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PHARMACOLOGICAL EVALUATION OF CRATEVA RELIGIOSA, LEAVES AQUEOUS EXTRACT FOR CENTRAL NERVOUS SYSTEM DEPRESSANT ACTIVITY IN MICE

Kanakam Vijayabhaskar*[1], Renukuntla Pranay[1], Karra Ujwala[1], Anagandhula Sushma Reddy[1], Uppari Naresh[1], Kalakota Chaitanya prasad[2]

[1]Department of Pharmacognosy and Phytochemistry. Department of Pharmaceutics Sahasra Institute of Pharmaceutical Sciences, Mulugu Road, Warangal, Telangana, India.
[2]Department of Pharmaceutical chemistry, Sri Ramananda Thirdha Institute of pharmaceutical Sciences, Nalgonda, Telangana, India.

***Corresponding Author: Kanakam Vijayabhaskar**
Department of Pharmacognosy & Phytochemistry. Department of Pharmaceutics Sahasra Institute of Pharmaceutical Sciences, Mulugu Road, Warangal, Telangana, India.

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ABSTACT
Crateva religiosa, is called the sacred garlic pear and, belongs to the family capparaceae, is a medicinal plant widely used in Ayurveda to treat painful conditions, The drug is well known for its various pharmacological properties like diuretic, antiinflammatory, laxative, antioxidant, antioxaluric, hepatoprotectant, lithonotriptic, antirehumatic, antiperiodic, antimycotic, contraceptive, antipyretic, antilithitic, antihelminthic, rubifacient and vesicant properties. The present study was carried out to evaluate the central nervous system depressant activity of different extracts of Crataeva religiosa leaves, extracted successively with petroleum ether, chloroform, methanol and water respectively depending upon their polarity. CNS depressant activity was evaluated using pentobarbitone induced sleeping time and by determining locomotor activity using actophotometer. Aqueous extract of Crataeva religiosa leaves (400 mg/kg) produced highest activity as it significantly ($P<0.01$) reduced the onset and prolonged of sleep duration induced by pentobarbitone, extract also decreased locomotor activity by 67.33%. Chloroform and methanol extract also produced dose dependent CNS depressant activity. Though petroleum ether extract does not produce significant CNS depressant activity in both models. The results indicate that different extracts of Crataeva religiosa leaves possesses CNS depressant activity.

KEYWORDS: Crataeva religiosa, Pentobarbitone, Locomotor activity, Aqueous extract.

INTRODUCTION
Crateva religiosa, is called the sacred garlic pear and temple plant, belongs to the family capparaceae. Crataeva religiosa is much branched deciduous tree, commonly called as Varuna.[2] The trade name given for this tree is three leaved capper.[3] The leaves are trifoliate, glabrous, and ovate. Flowers are whitish to milky white in colour in terminal dense corymbs.[2] Fruit is berry, globose or oblong with woody rind, embedding seeds in the yellow pulp.[1] The outer surface of bark is wrinkled and greywhite in colour, covered with large number of lenticells. Tree flowers and fruits in the month of Dec-May.[4] 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.[5] The bark is used in the urinary disorders including kidney and bladder stones, antiemetic, and calculous affections and as an antidote in snakebite.[6] 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.[7] A preparation called 'Varunal' contains Crataeva in combination with Eclipts, Picrorrhiza, Achillea, Cichorium, Solanum, Arjuna, and Cassia seeds is used against hepatitis, edema, ascites, urinary stones and arthritis.[8] The bark is contraceptive and cytotoxic and useful in kidney bladder stones, fever vomiting and gastric irritation.[9] Roots and bark are laxative and lithontipic and increase appetite and biliary secretion.[10] Traditionally, the plant is used as oxitosic, in rheumatic fever in kidney stones, bladder stone and as tonic.[11] It is useful as antipyretic, antilithitic, antihelminthic, demulcent, in blood and chest diseases.[12] young shoots and fruits of Crataeva religiosa are eaten and used in curries. Fruits of this tree are used as spice because of its garlic taste.[13] Showed presence of four compounds- Dodecanoic anhydride, methyl pentacosanoate, Kaemferol-3-O-α-D glucoside and querscitin- 3-O-α-D- glucoside. Leaves contain

isovitexin, proanthocyanidins, myricetin and phenolic acids, p- hydroxyl benzoic acid vanilic acid, ferulic acid and sinapic acid.[14] Sethi et al.[15] reported glucocaparin from the fruits of Crataeva religiosa. Gagandeep and Khadilkar.[16], first time reported four chemical compoundspentadecane, octanamide, 12-tricosanonoe and friedelin from the fruits of C. religiosa. The drug is well known for its various pharmacological properties like diuretic, antiinflammatory, laxative, antioxidant, antioxaluric, hepatoprotectant, lithonotriptic, antirehumatic, antiperiodic, antimycotic, contraceptive, antipyretic, antilithitic, antihelminthic, rubifacient and vesicant properties. The bark of the Crataeva religiosa is useful in the urinary disorders and kidney stone remover. The crude drug contains an active principle lupeol, a triterpenoid which is mainly involved in the pharmacological activities of this plant. Taking into account these findings and in view of alleged CNS depressant activity of the Crataeva religiosa, it was decided to evaluate the CNS depressant activity of Crataeva religiosa leaves using various experimental models.

