# STUDY REPORT

ACUTE ORAL TOXICITY STUDY OF ***RECOVEREEZ (CAPSULES)*** IN WISTAR ALBINO RATS

STUDY NO: CKL/TOX/IAEC/2021-1/140

Copy No: 1/3

**SPONSOR**

Zum Heilen Diagnostic & Therapeutics (ZH D&T)
No 143, Bethel Lane
Thrissur 680001 Kerala

**TEST FACILITY**

CARe KERALAM LTD
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## STATEMENT OF CONFIDENTIALITY

This report which contains **c**onfidential and proprietary information of **Zum Heilen Diagnostic & Therapeutics (ZH D&T), No 143, Bethel Lane, Thrissur 680001 Kerala** will not be disclosed to anyone except the employees of CARe Keralam Ltd wherever necessary or to persons authorized by law or judicial judgment without the expressed or written approval of sponsor.

## DECLARATION

The study director hereby declares that the work was performed under his supervision and in accordance with the described procedures. It is assured that the reported results faithfully represent the raw data obtained during the experimental work. No circumstances have been left unreported which may have affected the quality or integrity of the data or which might have a potential bearing on the validity and reproducibility of this study.

The study director accepts overall responsibility for the technical conduct of the study as well as the interpretation, analysis, documentation and reporting of the results.

_____

(Signature with date)
Dr. Sithara M.S.; MVSc.
Toxicologist

## 1. STUDY DETAILS

**1.1 Study Title** : Acute oral toxicity study of ***Recovereez (Capsules)*** in Wistar albino rats

**1.2 Study Number** : CKL/TOX/IAEC/2021-1/140

**1.3 Test Item** : ***RECOVEREEZ (CAPSULES)***

**1.4 Test Item Code** : Nil

**1.5 Testing Facility** : CARe KERALAM Ltd
KINFRA Small Industries Park
Koratty- 680 309, Thrissur Kerala.

**1.6 Sponsor** : Zum Heilen Diagnostic & Therapeutics (ZH D&T)
No 143, Bethel Lane, Thrissur 680001 Kerala

**1.7 Animal welfare** : The present study protocol has been reviewed and approved by Institutional Animal Ethics Committee (IAEC) of CARe Keralam Ltd.

**1.8 Study Schedule**

| | | | | | |
|---|---|---|---|---|---|
| **a.** | **Study start date** | : | 25-05-2021 | | |
| **b.** | **Acclimatization** | : | Start: 25-05-2021 End: 31-05-2021 | | |
| **c.** | **Date of treatment** | : | Sighting study | 300 mg/kg Bwt | 01-06-2021 |
| | | | | 2000 mg/kg Bwt | 02-06-2021 |
| | | | Main study | 2000 mg/kg Bwt | 03-06-2021 |
| **d.** | **Experiment completion** | : | 16-06-2021 | | |
| **e.** | **Date of Necropsy** | : | 17-06-2021 | | |
| **f.** | **Draft Report Review** | | 02-07-2021 | | |
| **g.** | **Final report review** | : | 06-07-2021 | | |

## 2. STUDY PERSONNEL

The following personnel were involved in the conduct of the study.

| No. | Designation | Personnel | Signature with date |
|---|---|---|---|
| 1. | Principal Investigator | Dr. Sithara M.S., MVSc.; Toxicologist | |
| 2. | Co-Investigator | Mrs. Biniya Balakrishnan, M. Pharm Junior toxicologist | |
| 3. | Animal House Keeper | Mrs. Litty Biju | |
| 4. | Animal House Keeper | Miss Simi K.T. | |

## 3. LIST OF COMMONLY USED SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| IAEC | Institutional Animal Ethics Committee |
| OECD | Organization for Economic Co-operation and Development |
| w/v | Weight / volume |
| N | Normal |
| NAD | No Abnormality Detected |
| n | No of animals |
| Bwt | Body weight |
| µl | Microliter |
| g | Gram |
| % | Percentage |
| mg | Milligram |
| mmol | Milli moles |
| h/hr | Hour |
| SD | Standard Deviation |
| kg | Kilogram |
| m | Minute |

## 4. INTRODUCTION

Polyherbal formulations are the mainstay of traditional system of medicine due to its increased efficacy and zero/negligible side effects when compared to the use of single herbs. However, scientific validation is very less in Polyherbal formulations when compared to single herbs.

**Recovereez** is such a Polyherbal formulation in a capsule form. Active ingredients in this formulation are Cardamom extract and Piperine. But, it does not have any scientific evidence on the safety aspects. So a preclinical experiment is required to scientifically validate its safety. Hence the objective of this study was to assess the toxic potential of **Recovereez capsules**, when administered by oral gavage, in a single dose to Wistar rats.

## 5. MATERIALS AND METHODS

### 5.1. Test Item Information

The test item information furnished by the sponsor is presented below:

| | |
|---|---|
| **Test Item name** | : ***Recovereez (Capsules)*** |
| **Name to be used in Report** | : ***Recovereez (Capsules)*** |
| **Test Item code by Test facility** | : None |
| **Physical appearance** | : Dark green colored capsule containing light green Viscous liquid |
| **Date of manufacture** | : March 2021 |
| **Batch No.** | : ZHEF 20-12 |
| **Solubility** | : Water soluble |
| **Sample quantity** | : One bottle comprising of 60 capsules |
| **Storage conditions** | : To be stored in temperature below 25°C |
| **Date of Expiry** | : March 2022 |
| **Name of the Supplier** | : Zum Heilen Diagnostic & Therapeutics (ZH D&T) No 143, Bethel Lane, Thrissur 680001 Kerala |

The responsibility for the correct identity of the test item rests with the sponsor.

