NEUROPHARMACOLOGICAL ACTIVITY OF *DOLICHANDRONE ATROVIRENS* (ROTH) LEAF EXTRACTS IN SWISS ALBINO MICE

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ABSTRACT

The aim of the present study was to evaluate the sedative and anxiolytic property of the leaves of the *Dolichandrone atrovirens* (*D. atrovirens*) The chloroform and ethanolic extracts of leaves were analysed for their phytochemical constituents by phytochemical screening and thin layer chromatography studies. The acute toxicity in albino mice showed no clinical signs of toxicity in the 2000 mg kg^{-1} dose administration orally. The extracts were orally administered to the group of animals to study the neuropharmacology activity, such as sleep induced by drugs methods, spontaneous motor co-ordination, locomotor activity, hole board test and light dark test. The extracts significantly ($p<0.001$) prolonged the sleeping time of mice, reduced motor co-ordination and anxiety. The results obtained from these experimental models confirmed that chloroform and ethanolic extracts of leaves of *D. atrovirens* possess central nervous depressant activity and anxiolytic activity. The ethanolic extract shows better activity compared to chloroform extract.

Keywords: *Dolichandrone atrovirens,* chloroform, ethanol extract, CNS depressant activity, anxiolytic activity

INTRODUCTION

Mental, neurological and behavioral disorders are familiar to all countries and cause vast suffering throughout the world. According to the WHO, around 450 million people currently suffer from serious brain/mental or behavioral disorders. In India, one in seven people were affected by mental disorders of varying severity in 2017[1].

People with these disorders are often subjected to social segregation, poor eminence of life and higher mortality. Habituation, dependence and the resulting potential for addiction are the greater disadvantages of modern synthetic psychopharmacological agents. The sudden discontinuation of long-term therapy with these drugs leads to serious withdrawal symptoms[1]. Therefore, modern society is now cautiously working on traditional herbal medicines, particularly the current studies for even better properties than the conventional medicines. In the search for new therapeutic agents for the treatment of neurological disorders, work on medicinal plants demonstrates the pharmacological effectiveness of plant species in different animal models[2].

Neuropharmacology has two primary divisions, behavioural and molecular neuropharmacology. A psychological disorder is a condition characterized by the behavioural neuropharmacological intervention through irregular thinking, emotions and actions. Even a small disturbance to a neuron's structural pathway can result in dysfunction. As a result, neurological disorders can result from a number of causes, such as lifestyle-related, infections, genetics, nutritional, environmental influences and physical injuries.

D. atrovirens (Family: Bignonciaceae) is commonly known as wavy trumpet flower tree in English. It is found abundantly in wild areas of Kerala, Tamil Nadu, Andhra Pradesh, Maharashtra and Karnataka. Mainly leaf, bark and flower have medicinal uses. *D. atrovirens* has been widely used in folk medicine for a long time. The anti-diabetic, anti-oxidant, anti-cancer, hypo lipidemic and wound healing activity of this plant has already been reported[3-4]. Traditional and alternative medicine research has gained massive interest over the past 20 years, as it is easily accessible in some regions[5]. However, till now very little attention has been paid to develop functionally active CNS active drugs from psychoactive plants.

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D. atrovirens has not been explored for neuropharmacological activity in a scientifically evidenced manner and the literature reports are limited. In this study, chloroform and ethanol leaf extracts of *D. atrovirens* were evaluated for their neuropharmacological activity in albino mice.

MATERIALS AND METHODS

Solvents and chemicals

Chloroform, ethanol, Tween 80 and silica gel (TLC grade) were obtained from the CDH (Central Drug House), New Delhi. Diazepam (Calmpose inj. 5mg mL^{-1}) from Ranbaxy Laboratories was used for the study.

Plant collection and extraction

The leaves of the plant *D. atrovirens* were collected locally from the campus of Kalasalingam University, Krishnankoil (Virudhunagar Dist, Tamil Nadu) during the month of July. The plant was identified and authenticated by Department of Botany, The American College, Madurai.

Drying and size reduction of the plant

The leaves of the plant *D. atrovirens* were subjected to shade drying for about 1 week. The dried plant material was further crushed to powder and the powder was passed through sieve mesh 44 and stored in air-tight container for further analysis[6].

