Siddhayu Ayurvedic Research Foundation Private Limited Office : Baidyanath Bhawan, Great Nag Road, Nagpur - 440 000, Maharashtra (INDIA) Ph. No.: (+91) - 0712 - 6644900 / 901 / 902 Website : www.siddhayu.com CIN No. : U24233MH1983PTC030020



Date :12.07.2021

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

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.

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 of Pharmscertical Education & Resource Borgnou (Meghe), Wardha

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:- 404, Chartered House, Dr. Cawasji Harmosji Street, Mumbai - 400002. Reg. Off. Factory At :-:- Post - Kalmana (Acharya), Umrer Road, Bahadura, - Dist, Nagpur - 441 204. Ph. No.: 07103-270115 Bahadura - Lakhandur Road, Desaiganj Wadsa, - Dist. Gadchiroli - 441 207 Ph. No.: 07137-272856 Wadsa

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 > PRINCIPAD antibile of Pharmeentical Education & Series Borganou (Megine), 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 Uc 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



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(Dr. R. O. Ganjiwale)
Principal
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(Dr. R. O. Ganjiwale) Principal PRINCIPAL Institute of Pharmacentical Education & Secures Borgnon (Megin), Wardha

ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD- OECD GUIDELINE 420¹

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 **Turmerle Advance Tablet** (Batch No. S218020001).

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



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmecetical Education & Research Borgnou (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 sul weight.



(Dr. R. O. Ganjiwale) Principal PRINCIPAD bastilede of Plarmeertical Education & Resserva Borgaou (Megine), 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 $< LD_{50} < 5 \text{ mg/kg}$
- Category 2 = 5 mg/kg $< LD_{50} < 50$ mg/kg
- Category 3 = $50 \text{ mg/kg} < \text{LD}_{50} < 300 \text{ mg/kg}$
- Category 4 = $300 \text{ mg/kg} < \text{LD}_{50} \le 2000 \text{ mg/kg}$
- Category 5 = $LD_{50} >$
- Category 5 or non classified

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

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Dr. R. O. Ganjiwale I/c Principal PRINCIPAL I.P.E.R. Wardha Pharmacoutical Education & Resources I.P.E.R. Wardha Nuteron (Meghe), Wardha

: Dr. B. R. Gandhare

Principal investigator



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmecerical Education & Research Borgmon (Maghe), 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/2022-23/02

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

2. UROSTONE TABLET

1 X 100 gm. 2 X 60 Tablets

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

Thanking you

Yours Faithfully

S.S.Dhurde (Authorised Signatory)

Reg. Off. :- 404, Chartered House, Dr. Caw

(Dr. R. O. Ganjiwale) Principal PrincipAD astitute of Parasceritical Education & ferocrab Borguou (Meghe), Wardha

) 07103-276115

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:- Post - Kalmana (Acharya), Umrer F

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

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) Principal PRINCIPAS Institute of Pharmacentical Idention & Research Borgnou (Megire), Wardha

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ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD-OECD GUIDELINE 420¹

LAIM & 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.



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 here at in 27.

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 I 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 subody

weight.



2001e (Dr. R. O. Ganjiwale) Principal PRINCIPAD untitute of Pharmacentical Education & Resourch Borgnou (Meghe), Wardha 13

V. OBSERVATIONS

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

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

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

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

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

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

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

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

- Category I = 0 < LD₅₀ < 5 mg/kg
- Category 2 = 5 mg/kg < LD₃₀ < 50 mg/kg
- Category 3 = 50 mg/kg < LD₃₀ < 300 mg/kg
- Category 4 = 300 mg/kg < LD₃₀ ≤ 2000 mg/kg
- Category 5 = LD₅₀ >
- Category 5 or non classified



2000 mg/kg

(Dr. R. O. Ganjiwale) Principal PrincipAD Institute of Pharmecerical Education & Research Borgmon (Maghe), Wardha

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance 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 LD₅₀ 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.

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Project coordinator

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

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PRINCIPAD Institute of Pharmaceutical Education Reserve Inalitate of Borgnon (Meghe), Wardha

(Dr. R. O. Ganjiwale)

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Principal PRINCIPAD untitute of Pharmacentical Education & Resource Borgnou (Meghe), Wardha

Principal investigator



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 (Maghe), Wardha



(Dr. R. O. Ganjiwale) Principal FRINCIPAD

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

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 PRINCIPAD

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III. TEST ANIMALS

Species: Albino rats weighing in range of 150-170 g
Strain: Wister
Age: 8-12 weeks
Number & Sex: 5 nulliparous and non-pregnant females.
Diet: Standard feed prepared in-house.
Water: Plain tap water *ad libitum*.

