Vidarbha Youth Welfare Society's

INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH

Borgaon (Meghe), Wardha (M.S.)

Representative documents for collaborative activities 2019- 20

Table of content

I. P. E. R. WARDHA

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

To,

The Principal

IPER Boregaon (Meghe)

Wardha

Subject: Regarding permission for preparation of 0.05% sodium hypochlorite gel and mouthwash

Respected Sir,

I, Dr. Kiran Sethiya Post-graduate student from Department of Periodontics, Sharad Pawar Dental College, Sawangi (Meghe), Wardha would like to request you to kindly grant me the permission for preparation of 0.05% sodium hypochlorite gel and mouthwash for my project.

Kindly oblige me the permission.

Thanking you.

9/7/19

Dr. Prasad Dhadse

Head of Department

Dept. of Periodontics

H. O. D.
Department of Periodontics
S. P. Dental College,
Sawangi (M.), Wardha

K Sethiya

Dr. Kiran Sethiya

MDS Part I

Signature :—

Dept. of Periodontics

Dr. Kiran Sethiya
P G STUDENT
Dept. of PERIODONTICS
R. No. A-37343
Date :- 9/7/19 Time :- 10:00 am

Dr. S. A. Akbar.

11.07.19

300 ml gel recieved 9/8/19

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

Estd : 1991

VIDARBHA YOUTH WELFARE SOCIETY'S

INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH

Borgaon (Meghe), Wardha, Maharashtra State, India - 442 001

Ph. 07152 -240284 Fax 07152-241684

Dr. Nitin R. Dhande, President | Adv. Uday S. Deshmukh, Vice President | Prof. (Dr.) Hemant M. Deshmukh, Treasurer | Shri. Yuvrajsingh V. Choudhary, Secretary

Dr. R. O. Ganjiwale, I/c Principal

E-mail: iper4160@gmail.com

Web Side: www.iperwardha.com

Conducting Degree, Post Graduate and Doctorate Programme in Pharmaceutical Sciences

Date: 03/08/2019

To WHOMSOEVER IT MAY CONCERN

This is to certify that Dr. Kiran Sethiya, a postgraduate student of Sharad Pawar Dental College, Sawangi, Wardha has successfully formulated dental gel using the facilities available at the Institute of Pharmaceutical Education and Research, Borgaon (Meghe) Wardha.

Dr. Shagufta Khan

Professor

Department of Pharmaceutics

Dr. R. O. Ganjiwale

I/c Principal

PRINCIPAL

Institute of Pharmaceutical Education & Research

Borgaon (Meghe), Wardha

(Dr. R. O. Ganjiwale)

To,

The Principal,

Institute of Pharmaceuticals Education and Research

Wardha

Subject: Permission for formulation of Research material.

Respected Sir,

I, Dr. Pranjali Deulkar, the undersigned Post Graduate Student of Department of Pediatric and Preventive dentistry, Sharad Pawar Dental College would like to request you to grant me permission for formulation of my research material in your institute. The materials required for the same will be procured by your institute.

Kindly grant me permission to use your institute facilities.

Thanking you in anticipation.

Yours Sincerely,

Dr. Pranjali Deulkar
Post Graduate Student
Department of Pedodontics
Sharad Pawar Dental College

Dr. Nilesh Rathi
Guide & Reader
Department of Pedodontics
Sharad Pawar Dental College

Dr. Sudhindra Baliga
Dean & Professor
Sharad Pawar Dental College

Dr. S. A. Khan.
Permitted to propose formulation. Ask to pay consultancy charges

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research

Estd : 1991

VIDARBHA YOUTH WELFARE SOCITY'S

INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH

Borgaon (Meghe), Wardha, Maharashtra State, India - 442 001

NAAC accredited Grade 'A'

Dr. R. O. Ganjiwale
I/C. Principal

Ph. 07152 -240284
Fax 07152-241684

E-mail: iper4160@gmail.com
Web Side: www.iperwardha.com

Conducting Degree, Post Graduate and Doctorate Programme in Pharmaceutical Sciences

Date –29.10.2020

TO WHOMSOEVER IT MAY CONCERN

This is to certify that Dr.Pranjali Vilas Deulkar, a postgraduate student of Department of Pediatric and Preventive Dentistry, Sharad Pawar Dental College and Hospital, Sawangi, Wardha has successfully formulated the Nano Silver fluoride varnish for her thesis titled **"Comparative evaluation of 5% Sodium Fluoride Varnish, Neutral Nano Silver Fluoride & Acidulated Nano Silver Fluoride in Remineralisation of artificially induced enamel caries of primary teeth: An in vitro study."**using facilities available at Institute of Pharmaceuticals Education and Research, Borgaon (Meghe), Wardha.