METERIALS AND METHODS

Plant material

The leaves of Crataeva religiosa were collected from tirupathi hills, chitoor district region of andhra Pradesh, India in the month of August, 2013, authenticated the botanical identity of the plant at dept. Of botany kakatiya university. The herbarium was prepared and a voucher specimen (Sample No 01, Ref no.Gen/09-10/2013) was deposited.

Animals

Albino mice (18-25 g) of either sex were used in these experiments. Animals were provided with standard food and water ad libitum and were maintained at a temperature of 25±2°C, humidity of 55±5% and with 12 h light - dark cycle. All animal procedures have been approved and prior permission from the Institutional Animal Ethical Committee was obtained as per the prescribed guidelines.

Preparation of extracts

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., petroleum ether (60-80°C), chloroform, methanol and water. Each time the marc was dried and later extracted with other solvents. All the extract were concentrated by distilling the solvent in a rotary vacuum evaporator and evaporated to dryness. The yield was found to be 7.99, 1.46, 12.15 and 12.90% w/w respectively with reference to the dried plant material.

Acute toxicity test

Acute oral toxicity was performed as per OECD-423 guidelines.[17] Mice's were fasted overnight with free excess of water. Chloroform, methanol and water extracts were administered to the different groups orally at the dose level of 5 mg/kg body weight and mortality was observed for 14 days. If mortality was not observed for any animal then the procedure was repeated again with higher doses such as 50, 300 and 2000 mg/kg. The animals were observed for toxic symptoms such as behavioural changes, locomotion, convulsions and mortality for 72 hours.

Determination of pentobarbitone induces sleeping time(18)

In this method, mice of either sex were randomly taken and divided into control, standard and different test groups, each group contain six animals. Group I served as control and treated with normal saline (10 ml/kg, i.p.), group II (standard) treated with standard drug chlorpromazine hydrochloride (1mg/kg, i.m.) 15 min before the administration of pentobarbitone (40mg/kg,i.p.). Test groups III-VIII were treated with CSI (200 and 400 mg/kg), MCS (200 and 400 mg/kg) and Aqueous (200 and 400 mg/kg) respectively. Pentobarbitone (40mg/kg, i.p.) was administered 30 min later. Onset of sleep and duration of sleep measured for all the group. Onset of action was recorded by noting the time of loss of reflex for three consecutive trials, duration of sleep recorded by time difference between loss of righting reflex and recovery time.

Locomotor activity using actophotometer

The CNS depressant activity of the various extracts of Crataeva religiosa was evaluated by studying locomotor activity of mice using actophotometer.[25] Briefly, Albino mice of either sex (20 - 25 g) were randomly divided into eight groups of six animals. The mice were placed individually inside the chamber of actophotometer for 10 min and basal activity score was noted. Group I was treated with vehicle (0.5% sod. CMC) and standard drug chlorpromazine (3 mg/kg, i.p.) administered to group II. The animals of the group III-VIII were treated with CCS (200 and 400 mg/kg), PCS, MCS (200 and 400 mg/kg) and Aqueous (200 and 400 mg/kg) respectively and after 30 min of mice are placed again in actophotometer for 10 min and the activity was monitored. Percent decrease in activities were calculated for each group using the formula, Percent decrease in activity=(1- Wa/Wb)X100, where Wa and Wb are average activity scores after and before drug administration respectively and average decrease in activity was calculated for all groups.

Statistical analysis

The results have been expressed as mean ± standard error mean (S.E.M) and analysed using statistical package for social science (SPSS) version 10.0 using ANOVA followed by Dunnett's test.

RESULTS

Acute toxicity studies

Different extracts like PCS, CCS, MCS and Aqueous CS administered separately up to 2000 mg/kg body weight, none of the extracts produced any toxic symptoms of mortality.

Table 1: Effect of Crataeva religiosa leaf aqueous extract on Pentobarbitone induced sleeping time

Treatment	Onset of action (min)	Duration of action (min)
Control (Normal saline)	8.81±0.91	34.34±3.15
Chlorpromazine (3 mg/kg i.p.)	3.10±0.65**	54.99±0.62**
petroleum ether extract 0f Crataeva religiosa (200 mg/kg, p.o)	8.59±0.88	35.44±2.59
petroleum ether extract of Crataeva religiosaa (400 mg/kg, p.o)	7.33±0.90	37.04±3.77*
Chloroform extract of Crataeva religiosa (200 mg/kg, p.o)	7.81±0.83	38.44±3.50
Chloroform extract of Crataeva religiosa (400 mg/kg, p.o)	5.32±0.71*	44.74±4.87*
Methanol extract of Crataeva religiosa (200 mg/kg, p.o)	6.10±0.65*	41.81±3.08*
methanol extract of Crataeva religiosa (400 mg/kg, p.o)	4.01±0.59**	48.91±5.05**
Aqueous extract of Crataeva religiosa (200 mg/kg, p.o)	5.85±0.44*	43.80±5.21*
Aqueous extract of Crataeva religiosa (400 mg/kg, p.o)	4.25±0.40**	50.09±4.22**

All values are mean ± SEM; Statistical analysis by one-way ANOVA followed by Dunnet's multiple comparison test; *P < 0.05 **P < 0.01; N =6).

