SIDDHAYU HEALTHCARE PRIVATE LIMITED

SIDDHAYU
REIMAGINING HEALTHCARE

Off: Flat No. 704, 7th Floor, Pramukh Plaza
Cardinal Gracious Road, Chakala, Mumbai 400099.
Corporate Office : Great Nag Road, Nagpur 440024
Ph. No. 9833709118 / 9970303437

Ref No : SHPL/2020-21/ Date: 17.08.2020.

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

Sub: Sample for acute toxicity study.

Dear Sir,

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

Sr. No.	Name of Product	Batch No.	Mfg.Dt.	Exp.Dt.	Qty in Nos
1	Diabo Yogue(5202390001)				1 Nos
2	Sleep Yogue(5202370001)				1 Nos
3	Cof Yogue(5202320001)				1 Nos
4	Spiruactiv(5202380002)				1 Nos
5	Winostress(5201030001)				1 Nos
6	Painquit(5202420001)				1 Nos
7	ChyawanYogue(5202460001)				1 Nos
8	Diabo Yogue(1301)				1 Nos
9	Heart Yogue(5202330001)				1 Nos
10	Immune Yogue(5202470001)				1 Nos
11	Digee Yogue(5202350001)				1 Nos
12	Turmeric Yogue(5202400001)				1 Nos

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

Thanking you.

Yours Faithfully

S. S. Dhurde
(Authorised Signatory)

Received
Mr. S. S. Dhurde

I.P.E.R. WARDHA

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

PROJECT REPORT

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

Submitted to

Siddhayu Healthcare Pvt. Ltd,
Nagpur 440024

Submitted By

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

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

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

SEPI

I.P.E.R. WARDHA

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

REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Healthcare Pvt. Ltd. Nagpur, 440024

Submitted to
Siddhayu Healthcare Pvt. Ltd. Nagpur, 440024

Date of commencement: 8th September 2020 to 24th September 2022

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

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

I.P.E.R. WARDHA

(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 Tablets identified as **Diabo Yogue** (Batch No. 5202390001).

The test substance was stored at am

I. P. E. R. WARDHA

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

III. TEST ANIMALS

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

Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum.*

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

IV. TEST PROCEDURE

Preparation of the test substance: 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 I formed with 5 animals receiving the test sub f body weight.

I.P.E.R. WARDHA

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

V. OBSERVATIONS

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

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

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

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

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

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

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

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

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

I.P.E.R. WARDHA

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

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **Diabo Yogue** (Batch No. 5202390001), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Diabo Yogue** (Batch No. 5202390001) 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 **Diabo Yogue** (Batch No. 5202390001) was more than 2000 mg/kg.

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

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

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

Co-Principal Investigator : [illegible]
I.P.E.R. Wardha

I. P. E. R. WARDHA

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

PROJECT REPORT

ACUTE ORAL TOXICITY TEST IN THE RATS

Submitted to

Siddhayu Healthcare Pvt. Ltd,
Nagpur 440024

Submitted By

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

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

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

SEPI

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

REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Submitted to
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Date of commencement: 7th September 2020 to 23th September 2020

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

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

Co-Principal Investigator
Mr. Jyotiranjan Roul
Assistant Professor
Departme
Institute of Pharmac
Borgaor

I.P.E.R. WARDHA

(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 Capsule identified as **Sleep Yogue** (Batch No. 5202370001).

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

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

III. TEST ANIMALS

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

Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum*.

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

IV. TEST PROCEDURE

Preparation of the test substance: The test substance was 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 sub ody weight.

I.P.E.R. WARDHA

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

V. OBSERVATIONS

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

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

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

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

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

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

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

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

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

I.P.E.R. WARDHA

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

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **Sleep Yogue** (Batch No. 5202370001), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Sleep Yogue** (Batch No. 5202370001) 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 **Sleep Yogue** (Batch No. 5202370001) was more than 2000 mg/kg.

