

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.

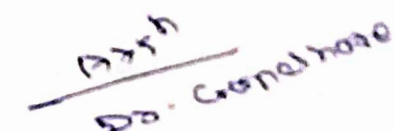
Thanking you.

Yours Faithfully



S. S. Dhurde  
(Authorised Signatory)



  
12.09.19  
(Dr. R. O. Ganjiwale)  
Principal  
PRINCIPAL  
Institute of Pharmaceutical Education & Research  
Borgaon (Wardha), Maharashtra

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

Test substance :- HEPASUPPORT 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  
Borgun (Moghe), Wardha

Date of commencement: 16<sup>th</sup> September 2019

ber 2019

## **CONTENTS**

<b>I. AIM AND OBJECTIVES</b>	<b>3</b>
<b>II. TEST SUBSTANCE</b>	<b>3</b>
<b>III. TEST ANIMALS</b>	<b>4</b>
<b>IV. TEST PROCEDURE</b>	<b>4</b>
<b>V. OBSERVATIONS</b>	<b>5</b>
<b>VI. RESULTS</b>	<b>6</b>
<b>VII. CONCLUSION</b>	<b>6</b>
<b>VIII. REFERENCES</b>	<b>7</b>
<b>APPENDICES</b>	

## **ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD- OECD GUIDELINE 420<sup>1</sup>**

### **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).<sup>2</sup>

### **II. TEST SUBSTANCE**

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

#### **HEPASUPPORT TABLET**

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



  
(Dr. R. O. Ganjivale)  
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



  
(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.<sup>3</sup>

**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<sub>50</sub> cut-off values, in compliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1 = 0 < LD<sub>50</sub> < 5 mg/kg
- Category 2 = 5 mg/kg < LD<sub>50</sub> < 50 mg/kg
- Category 3 = 50 mg/kg < LD<sub>50</sub> < 300 mg/kg
- Category 4 = 300 mg/kg < LD<sub>50</sub> ≤ 2000 mg/kg
- Category 5 = LD<sub>50</sub> > 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 HEPASUPPORT 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<sub>50</sub> higher than 2000 mg/kg in the Rat.

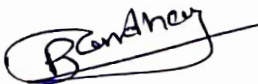
The Study has indicated that HEPASUPPORT TABLET (Batch No. TH-19-06) at dose of 2000mg/kg did not affect general health of Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this, minimal lethal dose of HEPASUPPORT TABLET (Batch No. TH-19-06) was more than 2000 mg/kg.

**Project coordinator**

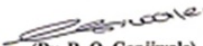
  
: Dr. R. O. Ganjiwale  
I/c Principal

**PRINCIPAL**  
I.P.E.R., Wardha  
**Institute of Pharmaceutical Education & Research,**  
**Borgaon (Meghe), Wardha,**

**Principal investigator**

  
: Dr. B. R. Gandhare  
Associate  
I.P.E.R.



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

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)

*Attn*  
*Dr. R. O. Ganjiwale*  
*07.10.19*



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

Reg. Off. :- 404, Chartered House, Dr. Cawasji Nanawasji Street, Mumbai - 400 001.

Factory At :-

Bahadura :- Post - Kalmna (Acharya), Umrer Road, Bahadura, - Dist. Nagpur - 441 204. Ph. No.: 07103-276115

Wadsa :- Lakhandur Road, Desai Ganj 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

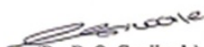
Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Dr. B. R. Gandhare

**Institute of Pharmaceutical Education & Research,  
WARDE**

Date of commencement: 10<sup>th</sup> October 20




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

October 2019

## CONTENTS

I. AIM AND OBJECTIVES	3
II. TEST SUBSTANCE	3
III. TEST ANIMALS	4
IV. TEST PROCEDURE	4
V. OBSERVATIONS	5
VI. RESULTS	6
VII. CONCLUSION	6
VIII. REFERENCES	7
APPENDICES	



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

## ***ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD- OECD GUIDELINE 420<sup>1</sup>***

### **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.

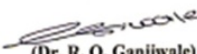
This test using pre-defined doses provides results allowing the test substance to be ranked according to the Globally Harmonised System (GHS) for classification of chemicals which cause acute toxicity (OECD 1998).<sup>2</sup>

### **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  
Borgnour (Moghe), 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.

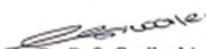
The volume per 100 g of body weight, defined to 2 ml/100g, the volume to administer was calculated for each rat.

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 level of 2ml/100g rat.



