



**MEERUT INSTITUTE OF ENGINEERING & TECHNOLOGY
(MIET Meerut)**

CPCSEA Registration Number: 711/PO/Re/S/02/CPCSEA
(Ministry of Environment, & Forest, Government of India)

ACUTE SYSTEMIC TOXICITY OF MADHUMEH_FORMULA1

(Batch No. 10201) (ISO 10993-11, Tests for Systemic Toxicity)

SPONSOR

JEENA SIKHO LIFECARE LIMITED and MITTAL AYURVED SANSTHAN

CLINICAL RESEARCH ORGANIZATION

MITTAL GLOBAL CLINICAL TRIAL SERVICES (MGCTS)

DATA REQUIREMENTS

ISO GUIDELINE 10993 PART 11 BIOLOGICAL EVALUATION OF MEDICAL DEVICES –
TEST FOR SYSTEMIC TOXICITY

TEST LABORATORY

PHARMACOLOGY DEPARTMENT, DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY, MIET
Meerut

NH-58, Delhi-Roorkee Highway, Baghpat Bypass Road Crossing, Meerut, U.P. – 20005

PROJECT NO	: 2023-09-086
REPORT NO.	: MIET/DPT/2023-19/086/AST
DATE	: 17-10-2023

Test Compound : MADHUMEH_FORMULA1
SPONSOR : Jeena Sikho Lifecare Limited and Mittal Ayurved Sansthan
CRO : Mittal Global Clinical Trial Services (MGCTS)
STUDY : ACUTE SYSTEMIC TOXICITY
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1. STATEMENT OF COMPLIANCE

I, the undersigned hereby declare that Project No. 2023-09-086/ Report No. MIET/DPT/2023-09/086/AST; entitled “**Acute Systemic Toxicity of MADHUMEH_FORMULA1**” was performed in accordance to the standard procedure of Pharmacology Department, Department of Pharmaceutical Technology, MIET Meerut, UP, as well as the approved study plan.

I hereby attest the authenticity of the study and guarantee that this report is a true and accurate record of the results obtained and shall not be reproduced except in full, without the written approval of the Sponsor.

The study was conducted in compliance with the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 11 Tests for Systemic Toxicity and OECD guidelines 423 – Acute Toxic Class Method. This study was conducted in accordance to the Good Laboratory Practices (GLP).

All original raw data including documentation, the draft report, a copy of the final report and the representative test sample are archived at the Pharmacology Department, Department of Pharmaceutical Technology, MIET Meerut, UP. There were no circumstances that may have affected the quality and integrity of the study.

The sponsor of the study is responsible for the necessary evaluation of the test sample concerning the chemicals, purity, identity, stability and other required data.



Principal Investigator

Dr. Vipin Kumar Garg (M.Pharm, PhD)

17-10-2023

Date

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2. STATEMENT BY TEST FACILITY MANAGEMENT

Management of the test facility has made available all the resources to the Principle Investigator necessary for conduct of the present study in compliance with the principles of GLP.

I, the undersigned, take overall responsibility for the reliability of the work described in the report with accordance to GLP.



Laboratory In-charge
Dr. Vipin Kumar Garg (M.Pharm, PhD)

17-10-2023
Date

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3. QUALITY ASSURANCE REPORT

This **Project No. 2023-09-086, Report No. MIET/DPT/2023-09/086/AST** entitled "**Acute Systemic Toxicity of MADHUMEH_FORMULA1** (ISO Guideline 10993 – 11) was subjected to inspection by Quality Assurance Unit.