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

IV. TEST PROCEDURE

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





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



2000 mg/kg

(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Planescentical Education & Research Borgnou (Megine), 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 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 LD₅₀ 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.

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Project coordinator

Dr. R. O. Ganjiwale Vc Principal I.P.E.R. Wardha

D.D. Candhard

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Principal investigator



Siddhayu Ayurvedic Research Foundation Private Limited 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. STRESCLINE COMPLEX TABLET
- 3. ASTHA 15 FORTE CAPSULE
- 4. ASTHA 15 FORTE SYRUP

2 X 100 Tablets 2 X 60 Tablets 2 X 60 Capsules 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)



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

:- 404, Chartered House, Dr. Cawasji Harmosji Street, Mumbai - 400002. Reg. Off. Factory At :-:- Post - Kalmana (Acharya), Umrer Road, Bahadura, - Dist. Nagpur - 441 204. Ph. No.: 07103-276115 Bahadura :- Lakhandur Road, Desaiganj Wadsa, - Dist. Gadchiroli - 441 207 Ph. No.: 07137-272856 22 Wadsa

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



	and a sould
-	(Dr. R. O. Ganjiwale)
	Principal
	PRINCIPAD
stitufe	of Pharmscentical Education & Resource Borgnou (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 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.



(Dr. R. O. Ganjiwale) Principal Principal Principal Battlefe of Pharmacentical Education & Research Borgmon (Magdia), Wardha

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(Dr. R. O. Ganjiwale) Principal > PRINCIPAD hestilate of Pharmscericial Education & Research Borgaou (Megho), Wardha

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ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD-OECD GUIDELINE 420⁴

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.



(Dr. R. O. Ganjiwale) Principal Principal Principal Institute of Planmeertical Education & Ressered Borgnon (Meghe), Wardha

III. TEST ANIMALS

Species: Albino rats weighing in range of 140-160 g
Strain: Wister
Age: 8-12 weeks
Number & Sex: 5 nulliparous and non-pregnant females.
Diet: Standard feed prepared in-house.
Water: Plain tap water *ad libitum*.

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

IV. TEST PROCEDURE

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

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

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

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

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

Main study: According to the methodology describe in the Annex 3 of the OECD guideline 420 (joined in Appendix I with 5 animals receiving the test sult with the test sult with the of Parimeter at Education & Frincipal bergaos (Maghe), Wardha

V. OBSERVATIONS

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

The clinical observations were made individually, each animal being examined

The different parameters observed were - changes in skin & fur, eyes & mucous membranes, respiratory function, tremors, convulsions, salivation, diarrhea, lethargy,

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

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

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

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

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

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

28- cole (Dr. R. O. Ganjiwale) Principal PRINCIPAD atitate of Pharmacentical Education & Resoured Borgnou (Meghe), Wardha

VI. RESULTS

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

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

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

VII. CONCLUSION

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 LD₅₀ 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.

Project coordinator

LP.E.R. Winthing (Meche), Wardba Bostilato Borenon (Meche), ----:



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

Principal investigator

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

> Principal investigator Dr. B. R. Gandhare Associate Professor Department of Pharmacology,

Institute of Pharmace

Borgaon

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(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Plasmacentical Education & Resource Berguou (Meglio), Wardha

ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURE METHOD- OECD GUIDELINE 420⁴

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.



(Dr. R. O. Ganjiwale) Principal V PRINCIPAD Institute of Planmeerical Education & Ressered Borgnon (Meghe), Wardha

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 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, diarthea, 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 LD₁₀ cut-off values, incompliance with Globally Harmonized System (GHS).²

- Category 1 = 0 < LD₃₀ < 5 mg/kg
- Category 2 ~ 5 mg/kg < LD₁₀ < 50 mg/kg
- Category 3 ~ 50 mg/kg < LD10 < 300 mg/kg
- Category 4 = 300 mg/kg < LD30 5 2000 mg/kg
- Category 5 = LDsa >
- Category 5 or non classifi



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

2000 mg kg

35

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.

