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The effect of *Wrightia tinctoria* leaves on behavioral health

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ABSTRACT

The study has been designed to get better understanding about the actions of aqueous, alcohol extract and crude powder of **Wrightia tinctoria** leaves on the behavioral health of wistar albino rat. Spontaneous motor activity of *Wistar albino* rats, Motor coordination in rats, Assessment of learning and memory in rats.

The animals were divided into 4 groups, each group with 6 animals. Following is the treatment allocation for the groups:

Group –I control group 1.0 ml-distilled water (vehicle) was administered

Group –II was administered aqueous extract (200 mg/kg) in 1.0 ml of vehicle.

Group -III was administered leaf powder (500mg/kg) in 1.0 ml of vehicle.

Group -IV was administered alcohol extract (200 mg/kg) in 1.0 ml of vehicle.

The data obtained were assessed for their statistical significance by using the tukey's test of one-way **ANOVA**.

There is no statistical significant difference ($p < 0.005$) occurs between control and study groups in all the three parameters under study. No significant alteration has been noticed in the motor activity of the animals subjected to treatment with the test substances as indicated by the results of the photoactometer analysis. Similarly motor coordination has been found to be unaltered following treatment with the leaf powder or extracts under investigation. Treatment with leaf extract or powder did not alter learning and memory in rats as indicated by the results of the maze test.

Absence of any alteration in the behavioral parameters are indicative of lack of CNS influence by the plant.

Keywords: *Wrightia tinctoria*, Behavioral health, *Wistar albino* rats.

INTRODUCTION

The medicinal plant "***Wrightia tinctoria***" quoted to possess a variety of medicinal properties, has been selected for the present study to evaluate its behavioral effect in wistar albino rat.

Plant description

Division	: Plant Kingdom
Sub division	: Gamopetalae
Class	: Dicotyledones
Subclass	: Sympetalae

Serius : Bicarpellatae
Order : Gentianales
Family : Apocynacae
Genus : *Wrightia*
Species : *tinctoria*

Chemical Properties

Wrightia tinctoria seeds contains*:* (TOSHIHIRO AKIHISA et al., 1988)

14-methylzymosterol, desmosterol, clerosterol, 24-methylene-25-methylcholesterol and 24-dehydropollinastanol

Acetate Composition Sterol

a. Cholesterol
b. Desmosterol (24-dehydrocholesterol)
c. 24-Methylcholesterol
d. 24-Methylenecholesterol
e. 24-Ethylcholesterol
f. 24-Ethyl-22E-dehydrocholesterol
g. Isofucosterol (24Z-ethylidenecholesterol)
h. Clerosterol
i. 24-Methylene-25-methylcholesterol
j. 24-Dehydropollinastanol
k. 14-Methylzymosterol

Wrightia tinctoria bark contains. (REDDY Y.S.R. et al 2002)

Triterpenes, triacontanol, tryptanthrin, steroids, triterpenoids, flavonoids and glycosides.

USES OF WRIGHTIA TINCTORIA

The leaves are applied as a poultice for mumps and herpes and sometimes, they are also munched to relieve toothache. *Wrightia tinctoria* is called *dhudi* (Hindi) because of its preservative nature. A few drops of its sap in milk prevent curdling and enhance its shelf life, without refrigeration. In folk medicine, the dried and powdered roots of *Wrightia* along with *Phyllanthus amarus* (*keezhanelli*) and *Vitex negundo* (*nochi*) is mixed with milk and orally administered to women for improving fertility. The bark and seeds are effective against psoriasis and non-specific dermatitis. It has anti-inflammatory and anti-dandruff properties and hence it is used in hair oil preparations(Krishnamoorthy JR, and Ranganathan, 2000) [1].

The ethnobotanical uses of *Wrightia tinctoria* in traditional medicine include; its use in tooth ache and abdominal pain; as a vermifuge, antidiarrhoeal, antibacterial (Jolly RBR and Mechery CI, 1996), antifungal (Vijayakumar R, et al 2006). antihaemorrhagic and as a hepatoprotective agent(Bhattacharyya D. et al 2003).

SCOPE OF THE PRESENT STUDY

The present study has been designed to get better understanding about the actions of aqueous, alcohol extract and crude powder of *Wrightia tinctoria* leaves on the behavioral health of rat [2].

MATERIALS AND METHOD

Method of Extraction From *Wrightia Tinctoria* Leaves

Immediately after collection, the leaves were washed in tap water twice and in distilled water once to remove all the external dust, dirt, and unwanted materials. The leaves were than dried under shade for 72 hrs. Small bits of plants materials; petioles, mid ribs and twins were removed after shade drying. The dried leaves were crushed by hand to crude powder.

