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Original Research Paper

Pharmacology

STUDY OF ACUTE AND SUB ACUTE TOXICITY OF PALA INDIGO (*WRIGHTIA TINCTORIA*) IN ANIMAL MODELS

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ABSTRACT

Introduction: *W.tinctoria* often referred to as Snowflake Tree or Pala Indigo used as a vermifuge, antidiarrhoeal, antihaemorrhagic and a hepatoprotective agent. The present study has been designed to get better understanding about the actions of *Wrightia tinctoria* leaves on acute and sub acute toxicity studies.

Materials and methods: Acute toxicity was evaluated in Wistar albino rats by oral administration of single dose of aqueous extract, crude powder and alcohol extract of plant *Wrightia tinctoria* leaves. Sub acute toxicity studies should always attempt to expose the animals by the same route that is likely to be exposed.

Results and discussion: Mortality was not observed in the three groups observed up to 72 hrs. Sub-acute toxicity study is indicative of the plant having no toxic effect on liver during repeated administrations as indicated by the absence of any alteration in the values of the biochemical parameters.

KEYWORDS : *Wrightia Tinctoria, Vermifuge,* Sub-acute Toxicity

INTRODUCTION:

Medicinal plants are still considered as the source of untapped reservoir of drugs and the structure of the component molecules make them valuable sources of novel drug discovery(Lahlou, 2013). *Wrightia tinctoria*, often referred to as the Snowflake Tree or Pala Indigo, Known as *paalai* in Tamil, Native to India and Burma. *Wrightia* belongs to the family Apocynaceae (Srivastava, 2014). Wrightia is named after a Scottish physician and botanist William Wright (1740 - 1827)(Chandrashekar, Adake, Rao, & Santanusaha, 2013). *Wrightia tinctoria* is a deciduous tree with a light grey, smooth bark, amenable for carving (Raj, Kumar, & Gandhimathi, 2009). The leaves of this tree yield a blue dye called Pala Indigo(Siva, 2007). *Wrightia tinctoria* seeds contains 14-methylzymosterol, desmosterol, clerosterol, 24-methylene-25-methylcholesterol and 24-dehydropollinastanol.

The ethno botanical uses of *w. Tinctoria* in traditional medicine include its use in toothache, abdominal pain, as a vermifuge, antidiarrhoeal, antihaemorrhagic and as a hepatoprotective agent (Duke, 2002). Lisanul asafir (seeds of *wrightia tinctoria*) is one of the unani drugs reported to possess some important central actions such as analgesic action and aphrodisiac action along with other actions(Khyade & Vaikos, 2014). The ethanolic extract has been reported to diminish alertness, grooming, and spontaneous locomotor activity (Kasture, Kasture, & Chopde, 2002). In addition, pain response and body tone of the animals in gross behavior studies. The other findings include straub's reaction, analgesia and anticonvulsant activity suggestive of central depressant, muscular relaxant and anticonvulsant activities (Turner, 2013).

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 following pharmacological profiles:

- Acute toxicity study
- Sub Acute toxicity study

MATERIALS AND METHODS:

COLLECTION AND EXTRACTION OF *WRIGHTIA TINCTORIA* LEAF MATERIALS:

Fresh leaves of *Wrightia tinctoria* were collected from vinny garden, Nachaloor , Trichy District ,TamilNadu. 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 then dried under shade for 72 hrs. The dried leaves were crushed by hand to make crude powder. 300 gm of this powder was soaked in 2 liters of rectified sprit for 1 day. The leaf extract was filtered using whatman no.1 filter paper and 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 leaf extract was filtered using whatman no.1 filter paper and the filtered aqueous extract was used in this study.

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. They were quarantined for one week and then allowed to get acclimatized to the conditions of animal Room. Young, healthy, adult rats weighing 150 – 260 g were used for the experimental studies of the present investigation. The animals were subjected to periodic clinical examinations. Daily feed and water consumption and weekly body weight changes were recorded.

ACUTE TOXICITY STUDY :

Acute toxicity was evaluated in Wistar albino rats by oral administration of single dose of aqueous extract, crude powder and alcohol extract of plant *Wrightia tinctoria* leaves.

STUDY DESIGN:

Animals were grouped into four groups, each group consisting of 4 animals. Group –I control group- 1.0 ml-distilled water (vehicle) was administered.The animals in the other three groups received single dose of aqueous extract, crude powder and alcohol extract of *Wrightia tinctoria* 2000 mg/kg at a constant volume of 1 ml. Observation, clinical examination, and mortality check were made for 72 hours.

SUB ACUTE TOXICITY STUDY

Sub acute toxicity studies should always attempt to expose the animals by the same route that is likely to be exposed.

STUDY DESIGN:

Repeated low dose exposure study by oral route was conducted for 21days in *Wistar albino* 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 (2000mg /kg) in 1.0 ml of vehicle.
General observations during the period of exposure were recorded.

FOOD AND WATER CONSUMPTION:

Food and water consumption was measured daily for 3 days before starting treatment and 21days after starting treatment.

COLLECTION OF SAMPLES FOR BIOCHEMICAL AND HEMATOLOGICAL ANALYSIS:

At the end of the experimental period 22nd day, all the animals were anaesthetized under mild ether anaesthesia and blood was collected by retro orbital vein puncture. Following biochemical and hematological investigations were done.