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

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

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

Co-Principal Investigator :

I. P. E. R. WARDHA

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

PROJECT REPORT

ACUTE ORAL TOXICITY TEST IN THE RATS

Submitted to

Siddhayu Healthcare Pvt. Ltd,
Nagpur 440024

Submitted By

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

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

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

SEPI

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

REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Submitted to
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Date of commencement: 7th September 2020 to 23th September 2020

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

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

Co-Principal Investigator
Mr. Jyotiranjan Roul
Assistant Professor
Department of Pharmacology,
Institute of Pharmace
Borgaon

I.P.E.R. WARDHA

(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 liquid preparation identified as **Cof Yogue** (Batch No. 5202320001).

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

I.P.E.R. WARDHA

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

III. TEST ANIMALS

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

Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum*.

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

IV. TEST PROCEDURE

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

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

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

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

Selection of starting dose: As the test substance was an Ayurvedic formulation the starting dose was selected as 2ml/100g rat.

Main study: According to the methodology describe in the Annex 3 of the OECD guideline 420 (joined in Appendix IV of the present report) the test was performed with 5 animals receiving the test s ` body weight.

I. P. E. R. WARDHA

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

V. OBSERVATIONS

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

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

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

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

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

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

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

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

- Category 1 = 0 $< LD_{50} <$ 5 mg/kg
- Category 2 = 5 mg/kg $< LD_{50} <$ 50 mg/kg
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PRINCIPAL
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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 **Cof Yogue** (Batch No. 5202320001), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Cof Yogue** (Batch No. 5202320001) 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 **Cof Yogue** (Batch No. 5202320001) was more than 2000 mg/kg.

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

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

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

Co-Principal Investigator :

I. P. E. R. WARDHA

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

ACUTE ORAL TOXICITY TEST IN THE RATS

Submitted to

Siddhayu Healthcare Pvt. Ltd,
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Institute of Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

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

Performing Department
Department of Pharmacology,
Institute of Pharmaceutical Education and Research,
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SEPI

I.P.E.R. WARDHA

(Dr. R. O. Ganjiwale)
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STUDY TITLE

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-Fixed Dose Procedure-

Sponsored by
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Date of commencement: 7th September 2020 to 23th September 2020

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

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Mr. Jyotiranjan Roul
Assistant Professor
Department of Pharmacology,
Institute of Pharmaceutical Education and Research,
Borgaon (

I. P. E. R. WARDHA

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

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 Capsule identified as **Spiruactiv** (Batch No. 5202380002).

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 190-210 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 s[illegible] f body weight.

I. P. E. R. WARDHA

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

V. OBSERVATIONS

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

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

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

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

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

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

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

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

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

I.P.E.R. WARDHA

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

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **Spiruactiv** (Batch No. 5202380002), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Spiruactiv** (Batch No. 5202380002) 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 **Spiruactiv** (Batch No. 5202380002) was more than 2000 mg/kg.

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

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

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

Co-Principal Investigator :

I. P. E. R. WARDHA

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

PROJECT REPORT

ACUTE ORAL TOXICITY TEST IN THE RATS

Submitted to

Siddhayu Healthcare Pvt. Ltd,
Nagpur 440024

Submitted By

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

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

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

SEAL

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

REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Submitted to
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Date of commencement: 7th September 2020 to 23th September 2020

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

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

Co-Principal Investigator
Mr. Jyotiranjan Roul
Assistant Professor
Department of Pharmacol
Institute of Pharmaceut
Borgaon (M

I.P.E.R. WARDHA

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

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 Capsule identified as **Winostress** (Batch No. 5201030001).

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

(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 LD_{50} cut-off values, incompliance with Globally Harmonized System (GHS).[2]

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

I.P.E.R. WARDHA

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

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **Winostress** (Batch No. 5201030001), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Winostress** (Batch No. 5201030001) 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 **Winostress** (Batch No. 5201030001) was more than 2000 mg/kg.