### 5.2. Vehicle

Distilled water was used as vehicle for formulation preparation

**Justification for Selection of Vehicle**

The test item forms good suspension with Distilled water. Hence Distilled water was used as a suspending agent for test item formulation. Distilled water is universally accepted and routinely used vehicle in oral (gavage) toxicity studies.

5.3.**Test System and Management**

| | | |
|---|---|---|
| Animal species | : | Rats |
| Strain | : | Wistar albino rats |
| Justification for selection of species | : | Rat is one of the recommended species by regulatory agencies for conducting acute toxicological studies among rodents. |
| Source | : | Small animal breeding station, College of Veterinary & Animal Sciences, Mannuthy, Thrissur. |
| No. of animals & Sex | : | 6 Female nulliparous and non-pregnant rats |
| Body weight range | : | 170-200 g |
| Age at treatment | : | 8 to 12 Weeks |
| Identification | : | Cage cards |

5.4.**Methods**

5.4.1. Husbandry

| | | |
|---|---|---|
| a. Conditions | : | Animals were housed under standard laboratory conditions: air-conditioned environment with adequate fresh air supply through IVC system, room temperature 21.0 to 24.0°C, relative humidity 50-70%, with 12 hours light and 12 hours dark cycle. The temperature and relative humidity were recorded daily. |
| b. Housing | : | Single animal was housed in a standard polysulfone cage (Size: L 300 x B 170 x H 140 mm) with stainless steel top grill mesh having facilities for holding pelleted food and drinking water in water bottle fitted with stainless steel sipper tube. Sterilized paddy husk was provided as bedding material. |
| c. Acclimatization | : | The animals were acclimatized for a minimum period of seven days to laboratory conditions and were observed for clinical signs daily. Veterinary examination of all the animals was recorded on the 1st and 7th day of acclimatization. |

d. Diet : The animals were fed *ad libitum* throughout the acclimatization and study period. Pelleted lab rodent feed (Manufactured by Feed plant of School of Animal Nutrition and Feed Technology, Kerala Veterinary & Animal Sciences University) was provided.

e. Water : Water was provided *ad libitum* throughout the acclimatization and study period. Deep bore-well water passed through activated charcoal filter and exposed to ultraviolet rays in Aquaguard water filter cum purifier (Manufactured by Eureka Forbes Ltd., Mumbai) was provided in plastic water bottles with stainless steel sipper tubes.

5.5. **Study Design**

The study was conducted following the OECD Guidelines for Testing of Chemicals (No. 420, Section 4: Health Effects) on conduct of "Acute Oral Toxicity – Fixed dose procedure" (Adopted: 17th December 2001). The test item was administered as a single dose at an equivolume of 1ml/100g body weight for all animals and the actual volume of administration was calculated based on the most recent body weight of the animals. Animals were treated in the following manner.

| Study particulars | Group | Dose |
|---|---|---|
| Sighting study | Group 1 | 1animal<br>administered single dose of **Recovereez Capsules**<br>300 mg/kg body weight, per oral |
| | Group 2 | 1animal<br>administered single dose of **Recovereez Capsules**<br>2000 mg/kg body weight, per oral |
| Main study | Group 3 | 4 animals<br>administered single dose of **Recovereez Capsules**<br>2000 mg/kg body weight, per oral |

In sighting study, dosing was sequential, allowing at least 24 hours before dosing the next animal. In the main study, a total of five animals were used for each dose level. The five animals were made up of one animal from the sighting study dosed at the 2000mg/kg dose level together with an additional four animals. The time interval between dosing at each level was determined by the onset, duration, and severity of toxic signs. All animals were observed individually at least once during the first 30 minutes after dosing, then periodically during the first 24 hours (with special attention during the first 4 hours) and thereafter, daily for a total period of 14 days.

On 15th day all animals were euthanized by CO2 Inhalation and were subjected to necropsy and gross pathological examination.

5.6.**Dose Formulation**

The weighed test item was formulated in vehicle to get desired concentration as per the dose (mg/kg body weight). Test item was formulated shortly before dosing.

5.7. **Administration of Test Item**

The animals were fasted overnight (water provided *ad libitum*) prior to dosing. The test item was administered orally by gavage to each rat as a single dose, using gavaging needle (animal feeding needle). The dosage volume administered to individual rat was adjusted according to its body weight recorded on the day of dosing. The dose volume was 1ml/100 g body weight for all animals. Food was offered 3-4 hours followed by dosing.

5.8.**Study compliance**

The study was performed in accordance with the following:

- OECD Principles of Good Laboratory Practices (1997)
- The standard operating procedures at CARe KERALAM Ltd and the mutually agreed study plan with the sponsor
- The recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility published in the gazette of India, January 7th 2010 and the protocol approved by Institutional Animal Ethics Committee (IAEC).

5.9.**Safety Precautions**

Gloves, cap and face mask were used in addition to protective body garments and rubber slipper to ensure adequate personal health and safety and to avoid inhalation and skin contact with the test item.

5.10. **Observations**

The following observations were made during the study.

