TOXINDIA

Study Report

Study No.	:	TXI/0144-NG
Title	:	Acute Oral Toxicity Test of RECOVEREEZ FORTE in Rats
Regulatory Guideline	:	OECD Guideline No. 423
Study Director	:	Satej Shegar

Sponsor

Zum Heilen Diagnostic & Therapeutics Pvt Ltd (ZH DAT)
Office number: 12/1543-C, SB Center, Second floor,
Museum road, Thissur 680020
Kerala

Test Facility

TOXINDIA,
Plot No. C-33 Part, Jejuri MIDC,
Jejuri, (Purandar),
Pune - 412 303,
(Maharashtra) INDIA

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ABBREVIATIONS

%	:	Percent
°C	:	Degree Celsius
CPCSEA	:	Committee for the Purpose of Control and Supervision of Experimental Animals
GLP	:	Good Laboratory Practice
IAEC	:	Institutional Animal Ethics Committee
kg	:	Kilogram
mg	:	Milligram
mL	:	Milliliter
mm	:	Millimeter
n	:	Number of Animals
No.	:	Number
OECD	:	Organization for Economic Co-Operation and Development
QAU	:	Quality Assurance Unit
SOP	:	Standard Operating Procedure
TIS	:	Test Item Information Sheet
TS	:	Terminal Sacrifice

Study Director's Statement of Compliance

Study No. : TXI/0144-NG

Study Title : Acute Oral Toxicity Test of RECOVEREEZ FORTE in Rats

I declare that the study was performed in its entirety in adherence to the OECD Principles of Good Laboratory Practice [ENV/MC/CHEM (98)17 (as revised in 1997)] and adopted on November 26th 1997 by the decision of the OECD Council [C(97)186/Final]. The study was performed as mentioned in the approved Study Plan and Standard Operating Procedures. No deviation was observed from the approved Study Plan.

The stability and homogeneity of the test item in the vehicle was not analysed.

This report is a complete, true and accurate representation of the study and its results and as Study Director I accept the responsibility for the validity of the data.

Satej Shegar, M.Sc.
Study Director

Date: 06/10/2021

Certificate of Affirmation

Study No. : TXI/0144-NG

Study Title : Acute Oral Toxicity Test of RECOVEREEZ FORTE in Rats

It is certified that a sufficient number of qualified personnel, appropriate facilities, equipment, and materials were provided in timely manner and proper conduct of this study in accordance with the OECD Principles of GLP.

A. Thomson Mathai, PhD, FAEB
Test Facility Management

Date: 06/10/2021

SUMMARY

Acute Oral Toxicity Test of RECOVEREEZ FORTE in Wistar Rats was conducted at TOXINDIA, Jejuri, Pune, Maharashtra, India. This study was performed as per OECD 423.

Six female Wistar rats were fasted for approximately 16.0 hours to 18.0 hours prior to dosing. The feed was withheld prior to dosing and approximately 3.0 hours to 3.5 hours post dosing but drinking water was provided *ad libitum*. The dose was calculated based on the fasted body weight of each rat and administered in a single dose by gavage using a stainless steel 18 G cannual. The time interval between dosing was determined by the onset, duration and severity of toxic signs.

Initially, Three rats of Step I were administered orally at dose of 5000 mg/kg body weight; no mortality was observed, hence, another set of three rats Step II, were dosed with 5000 mg/kg body weight. No mortality was observed at this dose level. Hence, further dosing was not performed.

The body weights were recorded on day 0 (prior to dosing), 7, 14. The mean body weight of all the animals were observed with gain on day 7 and 14, as compared to day 0. Food Consumption was measured weekly. Feed consumption was normal for all animal of step I and II at 5000 mg/kg body weight.

All animals were observed for mortality and morbidity twice daily. Animals were observed for clinical signs during acclimatization period and treatment period once daily. On the day of dosing, animals were observed during first 30 minutes and at 1 hour, 2 hour, 3 hour and 4 hour after dosing. No mortality was observed in any of the animals throughout the 14 days observation period.

In Step-I, animal no. 01, 02 and 03 were normal at 30 minutes and 1 hour observation. At 2nd hour observation all three animals showed lethargy. At 3rd hour observation animal number 01 showed lethargy, while animal number 02 and 03 showed lethargy along with ataxia. At 4th hour observation all three animal showed lethargy along with ataxia. From day 1 all three animals were normal till day 14.

In step-II, animal no. 04, 05, 06 were normal at 30 minutes and 1 hour observation. At 2nd hour observation all three animals showed lethargy. At 3rd hour observation animal number 04 showed lethargy, while animal number 05 and 06 showed lethargy along with ataxia. At 4th hour observation animal no. 04 showed lethargy and ataxia, while animal no. 05 and 06 showed lethargy, ataxia along with abdominal breathing. On day 1 animal no. 04 showed lethargy, while animal no. 05 and 06 showed lethargy and abdominal breathing. From day 2 all three animals were normal till day 14.

All animals blood was collected from retro orbital plexus. The haematological parameters like white blood

cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells, hemoglobin, platelets were analyzed and found within normal range.

The Clinical chemistry parameters like alkaline phosphatase, bilirubin total, creatinine, serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, Urea were analyzed and found within normal range.

At the end of 14 day observation period, all the rats were euthanised by overdose of CO2. All the animals were observed for external and internal gross pathology. No external and internal gross pathological changes were seen in any of the animals of Step I and II treated with 5000 mg/kg body weight.

For histopathological examination brain, heart, liver, lung, spleen and kidney were collected and and processed for histopathological examination. Microscopic examination of all the animals treated with test item at 5000 mg/kg body weight belonging to Set-I and Set-II did not show any lesion of pathological significance.

Under the conditions of this study, the acute Oral Toxicity Test of RECOVEREEZ FORTE in female Wistar rat is as given below:

The acute oral LD_{50} of RECOVEREEZ FORTE was greater than 5000 mg/kg body weight with 5000 mg/kg body weight as LD_{50} cut-off value and is classified under "**Category 5 or Unclassified**" as per Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

1. OBJECTIVE

The objective of the study was to determine the acute oral toxicity potential of RECOVEREEZ FORTE when administered once through the oral route to Wistar strain of rats. This study provides information on the lethal dose (LD_{50}) of the test item and to classify it as per the Globally Harmonized Classification System (GHS) based on its acute toxic effects.

2. GUIDELINES

2.1 Study Guideline

The design of this study was based on the requirements of the following guideline:

OECD Guideline for Testing of Chemicals: Acute Oral Toxicity – Acute Toxic Class Method (No 423, Adopted: 17th December 2001).

