PIJAR
Paryeshana International Journal of Ayuredic Reserach
www.pijar.org
ISSN:2456:4354
Research Article

ACUTE TOXICITY (LD_{50} CALCULATION) OF TALIKA VATI (3RD METHOD) IN MICE

C.S. Maladkar[1], Amol B. Dhulgade[2]

[1]Professor & HOD, [2]PG Schollar P.G.Department of Rasashastra & Bhaishajya kalpana. Shri J.G.Co-Operative Hospital Societies Ayurvedic Medical College & Ghataprabha.591306, Karnataka.India

ABSTRACT:

Talika vati (3rd method), a herbo-mineral formulation and use to treat the conditions like *Kanth roga* (Throat infectious diseases). *Talika vati* was prepared in accordance with description of *Rasa yoga sagar*. For acute toxicity study, the study was conducted in 7 groups of Mice (5 mice per group) were administered respective oral doses of 10, 100, 285, 500, 1000, 2000 & 5000 mg/kg body weight of *Talika vati* (3rd method) suspension. Signs of toxicity & possible death of mice were monitored for 24 hr to calculate LD_{50} of *Talika vati* susp. This was observed initially for 4 hrs and mortality checked for 24 hrs. Kerber arithmetic method for calculation of LD_{50} was used. It was observed that when the *Talika vati* suspension was administered up to the dose of 100 mg/kg body weight, no toxicity signs were recorded. It is thus concluded that administration of *Talika vati* to Mice is safe at the dose of 10 mg/kg & 100 mg/kg body weight.

Key words: *Talika vati* (3rd method) suspension, acute toxicity, Kerber scale.

INTRODUCTION:

In Rasashastra science, several herbo mineral formulations are mentioned. Owing to the presence of mineral drugs, there is constantly feared about its safety & toxicity. Safety of the formulation depends on its dose. In this context Charaka stated that "Poison can perform as medicine in appropriate dose while an efficient medicine can take action as poison in higher dose" [1]. Consequently the LD 50 study is required for herbo mineral formulation to decide the therapeutically effective dose.

Talika vati (3rd Method) is one of the considerable herbo-mineral medicine especially claimed for all types of throat infection (*Kanthgat Rog*)[2]. The ingredients of *Talika vati* are *Harital, Manasila*[3], *Daruharidra* (Berbers aristata), *Yavaksha* [4](Potassi Carbonas), *Madhu* [5](Honey), *Saindhav* (Rock salts) & Sudha churna (Lime stone). In the literature, properties of the ingredients have been reported that they possess *Krimighna* (Anti Bacterial) property. Harital & Manasila should undergo purification process before preparing the formulation (*Talika Vati*). After carrying out the *shodhana* process on *Harital & manasila*, there was embodiment of Sulfur (*Gandhak*) from inorganic state to organic matter. At this juncture Herbal media was used in *shodhana* process; Arsenic compound was bounded to other organic functional groups. Therefore organo-arsenic compounds are relatively safe in treatment if administered in appropriate dose[6]. Hence the present examination was undertaken to estimate the safety dose of Talika *vati* (3rd Method) in the animal models using OECD guidelines.

MATERIALS & METHODS:

Test drug: *Talika vati* (3rd method)

Source of Drug: the raw materials were procured from the local market & authenticated as per *Ayurvedic* criteria in *Rasashastra & Bhaishajy kalpana* department of Shri J.G.C.H.S.Ayurvedic Medical College, Ghataprabha.

***Talika vati* (3rd method) preparation:**

It was prepared and collected from Teaching Pharmacy of Shri J.G.C.H.S. Ayurvedic Medical College, Ghataprabha. *Harital & Manasila* were purified according to the classical texts mentioned.

Materials used:

Table no 1: Ingredients of *Talika vati* (3rd method)

Ingradients	Quantity
Purified *Harital* (Orpiment)	100 gm
Purified *Manasila* (Realgar)	100 gm
Daruharidra (Berbers aristata) stem	100 gm
Yavakshar (Potassi Carbonas)	100 gm
Saindhav (Rock salts)	100 gm
Sudha (Lime stone)	100 gm
Madhu (Honey)	Q.S.

