Original Article

Absence of central activity in *Wrightia tinctoria* bark ethanolic extract

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Abstract

Objective: The objective of the present study was to investigate the central activity profile of *Wrightia tinctoria* (Roxb) R.Br. Linn. (Family: Apocynaceae) in mice and rats using various models. **Materials and Methods:** The effects of ethanolic extract were observed in 3 different dose levels 300, 500, and 1000 mg/kg as the extract did not show any signs of toxicity up to 5000 mg/kg (p.o.) dose. Investigations were carried out for assessing the activity on pentobarbitone-induced hypnosis and maximum electro-shock (MES)- and leptazole-induced convulsions. **Results:** *W. tinctoria* ethanolic extract did not have any significant effect on pentobarbitone-induced hypnosis. The extract is devoid of any protective effect against leptazole- or MES-induced convulsions at any of the tested doses. **Conclusion:** *W. tinctoria* bark ethanolic extract had no central nervous system depressant or anticonvulsant activity.

Key words: Antiepileptic, sedative, *Wrightia tinctoria*, laptazole

INTRODUCTION

Wrightia tinctoria (Roxb.) R.Br. Linn. is a small deciduous tree belonging to the family Apocynaceae, distributed in Central India, Burma, and Timor. This plant is extensively used in the Indian system of medicine. Fresh leaves are pungent and are chewed for relief from toothache.[1] Bark and seeds are antidysenteric, carminative, astringent, aphrodisiac, and diuretic, and are used in flatulence, stomach pain, and bilious affections. Oil emulsion of *W. tinctoria* pods is used to treat psoriasis and they also have fungicidal activity against *Pityrosporum ovale*, which was recovered from dandruff.[2] Ethyl acetate, acetone, and methanol extracts of *W. tinctoria* bark showed antinociceptive activity in mice.[3] *W. tinctoria* bark ethanolic extract showed immunomodulatory and good antiulcer activity against experimentally induced acute gastric ulcers on rat along with moderate analgesic and anti-inflammatory activity. [4,5] Qualitative phytochemical investigation of crude plant extract revealed the presence of steroidal saponin, alkaloid, reducing sugar, tannins, and flavonoids.

A new sterol 14α-methylzymosterol in addition to 4 rare plant sterols, desmosterol, clerosterol, 24-methylene-25-methylcholesterol, and 24-dehydropollinastanol, have been isolated from *W. tinctoria* seeds.[6] The stem bark of *W. tinctoria* contains β-amyrin, lupeol, β-sitosterol, and a new triterpenoid.[7] In north and central India *W. tinctoria* is widely used to treat a number of ailments in traditional system of medicine; however, scientific data regarding its central effects are not available. Presence of steroid and triterpenoid in its ethanolic extract has provoked us to explore the possibilities of the central effects of *W. tinctoria*.

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MATERIALS AND METHODS

Collection and identification of plant material

W. tinctoria bark was collected from Hoshangabad district of Madhya Pradesh, India, during Sept–Nov 2003. The plants

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were identified with the help of the available literature and authenticated by Dr. A.P. Shrivastava, Principal, P.K.S. Govt. Ayurveda College and Institute, Bhopal, India. A voucher specimen was deposited in the herbarium department (*W. tinctoria*; No. 1084).

Preparation of ethanolic extract

Ethanolic (70%) extract of dried, milled coarse bark powder was prepared by cold maceration. The extract was filtered through muslin cloth and evaporated at 40°C up to one third of the initial volume, and the remaining solvent was completely evaporated using a rotary vacuum evaporator (Superfit, Mumbai, India). The extract was then weighed and percentage yield calculated. The color and consistency of the extract was noted and subjected to different tests to detect the presence of various phytoconstituents.[8]

Drugs

The drugs used in the study were obtained from the following sources: diazepam (Ranbaxy, Dewas, MP, India), pentobarbitone sodium (Sigma, St. Louis, USA), phenytoin (Parke Davis India Ltd, Mumbai, India), and phenobarbitone sodium (CDH, New Delhi, India). All the standard drugs were dissolved in water for injection and administered intraperitoneally (i.p.).