2.2 Classification Guideline

United Nations New York and Geneva, 2019: Globally Harmonized System of Classification and Labelling of Chemicals (GHS); ST/SG/AC.10/30/Rev.8, 2019.

3. GLP COMPLIANCE

This study was performed in accordance with the OECD Principles on Good Laboratory Practice ENV/MC/CHEM(98)17, as revised in 1997, Environment Directorate, Organization for Economic Co-operation and Development, Paris (1998).

4. STUDY SCHEDULE

Study Initiation Date	:	July 30, 2021
Experiment Start Date	:	July 31, 2021
Experiment Completion Date	:	September 22, 2021
Study Completion Date	:	October 06, 2021

5. LIST OF PERSONNEL INVOLVED IN THE STUDY

Study Director	: Satej Shegar, M.Sc.
Study Personnel	: Suraj Bhongale, M.Sc.
	Sanket Shirgaonkar, M.Sc.
Animal Veterinarian	: Dr. Sachin Bhalchim, M.V.Sc.
Pathologist	: Dr. Sagar Surjagade, M.V.Sc.
	Dr. Seema Shinde, M.V.Sc.
Peer review Pathologist	: Dr. Mahesh Brahmanakar, M.V.Sc.

6. TEST ITEM DETAILS

The information mentioned below is as per the TIS provided by sponsor, no test item characterization was carried out at the Test Facility to confirm it.

Name of the test item	:	RECOVEREEZ FORTE
Appearance	:	Green colored hard gelatin capsule containing cream colored powder
Batch No.	:	RE6WL6
Manufactured Date	:	June 20, 2021
Expiry Date	:	December 20, 2022
Supplied by	:	Zum Heilen Diagnostic & Therapeutics Pvt Ltd (ZH DAT) Office number: 12/1543-C, SB Center, Second floor, Museum road, Thissur 680020 Kerala
Storage Condition	:	Room Temperature
Receipt at the test facility	:	28/07/2021
Handling	:	Test item was handled with necessary PPE's (Apron, Face mask, gloves, head cover) and all recommended Safety measures were followed as per SOPs of TOXINDIA
Disposal	:	Remaining Test Item and Test Item formulation was disposed safely as per in-house SOPs

7. PREPARATION OF TEST ITEM FORMULATION

The test item was soluble in distilled water. The vehicle distilled water was added to the weighed quantity of test item (5000.01 mg and 5000.08 mg) slowly and mixed well to make the formulation. It was then transferred to calibrated measuring cylinders. The stock concentration of 500 mg/mL was achieved by making up the volume to 10 mL.

Dose Formulation was freshly prepared prior to dosing. The homogeneity was ensured using Magnetic Stirrer during dose administration.

8. REASON FOR CHOICE OF SPECIES AND ROUTE OF ADMINISTRATION

Rat is the commonly used species for oral toxicity studies and recommended by the International guidelines (i.e., OECD guideline 423) and it meets the regulatory requirement of the most regulatory agencies. The Oral route of administration is recommended by the regulatory guidelines.

9. TEST SYSTEM DETAILS

Species	: *Rattus Norvegicus*
Strain	: Wistar
Sex	: Females (Females were nulliparous and non - pregnant)
Number of animals	: 6 (3 females per step)
Supplier / Source	: Global Bioresearch Solution Pvt. Ltd. (CPCSEA Register No.:1899/PO/Bt/S/16/CPCSEA)
Health status	: Healthy young adult animals were used for study.
Body Weight	: Minimum: 165.66 g Maximum: 180.67 g
Age	: 9-11 weeks at the time of dosing
Acclimatisation	: Animals were acclimatized to the test conditions for 8 days (step I), 10 days for (step –II).

9.1 Animal Health

The health status of the animals used in this study were examined on receipt (initial examination) by Veterinarian. Animals only in good health were acclimatised to Laboratory conditions. Before initiation of the study, veterinary examination was carried out to assure that all animals are still in good condition.

9.2 Animal Identification

During acclimatization study animals were marked for individual identification by using nontoxic blue colour permanent marker with numbers starting from 101 onwards on tail. After acclimatization, all animals were marked by by nontoxic red colour permanent marker and cage cards with numbers starting from 01 onwards. Following allocation to the study, each animal cage was assigned an individual cage card labelled with Study number, Species, Group, Sex, Dose, Permanent animal number, Date of Necropsy and signed and date.

9.3 Animal Husbandry

Before the animals are brought in, the study room and cages were cleaned and disinfected. During the study, the floor of the experimental room and work tops were swept and mopped with disinfectant solution every day. Cages were cleaned at regular intervals.

The Rats were housed in groups of 3 animals per cage. Standard polypropylene cages (floor area 330 mm x 200 mm, height 140 [mm]).

Cages were placed on stainless steel racks. Clean and sterilized paddy husk was used as bedding material.

9.3.1 Experimental Condition

Room Temperature	: Minimum: 19.1°C Maximum: 23.4°C
Room Relative Humidity	: Minimum: 43% Maximum: 61%
Light-dark-rhythm	: 12 hours light: 12 hours dark
Air Changes	: More than 12 changes per hour

9.4 Water

RO (Reverse Osmosis) water was offered *ad libitum*. Samples of the RO water was subjected periodically to microbiological tests and to chemical contaminant analysis.

9.5 Feed

A conventional laboratory pelleted feed from Nutrivet Life Sciences (Batch No. 010621) offered *ad libitum*.

9.6 Animal Welfare and Approval

TOXINDIA is committed to enhancing animal welfare and registered for Breeding and experiments of animals by CPCSEA (922/PO/RcBiBt/S/05/CPCSEA). Use of live animals is inevitable to accomplish the purpose of this study. The recommendations regarding animal care and handling was followed in consistent with: Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for Laboratory Animal Facility, The Gazette of India, 1998.

The use of animals and the general procedures involved in the present study have been reviewed and approved by the Institutional Animal Ethics Committee of TOXINDIA (IAEC Approval Number: TXI/05/25 dated, 19/07/21).

10. EXPERIMENTAL DESIGN AND PROCEDURE

10.1 Vehicle

Vehicle distilled water was selected based on solubility test performed at TOXINDIA.

10.2 Test procedure and Dosing

Three animals were used for each step. As per information available for this compound, dose 5000 mg/kg body weight was administered in first set of animals.

