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

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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 antianxiolytic 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 Crataevus, 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, lithonotriptic, antirheuumatic, 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 (joint lubrication, skin moisture, wound healing, strength and vigour, memory loss, heart and lung weakness and weak immune system [9]. A preparation called 'Varunal' contains *Crataeva* in combination with *Eclipts, 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 lithontipic and increase appetite and biliary secretion [14]. Leaves are used as externally rubifacient and used in rheumatism. Leaves are given internally febrifuge and tonic [15], [16]. According to Gurrero [http/www.mbpi.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 rubifacient 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 oxitosic, in rheumatic fever in kidney stones, bladder stone and as tonic [17]. It is useful as antipyretic, antilithitic, antihelminthic, 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 Sacchaarum 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 the vehicle. Various strengths were prepared from a stock solution 100 mg/ml. the solutions were prepared freshly solutions were administered orally. **Acute toxicity study** The procedure was followed as per OECD 423 guidelines[4]. 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 with 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. All the experiments were conducted after obtaining permission from the Institutional Animal Ethics Committee (IAEC) Chalapathi Institute of Pharmaceutical Sciences, Guntur. Animals are 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)

Procedure

Open field

- Swiss albino mice (125-130g) were selected and divided into 4 groups.
- Mice were carried into the test room in their home cages and were 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.
- The photocells are spaced 90m apart and the last photocell in a row is spaced 25 mm from the wall.
- Measurements are made in the dark in a ventilated, sound-attenuating box.
- Interruptions of photocell beams can be collected by a microcomputer and the following variables can be evaluated Motor activity: All interruptions of photo beams in the lower rows.
- Peripheral motor activity: Activation of photo beams in the lower rows, provided that the photo-beams spaced 25 mm from the wall were also activated.
- Rearing: All interruption of the photo beams in the upper rows.
- Peripheral rearing: Interruption of photo beams in the upper rows, provided that the photo beams spaced 25 mm from the wall were also activated.
- Locomotion: Successive interruptions of photocells in the lower rows when the animal is moving in the same direction.
- Speed: The time between successive photo beam interruptions during locomotion collected in 0.1 s categories.
- The percentage of entries into the central square was also calculated for each animal.
- The percentage of time spent in the centre square was calculated over the 5 minute test

LIGHT AND DARK BOX

- Mice and rats tend to explore a novel environment, but to retreat from the aversive properties of a brightly-lit open field.
- In a two chambered system, where the animals can freely move between a brightly-lit open field and a dark corner, they show more crossings between the two chambers and more locomotor activity after treatment with anxiolytics.
- The numbers of crossings between the light and dark sites are recorded.
- The percentage of entries into the central square was also calculated for each animal.
- The percentage of time spent in the centre square was calculated over the 5 minute test**.**

RESULTS AND DISCUSSION

Open Field test:

Table 01.The anxiolytic activity of leaf extracts of *Crataeva religiosa* using the open field test.

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.

S.No	Treatment	Evaluation of parameters for 5min (After 3o 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

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

S.No	Treatment Dose(mg/kg) i.p	Total time (Min)	Time spent in closed arm (Sec)			Time spent in open arm (Sec)			Avg Time in closed arm	Avg Time in open arm	Mean ± SEM
			15 min	30 min	60 min	15 min	30 Min	60 min			
1	Control	5	242	239	228	36	41	54	236.3	44.66	44.66 ± 0.645
2	Diazepam		230	194	110	58	77	161	178	98.66	98.66 ± 0.912
3	Methanolic Extract		224	176	94	69	112	193	164.6	124.6	124.6 ± 1.108
4	Aqueous Extract		262	196	107	29	57	184	188.3	90	90 ± 1.080

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.

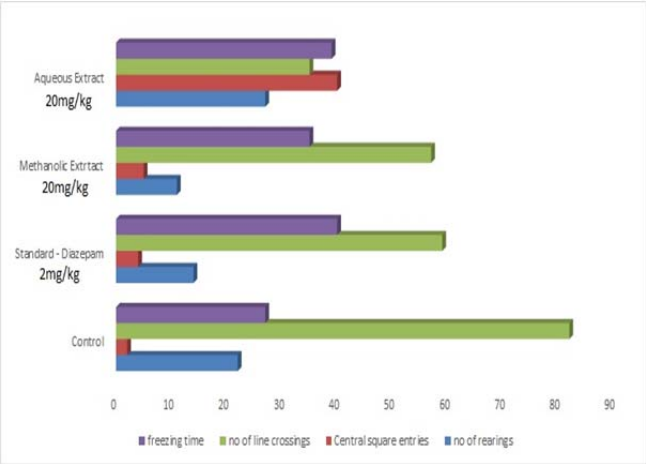

Fig .01 Evaluation of Anxiolytic activity by open field test

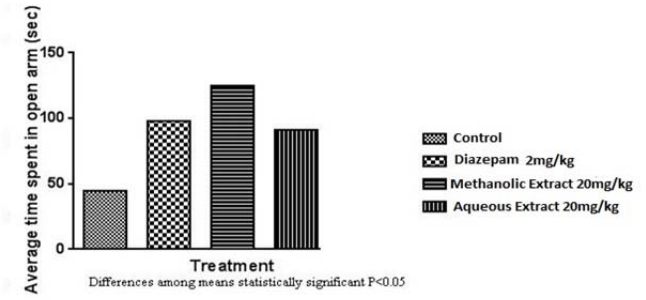

Fig.02 Evaluation of Anxiolytic activity by Light and Dark Box test

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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