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

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

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

Co-Principal Investigator : Mr
Ass
I.P.E.R. Wardha

I. P. E. R. WARDHA

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

PROJECT REPORT

ACUTE ORAL TOXICITY TEST IN THE RATS

Submitted to

Siddhayu Healthcare Pvt. Ltd,
Nagpur 440024

Submitted By

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

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

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

SEPI

I. P. E. R. WARDHA

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

REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Submitted to
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Date of commencement: 7th September 2020 to 23th September 2020

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

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

Co-Principal Investigator
Mr. Jyotiranjan Roul
Assistant Professor
Departme
Institute of Pharmace
Borgaon

I.P.E.R. WARDHA

(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 Tablets identified as **Painquit** (Batch No. 5202420001).

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

I.P.E.R. WARDHA

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

III. TEST ANIMALS

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

Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum.*

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

IV. TEST PROCEDURE

Preparation of the test substance: 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 sub body weight.

I. P. E. R. WARDHA

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

V. OBSERVATIONS

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

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

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

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

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

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

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

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

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

I.P.E.R. WARDHA

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

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **Painquit** (Batch No. 5202420001), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Painquit** (Batch No. 5202420001) 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 **Painquit** (Batch No. 5202420001) was more than 2000 mg/kg.

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

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

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

Co-Principal Investigator :

I.P.E.R. WARDHA

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

PROJECT REPORT

ACUTE ORAL TOXICITY TEST IN THE RATS

Submitted to

Siddhayu Healthcare Pvt. Ltd,
Nagpur 440024

Submitted By

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

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

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

SEPI

I.P.E.R. WARDHA

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

REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Submitted to
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Date of commencement: 4th September 2020 to 20th September 2020

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

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

Co-Principal Investigator
Mr. Jyotiranjan Roul
Assistant Professor
Departm
Institute of Pharma
Borga

(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
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 semi-solid viscous material identified as: **Chyawan Yogue** (Batch No. 520246001).

The test substance was stored at amb

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

III. TEST ANIMALS

Species: Albino rats weighing in range of 170-230 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 dissolve 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 [illegible] formed with 5 animals receiving the test su[illegible] of body weight.

I. P. E. R. WARDHA

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

V. OBSERVATIONS

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

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

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

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

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

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

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

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

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

I.P.E.R. WARDHA

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

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **Chyawan Yogue** (Batch No. 5202460001), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Chyawan Yogue** (Batch No. 5202460001) 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 **Chyawan Yogue** (Batch No. 5202460001) was more than 2000 mg/kg.

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

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

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

Co-Principal Investigator :

I.P.E.R. WARDHA

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

I.P.E.R. Wardha

PROJECT REPORT

ACUTE DERMAL TOXICITY STUDY OF DIABO YOGUE

Submitted to

Siddhayu Healthcare Pvt. Ltd,
Nagpur 440024

Submitted By

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

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

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

SEPI

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

REPORT

STUDY TITLE

ACUTE DERMAL TOXICITY STUDY OF DIABO YOGUE

Sponsored by
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Submitted to
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

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

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

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

Co-Principal Investigator
Mr. J
Assi
Departme
Institute of Pharmaceutical Education and Research,
Borgaon (Meghe), Wardha

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

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I.P.E.R. WARDHA

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

1. INTRODUCTION

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

OBJECTIVE:

To determine the acute dermal toxicity (if any) of the test sample **Diabo Yogue** (Batch No. 1301) supplied by Siddhayu Healthcare Private Limited Nagpur.

PRINCIPLE:

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

2. MATERIALS AND METHODS

Test Substance: The supplier provided for the test container containing cream identified as **Diabo Yogue** (Batch No. 1301)

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

Details of test animals

Species: Albino rats weighing in rang

Strain: Wistar

Sex: Female

Number of animals per dose level: 3

Number of groups: 2

I. P. E. R. WARDHA

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

Selected doses: 200 and 2000 mg/kg

Rationale of selection: As per OECD 402 guidelines

Housing and feeding conditions

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum*.