Tweleve female Wistar rats were fasted for approximately 16.0 hours to 18.0 hours prior to dosing. The feed was withheld prior to dosing and approximately 3.0 hours to 3.5 hours post dosing but drinking water was provided *ad libitum*. The dose was calculated based on the fasted body weight of each rat and administered in a single dose by gavage using a stainless steel 18G cannual. The time interval between dosing was determined by the onset, duration and severity of toxic signs. Initially, Three rats of Step I were administered orally at dose of 5000 mg/kg body weight; no mortality was observed, hence, another set of three rats Step II, were dosed with 5000 mg/kg body weight. No mortality was observed at this dose level. Hence, further dosing was not performed. Animal nos. 01 to 03 were dosed at 10.30 a.m., Animal nos. 04 to 06 were dosed at 10.30 a.m.

10.3 Dose Volume

The maximum dose volume was administered at 10 mL/kg body weight.

11. OBSERVATIONS

11.1 Mortality, Morbidity and Clinical Sign Observations

All animals were observed for mortality and morbidity twice daily.

Animals were observed for clinical signs during acclimatization period and treatment period once daily. On the day of dosing, animals were observed during first 30 minutes and at 1 hour, 2 hour, 3 hour and 4 hour after dosing. The observations were including conditions of skin and fur, eyes and mucus membrane, respiratory, circulatory, and autonomic and central nervous system, somato-motor activity and behavioural pattern. Specific observations were made for tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and other.

11.2 Body Weight and Feed consumption

Body weight of the individual animal to be dosed was observed and recorded on day 0 prior to dosing and in weekly intervals thereafter (day 7 and 14). Food Consumption was measured weekly.

11.3 Pathology

All animals blood was collected from retro orbital plexus and following Haematology parameters were analyzed **(Medonic Hematology analyzer Make: Boule Medical AB, Model: Medonic M51)**

Erythrocyte	Platelet
Haemoglobin	Leukocyte and Leukocyte differential count

The following Clinical Chemistry Parameters were analyzed **(Clinical Chemistry auto analyzer, Make: Elitech group B. V. Model: Selectra PRO S)**.

Serum Separation

The tubes of blood sample was placed in suitable position either upright or slant position for at least 30 minutes at room temperature for clotting, which facilitate for better separation of serum. The clot formation was ensured before centrifugation. The blood samples were Centrifuged for 10 to 15 minutes at 4000 rpm at room temperature. After centrifugation the tubes from the centrifuge

was gently removed and care was taken not to disturb the sediment. The serum was transferred to their respective sample tube for analysis using micro pipette.

Alanine aminotransferase	Alkaline phosphatase
Aspartate aminotransferase	Total bilirubin
Creatinine	Urea nitrogen

Animals were euthanatized after completion of 14 days of observation period by CO_2 exposure and gross pathology examination was performed.

The following organs were collected and preserved in 10% NBF and processed for histopathological examination. After tissue fixation trimmed the tissues to appropriate size to fit into cassettes with the help of microtome blade. After trimming, wash the tissue and then process for dehydration and clearing. The tissue were embedded in paraffin wax. Tissue sectioning was performed with microtome. The section was taken on clean slide and stain the slide with Hematoxylin and Eosin stain. Mount the stained slides with clean cover glass using DPX mountant.

Brain	Heart
Lungs	Liver
Spleen	Kidney

The slides were peer reviewed by external veterinary pathologist.

12. Calculation of LD_{50}

The LD_{50} was determined by using fixed LD_{50} cut-off values (refer **Annexure 1**).

13. RESULTS

13.1 Mortality, Morbidity and Clinical Sign Observations

No mortality was observed in any of the animals throughout the 14 days observation period (refer **Table 5**).

In Step-I, animal no. 01, 02 and 03 were normal at 30 minutes and 1 hour observation. At 2nd hour observation all three animals showed lethargy. At 3rd hour observation animal number 01 showed lethargy, while animal number 02 and 03 showed lethargy along with ataxia. At 4th hour

observation all three animal showed lethargy along with ataxia. From day 1 all three animals were normal till day 14.

In step-II, animal no. 04, 05, 06 were normal at 30 minutes and 1-hour observation, At 2nd hour observation all three animals showed lethargy. At 3rd hour observation animal number 04 showed lethargy, while animal number 05 and 06 showed lethargy along with ataxia. At 4th hour observation animal no. 04 showed lethargy and ataxia, while animal no. 05 and 06 showed lethargy, ataxia along with abdominal breathing. On day 1 animal no. 04 showed lethargy, while animal no. 05 and 06 showed lethargy and abdominal breathing. From day 2 all three animals were normal till day 14 (refer **Table 4**).

13.2 Body Weight

The mean body weight of all the animals were observed with gain on day 7 and 14, as compared to day 0 (refer **Table 1 and 2**).

13.3 Feed Consumption

Feed consumption was normal for all animal of step I and II at 5000 mg/kg body weight (refer **Table 3**).

13.4 Pathology

The haematological parameters like white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells, hemoglobin, platelets were found within normal range (refer **Table 6**).

The Clinical chemistry parameters like alkaline phosphatase, bilirubin total, creatinine, serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, Urea were found within normal range (refer **Table 7**).

At the end of 14 day observation period, all the rats were euthanised by overdose of CO_2. All the animals were observed for external and internal gross pathology. No external and internal gross pathological changes were seen in any of the animals of Step I and II treated with 5000 mg/kg body weight (refer **Table 8**).

For histopathological examination brain, heart, liver, lung, spleen and kidney were collected and and processed for histopathological examination. Microscopic examination of all the animals treated with test item at 5000 mg/kg body weight belonging to Set-I and Set-II did not show any

lesion of pathological significance (refer **Table 9**).

14. Conclusions

Under the conditions of this study, the acute Oral Toxicity Test of RECOVEREEZ FORTE in female Wistar rat is as given below:

The acute oral LD_{50} of RECOVEREEZ FORTE was greater than 5000 mg/kg body weight with 5000 mg/kg body weight as LD_{50} cut-off value and is classified under "**Category 5 or Unclassified**" as per Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

15. STUDY PLAN AMENDMENT(s) and DEVIATION(s)

There was no amendment to the study plan and no deviation from the study plan.

16. ARCHIVES

All the original raw data, approved study plan, final report and representative sample of the test item will be retained in the Archives of TOXINDIA for a period of 3 years. At the end of the archiving period, the Sponsor's instructions will be sought either to extend the archiving period or to return or disposal of the archived study related materials.

17. REFERENCES

- OECD series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1, "OECD Principles of Good Laboratory Practice" ENV/MC/CHEM(98)17 (as revised in 1997).
- OECD Guideline for Testing of Chemicals No. 423, "Acute Oral Toxicity- Toxic Class Method"; adopted on 17th December, 2001.
- Globally Harmonized System of Classification and Labeling of Chemicals (GHS), United Nations, New York and Geneva, 2019 (ST/SG/AC.10/30/Rev.8).