Procedure:

All ingredients (Table no 1) were mixed uniformly in khalwa yantra (Mortar & pestle). Then pounded in Khalwa yantra and made to soft paste with prescribed honey. Talika Vati was made by rolled in to circular in shape.

Pills were dried in the shade. These *Talika vati's* were kept in airtight container[7].

Toxicity study:

Conducting laboratory & location:

Experimental protocol was permitted by the institutional Ethical Committee (IAEC) under CPCSEA, approved no: Reg.No. 112/PO/RE/1999/CPCSEA, dated 2.6.2015 of Pharmacology Laboratory of SET'S Pharmacy College, Dharwad, Karnataka.

The study was carried out under prevailing husbandry conditions in the animal house as per OECD Guideline of CPASEA[8].

Selection of Animals:

30 Adult mice (20 - 25 g) of either sex were housed in polypropylene cages and fed with standard diet and water ad libitum. The animals were exposed to alternate cycle of 12 h of darkness and light each. Animals had been fasted for overnight.

Sample size: Animals were randomly divided into 7 groups of 5 per cage for seven days. Each animal was used only once.

Selection of different doses of *Talika vati* susp. : Different doses of *Talika vati* suspension were used as shown in table no 1. All doses were prepared by suspension form.

Sample Preparation: *Talika Vati* Suspension was prepared by weighing required quantity of Vati (Tablet) into a cleaned motor and pestle then crushed in to fine powder for 5 min. Two to three drops of Tween-80 was added and triturated to homogenous mass. Measured quantity of water was then added to mass with continuous triturating to suspend the particles. Resulted solution was filtered in muslin cloth to remove larger particles and filtrate was used for the administration to mice.

Test Procedure: *Talika Vati* Suspension was administered orally in graduated dose in 7 groups (Table no 2) & one dose being used per group.

OBSERVATIONAL PERIOD:

After the test, the mice were the sole occupant of the cage with free contact of food & water. Overnight fasted animals were treated with respective dose and were observed for initial 4 hrs and mortality checked for 24 hrs. Then it was noted (Table no 4). At the end of study period, expired animals were counted for the calculation of LD50. For ethical reason, all animals were sacrificed at the end of the study.

Table no 2: Observation of Mortality rate

Exp. Group	Dose of *Talika Vati* (mg/kg)	Mortality (x/N)
I	10 mg/kg	0/5
II	100 mg/kg	0/5
III	285 mg/kg	0/5
IV	500 mg/kg	0/5
V	1000 mg/kg	0/5
VI	2000 mg/kg	1/5
VII	5000 mg/kg	5/5

Table no 3: Mathematical method (Karber's Method)[9]

Exp. Group	Dose (mg/kg)	Dose difference (DD)	No. of animals (n)	No. of dead animals	Mean mortality (MM)	DD x MM
I	10	-	5	-	0	0
II	100	90	5	-	0	0
III	285	185	5	-	0	0
IV	500	215	5	-	0	0
V	1000	500	5	-	0	0
VI	2000	1000	5	1	0.5	500
VII	5000	3000	5	5	3	9000
Σ(DD x MM) = 9500						

Calculation[9] of LD_{50}

Mean mortality = difference of two adjacent no. of dead animals / 2

Σ(DD x MM) = 9500; value is divided by the no of animals in the group (n=5),

Hence value obtained is 9500/5= 1900

Finally, this value is subtracted from the minimum dose which produces the 100 % mortality, ie 5000 mg/kg.

So, LD_{50} = 5000-1900 = 3100 mg/kg

Table no 4: Observation during Acute toxicity of *Talika vati* suspension

Exp. Group	Dose of Talika Vati (mg/kg)	Observations for 4 hrs
I	10	Continuously drinking water, increased locomotor activity, Settled at corner, Trying to eat active,
II	100	Continuously drinking water, increased locomotor activity, active
III	285	Drinking water, Sitting at corner, Drowsy.