This report had been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed. In each case, the outcome of the QA evaluation is reported to the Principle Investigator and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

Standard Test Method Compliance Audit	: 24-09-2023
Animal Preparation	: 25-09-2023
Test Material Preparation	: 30-09-2023
Application of test compound	: 01-10-2023
Assessment of Response	: 14 Days (01-10-2023 to 14-10-2023)
Draft Report Audit	: 16-10-2023
Final Report Date	: 17-10-2023



QUALITY ASSURANCE OFFICER
Ms. Garima Agarwal (M.Pharm)

17-10-2023
Date

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4. STUDY PERSONNEL

Principle Investigator : Dr. Vipin Kumar Garg (M.Pharm, PhD)
Co – Investigators : Mr. Ankit Chaudhary (M.Pharm, Pharmacology)
Veterinarian : Dr. Sonia Sharma
Study Managers : Ms. Garima Agarwal (M.Pharm)
: Mr. Ankit Chaudhary (M.Pharm)

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5. SUMMARY

The study was conducted in accordance to the OECD Guideline for the testing chemical No. 423 to evaluate the acute oral toxicity of MADHUMEH_FORMULA1.

The test sample was dissolved in the purified water, and four pre specified fixed-doses (5, 50, 300, and 2000 mg/kg body weight) was orally administered in female Wistar rats (8-12 weeks) by oral gavage. The body weight of animals was measured at day 0 (before the drug administration) and on days 1, 7, and 14 of the study. The rate of mortality and general behavior of the animals were observed continuously for the initial 1, 4, and 24 h after the drug administration and then daily for 14 days. Cage side observations included variations in the skin and fur, eyes, and mucous membranes. Particular attention was directed to observations of tremor, convulsions, salivation, diarrhea, lethargy, sleep, and coma. Also, respiratory, circulatory, autonomic, and central nervous systems and somatomotor activity were examined.

In the study, no mortality was observed up to 14 days. No toxic symptoms were found in rats in all four doses. No abnormalities were been observed in general clinical observations or gross necropsy. The rats were found to behave normally with no variation in locomotor, behavioral, neurological, or secretory patterns. No significant changes were observed in the body weight of rats in all four treated doses.

Considering the data obtained from the study, the MADHUMEH_FORMULA1 is not found to be lethal up to a dose of 2000 mg/kg.

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6. INTRODUCTION

A. Purpose of the Study:

The test article identified below was extracted and evaluated to determine if the test substance provided would cause acute systemic toxicity following oral administration to rats.

B. Testing Guidelines:

The study was conducted based on the International Organization for Standardization (ISO) 10993, Biological Evaluation of Medical Devices, Part 11- Test for Systemic Toxicity, OECD guidelines 423-Acute Toxic Class Method and ISO 10993, Biological evaluation of Medical Devices, Part 12, Sample Preparation and Reference Materials (2012).

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7. MATERIALS

Sample Details

The test article provided by the sponsor was identified and handled as follows:

Test Compound	: MADHUMEH_FORMULA1
Batch No	: NA
Mfg Date	: NA
Physical Appearance	: dark color Tablet
Expiry Date	: NA
Storage Condition	: Room Temperature
Control Article	: NA
Control Article Stability Testing	: NA

Sample Preparation:

The sample was dissolved in the purified water just before its administration in rats.

Test System

Species	: Wistar rats
Source	: Lala Lajpat Rai University
Strain	: Albino
Sex	: Female
Body Weight Range	: 180-250 gm
Acclimatization	: Minimum 5 days
No. of Animals	: 12 Rats

Identification Method: Marked with permanent marker on the tail

Animal Management

Husbandary:

The conditions conformed to MIET Standard Operating System that are based on “*Guide for the Care and Use of Experimental Animals*”

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Food:

A commercially available Rat feed was provided daily

Water:

Portable water was provided *ad libitum* through species appropriate water container or delivered through an automatic watering system.

Environmental conditions:

Air conditioned rooms with 10-15 air changes per hour, Temperature between $22 \pm 3^{\circ}\text{C}$, relative humidity 40 – 60% and illumination cycle set to 12 hours artificial fluorescent light and 12 hours dark.

Selection:

Only healthy previously unused animals were selected.

Personnel:

Associates involved were appropriately qualified and well trained.

Veterinary Care:

Standard veterinary medical care was provided in the study.

IAEC:

This procedure has been approved by MIET's Institutional Animal Ethical Committee and is reviewed at least half-yearly by the same committee.