300 gm of this powder was soaked in 2 liters of rectified spirit for 1 day. The soaked leaves were allowed to undergo natural percolation under occasional shaking twice a day at an interval of 6 to 8 hours during daytime. The leaf extract was filtered using watman no.1 filter papers and the filtered extract was evaporated to dryness at 60 °C over a water bath. The yield of the alcoholic leaf extract was between 3 to 5 percent. This extract was found to be sparingly soluble in water; but soluble in alkalized water (0.05N NaOH). This alcoholic leaf extract preparation was used in this study.

400 gm of this powder was soaked in double distilled water for 2 days. The soaked leaves were allowed to undergo natural percolation under occasional shaking twice a day at an interval of 6 to 8 hours during daytime. The leaf extract was filtered using watman no.1 filter papers and the filtered extract was evaporated to dryness at 95 ° C over a water bath. The yield of the aqueous leaf extract was between 8 to 12 percent. This extract was found to be soluble in water [3].

Animals

Healthy Wistar albino rats of both sexes not previously used for other studies were procured from

central animal house facility. DR.A.L.M.PGIBMS, Chennai.

Study Design

The study was designed

➢ To test the learning and memory activity of rats on aqueous extract, crude powder and alcohol extract of *Wrightia tinctoria leaves.*

➢ To test the effect of drug (aqueous extract, crude powder and alcohol extract of *Wrightia tinctoria leaves*) on motor coordination in rats.

➢ To Determine the spontaneous motor activity of rats treated with aqueous extract, crude powder and alcohol extract of *Wrightia tinctoria leaves.*

➢ Wistar albino rats with a body weight of 150-260 gms were selected and divided into 4 groups. Each group consists of 6 animals as explained in Table 1 [4].

Table 1. Evaluation of behavioral effect on aqueous extract, crude powder and alcohol extract of plant
***Wrightia tinctoria* leaves**

Group (n=6)	Treatment (Day)
Group I	Normal control – Distilled water - 1ml/day for 21 days..
Group II	Aqueous extract of plant *Wrightia tinctoria* leaves – 200mg/kg/day for 21 days
Group III	Crude Powder of plant *Wrightia tinctoria* leaves – 500mg/kg/day for 21 days
Group IV	Alcohol extract of plant *Wrightia tinctoria* leaves – 200mg/kg/day for 21 days

Aqueous leaf extract: 200mg/ kg P.O. in distilled water
Crude powder: 500mg/ kg P.O. in distilled water
Alcohol leaf extract: 200mg/ kg P.O. in 0.05N NaOH

Statistical analysis

The control distilled water group values were used to compare the values obtained with the Aqueous extract, crude powder and alcohol extract of *Wrightia tinctoria* leaves. The data obtained were assessed for their statistical significance by using the tukey's test of one-way **ANOVA**.

Hebb – William's Maze

This instrument is used to test the learning and memory. The apparatus consists of a two chambers A and B, separated by a maze. The floor and the walls of chambers are wooden and painted white. The Maze used was elevated to a height of 100 cm above floor level and kept in a table.

PROCEDURE

Testing was conducted in a quiet room that was illuminated by a dim light. The treatments were randomized throughout the day, between 08:00 and 13:00 h, to control the diurnal variations in animal activity. A rat was placed in chamber A and allowed to explore the maze and the reward is provided at chamber B. Lights on the maze will indicate the location of the animal. The time taken by the animals to traverse the maze and to reach the food compartment will be the criterion for successful learning and memory activity. Trained animals are treated with the test drug or the vehicle before 90 minutes start of test. If the traversing time is shorter than control, it shows that memory has been improved or the activity of the animals is stimulated. If longer than the control, it shows that memory of the animal has been impaired or activity has been depressed. The animal may or may not attempt to traverse the maze if memory is totally impaired or the animal is depressed due to CNS depressant action of the drug or due to motor impairment or due to loss of appetite.

EFFECTS ON THE PERFORMANCE ON THE ROTA ROD

This method is also known as "Rota rod test". This method is used to assess the ability of the animals of control and treated groups of Wistar albino rats to maintain equilibrium and muscle control on a rotating rod.

PROCEDURE

Test period of 180 seconds is allowed to each animal. The number of animals standing successfully or falling down during the test period can be recorded. If there is any significant reduction in endurance time or if more number of animals in test groups is falling during the test then it may be concluded that the test compound has impaired motor system of animal.

MEASUREMENT OF SPONTANEOUS MOTOR ACTIVITY OF RATS

Any assessment of the behavior effects of drug must include information on its effect on spontaneous motor activity. In this study the spontaneous motor activities were measured by using the Actophotometer.

PROCEDURE

In this study, each animal activity was tested for 2 min before treatment and once weekly for 3 consecutive weeks after treatment. On the day of the test, after 90 minutes of last dosage the activity of animals was measured for their ambulatory behavior for 2 min after a 5-min habituation period in the cage, the activity meter was zeroed and counts were then taken. The measurements were taken between 08:00 and 13:00 h to reduce the confounding influence of diurnal variation in motility.

