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Acute oral toxicity of herbo-mineral combination: Talisadi yoga in Wistar albino rats

Case Report

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Abstract

Talisadi yoga (TY) has been used as an herbal contraceptive. Before any product is released into the market, the toxicity study parameters are necessary to generate data that is safe for scientific use. An in vivo study was carried out on female Wistar albino rats at the Institute for Industrial Research and Toxicology (IIRT), Ghaziabad. The study aimed to evaluate the acute oral toxicity (AOT) study of TY by following the guidelines of OECD 425: AOT-Up and Down Procedure. Healthy female Wistar albino rats, of body weight 200±20 g, were selected for study and used stepwise according to the guidelines. The study was conducted with a single starting dose of 2000 mg/kg body weight. The test sample was administered orally at dose volume of 10 ml/kg to one female rat. The treated animal was closely observed for clinical signs of intoxication during the first four hours of TY administration. Thereafter, the animal was observed periodically at a 12-hour interval for 48 hours. No sign of toxicity or mortality was observed in the 48 h; hence, another two animals were dosed at the same dose level and observed for 48 h and finally for 14 days after the administration of the TY, respectively. Since three animals survived at the tested dose level (2000 mg/kg b. wt.), the TY tested for AOT, was found non-hazardous to Wistar albino rats. According to the Globally Harmonised System of Classification and Labelling of Chemicals (GHS), it comes under Category 4 (LD50 > 2000 mg/kg).

Keywords: AOT, Herbo-mineral combination, Wistar rats, OECD 425, Ayurvedic contraceptive.

Introduction

Although *Ayurvedic* formulations are widely used, there is a lack of information regarding their safety and potential drug interactions. This is particularly true for compound polyherbal and herbo-mineral formulations. (1) The ingredients used in *Ayurveda* medicine formulations come from animal, mineral, and/or plant sources and are treated pharmaceutically to produce therapeutic effects. Safety is always a priority from a pharmaceutical perspective, even when the time, toxicity, and side effects of medicinal plants and mineral preparations used traditionally have passed. A common misconception is that anything related to plants is safe, and it is not necessary to research their safety. The various plant parts, such as the leaves, fruits, and barks, are the main sources of the toxin. (2) The World Health Organisation (WHO) reports that there is a growing public demand for research to close knowledge gaps on the preservation of medicinal plants. (3)

Talisadi yoga is one of the *garbhanirodhaka* (contraceptive) combinations described in *Ayurveda*. *Yogartanakar* has mentioned the combination of *Talispatra* (*Taxus baccata L.*) and *Gairika* (red oxide of iron) to be taken in equal quantity by the women as contraceptives. (4) Till date, no toxicity studies with this combination of herbo-minerals have been conducted. Thus, the goal of the current study was to look into acute oral toxicity in albino Wistar rats, as per Organisation for Economic Cooperation and Development guidelines (OECD) 425 (5) and classified according to the Globally Harmonized System for the classification of chemicals that cause acute toxicity.

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Materials and Methods

Test material

The test drug materials were purchased from an authentic source in Gurugram, Haryana, and prepared after authentication from SGT University in Gurugram, Delhi, NCR.

Animals and approval from animal ethical committee

From the Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, three female (nulliparous and non-pregnant) Wistar albino rats of age 8–12 weeks and weighing 200±20 g were obtained and maintained under standard environmental conditions of 22 ± 3°C and relative humidity of 30-70 %. The 12 h light and 12 h dark cycles were maintained manually throughout the study. Each day, the floor of the experimental room was cleaned and mopped twice with a disinfectant solution. A maximum of three animals were housed in standard polypropylene cages with a

stainless-steel grill top that had facilities for holding pelleted food and drinking water in a water bottle fitted with a stainless-steel sipper tube. Sterilised paddy husk was used to collect the excreta and urine and was changed every day. Animals were supplied with pelleted feed (supplied by San Biotech Pvt. Ltd., 27 Street No. 2, Madhu Vihar, IP Extn. 110092, Delhi, India). The portable purified water was provided to animals in polypropylene bottles (autoclavable). The study was conducted in compliance with the modified OECD Guidelines for Testing of Chemicals (No. 425, Section 4: Health Effects) on "AOT".

IAEC

This procedure has been approved by the IIRT Institutional Animal Ethical Committee and is reviewed at least annually by the same committee.

Toxicity study

The toxicity assessments were done in accordance with globally recognised standards (6).