Extraction of the plant material

The dried coarse powder of leaves, about 800 g, was subjected to extraction with 1 L, of chloroform by using Soxhlet apparatus. After 72 h, the extract was collected by filtration, the marc was separated for further extraction. After the extraction was completed, it was allowed to distill for the further separation of solvent and to concentrate the extract. Finally, a dark green color residue as chloroform extract of *D. atrovirens* (CEDA) was obtained. The marc left after chloroform extraction was dried and subsequently extracted with 1 L, of ethanol by using Soxhlet apparatus. After the completion of extraction, it was filtered and the solvent was redistilled. Finally, a dark green color residue was obtained called as ethanolic extract of *D. atrovirens* (EEDA).

Qualitative phytochemical analysis

The phytochemical analysis of the chloroform and ethanol extracts of *D. atrovirens* leaves was analyzed by standard methods. The extract was analyzed to obtain secondary metabolites such as alkaloids, terpenoids, tannins, flavonoids, aminoacids, saponins, aromatic acids, phenolic compounds, triterpenoids, xantho proteins, carbohydrates, reducing sugars and proteins.

Thin layer chromatography

Thin layer chromatography is an universal analytical technique and is widely used by analysts and research workers[7]. When a mixture containing different components is made to ascend in a TLC plate with the help of a solvent which acts as mobile phase,there will be a preferential adsorption of different components at different places on the plate. The result is the separation of components. The chloroform and ethanol extracts were added as spots on TLC plates using capillary tubes on the one end of the thin layer plate. Plate was allowed to air dry and then it was placed in a beaker containing solvent mixture of chloroform: ethanol in the ratio of 9:1(V/V). The samples were allowed to separate towards the other end of the plate. The sheet was removed, allowed to air dry and Dragendroff's reagent was sprayed. The plate was then visualized for presence of colour spot on the plate and the R_f value was calculated[7].

$$R_f \text{ value} = \frac{\text{Distance travelled by the solvent}}{\text{Distance travelled by the solute}}$$

Animals

Healthy Swiss albino mice of male sex weighing 20-25 g were used for these studies. The albino mice were obtained from the animal house of JIPMER Institutional animal house (JIPMER, Puducherry-6, Tamil Nadu.) India. The animals were kept in colony cages and maintained under standard environmental conditions. The animals were fasted overnight and during the experiment. The Institutional Animal Ethical Committee approved (approval no: AKCP/IAEC/011/16-17) the protocol of the study. In each experiment, apparatus was cleaned using 5% ethanol before introducing the next animal to preclude the possible cueing effects of odours left by previous subjects.

Selection of test doses and acute toxicity study

The acute toxicity study of herbal extracts was done according to the Organisation for Economic Co-operation and Development (OECD) guideline 423.The adverse effects should occur within 14 days of the administration of the substance. Overnight fasted, healthy rats (n = 6) were administered orally the chloroform and ethanolic extract of leaves of *D.atrovirens* in the dose of 2000 mg kg^{-1}(p.o.) body weight and observed continuously for 4

h and after 24 h for any abnormality and mortality for 7 days[8-10].

Neuropharmacological evaluation

Potentiation of diazepam-induced sleeping time

For evaluation of CEDA and EEDA for their sedative effects, diazepam induced sleep models was used. Swiss albino mice were randomly divided into six groups with each group having five mice. First group (Group I) served as the negative control (treated with vehicle, 2.5% Tween 80 orally), Group II served as a positive control which was treated with diazepam (2 mg kg^{-1}, i.p), Group III and IV were treated with CEDA with the dose of 200 and 400 mg kg^{-1}, per orally (p.o.) respectively. Groups V and VI were treated with EEDA with the dose of 200 and 400 mg kg^{-1}, p.o respectively. Thirty minutes later, the animals were post-treated with diazepam administered at 2 mg kg^{-1} (i.p.). The onset and duration of sleep were recorded for each animal. The loss and recovery of righting reflex was taken as the endpoint[11].

Spontaneous motor activity

The animal locomotor activity of experimental groups was determined using digital actophotometer. Animals were placed in the actophotometer individually, and basal activity score was recorded over the period of 5 min. Each group of animals was treated with respective drug, and the score was recorded at time intervals of 30 minutes. Decreased activity of score was taken as an endpoint[12].

Table I: The secondary metabolite constituents of chloroform and ethanolic leaf extracts

Phytochemical constituent	Chloroform extract	Ethanolic extract
Carbohydrates	+	+
Glycosides	+	+
Proteins & Amino acids	+	+
Tannins & phenolics	+	+
Terpenoids	+	+
Flavonoids	+	+
Phytosterols	-	+
Fixed oils & Fats	+	+
Alkaloids	-	+
Saponins	+	+
Gums & Mucilages	-	-

+ indicates the presence of constituents; Indicates the absence of constituents

Motor co-ordination

Rota rod is a biological research apparatus, used to evaluate the activity of drugs that interfere with motor co-ordination. The animals were placed on rod rotating at 16 rpm speed. The mice capable of remaining on the top for 3 min or more, in three successive trials, were selected for the study. The fall of time was recorded in all the groups upto 120 mins (at the time interval of 30 mins) and significant reduction in motor coordination was considered as end point[13].