RESULT & DISCUSSION

Table – 2: Behavioural Studies Parameters

S.No	Behavioral Parameters	Group – I	Group – II	Group –III	Group – IV
I	MAZE (Sec)				
a	Before drug Administration	237.33±56.17	148.33± 114.53	219.17±125.96	125.83±124.48
b	After treatment (7th day)	154.17±89.1	86.5± 88.14	233.5±60.74	166.17±101.98
c	After treatment (14th day)	131.5±132	102.17± 86.01	270.17± 73.08	82.5±111.76
d	After treatment (21st day)	80.67±108.45	204.5± 101.48	174± 128.9	159.33±123.92
II	ROTAROD (Sec)				
a	Before drug Administration	72±24.03	85.67± 63.98	97.33± 55.84	129.5± 57.91
b	After treatment (7th day)	59±29.77	133.5± 55.56	123.17± 63.72	137± 54.49
c	After treatment (14th day)	119.33±74.48	105± 59.65	67.83± 57.24	134± 51.82
d	After treatment (21st day)	123.83 ±62.53	140.33±52.59	101±56.57	135.17±50.50
III	PHOTOACTOMETER(Counts)				
a	Before drug Administration	107.71± 19.71	118.62± 13.71	103.17± 31.48	98.83±23.23
b	After treatment (7th day)	81± 37.91	107.33± 17.95	89.17±34.56	83.33±33.48
c	After treatment (14th day)	85.83± 42.97	115.83± 30.04	85.33± 47.18	92.83±23.58
d	After treatment (21st day)	91.83±36.31	92.17± 24.94	92.67± 51.04	76.17±15.2

Values presented are Mean ± SD; n = 6.
Group I = control 1ml of distilled water;   Group II = Aqueous extract 200 mg/kg;
Group III =Whole leaf powder 500 mg/kg;   Group IV = Alcohols extract 200 mg/kg

Table – 3: Behavioural Parameters After Treatment Expressed As Percent (Oral Administaration)
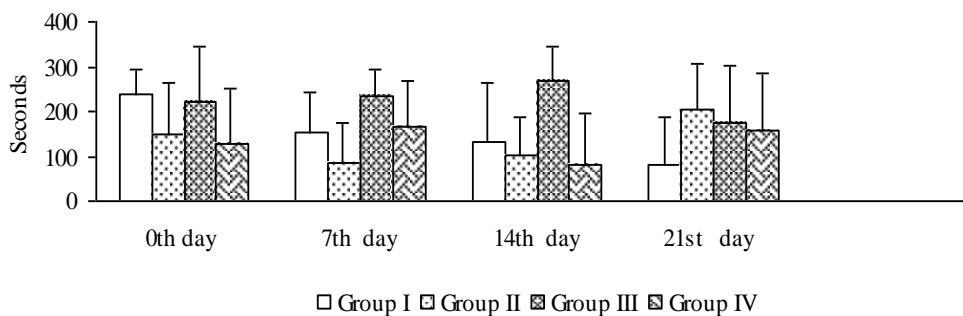
S.No	Behavioral Parameters	Group – I	Group – II	Group –III	Group – IV
I	MAZE (Sec)				
A	Before drug Administration	100.00	62.5	92.34	53.02
B	After treatment (7th day)	100.00	56.11	151.46	107.78
C	After treatment (14th day)	100.00	66.27	205.45	62.74
D	After treatment (21st day)	100.00	253.5	215.69	197.51
II	ROTAROD (Sec)				
A	Before drug Administration	100.00	118.99	135.18	179.86
B	After treatment (7th day)	100.00	226.27	208.76	232.20
C	After treatment (14th day)	100.00	87.99	56.84	111.29
D	After treatment (21st day)	100.00	113.32	81.56	109.16
III	PHOTOACTOMETER (Counts)				
A	Before drug Administration	100.00	110.13	95.78	91.76
B	After treatment (7th day)	100.00	132.51	110.09	102.88
C	After treatment (14th day)	100.00	134.95	99.42	108.16
D	After treatment (21st day)	100.00	100.37	100.91	82.95

