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Research Article

ACUTE ORAL TOXICITY OF VTH PILLS IN WISTAR RATS

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ABSTRACT

Objectives: To evaluate the acute oral toxicity of VTH pills in wistar rats

Methods: The study was conducted in two phases: initial and continuation phase. In the initial phase there were two groups: control and test, with ten rats (5 male and 5 female) in each group. The control group received 2% gum acacia (vehicle) 10mg/kg; the test group received 5000mg/kg of VTH pillssuspended in 2% gum acacia,orally as a single dose on day 1. All the animals were observed for clinical signs of toxicity and/or death at 1hour (±10min), 2hours (±10min), 4hours (±10min) and 6hours (±10min) on day 1 and thereafter once daily for 14 days. On day 15, the animals were subjected to necropsy. In the continuation study, 30 rats were divided into three groups receiving 2000, 1000 and 500mg/kg VTH pills respectively. Behavioural toxicity analysis was done at the end of the continuation study.

Results:There was no mortality andthere wasalso no biochemical, hematological or histopathological changes at any of the doses. However, VTH pills showed significant behavioral toxicity at 5000mg/kg which was reduced at 2000mg/kg. No behavioral toxicity was noted at 500 or 1000mg/kg body weight.

Conclusion: The test item, VTH pill is non-toxic upto and at 1000 mg/kg when administered by oral gavage in a single dose to wistar rats.

Keywords: VTH pills, Acute oral toxicity, Behavioral toxicity

INTRODUCTION

VTH pill is a poly herbal formulation containing well-known anti-oxidant herbs like *Strychnosnuxvomica*,*Syzygiumaromaticum* and *Centratherumanthelminticum*. Anti-lipid-peroxidative property of *Strychnosnuxvomica* extract has been reported on cumenehydroperoxide and ferrous sulphate induced models of lipid peroxidation [1]. It also possesses significant metal chelating property and chelates both forms of iron (Fe^{2+} and Fe^{3+})[1].The essential oil of *Syzygiumaromaticum* is a very good antioxidant *in vivo* and *in vitro* [2].*In vitro* antioxidant experiments with the fenton reaction and UV irradiation of hydrogen peroxide, both of which generate hydroxyl radicals, showed *Syzygiumaromaticum* extract to directly scavenge hydroxyl radicals. It is also a potent scavenger of superoxide anions [3]. Bitter cumin [*Centratheruman anthelminticum* (L.)Kuntze] is a medicinally important plant as it is a rich source of polyphenolic compounds. The phenolic extracts of *Centratherumant anthelminticum* showed significant scavenging of DPPH and ABTS radicals, reduced phosphomolybdenum {Mo (VI) to Mo(V)}, ferricyanide{Fe(III) to Fe(II)} and inhibited liposome oxidation and hydroxyl radical induced damage to prokaryotic genomic DNA. The results showed a direct correlation between phenolic acid content and antioxidant activity[4].This polyherbal combination has been postulated to be effective in coronary artery disease. However, preliminary toxicity studies of this combination have not been reported yet.The objective of this study was to assess the acute toxic potential of the test item - VTH pills, a poly herbal product when administered orally in a single dose to wistar rats of both sexes.

MATERIALS AND METHODS

The study was performed in accordance with the following:

1. The OECD Guidelines [5] for Testing of Chemicals (No. 420, Section 4: Health Effects) on conduct of "Acute Oral Toxicity – Fixed Dose Method" (Adopted: 17th December 2001).
2. In the spirit of OECD Principles of Good Laboratory Practices (1997).
3. The recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animals' experiments published in the gazette of India, December 15th 1998 and approved by Institutional Animal Ethics Committee (IAEC).

The test item was supplied by Ilaj Herbal Research Remedies, Malappuram, Kerala.

In-house bred wistar rats aged 10 – 12 weeks, weighing 150 – 200gm were housed under standard laboratory conditions, room temperature 22 ± 2°C, with 12 hours light and dark cycle. A maximum of three animals were housed in standard polypropylene cages (Size: L 430 x B 270 x H 150 mm) with stainless steel top grill mesh having facilities for holding pelleted food and drinking water in water bottle fitted with stainless steel sipper tube. The animals were acclimatized for seven days to laboratory conditions and were observed daily. Water and feeds were provided *ad libitum* throughout the acclimatization and study period except twelve hours before &four hours after dosing of the test drug.

Study Design

The study was conducted in two phases: initial and continuation phase.

Initial Phase

Rats were divided into two groups of ten rats each. Each group had 5 male & 5 female rats. Required quantity of test item was weighed as per the dose. The weighed test item was suspended in 2% solution of gum acacia to get the desired concentration as per the dose. Formulation of the test item was prepared shortly before dosing. The rats were dosed as shown in Table 1.

Table 1: Dosing of rats in the initial phase

Group	Drug	Dose	Route
G1 (Control group)	Vehicle	10 ml/kg body weight	Oral
G2 (Test group)	VTH pill	5000 mg/kg body weight	Oral

Continuation Phase

Rats were divided into three groups. Each group had 5 male & 5 female rats.The required quantity of test item was weighed as per the dose. The weighed test item was suspended in 2% solution of gum acacia to get the desired concentration as per the dose. Formulation of the test item was prepared shortly before dosing. The rats were dosed as shown in Table 2.Food was restricted 12 hours before & 4 hours after dosing. Access to water was continued *ad libitum*.

Table 2: Dosing of rats in the continuation phase

Group	Drug	Dose	Route
G3	VTH pill	2000 mg/kg body weight	Oral
G4	VTH pill	1000 mg/kg body weight	Oral
G5	VTH pill	500 mg/kg body weight	Oral

Parameters Observed

The following observations were undertaken during the study.