Dr.Shagufta Khan.
Dept. of Pharmaceutics,
Institute of Pharmaceuticals Education and Research, Wardha

Recieved on 29/10/2020

(Dr. Pranjali Deulkar)

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

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

Genetek Lifesciences Pvt. Ltd.
Admn. Office : 85, KT Nagar, Katol Road, Nagpur-440 013 (M.S.) India
Cell No. : +91-9371272375
E-mail : genetekpharma@gmail.com

Factory : Plot No. B-18, MIDC, Sevagram Road, Wardha-442 006. (India)
Drug Mfg. Lic No. ND/56, ND/57

CIN No. U51101MH2012PTC228652, GST No. 27AAECG6460D1ZQ
MSME No. MH34C0010223

Date: 25/01/2020

To whom so ever it may concern

This to certify that **sixty five** students of B. Pharm final year of the Institute of Pharmaceutical Education and Research Borgaon (Meghe) Wardha visited our industry on **25/01/2020** and got the opportunity to see the large scale production and quality control of parenterals. They learned the critical process controls that are exercised to get the reproducible quality batches.

Genetek Lifesciences Pvt. Ltd. WARDHA

Managing Director

Genetek Life SceincesPvt. Ltd, Wardha

(Dr. R. O. Ganjiwale)

Regd. No. : 11-109052

ALLWIN MEDICOT PVT. LTD.

REG. OFFICE Opp. Income Tax Office, Dr. Ambedkar Marg, Civil Lines, Wardha-442001
(07152) 230215, Fax (07152) 244005, Mobile - 9766144486
Email ID : allwinsurgical11@gmail.com

FACTORY Survey No 414/3, At Salod (Hirapur), Dist. Wardha Maharstra State, INDIA

Ref. No. AL/21022

Date 15/02/2020

CERTIFICATE

Date: 15/02/2020

To whom so ever it may concern

This to certify that **Thirty Two** students of B. Pharm final year of the Institute of Pharmaceutical Education and Research Borgaon (Meghe) Wardha visited our industry on **15/02/2020** and got an opportunity to see the large scale production and quality control of surgical cotton, Absorbent Gauze cloth F-II and Zig-Zag cotton wool. They learned the critical process controls that are exercised to get the reproducible quality batches.

Managing Director

AllwinMedicotPvt. Ltd, Wardha

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

Siddh:ayu

Ref No.: SARF/2019-20/ Date: 10.09.2019.

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

Sub: Sample for acute toxicity study.

Dear Sir,

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

1) **Hepasuport Tablet**
Batch No.:- TH-19-06
Mfg dt.:-08/2019
Exp.dt:-07/2022
Qty:-100 Tab
Weight:-433.00 mg

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

Thanking you.

Yours Faithfully

S. S. Dhurde
(Authorised Signatory)

Dr. Gopalkar
12.09.19

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-

Test substance :- **HEPASUPORT TABLET**
Supplied by :- **Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur**

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Dr. B. R. Gandhare

Institute of Pharmaceuti[illegible]
WARDHA

I. P. E. R. WARDHA

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

Date of commencement: 16th September 201[illegible] ber 2019

CONTENTS

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 pack containing Tablets identified as :

HEPASUPORT TABLET

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

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research

III. TEST ANIMALS

Species: Albino rats weighing in range of 170-220 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 dissolved in water

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 2000 mg/kg.

Main study: According to the methodology describe in the Annex 3 of 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 [illegible]

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research

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/kg $< LD_{50} \leq$ 2000 mg/kg
- Category 5 = $LD_{50} >$ 2000 mg/kg
- Category 5 or non classified

(Dr. R. O. Ganjiwale)

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 HEPASUPORT Tablet (Batch No. TH-19-06), 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 HEPASUPORT TABLET (Batch No. TH-19-06) 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 HEPASUPORT TABLET (Batch No. TH-19-06) 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. B. R. Gandhare
Associate
I.P.E.R.

I.P.E.R. WARDHA

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

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/2019-20/ **Date: 07.10. 2019.**

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

Sub: Sample for acute toxicity study.

Dear Sir,

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

Sr.No.	Brand Name	Dosage form	Pack Size
1	**Digivin-Pet**	Oral	100 ml
2	**Sidcof-Pet**	Oral	100 ml
3	**Wormswin Pet**	Oral	100 ml
4	**Oti Sid Ear oil**	External	25 ml

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

Thanking you.

Yours Faithfully

S. S. Dhurde
(Authorised Signatory)

Dr. R. D. Gundhote
07.10.19.

