# Vidarbha Youth Welfare Society's INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH

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

# Representative documents for collaborative activities 2017-18

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(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmacertical Education & Resource Borgnon (Megho), Wardha



Datta Meghe Institute of Medical Sciences (Deemed to be University) [Accredited by NAAC 'A+' Grade]

Mahatma Gandhi Ayurved College, Hospital & Research Centre Salod (Hirapur), Wardha, (MS) College .07152-202632, Hospital 202631, = Fax.287882 Email- mgayurvedcollege@gmail.com Web: www.mgachrc.org, www.dmims.edu.in



Date: - 1/02/2018

To, **The Principal** IPER Wardha

Subject:- External ship of Rushikesh Thakre Through:- Dean MGACH & RC

### **Respected Sir**

As per curriculum of fellow ship in ethanopharmacology, Rushikesh Thakre is posted as per following details for his research work

Sr no	Place	Duration	
1	IPER Borgaon Meghe, Wardha	1.2.2018- 20.2.2018	

Kindly grant the permission for the same and do the needful

Enclosed: - Research Protocol

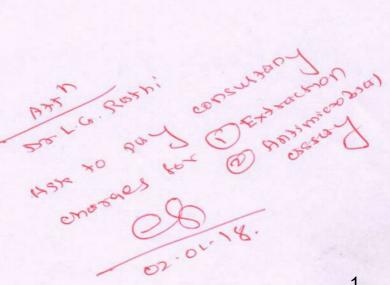
Copy to:- Rushikesh Thakre

Sincerely yours

Dr. Pramod Khobragade HOD Dravyaguna dept **MGACH & RC** 



en cole Dr. R. O. Ganjiwale) Principal PRINCIPAL of Pharmacentical Education & Resourch orgnos (Meghe), Wardha



Ref. No.: EDN/492-A/2017-18/1

Date: 16.03.2018

To,

Dr. Pramod Khobragade HOD, Dravyaguna Department MGACH & RC Sawangi, Wardha

Subject:- Extraction report of your given samples

Dear Sir,

Find enclosed herewith Extraction study of given samples received from

you on dated 1st February 2018.

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(Dr. R.O. Ganjiwale) I/c Principal

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



Datta Meghe Institute of Medical Sciences (Deemed to be University) [Accredited by NAAC 'A+' Grade] Mahatma Gandhi Ayurved College, Hospital & Research Centre Salod (Hirapur), Wardha, (MS)

> College .07152-202632, Hospital 202631, Fax.287882 Email- mgayurvedcollege@gmail.com Web: www.mgachrc.org, www.dmims.edu.in



Date: - 1/02/2018

To, The Principal IPER Wardha

Subject:- External ship of Rushikesh Thakre Through:- Dean MGACH & RC

### **Respected Sir**

As per curriculum of fellow ship in ethanopharmacology, Rushikesh Thakre is posted as per following details for his research work

Sr no	Place	Duration	
1	IPER Borgaon Meghe, Wardha	1.2.2018- 4.3.2018	

Kindly grant the permission for the same and do the needful

Enclosed: - Research Protocol

Copy to:- Rushikesh Thakre

Sincerely yours

Dr. Pramod Khobragade HOD and supervisor Dravyaguna dept MGACH & RC

Supervisor Fellowship Ethnopharmacology Dept. of Dravyaguna MGACH & RC SALOD (H.), WARDHA

Dr. Shyam Bhutada M.D.(AYU.) Reg. No. I-17076-A-1 DEAN / CMS MGACH & RC, SALOD(H.), Wardha Ref. No.: EDN/492-A/2018-19/2

Date: 13.04.2018

# CERTIFICATE

This is to certify that -

Dr. Rushikesh Thakare has performed the extraction and antimicrobial activity evaluation of Khandu Chakka at this Institute from 01.02.2018 to 04.03.2018. The results of the same are enclosed herewith.

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(Dr. R.O. Ganjiwale) I/c Principal I/s. Photocopha Photocopha Manual of Photocophics & Concepts

Borgaon (Maghe), Wiersho

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(Dr. R. O. Ganjiwale) Principal '> PRINCIPAD hastilede of Pharmecerical Idention & Sessered Bergnou (Meghe), Wardha

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Siddhayu Ayurvadic Research Foundation Private Limited Office : Baidyanath Bhawan, Great Nag Road, Nagpur - 440 024. Maharashtra (INDIA) Ph. No.: (+91) - 0712 - 6644900 / 901 / 902 Website : www.siddhayu.com CIN No. : U24233MH1983PTC030020



Ref No.:SARF/2017-18

Date : 17.03.2018

To,

#### Principal,

Institute of Pharmacutical Educational and Research Borgaon Meghe,Wardha.

Dear Sir,

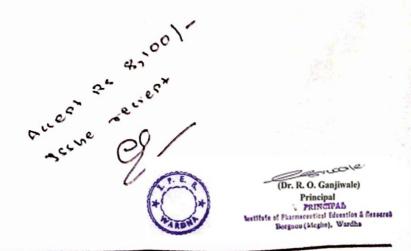
Enclosed herewith cheque of Yes Bank of Rs.8100/-[Rs,9000/- -{Rs900/ }as TDS] (RS.Eight Thousand One Hundred Only) No.878542 Dated 17.03.2018 as a advance towards the acute toxicity study of Mandur Bhasma.

Thanking You.

Yours Faithfully,

For, Siddhayu Ayurvedic Research foundation Pvt. Ltd.

Authorized Signatory



5

Reg. Off.:- 404, Chartered House, Dr. Cawasji Harmosji Street, Mumbai - 400002.Factory At:-Bahadura:- Post - Kalmana (Acharya), Umrer Road, Bahadura, - Dist. Nagpur - 441 204. Ph. No.: 07103-276115Wadsa:- Lakhandur Road, Desaiganj Wadsa, - Dist. Gadchiroli - 441 207 Ph. No.: 07137-272856

Acute toxicity of MANDUR BHASMA Study No.: PL - 08 (2017-18) IPER, Wardha

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

Test substance Supplied by :- MANDUR BHASMA

:- Shree-Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur Sidol hayu

# **TEST REPORT**

Project Coordinator : Dr. R. O. Ganjiwale

Principal Investigator : Mr. A. M. Patole

# Institute of Pharmaceutical Education & Research, WARDHA (M.S.) INDIA

Date of commencement: 17th March 2018



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planenscentical I densities & Sessered Borguou (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 : MANDUR BHASMA (Batch No. T – 171220001).

The test substance was stored at ambien

(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planenscentical Education & Resource Borgmon (Maghe), Wardha

Acute toxicity of MANDUR BHASMA Study No.: PL – 08 (2017-18) IPER, Wardha

#### **III. TEST ANIMALS**

Species: Albino rats weighing in range of 200-220 g
Strain: Wister
Age: 10-12 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 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 2000 mg/kg of body weight.



(Dr. R. O. Ganjiwale) Principal PRINCIPAD

#### 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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1 = 0 < LD50 < 5 mg/kg
- Category 2 = 5 mg/kg < LD50 < 50 mg/kg
- Category 3 = 50 mg/kg < LD50 < 300 mg/kg
- Category 4 =  $300 \text{ mg/kg} < \text{LD50} \le 2000 \text{ mg/kg}$
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified



(Dr. R. O. Ganjiwale) Principal PRINCIPAS Institute of Pharmscentical Education & Resource Borgmon (Maghe), 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 MANDUR BHASMA (Batch No. T - 171220001) supplied by Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur was classified in the hazard category 5 or unclassified with a LD50 higher than 2000 mg/kg in the Rat.

