Vidarbha Youth Welfare Society's

INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH

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

Representative documents for collaborative activities 2017- 18

Table of content

I. P. E. R. WARDHA

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

Ask to pay consultancy charges for ① Extraction ② Antimicrobial assay
02.01.18.

I.P.E.R. WARDHA
(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (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.

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

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

Ayu
Dr. L.G Rathi
01.02.18.
Issue certificate of extension of IPER Wardha

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

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

Received
13/4/18

L.P.E.R. WARDHA

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

Siddhayu Ayurvedic Research Foundation Private Limited
Office : Baidyanath Bhawan, Great Nag Road, Nagpur - 440 024. 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

Accept Rs 8,100/-
Issue receipt

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

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.: 07103-276115
Wadsa :- Lakhandur Road, Desaiganj Wadsa, - Dist. Gadchiroli - 441 207 Ph. No.: 07137-272856

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

Test substance :- **MANDUR BHASMA**
Supplied by :- **~~Shree Baidyanath~~ Siddhayu 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: 17th March 2018

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

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as : MANDUR BHASMA (Batch No. T – 171220001).

The test substance was stored at ambien

I. P. E. R. WARDHA

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

III. TEST ANIMALS

Species: Albino rats weighing in range of 200-220 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
PRINCIPAL

V. OBSERVATIONS

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

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

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

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

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

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).[2]

- 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 < LD50 ≤ 2000 mg/kg
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified

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

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance 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) 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 : Dr. R. O. Ganjiwale
I/c Principal
I.P.E.R. Wardha

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

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

I. P. E. R. WARDHA

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

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

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:

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

SIDDHAYU AYURVEDIC RESEARCH FOUNDATION NAGPUR

L.P.E.R. WARDHA

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

Accept Rs 8,100/-
05.06.18
DSSCMC is correct
05.06.18

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.: 07103-276116
Wadsa :- Lakhandur Road, Desaiganj Wadsa, - Dist. Gadchiroli - 441 207 Ph. No.: 07137-272856

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

Test substance :- **DIABETES SUPPORT Tablet.**
Supplied by :- **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
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

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

Date of commencement: 5th June 2018 Date of completion : 19th June 2018

CONTENTS

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

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as :
DIABETES SUPPORT Tablet (Batch No. DST – 01).

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

I. P. E. R. WARDHA

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

I. P. E. R. WARDHA

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

V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 2000 mg/kg
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified

I.P.E.R. WARDHA

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

VI. RESULTS

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

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

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

VII. CONCLUSION

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
I/c Principal
I.P.E.R. Wardha

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

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

I. P. E. R. WARDHA

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

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

Test substance :- **D-STRESS Capsules**
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 Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

CONTENTS

I.P.E.R. WARDHA

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

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as :
D-STRESS Capsules (Batch No. DSC – 01).

The test substance was stored at ambient te

I. P. E. R. WARDHA

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

III. TEST ANIMALS

Species: Albino rats weighing in range of 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 le

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Borgaon (Meghe), Wardha

V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 2000 mg/kg
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified

I.P.E.R. WARDHA

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

VI. RESULTS

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

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

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

VII. CONCLUSION

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
I.P.E.R. Wardha

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

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

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

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 Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

ne 2018

CONTENTS

(Dr. R. O. Ganjiwale)
Principal

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as :
VIGOUR Capsule (Batch No. VGC-01).

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 150-180 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.

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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.[3]

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 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 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.

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

Test substance :- **CARDIO Elixir**
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

Date of commencement: 5th June 2018

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

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

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 150-170 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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V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 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.

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

Test substance :- **DIGEST Elixir**
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

Date of commencement: 5th June 2018

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as :
DIGEST Elixir (Batch No. DE–01).

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 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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V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 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 DIGEST Elixir (Batch No. DE- 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 DIGEST Elixir (Batch No. DE- 01) was more than 2000mg/kg.

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Principal investigator : Mr. A. M. Patole
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REFERENCES

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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

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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° C (± 3°) and the relative humidity 30-70 per cent. Cages maintained at 12 hr of light and dark cycle in the animal house of the Institute.

Diet: Standard feed prepared in-house.

Water: Plain tap water ad libitum.

Allocation of animals to various groups:

Table No. 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	

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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^{th} day, 7^{th} day and 14^{th} day.

3. **Mortality:**

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

4. **Food consumption:**

   The 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.