Exploratory behavior pattern using hole board test

Test for exploratory behavior in mice was performed by (i) Open field test (ii) Hole cross test and (iii) Hole board test. The hole board test is an experimental method used in scientific research to measure anxiety, stress, and emotionality in animals. The study was carried out using a wooden box with 16 equidistant spaced holes in 3 cm diameter in the floor. The apparatus was elevated to the height of 25 cm. Each group was treated with the drugs under study. Decreased head dip response in mice at various drug treated group was determined[14-15].

Light-dark test

The light/dark transition test was based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors. Light–dark test consists of two compartments, namely light and dark. After the drug treatment to individual group, mice were placed in the centre of light compartment with their back to dark compartment and finally the number of transition behavior and the time spent in the dark area over 5 min period was observed[16].

STATISTICAL ANALYSIS

The results are expressed as mean ± SEM, (n=5), from six observations as compared to standard group the one way ANOVA followed by Dunnett's test by prism software. A level of significance ($p < 0.001$) was considered statistically significant.

RESULTS AND DISCUSSION

Insomnia, hallucination, anxiety, seizures and mental health issues, in general, and senile neurological disorders, in particular are widely prevailing in modern fast-paced life with a mass of stressful conditions. Nowadays, the available psychotherapeutic medications do not fulfill the therapeutic demands completely and the need for natural based psychotherapeutic products is growing.

In the present study, a total yield of 3.8 % (w/w) was obtained from the chloroform and ethanolic leaf extracts of *D. atrovirens*. The yield for the chloroform extract and ethanolic extract are 1.85% and 1.95% (w/w), respectively and the color of the extracts was observed. The preliminary phytochemical screening of chloroform and ethanolic extracts shows the presence of carbohydrates, glycosides, aminoacids, tannins, phenols, flavonoids and proteins. (Table I). For the extracts, thin layer chromatography was done for the presence of phytochemical constituents. Fig. 1, 2. The R_f value of the extracts is reported (Table II).

Table II: Thin layer chromatography R_f values of leaf extracts of *D. atrovirens*

S.No	Extracts	R_f value
1	CEDA	0.8
2	EEDA	0.88

CEDA-.Chloroform extracts of D.atrovirens; EEDA- Ethanolic extracts of D. atrovirens; R_f value-Retention factor value

Table III: Potentiation of diazepam-induced sleeping time

Group	Treatment	Onset of action (mins)	Duration of action (mins)
I	Control	7.5 ± 1.15	53.6 ± 5.6
II	Diazepam (2 mg kg^{-1}) i.p.	3.08 ± 0.79**	101.8 ±4.81**
III	Chloroform extract (200 mg kg^{-1}) p.o.+diazepam (2 mg kg^{-1}) i.p.	6.3 ± 0.82	51.4 ± 2.1
IV	Chloroform extract (400 mg kg^{-1}) p.o.+diazepam (2 mg kg^{-1}) i.p.	5.27 ± 0.17*	70.2 ±3.27
V	Ethanolic extract (200 mg kg^{-1}) p.o.+diazepam (2 mg kg^{-1}) i.p.	5.14 ± 0.38*	73.2 ±5.06*
VI	Ethanolic extract (400 mg kg^{-1}) p.o.+diazepam (2 mg kg^{-1}) i.p.	4.45 ± 0.36**	90.2 ±5.54**

*Results are expressed as mean ± SEM,(n=5), from six observations as compared to standard group the one way ANOVA followed by Dunnett's test.**Values are significantly different at P<0.001, * Values are significantly different at P<0.01*

As per OECD guideline 423, acute toxicity studies were carried out to evaluate the toxicity and to determine the minimum lethal dose of the drug extracts using mice. It was found that lethal dose was assigned to be more than 2000 mg kg^{-1}. The doses of 200 and 400 mg kg^{-1} were selected as experimental doses of extracts for the studies.

In the study, the ethanolic extract of *D. atrovirens* at both doses and chloroform extract (400 mg kg^{-1} dose) show the prolongation of diazepam – induced sleeping time. The prolongation of diazepam-induced sleeping time may be attributed to an action of extracts on the central mechanisms involved in the regulation of sleep. So both the extracts of *D.atrovirens* leaves possess significant (P<0.001) CNS depressant activity (Table III).

