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

Ref.No : SARF/T/2021-22/01

Date :12.07.2021

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 study.

1. Turmeric Advance tablet 1 X 30 Tablets

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

Thanking you

Yours Faithfully

S.S.Dhurde

(Authorised Signatory)

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

PROJECT REPORT

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

Submitted to

Siddhayu Ayurvedic Research Foundation Pvt. Ltd,
Nagpur 440009

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,
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Borgaon (Meghe), Wardha

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(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 Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Submitted to
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Date of commencement: 22nd July2021 to 7th August 2021

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

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

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I. P. E. R. WARDHA

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

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing Tablets identified as **Turmeric Advance Tablet** (Batch No. S218020001).

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 su f body weight.

I. P. E. R. WARDHA

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

V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral 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
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Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **Turmeric Advance Tablet** (Batch No. S218020001), supplied by Siddhayu Healthcare 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 Advance Tablet** (Batch No. S218020001) 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 Advance Tablet** (Batch No. S218020001) was more than 2000 mg/kg.

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

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Principal investigator : Dr. B. R. Gandhare
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CIN No. : U24233MH1983PTC030020

Siddh:ayu

Ref.No : SARF/T/2022-23/02 Date :12.04.2022

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 study.

1.	HEMOVERT GRANULES	1 X 100 gm.
2.	UROSTONE TABLET	2 X 60 Tablets

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

Thanking you

Yours Faithfully

S.S.Dhurde

(Authorised Signatory)

(Dr. R. O. Ganjiwale)
Principal
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Reg. Off. :- 404, Chartered House, Dr. Caw
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07103-276115

REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Submitted to
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Date of commencement: 18th April 2022 to 3rd May 2022

Project coordinator
Dr. R. O. Ganjiwale
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Principal investigator
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Associate Professor
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ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD- OECD GUIDELINE 420 [1]

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container identified as **Hemovert granules** (Batch No. HEGR-22-03).

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 170-200 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 suspended in olive oil.

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 cannula. 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 st body weight.

I.P.E.R. WARDHA

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V. OBSERVATIONS

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

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

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

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

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

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

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

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

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

I.P.E.R. WARDHA

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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 **Hemovert granules** (Batch No. HEGR-22-03), 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 **Hemovert granules** (Batch No. HEGR-22-03) 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 **Hemovert granules** (Batch No. HEGR-22-03) was more than 2000 mg/kg.

Project coordinator : Dr. R. O. Ganjiwale
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Principal investigator :

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Borgaon (Meghe), Wardha

REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Submitted to
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Date of commencement: 18th April 2022 to 3rd May 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

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

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(Dr. R. O. Ganjiwale)
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ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD- OECD GUIDELINE 420 [1]

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing Tablets identified as **Urostone tablet** (Batch No. UUAE-22-03).

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

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

III. TEST ANIMALS

Species: Albino rats weighing in range of 150-170 g

Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum.*

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

IV. TEST PROCEDURE

Preparation of the test substance: The test substance was 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 cannula. 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 f body weight.

I. P. E. R. WARDHA

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

V. OBSERVATIONS

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

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

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

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

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

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

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

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

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

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

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **Urostone tablet** (Batch No. UUAE-22-03), 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 **Urostone tablet** (Batch No. UUAE-22-03) 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 **Urostone tablet** (Batch No. UUAE-22-03) 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. R. O. Ganjiwale)
Principal
PRINCIPAL
Institute of Pharmaceutical Education & Research
Borgaon (Meghe), Wardha

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

Ref.No : SARF/T/2022-23/01

Date :23.03.2022

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 study.

1.	HEPASUPORT TABLET	2 X 100 Tablets
2.	STRESCLINE COMPLEX TABLET	2 X 60 Tablets
3.	ASTHA 15 FORTE CAPSULE	2 X 60 Capsules
4.	ASTHA 15 FORTE SYRUP	2 X 100 ml Syrup

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

Thanking you

Yours Faithfully

S.S.Dhurde

(Authorised Signatory)

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 Ayurvedic Research Foundation Pvt. Ltd,
Nagpur 440009

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

(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 Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Submitted to
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Date of commencement: 26th March 2022 to 11th April 2022

Project coordinator
Dr. R. O. Ganjiwale
I/c Principal
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Principal investigator
Dr. B. R. Gandhare
Associate Professor
Department of Pharmacology,
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Principal
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Borgaon (Meghe), Wardha

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(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 **Hepasuport tablet** (Batch No. HPAQ-22-01).

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

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III. TEST ANIMALS

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

Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum*.

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

IV. TEST PROCEDURE

Preparation of the test substance: The test substance was dissolved in water.

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

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

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

Selection of starting dose: As the test substance was an Ayurvedic formulation the starting dose was selected as 2000 mg/kg.

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

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V. OBSERVATIONS

Animals were observed individually after dosing during the first 30 minutes, periodically during the first 24 hours and daily thereafter for a total of 14 days. The clinical observations were made individually, each animal being examined outside the home cage.

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral 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

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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 **Hepasuport tablet** (Batch No. HPAQ-22-01), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Hepasuport tablet** (Batch No. HPAQ-22-01) at dose of 2000mg/kg did not affect general health of Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this, minimal lethal dose of **Hepasuport tablet** (Batch No. HPAQ-22-01) was more than 2000 mg/kg.

