Siddhayu Ayurvedic Research Foundation Private Limited
Office : Baidyanath Bhawan, Great Nag Road, Nagpur - 440 009. Maharashtra (INDIA)
Ph. No.: (+91) - 0712 - 6644900 / 901 / 902 Website : www.siddhayu.com
CIN No. : U24233MH1983PTC030020

Ref No.: SARF/2020-21

Date: 31.08.2020

To,
The Principal,
Institute of Pharmaceutical Education and Research,
Borgaon, Wardha.

Subject: Sample for acute toxicity study.

Dear Sir,

With reference to our discussion, we are sending herewith following samples for acute toxicity.

Sr No.	Name of Product	Batch No.	Mfg Date.	Expiry Date	Weight	Qty in No's
1	Termino Spot on	VTS04	03/2020	02/2023	30 ml	02
2	Respi-SID Poultry	VRPP04	03/2020	02/2023	100 ml	02
3	Renosid Poultry	VRP05	03/2020	02/2023	100 ml	02

Kindly acknowledge the receipt of the same and start the work.

Thanking you.

Yours Faithfully.

S.S. Dhurde
(Authorized Signatory)

Reg. Off. :- 404, Chartered House, Dr. Cawasji Ha
Factory At :-
Bahadura :- Post - Kalmana (Acharya), Umrer Road, B
Wadsa :- Lakhandur Road, Desaiganj Wadsa, - Dist. Gadchiroli - 441 2
276115

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

PROJECT REPORT

ACUTE DERMAL TOXICITY STUDY OF TERMINO SPOT ON

Submitted to

Siddhayu Ayurvedic Research Foundation Pvt. Ltd,
Nagpur 440 009

Submitted By

Project coordinator
Dr. R. O. Ganjiwale
I/c Principal
Institute of Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

Principal investigator
Dr. B. R. Gandhare
Associate Professor
Department of Pharmacology,
Institute of Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

Performing Department
Department of Pharmacology,
Institute of Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

SEPETM

I.P.E.R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

REPORT

STUDY TITLE

ACUTE DERMAL TOXICITY STUDY OF TERMINO SPOT ON

Sponsored by
Siddhayu Ayurvedic Research Foundation Pvt. Ltd,
Nagpur 440 009

Submitted to
Siddhayu Ayurvedic Research Foundation Pvt. Ltd,
Nagpur 440 009

Date of commencement: 8th September 2020 to 24th September 2020

Project coordinator
Dr. R. O. Ganjiwale
I/c Principal
Institute of Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

Principal investigator
Dr. B. R. Gandhare
Associate Professor
Department of Pharmacology,
Institute of Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

Co-Principal Investigator
Mr. Jyotiranjan Roul
Assistant Professor
Department of Pharmacology,
Institute of Pharmaceutical Education and Research
Borgaon (M

I.P.E.R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

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I. P. E. R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

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1. INTRODUCTION

Acute dermal toxicity is the adverse effects occurring within a short time of dermal application of a single dose of a test substance. In the assessment and evaluation of the toxic characteristics of a substance, determination of acute dermal toxicity is useful where exposure by the dermal route is likely. It provides information on health hazards likely to arise from a short-term exposure by the dermal route. Data from an acute dermal toxicity study may serve as a basis for classification and labelling. It is an initial step in establishing a dosage regimen in subchronic and other studies and may provide information on dermal absorption and the mode of toxic action of a substance by this route. (OECD Guideline 402)

OBJECTIVE:

To determine the acute dermal toxicity (if any) of the test sample **Termino Spot on** (Batch No. VTS04) supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd, Nagpur.