IV	500	Sitting at corner, Drowsy, Resting position, No movement
V	1000	muscle cramps, Settled at corner, less active
VI	2000	Initial movement, Sitting at corner, Salivation, muscle cramps, Almost in rest position, **(1 death)**
VII	5000	Sitting at corner, Salivation, muscle cramps, No movement, Rest position, **Death of all animals.**

RESULTS

In this study, the results notified that *Talika vati* susp has not been found toxic at the dose of 10 & 100 mg/kg body weight (Group 1 &II). The mice received up to the dose of 1000 mg/kg body weight orally did not show any mortality (Group I to V). The mortality rate was 1 by the dose of 2000 mg/kg body weight in Group VI. The mortality rate was 5 by the dose of 5000 mg/kg body weight in Group VII (Table no 2). Toxic responses were observed in Group III, IV, V & VI. Such as muscle cramps, salivation, sitting at corner (Table no 4).

DISCUSSION

In this study, *Talika vati* susp was administered to the mice orally up to 5000 mg / kg body weight in different doses (Table no 1). Whereas there was no indication of death occurred at the dose of 285, 500 & 1000 mg / kg body weight & shown abnormal responses such as corner sitting, drowsy, muscle cramps. This may be due to the damage of cells and suffered from deficiency of oxygen needed by the body[10] (Table no 4). Given *Talika vati* suspension at the dose of 5000 mg/kg body weight has shown maximum toxicity in which all the animals were died (Table no 4). Death typically occurred by higher dose. The mice that were received 10 & 100 mg / kg body weight of *Talika vati* susp was survived and did not show any toxic signs correlated to the other dose. That means *Talika vati* susp could be non toxic at the dose of 10 & 100 mg / kg body weight (Table no 4).

CONCLUSION

- The results of the present study conclude that given *Talika vati* suspension at the dose of 5000 mg/kg body weight has revealed utmost toxicity.
- Whereas, 10 & 100 mg/kg body weight was found to be safer. Hence these doses can be taken for pre-clinical studies.

REFERENCE

1. Agnivesh. Charaka samhita. Elaborated by Charak & Dridhbala. Sutrasthan. Chapter no 1. Verse 127-128. Vidyotini hindi commentary. Edited by kashinath shastri. Varanasi, Choukambha Bharati Acaemy; 18th edition.1992. p.no 49.
2. Hariprapanna Sharma, Rasayogasagar. 1st volume. Verse 71. 1st edition. Varanasi, Krishnadas academy; reprint 2010. P no 547.
3. Chandabhushana Zha, Ayurvediya Rasashasastra. 6th Lesson, 1st edition, Varanasi, Choukambha surabharati prakashana; 2003. Page no 265.
4. P.C.Sharma et al, Data Base of Medicinal Plants in Ayurveda. Vol I. 1st edition. New Delhi, CCRAS; 2002. P no 120-126
5. Sadanand Sharma, Rasa Tarangini. Edited by Haridatta shastri. 13th Taranga. 11th Edition. Varanasi,Motilal Banarasi Das; 1979. Page no 308.
6. https://www.quora.com/What-are-the-differences-between-organic-and-inorganic-arsenic what are the differences between organic and inorganic arsenic? Retrieved on: 17-02-2017, 2.28pm
7. Hariprapanna Sharma, Rasayogasagar. 1st volume. Verse 71. 1st edition. Varanasi, Krishnadas academy; reprint 2010. P no 547.
8. Ghosh MN. Fundamentals of Experimental Pharmacology. 2nd ed. Calcutta: Scientific Book Agency; 1984. p. 154-8. (OECD Guideline 425)
9. Bikash Medhi et al, Practical manual of Experimental & Clinical Pharmacology Part I. 9th lesson.1st edition. New delhi, Jaypee brothers medical publishers; 2010. P. no 118.
10. Ghosh MN. Fundamentals of Experimental Pharmacology. 2nd ed. Calcutta: Scientific Book Agency; 1984. p. 154-8. (OECD Guideline 425)

Corresponding author:
Dr Chidambar.S Maladkar.
Professor, P.G.Dept of Rasashastra & Bhaishajya kalpana Shri J.G.Co-Operative Hospital Societies Ayurvedic Medical College & Ghataprabha.591306, Karnataka. Email:csmaladkar@yahoo.in

Source of Support: NIL
Conflict of Interest : None declared

ACKNOWLEDGEMENT

Author is thankful to Dr Prasana V. Habbu. Head of Department. Dept of Pharmacognosy. SET'S College of Pharamacy. S.R.Nagar, Dharwad, Karnataka.