5.10.1 **Clinical Signs and Mortality**

All the animals were observed for clinical signs and mortality at 30-40 min, 1 hr. (±10 min),2 hr. (±10min), 3 hr. (±10min) and 4 hr. (±10min) on day 1 followed by dosing and thereafter once daily for clinical signs and twice daily for mortality/morbidity during the 14 day observation period. Observations were made including changes in skin and fur, eyes and mucous membranes,

tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma and also respiratory, somatomotor activity and behaviour pattern in the animals.

5.10.2 **Body Weight, Food and Water intake**

The body weight of each rat was recorded prior to treatment on Day 1, then weekly and at the terminal sacrifice time. Individual body weights and body weight changes were calculated. Daily food and water intake were recorded during the study period.

5.10.3 **Gross pathology**

All the animals euthanized by CO2 Inhalation were subjected to necropsy. Detailed gross pathological examination was done and the observations were recorded.

## 6. DATA COMPILATION

The computer printouts of the data (in the form of appendix) were verified against raw data. All individual animal data were summarized and presented as tables. All findings were presented in the report as per the standard reporting procedure.

## 7. INTERPRETATION OF RESULTS

The test was conducted as per OECD Guideline 420. The test substance was classified according to the Globally Harmonized System (GHS) for classification of chemicals which cause acute toxicity (OECD series on testing and assessment, Number 33; Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures; ENV/JM/MONO (2001) 6).

## 8. AMENDMENTS AND DEVIATIONS

There were no amendments and deviations during the conduct of the study.

## 9. REPORT DISTRIBUTION

Three copies of the Study Reports were distributed as mentioned below;

a). Copy No. 1/3 & 2/3 - Sponsor's copy
b). Copy No. 3/3 -Archives, CARe Keralam Ltd

## 10. AUDITING OF THE REPORT

After completion of the report writing, draft report along with the raw data was sent to the QAU for auditing. After complying with the QAU findings final report was prepared and again sent to QAU for final audit.

## 11. ARCHIVING

All materials and data generated from the experiment was stored at archives of the test facility. CARe Keralam Ltd, archives will maintain the materials for 3 years after completion of the study. Study plan, raw data, draft report and final report were archived. A sample of the test

item was sent from the storage and disposal to the archives after the receipt of test item. This sample was stored for a period of 3 months from the date of completion of the study or till its expiry, whichever is earlier.

## 12. RESULTS AND DISCUSSION

### 12.1. Clinical Signs and Mortality

There were no clinical signs of toxicity and mortalities at the dose tested. The test item had no adverse effect on the behavioral responses of the tested rats up to 14 days of observation. Physical observations indicated no signs of changes in the skin, fur, eyes mucous membrane and behavior patterns of the rats.

Refer Table - 1 and Appendix - 1

### 12.2. Body Weight, Food and Water intake

There were no significant treatment-related changes in body weight, percent body weight gain, food and water intake during the study period at the tested doses.

Refer Table – 2, 3, 4 and Appendix - 2, 3, 4

### 12.3. Gross pathology

There were no gross pathological changes in any of the animals sacrificed at the end of the study.

Refer Table – 5 and Appendix – 5

In general, acute toxicity test is utilized to study the harmful effects of an agent to the organism given as a single exposure [Krishnaraju *et. al.*, 2005]. Mainly, the study evaluates the mortality, changes in behavior, body weight, and other spontaneous changes in the overall well-being of the rodents. Body weight is an important factor to monitor the health of the animal. The loss of bodyweight is frequently the first indicator of the onset of an adverse effect. A dose, which causes 10% or more reduction in body weight, is considered to be toxic. It is considered to be the dose, which produces minimum toxic effect, irrespective of whether or not it is accompanied by any other changes [Feres *et. al.*, 2006; Pingale, 2008 and Lobo *et. al.*, 2010]. However in the present acute toxicity study of ***Recovereez (capsules)***, all the animals from treated groups did not show any toxicologically significant decrease in body weights for all the 14 days as compared with the first day values.

## 13. CONCLUSION

The current acute oral toxicity study of test item, ***Recovereez (capsules)***, in Wistar rats did not reveal any clinical signs, mortalities and gross pathological changes when administered once orally upto dose of 2000 mg/kg. So the $LD_{50}$ of the test item was estimated to be more than 2000 mg/kg. Hence, it can be concluded that ***Recovereez (capsules)***, is safe and non-toxic in Wistar rats up to a dose of 2000 mg/kg bwt.

## 14. SUMMARY

**Recovereez** is a Polyherbal formulation in a capsule form. Active ingredients in this formulation are Cardamom extract and Piperine. But, it does not have any scientific evidence on *in vivo* safety aspects. Hence the objective of this study was to assess the toxic potential of **Recovereez capsules**, when administered by oral gavage, in a single dose to Wistar rats. The study was conducted following the OECD Guidelines for Testing of Chemicals (No. 420, Section 4: Health Effects) on conduct of "Acute Oral Toxicity – Fixed dose procedure" (Adopted: 17th December 2001). The test item was administered as a single dose at an equivolume of 1ml/100g body weight for all animals and the actual volume of administration was calculated based on the most recent body weight of the animals. Using the normal procedure, a sighting study with starting dose of 300mg/kg followed by 2000mg/kg and dosing of a further four animals at 2000mg/kg served as main test.