BIOCHEMICAL INVESTIGATION:

- Assay of serum aspartate aminotransferase (AST)
- Assay of serum alanine aminotransferase (ALT)
- Assay of serum alkaline phosphatase (ALP)
- Estimation of serum protein

HEMATOLOGICAL INVESTIGATION:

- Erythrocyte Count
- Leukocyte count
- Differential Leukocyte count
- Determination of Hemoglobin
- Determination of clotting time

RESULTS

ACUTE TOXICITY STUDY:

Aqueous extract, crude powder and alcohol extract of *Wrightia tinctoria* leaves were subjected to acute toxicity testing in rats. A dose of 2000mg /kg of test substance administered to four rats in each group. Mortality was not observed in the three groups observed up to 72 hrs. Signs of neurotoxicological symptoms such as convulsions, myoclonus, tremor and other physiological alterations such as elevation in heart rate and respiration rate were absent in all animals treated with three test substance at 2000 mg/kg (Table 1). Since toxicity was not observed, a dose of 500 mg /kg for crude powder and 200mg/kg (one tenth of the maximum tested dose) of aqueous and alcohol extracts was chosen for evaluation of sub acute toxicity studies in *Wistar albino rats*.

Table 1. Acute toxicity study

Group	Treatment	No. of animals	Hours of observation	No. of animal survived	No. of animal died	Mortality rate
1	Control	4	72	4	Nil	Nil
2	Aqueous extract2000 mg/kg.	4	72	4	Nil	Nil
3	Alcoholic extract 2000 mg/kg.	4	72	4	Nil	Nil
4	Crude powder 2000 mg/kg.	4	72	4	Nil	Nil

SUB-ACUTE TOXICITY STUDY:

Feed and water consumption of rats exposed to the test substances by oral route for 21 days did not differ significantly from the corresponding control animals (Table-2). No mortality was seen during the test period in all the groups.

TABLE –2 FEED AND WATER INTAKE AFTER TREATMENT EXPRESSED AS PERCENT

S.No	Feed and Water intake Parameters	Group – I	Group – II	Group -III	Group – IV
I	Feed intake in Wistar albino rats				
1	Before Treatment	100%	100%	100%	100%
2	After Treatment (1 to 7 days)	86.50%	90.18%`	80.61%	107.87%
3	After Treatment (8 to 14 days)	81.44%	85.14%	72.30%	110.61%
4	After Treatment (15 to 21 days)	76.29%	77.91%	67.36%	100.72%
II	Water intake in Wistar albino rats				
1	Before Treatment	100.%	100%	100%	100%
2	After Treatment (1 to 7 days)	78.25%	114.96%	68.40%	69.31%
3	After Treatment (8 to 14 days)	85.99%	85.27%	75.54%	69.92%
4	After Treatment (15 to 21 days)	83.15%	78.28%	62.36%	59.24%

BIOCHEMICAL INVESTIGATION:

Rats treated with the aqueous extract, crude powder and alcohol extract of *Wrightia tinctoria* leaves did not show any significant difference in serum AST and ALT when compared to the control group, whereas rats exposed to aqueous leaf extract and crude leaf powder had a significantly higher ($p<0.05$) ALP level as compared to distilled water treated control group. The level of serum total protein was significantly lower ($p<0.05$) in rats treated with aqueous leaf extract compared to control group, whereas animals treated with crude leaf powder and alcoholic extract did not show any difference significantly (Table-3).

TABLE – 3

BIOCHEMICAL PARAMETERS AFTER TREATMENT EXPRESSED AS PERCENT

	Biochemical Parameters	Group – I	Group - II	Group -III	Group – IV
1	ALT (IU/L)	100.00	103.7	101.46	116.3
2	AST (IU/L)	100.00	80.88	103.37	88.23
3	ALP (IU/L)	100.00	132.12	134.64	113.42
4	Protein (g/dl)	100.00	91.54	97.94	95.16

HEMATOLOGY

Rats exposed to alcoholic leaf extract had a significantly lower ($p<0.05$) erythrocyte count as compared to that of the control group and rats exposed to crude leaf powder had a significantly lower ($p<0.05$) circulating leukocyte count as compared to that of the control group. The differential count of the test group did not show any difference significantly when compared to the control group. There was no significant difference in the haemoglobin content and clotting time (Table-4)

TABLE – 4 HAEMATOLOGICAL PARAMETERS AFTER TREATMENT EXPRESSED AS PERCENT

S. No	Hematological Parameters	Group – I (% Change)	Group – II (% Change)	Group –III (% Change)	Group – IV (% change)
1	Hb (g)	100.00	89.34	87.75	109.35
2	Clotting Time (sec)	100.00	129.42	101.47	89.47
3	RBC Mil/cu.mm	100.00	94.32	74.43	134.69
4	WBC Cells/cumm	100.00	74.76	64.34	110.21
5	Differential counts				
5(a)	Neutrophils (%)	100.00	79.63	92.77	81.71
5(b)	Esinophils (%)	100.00	178	88.67	90.36
5(c)	Basophils (%)	100.00	167	167	150
5(d)	Lymphocytes (%)	100.00	101.73	98.51	87.84
5(e)	Monocytes (%)	100.00	143.4	146.6	136.6

DISCUSSION:

The plant *Wrightia tinctoria* investigated in the present study seems to be devoid of having any toxic principle, as no mortality has been noticed with crude powder as well as aqueous and alcoholic extracts of the leaves of the plant up to the dose of 2000 mg/kg body weight administrated orally in *Wistar albino rats* in the acute toxicity study. This finding is substantiated by the lack of any alteration in the feed and water consumption in *Wistar albino rats* subjected to repeated treatment in the sub-acute toxicity study.

CONCLUSION:

The powder as well as aqueous/alcoholic extract of leaves of *Wrightia tinctoria*, has been found to cause no mortality up to 2000 mg/kg in *Wistar albino* rats by oral administration. Sub-acute toxicity study is indicative of the plant having no toxic effect on liver during repeated administrations as indicated by the absence of any alteration in the values of the biochemical parameters. Absence of any alteration in the hematological and behavioral parameters as well as feed and water intake are indicative of lack of any toxic influence by the plant.

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