PRINCIPLE:

The test substance is applied to the skin in graduated doses to several groups of experimental animals, one dose being used per group. Subsequently, observations of effects and deaths are made. Animals which die during the test are necropsed. The surviving animals are sacrificed and necropsied. Animals showing severe and enduring signs of distress and pain may need to be humanely killed. Dosing test substances in a way known to cause marked pain and distress due to corrosive or irritating properties need not be carried out. (OECD Guideline 402)

2. MATERIALS AND METHODS

Test Substance: The supplier provided for the test container containing cream identified as **Termino Spot on** (Batch No. VTS04)

The test substance was stored at ambient temperature and out of the light

Details of test animals

Species: Albino rats weighing in range of 220-240 g

Strain: Wistar

Sex: Female

Number of animals per dose level: 3

I. P. E. R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

Number of groups: 2

Selected doses: 200 and 2000 mg/kg

Rationale of selection: As per OECD 402 guidelines

Housing and feeding conditions

Animals were caged individually. The temperature of the experimental animal room was 22°C (± 3°) and the relative humidity 30-70 per cent. Cages maintained at 12 hr of light and dark cycle in the animal house of the Institute.

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum*.

Allocation of animals to various groups:

Table No. 1. GROUPS OF ANIMALS

Group No.	Dose (mg/kg b. Wt) Topically	Animal Numbers
I	200	1 – 3
II	2000	4 – 6

Preparations

Healthy young adult rats were acclimatized to the laboratory conditions for 5 days prior to the test. Before the test, animals were randomized and assigned to the treatment groups. Approximately 24 hours before the test, fur was removed from the dorsal area of the trunk of the test animals by shaving. Care was taken to avoid abrading the skin, which could alter its permeability.

About 10 per cent of the body surface area was clear for the application of the test substance.

EXPERIMENTAL:

The animals were fasted overnight prior to dosing but water was not withheld. Following the period of fasting, the animal[illegible] was applied on the fur-removed skin. After t[illegible] was withheld for 1-2 hours. If the compound are[illegible] ity

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

only above regulatory limit dose then, the dose level to be used as the starting dose is selected as 200 mg/kg b. wt. Accordingly, the first dose in this study was 200 mg/kg (topically) and after that as no toxicity occurred; a limit test dose of 2000 mg/kg b. wt. (topically) was taken. Body weight of the entire test animal was recorded before and periodically (weekly) after administration of the test sample. The animal were observed for 24 hours, then for further 1 days for death and manifestation of toxic effects like changes in skin and fur, eyes and mucous membranes and also any changes in respiratory, circulatory, CNS, autonomic, somatic activity and behaviour pattern if any was recorded. The important clinical sign like muscular tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma if any observed during study period were recorded.

Acute Dermal Toxicity (OECD-402)

For the product of topical application, the testing was undertaken as per OECD-402 guidelines to conduct acute dermal toxicity study test. The Original acute dermal toxicity guideline 402 was adopted in 1987. In this study, both local and systemic effects were investigated. For acute dermal toxicity test 06 Wister rats were procured from CPCSEA registered breeding source i.e. Small Animal Facility, of IPER, Wardha and the test was conducted as per OECD-402 guidelines.

OBSERVATIONS:

The animals were clinically observed twice on the day of the dosing and once daily thereafter for 14 days.

1. Clinical signs:

All animals were observed daily for clinical signs such as gross changes in the activity and the behavioral pattern. They were also observed for the presence of tremors, convulsions, salivation, diarrhea and lethargy

2. Body weight:

The mean group body weight of recorded on 0^{th} day, 7^{th} day and 14^{th} day.

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

3. Mortality:

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

4. Food consumption:

The quantities of food consumed by control and test groups were recorded on 0^{th} day, 7^{th} day and on 14^{th} day.

5. Biochemical Parameters:

- Aspartate aminotransferase (AST),
- Alanine transaminase (ALT),
- Alkaline phosphatase (ALP),
- Creatinine

6. Gross pathology and Histopathology:

All the animals surviving at the end of the 14 days of observation were scarified by intraperitoneal injection of 6% sodium pentobarbital solution at the rate of 0.12 ml/100g and bled at the femoral artery. They were necropsied and the main organs were examined macroscopically. Gross pathology and histopathological examination of lung, Liver, Kidney, and heart were conducted.

Interpretation of the results: The assessment criteria of the toxicity of the test substance taken into account were:

- Body weight change
- Clinical and behavioral signs
- Necropsy findings histopathological examination
- The mortality expressed in % of compound related deaths.