The ethanolic and chloroform extracts of *D.atrovirens* produced central inhibitory effects in mice. The ethanolic extracts significantly (P<0.001) reduced the spontaneous motor activity in mice.The decrease in locomotor activity indicates the level of excitability of the CNS and this decrease may be closely related to sedation resulting from depression of the CNS (Table IV). The lack of co-ordination in the rota rod test is characteristic of a drug that reduces the central nervous activity, such as neuroleptics, anxiolytics, sedatives and hypnotics. The ethanolic extract at both dose levels shows significant (P<0.001) changes in motor activity. The chloroform extract at the dose of 400 mg kg^{-1} also shows activity (Table V).

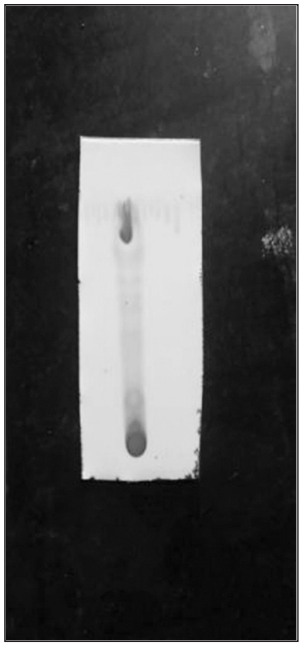

Fig. 1: TLC of chlroform extract

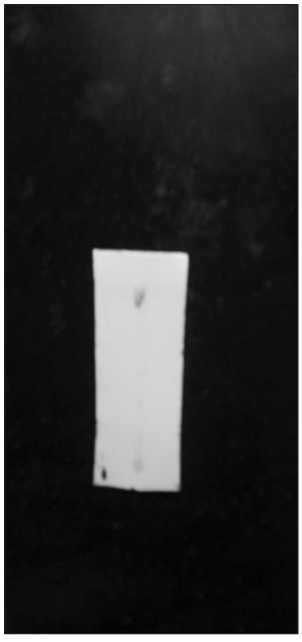

Fig. 2: TLC of ethanolic extract

Table IV: Spontaneous motor activity

Group	Treatment	Experimental mean time (5 min)				
		0	30	60	90	120
I	Control	315.25±8.63	314.05±5.22	305.95±6.73	311.75±8.33	305.7±14.09
II	Vehicle and diazepam (2 mg kg^{-1}) i.p.	312.6±9.34	118.4±12.91**	59.15±7.35**	42.05±8.05**	64.4±15.24*
III	Chloroform extract (200 mg kg^{-1}) p.o.	319.4±3.64	221.8±3.70	145.50±13.0*	131.65±7.19	162.3±5.26
IV	Chloroform extract (400 mg kg^{-1}) p.o.	322.8±6.49	183.4±8.08*	138.6±14.79	123.6±13.09	145.45±10.26
V	Ethanolic extract (200 mg kg^{-1}) p.o.	321.1±4.56	176.4±10.94*	126±28.01**	89.1±7.07	120.4±8.93
VI	Ethanolic extract (400 mg kg^{-1}) p.o.	315.4±11.56	148.65±12.2**	111.35±6.84**	55.9±9.48**	108.1±11.31

Table V: Time spent by mice in the rota–rod apparatus for different groups

Group	Treatment	Time spent on rods (5min)				
		0	30	60	90	120
I	Control	212.7±9.91	214.6±5.60	212.6±2.54	216.3±5.41	217±11.84
II	Vehicle and diazepam (2 mg kg^{-1})i.p.	212.5±5.89	48.95±11.31**	25.75±6.41**	74±7.24**	106.4±7.40**
III	Chloroform extract (200 mg kg^{-1})p.o.	216.1±3.47	132±9.10	91.6±14.1	125.8±6.49	162.4±7.43
IV	Chloroform extract (400 mg kg^{-1})p.o.	215.85±4.92	120±15.85*	74±12.38*	113.3±9.73	150.6±5.94
V	Ethanolic extract (200 mg kg^{-1})p.o.	213.25±8.36	85±21.38	63.8±8.46**	101.95±9.5	123.4±6.06
VI	Ethanolic extract (400 mg kg^{-1})p.o.	208.2±4.95	77.4±51.28	45±7.84**	88.1±4.97**	114±7.34

Table VI: Exploratory behavior pattern (Hole board test)

