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/2018-19/ **Date: 28.03.2019.**

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.

1) STRESSCLINE COMPLEX 2 Nos
(Tablet Coated)

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

Thanking you.

Yours Faithfully

S. S. Dhurde
(Authorised Signatory)

Received (Unpaid)

I.P.E.R. WARDHA

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

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

Test substance :- **STRESCLIN COMPLEX Tablet - Coated**
Supplied by :- **Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur**

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

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

eutical Education & Research,
HA (M.S.) INDIA

Date of completion: 29th April 2019

CONTENTS

I. AIM AND OBJECTIVES	3
II. TEST SUBSTANCE	3
III. TEST ANIMALS	4
IV. TEST PROCEDURE	4
V. OBSERVATIONS	5
VI. RESULTS	6
VII. CONCLUSION	6
VIII. REFERENCES	7

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 pack containing Tablets identified as :
STRESCLIN COMPLEX Coated Tablet

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

(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 substance at the dose level of 2000mg/kg of body weight.

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

V. OBSERVATIONS

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

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

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

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

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

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

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

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

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

I.P.E.R. WARDHA

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

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance STRESCLIN COMPLEX Tablet – Coated (Batch No. T- 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 STRESCLIN COMPLEX Tablet – Coated (Batch T-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 STRESCLIN COMPLEX Tablet - Coated (Batch No. T-01) was more than 2000 mg/kg.

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

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

atole

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

rdha

I.P.E.R. 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

Siddh:ayu

Ref.No : SARF/2019-20/101

Date : 11.06.2019

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.

1.	Sidpiles DS tablet	2 X 60 Tabs
2.	Sidpiles Cream	2 X 100 gm
3.	Nasiyam Nasal drop	2 X 100 ml
4.	Clearactiv syrup	2 X 200 ml

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

11.06.19

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

Test substance :- **SIDPILES DS TABLET**
Supplied by :- **Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur**

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

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

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

Date of completion: 19th April 2019

CONTENTS

I. AIM AND OBJECTIVES	**3**
II. TEST SUBSTANCE	**3**
III. TEST ANIMALS	**4**
IV. TEST PROCEDURE	**4**
V. OBSERVATIONS	**5**
VI. RESULTS	**6**
VII. CONCLUSION	**6**
VIII. REFERENCES	**7**
APPENDICES	

(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 pack containing Tablets identified as :

SIDPILES DS TABLET

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

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

III. TEST ANIMALS

Species: Albino rats weighing in range of 190-200 g

Strain: Wister

Age : 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum.*

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

IV. TEST PROCEDURE

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

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

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

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

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

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

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

V. OBSERVATIONS

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

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

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

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

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

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

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

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

- Category 1 = 0 $< LD_{50} <$ 5 mg/kg
- Category 2 = 5 mg/kg $< LD_{50} <$ 50 mg/kg
- Category 3 = 50 mg/kg $< LD_{50} <$ 300 mg/kg
- Category 4 = 300 mg/kg $< LD_{50} \leq$ 2000 mg/kg
- Category 5 = $LD_{50} >$ 2000 mg/kg
- Category 5 or non 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 Sidpiles DS Tablet (Batch No. TST-19-01), 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 SIDPILES DS TABLET (Batch No. TST-19-01) 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 SIDPILES DS TABLET (Batch No. TST-19-01) 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.

Patole
Professor
Wardha

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

ACUTE TOXICITY STUDY OF SIDPILES CREAM

Supplied by :- Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur.

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceutical Education & Research, WARDHA

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

Date of completion: 19th July 2019

Acute toxicity study of Sidpiles Cream

OBJECTIVE: To determine the acute toxicity (if any) of the test sample Sidpiles Cream (Batch No. TSC-19-01) supplied by Siddhayu Ayurvedic Research Foundation Private Limited Nagpur.

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

TEST PROCEDURE

Preparations

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

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

Details of test animals

Species: Albino rats weighing in range of 140-170 g

Strain: Wistar

Sex: Male

Number of animals per dose level: 5

Number of groups: Five

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

kg

)2 guidelines

Housing and feeding conditions

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum.*

Allocation of animals to various groups:

Table No. 1. GROUPS OF ANIMALS

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

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

EXPERIMENTAL:

The animals were fasted overnight prior to dosing but water was not withheld. Following the period of fasting, the animals were weighed and the test sample was applied on the fur removed skin.

OBSERVATIONS:

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

1. Clinical signs:

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

2. Body weight:

The mean group body weight of the control and test group animals was recorded on 0^{th} day, 7^{th} day and 14^{th} day.

3. Mortality:

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

4. Food consumption:

The quantity of food consumed by control and test groups was recorded on 0^{th} day, 7^{th} day and on 14^{th} day.

5. Pathology:

All the animals surviving at the end of the 14 days of observation were scarified by 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.