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PROJECT REPORT

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

Submitted to

Siddhayu Ayurvedic Research Foundation Pvt. Ltd,
Nagpur 440009

Submitted By

Project coordinator
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REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Submitted to
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Date of commencement: 26th March 2022 to 11th April 2022

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing Tablets identified as **Strescline Complex tablet** (Batch No. STAQ-22-01).

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

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III. TEST ANIMALS

Species: Albino rats weighing in range of 130-160 g

Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum*.

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

IV. TEST PROCEDURE

Preparation of the test substance: The test substance was dissolved in water.

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

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

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

Selection of starting dose: As the test substance was an Ayurvedic formulation the starting dose was selected as 2000 mg/kg.

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

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V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral LD_{50} cut-off values, incompliance with Globally Harmonized System (GHS).[3]

- 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} >		2000 mg/kg
- Category 5 or non classifi			

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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 **Strescline Complex tablet** (Batch No. STAQ-22-01), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Strescline Complex tablet** (Batch No. STAQ-22-01) at dose of 2000mg/kg did not affect general health of Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this, minimal lethal dose of **Strescline Complex tablet** (Batch No. STAQ-22-01) was more than 2000 mg/kg.

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PROJECT REPORT

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

Submitted to

Siddhayu Ayurvedic Research Foundation Pvt. Ltd,
Nagpur 440009

Submitted By

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REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Submitted to
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Date of commencement: 26th March 2022 to 11th April 2022

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing Capsule identified as **Astha-15 Forte Capsule** (Batch No. ASTH-02).

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

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III. TEST ANIMALS

Species: Albino rats weighing in range of 120-150 g

Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum.*

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

IV. TEST PROCEDURE

Preparation of the test substance: The test substance was dissolved in water.

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

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

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

Selection of starting dose: As the test substance was an Ayurvedic formulation the starting dose was selected as 2000 mg/kg.

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

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V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral 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 classifie

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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 **Astha 15 Forte Capsule** (Batch No. ASTH-02), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd., Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Astha-15 Forte Capsule** (Batch No. ASTH-02) at dose of 2000mg/kg did not affect general health of Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this, minimal lethal dose of **Astha-15 Forte Capsule** (Batch No. ASTH-02) was more than 2000 mg/kg.

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PROJECT REPORT

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

Submitted to

Siddhayu Ayurvedic Research Foundation Pvt. Ltd,
Nagpur 440009

Submitted By

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REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Submitted to
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Date of commencement: 26th March 2022 to 11th April 2022

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing liquid preparation identified as **Astha-15 Forte** (Batch No. AFS-22-03).

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

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III. TEST ANIMALS

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

Strain: Wister

Age: 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum*.

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

IV. TEST PROCEDURE

Preparation of the test substance: The test substance was 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 I rmed with 5 animals receiving the test su body weight.

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V. OBSERVATIONS

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

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

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

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

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

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

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

These results enabled to classify the test substance to one of a series of toxicity classes defined by fixed oral 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

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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 **Astha-15 Forte** (Batch No. AFS-22-03), 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 **Astha-15 Forte** (Batch No. AFS-22-03) 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 **Astha-15 Forte** (Batch No. AFS-22-03) was more than 2000 mg/kg.

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Borgaon (Meghe), Wardha

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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 study.

1. ZENBLEU TABLET 1 X 60 Tablets

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

Thanking you

Yours Faithfully

for Baidyanath 13/5/2022

(Authorised Signatory)

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(Dr. R. O. Ganjiwale)
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PROJECT REPORT

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

Submitted to

Siddhayu Ayurvedic Research Foundation Pvt. Ltd,
Nagpur 440009

Submitted By

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REPORT

STUDY TITLE

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

Sponsored by
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Submitted to
Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur, 440009

Date of commencement: 17th May 2022 to 31st May 2022

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing Tablets identified as **Zenbleu tablet** (Batch No. ZBLU-22-01).

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

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III. TEST ANIMALS

Species: Albino rats weighing in range of 175-210 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 cannula. After administration, animals were fasted for 3-4 hours.

Selection of starting dose: As the test substance was an Ayurvedic formulation the starting dose was selected as 2000 mg/kg.

Main study: According to the methodology describe in the Annex 3 of the OECD guideline 420 (joined in Appendix IV of the present report) the test was performed with 5 animals receiving the test substance at the dose level of 2000 mg/kg of body weight.

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V. OBSERVATIONS

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

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

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

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

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

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

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

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

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

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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 **Zenbleu tablet** (Batch No. ZBLU-22-01), supplied by Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur was classified in the **hazard category 5 or unclassified** with a LD_{50} higher than 2000 mg/kg in the Rat.

The Study has indicated that **Zenbleu tablet** (Batch No. ZBLU-22-01) at dose of 2000mg/kg did not affect general health of Wistar rats. There were no gross abnormalities observed in necropsied rats. Based on this, minimal lethal dose of **Zenbleu tablet** (Batch No. ZBLU-22-01) was more than 2000 mg/kg.

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