18. TABLES

Table 1: Individual Animal Body Weight (g) and Body Weight Changes (%)

Sex: Female

Animal No.	Step/ Dose (mg/kg body weight)	Dose Volume (mL)*	Body Weight (gram)			Body Weight Change (%)	
			Day 0	Day 7	Day 14	Day 0-7	Day 0-14
01	Step-I / 5000	1.8	179.83	190.21	199.74	5.77	11.07
02		1.7	169.98	192.53	200.91	13.27	18.20
03		1.7	166.26	188.34	198.21	13.28	19.22
04	Step-II/ 5000	1.8	180.67	208.51	217.72	15.41	20.51
05		1.7	170.02	178.18	188.24	4.80	10.72
06		1.7	165.66	172.25	182.36	3.98	10.08

Key: *= Dose volume calculated based on day 0 body weight, g = gram, mL = milliliter, mg = milligram, kg = kilogram, % = percent, No. = Number

Table 2: Summary of Animal Body Weight (g) and Body Weight Changes (%)

Sex: Female

Step/ Dose (mg/kg body weight)		Rats Body Weight (gram)			Body Weight Changes (%)	
		Day 0	Day 7	Day 14	0-7	0-14
Step-I/ 5000	Mean	172.02	190.36	199.62	10.77	16.16
	SD	7.01	2.10	1.35	4.33	4.44
	n	3	3	3	3	3
Step-II / 5000	Mean	172.12	186.31	196.11	8.06	13.77
	SD	7.72	19.45	18.95	6.38	5.84
	n	3	3	3	3	3

Key: SD = Standard Deviation, n = Number of Animals, g = gram, mg = milligram, kg = kilogram, % = Percent

Table 3: Summary of Feed Consumption (gram/animal/day)

Sex: Female

Step	Dose (mg/kg body weight)	Cage No.	Animal No.	Feed Consumption (gram/animal/day)	
				(0-7)	(7-14)
I	5000	1	01 to 03	15.62	15.90
II	5000	2	04 to 06	15.47	15.80

Table 4: Individual Animal Clinical Signs and Symptoms

Sex: Female

Animal No.	Step/ Dose (mg/kg body weight)	Min & Hours (Day 0)				
		30 Min	1 h	2 h	3 h	4 h
01	Step-I/5000	1	1	15	15	15, 10
02		1	1	15	15, 10	15, 10
03		1	1	15	15, 10	15, 10
04	Step-II /5000	1	1	15	15	15,10
05		1	1	15	15,10	15,10,37
06		1	1	15	15,10	15,10,37

Animal No.	Step/ Dose (mg/kg body weight)	Days post dosing												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
01	Step-I/ 5000	1	1	1	1	1	1	1	1	1	1	1	1	1	1
02		1	1	1	1	1	1	1	1	1	1	1	1	1	1
03		1	1	1	1	1	1	1	1	1	1	1	1	1	1
04	Step-II/ 5000	15	1	1	1	1	1	1	1	1	1	1	1	1	1
05		15, 37	1	1	1	1	1	1	1	1	1	1	1	1	1
06		15, 37	1	1	1	1	1	1	1	1	1	1	1	1	1

Key: 1 = Normal, 15 = Lethargy, 10 = Ataxia, 37 = Abdominal breathing, No. = Number, mg = Milligram, kg = Kilogram, Min = Minutes, h = Hour

Table 2: Summary of Animal Body Weight (g) and Body Weight Changes (%)

Sex: Female

Step/ Dose (mg/kg body weight)		Rats Body Weight (gram)			Body Weight Changes (%)	
		Day 0	Day 7	Day 14	0-7	0-14
Step-I/ 5000	Mean	172.02	190.36	199.62	10.77	16.16
	SD	7.01	2.10	1.35	4.33	4.44
	n	3	3	3	3	3
Step-II / 5000	Mean	172.12	186.31	196.11	8.06	13.77
	SD	7.72	19.45	18.95	6.38	5.84
	n	3	3	3	3	3

Key: SD = Standard Deviation, n = Number of Animals, g = gram, mg = milligram, kg = kilogram, % = Percent

Table 6: Hematology Parameters Observation

Sex: Female

Animal No.	WBC (10^9/L)	Neu%	Lym%	Mon%	Eos%	Bas%	RBC (10^12/L)	HGB (g/dL)	PLT (10^9/L)
01	9.26	16.2	81.2	2.2	0.1	0.3	7.18	14.3	934
02	7.74	29.3	63.4	6.7	0.1	0.5	7.14	14.6	772
03	10.91	9.2	88.4	2	0	0.4	7.64	14.2	947
04	7.74	37.5	51.3	10.3	0.3	0.6	7.12	13.4	1138.0
05	6.45	39.7	54	5.6	0.3	0.4	7.49	14.2	1003
06	10.42	30.7	55.8	12.9	0	0.6	5.93	11.1	1386

Key: WBC= White Blood Cells, Neu= Neutrophils, Lym= Lymphocytes, Mon= Monocytes, Eos= Eosinophils, Bas= Basophils, RBC= Red blood cells, HGB= Hemoglobin, PLT= Platelets

Table 7: Clinical Chemistry Parameters

Sex: Female

Animal No.	ALP (U/l)	BILI T (mg/dl)	CREAT (mg/dl)	SGOT (U/l)	SGPT (U/l)	UREA (mg/dl)	BUN
01	323.1	0.09	0.70	171.7	145.4	62.3	29.1
02	389.9	0.11	0.77	184.4	180.2	64.0	29.9
03	369.5	0.08	0.79	173.2	139.2	67.4	31.5
04	319.9	0.12	0.60	197.9	104.7	70.1	32.8
05	503.3	0.08	0.56	178.9	68.2	80.0	37.4
06	699.9	0.10	0.58	220.8	109.8	73.8	34.5

Key: ALP= Alkaline phosphatase, BILI T= Bilirubin Total, CREAT= Creatinine, SGOT = serum glutamic oxaloacetic transaminase, SGPT= Serum Glutamic Pyruvic Transaminase, BUN = Blood Urea Nitrogen.