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD_{50} cut-off values, incompliance with Globally Harmonized System (GHS).

- Category 1 = 0 < LD_{50} < 5 mg/kg
- Category 2 = 5 mg/k
- Category 3 = 50 mg/
- Category 4 = 300 mg
- Category 5 = LD_{50} > 2000 mg/kg
- Category 5 or non classified

I. P. E. R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

3. RESULTS

Clinical signs:

All the groups were free of intoxication signs throughout the period of study.

Body weight:

Animals from control and test groups exhibited normal and comparable body weight gain throughout the period of study. (Table No. 2)

Table No. 2 INDIVIDUAL BODY WEIGHT

Group No.	Dose (mg/kg) Topically	Rats No.	Body weight (g)		
			0^{th} day	7^{th} day	14^{th} day
I	200	1	220	228	234
		2	225	234	240
		3	220	229	240
		Mean±SD	221.67±289	230.33±3.21	238.00±3.46
II	2000	4	234	242	249
		5	230	238	245
		6	221	231	240
		Mean±SD	228.33±6.66	237±5.57	244.67±4.51

Values expressed as mean ± standard deviation

Mortality: (Table No. 3)

All animals from control group and test group survived throughout the study for 14 days.

Table No. 3 MORTALITY

Under the condition of the present study, the following mortality rates were recorded

Group No.	Dose (mg/kg) Body weight (g)	Mortality
	t	
I		
II		

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

Food consumption

Table No. 4 GROUP MEAN FOOD CONSUMPTION (g / animal)

Group No.	Dose (mg/kg)	Day		
		0^{th}	7^{th}	14^{th}
I	200	11	14	15
II	2000	12	14	13

Biochemical Parameter

Table No. 5: BIOCHEMICAL FINDING

Group No.	Dose	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creatinine (mg/dL)
I	200 mg/ kg b. wt topically	49.67±2.52	68.00±5.29	77.67±7.51	1.37±0.15
II	2000 mg/ kg b. wt topically	59.67.33±2.52	81.67±2.08	84.67±4.93	1.47±0.32
Significance /NS		NS	NS	NS	NS

Values are expressed as mean ± SD, NS=Non significant

Gross pathology: All main organs subjected to necropsy were found normal in all animals. The result of the necropsy findings are summarized in the table 5.

Table No. 6 PATHOLOGY FINDINGS

Group No.	Death		Comments
	Day	Reason	
I	Day 15	Sacrifice	NTR
II	Day 15	Sacrifice	NTR

NTR = nothing to report

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

Histopathology: Section from live n with mild perivascular lymphoid aggregation. Section from kidney and heart are unremarkable. Section from Lung shows mild pneumonitis.