The study has indicated that MANDUR BHASMA (Batch No. T - 171220001)<sup>A</sup> 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 MANDUR BHASMA (Batch No. T - 171220001) was more than 2000mg/

**Project coordinator** 

Swool-e

Dr. R. O. Ganjiwale PRINCIPAL I/c Principal Borgaon (Meghe), Wardha.

Principal investigator : Mr. A. M. Patole

Mr. A. M. Patole Assistant Professor I.P.E.R. Wardha



200010 Dr. R. O. Ganjiwale) Principal PRINCIPAN of Pharmacentical Education & Resource Borgnou (Meghe), Wardha

Siddhayu Ayurvedic Research Foundation Private Limited Ph. No.: (+91) - 0712 - 6644900 / 901 / 902 Website : www.siddhayu.com CIN No. : U24233MH1983PTC030020



Date: 12.05.2018

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Accept

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### Ref No. SARF/2018-19/01

То

The Principal Institute of Pharmaceutical Education & Research Borgaon Meghe, Wardha

### Dear Sir,

We are sending herewith following samples for acute toxicity study:

- 1. Diabetes Support Tablet
- 2. D-Stress Capsule
- 3. Vigour Capsule
- 4. Cardio Elixir
- 5. Digest Elixir
- 6. Spotless Cream
- 7. Calcium Plus Tablet

Kindly acknowledge the receipt of the same.

**Thanking You** 

**Yours Sincerely** 

For, Siddhayu Ayurvedic Research Foundation Pvt. Ltd.

**Authorized Signatory** 





Reg. Off.	- 404, Chartered House, Dr. Cawasji Harmosji Street, Mumbai - 400002.	1 March 1
Factory A		07A116
Bahadura	- Post - Kalmana (Acharya), Umrer Road, Bahadura, - Dist. Nagpur - 441 204. Ph. No.: 07103-	11
Wadsa	- Lakhandur Road, Desaiganj Wadsa, - Dist. Gadchiroli - 441 207 Ph. No.: 07137-272856	A L

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

Test substance Supplied by

:- DIABETES SUPPORT Tablet.

:- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole



(Dr. R. O. Ganjiwale) Principal > PRINCIPAD Beneratical Edention & Generate Beorgoou (Megho), Wardha

# Institute of Pharmaceutical Edu WARDHA (M.S.) INDIA

Date of commencement: 5<sup>th</sup> June 2018

Date of completion : 19th June 2018

Acute toxicity of DIABETIES SUPPORT Tables. Study No.: PL - 01 (2018-19)

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# 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 : DIABETES SUPPORT Tablet (Batch No. DST - 01).

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



(Dr. R. O. Ganjiwale) Principal Principal Principal Bastitute of Pharmacerical Education & Research Borgaou (Ategho), Wardha Acute toxicity of DIABETIES SUPPORT Tablet. Study No.: PL - 01 (2018-19)

IPER, Wardha

## **III. TEST ANIMALS**

Species: Albino rats weighing in range of 160-180 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



(Dr. R. O. Ganjiwale) Principal ', PRINCIPAD Sustitute of Pharmaceuticat Idention & Research Borgmon (Magho), 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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	< LD50 <	5 mg/kg
- Category 2	= 5 mg/kg	< LD50 <	50 mg/kg
- Category 3	= 50 mg/kg	< LD50 <	300 mg/kg
- Category 4	= 300 mg/kg	$g < LD50 \leq$	2000 mg/kg
- Category 5	= LD50 >		2000 mg/kg

- Category 5 or non classified



10012 Dr. R. O. Ganjiwale) Principal PRINCIPAD untitute of Pharmacertical Education & Resource Borgnou (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**

The toxicity study has indicated that DIABETES SUPPORT Tablet (Batch No. DST - 01) supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur at dose of 2000mg/kg did not affect the general health in Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this the (minimal) lethal dose of Diabetes support tablet (Batch No. DST- 01) more than 2000 mg/kg in the Rat.

Project coordinator

: Dr. R. O. Ganjiwale **PRINCIPAL** I/c Principalitute of Pharmaceutical Education & Researed I.P.E.R. Wardha

Principal investigator : Mr. A. M. Patole Assistant P I.P.E.R. W

(Dr. R. O. Ganjiwale) Principal Principal Principal Beneratical Idention & Research Bergoou (Megho), Wardha

IPER, Wardha

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

### Test substance Supplied by

:- D-STRESS Capsules

:- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceutical Education & Descaped WARDHA (M.S.) INDIA Date of commencement: 5<sup>th</sup> June 2018

5 Julie 2018

Acute toxicity of D-STRESS Capsules Study No.: PL – 02 (2018-19)

IPER, Wardha

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# 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 : D-STRESS Capsules (Batch No. DSC - 01).

The test substance was stored at ambient te



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Plasmacentical Education & Research Borgnou (Maghe), Wardha Acute toxicity of D-STRESS Capsules Study No.: PL - 02 (2018-19)

IPER, Wardha

#### **III. TEST ANIMALS**

Species: Albino rats weighing in range of 140-210 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  $|\epsilon|$ 



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planescentical Education & Research Bergmon (Maghe), Wardha

Acute toxicity of D-STRESS Capsules Study No.: PL – 02 (2018-19)

IPER, 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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	< LD50 <	5 mg/kg
- Category 2	= 5 mg/kg	< LD50 <	50 mg/kg

- Category 3 = 50 mg/kg < LD50 < 300 mg/kg
- Category 4 =  $300 \text{ mg/kg} < \text{LD50} \le 2000 \text{ mg/kg}$
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmeentical Education & Research Borgnou (Megine), Wardha Acute toxicity of D-STRESS Capsules Study No.: PL - 02 (2018-19)

IPER, 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**

The toxicity study has indicated that D-STRESS Capsule (Batch No. DSC - 01) supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur at dose of 2000mg/kg did not affect the general health in Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this the (minimal) lethal dose of D-STRESS Capsule (Batch No. DSC- 01) more than 2000 mg/kg in the Rat.

Project coordinator

: Dr. R. O. Ganjiwale I/c Principal *pRINCIPAL* I.P.E.R. Wardhaeutical Education & Research. Borgaon (Meghe), Wardha,

Pable

Principal investigator : Mr. A. M. Patole Assistant Professor I.P.E.R. Wardha



2001e Dr. R. O. Ganjiwale) Principal PRINCIPAD atitute of Pharmacentical Education & Resourch

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

Test substance :- VIGOUR Capsule Supplied by :- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

# Institute of Pharmaceutical Education & Research, WARDHA (M.S.) INDIA

Date of commencement: 5th June 2018



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

re 2018

Acute toxicity of VIGOUR Capsule Study No.: PL - 03 (2018-19) IPER, Wardha

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#### APPENDICES



(Dr. R. O. Ganjiwale) Principal

# 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 : VIGOUR Capsule (Batch No. VGC-01).

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



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Acute toxicity of VIGOUR Capsule Study No.: PL - 03 (2018-19) IPER, Wardha

#### **III. TEST ANIMALS**

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

Species: Albino rats weighing in range of 150-180 g

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.