  
(Dr. R. O. Ganjivale)  
Principal  
PRINCIPAL  
Institute of Pharmaceutical Education & Research  
Borgun (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.<sup>3</sup>

**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<sub>50</sub> cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1 = 0 < LD<sub>50</sub> < 5 mg/kg
- Category 2 = 5 mg/kg < LD<sub>50</sub> < 50 mg/kg
- Category 3 = 50 mg/kg < LD<sub>50</sub> < 300 mg/kg
- Category 4 = 300 mg/kg < LD<sub>50</sub> ≤ 2000 mg/kg
- Category 5 = LD<sub>50</sub> > 2000 mg/kg
- Category 5 or non classified



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

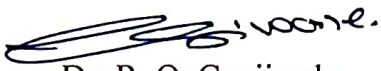
**Clinical Observations:** All animals were free of intoxication signs throughout the period of study. The results of the clinical observations are summarized in the table enclosed in Appendix II.

**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.

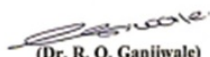
## VII. CONCLUSION

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<sub>50</sub> 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 **PRINCIPAL**  
I/c Principal Institute of Pharmaceutical Education & Research  
I.P.E.R. Wardha Bargaon (Meghe), Wardha.

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

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

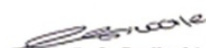
**Project coordinator** : Dr. R. O. Ganjiwale

**Principal investigator** : Dr. B. R. Gandhare

**Institute of Pharmaceutical Education & Research,  
WARDHA**



Date of commencement: 10<sup>th</sup> October 2019

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


er 2019

## CONTENTS

I. AIM AND OBJECTIVES	3
II. TEST SUBSTANCE	3
III. TEST ANIMALS	4
IV. TEST PROCEDURE	4
V. OBSERVATIONS	5
VI. RESULTS	6
VII. CONCLUSION	6
VIII. REFERENCES	7

## APPENDICES



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

## **ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD- OECD GUIDELINE 420<sup>1</sup>**

### **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).<sup>2</sup>

### **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 temperature.



*(Signature)*  
(Dr. R. O. Ganjiwale)  
Principal  
PRINCIPAL  
Institute of Pharmaceutical Education & Research  
Baramon (Mehar), 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.

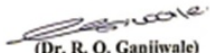
The volume per 100 g of body weight, defined to 2 ml/100g, the volume to administer was calculated for each rat.

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.



  
(Dr. R. O. Ganjwale)  
Principal  
PRINCIPAL  
Institute of Pharmaceutical Education & Research  
Borgun (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.<sup>1</sup>

**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 sacrificed 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.

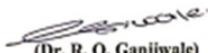
**Interpretation of the results:** The assessment criteria of the toxicity of the test substance taken into account were:

- 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<sub>50</sub> cut-off values, in compliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1 = 0 < LD<sub>50</sub> < 5 mg/kg
- Category 2 = 5 mg/kg < LD<sub>50</sub> < 50 mg/kg
- Category 3 = 50 mg/kg < LD<sub>50</sub> < 300 mg/kg
- Category 4 = 300 mg/kg < LD<sub>50</sub> ≤ 2000 mg/kg
- Category 5 = LD<sub>50</sub> > 2000 mg/kg
- Category 5 or non classified



  
(Dr. R. O. Ganjiwale)  
Principal  
PRINCIPAL  
Institute of Pharmaceutical Education & Research  
Borgun (Moghe), 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 Sidecof-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<sub>50</sub> higher than 2000 mg/kg in the Rat.

The Study has indicated that Sidecof-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 Sidecof-Pet (Batch No. VSIPO4) was more than 2000 mg/kg.

**Project coordinator**

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

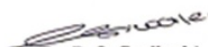
**PRINCIPAL**

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

**Principal investigator :** Dr. B. R. Gandhare  
Associate P  
I.P.E.R. 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 Pharmaceutical Education & Research  
WARDHA



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

h,

Date of commencement: 10<sup>th</sup> October 2019

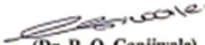
ber 2019

## CONTENTS

I. AIM AND OBJECTIVES	3
II. TEST SUBSTANCE	3
III. TEST ANIMALS	4
IV. TEST PROCEDURE	4
V. OBSERVATIONS	5
VI. RESULTS	6
VII. CONCLUSION	6
VIII. REFERENCES	7

## APPENDICES



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

## ***ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD- OECD GUIDELINE 420<sup>1</sup>***

### **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).<sup>2</sup>

### **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  
Institute of Pharmaceutical Education & Research



### 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.

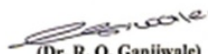
The volume per 100 g of body weight, defined to 2 ml/100g, the volume to administer was calculated for each rat.