PLATES

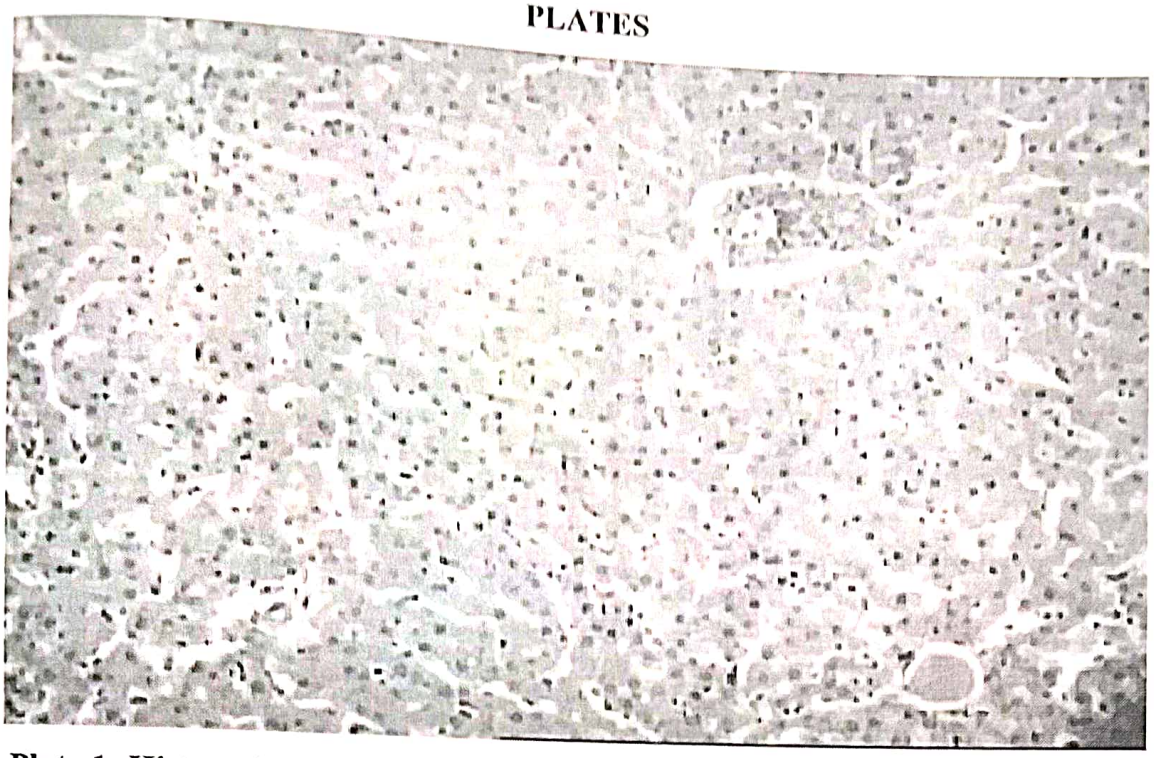


Plate 1: Histopathological Studies of Liver (Group II (2000 mg/kg) (H&E) X100

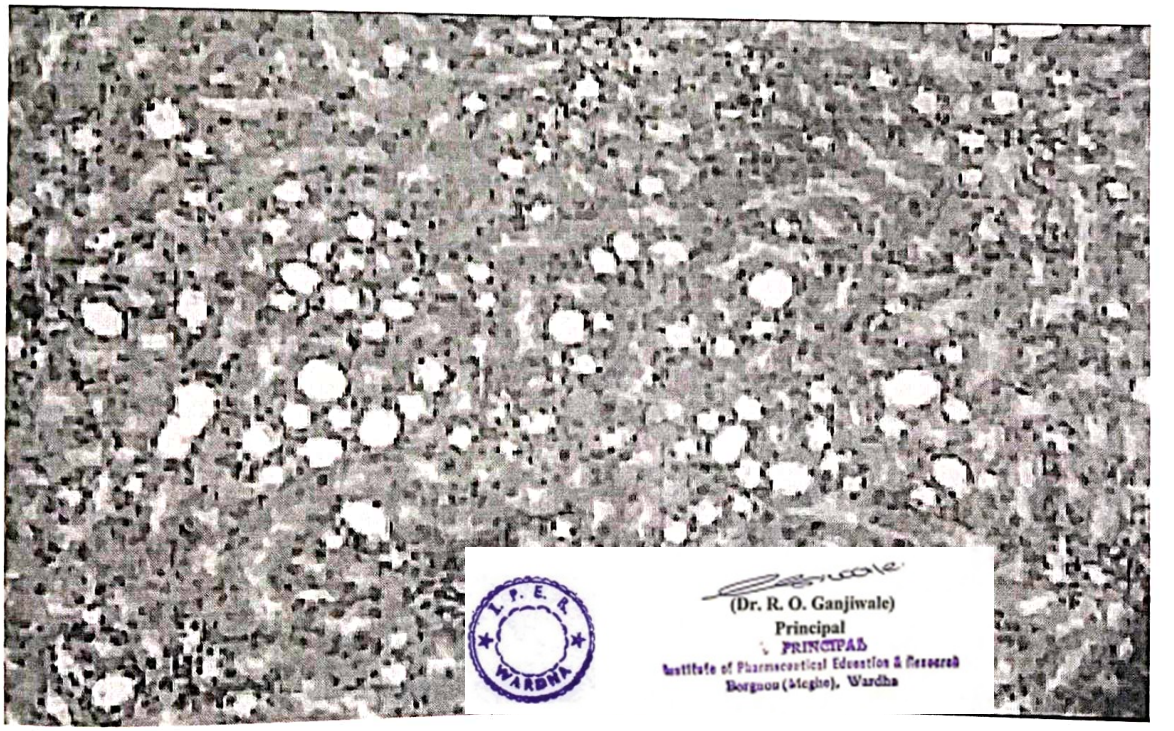
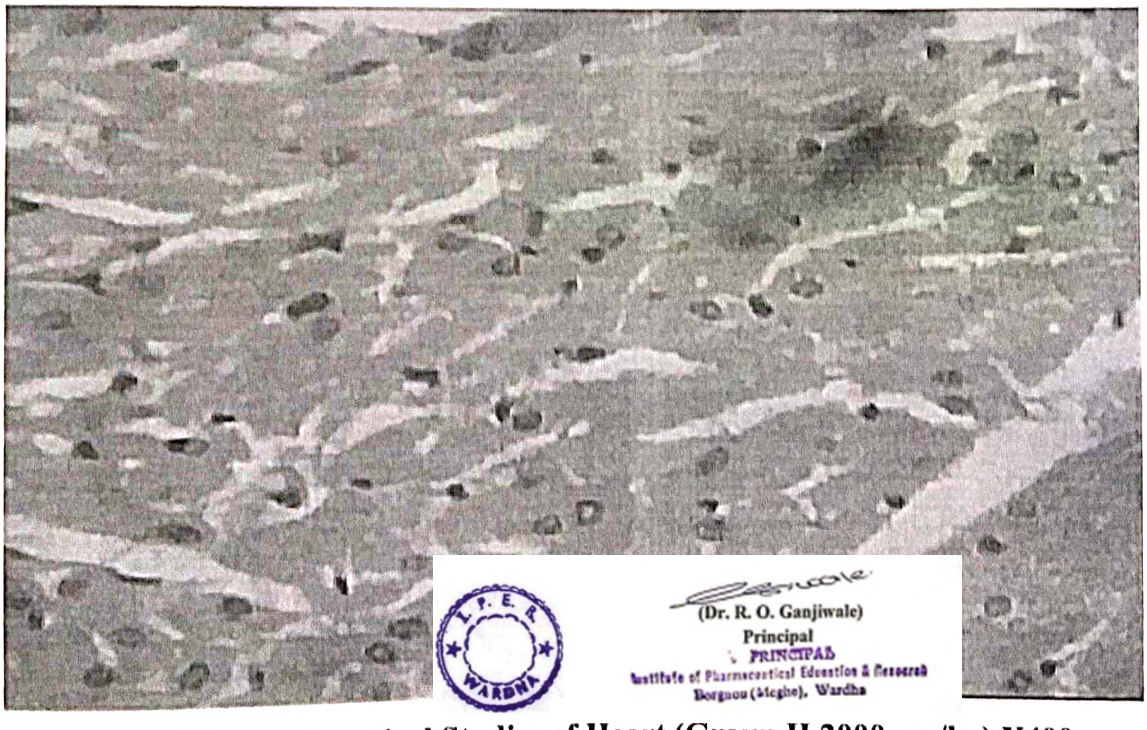


Plate 2: Histopathological Studies of Kidney (Group II (2000 mg/kg) (H&E) X100

Plate 3: Histopathological Studies of Lung (Group II (2000 mg/kg) (H&E) X400



(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

Plate 4: Histopathological Studies of Heart (Group II 2000 mg/kg) X400

4. DISCUSSION:

1. The animals treated at different dose levels with the above test compound Termino Spot on (Batch No. VTS04) in all the groups exhibited normal activity and behavioral pattern.
2. There were no clinical signs of tremors, convulsions, exophthalmia, salivation, diarrhea and lethargy.
3. Animals exhibited normal and comparable body weight gain throughout the period of study.
4. Food intake of normal and animals treated at different dose levels was found to be comparable throughout the tenure of the study.
5. The pathology finding shows no abnormality in any group of animals.