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Acute toxicity of VIGOUR Capsule Study No.: PL - 03 (2018-19)

#### IPER, 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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	< LD50 <	5 mg/kg
- Category 2	= 5 mg/kg	< LD50 <	50 mg/kg
- Category 3	= 50 mg/kg	< LD50 <	300 mg/kg
- Category 4	= 300 mg/kg	g < LD50 <u>&lt;</u>	2000 mg/kg
- Category 5	= LD50 >		2000 mg/kg

- Category 5 or non classified



(Dr. R. O. Ganjiwale) Principal PRINCIPAD

#### 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

The toxicity study has indicated that VIGOUR Capsule (Batch No. VGC - 01) supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur at dose of 2000 mg/kg did not affect the general health in Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this the (minimal) lethal dose of VIGOUR Capsule (Batch No.VGC- 01) more than 2000 mg/kg in the Rat.

Project coordinator

: Dr. R. O. Ganjiwale I/c Principal I.P.E.R. Wardha institute of Pharmacentical Education & Research, Borgaon (Meghe), Wardha,

Principal investigator : Mr. A. M. PAtole Assistant Professor I.P.E.R. Wardha



(Dr. R. O. Ganjiwale) Principal , PRINCIPAD antibule of Pharmeentical Education & Research Borguou (Megho), Wardha

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

Test substance Supplied by

:- CARDIO Elixir

:- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceutical Education & Research,

Date of commencement: 5<sup>th</sup> June 2018



(Dr. R. O. Ganjiwale) Principal PRINCIPAD bestliftefe of Flarencesetical Education & Research Borgnon (Meghe), Wardha Acute toxicity of CARDIO Elixir Study No.: PL - 04(2018-19)

IPER, Wardha

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(Dr. R. O. Ganjiwale) Principal PRINCIPAL Institute of Pharmacentical Education & Resserab Borgnon (Megico), 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 : CARDIO Elixir (Batch No. CE-01).

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



(Dr. R. O. Ganjiwale) Principal V PRINCIPAD Institute of Pharmacentical Education & Resources Borgmon (Magdue), Wardha Acute toxicity of CARDIO Elixir Study No.: PL – 04(2018-19)

IPER, 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 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.



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IPER, 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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	< LD50 <	5 mg/kg
- Category 2	= 5 mg/kg	< LD50 <	50 mg/kg
- Category 3	= 50 mg/kg	< LD50 <	300 mg/kg
- Category 4	= 300 mg/kg	$g < LD50 \leq$	2000 mg/kg
- Category 5	= LD50 >		2000 mg/kg

- Category 5 or non classified



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#### 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

The toxicity study has indicated that CARDIO Elixir (Batch No. CE - 01) supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur at dose of 2000mg/kg did not affect the general health in Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this the (minimal) lethal dose of CARDIO Elixir (Btach No. CE-01) more than 2000 mg/kg in the Rat.

Project coordinator

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Principal investigator : Mr. A. M. PAtole Assistant Professor I.P.E.R. Wardha

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

Test substance Supplied by

:- DIGEST Elixir

:- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceutical Education & Research,

Date of commencement: 5th June 2018



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Acute toxicity of DIGEST Elixir Study No.: PL - 05 (2018-19)

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(Dr. R. O. Ganjiwale) Principal

## 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 : DIGEST Elixir (Batch No. DE-01).

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



Acute toxicity of DIGEST Elixir Study No.: PL – 05 (2018-19)

#### **III. TEST ANIMALS**

Species: Albino rats weighing in range of 150-160 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 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



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Acute toxicity of DIGEST Elixir Study No.: PL – 05 (2018-19)

IPER, 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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	< LD50 <	5 mg/kg
- Category 2	= 5 mg/kg	<ld50<< td=""><td>50 mg/kg</td></ld50<<>	50 mg/kg
- Category 3	= 50 mg/kg	< LD50 <	300 mg/kg
- Category 4	= 300 mg/kg	$g < LD50 \leq$	2000 mg/kg
- Category 5	= LD50 >		2000 mg/kg

- Category 5 or non classified



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Acute toxicity of DIGEST Elixir Study No.: PL – 05 (2018-19) IPER, 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

The toxicity study has indicated that DIGEST Elixir (Batch No. DE- 01) supplied by Siddhayu Ayurvedic Research Foundation Pv4. Ltd., Nagpur at dose of 2000mg/kg did not affect the general health in Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this the (minimal) lethal dose of DIGEST Elixir (Batch No. DE- 01) was more than 2000mg/kg.

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**Project coordinator** 

: Dr. R. O. Ganjiwale I/c Principal I.P.E.R. Wardha Heatitute of Pharmaceutical Education & Research, Borgaon (Meghe), Wardha,

Principal investigator : Mr. A. M. Patole Assistant Professor I.P.E.R. Wardha



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REFERENCES

#### ACUTE TOXICITY STUDY OF SPOTLESS CREAM

- Test substance :- SPOTLESS Cream
- Supplied by
- :- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur.

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole



## Institute of Pharmaceutical Education & Research, WARDHA (M.S.) INDIA

Date of commencement: 5th June 2018

Date of completion : 19th June 2018

# ACUTE TOXICITY STUDY OF SPOTLESS CREAM

**OBJECTIVE:** To determine the acute toxicity (if any) of the test sample Spotless Cream (Batch No. 1101) 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 sacrified and necropsied. Animal 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 150-180g Strain: Wistar Sex: Male Number of animals per dose level: 5 Number of groups: Five Selected doses: 5, 50, 300 and 2000 mg/l Rationale of selection: As per OECD 40

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# Housing and feeding conditions

Animal were caged individually. The temperature of the experimental animal room was  $22^{\circ}$  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. 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	Carole.
		(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Parameerical Education & Serence Borgnou (Magda), Wardha

IPER, Wardha

# 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.

## **OBSERVATION:**

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 quality 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 intraperitonial 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 ; PRINCIPAD antitate of Pharmacentical Education & Sensered

IPER, Wardha

## **RESULTS:**

# **Clinical signs:**

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

# **Body weight:**

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

Group	Dose	Mean body weight ± SD (g)				Mean body weight ± SD (g)	
No.	(mg/kg)	0 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day			
Ι	Control	$162.2 \pm 5.40$	167.4 ± 6.26	172.4± 5.12			
II	5	161 ± 4.74	166.4 ± 4.39	171.2 ± 3.70			
III	50	$165.4 \pm 7.72$	170.6 ± 8.32	$176.2 \pm 6.76$			
IV	300	$159.2 \pm 4.32$	$166.2 \pm 5.16$	$172.3 \pm 4.96$			
V	2000	$158 \pm 6.28$	$164.6 \pm 6.02$	$170.8 \pm 6.90$			

# Table No. 2 MEAN BODY WEIGHT

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	
Ι	Control	0/5	
II	5	0/5	
III	50	0/5	
IV	300	100	sole.
V	2000	(Dr. R. O. Gan Principa > PRINCIPA Institute of Pharmacertical E Borgmon (Meghe),	njiwale) d PAD idention & firstersb

# 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	Dose		Dose	Day		
No.	(mg/kg)	0 <sup>th</sup>	7 <sup>th</sup>	14 <sup>th</sup>		
I	Control	14	16	15		
II	5	13	13	14		
III	50	12	15	14		
IV	300	13	12	14		
V	2000	14	14	15		

**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**

	Death		
Group No.	Day	Reason	Comments
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



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#### **DISCUSSSION:**

- 1. The animals treated at different dose levels with the above test compound Spotless Cream (Batch No. 1101) 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. Animal 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 results of Toxicity Study has indicated that the Spotless Cream (Batch No. 1101) supplied by **Siddhayu Ayurvedic Research Foundation Private Limited, Nagpur** at doses 5, 50, 300, 2000 mg/kg when applied locally did not affect general health in Wistar rats. There were no gross abnormalities observed in necropsied rats, based on this fact it is concluded that the minimal dose of Spotless Cream (Batch No. 1101) when applied locally was more than 2000 mg/kg.