Table 8: Gross Necropsy Observation

Sex: Female

Animal No.	Step/ Dose (mg/kg body weight)	Mode of Death	Gross Observation	
			External	Internal
01	Step-I/ 5000	TS	No abnormality detected	No abnormality detected
02		TS	No abnormality detected	No abnormality detected
03		TS	No abnormality detected	No abnormality detected
04	Step-II / 5000	TS	No abnormality detected	No abnormality detected
05		TS	No abnormality detected	No abnormality detected
06		TS	No abnormality detected	No abnormality detected

Keys: TS= Terminal Sacrifice, No. = Number, mg = Milligram, kg = Kilogram

Table 9: Histopathology Observations

Sex: Female

Organ/ Microscopic Finding	Animal Number					
	Step I/ 5000 mg/kg body weight			Step II/ 5000 mg/kg body weight		
	01	02	03	04	05	06
Liver	NAD	NAD	NAD	NAD	NAD	NAD
Lungs	NAD	NAD	NAD	NAD	NAD	NAD
Kidneys	NAD	NAD	NAD	NAD	NAD	NAD
Heart	NAD	NAD	NAD	NAD	NAD	NAD
Brain	NAD	NAD	NAD	NAD	NAD	NAD
Spleen	NAD	NAD	NAD	NAD	NAD	NAD

Key: NAD = No Abnormality Detected

Table 10: Histopathology Images

Organ : Lungs

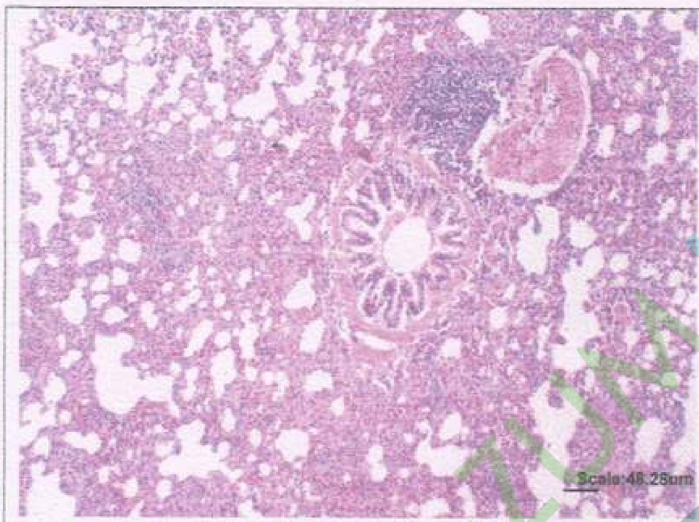
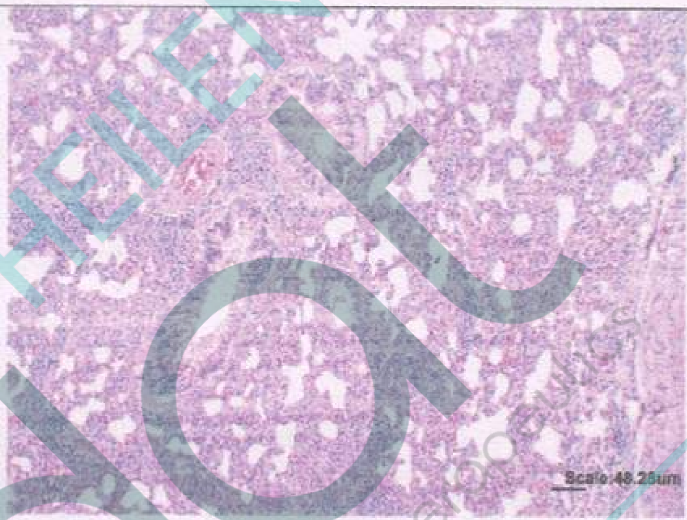
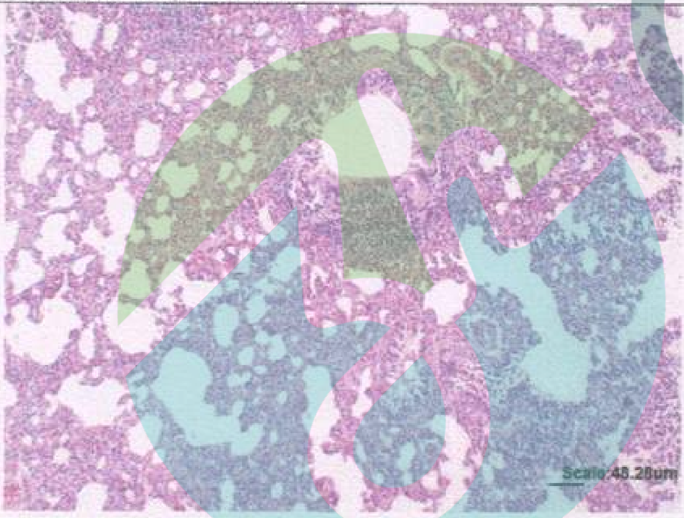
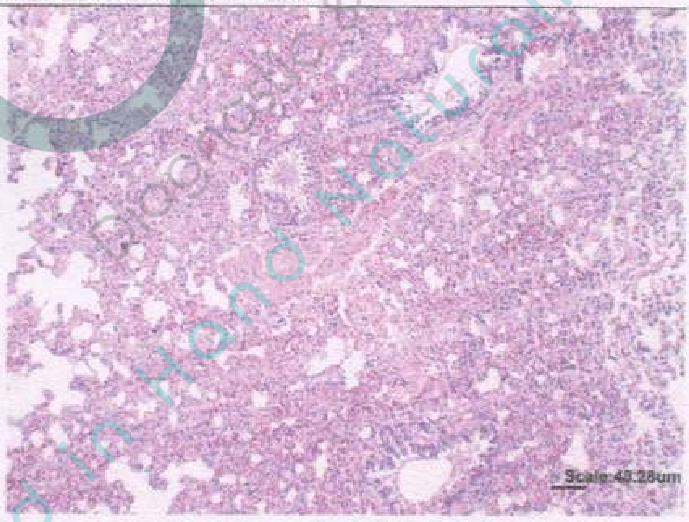
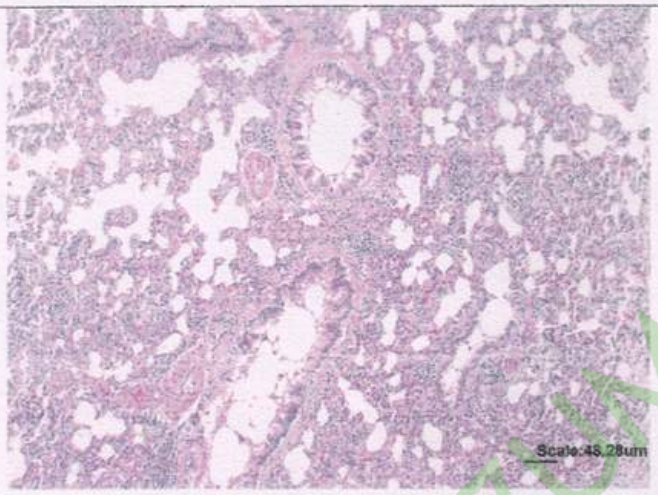
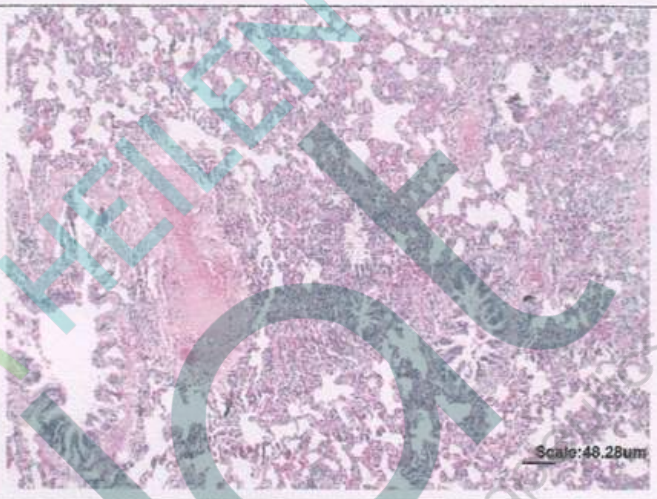
	