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

Reg. Off. :- 404, Chartered House, Dr. Cawasji Hormusji Street, Mumbai.
Factory At :-
Bahadura :- Post - Kalmana (Acharya), Umrer Road, Bahadura, - Dist. Nagpur - 441 204. Ph. No.: 07103-276115
Wadsa :- Lakhandur Road, Desaiganj Wadsa, - Dist. Gadchiroli - 441 207 Ph. No.: 07137-272856

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

Test substance :- **Digivin-Pet**
Supplied by :- **Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur**

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Dr. B. R. Gandhare

Institute of Pharmaceutical Education & Research, WARDH

Date of commencement: 10th October 20 ober 2019

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

CONTENTS

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 solution identified as:
Digivin-Pet (Batch No. DGS02).

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

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

III. TEST ANIMALS

Species: Albino rats weighing in range of 140-160 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 2ml/100g rat.

Main study: According to the methodology describe in the Annex 3 of 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 lev

I. P. E. R. WARDHA

(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/kg $< LD_{50} \leq$ 2000 mg/kg
- 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

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 **Digivin-Pet** (Batch No.DGS02), 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 **Digivin-Pet** (Batch No.DGS02) 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 **Digivin-Pet** (Batch No.DGS02) 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. B. R. G
Associate P
I.P.E.R. Wa

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-

Test substance :- **Sidcof-Pet**
Supplied by :- **Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur**

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Dr. B. R. Gandhare

Institute of Pharmaceutical Education & Research, WARDHA

Date of commencement: 10th October 2019

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

CONTENTS

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 solution identified as:
Sidcof-Pet (Batch No. VSIPO4).

The test substance was stored at ambient te

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

III. TEST ANIMALS

Species: Albino rats weighing in range of 150-170 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 2ml/100g rat.

Main study: According to the methodology describe in the Annex 3 of 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.

I. P. E. R. WARDHA

(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/kg $< LD_{50} <$ 300 mg/kg
- Category 4 = 300 mg/kg $< LD_{50} \leq$ 2000 mg/kg
- Category 5 = $LD_{50} >$ 2000 mg/kg
- Category 5 or non classified

(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 **Sidcof-Pet** (Batch No. VSIPO4), 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 **Sidcof-Pet** (Batch No. VSIPO4) 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 **Sidcof-Pet** (Batch No. VSIPO4) 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. B. R. Gandhare
Associate P
I.P.E.R. Wa

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-

Test substance :- **Wormswin Pet**
Supplied by :- **Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur**

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Dr. B. R. Gandhare

Institute of Pharmaceu h,
WARDH

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

Date of commencement: 10th October 201 ber 2019

CONTENTS

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 solution identified as:
Wormswin Pet (Batch No. VWP04).

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

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

III. TEST ANIMALS

Species: Albino rats weighing in range of 150-170 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 2ml/100g rat.

Main study: According to the methodology describe in the Annex 3 of 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

I.P.E.R. WARDHA

(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/kg $< LD_{50} \leq$ 2000 mg/kg
- 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

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 **Wormswin Pet** (Batch No. VWP04), 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 **Wormswin Pet** (Batch No. VWP04) 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 **Wormswin Pet** (Batch No. VWP04) 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. B. R. Gandhare
Associate
I.P.E.R. W

I.P.E.R. WARDHA

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

ACUTE TOXICITY STUDY OF OTI SID EAR OIL

**Supplied by :- Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur.**

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Dr. B. R. Gandhare

Institute of Pharmaceutical Education & Research, WARDHA

Date of commencement: 10th October 2 tober 2019

I.P.E.R. WARDHA

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

Acute toxicity study of Oti Sid Ear oil

OBJECTIVE: To determine the acute toxicity (if any) of the test sample **Oti Sid Ear oil** (Batch No. VOT04) supplied by Siddhayu Ayurvedic Research Foundation Private Limited 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.

TEST PROCEDURE

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.

Details of test animals

Species: Albino rats weighing in range of 140-160 g

Strain: Wistar

Sex: Male

Number of animals per dose level: 5

Number of groups: Five

Selected doses: 5, 50, 300 and 2000 mg/kg

Rationale of selection: As per OECD 402 g

I.P.E.R. WARDHA

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

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)	Animal Numbers
I	Control	1 – 5
II	5	1 – 5
III	50	1 – 5
IV	300	1 – 5
V	2000	1 – 5

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research

EXPERIMENTAL:

The animals were fasted overnight prior to dosing but water was not withheld. Following the period of fasting, the animals were weighed and the test sample was applied on the fur removed skin.

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 the control and test group animals was recorded on 0^{th} day, 7^{th} day and 14^{th} day.

