antibacterial Susceptibility Test

Invitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA). The MHA plates were prepared by pouring 15mL of molten media in sterile petriplates. The plates were allowed to solidify for 5 min and 0.1% innoculum suspension was swabbed uniformly and the innoculum was allowed to dry for 5 min. The different concentrations of extracts (50 & 100 mcg/disc) were loaded on 6mm sterile disc. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the discs were measured with transparent ruler in mm.

RESULTS AND DISCUSSION

Toxicity Testing

No mortality was observed in experimental animals at the dose of 5000 mg/kg body weight. There was no significant difference in body weight of all the mice. There were no signs of any systemic toxicity. Necropsy findings also did not reveal any lesions of toxicity suggesting that these plant extracts seem to be non-toxic and could be tested in future on human volunteers. Therefore, according to OECD guidelines no. 420, the drug falls under Unclassified Category of Globally Harmonized System (GHS).
Antibacterial Susceptibility Testing

As shown in Table I, all the extracts (alcohol, water & methanol) showed promising activity against the gram-positive (S. aureus) microorganisms. On the other hand, none of the extracts showed activity against gram-negative (E. coli) microorganisms. In the previous findings leaves have shown antimicrobial activity. Further studies can be taken up to isolate and identify the active constituents. Effectiveness of the extracts can also be tested on clinical samples.

Table I: Antibacterial Activity of Alcohol, Water & Crude Calyx extracts of N. arbor-tristis

<table>
<thead>
<tr>
<th>Groups</th>
<th>S. aureus 50 mcg/mL</th>
<th>S. aureus 100 mcg/mL</th>
<th>E. coli 50 mcg/mL</th>
<th>E. coli 100 mcg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>7 mm</td>
<td>10 mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>6 mm</td>
<td>9 mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol</td>
<td>7 mm</td>
<td>9 mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Std (Ciprofloxacin)</td>
<td>13 mm</td>
<td>8 mm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES


6. www.google.com