Allocation of animals to various groups:

Table No. 1. GROUPS OF ANIMALS

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

Preparations

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

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

EXPERIMENTAL:

The animals were fasted overnight I ithheld. Following the period of fasting, the a le was applied on the fur-removed skin. A d was withheld for 1-2 hours. If the compound are likely to be non-toxic i.e. having toxicity only above regulatory limit dose then, the dose level to be used as the starting dose is

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

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

Acute Dermal Toxicity (OECD–402)

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

OBSERVATIONS:

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

1. Clinical signs:

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

2. Body weight:

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

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

5. Biochemical Parameters:

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

6. Gross pathology and Histopathology:

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

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

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

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

- Category 1 = 0 $< LD_{50} <$ 5 mg/kg
- Category 2 = 5 mg/kg $< LD_{50} <$ 50 mg/kg
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- Category 5 = $LD_{50} >$
- Category 5 or non classified

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

3. RESULTS

Clinical signs:

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

Body weight:

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

Table No. 2 INDIVIDUAL BODY WEIGHT

Group No.	Dose (mg/kg) Topically	Rats No.	Body weight (g)		
			0^{th} day	7^{th} day	14^{th} day
I	200	1	208	210	221
		2	212	215	225
		3	202	202	206
		Mean±SD	207.33±5.03	209±6.56	217.33±10.02
II	2000	4	221	222	225
		5	236	236	226
		6	226	230	241
		Mean±SD	227.67±7.64	229.33±7.02	230.67±8.96

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) topically	Mortality
I	200	0/3
II		

I. P. E. R. WARDHA

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

Food consumption

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

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

Biochemical Parameter

Table No. 5: BIOCHEMICAL FINDING

Group No.	Dose	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Creatinine (mg/dL)
I	200 mg/ kg b. wt topically	50.33±4.16	70.33±5.03	76.33±.51	1.33±0.21
II	2000 mg/ kg b. wt topically	62.33±5.51	81.33±3.79	84.7±4.16	1.57±0.32
Significance /NS		NS	NS	NS	NS

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

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

Table No. 6 PATHOLOGY FINDINGS

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

NTR = nothing to report

Histopathology: Section from live th mild perivascular lymphoid aggregation. rkable.

Section from Lung shows mild pneumonitis.