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.



  
(Dr. R. O. Ganjiwale)  
Principal  
PRINCIPAL  
Institute of Pharmaceutical Education & Research  
Borgun (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.<sup>3</sup>

**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.

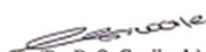
**Interpretation of the results:** The assessment criteria of the toxicity of the test substance taken into account were:

- 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<sub>50</sub> cut-off values, in compliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1 = 0 < LD<sub>50</sub> < 5 mg/kg
- Category 2 = 5 mg/kg < LD<sub>50</sub> < 50 mg/kg
- Category 3 = 50 mg/kg < LD<sub>50</sub> < 300 mg/kg
- Category 4 = 300 mg/kg < LD<sub>50</sub> ≤ 2000 mg/kg
- Category 5 = LD<sub>50</sub> > 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 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<sub>50</sub> 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.

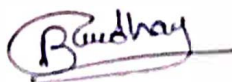
Project coordinator

  
Dr. R. O. Ganjivale  
I/c Principal  
I.P.E.R. Wardha

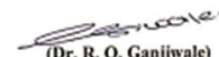
PRINCIPAL

Institute of Pharmaceutical Education & Research  
Borgnur (Meghe), Wardha

Principal investigator : Dr. B. R. Gandhure  
Associate  
I.P.E.R. W





  
(Dr. R. O. Ganjivale)  
Principal  
PRINCIPAL  
Institute of Pharmaceutical Education & Research  
Borgnur (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: 10<sup>th</sup> October 2019



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

tober 2019

## 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 necropsied. 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

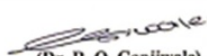
**Number of animals per dose level:** 5

**Number of groups:** Five

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

**Rationale of selection:** As per OECD 402



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

### Housing and feeding conditions

Animals were caged individually. The temperature of the experimental animal room was 22°C ( $\pm 3^\circ$ ) 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





## 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<sup>th</sup> day, 7<sup>th</sup> day and 14<sup>th</sup> 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<sup>th</sup> day, 7<sup>th</sup> day and on 14<sup>th</sup> 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 $\pm$ SD (g) 0 <sup>th</sup> day	Mean body weight $\pm$ SD (g) 7 <sup>th</sup> day	Mean body weight $\pm$ SD (g) 14 <sup>th</sup> day
I	Control	151 $\pm$ 2.65	156.4 $\pm$ 2.07	162.4 $\pm$ 2.30
II	5	152.4 $\pm$ 2.70	158.2 $\pm$ 2.95	162.8 $\pm$ 2.17
III	50	156 $\pm$ 3.08	164.4 $\pm$ 4.04	169.4 $\pm$ 2.97
IV	300	149.2 $\pm$ 4.09	158.6 $\pm$ 4.98	166.2 $\pm$ 2.68
V	2000	151.2 $\pm$ 2.28	156.6 $\pm$ 2.70	162.8 $\pm$ 3.03

Values expressed as mean  $\pm$  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	



*(Signature)*  
(Dr. R. O. Ganjiwale)  
Principal  
PRINCIPAL  
Institute of Pharmaceutical Education & Research  
Borgnur (Moghe), 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 <sup>th</sup>	7 <sup>th</sup>	14 <sup>th</sup>
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*



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

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

Reg. Off. :- 404, Chartered House, Dr. C. S. Jadhav, Nagpur, Maharashtra - 440 009

Factory At :-

Bahadura :- Post - Kalmana (Acharya), Umrer Road, Bahadura, - Dist. Nagpur - 441 204. Ph. No. 07103-276115

Wadsa :- Lakhandur Road, Desaijanj Wadsa, - Dist. Gadchiroli - 441 207 Ph. No. 07137-272856



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

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

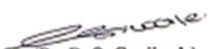
### TEST REPORT

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  
Borgun (Moghe), Wardha

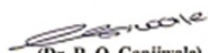
Date of commencement: 05<sup>th</sup> December 2019

er 2019

## CONTENTS

<b>I. AIM AND OBJECTIVES</b>	<b>3</b>
<b>II. TEST SUBSTANCE</b>	<b>3</b>
<b>III. TEST ANIMALS</b>	<b>4</b>
<b>IV. TEST PROCEDURE</b>	<b>4</b>
<b>V. OBSERVATIONS</b>	<b>5</b>
<b>VI. RESULTS</b>	<b>6</b>
<b>VII. CONCLUSION</b>	<b>6</b>
<b>VIII. REFERENCES</b>	<b>7</b>
<b>APPENDICES</b>	