Acute oral toxicity

The toxicity of the test compound following oral administration was assessed. One female rat at a time was used at each dose level. The next dose level (upper or lower at a factor of 3.2-fold) had to be used after the survival or death of the currently dosed animal after 48 h (up and down procedure). At each dose level, the rat was observed for the incidence of mortality and signs of intoxication for 48 h, and finally for 14 days after the administration of the test article. The study design is detailed and described in Table 1.

Table 1: Study Design

Step	Dose (mg/kg)	Number of animal per dose level	Animal ID
Step-1	2000 mg/kg b. wt.	1	202308-085-01
Step-2	2000 mg/kg b. wt.	1	202308-085-02
Step-3	2000 mg/kg b. wt.	1	202308-085-03

Dose preparation

Dose preparation of the test article TY was done freshly, a few minutes prior to dosing. A suitable amount of sample was weighed, and some amount of vehicle was added. The sample was triturated well, followed by the addition of more vehicles to make the desired volume.

Administration of test compound

The test compound was administered by oral route with the help of an oral cannula at a dose volume of 10 ml/kg b. wt.

Observations

Bodyweight

The body weight of all the animals was observed weekly on days 0 (pretreatment), 7, and 14 (post-treatment).

Mortality

All the animals were observed for mortality at a 30 min interval for the first 6 hour the day of test compound administration and there after twice a day for14 days.

Clinical signs

The treated animals were closely observed for clinical signs of intoxication for the first 4 h and every 12 h interval for 48 h after dosing, and thereafter twice a day for 14 days. All the rats were observed at least twice daily to observe any clinical signs or behavioural changes. These observations included changes in skin and fur, in the eyes and mucous membranes, respiratory, circulatory, central nervous, and autonomic nervous systems, somatomotor activity, and behavioural changes. The following clinical signs were observed in rats to characterise the various systemic studies: salivation, lacrimation, pale mucous membrane, diarrhoea faeces, hunched posture, scratching, polyuria, hypoactivity, etc. The clinical signs were graded as

0=normal,
+= mild,
++ = moderate,
+++ = high, and
++++=severe.

Necropsy

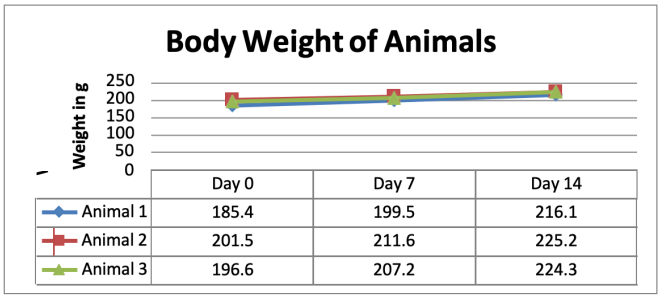
All the animals tested during the study were sacrificed at the end of the study, and necropsy was carried out to observe any gross pathological changes.

Results

Body weight

Wistar albino rats treated with the test compound TY at a dose level of 2000 mg/kg body weight showed normal gains in body weight on days 7 and 14 (post-treatment) as shown in Figure 1.

Figure 1: Summary of bodyweight (g) of animals



Clinical signs

The test compound TY did not produce any clinical signs throughout the observational period at the tested dose level of 2000 mg/kg body weight.

Mortality

No mortality was recorded in any of the experimental rats after administration of the test compound at the dose level of 2000 mg/kg body weight throughout the period of observation (Table 2).

Table 2: Clinical signs and mortality Dose: 2000 mg/kg b. wt.

AnimalIDs:202308-085-01, 202308-085-02, 202308-085-03																				
Parameters	**Incidence of clinical signs observed after dosing**																			
	Day0					**Day**														**Mortality**
	Min	**Hour**																		
	30	1	2	4	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total*
Mortality (total)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3
Clinical Signs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Were (0) Normal; (+) = Mild;(++) = Moderate; (+++) = High;(++++) = Severe;(-) = Can't be observed as animal was found dead																				

Necropsy finding

External: Skin and all external orifices were normal.

Internal: Subcutaneous-Nochange was observed; Superficial and deep lymph nodes-No change in mesenteric lymph node.

Abdominal cavity: Opening and general examination: In the abdominal cavity, all the organs were present in a normal position. Spleen was observed as normal. No change was observed in the digestive system. The liver and biliary ducts appeared normal. No gross pathological changes were observed in the excretory system. Adrenal Observed Normal; Female genital organs showed normal colour, consistency, and no inflammatory changes up to the highest tested dose level.

