Obesidat

Detailed Research Report

Guduchi
the ayurvedism

RADIANT RESEARCH

- **Acute Oral Toxicity Study**
- **Microbial Analysis**
- **HPTLC analysis**
- **Lipid lowering activity in high fat diet induced Obesity in C57 mice**
- **Test for Aflatoxins: B1, B2, G1, G2**

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D R A F T R E P O R T

Copy No. 1/2

Study Title

ACUTE ORAL TOXICITY

Study Director

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Test Facility

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COMPLIANCE STATEMENT

The Study Director hereby declares that the work was performed under his supervision and in accordance with the mutually agreed study plan and the in house procedures. It is assured that the reported results 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, documentation and reporting of the results.

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Study Director

Date: 31/ 08 /2016

CERTIFICATE OF AFFIRMATION AND CONFIDENTIALITY

The Management hereby attests to the originality, accuracy and authenticity of the study to the best of their knowledge. This report contains confidential and proprietary information of Guduchi" The Ayurvedism", Bangalore, which will not be disclosed to anyone without the expressed or written approval of authorized personnel.

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Management

Date: 31/08 /2016

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.

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Head, QA

Date: 31/08/2016

ABBREVIATION USED

Cell Biology : CB

Molecular Biology : MB

Microbiology : MCR

Biochemistry : BC

DTL : DTL

Preclinical : PC

Clinical : CL

1. STUDY DETAILS

1.1. Study title : ***Acute Oral Toxicity Study in rats***

1.2. Study number : RR160235

1.3. Test Substance : Obesidat Tablet

1.4. Sponsor : **Guduchi "The Ayurvedism"**

#2,3rd A Cross, 2nd Block, HRBR Layout,
Kammanahalli, Bangalore, Karnataka

1.5. Test facility : Radiant Research Services Pvt. Ltd

No: 99/A, 8th Main, 3rd Phase,

Peenya industrial area,

Bangalore-560 058

1.6. Test schedule

Study Initiation Date	:	17/08/2016
Experimental Start Date	:	17/08/2016
Experimental Completion Date	:	30/08/2016
Study Completion Date	:	31/08/2016

1.7. Study Responsibilities

Study Director	:	Dr. Ashok Godavarthi
Study Co-ordinator	:	Mr. Atul Chandra Jha
Study Scientist	:	Mr. Dinesh. Mr. Gopal

2. OBJECTIVE

The objective of this study is to assess the toxicity of the test compound.

3. MATERIALS AND METHODS

Details of the materials and methods that are not specified in the subsequent sections of this study plan are contained in the appropriate Radiant Research standard operating procedures.

4. TYPE OF STUDY

The test was carried out with the aid of "Limit test", according to the requirements of OECD guideline "Guidelines for testing of chemicals 423".

5. TEST SYSTEM

Test system	:	Rat, Wistar
Justification	:	Recommended by the guideline.
Source	:	In-house breaded animals
Total number of animals	:	06
Age when treated	:	8 – 9 weeks
Body weight when treated	:	Females: 180.0 to 200.0 grams
Identification	:	By Unique cage number and individual animal number markeds with indelible marker pen on the tail. The animals were marked (towards the tip of tail) with the temporary animal numbers at start of acclimatization. The animals were

	marked with permanent animal numbers (towards the base of tail) with different color indelible marker pen before the start of test item administration.
Randomization	Animals were selected and grouped manually. No computer generated randomization program was used.
Acclimatization	Under laboratory conditions for 10 days after veterinary examination. Only animals without any visible signs of illness were used for the study
Safety precautions	Routine hygienic procedures: protective gloves face mask, aprons/protective suit and goggles will be used to ensure the health and safety of the personnel

6. ALLOCATION:

GROUPS / STEPS	NO. OF ANIMALS	ANIMAL NUMBER
1	3	RA001 - RA003
2	3	RA004 - RA006

7. HUSBANDRY

Total 6 female wistar rats were used in present study. Weight of the animals was in the range of 180 to 200 gram. After veterinary examination, only animals without any visible signs of illness were used for the study. The animals were housed in groups of three in polycarbonate cages with paddy husk bedding and given a unique number on cage within the study and individual animal numbers were marked with different indelible marker pen towards the base of tail. The diet and drinking water were free from any contaminant, which might affect the

purpose or integrity of the study. The temperature and humidity were set to achieve limits of 18-23^{0}C and 40-70%, respectively with 12 hr light and dark cycle.

