

SUBCHRONIC TOXICITY ANALYSIS OF CLERODANE USING RATS

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ABSTRACT

Objective: To assess the Sub, chronic toxicity of Clerodane administration orally to rats for 28 days and to determine (i) Target organ toxicity (ii) "No observed effect level (NOEL)" and reversibility of signs of toxicity after recovery period.

Methods: This animal toxicity study we were used OECD Guidelines No. 407, and WHO GCP Guidelines. As per these guidelines the rats of both sexes in controlled age and body weight were selected. 2) Clerodane was administration at 25, 50 and 100mg/kg body weight as aqueous solution along with a blank 3) The results were recorded on day 0, 28th day and 43rd day i.e. recovery period.

Results: I found to be Ophthalmoscopes examination on days 0+29, 2) Organ weights, Hematological analysis, biochemical analysis and urine analysis on days 0+29+43. 3), Histopathological and gross pathological observations of sacrificed animals recorded after recovery period it was normal.

Conclusions: There was no toxicity effect in food consumption, Ophthalmoscopes examination, Organ weights, Hematological analysis, biochemical analysis and urine analysis.

Keywords: NTP, Clerodane, OECD Guidelines No.407, 28-Day Sub chronic oral toxicity.

INTRODUCTION

The genus *Tinospora* (Menispermaceae) has about thirty species, mainly distributing in the tropical regions of Asia and Africa, and many of the species have been used in traditional medicines, e.g., *T. cordifolia* has been used in Ayurveda (Indian traditional medicines) as a hepatoprotectant [1,2]. The stem of *T. sinensis* has been used in Traditional Chinese Medicine in the treatment of rheumatism, diabetes and muscle pain. A variety of constituents like alkaloids, glycosides, diterpenoid lactones, sesquiterpenoids and steroids have been isolated from *T. cordifolia*. *T. cordifolia* contains about 11.2% protein and is rich in Ca and phosphorus [3,4,5,6]. The antibacterial and immunomodulation effect of the plant was also studied [7,8]. *T. cordifolia* had cardioprotective and anti diuretic effects [9,10]. Toxicity analysis of plant extracts on rats has been extensively studied [11,12]. The organization for Economic and Development mentioned acute toxicity as the advance effect occurring within short time of oral administration of a simple dose of substance or a multiple doses given within 24 hrs [13]. The purpose of this study was to evaluate the safety of water extract of *T. cordifolia* in rats by determining both oral acute and sub chronic toxicities [14].

MATERIALS AND METHODS

Preparation of sample

Stems of *T. cordifolia* were shade dried powdered (1 kg) and extracted with alcohol by cold percolation method. The extract was evaporated to dryness in vacuum (25 gm) and chromatographed using silica gel (100–200 mesh). The column was eluted by increasing solvent polarity (hexane to methanol). When increasing the solvent polarity at chloroform: methanol (19:1), a white color compound was eluted which was confirmed as terpenoid. The compound upon repeated crystallization from methanol yielded transparent crystals. Crystal of dimensions 0.22×0.20×0.16 mm was selected for data collection (Indian Institute of Technology, Chennai) using graphite radiation monochromatic [15]. The isolated crystal was confirmed as (5R, 10R)-4R, 8R-dihydroxy-2S, 3R:15,16-diepoxycleroda- 13(16),17,12S,18,1S-dilactone.

Experimental animals

Albino Wistar rats weighing 100-120 gm which were procured from the Animal house of Shadan College of Pharmacy were used throughout the experiment. The experimental animals were

maintained under standard laboratory conditions with 12-h light/dark cycle under controlled temperature. All the animals were acclimatized to the laboratory conditions for at least one week before the commencement of the experiment. All the experiments were performed in accordance with the CPCSEA guidelines.

Acute toxicity studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for economic co-operation and development (OECD) guidelines 425. The animals were fasted overnight prior to the experiment. The first group was treated after fasting overnight with oral dose 1000 mg/kg body weight of clerodane. The test solutions were given in two different groups and the animals were observed continuously for 2-3 hrs for general, behavioral, neurological, autonomic profiles and finally death after 24 hr. There were signs of toxicity like C.N.S stimulant actions and the test solution was found to be toxic at this dose level. Then a lower dose of 500 mg/kg body weight of the clerodane test solution was administered in another 2 groups. No mortality and no sign of toxicity were observed. The maximum tested dose level was taken as 500 mg/kg body weight. The doses for pharmacological studies were taken as 25, 50, and 100 mg/kg body weight i.e. 1/5th, 1/10th, 1/20th of the maximum tolerable dose (i.e. 500 mg/kg). [16,17].

Randomization, Numbering and Grouping of Animals

Sixty rats (30 male and 30 female healthy animals) were randomly divided into four groups of 5 animals per sex for dosing up to 28 days and 5 animals per sex as reversal groups for and high dose i.e 0 mg/kg, 0 mg/kg (Rev), 25 mg/kg, 50 mg/kg, and 100 mg/kg and 100 mg/kg (Rev.) body weight. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Five rats of same sex were housed per cage. Each animal fur was marked with picric acid. The females were nulliparous and non pregnant.