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

PLATES

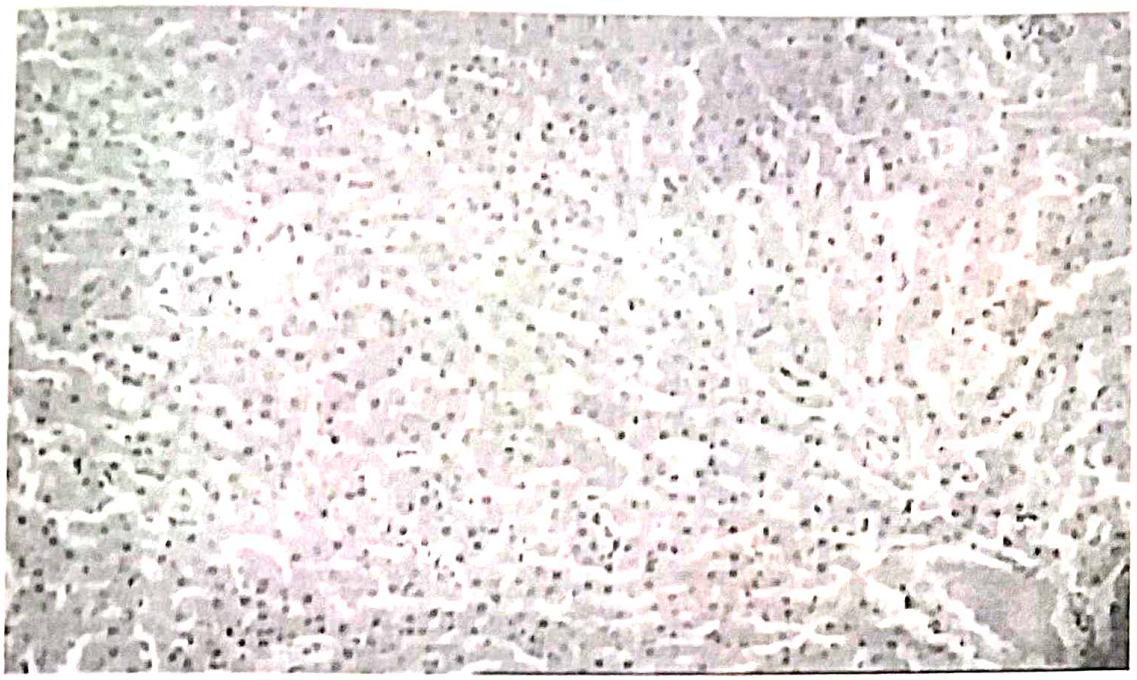

Plate 1: Histopathological Studies of Liver (Group II (2000 mg/kg) (H&E) X100

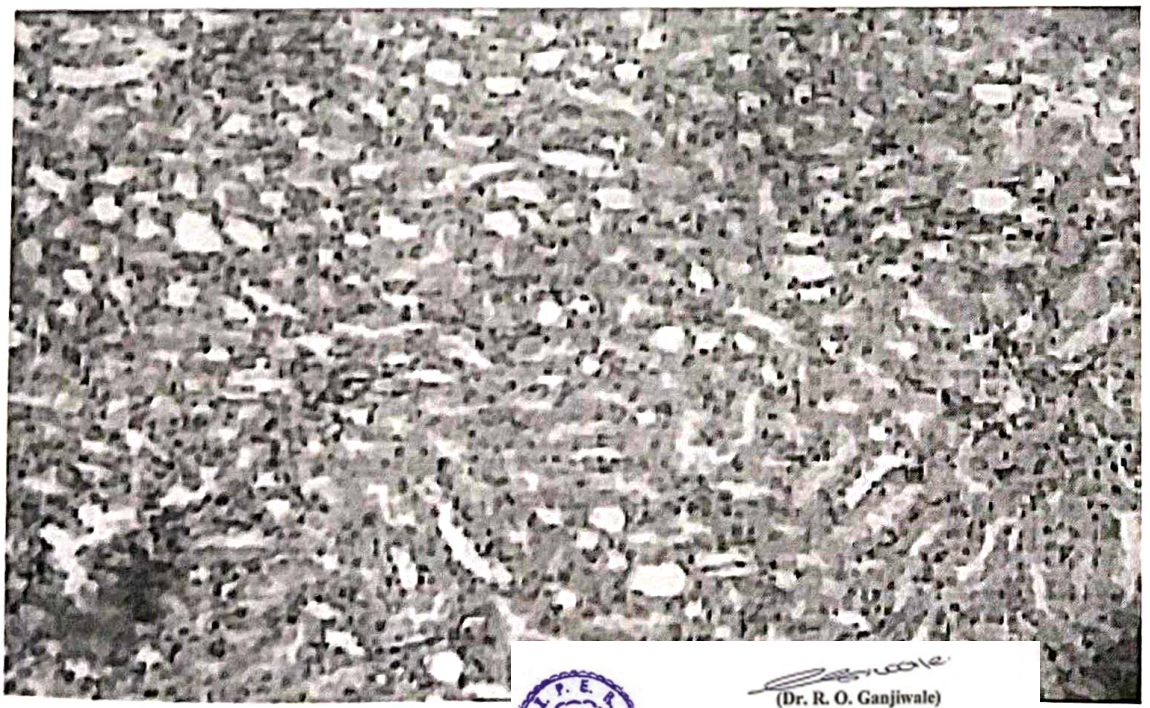

Plate 2: Histopathological Stud

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

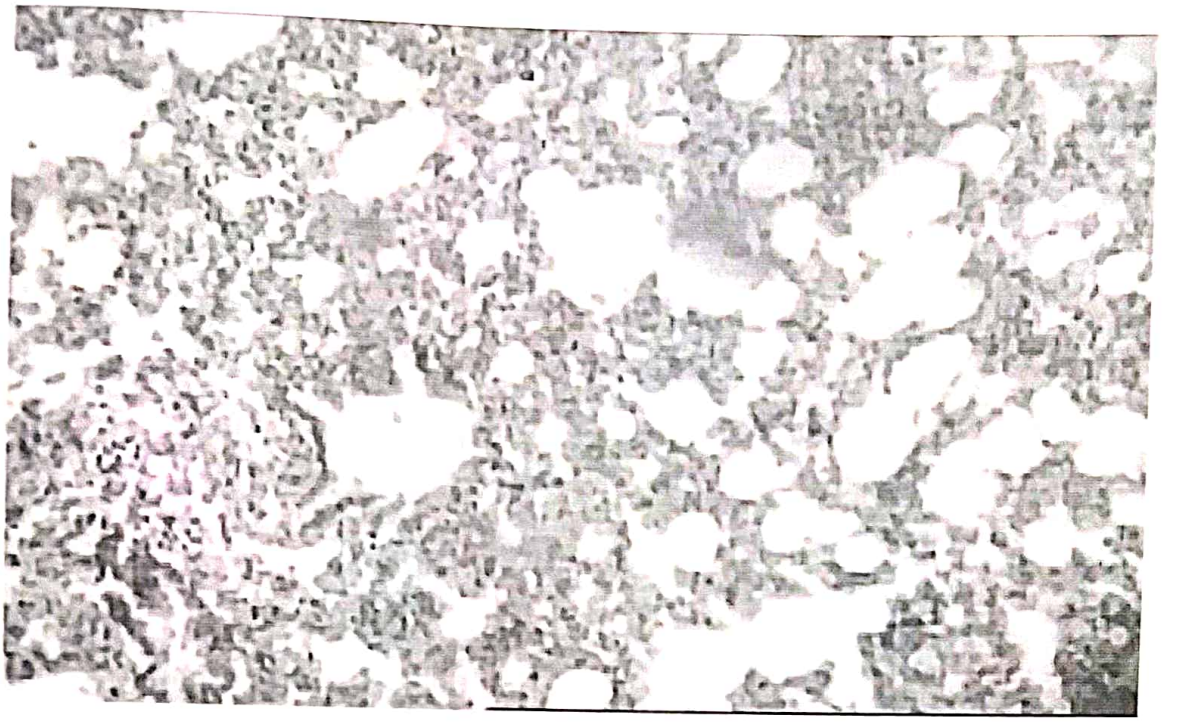

Plate 3: Histopathological Studies of Lung (Group II (2000 mg/kg) (H&E) X400

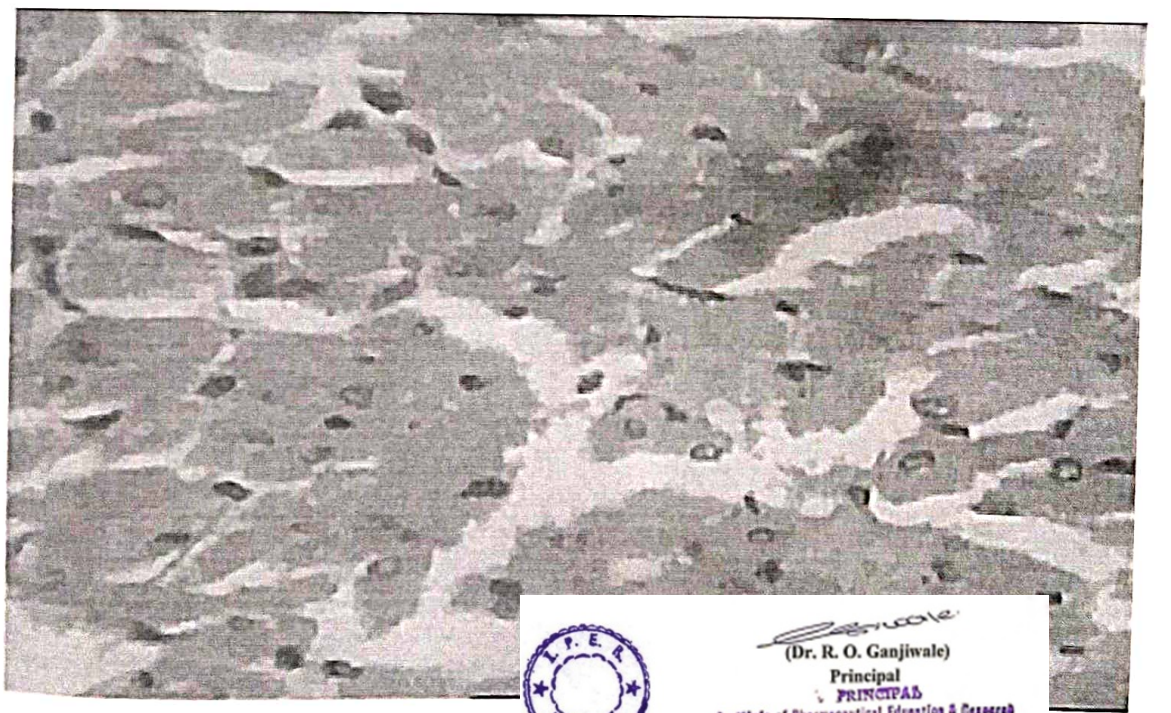

Plate 4: Histopathological Studies

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

4. DISCUSSION:

1. The animals treated at different dose levels with the above test compound Diabo Yogue (Batch No. 1301) in all the groups exhibited normal activity and behavioral pattern.
2. There were no clinical signs of tremors, convulsions, exophthalmia, salivation, diarrhea and lethargy.
3. Animals exhibited normal and comparable body weight gain throughout the period of study.
4. Food intake of normal and animals treated at different dose levels was found to be comparable throughout the tenure of the study.
5. The pathology finding shows no abnormality in any group of animals.

5. CONCLUSION:

The above findings revealed that the Diabo Yogue (Batch No. 1301) supplied by Siddhayu Healthcare Private Limited, Nagpur was found to be safe at doses 200 and 2000 mg/kg when applied locally. None of the animals were found to be moribund condition in all the groups until the 14 days of the study.

The Study has indicated that Diabo Yogue (Batch No. 1301) at dose of 2000 mg/kg did not affect general health of Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this, minimal lethal dose of Diabo Yogue (Batch No. 1301) was more than 2000 mg/kg.

(Dr. R. O. Ganjiwale)

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

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

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

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