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Project coordinator

: Dr. R. O. Ganjiwale I/c Principal I.P.E.R. Wardha Borgaon (Meghe), Wardha,

Principal investigator : Mr. A. M. Patole Assistant Professor I.P.E.R. Wardha



(Dr. R. O. Ganjiwale) Principal PRINCIPAD

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

Test substance Supplied by

:- CALCIUM PLUS Tablet

:- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

# Institute of Pharmaceutical Education & Research.

WARDHA

(Dr. R. O. Ganjiwale) Principal Principal Principal Bergnos (Meghe), Wardha

Date of commencement: 5th June 2018

Acute toxicity of CALCIEM PLE'S Tables Study No.: PL - 07 (2013-19)

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(Dr. R. O. Ganjiwale) Principal Principal Bastitute of Pharmacertical Education & Serganou Eorganou (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 : CALCIUM PLUS Tablet (Batch No. CPT - 01).

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



(Dr. R. O. Ganjiwale) Principal ', PRINCIPAD bastitute of Pharmacovical Idention & Ressered Berguou (Ategho), Wardha Acute toxicity of CALCIUM PLUS Tablet Study No.: PL - 07 (2018-19)

IPER, 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 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 V PRINCIPAD Institute of Pharmecerical Education & Research Borgmon (Meghe), Wardha

Acute toxicity of CALCIUM PLUS Tablet Study No.: PL – 07 (2018-19)

IPER, Wardha

### V. OBSERVATIONS

Animals were made individually, each animal being examined outside the home cage. 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

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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	< LD50 <	5 mg/kg
- Category 2	= 5  mg/kg	< LD50 <	50 mg/kg
- Category 3	= 50 mg/kg	< LD50 <	300 mg/kg
- Category 4	= 300 mg/kg	$g < LD50 \leq$	2000 mg/kg
- Category 5	= LD50 >		2000 mg/kg

- Category 5 or non classified



es cole Dr. R. O. Ganjiwale) Principal TPAN

Acute toxicity of CALCIUM PLUS Tablet Study No.: PL – 07 (2018-19) IPER, 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

The toxicity study has indicated that CALCIUM PLUS Tablet (Batch No. CPT- 01) at dose of 2000mg/kg did not affect the general health in Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this the (minimal) lethal dose of CALCIUM PLUS Tablet (Batch No. CPT- 01) was more than 2000mg/kg.

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Project coordinator

Dr. R. O. Ganjiwale I/c Principal PRINCIPAL I.P.E.R. Wardha institute of Pharmareutical Education & Bossarob, Borgaon (Meghe), Wardha,

Principal investigator : Mr. A. M. Patole Assistant Professor I.P.E.R. Wardha



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Phareneerical Education & Research Berrange (Meighe), Wardha





Date: 12.05.2018

### Ref No. SARF/2018-19/01

To

The Principal Institute of Pharmaceutical Education & Research Borgaon Meghe, Wardha

## Dear Sir,

We are sending herewith following samples for acute toxicity study:

- Diabetes Support Tablet
- 2. D-Stress Capsule
- 3. Vigour Capsule
- 4. Cardio Elixir
- 5. Digest Elixir
- 6. Spotless Cream
- 7. Calcium Plus Tablet

Kindly acknowledge the receipt of the same.

## **Thanking You**

**Yours Sincerely** 

For, Siddhayu Ayurvedic Research Foundation Pvt. Ltd.

**Authorized Signatory** 





Acces

Qs 8,001

:- 404, Chartered House, Dr. Cawasji Harmosji Street, Mumbai - 400002. Reg. Off. Factory At :-:- Post - Kalmana (Acharya), Umrer Road, Bahadura, - Dist. Nagpur - 441 204. Ph. No.: 07103-276115 Bahadura :- Lakhandur Road, Desaiganj Wadsa, - Dist. Gadchiroli - 441 207 Ph. No.: 07137-272856 55 Wadsa

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

Test substance Supplied by :- DIABETES SUPPORT Tablet.

:- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceutic I The WARDHA (M.S.) INDIA

Date of commencement: 5<sup>th</sup> June 2018

(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planmeertical Education & Resserved Borgmon (Meghe), Wardha Acute toxicity of DIABETIES SUPPORT Tablet. Study No.: PL - 01 (2018-19)

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(Dr. R. O. Ganjiwale) Principal > PRINCIPAD hestifiste of Pharmecentical Education & Bergmon (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 : DIABETES SUPPORT Tablet (Batch No. DST - 01).

The test substance was stored at ambient



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planmeerical Education & Sesserab Bergnou (Meghe), Wardha

### III. TEST ANIMALS

Species: Albino rats weighing in range of 160-180 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 lev



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Plasmacentical Education & Research Borguou (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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1 = 0 < LD50 < 5 mg/kg
- Category 2 = 5 mg/kg < LD50 < 50 mg/kg
- Category 3 = 50 mg/kg < LD50 < 300 mg/kg
- Category 4 =  $300 \text{ mg/kg} < \text{LD50} \le 2000 \text{ mg/kg}$
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified



200 cole Dr. R. O. Ganjiwale) Principal PRINCIPAD untitute of Pharmacentical Education & Resource Borgnou (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

The toxicity study has indicated that DIABETES SUPPORT Tablet (Batch No. DST - 01) supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur at dose of 2000mg/kg did not affect the general health in Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this the (minimal) lethal dose of Diabetes support tablet (Batch No. DST- 01) more than 2000 mg/kg in the Rat.

**Project coordinator** 

CHOOLS BRIN

: Dr. R. O. Ganjiwale **PRINCIPAL** I/c Principalitute of Pharmaceutical Education & Researed I.P.E.R. Wardha

Patole

Principal investigator : Mr. A. M. Patole Assistant Professor I.P.E.R. Wa



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmscentical Education & Resource Borgmon (Maghe), Wardha

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

Test substance Supplied by

:- D-STRESS Capsules

:- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceu WARDHA (M.S.) IND



(Dr. R. O. Ganjiwale) Principal , PRINCIPAD hattlate of Plarencentical Idention & Research Borguou (Megho), Wardha

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(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmscentical Education & Research Borgmon (Megho), 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 : D-STRESS Capsules (Batch No. DSC - 01).