Observations were made including changes in skin and fur, eyes and mucous membranes, tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma and also respiratory, autonomic and somatomotor activity and behavior pattern in the animals. The body weight of each rat was recorded prior to treatment on day 1, then weekly and at the terminal sacrifice time. Group mean bodyweights and body weight gains were calculated. The quantity of food and water consumed by rats in each cage was measured and recorded from the day of commencement of treatment till end of experiment period. On 15$^{th}$ day all animals were sacrificed under euthanasia condition and subjected to gross pathological examinations.

The acute oral toxicity study of ***Recovereez (capsules)***, in Wistar rats did not reveal any clinical signs, mortalities and gross pathological changes when administered once orally upto dose of 2000 mg/kg. So the $LD_{50}$ of the test item was estimated to be more than 2000 mg/kg. Hence, it can be concluded that ***Recovereez (capsules)***, is safe and non-toxic in Wistar rats up to a dose of 2000 mg/kg bwt.

## 15. REFERENCES

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## TABLES

TABLE 1.**SUMMARY OF CLINICAL SIGNS AND MORTALITY**

**Refer Appendix – 1**

| Dose (mg/kg) | No. of Animals | Sex | Clinical Signs | Mortality |
|---|---|---|---|---|
| 300 | 1 | F | Nil | 0/1 |
| 2000 | 1 | F | Nil | 0/1 |
| 2000 | 4 | F | Nil | 0/4 |

F= Female

TABLE 2. **SUMMARY OF BODY WEIGHT (g) AND BODY WEIGHT GAIN (%)**

**Refer Appendix – 2**

| Dose (mg/kg) | No. of Rats | Sex | Average Body weight on days | | | Body weight gain (%) | |
|---|---|---|---|---|---|---|---|
| | | | Day 1 | Day 7 | Day 14 | 1 to 7 | 1 to 14 |
| 300 | 1 | F | 190.00±0.00 | 190.00±0.00 | 195.00±0.00 | 0.00±0.00 | 2.60±0.00 |
| 2000 | 1 | F | 180.00±0.00 | 175.00±0.00 | 175.00±0.00 | -2.80±0.00 | -2.80±0.00 |
| 2000 | 4 | F | 182.50±15.00 | 178.80±16.00 | 183.80±7.50 | -2.10±1.30 | 1.00±3.38 |

F= Female, Values are expressed as mean ± SD

TABLE 3. **SUMMARY OF FOOD INTAKE (g)**

**Refer Appendix – 3**

| Dose (mg/kg) | No. of Animals | Sex | Average Food intake (g) | | |
|---|---|---|---|---|---|
| | | | 1 to 7 days | 8 to 14 days | 1 to 14 days |
| 300 | 1 | F | 12.86±2.67 | 14.29±1.89 | 13.57±2.34 |
| 2000 | 1 | F | 12.14±2.67 | 14.29±3.45 | 13.21±3.17 |
| 2000 | 4 | F | 12.32±0.68 | 13.75±1.22 | 13.04±0.68 |

F= Female, Values are expressed as mean ± SD

TABLE 4. **SUMMARY OF WATER INTAKE (ml)**

**Refer Appendix – 4**

| Dose (mg/kg) | No. of Animals | Sex | Average water intake (ml) | | |
|---|---|---|---|---|---|
| | | | 1 to 7 days | 8 to 14 days | 1 to 14 days |
| 300 | 1 | F | 24.14±4.18 | 20.86±2.27 | 22.50±3.65 |
| 2000 | 1 | F | 19.00±3.32 | 19.00±3.32 | 19.00±3.19 |
| 2000 | 4 | F | 20.54±4.24 | 18.96±1.58 | 19.75±2.35 |

F= Female, Values are expressed as mean ± SD

TABLE 5. **SUMMARY OF GROSS PATHOLOGICAL FINDINGS**

| **Dose (mg/kg)** | **No. of Animals** | **Sex** | **Gross pathology** | |
|---|---|---|---|---|
| | | | **External** | **Internal** |
| 300 | 1 | F | NAD | NAD |
| 2000 | 1 | F | NAD | NAD |
| 2000 | 4 | F | NAD | NAD |

F= Female, NAD= No abnormalities detected

## APPENDICES

APPENDIX 1. **INDIVIDUAL ANIMAL CLINICAL SIGNS AND MORTALITY RECORD**

| Animal No. | Sex | Dose (mg/kg) | Study Day 1 | | | | | Study Days | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 30-40 m | 1 hr | 2 hr | 3 hr | 4 hr | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| 1 | F | 300 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| 2 | F | 2000 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| 3 | F | 2000 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| 4 | F | | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| 5 | F | | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| 6 | F | | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |

N= Normal, F= Female

APPENDIX 2. **INDIVIDUAL ANIMAL BODY WEIGHT (g)**

| **Animal no.** | **Sex** | **Dose(mg/kg)** | **Body Weight on days (g)** | | |
|---|---|---|---|---|---|
| | | | **1** | **7** | **14** |
| 1 | F | 300 | 190 | 190 | 195 |
| 2 | F | 2000 | 180 | 175 | 175 |
| 3 | F | 2000 | 170 | 165 | 180 |
| 4 | F | 2000 | 190 | 190 | 190 |
| 5 | F | 2000 | 170 | 165 | 175 |
| 6 | F | 2000 | 200 | 195 | 190 |