5. CONCLUSION:

The above findings revealed that the Termino Spot on (Batch No. VTS04) supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd, Nagpur was found to be safe at doses 200 and 2000 mg/kg when applied locally. None of the animals were found to be moribund condition in all the groups until the 14 days of the study.

The Study has indicated that Termino Spot on (Batch No. VTS04) at dose of 2000 mg/kg did not affect general health of Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this, minimal lethal dose of Termino Spot on (Batch No. VTS04) was more than 2000 mg/kg.

I. P. E. R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

REFERENCES

1. OECD Guideline for Testing of Chemicals, Acute oral Toxicity – Fixed Dose Procedure, 402. Organization for Economic Co-operation and Development, Paris, 9th October 2017.
2. OECD – Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances – as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, November 1998.
3. OECD (2000) – Guidance Document on the Recognition Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. Environmental Health and Safety Monograph Series on Testing and Assessment No. 19.

Project coordinator : Dr. R. O. Ganjiwale
I/c Principal
I.P.E.R. Wardha

PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha.

Principal investigator : Dr. B. R. Gandhare
Associate Professor
I.P.E.R. Wardha

Co-Principal Investigator

I.P.E.R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

PROJECT REPORT

ACUTE ORAL TOXICITY TEST IN THE RATS
-Fixed Dose Procedure-

Submitted to

Siddhayu Ayurvedic Research Foundation Pvt. Ltd,
Nagpur 440009

Submitted By

Project coordinator
Dr. R. O. Ganjiwale
I/c Principal
Institute of Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

Principal investigator
Dr. B. R. Gandhare
Associate Professor
Department of Pharmacology,
Institute of Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

Department of Pharmacology
Institute of Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

SEPETMBER 2020

REPORT

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ACUTE ORAL TOXICITY TEST IN THE RATS
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Sponsored by
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Submitted to
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Nagpur, 440009

Date of commencement: 14th September 2020 to 28th September 2020

Project coordinator
Dr. R. O. Ganjiwale
I/c Principal
Institute o Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

Principal investigator
Dr. B. R. Gandhare
Associate Professor
Department of Pharmacology,
Institute o Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

C

Dep
Institute o Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

I. P. E. R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

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I.P.E.R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD- OECD GUIDELINE 420 [1]

I. AIM & OBJECTIVE OF THE TEST

The aim of the test is to obtain, using a minimum number of animals, sufficient information on the acute toxicity after single administration, by oral route in the rat, of a test substance, for its classification.

The test substance is administered to a group of experimental animals, by oral route at one defined dose (5 mg/kg, 50 mg/kg, 300 mg/kg or 2000 mg/kg) according to the available information on the test substance.

The animals are observed each day for 14 days at least, after administration, to detect the signs of toxicity. Animals which die are necropsied; the animals which are intercurrently sacrificed as well as the ones which survive until the end of the test are necropsied.

This test using pre-defined doses provides results allowing the test substance to be ranked according to the Globally Harmonised System (GHS) for classification of chemicals which cause acute toxicity (OECD 1998).[2]

II. TEST SUBSTANCE

The supplier provided for the test container containing liquid preparation identified as **Respi-SID Poultry** (Batch No. VRPP04).

The test substance was stored at ambient temperature and out of the light.

I. P. E. R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

III. TEST ANIMALS

Species: Albino rats weighing in range of 180-200 g

Strain: Wister

Age: 8-12 weeks

Number & Sex: 5 nulliparous and non-pregnant females.

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum*.

Housing: The animals were housed in 37cm x 23cm x 16cm polypropylene cages with maximum 3 animals per cage. The cages were placed in limited-access premises of animal house with controlled temperature and humidity. The artificial lighting ensured a sequence of 12 hours light and 12 hours dark.