Group	Treatment	Number of head dippings in 5 mins	
		Before treatment	After treatment
I	Control	18±2.34	18±0.89
II	Vehicle and diazepam (2 mg kg^{-1}) i.p.	18±1.48	24±1.51**
III	Chloroform extract (200 mg kg^{-1}) p.o.	16±1.67	23±1.14
IV	Chloroform extract (400 mg kg^{-1}) p.o.	18±1.58	25±2.23*
V	Ethanolic extract (200 mg kg^{-1}) p.o.	17±1.73	29±1.14**
VI	Ethanolic extract (400 mg kg^{-1}) p.o.	19±1.92	33±2.04**

*Results are expressed as mean ± SEM,(n=5),from six observations as compared to standard group the one way ANOVA followed by Dunnett's test.** Values are significantly different at P<0.001, * Values are significantly different at P<0.01*

Table VII: Time spent in light area and number of transitions between light/dark compartments for different groups

Group	Treatment	Time spent in light area (sec)	Number of transition between light and dark(or)tunnel crossing
I	Control	97.2± 11.82	15±2.730
II	Vehicle and diazepam (2 mg kg^{-1})i.p.	186.2± 9.17**	15.4± 2.30**
III	Chloroform extract (200 mg kg^{-1})p.o.	129.8± 7.85	25± 1.58
IV	Chloroform extract (400 mg kg^{-1})p.o.	141.6± 8.44	22± 1.58
V	Ethanolic extract (200 mg kg^{-1})p.o.	157± 7*	18± 0.83**
VI	Ethanolic extract (400 mg kg^{-1})p.o.	163.4± 8.20**	17.6± 2.07**

It might be acting as a mild neurosedative. The reduction in motor co-ordination might also be a result of skeletal muscle relaxation. Therefore, the observed skeletal muscle relaxation activity may be attributed to flavanoids present in the extracts.

The hole board test is useful for modeling anxiety in animals. The head dipping behavior is sensitive to changes in the emotional state of the animal. The extracts of *D.atrovirens* significantly (P<0.001) increased the head dipping behavior compared to control. These results shows that both the extracts of *D. atrovirens* cause their sedative behavior. The chloroform extract (400 mg kg^{-1}) produced less effect, compared to the ethanolic extract of the plant (Table VI).

In the light / dark test, anxiety is generated by the conflict between the tendency to explore and the initial tendency to avoid the unfamiliar and can be evaluated according to the number of transitions into and the time spent in the light chamber. The increase in these parameters was considered to reflect anxiolytic-like properties. The *D. atrovirens* extracts show the increased time spent in the light chamber, suggesting significant (P<0.001) anxiolytic action (Table VII).

Sedation and anxiety are primarily mediated in the CNS by the γ aminobutyric acid (GABA)-A receptor complex, which is also involved in other physiological and neurological disorders such as epilepsy, depression, Parkinson syndrome and Alzheimer's disease. Diverse drugs that are used in these pathologies might modify the phenomena of GABA system at various levels, including the synthesis of GABA mediators, release or re-uptake or metabolism[17,18].

Several experiments with some natural and synthetic flavones have shown that they can modulate GABA-generated chloride current, either positively or negatively. The preliminary phytochemical studies about the leaf extracts of the *D. atrovirens* confirms the presence of flavanoids, glycosides, polyphenols etc. Existing literature support for *D. atrovirens* also confirms the presence of flavones, rutin, quercetin, ferulic acid, gallic acid, flavononones and others. Other components contributing to CNS depression reported in the literature for *D. atrovirens* are sterols[19-21].

Many flavonoids were found to be ligands for the GABA -A receptors in the CNS, which led to the hypothesis that they act as benzodiazepine like molecules[22]. This is supported by their behavioral effects in animal models of anxiety and sedation activity. Therefore, they may exhibit CNS depressant activity[23,24].

CONCLUSION

The results of this report, suggest that herbal remedies remain the ultimate therapeutic hope for safe neuro pharmacological activity. Chloroform and ethanolic leaf extracts of *D. atrovirens* potentiated diazepam induced sleep and they decreased spontaneous motor activity, indicating a central depressant effect. Both the extracts of *D. atrovirens* reduced motor co-ordination in mice. This motor coordination helped to conclude that the extracts are having neuromuscular blocking activity. Finally, the study shows the marked effect on anxiety behavioural parameters on exposure to light /dark test and in the hole board test in mice. The results obtained from these experimental models clearly confirmed that the chloroform and ethanolic extract of leaves of *D. atrovirens* possess CNS depressant activity and anxiolytic activity. Further thorough research into isolation and characterization of active principles from *D.atrovirens* are required for the successful drug development for clinical use. In depth studies are also required to determine the possible mechanisms of action.

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