Experimental animals

Swiss albino mice (weighing 18–25 g) and Wister albino rats (weighing 150–200 g) of either sex bred in Animal House facility at the Department of Pharmacology, Radharaman College of Pharmacy, Bhopal, were used. The animals were housed under standard laboratory conditions and maintained at 24°C ± 1°C, relative humidity 50% ± 15%, and under 12:12 h light:dark cycle. Commercial pellet diet (Hindustan Lever, Delhi, India) and water were provided *ad libitum*. All the experiments were performed between 0900 and 1700 hours. Ethical Committee approval was obtained before carrying out these experiments on rats and mice.

Dried crude extract was freshly suspended in 2% (w/v) carboxy methyl cellulose prepared in distilled water and used as vehicle control. On the basis of the OECD guidelines, a *Limit test* was performed to categorize the toxicity class (LD_{50}) of the compound.[9] *Limit test* was performed at 2000 mg/kg, per oral (p.o.) and repeated at 5000 mg/kg in which it did not show mortality in rats. LD_{50} is greater than 5000 mg/kg. A dose range of 300, 500, and 1000 mg/kg were selected for evaluation of the pharmacologic activity. For all the studies, overnight fasted animals of either sex were divided randomly into 6 per group.

Effect on pentobarbitone-induced hypnosis in mice

Pentobarbitone (45 mg/kg, i.p.) was administered to the control and extract-treated animals after 30 min. Onset of sleep (loss of righting reflux) was noted and duration of sleep measured, which is the period between loss of righting reflux and its revival.[10] Diazepam (2 mg/kg, i.p) and the extract at different doses were given subsequently 30 and 45 min prior to pentobarbitone injection.

Effect on leptazole-induced convulsions in rats

All the animals were injected subcutaneously with 80 mg/kg of leptazole in the loose skin over the back, 1 h after the administration of the extracts and the standard drug diazepam (2 mg/kg, i.p.). The animals were observed for a further 1 h and the presence or absence of convulsions was recorded. The occurrence of facial or forelimb clonuses for more than 5 s was taken as the convulsion threshold.[11]

Effect on maximum electro-shock convulsions in rats

The test extracts, phenobarbitone sodium (45 mg/kg, i.p.) and phenytoin (120 mg/kg, i.p.), were given to the respective groups of animals 1 h before electro-shock and the time taken for each phase was observed. Antiepileptic activity on rats was measured using electro-convulsiometer (Techno, Haryana, India). A current of 150 mA strength was delivered to the animals using corneal electrodes for 0.2 s. The animals were placed on a table and its head was fixed. The eyes were made wet with normal saline solution and corneal electrodes were placed gently on the cornea. The shock was delivered by putting on the switch of the instrument and the animals were observed for the following: flexor component of tonic phase (extreme tonic flexion at limb joints with slight superimposed tremor), extensor component of tonic phase (extreme extension at all limb joints), intermittent jerky movements (clonic phase), and stupor phase. Time for each phase was noted using a stopwatch.[12]

Statistical analysis

Experimental data were analyzed using one-way ANOVA followed by Tukey–Kramer multiple comparison test. A P value less than 0.05 was considered statistically significant. GraphPad Prism Version 3.02 software (San Diego, CA, USA) was used for statistical calculations.

RESULTS AND DISCUSSION

Qualitative phytochemical investigation of crude plant extract (dark brown in color, yield 19.145% w/w) revealed the presence of steroidal saponin, alkaloid, reducing sugar, tannins, flavonoids, and absence of glycoside. *W. tinctoria* bark extract at a dose range of 300, 500, and 1000 mg/kg orally, had no effect on the general behavior of rats. All the activities of the test animals were normal.

The study of unstrained behavioral pattern of animals is

Table 1: Effect of *Wrightia tinctoria* ethanolic extract on pentobarbitone sodium-induced hypnosis on rats

Treatment (mg/kg)	Onset of action (min)	Average sleeping time (min)	Increase in sleeping time (%)
Vehicle control	6.45 ± 0.75	97.54 3.75	—
Diazepam (2) i.p.	2.06 ± 0.15**	240.75 ± 9.25***	146.82
W. tinctoria extract (300)	8.62 ± 0.90^{ns}	98.33 ± 4.24^{ns}	0.80
W. tinctoria extract (500)	6.17 ± 0.68^{ns}	100.54 ± 6.91^{ns}	3.07
W. tinctoria extract (1000)	6.32 ± 0.93^{ns}	104.06 ± 5.85^{ns}	6.68

****P* < 0.001 and ns = not significant when compared with control values. Values are expressed as mean ± SEM. i.p., intraperitoneally; p.o., per oral.