The test substance was stored at ambient to an and the first store at a store



(Dr. R. O. Ganjiwale) Principal ', PRINCIPAL Institute of Pharmacontical Identition & Ressorab Bergmon (Ategho), Wardha Acute toxicity of D-STRESS Capsules Study No.; PL - 02 (2018-19)

IPER, Wardha

#### III. TEST ANIMALS

Species: Albino rats weighing in range of 140-210 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 libltum*.
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 let



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmeerical Education & Ressarah Borgnon (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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	< LD50 <	5 mg/kg
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- Category 2 = 5 mg/kg < LD50 < 50 mg/kg
- Category 3 = 50 mg/kg < LD50 < 300 mg/kg
- Category 4 =  $300 \text{ mg/kg} < \text{LD50} \le 2000 \text{ mg/kg}$
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planneerstical Education & Research

IPER, 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**

The toxicity study has indicated that D-STRESS Capsule (Batch No. DSC - 01) supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur at dose of 2000mg/kg did not affect the general health in Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this the (minimal) lethal dose of D-STRESS Capsule (Batch No. DSC- 01) more than 2000 mg/kg in the Rat.

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Project coordinator

: Dr. R. O. Ganjiwale I/c Principal PRINCIPAL I.P.E.R. Wardhaeutical Education & Research. Borgaon (Meghe), Wardha,

Brable.

Principal investigator : Mr. A. M. Patole Assistant Professor I.P.E.R. Wardha



2000le (Dr. R. O. Ganjiwale)

(Dr. R. O. Ganjiwate) Principal PRINCIPAD Institute of Planmeertical Education & Sesserab Borgnon (Meghe), Wardha

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

Test substance Supplied by

:- VIGOUR Capsule

:- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceut WARDHA

Date of commencement: 5<sup>th</sup> June 2018

(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmeentein Edensition & Sesserab Borguou (Megin), Wardha

: 2018

IPER, Wardha

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(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Placencertical Education & Research Borgnou (Meglio), Wardha

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# 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 : VIGOUR Capsule (Batch No. VGC-01).

The test substance was stored at ambient t



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planescentical Education & Research Bergmon (Maghe), Wardha Acute toxicity of VIGOUR Capsule Study No.: PL - 03 (2018-19) IPER, Wardha

#### III. TEST ANIMALS

Species: Albino rats weighing in range of 150-180 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 PRINCIPAD Institute of Pharmaceutical Education & Research Borgnon (Maghe), 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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	< LD50 <	5 mg/kg
- Category 2	= 5 mg/kg	< LD50 <	50 mg/kg
- Category 3	= 50 mg/kg	< LD50 <	300 mg/kg
- Category 4	= 300 mg/kg	g < LD50 ≤	2000 mg/kg

- Category 5 = LD50 > 2000 mg/kg

- Category 5 or non classified



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmecerical Education & Ressered Borganos (Magine), 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**

The toxicity study has indicated that VIGOUR Capsule (Batch No. VGC - 01) supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur at dose of 2000 mg/kg did not affect the general health in Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this the (minimal) lethal dose of VIGOUR Capsule (Batch No.VGC-01) more than 2000 mg/kg in the Rat.

Project coordinator

Dr. R. O. Ganjiwale I/c Principal I.P.E.R. Wardinat Votical Education & Research. Borgaon (Meghe). Wardha.

Principal investigator : Mr. A. M. PAtole Assistant Professor I.P.E.R. Wardha



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmeentical Education & Ressered Borgmon (Megho), Wardha

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

Test substance Supplied by

:- CARDIO Elixir :- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceutical Education & Research,



(Dr. R. O. Ganjiwale) Principal Principal

Date of commencement: 5<sup>th</sup> June 2018

Acute toxicity of CARDIO Elixir Study No.: PL – 04(2018-19) IPER, Wardha

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(Dr. R. O. Ganjiwale) Principal PRINCIPAL Institute of Pharmecentical Education & Research Borgnou (Megice), 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).<sup>2</sup>

#### **II. TEST SUBSTANCE**

The supplier provided for the test container containing solution identified as : CARDIO Elixir (Batch No. CE-01).

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



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmeentical Education & Bessered

Acute toxicity of CARDIO Elixir Study No.: PL ~ 04(2018-19) IPER, 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.
Dict: 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 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 Placenscentical Education & Research Borgmon (Ategho), 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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category $1 = 0$	< LD50 <	5 mg/kg
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- Category 2 = 5 mg/kg < LD50 < 50 mg/kg
- Category 3 = 50 mg/kg < LD50 < 300 mg/kg
- Category 4 =  $300 \text{ mg/kg} < \text{LD50} \le 2000 \text{ mg/kg}$
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmeerical Education & Research Borgnou (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

The toxicity study has indicated that CARDIO Elixir (Batch No. CE - 01) supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur at dose of 2000mg/kg did not affect the general health in Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this the (minimal) lethal dose of CARDIO Elixir (Btach No. CE-01) more than 2000 mg/kg in the Rat.

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Project coordinator : Dr. R. O. Ganjiwale I/c Principal PRINCIPAL I.P.E.RiguWardhamaceutical Education & Bossaroa, Borgaon (Meghe), Wardha,

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Principal investigator : Mr. A. M. PAtole Assistant Professor



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(Dr. R. O. Ganjiwale) Principal PRINCIPAD untitute of Pharmacentical Education & Resourch Borgnou (Meghe), Wardha

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

Test substance :- DIGEST Elixir Supplied by :- Siddbayu Ayurvedic Research Foundation Pvi. Ltd., Nagpur

# TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Parole

Institute of Pharmaceutical Education & Research.

Date of commencement: 5th June 2018



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(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planescentical Education & Sessered Bergmon (Meghe), Wardha

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#### **APPENDICES**



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmscertical Education & Research Borgmon (Megine), 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 : DIGEST Elixir (Batch No. DE-01).

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



(Dr. R. O. Ganjiwale) Principal ', PRINCIPAD Institute of Pharmacontical Identition & Research Bergmon (Magho), Wardha Acute toxicity of DIGEST Elixir Study No.: PL – 05 (2018-19) IPER, Wardha

#### **III. TEST ANIMALS**

Species: Albino rats weighing in range of 150-160 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 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 ', PRINCIPAD Sentitute of Pharmacertical Education & Resources Borgmon (Magho), Wardha Acute toxicity of DIGEST Elixir Study No.: PL - 05 (2018-19)

IPER, 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, cycs & 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 m<sup>1/</sup>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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	< LI)50 <	5 mgkg
- Category 2	≡ 5 mg/kg	< L1)50 <	50 mg/kg
- Category 3	= 50 mg/kg	<ld50 <<="" td=""><td>300 m g/kg</td></ld50>	300 m g/kg
- Category 4	= 300 mg/kg	s ≤LD50 ≤	2000 mg/kg
- Category 5	= LD50 >		2000 mg/kg

- Category 5 or non classified



(Dr. R. O. Ganjiwale)

Acute toxicity of DIGEST Elixir Study No.: PL – 05 (2018-19)

IPER, 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

The toxicity study has indicated that DIGEST Elixir (Batch No. DE- 01) supplied by Siddhayu Ayurvedic Research Foundation Pv4. Ltd., Nagpur at dose of 2000mg/kg did not affect the general health in Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this the (minimal) lethal dose of DIGEST Elixir (Batch No. DE- 01) was more than 2000mg/kg.