Co-Principal Investigator : Mr. Jyotiranjan Roul
Assistant Professor

I. P. E. R. WARDHA

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

PROJECT REPORT

ACUTE ORAL TOXICITY TEST IN THE RATS

Submitted to

Siddhayu Healthcare Pvt. Ltd,
Nagpur 440024

Submitted By

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

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

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

SEPE

I.P.E.R. WARDHA

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

REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Submitted to
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Date of commencement: 5th September 2020 to 21st September 2020

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

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

Co-Principal Investigator
Mr. Jyotiranjan Roul
Assistant Professor
Depa
Institute of Phar
Bo

I.P.E.R. WARDHA

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

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 liquid preperation identified as: **Heart Yogue** (Batch No. 520233001).

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

I. P. E. R. WARDHA

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

III. TEST ANIMALS

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

Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum.*

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

IV. TEST PROCEDURE

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

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

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

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

Selection of starting dose: As the test substance was an Ayurvedic formulation the starting dose was selected as 2ml/100g rat.

Main study: According to the methodology describe in the Annex 3 of the OECD guideline 420 (joined in Appendix ormed with 5 animals receiving the test s f body weight.

I.P.E.R. WARDHA

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

V. OBSERVATIONS

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

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

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

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

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

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

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

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

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

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

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **Heart Yogue** (Batch No. 520233001), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Heart Yogue** (Batch No. 520233001) 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 **Heart Yogue** (Batch No. 520233001) was more than 2000 mg/kg.

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

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

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

Co-Principal Investigator

I.P.E.R. WARDHA

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

I.P.E.R. Wardha

PROJECT REPORT

ACUTE ORAL TOXICITY TEST IN THE RATS

Submitted to

Siddhayu Healthcare Pvt. Ltd,
Nagpur 440024

Submitted By

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

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

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

SEP

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

REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Submitted to
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Date of commencement: 4th September 2020 to 20th September 2020

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

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

Co-Principal Investigator
Mr. Justinian Paul
Ass
Departm
Institute of Pharmac
Borgaon (Meghe), Wardha

(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.