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RESULTS:

Clinical signs:

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

Body weight:

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

Table No. 2 MEAN BODY WEIGHT

Group No.	Dose (mg/kg)	Mean body weight ± SD (g)		
		0^{th} day	7^{th} day	14^{th}day
I	Control	162.2 ± 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 ± 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

Values expressed as mean ± standard deviation

Mortality: (Table No. 3)

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

Table No. 3 MORTALITY

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

Group No.	Dose (mg/kg) Body weight (g)	Mortality
I	Control	0/5
II	5	0/5
III	50	0/5
IV	300	
V	2000	

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Food consumption: (Table No. 4)

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

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

Group No.	Dose (mg/kg)	Day		
		0^{th}	7^{th}	14^{th}
I	Control	14	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

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

NTR = nothing to report

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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.

Project coordinator : Dr. R. O. Ganjiwale
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Principal investigator : Mr. A. M. Patole
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ACUTE ORAL TOXICITY TEST IN THE RATS
-Fixed Dose Procedure-

Test substance :- **CALCIUM PLUS Tablet**
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

Date of commencement: 5th June 2018

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as :
CALCIUM PLUS Tablet (Batch No. CPT – 01).

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 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

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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.[3]

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 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 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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Ph. No.: (+91) - 0712 - 6644900 / 901 / 902 Website : www.siddhayu.com
CIN No. : U24233MH1983PTC030020

Siddh:ayu

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:

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

SIDDHAYU AYURVEDIC RESEARCH FOUNDATION PVT. LTD. NAGPUR

Accept Rs 8,100/-
05.06.18

Receipt
05.06.18

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I. P. E. R. WARDHA

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

Test substance :- **DIABETES SUPPORT Tablet.**
Supplied by :- **Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur**

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

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Date of commencement: 5th June 2018

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as :
DIABETES SUPPORT Tablet (Batch No. DST – 01).

The test substance was stored at ambient

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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 le

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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.[3]

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 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 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.

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

Test substance :- **D-STRESS Capsules**
Supplied by :- **Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur**

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as :
D-STRESS Capsules (Batch No. DSC – 01).

The test substance was stored at ambient [illegible]

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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 le

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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.[3]

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 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 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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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

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Date of commencement: 5th June 2018 : 2018

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as :
VIGOUR Capsule (Batch No. VGC-01).

The test substance was stored at ambient t

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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.

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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.[3]

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 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 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.

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

Test substance :- CARDIO Elixir
Supplied by :- Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

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Date of commencement: 5th June 2018 ... ne 2018

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

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

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 150-170 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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V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 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 : Dr. R. O. Ganjiwale
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ACUTE ORAL TOXICITY TEST IN THE RATS
-Fixed Dose Procedure-

Test substance :- DIGEST Elixir
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

Date of commencement: 5th June 2018

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CONTENTS

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as :
DIGEST Elixir (Batch No. DE–01).

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 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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V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < 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

The toxicity study has indicated that DIGEST Elixir (Batch No. DE- 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 DIGEST Elixir (Batch No. DE- 01) was more than 2000mg/kg.

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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

Date of commencement: 5th June 2018

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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

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Housing and feeding conditions

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

Diet: Standard feed prepared in-house.

Water: Plain tap water ad libitum.

Allocation of animals to various groups:

Table No. GROUPS OF ANIMALS

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

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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^{th} day, 7^{th} day and 14^{th} day.

3. **Mortality:**

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

4. **Food consumption:**

   The 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.

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RESULTS:

Clinical signs:

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

Body weight:

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

Table No. 2 MEAN BODY WEIGHT

Group No.	Dose (mg/kg)	Mean body weight ± SD (g)		
		0^{th} day	7^{th} day	14^{th} day
I	Control	162.2 ± 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 ± 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

Values expressed as mean ± standard deviation

Mortality: (Table No. 3)

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

Table No. 3 MORTALITY

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

Group No.	Dose (mg/kg) Body weight (g)	Mortality
I	Control	0/5
II	5	0/5
III	50	0/5
IV	300	
V	2000	

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Food consumption: (Table No. 4)

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

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

Group No.	Dose (mg/kg)	Day		
		0^{th}	7^{th}	14^{th}
I	Control	14	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

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

NTR = nothing to report

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

Test substance :- CALCIUM PLUS Tablet
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

Date of commencement: 5th June 2018

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CONTENTS

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test 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.