I. P. E. R. WARDHA

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

RESULTS:

Clinical signs:

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

Body weight: (Table No. 2)

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

Table No. 2 MEAN BODY WEIGHT

Group No.	Dose (mg/kg)	Mean body weight ± SD (g) 0^{th} day	Mean body weight ± SD (g) 7^{th} day	Mean body weight ± SD (g) 14^{th} day
I	Control	156 ± 6.16	160 ± 6.00	165.8 ± 5.26
II	5	157.6 ± 3.84	162.4 ± 3.04	166.8 ± 3.56
III	50	152.6 ± 3.97	157.4 ± 3.91	161.6 ± 4.15
IV	300	155 ± 3.16	159.6 ± 3.36	162. 6± 2.07
V	2000	148.8 ± 5.89	153.0 ± 6.32	158.2 ± 5.97

Values expressed as mean ± standard deviation

Mortality: (Table No. 3)

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

Table No. 3 MORTALITY

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

Group No.	Dose (mg/kg) Body weight (g)	Mortality
I	Control	0/5
II	5	0/5
III	50	0/5
	)0	0/5
	00	0/5

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

Food consumption: (Table No. 4)

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

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

Group No.	Dose (mg/kg)	Day		
		0^{th}	7^{th}	14^{th}
I	Control	12	14	15
II	5	13	16	15
III	50	12	14	17
IV	300	13	16	15
V	2000	11	13	14

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

Table No. 5 PATHOLOGY FINDINGS

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

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

DISCUSSION:

1. The animals treated at different dose levels with the above test compound Sidpiles Cream (Batch No. TSC-19-01) in all the groups exhibited normal activity and behavioral pattern.
2. There were no clinical signs of tremors, convulsions, exophthalmia, salivation, diarrhea and lethargy.
3. Animals from control and test groups exhibited normal and comparable body weight gain throughout the period of study.
4. Food intake of normal and animals treated at different dose levels was found to be comparable throughout the tenure of the study.
5. The pathology finding shows no abnormality in any group of animals.

CONCLUSION:

The above findings revealed that the Sidpiles Cream (Batch No. TSC-19-01) supplied by Siddhayu Ayurvedic Research Foundation Private Limited, Nagpur was found to be safe at doses 5, 50, 300, and 2000 mg/kg when applied locally. None of the animals were found to be moribund condition in all the groups until the 14 days of the study.

The Study has indicated that Sidpiles Cream (Batch No. TSC-19-01) 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 Sidpiles Cream (Batch No. TSC-19-01) 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 : Mr. A. M
Assistant
I.P.E.R. W

I. P. E. R. WARDHA

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

DISCUSSION:

1. The animals treated at different dose levels with the above test compound Sidpiles Cream (Batch No. TSC-19-01) in all the groups exhibited normal activity and behavioral pattern.
2. There were no clinical signs of tremors, convulsions, exophthalmia, salivation, diarrhea and lethargy.
3. Animals from control and test groups exhibited normal and comparable body weight gain throughout the period of study.
4. Food intake of normal and animals treated at different dose levels was found to be comparable throughout the tenure of the study.
5. The pathology finding shows no abnormality in any group of animals.

CONCLUSION:

The above findings revealed that the Sidpiles Cream (Batch No. TSC-19-01) supplied by Siddhayu Ayurvedic Research Foundation Private Limited, Nagpur was found to be safe at doses 5, 50, 300, and 2000 mg/kg when applied locally. None of the animals were found to be moribund condition in all the groups until the 14 days of the study.

The Study has indicated that Sidpiles Cream (Batch No. TSC-19-01) 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 Sidpiles Cream (Batch No. TSC-19-01) 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 : Mr. A. M.
Assistant P
I.P.E.R. W

I. P. E. R. WARDHA

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

ACUTE TOXICITY STUDY OF NASIYAM NASAL DROP

**Supplied by :- Siddhayu Ayurvedic Research Foundation Pvt. Ltd.
Nagpur.**

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceutical Education & Research, WARDHA

Date of commencement: 5th July 2019

I.P.E.R. WARDHA

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

Acute toxicity study of Nasiyam Nasal Drop

OBJECTIVE: To determine the acute toxicity (if any) of the test sample NASIYAM Nasal Drop (Batch No. TND-19-01) supplied by Siddhayu Ayurvedic Research Foundation Private Limited Nagpur.

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

TEST PROCEDURE

Preparations

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

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

Details of test animals

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

Strain: Wistar

Sex: Male

Number of animals per dose level: 5

Number of groups: Five

Selected doses: 5, 50, 300 and 2000 mg/kg

Rationale of selection: As per OECD 402 gı

I. P. E. R. WARDHA

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

Housing and feeding conditions

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum.*

Allocation of animals to various groups:

Table No. 1. GROUPS OF ANIMALS

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

I. P. E. R. WARDHA

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

EXPERIMENTAL:

The animals were fasted overnight prior to dosing but water was not withheld. Following the period of fasting, the animals were weighed and the test sample was applied on the fur removed skin.

OBSERVATIONS:

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

1. Clinical signs:

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

2. Body weight:

The mean group body weight of the control and test group animals was recorded on 0^{th} day, 7^{th} day and 14^{th} day.

3. Mortality:

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

4. Food consumption:

The quantity of food consumed by control and test groups was recorded on 0^{th} day, 7^{th} day and on 14^{th} day.

