Pharmacological evaluation of leaf extracts of *Crataeva religiosa* for its anxiolytic activity in Albino mice

N.Rama Lakshmi*, P.Hemasoundarya¹, B.Ganga Rao¹, D. Eswar Tony¹, Ramarao .N¹
Department of pharmacology, Chalapathi institute of pharmaceutical sciences, Guntur-522634, Andhra Pradesh.

Abstract

**Objective:** To study the anxiolytic activity of ethanolic extract and aqueous extract of *Crataeva religiosa* in mice.

**Methods:** The anxiolytic activity of ethanolic extract and aqueous extract of leaves of *Crataeva religiosa* (20 mg/kg) in mice assessed by using open field test and light and dark test (behavioural test) Diazepam standard drug.

**Results:** Aqueous leaf extract showed significant anxiolytic activity when compared with methanolic leaf extract, standard and control treatment groups using open field test and light and dark test.

**Conclusion:** The ethanolic extract and aqueous extract of *Crataeva religiosa* possess antianxiety activity since it reduced the duration of anxiety produced by open field test and light and dark test.

**Keywords:** *Crataeva religiosa*, Open field test, light and dark box test.

INTRODUCTION

The name *Crataeva* is given in the honor of Crataenus, a Greek botanist, who was living in the time of Hippocrates and the name *religiosa* indicates its growth near the places of worship (1). *Crataeva religiosa* is much branched deciduous tree belonging to the family capparidaceae commonly called as Varuna (2). The trade name given for this tree is three leaved capper [3]. The leaves are trifoliate, glabrous, and ovate.

2. Distribution

*Crataeva religiosa* is globally distributed in India, Myanmar, Sri Lanka, Malaysia, Indonesia and China. In India, it is found in Peninsular India, Western India, Gangetic Plains, and Eastern India, up to Tripura and Manipur [2]. It is also found in Sikkim and Andman and Nicobar Island [3]. It is found mostly along the bank of the river and streams and near to temple side [5], [6].

3. Ethnobotany

The plant part used for the medicinal purpose includes Leaves, stem bark and Root bark [7], [8], [9]. These parts of *C. nurvala* are commonly applied to regulate equilibrium among Vata, Pitta and Kapha in Ayurvedic system while the stem bark is used to promote the appetite and to decrease the secretion of the bile in unani medicines [10]. Recently Bopana and Saxena [11] critically reviewed *C. nurvala* for its ethno botanical and pharmacological properties. Plant is used ethno pharmacologically as diuretic, laxative, lithontriptic, antihemorrhagic, antiperiodic, bitter tonic, rubifacient and counterirritant [7], [8]. The bark is used in the urinary disorders including kidney and bladder stones, antiemetic, and calculous affections and as an antidote in snakebite [7]. *C. religiosa* is valuable in treating vata (blood flow, waste elimination and breathing), Pitta- (fever and metabolic disorder) and Kapha (strength and vigour, memory loss, heart and lung weakness and weak immune system [9]. A preparation called ‘Varunal’ contains *Crataeva in combination with Eclips, Picrorrhiza, Achillea, Cichorium, Solanum, Arjuna*, and *Cassia* seeds are used against hepatitis, edema, ascites, urinary stones and arthritis [12]. The bark is contraceptive and cytotoxic and useful in kidney bladder stones, fever vomiting and gastric irritation [13]. Roots and bark are laxative and lithontriptic and increase appetite and biliary secretion [14]. Leaves are used as externally rubificant and used in rheumatism. Leaves are given internally febrifuge and tonic [15], [16]. According to Gurrero [http/www.bpi.da.gov.ph. 2009], in Philippines, leaves are useful in irregular menstruation and also in stomachic, whereas the bark is used to cure convulsions and tympanites. Sanyal and Ghose [http/www.bpi.da.gov.ph. 2009] speculated that the crushed leaves are applied in the form of paste for swelling of feet and also for a burning -sensation in the soles of feet. The bark and the leaves are pounded and applied in the form of a poultice in rheumatism. The fresh leaves bruised with little vinegar, applied to skin. Bark and roots are rubificant and vesicant. Decoction of bark is used in the disorders of urinary organs and urinary calculi. Roots and bark in the form of decoction are used as calculus affections [http/www.bpi.da.gov.ph. 2009]. Traditionally, the plant is used as oxitoxic, in rheumatic fever in kidney stones, bladder stone and as tonic [17]. It is useful as antipyretic, antilithic, antihelmintic, demulcent in blood and chest diseases [18]. NR-AG-I is a polyherbal formulation containing *Crataeva religiosa, Dollichos biflorus, Tribulus terrestris and Shilajit*. NRAG-II is another herbal formulation containing *Crataeva religiosa, Boerrhavia diffusa, and Saccharum officinarum. and Butea frondosa*. Between these two, NR-AG-II is having good diuretic potential than NR-AG-I [19]. A mixture containing- *Tribulus terrestris* fruits (25%); *Zinziber officinalis* roots(10%); *Solanum xanthocarpum* whole plant (10%); *Asparagus racemosus* roots (10%); *Tephrosia purpurea* leaves (10%) and *Crataeva religiosa* bark (25%) was prepared and 4gm of mixture given to patient twice daily with water in urinary disorder [20]. Drugs obtained from natural sources are perceived to have fewer side effects while having same ability to cure disorders in much the same way as their synthetic counterparts. Therefore, present study was undertaken to evaluate anxiolytic activity of ethanolic extract and aqueous extracts of *Crataeva religiosa* leaves.