Lungs; Animal No. 01: Showing normal bronchial epithelium and alveoli. 10X, H & E Stain.	**Lungs;** Animal No. 02: Showing normal bronchial epithelium and alveoli. 10X, H & E Stain.
	
Lungs; Animal No. 03: Showing normal bronchial epithelium and alveoli. 10X, H & E Stain.	**Lungs;** Animal No. 04: Showing normal bronchial epithelium and alveoli. 10X, H & E Stain.

Table 10: Histopathology Images (Continued)

Organ : Lungs

	
Lungs; Animal No. 05: Showing normal bronchial epithelium and alveoli. 10X, H & E Stain.	**Lungs;** Animal No. 06: Showing normal bronchial epithelium and alveoli. 10X, H & E Stain.

Organ : Liver

	
Liver; Animal No. 01: Showing normal periportal area and hepatic parenchyma. 10X, H & E Stain.	**Liver;** Animal No. 02: Showing normal periportal area and hepatic parenchyma. 10X, H & E Stain.

Table 10: Histopathology Images (Continued)

Organ : Liver

	
Liver; Animal No. 03: Showing normal periportal area and hepatic parenchyma. 40X, H & E Stain.	**Liver;** Animal No. 04: Showing normal periportal area and hepatic parenchyma. 10X, H & E Stain.
	
Liver; Animal No. 05: Showing normal periportal area and hepatic parenchyma. 10X, H & E Stain.	**Liver;** Animal No. 06: Showing normal periportal area and hepatic parenchyma. 10X, H & E Stain.

Table 10: Histopathology Images (Continued)

Organ : Kidneys

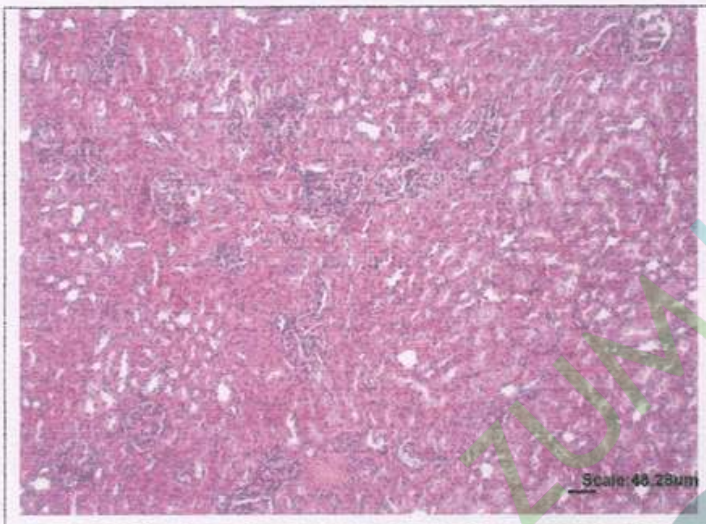
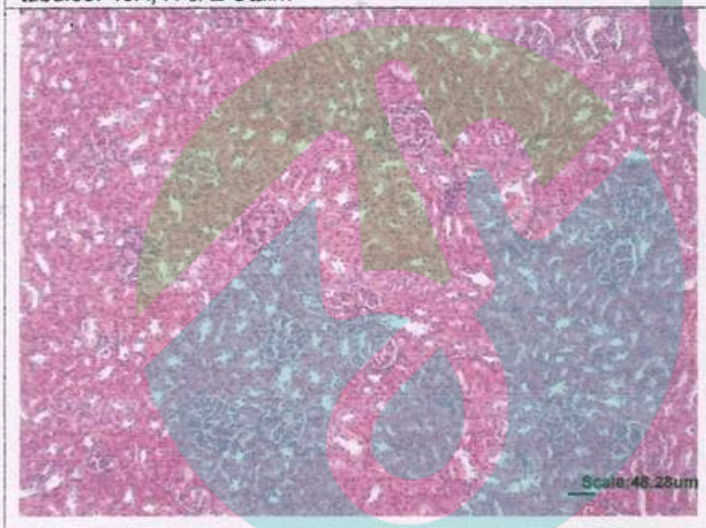
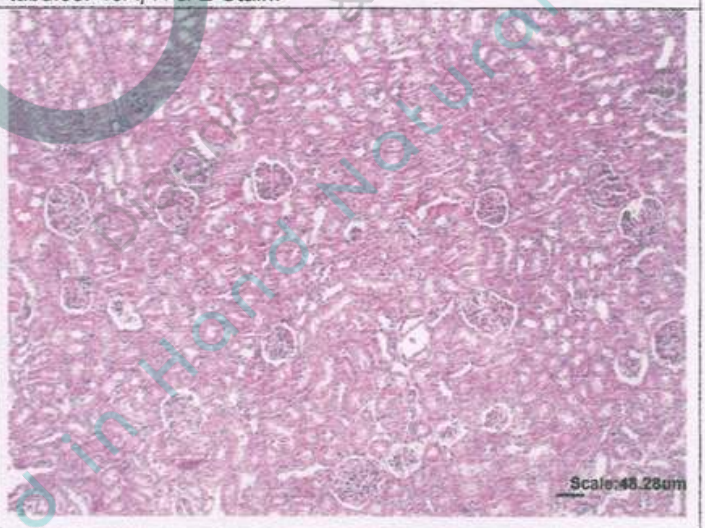
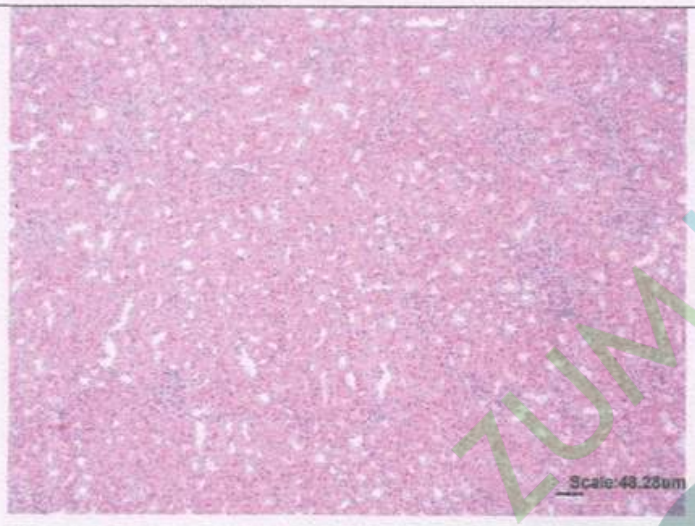
	