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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

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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.[3]

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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	< LD50 ≤	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 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 : Dr. R. O. Ganjiwale
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Principal investigator : Mr. A. M. Patole
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Baidyanath
Trusted since 1917

Shree Ayurved Bhawan Pvt. Ltd.
Great Nag Road, Nagpur (M.S.) 440 024
Ph.: 6644900/01/02/03/04/6644934,
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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. 1 Vitex-10 Capsule (Marron-Marron), 2 Baidyanath Shatavari Granules, 3 Clearpile Tablet (Red coating) 4 Baidyanath Ashwagandha Capsule(Vegi Capsule).

Thanking You.

Yours Faithfully,

***For,* Shree Baidyanath Ayurved Bhawan Pvt. Ltd.**

Authorized Signatory

Accept Rs 35280/-
Issue received
01.12.17

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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

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Date of commencement: 4th December 2017 Date of completion : 22nd December 2017

CONTENTS

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test 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.

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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

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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.[3]

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 2000 mg/kg
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System 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.

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

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

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

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

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

Test substance :- **BAIDYANATH SHATAVARI Granules**
Supplied 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

I.P.E.R. WARDHA

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

Date of commencement: 28th December 201

17

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I.P.E.R. WARDHA

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

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as : BAIDYANATH SHATAVARI Granules (Batch No. 176940066).

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

(Dr. R. O. [illegible])
Principal

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
PRINCIPAL
Institute of Pharmaceutical Education & Research

V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 2000 mg/kg
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance 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.

Project coordinator : Dr. R. O. Ganjiwale
I/c Principal
I.P.E.R. Wardha
I/c. PRINCIPAL
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17/07/18

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

(Dr. R. O. Ganjiwale)
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ACUTE ORAL TOXICITY TEST IN THE RATS
-Fixed Dose Procedure-

Test substance :- **CLEARPILE Tablet**
Supplied 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: 4th December 201 er 2017

I. P. E. R. WARDHA

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

CONTENTS

(Dr. R. O. Ganjiwale)
Principal

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as : CLEARPILE Tablet (Batch No. T – 02).

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

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

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.

I.P.E.R. WARDHA

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Borgaon (Meghe), Wardha

V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < 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.

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

I/c. PRINCIPAL
Institute of Pharmaceutical Education & Researc
Borgaon (Meghe), Wardha

17/01/18

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

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(Dr. R. O. Ganjiwale)
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Borgaon (Meghe), Wardha

2017-18/02

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

Test substance :- **BAIDYANATH ASHWAGANDHA Capsules**
Supplied 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: 28th December 2018

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

CONTENTS

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

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as : BAIDYANATH ASHWAGANDHA Capsules (Batch No. T – 01).

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

(Dr. R. O. Ganjiwale)
Principal

III. TEST ANIMALS

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

(Dr. R. O. Ganjiwale)

V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 2000 mg/kg
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified

(Dr. R. O. Ganjiwale)

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance 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 : Dr. R. O. Ganjiwale
I/c Principal
I.P.E.R. Wardha

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

17/01/18

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

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

Baidyanath
Trusted since 1917

Shree Baidyanath 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

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

Accepted Rs 17,640/-
Issue receipt
16.02.18.

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

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

Test substance :- **BASANT KUSUMAKAR RAS Tablet**
Supplied 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: 5th February 20 ary 2018

I. P. E. R. WARDHA

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Principal
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CONTENTS

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II.

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.

I. P. E. R. WARDHA

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

III. TEST ANIMALS

Species: Albino rats weighing in range of 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-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

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

V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 2000 mg/kg
- Category 5 = LD50 > 2000 mg/kg
- Category 5 or non classified

(Dr. R. O. Ganjiwale)

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance 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.

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

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

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

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

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

Test substance :- **VATCHINTAMANI RAS (Brihat) Tablet**
Supplied 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: 5th February 2018

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

I.P.E.R. WARDHA

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

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as : VATCHINTAMANI (Brihat) Tablet (Batch No. T – 173010007).

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

I. P. E. R. WARDHA

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

III. TEST ANIMALS

Species: Albino rats weighing in range of 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.

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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.[3]

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD50 cut-off values, incompliance with Globally Harmonized System (GHS).[2]

- 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 < LD50 ≤ 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

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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