Table 2: Effect of Crataeva religiosa leaf extracts on locomotor activity, accessed using Actophotometer

Treatment	Locomotor activity for 10 min.	
	Before treatment	After treatment
Control (Normal saline)	416.11±42.90	401.94±39.25
Chlorpromazine (3 mg/kg i.p.)	461.77±40.36	119.91±19.06**
PCS (200 mg/kg, p.o)	404.23±34.08	340.78±31.56
PCS (400 mg/kg, p.o)	386.91±42.49	264.32±27.01
CCS (200 mg/kg, p.o)	377.32±32.89	266.45±15.77*
CCS (400 mg/kg, p.o)	426.91±48.12	201.59±20.42**
MCS (200 mg/kg, p.o)	460.05±41.47	247.21±25.71*
MCS (400 mg/kg, p.o)	391.81±32.09	126.73±21.66**
Aq.CS (200 mg/kg, p.o)	429.91±30.08	253.65±22.34*
Aq.CS (400 mg/kg, p.o)	451.20±39.14	145.43±18.62**

All values are mean ± SEM; Statistical analysis by one-way ANOVA followed by Dunnet's multiple comparison test; *P < 0.05 **P < 0.01; N =6.

Hence the drugs were considered safe for further pharmacological screening. So, according to the OECD-423 guidelines for acute oral toxicity, the LD50 dose of 2000 mg/kg and above is categorized as unclassified.

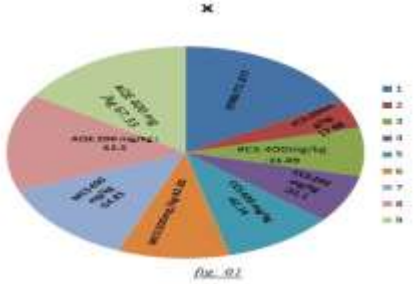

Figure: 1

Results of pentobarbitone induced sleeping time

The results showed that Aqueous extract of Crataeva religiosa leaf possess CNS depressant activity. Table 1 shows the effect of different extracts of Crataeva religiosa leaf. PCS (200 and 400mg/kg), MCS (200 and 400 mg/kg), CSI (400 mg/kg) produced significant reduction in the onset and prolongation of sleep duration induced by pentobarbitone. Aqueous extract (400 mg/kg) showed most potent effect (P<0.01) as it followed by the effect of methanol and chloroform extracts. Effects of Aqueous extract of Crataeva religiosa dose of 400 mg/kg are comparable with the effect produced by standard drug chlorpromazine. But CCS (200 mg/kg) and PCS (200 and 400 mg/kg) did not produce significant

decrease in onset of action and increase in duration of action.

Results of Locomotor Activity By Actophotometer

Results of locomotor activity were tabulated in Table 2. Extracts of Crataeva religiosa significantly decreased the locomotor activity in mice. The activity was found to be maximum for Aqueous extract at a dose of 400 mg/kg (67.33), and PSI (200 mg/kg) did not produced significant reduction in locomotor activity. MCS (200 and 400 mg/kg)-42.65&54.85, CCS (200 and 400 mg/kg) and PCS (200 and 400 mg/kg) produced 31.10, 42.160, 31.09, 17.98% decreased in locomotor activity (Fig.1), where standard drug chlorpromazine produced71.37% decreased in activity.

DISCUSSION AND CONCLUSION

This study has established the central nervous system depressant properties of Crataeva religiosa leaf. The study demonstrated that different extracts of Crataeva religiosa leaves caused an earlier onset of the effect of phenobarbitone (sleep latency) when compared with the control and it also increased the duration of action of pentobarbitone (sleeping time) significantly ($P<0.01$). Locomotor activity considered as an increase in alertness and decrease in locomotor activity indicated sedative effect.[19] Extracts of Crataeva religiosa leaf decreased locomotor activity indicates its CNS depressant activity. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidation their action through GABAA, therefore it is possible that extracts of Crataeva religiosa may acts by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts.[20] Many research showed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders.[21] Earlier investigation on phytoconstituents and plants suggests that many flavonoids and neuroactive steroids were found to be ligands for the GABAA receptors in the central nervous system; which led to the assume that they can act as benzodiazepine like molecules.[22] we conclude that Crataeva religiosa leaf possess CNS depressant activity and successive studies are mandatory to establish the precise nature of active constituents as well as their mechanism of action.

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