Table 2: Effect of *Wrightia tinctoria* ethanolic extract on leptazole-induced convulsion on rats

Treatment (mg/kg)	Convulsions	No. of dead animals
Vehicle control	Severe	2
Diazepam (2) i.p.	Protected	0
W. tinctoria extract (300)	Severe	2
W. tinctoria extract (500)	Severe	1
W. tinctoria extract (1000)	Severe	1

i.p., intraperitoneally; p.o., per oral.

Table 3: Effect of *Wrightia tinctoria* ethanolic extract on MES-induced convulsion on rats

Treatment (mg/kg)	Tonic convulsion (s)		Clonic convulsion (s)	Stupor phase (s)	Recovery or death
	Flexor	Extensor			
Vehicle control	7.73 ± 1.09	24.52 ± 2.17	31.46 ± 2.39	42.59 ± 3.17	R
Phenobarbitone (45) i.p.	1.12 ± 0.08***	2.37 ± 0.76***	3.76 ± 0.07***	14.15 ± 1.42***	R
Phenytoin (120) i.p.	Absent	Absent	6.23 ± 0.72***	10.45 ± 1.92***	R
W. tinctoria extract (300)	7.12 ± 1.43^{ns}	25.62 ± 3.67^{ns}	30.45 ± 5.36^{ns}	44.15 ± 6.42^{ns}	R
W. tinctoria extract (500)	6.34 ± 0.98^{ns}	23.78 ± 2.63^{ns}	32.61 ± 1.57^{ns}	40.72 ± 4.24^{ns}	R
W. tinctoria extract (1000)	6.75 ± 2.43^{ns}	22.47 ± 2.50^{ns}	28.35 ± 3.63^{ns}	43.89 ± 3.27^{ns}	R

****P* < 0.001 and ns = not significant when compared with control values. Values are expressed as mean ± SEM. MES, maximum electro-shock; SEM, standard error of mean; i.p., intraperitoneally; p.o., per oral.

one of the preliminary screening methods to investigate the effects of a new drug on the central nervous system (CNS). Depending on the effect produced by the drug, conclusion can be easily drawn about the nature of the drug. These observations help to plan other experiments to confirm the pharmacologic aspect of the drug. The fact that many neurosedative drugs tend to decrease sleep latency and increase sleeping time led us to assay the effect of the extract on pentobarbitone-induced hypnosis. The extract did not have any significant effect on pentobarbitone-induced hypnosis at any tested dose level. The standard drug diazepam extreme significantly potentiated pentobarbitone-induced hypnosis ($P < 0.001$) but has insignificant effect on the onset of sleep [Table 1].

W. tinctoria does not have protective effect against leptazole-induced convulsion at any tested doses [Table 2]. *W. tinctoria* extract was shown to be devoid of anticonvulsant activity against maximum electro-shock convulsions at any tested dose levels [Table 3]. Interpretation of the study data showed negative implications of *W. tinctoria*'s central effect. Pentobarbitone-induced hypnosis is used as a preliminary screening method to establish CNS activity pattern of a substance under study, categorizing it as a stimulant or a depressant. This study was a preliminary trial to establish unexplored CNS effects of *W. tinctoria*, although other exploratory trials can also be performed for confirmation. In the traditional system of medicine, *W. tinctoria* is used for treating a number of ailments but not related to CNS, signifying a lack of central activity in this plant. The results of this study are also in accordance with the traditional belief. The present finding will be helpful for the future researcher discouraging repetitive study on CNS activity profile of *W. tinctoria*. Change in phytochemical profiling of *W. tinctoria* using nonpolar solvent system or different extraction method can be explored for central activity.

CONCLUSION

W. tinctoria bark extract when administered orally, up to a dose of 1000 mg/kg in albino rats, showed no sign of CNS depression or stimulation. *W. tinctoria* bark ethanolic extract did not have any effect on pentobarbitone-induced sleeping latency. Although several phytosterols have been

isolated from *W. tinctoria* bark, the ethanolic extract had no anticonvulsant activity, which revealed that it is devoid of any CNS activity.

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