EVALUATION OF ANTI-DEPRESSANT ACTIVITY OF ETHANOLIC ROOT EXTRACT OF ANNONA SQUAMOSA L

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ABSTRACT
This work has been done for the investigation of the anti-depressant activity of Ethanolic root extract of Annona squamosa (EREAS) belongs to the family Annonaceae by oral administration at dose according to the body weight in a sequential manner in healthy albino rats. Extracts were studied for its anti-depressant activity by using Imipramine as standard drug. Forced swim test (FST) and Tail suspension tests (TST) are used for the study. Imipramine decreases the immobility time of the animals. Plant extract given in three doses (100, 200 and 300mg/kg) were in a dose dependent manner. 300mg/kg plant extract showed decreased immobility time near as to that of the standard drug. The decrease in immobility period in both the models was observed starting from 200 mg/kg. But the increase in dose from 200 to 300 mg/kg produces further reduction in immobility period. At the dose 100 & 200mg/kg, ASRE showed a little antidepressant effect when comparable to that of Imipramine. At the dose 300mg/kg, ASRE showed antidepressant effect which is comparable to that of Imipramine. This evaluation may suggest that the antidepressant action is because of the possible involvement of either nor-adrenergic or serotonergic system.

KEYWORDS: EREAS, Anti-depressant, Immobility time, Imipramine.

INTRODUCTION
Annona squamosa L.¹² is a species of the plant genus Annona, belongs to the family Annonaceae. The phytochemical analysis of Annona squamosa showed that it contained carbohydrates, alkaloids, flavonoids The preliminary pharmacological studies revealed that Annona squamosa possessed analgesic anti-inflammatory, antibacterial, cytotoxic, anti oxidant, antilipidimic, antiulcer, hypoglycemic, antidiabetic, hepatoprotective, insecticidal, anthelmintic and also used for the regulation of hyperthyroidism and lipid peroxidation.¹³ This study therefore, intends to investigate the anti depressant activities of the roots of Annona squamosa L. by studying the effects of ethanol extracts of the plant on forced swim test and tail suspension test induced depression in experimental animal models, in order to confirm the medicinal properties of the plant. Depression, a state of low mood and aversion to activity, can affect a person's thoughts, behavior, tendencies, feelings, and sense of well-being. A multiple biological, psychological and social factors that have been identified as being related to the development of depression. Depression has been linked to problems or imbalances in the brain with regard to the neurotransmitters serotonin, norepinephrine, and dopamine. Symptoms of the mood disorder is marked by sadness, inactivity, difficulty in thinking and concentration and a significant increase/decrease in appetite and time spent sleeping.¹⁶ Many types of antidepressants are available, they include Selective serotonin reuptake inhibitors (SSRIs). Serotonin-nor epinephrine reuptake inhibitors (SNRIs). A typical antidepressants, Tricyclic antidepressants, Monoamine oxidase inhibitors (MAOIs). Anti depressants by keeping the levels of the neurotransmitters higher improves communication between the nerve cells and which strengthens the circuits in the brain which regulates mood.ECT (Electroconvulsive Therapy) is an effective treatment for patients who do not respond to drug therapy.¹⁵

Plant profile
Botanical Name: Annona squamosa.
Classification
Kingdom: Plantae
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Magnoliidea
Order: Magnoliales
Family: Annonaceae
Genus: Annona L.

Vernacular Names
Hindi: sarifa, sherifa, sitaphal.
Telugu: seetapandu, seethapandu, seethaphalam, sitapandu, sitaphalamu.
English: custard apple, sweetsop.
Tamil: annila, atta, cintamaram.
Sanskrit: krishnabija, krisnabija, shubha, sitapalam, sitaphala.
Malayalam: atha, atta, sirpa, sirpha, sitapalam, sitappalam, sutakanni. [12]

Geographical Distribution
Annona squamosa is native to the tropical Americas and West Indies, but the exact origin is unknown. It is now the most widely cultivated of all the species of Annona, being grown for its fruit throughout the tropics and warmer subtropics, such as Indonesia, Thailand, and Taiwan; it was introduced to southern Asia before 1590. It is naturalized as far north as southern Florida in the United States and as south as Bahia in Brazil, and is an invasive species in some areas.

METHODS AND MATERIALS
Plant material
The fresh plant of Annona squamosa L. roots were collected from Nandivelugu, Guntur, Andhra Pradesh, India. The plant roots are dried under sunlight for one month and chopped into small pieces. The powdered plant was used for the preparation of ethanolic root extract.

Preparation of Ethanolic roots extract of Annona squamosa by maceration Method
The plant material was dried under sun light and powdered mechanically. The 50 gm of powder sample was extracted with ethanol by using maceration method. The extraction was continued till a few drops of the last portion of the extract left no residue on drying. The solvent was removed by heat evaporation and dried under reduced pressure. The yield of the ethanol extract was 9.4%. The dried extract was stored in refrigerator until further studies.

Preliminary Phytochemical Analysis
Phytochemicals of the selected plants were carried out by using ethanolic and powdered form of the plant following Harborne (1973) Trease and Evans (1989).