Values presented are Mean ± SD; n = 6. Significance at $*p < 0.05$

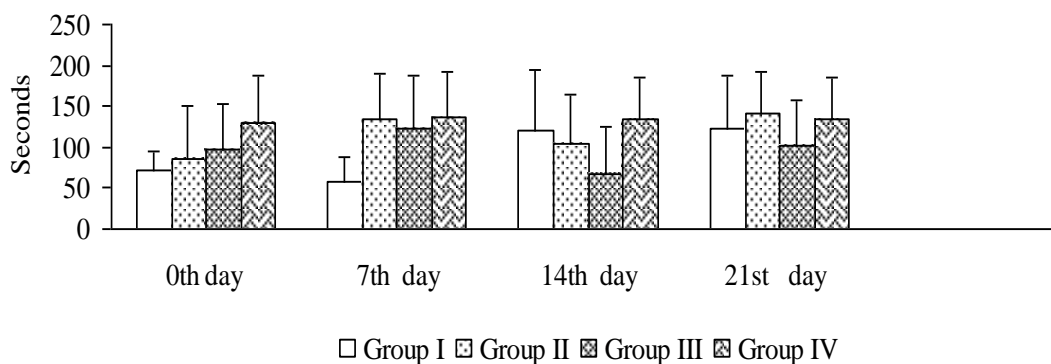Group I = control 1ml of distilled water; Group II = Aqueous extract 200 mg/kg;

Group III =Whole leaf powder 500 mg/kg; Group IV = Alcohols extract 200 mg/kg

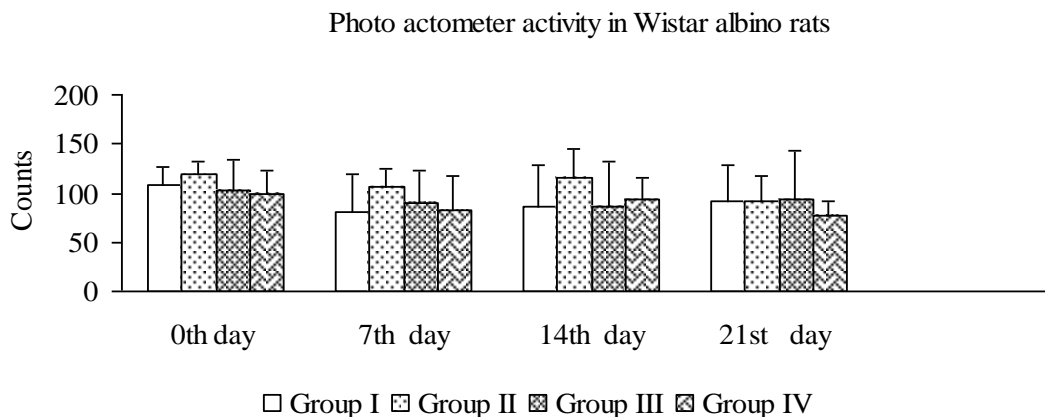Maze Behavioural changes in Wistar albino rats

□ Group I ⊡ Group II ⊠ Group III ⊠ Group IV

Fig- 5.3.1

Rota rod activity in Wistar albino rats

□ Group I ⊡ Group II ⊠ Group III ⊠ Group IV

Fig- 5.3.2

Photo actometer activity in Wistar albino rats

□ Group I ⊞ Group II ⊠ Group III ⊠ Group IV

Fig- 5.3.3

HEBB – WILLIAM'S MAZE

The transit time of the animals of test groups did not differ significantly when compared to the control group (Table.2, 3)

ROTAROD

Before commencing the experiment, each animal was tested for its balancing time on the rota-rod and this time was noted down for each animal separately. After $1^{st}, 2^{nd}, 3^{rd}$ week of treatment the balancing time of the individual animals were tested by placing them on the rota-rod. The difference between control and treatment group and pre and post treatment balancing time was calculated for every animal belonging to each group. The mean balancing time for the control group was considered as 100% (Table.2, 3). The staying time above this indicated a positive enhancement of muscular co-ordination and the reduction in staying time on the rota-rod was taken as an indication of muscular in-coordination. The data were treated statistically and presented in the table 3. Animals of test groups did not show any difference significantly when compared with the control group

ACTOPHOTOMETER

The result obtained were tabulated and presented in the table.2, 3. The spontaneous motor activity values were converted into percentages (taking the control values as 100%) for the easy handling of the data. The statistically significant values above control values indicate stimulation of CNS and statistically significant values below control values indicate CNS depression. Animals of the test groups did not show any significant difference when compared to the control group.

DISCUSSION

Treatment with leaf extract or powder did not alter learning and memory in rats as indicated by the results of the maze test. Similarly motor coordination has been found to be unaltered following treatment with the leaf powder or extracts under investigation. These observations indicate the lack of any toxic impact on nervous system. No significant alteration has been noticed in the motor activity of the animals subjected to treatment with the test substances as indicated by the results of the photoactometer analysis. This observation is in contradiction to the earlier report of diminished locomotor, central depressant and muscle relaxant effects reported with alcoholic extract by Jafri M.A and Amine K.M.Y., (2002).

CONCLUSION

Absence of any alteration in the behavioral parameters are indicative of lack of CNS influence by the plant.

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