1. Clinical signs and/or deaths

All the animals were observed for clinical signs of toxicity and pre-terminal deaths at 1hour (±10min), 2hours (±10min), 4hours (±10min) and 6hours (±10min) following dosing on day 1 and thereafter once daily for 14 days.

The following physical parameters were assessed.

1. Signs of toxicity

- Increased motor activity like tremors, convulsions, spasticity, muscle spasm
- Decreased motor activity like ataxia, sedation, muscle relaxation, anaesthesia
- Ptosis, lacrimation, exophthalmos
- Effect on respiration

2. Mortality

3. Body weight

- Body weight was measured before and after the study period

2. Haematological and biochemical parameters

At the end of the initial study, all rats were fasted overnight and anesthetized for blood collection. Heparinized blood samples were taken for determining the haematological parameters. Non heparinized blood samples were used for assessment of biochemical parameters.

3. Pathology

At the completion of the initial study, the animals were subjected to necropsy andhistopathological examinations.On the 15th day, all rats were fasted for 16 to 18 hours and then sacrificed by over dosage of sodium pentobarbitone (100mg/kg body weight) intra-peritoneally for necropsy examination.The internal organs i.e. liver, stomach, heart, kidney were excised and sent for histopathological examination after preserving in 10% neutral buffered formalin. These tissues were embedded in paraffin wax, sectioned at five micrometers and stained with haematoxylin and eosin.

RESULTS AND DISCUSSION

Initial Phase

No mortality was noted in any of the groups. In Group 2 (5000 mg/kg),hyperactivity was seen in 2 rats, more consistently in one rat throughout the 14 days observation period. Grooming was seen in 6 rats and sedation was observed in 4 rats. Overall 9 out of the 10 rats (90%) which received VTH pill developed some behavioural toxicity at least once during the 14 day observation period at 5000mg/kg. This suggests a CNS stimulatory effect with VTH pill. This could be due to the effect of strychnine, an indolomonoterpenic alkaloid of *Strychnosnuxvomica* which is a known CNS stimulant [6]. No significant changes in biochemical &haematological parameters were noted in the test group. No significant changes in the body weights were noted in either group (Table3 and 4).

Histopathological examination revealed mild to moderate eosinophilic infiltration in the mucosa of stomach in both the groups. There was no significant difference in the histopathology of control and test groups.

Table 3: Initial study - summary of clinical signs and mortality

Group	Dose (mg/kg)	Number of animals	Clinical Signs	Mortality
G1	10	10	Normal	Nil
G2	5000	10	2 – hyperactivity 6 – grooming 4 - sedation	Nil

Table 4: Initial study - biochemical and haematological parameters

Parameters	G1	G2	Normal Range
Total serum protein(g%)	6.7±0 .4497	6.58±0 .2821	6.0-8.3
BUN(mg%)	43.5± 6.964	39.1± 6.297	10-45
SGPT(IU/L)	43.7± 9.031	39.1± 8.999	5.0-40.0
SGOT(IU/L)	213.9± 45.812	196.6± 64.012	5.0-40.0
ALP(IU/L)	96.7± 22.000	111.4± 30.460	Upto 462
Glucose (mg%)	108.4± 7.121	97.6± 14.879	70-140
Creatinine (mg/dl)	0.32±0 .0789	0.29±0 .0568	0.2-1.4
Bilirubin(mg/dl)	0.11±0 .0316	0.11±0 .0316	0.1-1.2
Hb(g%)	15.91±0 .7622	15.49±0 .4358	12-16
Total RBC(millions/cu mm)	7.87±0 .49416	7.77±0 .23650	4.5-7.5
Total Platelets(cells/cu.mm)	881900± 151791.853	944100± 40487.172	1,50,000-5,00,000
Total Leucocytes	5760± 1350.062	8000± 1751.824	6000-18000

Data expressed as Mean ±SD

Continuation Phase

No mortality was noted in any of the groups. In the G3 (2000mg/kg) hyperactivity was seen in 1 rat during the first day of the observational period. The hyperactivity subsided during the subsequent days of observation. Grooming was seen in 3 rats. At 2000mg/kg, 40% rats developed some behavioural toxicity at least once during the 14 day observation period. No behavioural abnormalities were noted in any of the rats which received 500 & 1000mg/kg VTH pills. These findings suggest that VTH pill was found to be safe and did not reveal any toxicological symptoms at and below 1000 mg/kg dose(Table – 5). There were no significant changes in body weight in any of the rats.

Table 5: Continuation study - summary of clinical signs and mortality

Group	Dose mg/kg	Number of animals	Clinical Signs	Mortality
G3	2000	10	3-grooming 1-hyperactivity	Nil
G4	1000	10	Nil	Nil
G5	500	10	Nil	Nil

CONCLUSIONS

VTH pill at 5000 mg/kg was found to be safe on the hematopoietic parameters, liver and renal functioning as evidenced by the biochemical laboratory findingswhich were within the normal range. Nevertheless, it revealed to have some behavioural toxicity in the form of central nervous system stimulatory activity as evidenced by

grooming & hyperactivity. At 2000mg/kg too VTH pill showed somebehavioural toxicity. However atlower doses of 1000mg/kg and 500mg/kg (which is approximately 1000 and 500 times the recommended human dose respectively) it showed no signs of behavioural toxicity. Hence it can be concluded that VTH pill is safe and nontoxic in albino wistar rats at and belowthe dose of 1000mg/kg.

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