Project coordinator

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: Dr. R. O. Ganjiwale I/c Principal I.P.E.R. Wardha Institute of Phatmaceutical Education & Rossarch, Borgaon (Meghe), Wardha,

Principal investigator : Mr. A. M. Patole Assistant Professor I.P.E.R. Wardhe



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planescentical Education & Ressered Borgnou (Meghe), Wardha

# ACUTE TOXICITY STUDY OF SPOTLESS CREAM

## Test substance :- SPOTLESS Cream

Supplied by

:- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur.

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceutical Education & Research.



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmacontical Education & Resserved Borgmon (Meglice), Wardha

Date of commencement: 5<sup>th</sup> June 2018

#### ACUTE TOXICITY STUDY OF SPOTLESS CREAM

**OBJECTIVE:** To determine the acute toxicity (if any) of the test sample Spotless Cream (Batch No. 1101) 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 sacrified and necropsied. Animal 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 150-180g Strain: Wistar Sex: Male Number of animals per dose level: 5 Number of groups: Five Selected doses: 5, 50, 300 and 2000 mg/k Rationale of selection: As per OECD 402

(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Placencertical Education & Research Borgnou (Meghe), Wardha

## Housing and feeding conditions

Animal were caged individually. The temperature of the experimental animal room was  $22^{\circ}$  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:

Group No.	Dose (mg/kg)	Animal Numbers
1	Control	1-5
11	5	1-5
111	50	1-5
IV	300	1-5
V	2000	1-5

#### Table No. GROUPS OF ANIMALS



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Acute toxicity of SPOTLESS Cream Study No.: PL-02 2018-19

IPER Wardha

#### 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.

#### **OBSERVATION:**

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 quality 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 intraperitonial 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)

Acute toxicity of SPOTLESS Cream Study No.: PL-02 2018-19

IPER. Wardha

#### **RESULTS:**

#### **Clinical signs:**

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

#### Body weight:

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

Group	Dose	Mean body weig	ht ± SD (g)	
No.	(mg/kg)	0 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day
]	Control	162.2 ± 5.40	167.4 ± 6.26	172.4± 5.12
11	5	161 ± 4.74	166.4 ± 4.39	171.2 ± 3.70
111	50	165.4 ± 7.72	170.6 ± 8.32	176.2 ± 6.76
IV	300	159.2 ± 4.32	166.2 ± 5.16	172.3 ± 4.96
v	2000	158 ± 6.28	164.6 ± 6.02	170.8 ± 6.90

#### Table No. 2 MEAN BODY WEIGHT

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	
1	Control	0/5	
11	5	0/5	
111	50	0/5	
IV	300		Dr. R. O. Ganjiwale)
V	2000	At hand had a	Principal PRINCIPAD Charmacertical Education & ferenced agnon (Megho), 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	Day		
	(mg/kg)	0 <sup>th</sup>	7th	14 <sup>di</sup>
I	Control	14	16	15
II	5	13	13	1-4
111	50	12	15	14
IV	300	13	12	14
V	2000	1.4	[4	15

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.	D	eath	
	Day	Reason	Comments
I	Day 15	Sacrifice	NTR
11	Day 15	Sacrifice	NTR
111	Day 15	Sacrifice	NTR
IV	Day 15	Sacrifice	NIR
V	Day 15	Sacrifice	NTR

NTR = nothing to report



(Dr. R. O. Ganjiwale) Principal PRINCIPAS Institute of Planmeertical Education & Research Borgnon (Meghe), Wardha Acute toxicity of SPOTLESS Cream Study No.: PL-02 2018-19

IPER, Wardha

#### **DISCUSSSION:**

- The animals treated at different dose levels with the above test compound Spotless Cream (Batch No. 1101) 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. Animal 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 results of Toxicity Study has indicated that the Spotless Cream (Batch No. 1101) supplied by Siddhayu Ayurvedic Research Foundation Private Limited, Nagpur at doses 5, 50, 300, 2000 mg/kg when applied locally did not affect general health in Wistar rats. There were no gross abnormalities observed in necropsied rats, based on this fact it is concluded that the minimal dose of Spotless Cream (Batch No. 1101) when applied locally was more than 2000 mg/kg.

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Project coordinator

: Dr. R. O. Ganjiwale I/c Principal I.P.F.R. Wardha Borgaon (Meghe), Wardha,

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Principal investigator : Mr. A. M. Patole Assistant P I.P.E.R. W

(Dr. R. O. Ganjiwale) Principal 's PRINCIPAD Institute of Pharmecertical Idention & Research Borgaou (Magho), Wardha

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

### Test substance Supplied by

:- CALCIUM PLUS Tablet :- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur

# **TEST REPORT**

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

Principal investigator : Mr. A. M. Patole

# Institute of Pharmaceutical Education & Research,

Date of commencement: 5th June 2018



(Dr. R. O. Ganjiwale) Principal Principal Principal Bergnos (Meghe), Wardha

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(Dr. R. O. Ganjiwale) Principal Principal Principal Bustilate of Pharmacestical Education & Resource Borgnou (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 : CALCIUM PLUS Tablet (Batch No. CPT - 01).

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



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmscerical Education & Resource Borgmon (Megine), 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

Housing: The animals were housed in 37cm x 23cm x 16cm polypropyrine eages 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 PRINCIPAD Institute of Pharmecerical Education & Research Borgnou (Moghe), Wardha

#### **V. OBSERVATIONS**

Animals were made individually, each animal being examined outside the home cage. 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

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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1 = 0 < LD50 < 5 mg/kg
- Category 2 = 5 mg/kg < LD50 < 50 mg/kg
- Category 3 = 50 mg/kg < LD50 < 300 mg/kg
- Category 4 =  $300 \text{ mg/kg} < \text{LD50} \leq 2000 \text{ mg/kg}$
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified



200010 (Dr. R. O. Ganjiwale) Principal PRINCIPAD untitute of Pharmacentical Education & Resourch Borgnou (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**

The toxicity study has indicated that CALCIUM PLUS Tablet (Batch No. CPT- 01) at dose of 2000mg/kg did not affect the general health in Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this the (minimal) lethal dose of CALCIUM PLUS Tablet (Batch No. CPT- 01) was more than 2000mg/kg.

Project coordinator

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Dr. R. O. Ganjiwale I/c Principal PRINCIPAL I.P.E.R. Wardharantical Education & Bossards, Gestitute of Pharmarentical Education & Bossards, Borgaon (Meghe), Wardha,

Principal investigator : Mr. A. M. Patole Assistant Professor I.P.E.R. Wardha



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 Shree
 Ayurved Bhawan Pvt. Ltd.

 Great Nag Road, Nagpur (M.S.) 440 024

 Ph.: 6644900/01/02/03/04/6644934,

 Fax: 0712-2743453, Email: info@baidyanath.info

 Regd. Office: 1, Gupta Lane, Kolkata - 700 006

 CIN No.: U24233WB1947PTC015374

## Ref No.:SARF/2017-18

Date: 02.12.2017

## To, Principal,

Institute of Pharmacutical Educational and Research Borgaon Meghe,Wardha.