F=Female

APPENDIX 3. **INDIVIDUAL ANIMAL FOOD INTAKE (g)**

| Animal No. | Dose (mg/kg) | Sex | Food intake on days (g) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| 1 | 300 | F | 10 | 15 | 10 | 15 | 15 | 10 | 15 | 15 | 15 | 15 | 15 | 15 | 10 | 15 |
| 2 | 2000 | F | 10 | 10 | 10 | 10 | 15 | 15 | 15 | 10 | 15 | 15 | 10 | 15 | 20 | 15 |
| 3 | 2000 | F | 10 | 10 | 10 | 10 | 10 | 15 | 15 | 15 | 15 | 10 | 15 | 10 | 15 | 15 |
| 4 | 2000 | F | 10 | 10 | 10 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 20 | 10 | 15 | 15 |
| 5 | 2000 | F | 15 | 10 | 15 | 10 | 10 | 10 | 15 | 15 | 10 | 15 | 15 | 20 | 15 | 10 |
| 6 | 2000 | F | 10 | 15 | 10 | 10 | 15 | 15 | 15 | 15 | 15 | 10 | 10 | 10 | 10 | 15 |

F=Female

APPENDIX 4. **INDIVIDUAL ANIMAL WATER INTAKE (ml)**

| **Animal No.** | **Dose (mg/kg)** | **Sex** | **Water intake on days (ml)** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| 1 | 300 | F | 30 | 26 | 22 | 24 | 21 | 28 | 18 | 20 | 24 | 21 | 20 | 17 | 23 | 21 |
| 2 | 2000 | F | 16 | 20 | 18 | 21 | 24 | 20 | 14 | 16 | 20 | 21 | 24 | 18 | 20 | 14 |
| 3 | 2000 | F | 14 | 16 | 20 | 18 | 16 | 14 | 20 | 18 | 20 | 19 | 16 | 14 | 18 | 20 |
| 4 | 2000 | F | 24 | 26 | 21 | 24 | 14 | 12 | 20 | 24 | 26 | 22 | 18 | 21 | 20 | 18 |
| 5 | 2000 | F | 23 | 20 | 18 | 21 | 16 | 14 | 18 | 20 | 20 | 17 | 22 | 18 | 16 | 14 |
| 6 | 2000 | F | 36 | 28 | 30 | 24 | 28 | 18 | 22 | 26 | 20 | 16 | 23 | 18 | 15 | 12 |

F=Female

APPENDIX 5. **INDIVIDUAL ANIMAL GROSS PATHOLOGICAL FINDINGS**

| **Animal No.** | **Dose** (mg/kg) | **Sex** | **Gross Pathological findings** | |
|---|---|---|---|---|
| | | | **External** | **Internal** |
| 1 | 300 | F | NAD | NAD |
| 2 | 2000 | F | NAD | NAD |
| 3 | 2000 | F | NAD | NAD |
| 4 | 2000 | F | NAD | NAD |
| 5 | 2000 | F | NAD | NAD |
| 6 | 2000 | F | NAD | NAD |

NAD= No abnormalities detected, F= Female

**Acute oral toxicity of Recovereez capsules in Wistar rats**

**Histopathological analysis**

Light microscopic examination of sections of various organs like liver, heart, brain and kidney of treated animals showed a normal histology (Figures 1-4).