Project coordinator

Dr. R. O. Ganjiwale RINCIPAD

I/c Principal I.P.E.Ruw al pharmaceutical Education & Research I.P.E.Ruw al chagon (Meghe), Wardha

Principal investigator



(Dr. R. O. Ganjiwale) Principal '> PRINCIPAD hastitute of Pharmecerical Idention & Sesserab Bergnou (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 PrincipAD Institute of Pharmacentical Education & Screece Borguou (Meglio), 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 Institute of Pharmaceutical Education and Research, Borgaon (Meghe), Wardha

> Principal investigator Dr. B. R. Gandhare Associate Professor

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ACUTE ORAL TOXICITY TEST IN THE RATS – FINED DOSE PROCEDURE METHOD- OECD GUIDELINE 420⁻¹

1. 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

11. 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.



(Dr. R. O. Ganjiwale) Principal V PRINCIPAD Institute of Planmeertical Education & Resource Borgmon (Meghe), Wardha

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 JV of the accord and the test and a formed

with 5 animals receiving the test sul weight.



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

body

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 $< LD_{50} < 5 \text{ mg/kg}$
- Category 2 = 5 mg/kg $< LD_{50} < 50$ mg/kg
- Category 3 = $50 \text{ mg/kg} < LD_{50} < 300 \text{ mg/kg}$
- Category 4 = $300 \text{ mg/kg} < \text{LD}_{50} \le 2000 \text{ mg/kg}$
- Category 5 = LD_{50} >

- Category 5 or non classifie

(Dr. R. O. Ganjiwale) Principal V PRINCIPAD Institute of Pharmscertical Education & Ressered Borgmon (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 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 LD50 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.

Project coordinator

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I.P.E.R. Wandin acoutical Education & Research Institute Region (Meghe), Wardha : Borgaon (Meghe), Wardha

> 2000le (Dr. R. O. Ganjiwale) Principal

PRINCIPAD untitute of Pharmscortical Education & Resourch Borgnou (Micghe), Wardha



Principal investigator

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



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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

Project coordinator Dr. R. O. Ganjiwale

I/c Principal Institute o Pharmaceutical Education and Research, Borgaon (Meghe), Wardha

> Principal investigator Dr. B. R. Gandhare

Associate Professor Department of Pharmacology, Institute of Pharmaceutical Education and Personal

Borgaon "



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(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmacentical Education & Research Borgmon (Megine), Wardha

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ACUTE ORAL TOXICITY TEST IN THE RATS – FIXED DOSE PROCEDURI METHOD-OECD GUIDELINE 420¹

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.



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

III. TEST ANIMALS

Species: Albino rate 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 dellion.

Administration of the test substance: The animals fauted overnight prior to the test substance administration. All animals were weighed again on day I 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 with 5 animals receiving the test s

weight.

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V. OBSERVATIONS

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

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

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

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

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

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

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

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

- Category 1 = 0 < LD₅₀ < 5 mg/kg
- Category 2 = 5 mg/kg < LD₅₀ < 50 mg/kg
- Category 3 = 50 mg/kg < LD₅₀ < 300 mg/kg
- Category 4 = 300 mg/kg < I D., < 2000 ma/ca
- Category 5 = LD₅₀ >
- Category 5 or non classified



(Dr. R. O. Ganjiwale) Principal PRINCIPAD Institute of Pharmacentical Education & Research Borgnou (Megine), 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 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 LD50 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.

Project coordinator

Dr. R. O. Ganjinantelical Education & Research goon (Meghe), Wardha

I.P.E.R. Wardha



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

Principal investigator

Siddhayu Ayurvedic Research Foundation Private Limited Siddhayu 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/2022-23/03

Date :13.05.2022

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. ZENBLEU TABLET

1 X 60 Tablets

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

Thanking you

Yours Faithfully

13/5/2022

(Authorised Signatory)



..... 100 (Dr. R. O. Ganijwale) Principal PRINCIPAL untitute of Pharmacentical Education & Resource Borgnou (Mardha

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

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 PRINCIPAD Institute of Plasmoortical Education & Resource Borguou (Megleo), 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: 17th May 2022 to 31st 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,



(Dr. R. O. Ganjiwale) Principal Sentitude of Flarmerentical Education & Sentences Borgnon (Megine), Wardha

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APPENDICES



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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 data level of 2000 mailes of body

weight.



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



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2000 mg/kg

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 LD₅₀ 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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