Kidneys; Animal No. 01: Showing normal glomeruli and renal tubules. 40X, H & E Stain.	**Kidneys;** Animal No. 02: Showing normal glomeruli renal tubules. 40X, H & E Stain.
	
Kidneys; Animal No. 03: Showing normal glomeruli and renal tubules. 40X, H & E Stain.	**Kidneys;** Animal No. 04: Showing normal glomeruli and renal tubules. 40X, H & E Stain.

Table 10: Histopathology Images (Continued)

Organ : Kidneys

	
Kidneys; Animal No. 05: Showing normal glomeruli and renal tubules. 40X, H & E Stain.	**Kidneys;** Animal No. 06: Showing normal glomeruli renal tubules. 40X, H & E Stain.

Organ : Heart

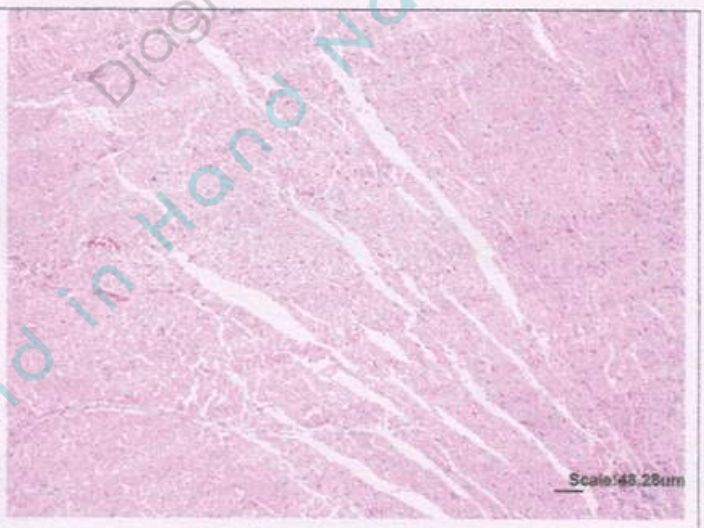
	
Heart; Animal No. 01: Showing normal cardiac muscles and myocytes. 10X, H & E Stain.	**Heart;** Animal No. 02: Showing normal cardiac muscles and myocytes. 10X, H & E Stain.

Table 10: Histopathology Images (Continued)

Organ : Heart

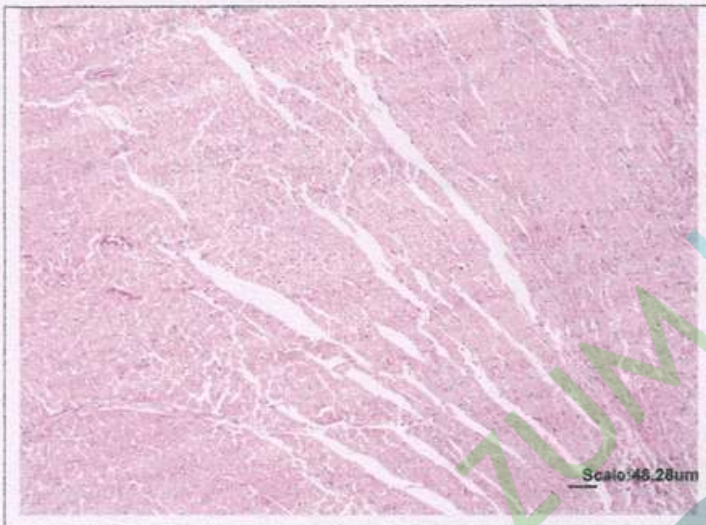
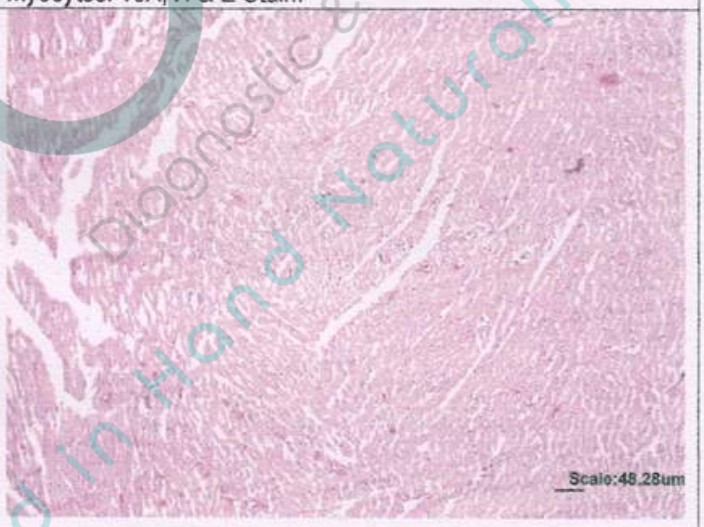
	
Heart; Animal No. 03: Showing normal cardiac muscles and myocytes. 10X, H & E Stain.	**Heart;** Animal No. 04: Showing normal cardiac muscles and myocytes. 10X, H & E Stain.
	
Heart; Animal No. 05: Showing normal cardiac muscles and myocytes. 10X, H & E Stain.	**Heart;** Animal No. 06: Showing normal cardiac muscles and myocytes. 10X, H & E Stain.