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing liquid preparation identified as: **Immune Yogue** (Batch No. 5202470001).

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

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

III. TEST ANIMALS

Species: Albino rats weighing in range of 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.

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I.P.E.R. WARDHA

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

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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.

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

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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 **Immune Yogue** (Batch No. 5202470001), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Immune Yogue** (Batch No. 5202470001) 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 **Immune Yogue** (Batch No. 5202470001) was more than 2000 mg/kg.

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

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

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

Co-Principal Investigator :

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

PROJECT REPORT

ACUTE ORAL TOXICITY TEST IN THE RATS

Submitted to

Siddhayu Healthcare Pvt. Ltd,
Nagpur 440024

Submitted By

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

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

Performing Department
Department of Pharmacology,
Institute of Pharmaceutical Education and Research,
Bor

S

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

REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Submitted to
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Date of commencement: 5th September 2020 to 21st September 2020

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

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

Co-Principal Investigator
Mr. Jyotiranjan Roul
As
Departm
Institute of Pharma
Borgac

I.P.E.R. WARDHA

(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 liquid preparation identified as: **Digee Yogue** (Batch No. 5202350001).

The test substance was stored at an

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

III. TEST ANIMALS

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

Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum*.

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

IV. TEST PROCEDURE

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

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

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

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

Selection of starting dose: As the test substance was an Ayurvedic formulation the starting dose was selected as 2ml/100g rat.

Main study: According to the methodology describe in the Annex 3 of the OECD guideline 420 (joined in Appen erformed with 5 animals receiving the te of body weight.

I. P. E. R. WARDHA

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

V. OBSERVATIONS

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

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

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

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

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

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

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

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

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

I. P. E. R. WARDHA

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

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **Digee Yogue** (Batch No. 5202350001), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Digee Yogue** (Batch No. 5202350001) 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 **Digee Yogue** (Batch No. 5202350001) was more than 2000 mg/kg.

Project coordinator	:	Dr. R. O. Ganjiwale I/c Principal I.P.E.R. Wardha
Principal investigator	:	Dr. B. R. Gandhare Associate Professor I.P.E.R. Wardha
Co-Principal Investigator	:	Mr. Jyotiranjan Roul Assistant Professor I.P.E.R. Wardha

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

PROJECT REPORT

ACUTE ORAL TOXICITY TEST IN THE RATS

Submitted to

Siddhayu Healthcare Pvt. Ltd,
Nagpur 440024

Submitted By

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

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

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

SEPI

I.P.E.R. WARDHA

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

REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

Submitted to
Siddhayu Healthcare Pvt. Ltd.
Nagpur, 440024

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

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

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

Co-Principal Investigator
Mr. Jyotiranjan Roul
Assis
Departmen
Institute of Pharmaceu
Borgaon

I.P.E.R. WARDHA

(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 powder identified as **Turmeric Yogue** (Batch No. 5202400001).

The test substance was stored at ambie

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

III. TEST ANIMALS

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

Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum.*

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

IV. TEST PROCEDURE

Preparation of the test substance: The test substance was 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 I [illegible] formed with 5 animals receiving the test sul[illegible] f body weight.

I. P. E. R. WARDHA

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

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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]

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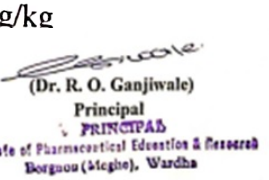
- Category 5 or non classified



VI. RESULTS

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

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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 **Turmeric Yogue** (Batch No. 5202400001), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Turmeric Yogue** (Batch No. 5202400001) 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 **Turmeric Yogue** (Batch No. 5202400001) was more than 2000 mg/kg.

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

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

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

Co-Principal Investigator : M
I.P.E.R. Wardha

I. P. E. R. WARDHA

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