MATERIALS AND METHODS:

**Plant collection:**

The leaves of *Crataeva religiosa* were collected from medicinal plant garden of Chalapathi institute of pharmaceutical sciences, Guntur. The plant was authenticated by Dr.P.Raghu Ram, Department of botany, Acharya Nagarjuna University, Guntur and voucher specimen was deposited in herbarium for further reference.

**Extraction procedure:**

The leaves of Crataeva religiosa were washed thoroughly and dried under shade and then made into a coarse powder using dry grinder. The powder leaves was passed through sieve no. 40 and stored in an air tight container at 25°C, used for further study. Powdered plant material (1.2 kg) were successively extracted using Soxhlet apparatus using the solvents in order of increasing polarity viz. methanol and water.
Drugs
Diazepam hydrochloride (Ranbaxy laboratory ltd, Mumbai) was used as reference drug. It was diluted with saline to the required strength before use.

**Preparation of test doses**
The extracts were suspended in vehicle. Various strengths were prepared from a stock solution 100 mg/ml. The solutions were prepared freshly to ensure that extracts were administered orally. 

**Acute toxicity study**
The procedure was followed as per OECD 423 guidelines. The extracts was administered orally at a dose of 100, 200, 400, 600, 800, 1000, 2000, mg/kg body weight. Animals were observed for 10 days to study their behavioral neurological toxicity.

**Experimental animals:**
Swiss albino mice (125-130g) were maintained for 7 days in the animal house of Chalapathi Institute of Pharmaceutical Sciences, Guntur under standard conditions temperature (24 ± 10 C), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were fed standard rat pellet and water ad libitum.

The animals were allowed to acclimatize to laboratory conditions 48 hours before the start of the experiment. 6 rats in a group were used in all sets of experiments. Chalapathi Institute of Pharmaceutical Sciences, Guntur under standard conditions temperature (24 ± 10 C), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were fed standard rat pellet and water ad libitum. All the experiments were carried out in a sound-attenuating box.

**Procedure**

**Group-4**
Aqueous extract (20mg kg, orally)

**Group-3**
Methanolic extract (20mg kg, orally)

**Group-2**
Standard – Diazepam (2 mg/kg i.p)

**Group I** (control group)
0.9% Normal saline 2ml/kg orally

The experiment was divided into four groups containing six animals in each group.

**Treatment design**

**Group I:** - Control group (0.9% Normal saline 2ml/kg orally)
**Group-2** – Standard (Diazepam at a dose of 2 mg/kg i.p)
**Group-3** – Methanolic extract (20mg kg, orally)
**Group-4** – Aqueous extract (20mg kg, orally)

**Open field**
- Swiss albino mice (125-130g) were selected and divided into four categories.
- Mice were carried into the test room in their home cages and handled by the base of their tails at all times.
- The rats are observed in a square open field arena (68 × 68 × 45 cm) equipped with 2 rows of 8 photocells, sensitive to infrared light, placed 40 and 125 mm above the floor, respectively.