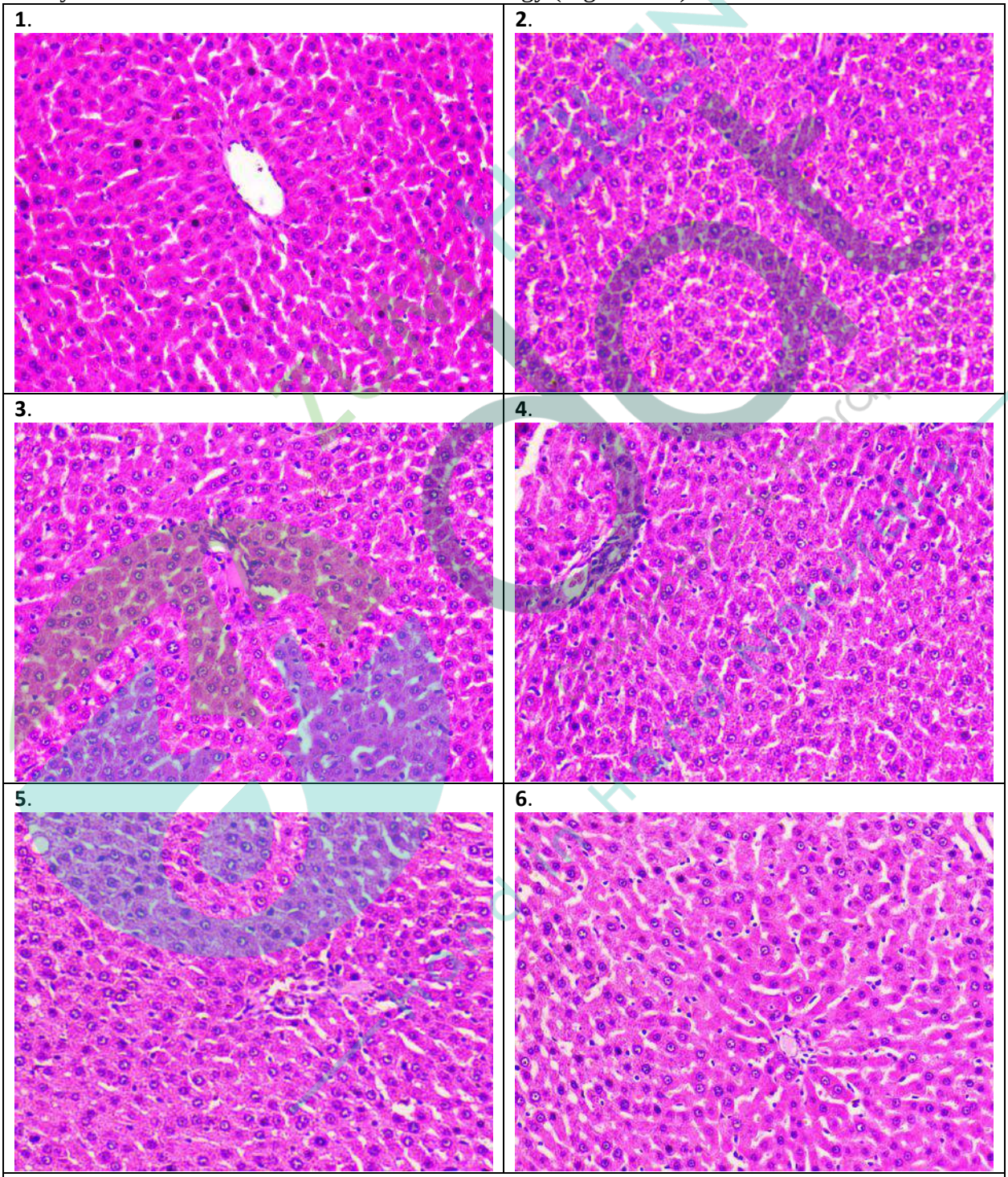



**Fig 1. Photomicrographs of Liver from rats (nos. 1-6):**Normal cytoarchitecture showing radiating appearance of hepatocytes around the central vein. Potal triads also appear normal(H&E).