8. TEST PRODUCT

Reference Number	: RR160235
Test Product	: Obesidat Tablet
Manufacture date	: JUN-2016
Expiry date	: JUN-2019
Batch/lot number	: G002
Description	: Brown coloured round shaped tablets
Purity	: -
Stability of test item dilution	: -
Storage conditions	: RT
Safety precautions	: Routine hygienic procedures: protective gloves face mask, aprons/protective suit and goggles were used to ensure the health and safety of the personnel.

Note: The identity and composition of the item is the responsibility of the sponsor. No analysis was performed at Radiant Research Services Pvt Ltd to confirm it.

9. EXPERIMENTAL CONDITIONS

Mode of application	: Per oral, via gastric tube
Frequency of treatment	: Single dose
Dosage level	: 2000mg/kg
Post treatment examination period	: 14 days

10. REASON FOR DOSE SELECTION

As to the information of sponsor the substance was of natural origin and non-toxic, therefore we applied a test dose of 2000 mg/kg, as per OECD guideline "Acute oral toxicity" 423.

10.1. BRIEF PREPARATION OF DOSING SOLUTION

A known amount of test substance added to a mortar slowly and small quantity of vehicle (quantity sufficient to make a uniform suspension) was added with continuous triturating. Then sufficient volume of vehicle was added to make up the appropriate dose concentration and stirred for 15 – 20 mins to get uniform suspension.

11. TREATMENT

The animals received a single dose of the test item by oral administration at 2000 mg/kg body weight after being fasted for approximately 18.0 hours but with free access to water. Food was provided again at approximately 3.0 hours after dosing for all the Steps. The administration volume was 10 ml / Kg body weight.

Justification: oral administration was considered as recommended by the guideline.

12. OBSERVATIONS

Mortality / Viability : Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 4 hours after administration on test day 0 (in common with the clinical signs) and twice daily during days 1-14 (at least once on day of sacrifice).

Body weights : On test days 0 (prior to administration), day 7, day 14.

Clinical signs : Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 4 hours after administration on test day 0. Once daily during days 1-14.

13. STATISTICAL ANALYSIS

Column statistics were applied for the data analysis.

14. DATA COMPILATION

Body weight, clinical signs, mortality/viability, and macroscopic findings were recorded.

15. RESULTS

15.1. MORTALITY

No mortalities were observed in the animals (2000 mg/kg body weight).

15.2. CLINICAL SIGNS AND BEHAVIORAL OBSERVATION

All the animals appeared normal throughout the experimental period (Refer Table 2).

Behavioral changes were observed carefully after the dose administration. There were not any abnormal signs observed and even throughout the study in all the animals.

15.3. BODY WEIGHT

All surviving animals had gained body weight by 7^{th} day as compared to day 0 (Refer Table 3, Figure 1) Increased body weight in animals during the study was observed in all the animals and it is a normal pattern with healthy animals.

15.4. MACROSCOPIC FINDINGS

No abnormalities were detected for any of the animals of terminal sacrifice (Refer Table 4).

15.5. PATHOLOGY

All surviving animals were sacrificed at the end of the observation period and discarded after the gross/macroscopic pathological changes were observed and recorded (Refer Figure 2).

No organs or tissues were retained.

16. EVALUATION OF RESULTS

The test group at a single oral dose of 2000 mg/kg did not cause death or clinical symptoms in rats observed over a period of 14 days. The median lethal dose (LD_{50}) of test substance is more than 2000mg/kg body weight.

17. CONCLUSION

The LD_{50} value of test substance in female rats after single oral treatment is above 2000 mg/kg body weight and is classified as Category 5.

NOTE: According to the OECD guideline 423, if the test compound could not be toxic in the level 2000 mg/kg of body weight in an animal, such level classified as **category 5**.