Thoracic cavity: Opening and general examination: the thoracic cavity was found to be normal without any fluid, mucous, or blood, etc.; the lungs appeared normal; the heart was found to be normal; no changes were observed in colour or consistency.

Cranial Cavity: Brain: normal in shape and size.

A summary of necropsy findings and individual animal fate along with the necropsy findings are given in Tables 3 and 4, respectively.

Table 3: Summary of necropsy findings

Sr. No.	Fate	Dose 2000 (mg/kg b. wt.)
1	Terminal sacrifice (TS)	3/3
2	Found Dead (FD)	0/3
3	Abnormalities detected	NAD

Table 4: Individual animal fate & necropsy findings

Animal ID	Fate	Time	Gross necroscopy findings
202308-085-01	TS	Day15	NAD
202308-085-02	TS	Day15	NAD
202308-085-03	TS	Day15	NAD

Discussion

Contraception refers to methods other than abstinence from coitus that prevent conception. The most often prescribed method of birth control is the pill. Although many people take birth control tablets to prevent pregnancy, there are a few other forms of contraception that are also in use due to the side effects that birth control pills can have on populations that are at risk. Other methods of contraception are taken into account with reference to past medical history, present medications, and coexisting conditions, including clotting problems. (7) Bleeding breakthrough is the most frequent side effect of combined OCP therapy. In addition, women may report experiencing headaches, nausea, cramps in the abdomen, breast tenderness, and increased vaginal discharge, or diminished libido. When compared to non-users, COC users had a greater risk of ischemic stroke (relative risk 1.7, 95% CI 1.5 to 1.9) and myocardial infarction (relative risk 1.6, 95% CI 1.2 to 2.1), according to a meta-analysis that included 28 publications. (8) So, women are seeking out more natural ways for contraception. As *Ayurveda* is also advanced in the practice of contraceptives, *Yogaratnakara* mentioned different combinations of drugs for local and oral use of contraceptives. TY is one of the combinations mentioned in Yogaratnakara containing the herbal drug *Talispatra* and metalo-mineral *Gairika* that needs to be evaluated for toxicity before being practiced clinically.

A drug's safety and toxicity profile are crucial because they give physicians the information, they need to determine whether or not to take a particular level of risk while treating a patient. Primarily, this can be produced by using animals in subclinical experiments. Acute toxicity was measured in the current study to document the immediate negative signs and symptoms following the administration of a single dose of drugs at dose levels that are several times higher than the therapeutic equivalent dose. In the present study, a single sex (ideally female) was employed in accordance with OECD Guideline 425 to minimise the number of animals used and reduce variability. Additionally, studies of the literature on conventional LD50 testing have shown that, in most cases, there is a small difference in sensitivity between the sexes; however, in those cases, female sensitivity was generally slightly higher. (9) Therefore, acute toxicity was assessed in female rats in the current study. Variations in an animal's body weight are a crucial indicator of its health. Weight loss is often the first sign that a negative consequence is happening. Regardless of whether there are any additional changes or not, the dosage at which body weight decreases by 10% or more is considered hazardous. (10) In the current investigation, the administration of test medications did not decrease the body weight of the subjects as compared to the initial

weight of the animals. However, there was not a significant difference in the normal increase in body weight observed in any of the animals. Furthermore, during the whole study period, neither test drug produced any mortality at the dose of 2000 mg/kg. No clinical signs (behavioural changes) were noticed. This suggests that the lethal dose of either test drug is higher than 2000 mg/kg, indicating that the test preparations are unlikely to cause significant degenerative changes at the therapeutic doses used in clinical trials. The necropsy study done after day 14 was also found to be normal, showing a nontoxic effect of TY.

Conclusion

Based on observations of behavioural changes (clinical signs), body weight changes, histological findings, and mortality documented in the acute oral toxicity research, it is evident that the TY sample containing an equal combination of *Talispatra* (*Taxcus baccata L.*) and *Gairik* (Red oxide) has LD50s greater than 2000 mg/kg, indicating their safety for therapeutic use. Further chronic toxicity studies can be conducted for the safety profile of the drug sample.

Abbreviations

OECD	Organization for Economic Cooperation and Development
AOT	Acute oral toxicity
TY	Talisadi yoga
IAEC	Institutional animal ethics committee
TS	Terminal Sacrifice
FD	Found dead
NAD	No abnormality detected
G	Gram
Mg	Milligram
Kg	Kilogram
°C	Degree Celsius
%	Percent
H	Hour
Min	Minute

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