IV. TEST PROCEDURE

Preparation of the test substance: As per information provided by sponsor, the provided liquid formulation is concentrated and should be used with dilution. The highest dose of formulation that can be used is 250 ml in 1000 liter water. So the test substance was diluted accordingly.

Administration of the test substance: The animals fasted overnight prior to the test substance administration. All animals were weighed again on day 1 before administration.

The volume per 100 g of body weight, defined to 2 ml/100g, the volume to administer was calculated for each rat.

The test substance was administered in a single dose, orally by gavage using a syringe fitted with suitable sized canula. After administration, animals were fasted for 3-4 hours.

Selection of starting dose: As nulation the starting dose was selected as 2m

Main study: According to the the OECD guideline 420 (joined in Appendix IV of the present report) the test was performed with 5 animals receiving the test substance at the dose level of 2ml/100g of body weight.

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

V. OBSERVATIONS

Animals were observed individually after dosing during the first 30 minutes, periodically during the first 24 hours and daily thereafter for a total of 14 days.

The clinical observations were made individually, each animal being examined outside the home cage.

The different parameters observed were – changes in skin & fur, eyes & mucous membranes, respiratory function, tremors, convulsions, salivation, diarrhea, lethargy, sleep & coma.[3]

Body weight: The animals were regularly weighed on day 1 before administration, then on day 8 and day 15 i.e. 7 and 14 days after administration of the test substance.

Pathology: All the animals surviving at the end of the 14 days of observation were scarified by intraperitoneal injection of 6% sodium pentobarbital solution at the rate of 0.12 ml/100g and bled at the femoral artery. They were necropsied and the main organs were examined macroscopically.

Interpretation of the results: The assessment criteria of the toxicity of the test substance taken into account were:

- Body weight change
- Clinical and behavioral signs
- Necropsy findings
- The mortality expressed in % of compound related deaths.

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD_{50} cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- Category 1 = 0 < LD_{50} < 5 mg/kg
- Category 2 = 5 mg/kg < LD_{50} < 50 mg/kg
- Category 3 = 50 mg/kg < LD_{50} < 300 mg/kg
- Category 4 = 300 mg/
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I. P. E. R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

VI. RESULTS

Body weight: All animals exhibited normal and comparable body weight gain throughout the period of study. The individual weights of the animals assessed during the test are supplied in Appendix I.

Clinical Observations: All animals were free of intoxication signs throughout the period of study. The results of the clinical observations are summarized in the table enclosed in Appendix II.

Pathology: All main organs subjected to necropsy were found normal in all animals. The result of the necropsy findings are summarized in the table enclosed in Appendix III.

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **Respi-SID Poultry** (Batch No. VRPP04), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Respi-SID Poultry** (Batch No. VRPP04) at dose of 2000mg/kg did not affect general health of Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this, minimal lethal dose of **Respi-SID Poultry** (Batch No. VRPP04) was more than 2000 mg/kg.

Project coordinator : Dr. R. O. Ganjiwale
I/c Principal
I.P.E.R. Wardha

PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

Principal investigator : Dr. R. R. Gandhare

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

I.P.E.R. WARDHA

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Principal investigator
Dr. B. R. Gandhare
Associate Professor
Department of Pharmacology,
Institute of Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

Department of Pharmacology
Institute of Pha , ,
B

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
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Borgaon (Meghe), Wardha

Principal investigator
Dr. B. R. Gandhare
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Department of Pharmacology,
Institute o Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

Co-
M
Dep
Institute o Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

I.P.E.R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

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APPENDICES

I. P. E. R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD- OECD GUIDELINE 420 [1]

I. AIM & OBJECTIVE OF THE TEST

The aim of the test is to obtain, using a minimum number of animals, sufficient information on the acute toxicity after single administration, by oral route in the rat, of a test substance, for its classification.

The test substance is administered to a group of experimental animals, by oral route at one defined dose (5 mg/kg, 50 mg/kg, 300 mg/kg or 2000 mg/kg) according to the available information on the test substance.