Animals
Adult Wistar Albino male rats (150-180g) were procured from the laboratory animal model house, Hindu College of Pharmacy, Guntur, Andhra Pradesh, India and used in the study. The animals were kept under standard environmental conditions of room temperature (220 ±20°C), relative humidity (50%±5%) and 12h light and dark cycle. The animals were housed in the colony cages (three rats per cage) and provided feed (commercial pellets contain a balanced ration obtained from the Vyas Enterprises, Hyderabad) and water ad libitum.

Medicinal uses
- Strongly astringent
- Used to treat diarrhoea and dysentery.
- They have effective vermicidal properties.
- To relieve cold and chills.
- To lower uric acid levels in the blood.
- Gastric purgative.
- Used in a sedative infusion.
- To aid digestion
- To treat rheumatism.
- For treatment of sleeplessness.
- Excellent vermifuge.
Mahesh et al. World Journal of Pharmaceutical and Life Sciences

Table 1: Phytochemical tests for constituents.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phytochemical Tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Test for Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Test for Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Test for Steroids</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Test for Proteins</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Test for Tannins</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Test for Flavanoids</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Test for Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Test for Saponins</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Test for Phenol</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Test for Triterpenoids</td>
<td>-</td>
</tr>
</tbody>
</table>

All the animals were acclimatized to the laboratory environment 5 days prior to experiment. The animal were fasted overnight just prior to the experiment but allowed free access to drinking water.

Chemicals: Imipramine, PEG.

Grouping of Animals

Table 2: Grouping of animals based on dose.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Dose and route of Drug Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control - treated with PEG</td>
<td>According to the body wt.</td>
</tr>
<tr>
<td>II</td>
<td>Standard drug: Imipramine 15 mg/kg</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Low dose of A. squamosa 100 mg/kg p.o.</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Mid dose of A. squamosa 200 mg/kg p.o.</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>High dose of A. squamosa 300 mg/kg p.o.</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Forced Swim Test (FST)

FST or behaviour despair was proposed as a model to test for antidepressant activity by Porsolt et al. Depression was produced by forcing the animal to swim individually in a glass jar containing fresh water of 15 cm height and maintained at 25°C. This constituted pretest session. Twenty-four hour later each animal was again forced to swim. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. The total duration of immobility was recorded in next 4 min of a total 6 min test. The change in the immobility period was calculated after administering drugs to the groups.

Table 3: Effect of ASRE on Immobility Period (Secs) of rats using Forced Swim Test.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DRUG</th>
<th>DOSE</th>
<th>IMMObILITY TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PEG 1 ml/100 gm</td>
<td>120.60 ± 3.88</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Imipramine 15 mg/kg</td>
<td>28.00 ± 1.58***</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>A. squamosa 100 mg/kg</td>
<td>60.60 ± 2.65*</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>A. squamosa 200 mg/kg</td>
<td>45.60 ± 2.73*</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>A. squamosa 300 mg/kg</td>
<td>31.40 ± 2.42**</td>
<td></td>
</tr>
</tbody>
</table>

Tail Suspension Test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. Depression was produced by suspending the animal from the edge of a table 50 cm above the floor by an adhesive tape placed approx. 1 cm.

Fig. 1: Forced swim test result analysis.
from the tip of the tail. Immobility time was recorded during a 6 min. period. Changes in the immobility duration were studied after administering drugs in separate groups of animals. The antidepressant activity was expressed as reduction in the immobility duration between the control, standard and animals treated with test drug.

### Table 4: Effect of ASRE on immobility period (sec’s) of rats using tail suspension test.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DRUG</th>
<th>DOSE</th>
<th>IMMObILITY TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PEG</td>
<td>1ml/100gm</td>
<td>109.40 ± 2.65</td>
</tr>
<tr>
<td>2.</td>
<td>Imipramine</td>
<td>15mg/kg</td>
<td>19.00 ± 2.28***</td>
</tr>
<tr>
<td>3.</td>
<td>A. squamosa</td>
<td>100mg/kg</td>
<td>55.80 ± 2.35*</td>
</tr>
<tr>
<td>4.</td>
<td>A. squamosa</td>
<td>200mg/kg</td>
<td>44.40 ± 2.46*</td>
</tr>
<tr>
<td>5.</td>
<td>A. squamosa</td>
<td>300mg/kg</td>
<td>32.00 ± 1.87**</td>
</tr>
</tbody>
</table>

### Statistical analysis
Statistical significance was determined by analysis of variance. The analysis was performed using INSTAT statistical software.

### CONCLUSION
The observation of acute toxicity study indicated that there was no death in 2000mg/kg dose after 72hrs. ASRE at the dose of 100 mg/kg had no beneficial effect on immobility period of rats in both the models of depression i.e. FST & TST. The decrease in immobility period in both the models was observed starting from 200 mg/kg. But the increase in dose from 200 to 300 mg/kg produces further reduction in immobility period. At the dose 100 & 200mg/kg, ASRE showed a little antidepressant effect when comparable to that of Imipramine. At the dose 300 mg/kg, ASRE showed antidepressant effect which is comparable to that of imipramine. This evaluation may suggest that the antidepressant action is because of the possible involvement of either nor-adrenergic or serotonergic system.

### REFERENCES