Table 10: Histopathology Images (Continued)

Organ : Spleen

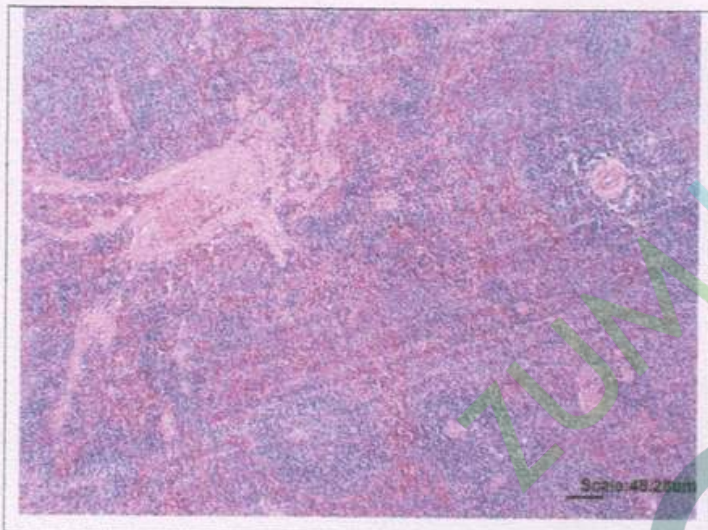
	
Spleen; Animal No. 01: Showing normal white pulp and red pulp. 10X, H & E Stain	**Spleen;** Animal No. 02: Showing normal white pulp and red pulp. 10X, H & E Stain
	
Spleen; Animal No. 03: Showing normal white pulp and red pulp. 10X, H & E Stain	**Spleen;** Animal No. 04: Showing normal white pulp and red pulp. 10X, H & E Stain

Table 10: Histopathology Images (Continued)

Organ : Spleen

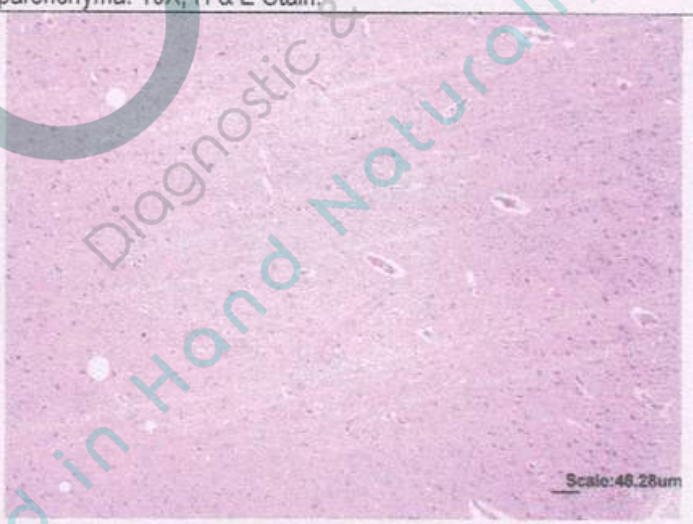
	
Spleen; Animal No. 05: Showing normal white pulp and red pulp. 10X, H & E Stain	**Spleen;** Animal No. 06: Showing normal white pulp and red pulp. 10X, H & E Stain

Organ : Brain

	
Brain; Animal No. 01: Showing normal neuron and cerebral parenchyma. 10X, H & E Stain.	**Brain;** Animal No. 02: Showing normal neuron and cerebral parenchyma. 10X, H & E Stain.

Table 10: Histopathology Images (Continued)

Organ : Brain

	
Brain; Animal No. 03: Showing normal neuron and cerebral parenchyma. 10X, H & E Stain.	**Brain;** Animal No. 04: Showing normal neuron and cerebral parenchyma. 10X, H & E Stain.
	
Brain; Animal No. 05: Showing normal neuron and cerebral parenchyma. 10X, H & E Stain.	**Brain;** Animal No. 06: Showing normal neuron and cerebral parenchyma. 10X, H & E Stain.

Annexure 1 Globally Harmonized System of Classification and Labelling of Chemicals

Acute toxicity	Cat. 1	Cat. 2	Cat. 3	Cat. 4	Category 5
Oral (mg/kg)	≤ 5	> 5 ≤ 50	> 50 ≤ 300	> 300 ≤ 2000	Criteria: • Anticipated oral LD_{50} between 2000 and 5000 mg/kg; • Indication of significant effect in humans;* • Any mortality at class 4;* • Significant clinical signs at class 4;* • Indications from other studies;* *If assignment to more hazardous class is not warranted.

Five GHS categories have been included in the GHS Acute Toxicity scheme from which the appropriate elements relevant to transport, consumer, worker and environment protection can be selected. Substances are assigned to one of the five toxicity categories on the basis of LD_{50} (oral, dermal) or LC_{50} (inhalation).

Category 1, the most severe toxicity category, has cut-off values currently used primarily by the transport sector for classification for packing groups. Some Competent Authorities may consider combining Acute Categories 1 and 2. Category 5 is for chemicals which are of relatively low acute toxicity but which, under certain circumstances, may pose a hazard to vulnerable populations. Criteria other than LD_{50}/LC_{50} data are provided to identify substances in Category 5 unless a more hazardous class is warranted.

20. APPENDIX

Appendix 1 TIS of RECOVEREEZ FORTE

Title: Test/ Reference Item Information Sheet

Format No: [illegible] Version No: [illegible] Effective Date: [illegible]

For Sponsor:

1	Name of the Test item	RECOVEREEZ FORTE
2	Sponsor Name and Address	Zum Heilen Diagnostic and Therapeutics Pvt Ltd (ZHDAT) Office number 12/543 [illegible] SB Center, [illegible] Museum Road, Thrissur 680020, Kerala GST number: 32AABCZ5396B1ZF
3	Supplied/ Manufactured by (If different from sponsor)	as given Above
4	Date of Manufacturing	20/06/2021
5	Date of Expiry	Best Before – 18 months from the manufacture
6	Batch No/ Lot No	RE6WL6
7	CAS No	-
8	Purity	
9	Composition	Cardamom extract 200mg; Rosemary extract [illegible]; Pepper extract - 10 mg
10	Concentration	as given Above
11	Quantity, Type and No. of Container sent	60 Capsules in 1 Plastic Container
12	Type of the Test item (Tick (√) at applicable option)	√ Pharmaceutical Industrial Chemical ☐Agrochemical Cosmetics Product ☐Food/Feed additives ☐Other [illegible]
13	Storage Condition (Tick (√) at applicable option)	√ Room Temperature Refrigerator (2-8°C) ☐Deep Freezer (Below -20°C) Others [illegible]
14	Specific Safety Precaution for Handling (If any)	-
15	Documents Sent (Tick (√) at applicable option)	☐Certificate of Analysis (COA) √ Test/ Reference Item Information Sheet (TIS) ☐Material Safety Data Sheet (MSDS)
16	Fate of Test Item/Reference item (Tick (√) at applicable option)	Sent back to Sponsor √ Dispose at TF after study completion
17	Sponsor Representative (Name, Sign and Date)	Dr. [illegible] 7.7.2021

Key: NA: Not Applicable; CAS No: Chemical Abstract Service number; TF: Test Facility

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