## Dear Sir,

Enclosed herewith cheque of Bank of Maharashtra of Rs.35280/- (Thirty Five Thousand Two Hundred Eighty Only) bearing cheque No.77107 Dated 02.12.2017 as a advance towards the acute toxicity study of Four products Vitex-10 Capsule (Marron-Marron), Baidyanath Shatavari Granules, Clearpile Tablet (Red coating) Baidyanath Ashwagandha Capsule(Vegi Capsule).

Thanking You.

Yours Faithfully,

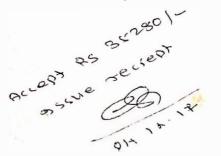
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# For, Shree Baidyanath Ayurved Bhawan Pvt. Ltd.



**Authorized Signatory** 







(Dr. R. O. Ganjiwale) Principal PRINCIPAD estitute of Parenecerical Education & Research Barranou (Maghen), Wardha

- Kolkata 🕐 Patna • Jhansi • Nagpur • Allahabad •

Blending the ancient knowledge with modern science, we offer more than 700 products - the world's largest Ayurvedic range

2017-18/05

Acute toxicity of VITEX-10 Capsules Study No.: PL - 05 (2017-18)

IPER, Wardha

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

Test substance :- VITEX 10 Capsules Supplied by :- Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole



and a le Dr. R. O. Ganjiwale) Principal PRINCIPAD Borgnou (Meghe), Wardha

Date of commencement: 4<sup>th</sup> December 2017

Date of completion : 22 December 2017 100

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Acute toxicity of VITEX-10 Capsules Study No.: PL - 05 (2017-18)

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(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Placencertical Education & Research Borgnou (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).<sup>2</sup>

#### **II. TEST SUBSTANCE**

The supplier provided for the test container containing solution identified as : VTEX-10 Capsule (Batch No. T - 03).

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



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Acute toxicity of VITEX-10 Capsules Study No.: PL - 05 (2017-18) IPER, Wardha

#### **III. TEST ANIMALS**

Species: Albino rats weighing in range of 160-180 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 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



(Dr. R. O. Ganjiwale) Principal > PRINCIPAD hastilate of Pharmacertical Education & Sessered Borgnou (Megho), Wardha

Acute toxicity of VITEX-10 Capsules Study No.: PL - 05 (2017-18)

IPER, 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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

kg
kg
kg
kg
1

- Category 5 or non classified



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#### 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 HS) for the classification of substances which cause acute toxicity, the substance VITEX-10 Capsules (Batch No. T - 03) supplied by Shree Biadyanath Ayurved Bhavan Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD50 higher than 2000 mg/kg in the Rat.

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Project coordinator : Dr. R. O. Ganjiwale I/c Principal (aleased & College (aleased & Co

Principal investigator : Mr. A. M. Patole Assistant Professor I.P.E.R. Wardha



20010 (Dr. R. O. Ganjiwale)

Principal PRINCIPAD Institute of Pharmacertical Education & Research Borgnon (Meghe), Wardha

Acute toxicity of BAIDYANATH SHATAVARI Granules Study No.: PL - 03 (2017-18)

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IPER, Wardha

2017-18/04

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

# Test substance:- BAIDYANATH SHATAVARI GranulesSupplied by:- Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceut WARDHA Date of commencement: 28<sup>th</sup> December 201 (Dr. R. O. Ganjiwale) Principal , PRINCIPAD atthe of Pharmeentical Education & Research Borgnos (Megho), Wardha Acute toxicity of BAIDYANATH SHATAVARI Granules Study No.: PL - 03 (2017-18)

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(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planenscentical Education & Research Bergmon (Megho), Wardha

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Acute toxicity of BAIDYANATH SHATAVARI Granules Study No.: PL - 03 (2017-18) IPER, 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 : BAIDYANATH SHATAVARI Granules (Batch No. 176940066).

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



#### **III. TEST ANIMALS**

Species: Albino rats weighing in range of 130-150 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 PRINCIPAD

#### **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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	<ld50 <<="" th=""><th>5 mg/kg</th></ld50>	5 mg/kg
- Category 2	= 5 mg/kg	<ld50<< td=""><td>50 mg/kg</td></ld50<<>	50 mg/kg
- Category 3	= 50 mg/kg	<ld50<< td=""><td>300 mg/kg</td></ld50<<>	300 mg/kg
- Category 4	= 300 mg/kg	$s < LD50 \leq$	2000 mg/kg
- Category 5	= LD50 >		2000 mg/kg

- Category 5 or non classified



(Dr. R. O. Ganjiwale) Principal PRINCIPAD

#### 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 BAIDYANATH SHATAVARI Granules (Batch No. T - 02) supplied by Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD50 higher than 2000 mg/kg in the Rat.

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**Project coordinator** 

inator : Dr. R. O. Ganjiwale I/c Principal I.P.E.R. Wardha I/c. PRINCIPAL Institute of Pharmacentical Education & Research Borgaon (Meghe), Wardha

Principal investigator : Mr. A. M. PAtole Assistant Professor I.P.E.R. Wardha



(Dr. R. O. Ganjiwale) Principal

Acute toxicity of CLEARPILE tablets Study No.: PL - 04 (2017-18)

IPER, Wardha

2017-18/04

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

Test substance:- CLEARPILE TabletSupplied by:- Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur

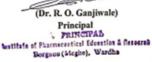
# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceutical Education & Research, WARDH

Date of commencement: 4<sup>th</sup> December 201



er 2017

Acute toxicity of CLEARPILE tablets Study No.: PL - 04 (2017-18)

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(Dr. R. O. Ganjiwale) Principal

Acute toxicity of CLEARPILE tablets Study No.: PL - 04 (2017-18)

IPER, 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 : CLEARPILE Tablet (Batch No. T - 02).

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



(Dr. R. O. Ganjiwale) Principal Principal Principal Education & Secored

Acute toxicity of CLEARPILE tablets Study No.: PL - 04 (2017-18)

#### **III. TEST ANIMALS**

Species: Albino rats weighing in range of 150-160 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

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 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 2000 mg/kg of body weight.



(Dr. R. O. Ganjiwale) Principal V PRINCIPAD Institute of Planemeentical Education & Research Borgnou (Meghe), Wardha Acute toxicity of CLEARPILE tablets Study No.: PL – 04 (2017-18)

#### IPER, 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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	< LD50 <	5 mg/kg
- Category 2	= 5  mg/kg	< LD50 <	50 mg/kg
- Category 3	= 50 mg/kg	< LD50 <	300 mg/kg
- Category 4	= 300 mg/kg	g < LD50 ≤	2000 mg/kg
- Category 5	= LD50 >		2000 mg/kg

- Category 5 or non classified



(Dr. R. O. Ganjiwale) 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 CLEARPILE Tablet (Batch No. T - 02) supplied by Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur was classified in the **hazard category 5** or unclassified with a LD50 higher than 2000 mg/kg in the Rat.