Preparation and administration of dose

Clerodane was dissolved in distilled water to obtain concentration of 25, 50 and 100 mg/ml. it was administered to animals at the dose levels of 25mg/kg, 50mg/kg and 100mg/kg in the dose volume of 10 ml/kg. The test substance solutions were freshly prepared everyday for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 days.

i) Body Weight

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

ii) Food Consumption

The quantity of food consumed by groups consisting of five animals of each sex for different doses (0 mg/kg, 25 mg/kg, 50 mg/kg and 100 mg/kg) and five animals of each sex for different doses (0 mg/kg Rev. and 100 mg/kg Rev.) was recorded at weekly interval. Food consumed per animal is calculated for control and the treated dose groups.

iii) Clinical signs

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

(iv) Mortality

All animals were observed twice daily for mortality during the entire course of study.

(v) Ophthalmoscopy

The eyes of experimental animals in control as well as treated groups given different dose levels were examined prior to the initiation of the dosing and in 4th and the 6th week (for reversal group animals) of the study. Eye examination is carried out using a hand slit lamp after induction of mydriasis with 0.5% solution of tropicamide.

(vi) Functional Observations

At the end of the 4th week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' [using Grip Strength Meter Single Digital

Terminal Studies

Laboratory Investigations: Following laboratory investigations were carried out on 0day, 29th and on day43, in animals:- Fasted over night. Blood samples were collected from orbital sinus on the following morning using sodium heparin (200 IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Haematological Investigations

Haematological parameters were determined by using Haematology analyzer.

Biochemical Investigations

Biochemical parameters were determined using Auto analyzer

Urine analysis

Urine samples were collected in week 0 day and in week 7 and for estimation of normal parameters. The estimations were being performed using appropriate methodology.^[4]

Necropsy

All the animals sacrificed on day 29 except for reversal group animals were sacrificed on day 43 i.e. post, dosing period of 14 days, using CO₂ asphyxiation technique Necropsy of all animals were carried out and the weights of the organs including liver, kidneys, adrenals, epididymis, thymus, spleen, brain, heart, uterus and testes/ovaries and the relative values as percent body weight] were recorded.

Histopathology

Tissue samples of organs from control and treated animals at the highest dose level of clerodane were preserved in 10% formalin. The organs included adrenals, brain, duodenum, gonads, heart, ileum,

kidneys, liver, lungs, oesophagus, spleen, stomach, seminal vesicles, spinal cord, thymus, thyroid, trachea, urinary bladder and uterus. They were subjected to histopathological examination. ^[5] Tissue samples of liver, kidneys, adrenals, epididymis, thymus, spleen, brain, heart, uterus and testes/ovaries of low and intermediate dose groups' animals were preserved for histopathology examination.

RESULTS AND DISCUSSION**Acute toxicity study**

The Aqueous solution of Clerodane was found to be safe at the maximum tolerable dose of 500 mg/ kg body weight by oral route. After 24 h animals were found to be well tolerated. There was no mortality and no signs of toxicity. General behaviors, neurological, autonomic profiles were found to be normal and the extract was found to be safe (Table-1).

Food Consumption

During dosing and the post-dosing recovery period, the quantity of food consumed by animal from different dose groups was found to be comparable with that by control animals (Table-2).

Table 1: Acute toxicity analysis

S. No.	Behavioral Response	Clerodane 500 mg/kg
1.	Alertness	N
2.	Stereotype	N
3.	Irritability	-
4.	Fearfulness	-
5.	Touch Response	N
6.	Pain Response	N
7.	Spontaneous activity	N
8.	Grooming	-
9.	Restlessness	-
Neurological Response		
1.	Righting Reflex	N
2.	Limb Tone	N
3.	Grip Strength	N
4.	Twitching	N
5.	Abdominal Tone	N
6.	Pinna Reflex	N
7.	Corneal Reflex	N
8.	Tremors	-
9.	Convulsions	-
10.	Straub Tail	-
Autonomic Response		
1.	Writhing	N
2.	Defecation	N
3.	Urination	N
4.	Pilo Erection	-
5.	Heart Rate	N
6.	Respiration	N
7.	Pupil Size	N
8.	Skin Colour	N

Table 2: Hematology, Blood chemistry, Urine analysis and Organ weight

Test	Sex	Results
Hematology	Male	Normal
Hematology	Female	Normal
Blood chemistry	Male	Normal
Blood chemistry	Female	Normal
Urine analysis	Male	Normal
Urine analysis	Female	Traces
Organ weight	Male	Increased weight
Organ weight	Female	Increased weight

CONCLUSIONS

All the male and female animals from control and all the treated dose groups up to 1000 mg/kg survived throughout the dosing

period of 28 days and the recovery period of 14 days. No signs of intoxication were observed in male and female animals from different dose groups during the dosing period of 28 days and during the recovery period of 14 days. Male and female animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days and the recovery period of 14 days. Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days and the recovery period of 14 days. Ophthalmoscopic examination, conducted prior to and at the end of dosing period on animals from control and all the treated dose groups did not reveal any abnormality. Hematological analysis conducted at the end of the dosing period on day 29 and at the end of recovery period on day 43, revealed no abnormalities attributable to the treatment. Biochemical analysis conducted at the end of the dosing period on day 29 and at the end of recovery period on day 43, revealed no abnormalities attributable to the treatment. Functional battery observation tests conducted at termination revealed no abnormalities. Urine analysis, conducted at the end of the dosing period in week 4 and at the end of recovery period in week 6, revealed no abnormality attributable to the treatment. Organ weight data of male and female sacrificed at the end of the dosing period and at the end of the recovery period was found to be comparable with that of respective controls. Gross pathological examination did not reveal any abnormality. Histopathological examination did not reveal any abnormality.

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