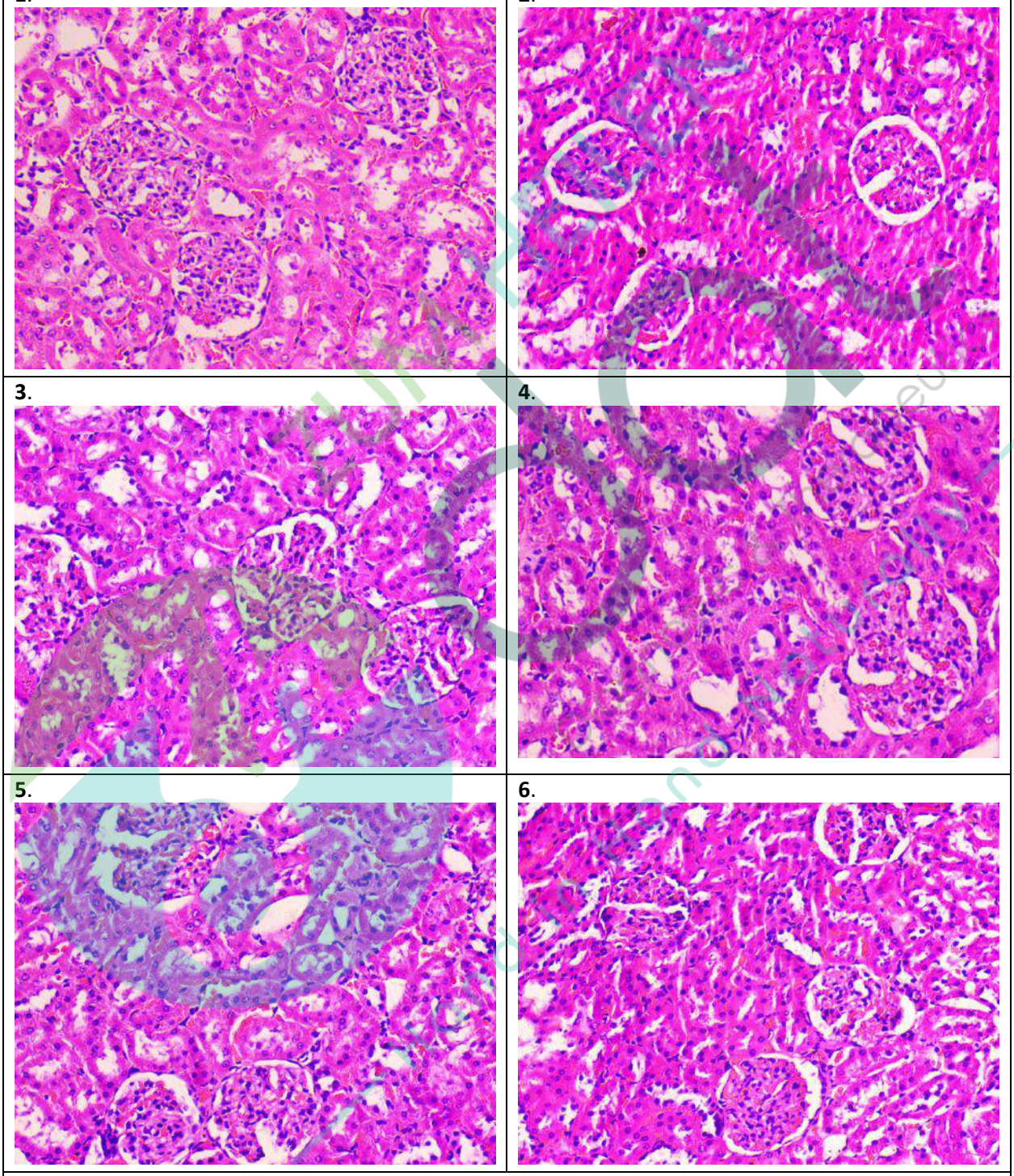



**Fig 2. Photomicrographs of Kidney from rats (nos. 1-6):**Normal appearance of glomerulus and tubules(H&E).

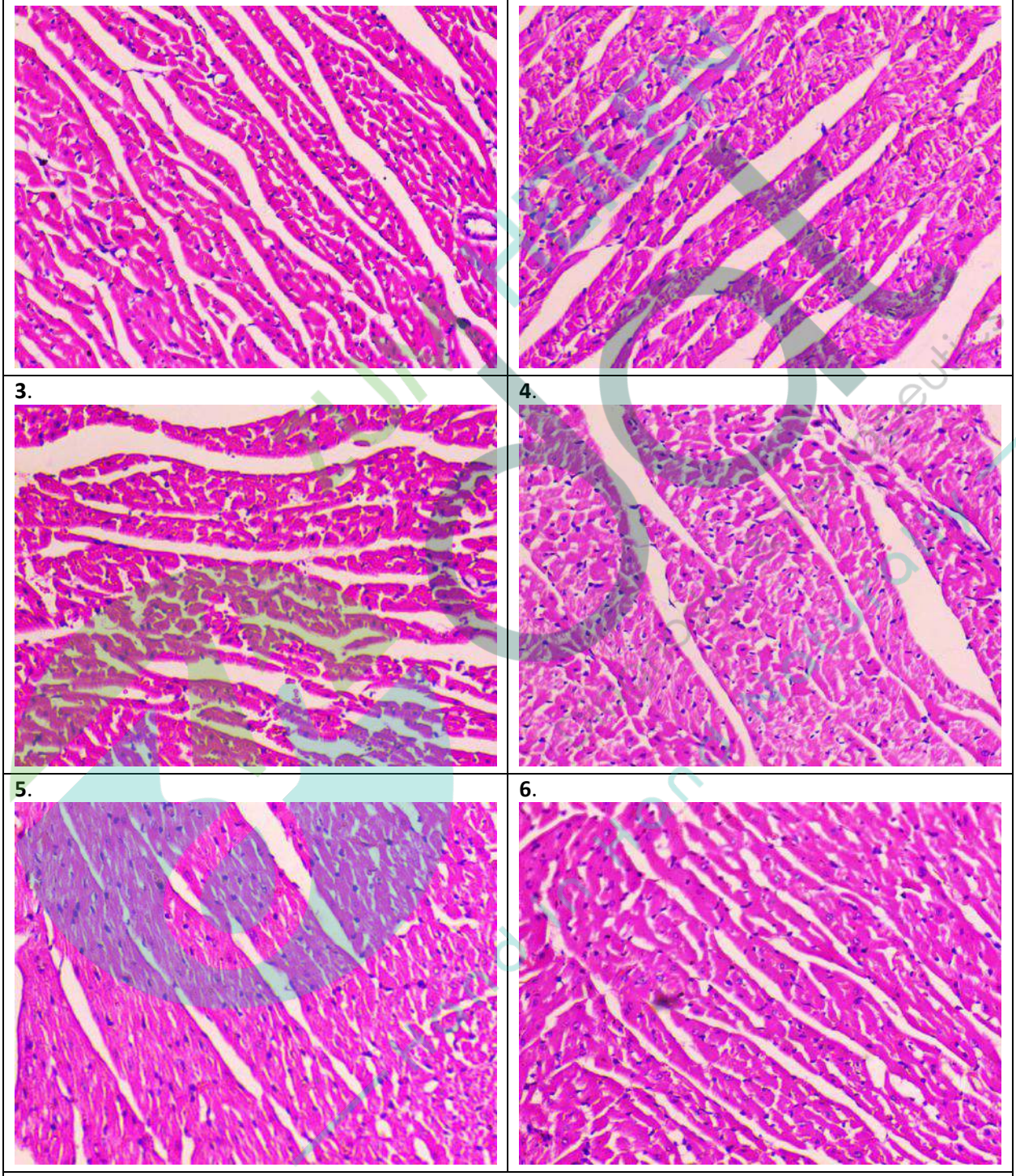



**Fig 3. Photomicrographs of Heart from rats (nos. 1-6):**Normal architecture of cardiac muscle tissue. (H&E).

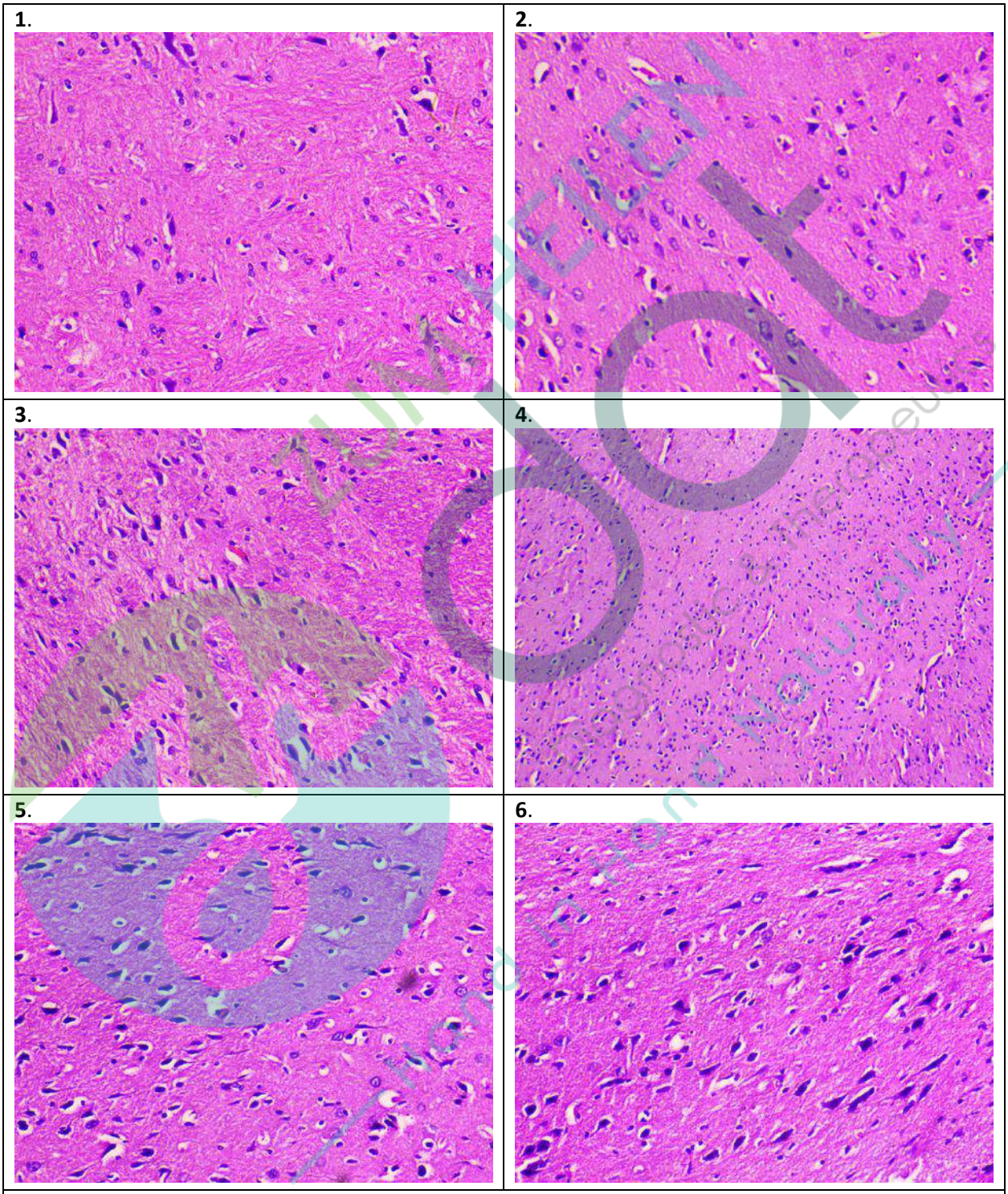



**Fig 4. Photomicrographs of Brain from rats (nos. 1-6):**Normal appearance of parts of cerebral tissues (H&E).

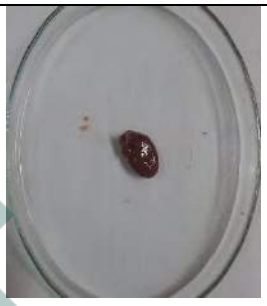
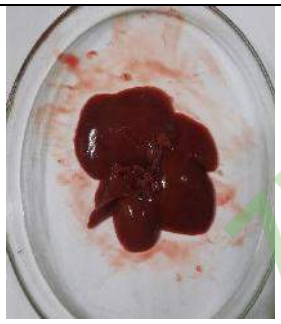
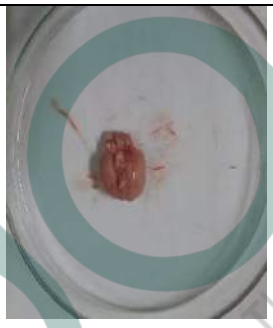
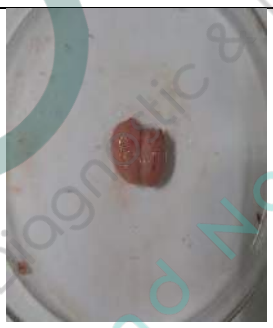
| Group & Dose | Liver | Kidney | Brain | Heart |
|---|---|---|---|---|
| Group 1 300 (mg/kg) |  |  |  |  |
| Group 2 2000 (mg/kg) |  |  |  |  |
| Group 3 2000 (mg/kg) |  |  |  |  |

**Fig 5.** Gross images of vital organs

**Summary of organ weights (g) after single exposure of Recovereez capsule**

| Animal No. | Organ weights (g) | | | |
|---|---|---|---|---|
| | Heart | Kidney | Liver | Brain |
| 1 | 0.65 | 1.65 | 7.92 | 1.52 |
| 2 | 0.65 | 1.48 | 5.94 | 1.1 |
| 3 | 0.67 | 1.59 | 6.16 | 1.34 |
| 4 | 0.74 | 1.61 | 6.84 | 1.38 |
| 5 | 0.71 | 1.61 | 6.54 | 1.26 |
| 6 | 0.64 | 1.6 | 5.89 | 1.22 |