Table 1: CLINICAL AND BEHAVIORAL SIGNS/SYMPTOMS

CODES	OBSERVATIONS	SIGNS/SYMPTOMS
01	NAD	02-40 codes observations are not seen
02	Accidental death	
03	Partial Cannibalism	An animal of a species consuming part of another animal of the same species
04	Total Cannibalism	An animal of a species consuming the major organs of another animal of the same species
05	Dead	Irreversible cessation of all body functions, manifested by absence of spontaneous breathing and total loss of cardiovascular and cerebral functions
06	Moribund condition	Approaching death animal will not be available for examination for next day
07	Weakness	A weak bodily state as expressed by difficulty in rising, a shuffling, disinclination to move, eating slowly and a drooping posture
08	Lethargy	A level of consciousness characterized by decreased interaction with objects in the environment, sluggishness, abnormal drowsiness
09	Salivation	Flow of saliva, Drooling(Abnormally abundant flow of saliva)
10	Lacrimation	Flow of tears
11	Discharge	Abnormal discharge
12	Snuffling(Unusual respiratory pattern)	A bubbling sound from the nasal cavities
13	Bronchial rales	An abnormal respiratory sound (crackles) in auscultation of lungs
14	Cough	A forceful release of air from the lungs
15	Dyspnea (Unusual respiratory pattern)	Shortness of breath
16	Corneal opacity	Opaque white spot on the cornea
17	Cataract	Opacity of the crystalline lens of the eye
18	Diarrhea	Diarrhea is the frequent passage of loose, watery, soft stools.
19	Hematuria	Presence of blood in the urine
20	Piloerection	Erection of hair

21	Response to handling	Normal response to approach.
22	Convulsions	Violent involuntary contraction of a muscle or muscles
23	Repetitive Circling	Continuous circling
24	Head tilted on one side	Head facing towards some other direction other than straight
25	Ataxia	Inability to control voluntary muscle movement
26	Dermatitis	Inflammation of the skin
27	Blister	A local swelling of the skin that contains watery fluid
28	Urticaria	An itchy skin eruption characterized by weal's with pale interiors and well-defined red margins
29	Necrosis	Death of a portion of tissue differentially affected by local injury
30	Erythema	Redness of the skin
31	Oedema	A swelling from effusion of watery fluid in the cellular tissue beneath the skin or mucous membrane
32	Cyanosis	Bluish discoloration of the skin and mucous membranes
33	Paralysis	Loss of sensation over a region of the body.
34	Edema	An excessive accumulation of serous fluid in tissue spaces or a body cavity.
35	Crepitation	A dry, crackling sound or sensation
36	Dehydration	Loss of water and salts. The skin turns pale and cold, the mucous membranes lining lose their natural moisture
37	Dull	Lacking responsiveness or alertness
38	Posture	Position of the body or of body parts
39	Epistaxis	Bleeding from the nose
40	Urine dribbling	Leaking of urine

Table 2: Clinical signs and Behavioral observation during the study in rats

OBSERVATION															
Animal Id	***After Treatment****	**Days of post treatment examination**													
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
RA 001	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01
RA 002	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01
RA 003	01	01	01	01	01	01	01	01	01	01	01	01	01	01	01

*** Observation of first four hours after treatment**
01 – NAD (02-40 codes observations are not seen)

Table 3: Body weight of Rats during the study

Animal ID	**Dose**	**Treatment**		
		Before	**After**	
		Day 0	**Day 7**	**Day 14**
RA 001	2000 mg/kg	180	185	191
RA 002		185	189	191
RA 003		190	194	198

Table 4: The result of pathological examinations

Animal ID No	**Dose**	**Macroscopic lesions**
RA 001	2000 mg/kg	No macroscopic alteration occurred
RA 002		No macroscopic alteration occurred
RA 003		No macroscopic alteration occurred

Figure 1: Body weight of animals from Day 0 to Day 14

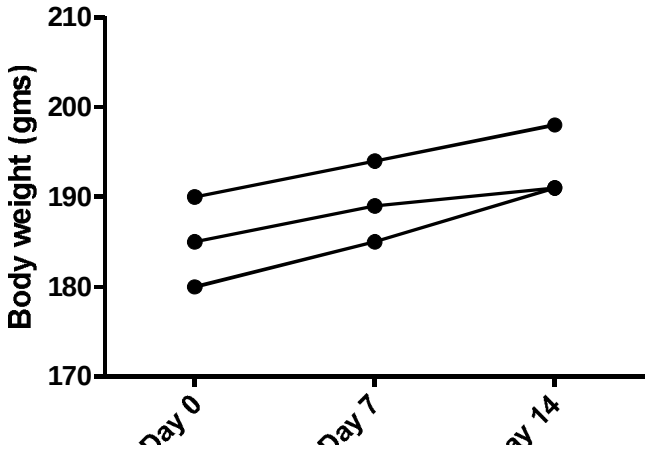

Figure 2: Gross pathological examination of treated animals (Macroscopic observation)