The animals are observed each day for 14 days at least, after administration, to detect the signs of toxicity. Animals which die are necropsied; the animals which are intercurrently sacrificed as well as the ones which survive until the end of the test are necropsied.

This test using pre-defined doses provides results allowing the test substance to be ranked according to the Globally Harmonised System (GHS) for classification of chemicals which cause acute toxicity (OECD 1998).[2]

II. TEST SUBSTANCE

The supplier provided for the test container containing liquid preparation identified as **Renosid Poultry** (Batch No. VI

The test substance was stored at

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

III. TEST ANIMALS

Species: Albino rats weighing in range of 180-200 g

Strain: Wister

Age: 8-12 weeks

Number & Sex: 5 nulliparous and non-pregnant females.

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum*.

Housing: The animals were housed in 37cm x 23cm x 16cm polypropylene cages with maximum 3 animals per cage. The cages were placed in limited-access premises of animal house with controlled temperature and humidity. The artificial lighting ensured a sequence of 12 hours light and 12 hours dark.

IV. TEST PROCEDURE

Preparation of the test substance: The test substance was administered without dilution.

Administration of the test substance: The animals fasted overnight prior to the test substance administration. All animals were weighed again on day 1 before administration.

The volume per 100 g of body weight, defined to 2 ml/100g, the volume to administer was calculated for each rat.

The test substance was administered in a single dose, orally by gavage using a syringe fitted with suitable sized canula. After administration, animals were fasted for 3-4 hours.

Selection of starting dose: As the test substance was an Ayurvedic formulation the starting dose was selected as 2m

Main study: According to the the OECD guideline 420 (joined in Appen performed with 5 animals receiving the test substance at the dose level of 2ml/100g of body weight.

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

V. OBSERVATIONS

Animals were observed individually after dosing during the first 30 minutes, periodically during the first 24 hours and daily thereafter for a total of 14 days.

The clinical observations were made individually, each animal being examined outside the home cage.

The different parameters observed were – changes in skin & fur, eyes & mucous membranes, respiratory function, tremors, convulsions, salivation, diarrhea, lethargy, sleep & coma.[1]

Body weight: The animals were regularly weighed on day 1 before administration, then on day 8 and day 15 i.e. 7 and 14 days after administration of the test substance.

Pathology: All the animals surviving at the end of the 14 days of observation were scarified by intraperitoneal injection of 6% sodium pentobarbital solution at the rate of 0.12 ml/100g and bled at the femoral artery. They were necropsied and the main organs were examined macroscopically.

Interpretation of the results: The assessment criteria of the toxicity of the test substance taken into account were:

- Body weight change
- Clinical and behavioral signs
- Necropsy findings
- The mortality expressed in % of compound related deaths.

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD_{50} cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- Category 1 = 0 < LD_{50} < 5 mg/kg
- Category 2 = 5 mg/kg < LD_{50} < 50 mg/kg
- Category 3 = 50 mg
- Category 4 = 300 m
- Category 5 = LD_{50}
- Category 5 or non classified

I. P. E. R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

VI. RESULTS

Body weight: All animals exhibited normal and comparable body weight gain throughout the period of study. The individual weights of the animals assessed during the test are supplied in Appendix I.

Clinical Observations: All animals were free of intoxication signs throughout the period of study. The results of the clinical observations are summarized in the table enclosed in Appendix II.

Pathology: All main organs subjected to necropsy were found normal in all animals. The result of the necropsy findings are summarized in the table enclosed in Appendix III.

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **Renosid Poultry** (Batch No. VRP05), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Renosid Poultry** (Batch No. VRP05) at dose of 2000mg/kg did not affect general health of Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this, minimal lethal dose of **Renosid Poultry** (Batch No. VRP05) was more than 2000 mg/kg.

Project coordinator : Dr. R. O. Ganjiwale
I/c Principal
I.P.E.R. Wardha

PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

Principal investigator

I.P.E.R. WARDHA

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

Co-Principal Investigator : Mr. Jyotiranjan Roul
Assistant Professor
I.P.E.R. Wardha