**RESULTS AND DISCUSSION**

**Open Field test:**

| Table 01. The anxiolytic activity of leaf extracts of *Crataeva religiosa* using the open field test. |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-----------------------------------------------|
| S.No   | Treatment                                      | Evaluation of parameters for 5min (After 30 min of treatment) |                                               |                                               |                                               |
|        |                                                | No. of Rearings | Central square entries | No. of line crossings | Freezing time (Sec) |
| 1      | Control                                        | 22             | 2±0.03                | 82±0.02              | 27               |
| 2      | Standard – Diazepam(2mg/kg)                   | 14             | 4±0.06                | 59±0.04              | 40               |
| 3      | Methanolic extract(20mg/kg)                   | 11             | 5±0.04                | 57±0.03              | 35               |
| 4      | Aqueous extract(20mg/kg)                      | 13             | 4±0.03                | 52±0.02              | 39               |

Values represent Mean ± SEM, n = 4. One way ANOVA followed by Dunnett’s multiple comparison tests. Aqueous leaf extract showed significant anxiolytic activity when compared with methanolic leaf extract, standard and control treatment groups using open field test.

**Table 02. The anxiolytic activity of leaf extracts of *Crataeva religiosa* using Light And Dark Box test**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment Dose(mg/kg) Lp</th>
<th>Total time (Min)</th>
<th>Time spent in closed arm (Sec)</th>
<th>Time spent in open arm (Sec)</th>
<th>Avg Time in closed arm</th>
<th>Avg Time in open arm</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>242</td>
<td>239 30 60 15 30 60</td>
<td>236.3</td>
<td>44.66 ± 0.645</td>
<td>44.66 ± 0.645</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Diazepam</td>
<td>230</td>
<td>194 110 58 77 161 98.66</td>
<td>178</td>
<td>98.66 ± 0.912</td>
<td>98.66 ± 0.912</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Methanolic Extract</td>
<td>224</td>
<td>176 94 69 112 193 124.6</td>
<td>164.6</td>
<td>124.6 ± 1.108</td>
<td>124.6 ± 1.108</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Aqueous Extract</td>
<td>262</td>
<td>196 107 29 57 184 183.3</td>
<td>183.3</td>
<td>90 ± 1.080</td>
<td>90 ± 1.080</td>
<td></td>
</tr>
</tbody>
</table>

Values represent Mean ± SEM, n = 4. One way ANOVA followed by Dunnett’s multiple comparison tests. Methanolic leaf extract showed significant anxiolytic activity when compared with and aqueous leaf extract, standard and control treatment groups using light and box test.
further chronic toxicity testing should be conducted to confirm its 
knowledge. The results obtained in this study showed the safety of 
natural treatment and exhibits no side effect up to our studies and 
parameters more than the plant extract, but 

Although the present study showed that Diazepam improved some 
anxiolytic effect of 
mediated its anxiolytic potential probably through its ROS-scavenging activity and protective 
effect against brain effect. Although some studies have been done to 
investigate the major active compound in Crataeva religiosa but more is still required, to fully illustrate the anti anxiolytic potential of Crataeva religiosa leaves.

CONCLUSION

Although the present study showed that Diazepam improved some 
parameters more than the plant extract, but Crataeva religiosa is a 
natural treatment and exhibits no side effect up to our studies and 
knowledge. The results obtained in this study showed the safety of 
Crataeva religiosa in rats, even at the highest dose. However, 

further chronic toxicity testing should be conducted to confirm its safe usage. In vivo study in rats demonstrated the promising anxiolytic effect of Crataeva religiosa against anxiety induced by ethanol. Crataeva religiosa mediated its anxiolytic potential probably through its ROS-scavenging activity and protective effect against brain effect. Although some studies have been done to investigate the major active compound in Crataeva religiosa but more is still required, to fully illustrate the anti anxiolytic potential of Crataeva religiosa leaves.

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