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**Project coordinator** 

inator : Dr. R. O. Ganjiwale I/c Principal I.P.E.R. Wardha I/o. PRINCIPAD Institute of Pharmacestical Education & Researc Borgaon (Meghe), Wardha

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Principal investigator : Mr. A. M. Patole Assistant Professor I.P.E.R. Wardha



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmacentical Education & Ressered Borgnou (Maghe), Wardha

2017-18/02

Acute toxicity of BAIDYANATH ASHWAGANDHA Capsules Study No.: PL - 02 (2017-18)

IPER, Wardha

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

# Test substance:- BAIDYANATH ASHWAGANDHA CapsulesSupplied by:- Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur

# **TEST REPORT**

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceutics | Education & Desearch WARDHA (M.S.) INDIA

Date of commencement: 28<sup>th</sup> December 2

(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmecerical Education & Resserab Borgmon (Megine), Wardha Acute toxicity of BAIDYANATH ASHWAGANDHA Capsules Study No.: PL - 02 (2017-18) IPER, Wardha

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(Dr. R. O. Ganjiwale) Principal > PRINCIPAD within of Planenscentical Education & Reserved

#### 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 : BAIDYANATH ASHWAGANDHA Capsules (Batch No. T - 01).

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



(Dr. R. O. Ganjiwale)

Species: Albino rats weighing in range of 150-200 g Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water ad libitum.

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

#### **IV. TEST PROCEDURE**

**Preparation of the test substance:** The test substance was 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



IPER. 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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1 = 0 < LD50 < 5 mg/kg
- Category 2 = 5 mg/kg < LD50 < 50 mg/kg
- Category 3 = 50 mg/kg < LD50 < 300 mg/kg
- Category 4 =  $300 \text{ mg/kg} < \text{LD50} \le 2000 \text{ mg/kg}$
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified





Acute toxicity of BAIDYANATH ASHWAGANDHA Capsules Study No.: PL – 02 (2017-18)

#### **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 BAIDYANATH ASHWAGANDHA Capsules (Batch No. T - 01) supplied by **Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur** was classified in the **hazard category 5 or unclassified** with a LD50 higher than 2000 mg/kg in the Rat.

**Project coordinator** 

inator : Dr. R. O. Ganjiwale I/c Principal I.P.E.R. Wardha I/c. PRINCIPAL Institute of Pharmacentical Education & Bussearc Borgaon (Meghe), Wardha

Principal investigator : Mr. A. M. Patole Assistant Professor I.P.E.R. Wardha



(Dr. R. O. Ganjiwale) Principal PRINCIPAD titute of Pharmecetical Education & Research

toshe), Wardha





Shree Beidyanam Ayurved Bhewan Pvt. Ltd. Great Nag Road, Nagpur (M.S.) 440 024 Ph.: 6644900/01/02/03/04/6644934, Fax: 0712-2743453, Email: info@baidyanath.info Regd. Office: 1, Gupta Lane, Kolkata - 700 006 CIN No.: U24233WB1947PTC015374

Date : 16-01-2018

To,

### Principal Institute of Pharmaceuticals Education & Research Borgaon Meghe, Wardha

#### Dear Sir,

Enclosed herewith cheque of Bank of Maharashtra, Laxmi Bhawan Square, Nagpur-440 010 of **Rs. 17,640=00/-** (Rs. Seventeen Thousand Six Hundred & Forty only) No.077467 Date: 15.01.2018 to towards Toxicity Study of medicine - Vasant Kusumakar Ras & Vatchintamani Ras (Brihat).

Thanking You

Your faithfully, For SHREE BAIDYANATH AYURVED BHAWAN PVT. LTD.

Dr. Veena Deo Head, Clinical Research.

Encl. As above

Access PS strent O 28-100le Dr. R. O. Ganjiwale) Principal Pharmacentical Education & Resoured attale ou (Meghe), Wardha

KOLKATA · PATNA · JHANSI · NAGPUR · ALLAHABAD ·

Blending the ancient knowledge with modern science, we offer more than 700 products - the world's largest Ayurvedic range

2017-18/06

Acute toxicity of Basant Kusumakar Ras Tablets Study No.: PL – 06 (2017-18)

IPER, Wardha

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

Test substance:- BASANT KUSUMAKAR RAS TabletSupplied by:- Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur

# **TEST REPORT**

Project Coordinator : Dr. R. O. Ganjiwale

Principal Investigator : Mr. A. M. Patole

# Institute of Pharmaceutical Education & Research, WARDHA (M.S.) INDIA

Date of commencement: 5<sup>th</sup> February 20



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planmeertical Idention & Resource Bergmon (Meghe), Wardha

ary 2018

Acute toxicity of Basant Kusumakar Ras Tablets Study No.: PL – 06 (2017-18)

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Acute toxicity of Basant Kusumakar Ras Tablets Study No.: PL - 06 (2017-18)

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.

The supplier provided for the test container containing solution identified as : BASANT KUSUMAKAR RAS Tablet (Batch No. T – 172590001).

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



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Acute toxicity of Basant Kusumakar Ras Tablets Study No.: PL - 06 (2017-18)

IPER, Wardha

### **III. TEST ANIMALS**

Species: Albino rats weighing in range of 175-200 g
Strain: Wistar
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 second prepared of animals

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 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



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#### **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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	< LD50 <	5 mg/kg
- Category 2	= 5 mg/kg	< LD50 <	50 mg/kg
- Category 3	= 50 mg/kg	< LD50 <	300 mg/kg
- Category 4	= 300 mg/kg	g < LD50 <u>&lt;</u>	2000 mg/kg
- Category 5	= LD50 >		2000 mg/kg

- Category 5 or non classified



#### **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 BASANT KUSUMAKAR RAS Tablet (Batch No. T - 172590001) supplied by Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur was classified in the hazard category 5 or unclassified with a LD50 higher than 2000 mg/kg in the Rat.

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Project coordinator

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Principal investigator : Mr. A. M. Patole Assistant Professor I.P.E.R. Wardha



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

# Test substance:- VATCHINTAMANI RAS (Brihat) TabletSupplied by:- Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur

# **TEST REPORT**

Project Coordinator : Dr. R. O. Ganjiwale

Principal Investigator : Mr. A. M. Patole

Institute of Pharmaceutical Education & Research,

WARDHA Date of commencement: 5<sup>th</sup> February 2018

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#### APPENDICES



(Dr. R. O. Ganjiwale) Principal PRINCIPAL Institute of Planescentical Education & Resserve Borgnou (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 VATCHINTAMANI (Brihat) Tablet (Batch No. T - 173010007).

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



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#### III. TEST ANIMALS

Species: Albino rats weighing in range of 150-160 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 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 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 2000 mg/kg of body weight.



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planescentical Education & Resource Borgmon (Maghe), Wardha Acute toxicity of VATCHINTAMANI RAS (Brihat) Tablets Study No.: PL – 07 (2017-18)

#### **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 LD50 cut-off values, incompliance with Globally Harmonized System (GHS).<sup>2</sup>

- Category 1	= 0	< LD50 <	5 mg/kg
- Category 2	= 5 mg/kg	< LD50 <	50 mg/kg
- Category 3	= 50 mg/kg	< LD50 <	300 mg/kg

- Category 4 =  $300 \text{ mg/kg} < \text{LD50} \le 2000 \text{ mg/kg}$
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified



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#### 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 VATCHINTAMANI RAS (Brihat) Tablet (Batch No. T - 173010007) supplied by Shree Baidyanath Ayurved Bhavan Pvt. Ltd., Nagpur was classified in the hazard category 5 or unclassified with a LD50 higher than 2000 mg/kg in the Rat.

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