5. Pathology:

All the animals surviving at the end of the 14 days of observation were scarified by 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.

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

RESULTS:

Clinical signs:

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

Body weight: (Table No. 2)

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

Table No. 2 MEAN BODY WEIGHT

Group No.	Dose (mg/kg)	Mean body weight ± SD (g) 0^{th} day	Mean body weight ± SD (g) 7^{th} day	Mean body weight ± SD (g) 14^{th} day
I	Control	154 ± 2.23	158.2 ± 1.92	161.8 ± 1.30
II	5	152.6 ± 2.07	157.8 ± 2.58	163.0 ± 1.73
III	50	166.0 ± 3.08	169.8 ± 3.03	173.4 ± 3.43
IV	300	164.6 ± 5.27	169.2 ± 5.26	172.6± 4.27
V	2000	153.2 ± 2.28	156.6 ± 2.07	160.8 ± 2.16

Values expressed as mean ± standard deviation

Mortality: (Table No. 3)

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

Table No. 3 MORTALITY

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

Group No.	Dose (mg/kg) Body weight (g)	Mortality
I	Control	0/5
II	5	0/5
III	50	0/5
IV	30	
V	20(	

I. P. E. R. WARDHA

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

Food consumption: (Table No. 4)

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

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

Group No.	Dose (mg/kg)	Day		
		0^{th}	7^{th}	14^{th}
I	Control	13	14	15
II	5	13	15	15
III	50	11	15	16
IV	300	11	15	14
V	2000	13	15	12

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

Table No. 5 PATHOLOGY FINDINGS

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

NTR = nothing to report

I.P.E.R. WARDHA

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

DISCUSSION:

1. The animals treated at different dose levels with the above test compound NASIYAM Nasal drop (Batch No. TND-19-01) in all the groups exhibited normal activity and behavioral pattern.
2. There were no clinical signs of tremors, convulsions, exophthalmia, salivation, diarrhea and lethargy.
3. Animals from control and test groups exhibited normal and comparable body weight gain throughout the period of study.
4. Food intake of normal and animals treated at different dose levels was found to be comparable throughout the tenure of the study.
5. The pathology finding shows no abnormality in any group of animals.

CONCLUSION:

The above findings revealed that the NASIYAM Nasal Drop (Batch No. TND-19-01) supplied by Siddhayu Ayurvedic Research Foundation Private Limited, Nagpur was found to be safe at doses 5, 50, 300, and 2000 mg/kg when applied locally. None of the animals were found to be moribund condition in all the groups until the 14 days of the study.

The Study has indicated that NASIYAM Nasal Drop (Batch No. TND-19-01) 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 NASIYAM Nasal Drop (Batch No. TND-19-01) 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 : Mr. A. M. Pat
Assistant Prof
I.P.E.R. Ward

I. P. E. R. WARDHA

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

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

Test substance :- **CLEARACTIV Syrup**
Supplied by :- **Siddhayu Ayurvedic Research Foundation Pvt. Ltd. Nagpur**

TEST REPORT

Project coordinator : Dr. R. O. Ganjiwale

Principal investigator : Mr. A. M. Patole

Institute of Pharmaceut[illegible],
WARDHA

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

Date of commencement: 05th July 2019 [illegible]ly 2019

CONTENTS

I. AIM AND OBJECTIVES 3

II. TEST SUBSTANCE 3

III. TEST ANIMALS 4

IV. TEST PROCEDURE 4

V. OBSERVATIONS 5

VI. RESULTS 6

VII. CONCLUSION 6

VIII. REFERENCES 7

APPENDICES

I. P. E. R. WARDHA

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

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

I. AIM & OBJECTIVE OF THE TEST

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

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

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

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

II. TEST SUBSTANCE

The supplier provided for the test container containing solution identified as :
CLEARACTIV Syrup (Batch No. TCA-19-01).

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

(Dr. R. O. Ganjiwale)
Principal
PRINCIPAL

III. TEST ANIMALS

Species: Albino rats weighing in range of 160-190 g

Strain: Wister

Age : 8-12 weeks

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

Diet: Standard feed prepared in-house.

Water: Plain tap water *ad libitum.*

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

IV. TEST PROCEDURE

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

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

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

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

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

Main study: According to the methodology describe in the Annex 3 of the OECD guideline 420 (joined in Appendix IV of the present report) the test was performed with 5 animals receiving the test substance at the dose level of 2ml/100g of body weight.

I. P. E. R. WARDHA

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

V. OBSERVATIONS

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

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

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

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

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

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

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

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

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

VI. RESULTS

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

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

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

VII. CONCLUSION

According to the Globally Harmonized System (GHS) for the classification of substances which cause acute toxicity, the substance **CLEARACTIV Syrup** (Batch No.TCA-19-01), 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 **CLEARACTIV Syrup** (Batch No.TCA-19-01) 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 **CLEARACTIV Syrup** (Batch No.TCA-19-01) 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 : Mr. A. M. P
Assistant Pr
I.P.E.R. War

I. P. E. R. WARDHA

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