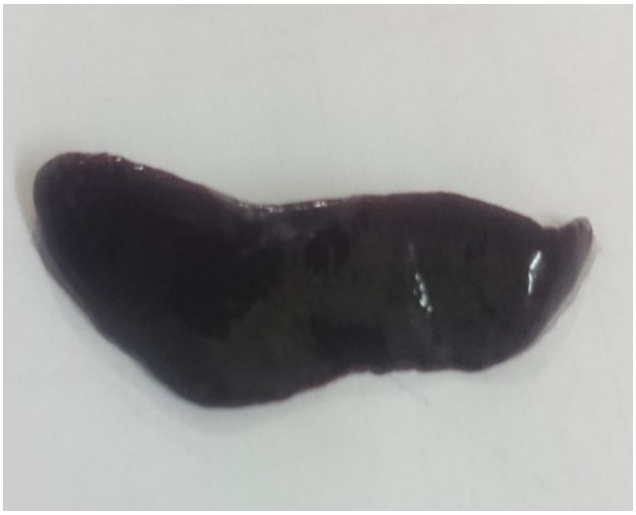

Figure-2a: Spleen

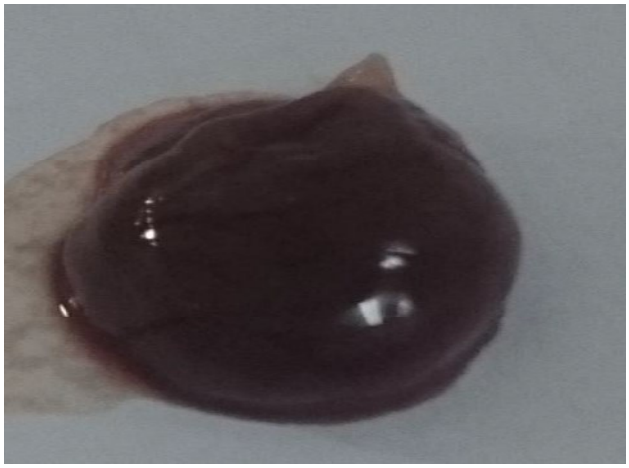

Figure-2b: Heart

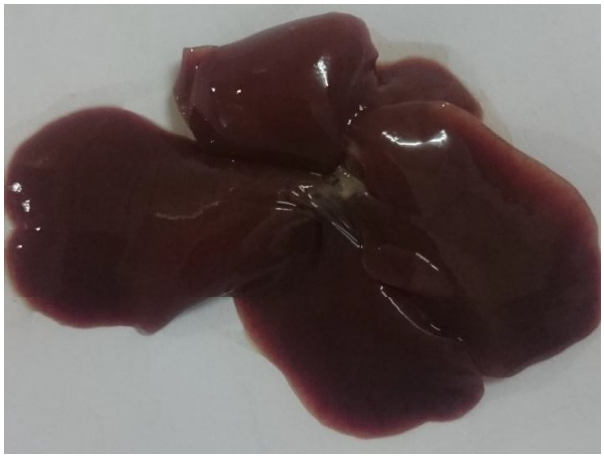

Figure-2c: Liver

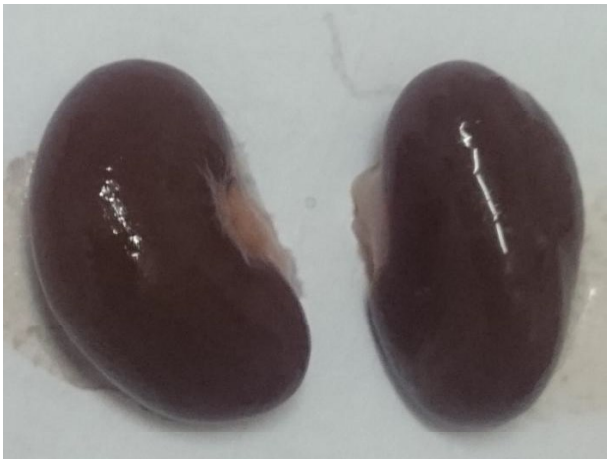

Figure-2d: Kidney

----------END OF REPORT----------

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