3. Mortality:

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

4. Food consumption:

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

5. 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.

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

RESULTS:

Clinical signs:

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

Body weight: (Table No. 2)

Animals from control and test groups exhibited normal and comparable body weight gain throughout the period of study.

Table No. 2 MEAN BODY WEIGHT

Group No.	Dose (mg/kg)	Mean body weight ± SD (g) 0^{th} day	Mean body weight ± SD (g) 7^{th} day	Mean body weight ± SD (g) 14^{th} day
I	Control	151±2.65	156.4±2.07	162.4±2.30
II	5	152.4±2.70	158.2±2.95	162.8±2.17
III	50	156±3.08	164.4±4.04	169.4±2.97
IV	300	149.2±4.09	158.6±4.98	166.2±2.68
V	2000	151.2±2.28	156.6±2.70	162.8±3.03

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
I	Control	0/5
II	5	0/5
III	50	0/5
IV	300	0/5
V	2000	

I.P.E.R. WARDHA

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

Food consumption: (Table No. 4)

During the course and at the termination of the study the quantity of food consumed by animals of test groups was comparable with that by control animals.

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

Group No.	Dose (mg/kg)	Day		
		0^{th}	7^{th}	14^{th}
I	Control	14	13	15
II	5	12	14	15
III	50	11	13	15
IV	300	11	14	15
V	2000	13	15	14

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. 5 PATHOLOGY FINDINGS

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

NTR = nothing to report

I. P. E. R. WARDHA

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

DISCUSSION:

1. The animals treated at different dose levels with the above test compound Oti Sid Ear oil (Batch No. VOT04) 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 from control and test groups 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.

CONCLUSION:

The above findings revealed that the Oti Sid Ear oil (Batch No. VOT04) supplied by Siddhayu Ayurvedic Research Foundation Private Limited, Nagpur was found to be safe at doses 5, 50, 300, 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 Oti Sid Ear oil (Batch No. VOT04) 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 Oti Sid Ear oil (Batch No. VOT04) 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. B. R. C
Associate
I.P.E.R. W

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

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/2019-20/ **Date: 03.12.2019.**

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

Sub: 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.Dt.	Exp.Dt.	Weight	Qty in Nos
1	HEMOVART TABLET	HT-01	11/2019	10/2022	927 mg	2 Nos
2	HEMOVART GRANULES	HG-02	12/2019	11/2022	60 gm	2 Nos

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

Thanking you.

Yours Faithfully

S. S. Dhurde
(Authorised Signatory)

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

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

Test substance :- HEMOVERT TABLET
Supplied by :- Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Dr. B. R. Gandhare

Institute of Pharmaceuti
WARDHA

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

Date of commencement: 05th December 2019 ... 2019

CONTENTS

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 pack containing Tablets identified as :

HEMOVART TABLET

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 160-210 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 dissolved in water

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 2000 mg/kg.

Main study: According to the methodology describe in the Annex 3 of 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 2000 mg/kg 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/kg $< LD_{50} \leq$ 2000 mg/kg
- Category 5 = $LD_{50} >$ 2000 mg/kg
- Category 5 or non classified

(Dr. R. O. Ganjiwale)
Principal

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.

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(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research

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 HEMOVART Tablet (Batch No. HT-01), 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 HEMOVART TABLET (Batch No. HT-01) 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 HEMOVART TABLET (Batch No. HT-01) 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. B. R. Gandhare
Associate Professor
I.P.E.R. W

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-

Test substance :- **HEMOVERT GRANULES**
Supplied by :- **Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur**

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Dr. B. R. Gandhare

Institute of Pharmaceu[illegible]h,
WARDH[illegible]

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

Date of commencement: 05th December 20[illegible]nber 2019

CONTENTS

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 classificarion of chemicals which cause acute toxicity (OECD 1998).[2]

II. TEST SUBSTANCE

The supplier provided for the test pack containing substance identified as :

HEMOVART GRANULES

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 200-260 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 dissolved in water

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 2000 mg/kg.

Main study: According to the methodology describe in the Annex 3 of 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 2000 mg/kg of body weight.

I.P.E.R. WARDHA

(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/kg $< LD_{50} \leq$ 2000 mg/kg
- Category 5 = $LD_{50} >$ 2000 mg/kg
- Category 5 or non classified

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

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 HEMOVART GRANULES (Batch No. HG-02), 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 HEMOVART GRANULES (Batch No. HG-02) 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 HEMOVART GRANULES (Batch No. HG-02) 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. B. R. Gandhare
Associate Professor
I.P.E.R. Wardha

I.P.E.R. WARDHA

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