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



## ***ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD- OECD GUIDELINE 420<sup>1</sup>***

### **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).<sup>2</sup>

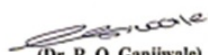
### **II. TEST SUBSTANCE**

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



  
(Dr. R. O. Ganjiwale)  
Principal  
PRINCIPAL  
Institute of Pharmaceutical Education & Research  
Borgun (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. Ganjivale)  
Principal  
PRINCIPAL  
Institute of Pharmaceutical Education & Research  
Borgoo (Moghe), 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.<sup>3</sup>

**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<sub>50</sub> cut-off values, in compliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1 = 0 < LD<sub>50</sub> < 5 mg/kg
- Category 2 = 5 mg/kg < LD<sub>50</sub> < 50 mg/kg
- Category 3 = 50 mg/kg < LD<sub>50</sub> < 300 mg/kg
- Category 4 = 300 mg/kg < LD<sub>50</sub> ≤ 2000 mg/kg
- Category 5 = LD<sub>50</sub> > 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.<sup>3</sup>

**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.

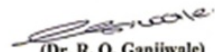
**Interpretation of the results:** The assessment criteria of the toxicity of the test substance taken into account were:

- 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<sub>50</sub> cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1 = 0 < LD<sub>50</sub> < 5 mg/kg
- Category 2 = 5 mg/kg < LD<sub>50</sub> < 50 mg/kg
- Category 3 = 50 mg/kg < LD<sub>50</sub> < 300 mg/kg
- Category 4 = 300 mg/kg < LD<sub>50</sub> ≤ 2000 mg/kg
- Category 5 = LD<sub>50</sub> > 2000 mg/kg
- Category 5 or non classified



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

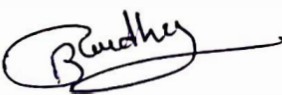
## VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance 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<sub>50</sub> higher than 2000 mg/kg in the Rat.

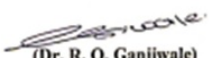
The Study has indicated that HEMOVART TABLET (Batch No. HT-01) at dose of 2000mg/kg did not affect general health of Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this, minimal lethal dose of HEMOVART TABLET (Batch No. HT-01) was more than 2000 mg/kg.

**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



  
(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 Pharmaceutical Education & Research, WARDHA



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

Date of commencement: 05<sup>th</sup> December 20


number 2019

## CONTENTS

I. AIM AND OBJECTIVES	3
II. TEST SUBSTANCE	3
III. TEST ANIMALS	4
IV. TEST PROCEDURE	4
V. OBSERVATIONS	5
VI. RESULTS	6
VII. CONCLUSION	6
VIII. REFERENCES	7

## APPENDICES



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



## **ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD- OECD GUIDELINE 420<sup>1</sup>**

### **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).<sup>2</sup>


### **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.



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

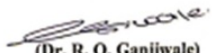
The volume per 100 g of body weight. defined to 2 ml/100g, the volume to administer was calculated for each rat.

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.



  
(Dr. R. O. Ganjiwale)  
Principal  
PRINCIPAL  
Institute of Pharmaceutical Education & Research  
Borgun (Moghe), 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.<sup>3</sup>

**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.

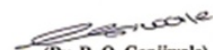
**Interpretation of the results:** The assessment criteria of the toxicity of the test substance taken into account were:

- 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<sub>50</sub> cut-off values, in compliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1 = 0 < LD<sub>50</sub> < 5 mg/kg
- Category 2 = 5 mg/kg < LD<sub>50</sub> < 50 mg/kg
- Category 3 = 50 mg/kg < LD<sub>50</sub> < 300 mg/kg
- Category 4 = 300 mg/kg < LD<sub>50</sub> ≤ 2000 mg/kg
- Category 5 = LD<sub>50</sub> > 2000 mg/kg
- Category 5 or non classified



  
(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.

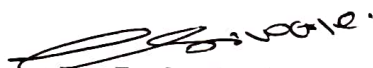
**Pathology:** All main organs subjected to necropsy were found normal in all animals. The result of the necropsy findings are summarized in the table enclosed in Appendix III.

## VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance HEMOVART 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<sub>50</sub> 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.

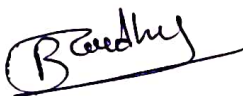
**Project coordinator**

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

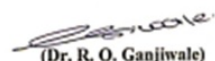
**PRINCIPAL**

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

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





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