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8. METHODS

Preparation of Animals:

Female rats were randomly selected, weighed, and marked individually for the identification. A total of four groups were formed containing three rats in each group. The animals were kept in the cages for five days prior to dosing to allow for acclimatization to the laboratory conditions.

Preparation of test drug:

Test drug was dissolved in the purified water just prior to the dose administration.

Drug administration:

Animals were kept on overnight fasting before the drug administration and three to four hours post drug administration. During the time, the animals had free access to the water. Prior to the drug administration, the animals were weighed. The drug in fixed-dose (5, 50, 300, and 2000 mg/kg body weight) was administered through oral gavage in the respective groups.

Mortality observation:

The numbers of animal deaths were observed at 1, 4, 24 h, and upto 14 days after drug administration.

General Clinical observation:

Post drug administration, the animals were continuously observed for the muscle activity (Locomotion, muscle coordination, catatonia, tremor and convulsive episode), reflex activity (Visual place response, writhing response, Tail pinch response, piloerection), and secretory activity (Lacrimation, salivation, sniffing and defecation). Also, respiratory and heart rate were examined.

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Body weight Measurement:

The body weight was measured on day 0 (prior to drug administration) and day 1, 7, and 14 of the drug administration using electric balance.

Gross Necropsy:

At the end of the observation period the survived animals were sacrificed by an over dosage of pentobarbital and were subjected to gross necropsy. All gross pathological changes were recorded.

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9. Acceptance Criteria and Statistical Analysis

According to the OECD Guidelines 423, the number of animal mortalities and evident toxicity, the hazard class of acute oral toxicity was classified to the category of Globally Harmonized Classification System.

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10. RESULTS & DISCUSSION

Mortality :

No mortality was observed in any animals until 14 days post drug administration.

Clinical signs:

No abnormalities were observed in any animals until 14 days after the drug administration.

Body Weight:

No significant variation has been observed. Weight gain or weight loss in the study cannot be considered a sign of evident toxicity as no abnormalities were observed in clinical and gross necropsy findings.

Macroscopic Findings:

No abnormalities were observed in any animals at 5, 50, 300, and 2000 mg/kg dose.

Table:

Dose (mg/kg)	Animals No.	Mortality Observed	Initial Body weight	Body weight post drug administration			Macroscopic Findings (Abnormalities detected)
			Day 0	Day 1	Day 7	Day 14	
5 mg/kg	1	No	196	196	199	201	None
	2	No	201	202	204	205	None
	3	No	187	188	191	194	None
50 mg/kg	1	No	224	224	226	230	None
	2	No	201	201	205	208	None
	3	No	197	197	200	204	None
300 mg/kg	1	No	194	192	195	193	None
	2	No	189	187	189	192	None
	3	No	219	220	222	226	None
2000 mg/kg	1	No	204	204	206	207	None
	2	No	199	200	202	211	None
	3	No	223	223	221	228	None

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11. ARCHIVE

On completion of the study, the raw data and other material, sample of the test substance and the study report are being retained for 09 years at the Pharmacology Department, Department of Pharmaceutical Technology, MIET Meerut, UP.

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12. CERTIFICATE

This is to certify that the “**Acute Systemic Toxicity of MADHUMEH_FORMULA1**” sponsored by **Jeena Seekho Lifecare Limited and Mittal Ayurved Sansthan** and the testing material for the study was provided by Clinical Research Organization: **MITTAL GLOBAL CLINICAL TRIAL SERVICES (MGCTS)** was performed according to the International Organization for Standardization 10993: Biological Evaluation of Medical Devices Part 11: Tests for Systemic Toxicity. **The test sample was found to be non-lethal up to dose 2000 mg/kg.**

Note: Results and conclusions apply only to the test article tested. Any extrapolation of these data to other samples is the sponsor's responsibility.



Principal Investigator
Dr. Vipin Kumar Garg (M.Pharm, PhD)

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13. ANNEXURE – I: REFERENCES

- International Organization for Standardization (ISO) 10993, Biological evaluation of Medical Devices Part -2, Animal Welfare Requirements (2006)
- International Organization for Standardization (ISO) 10993, Biological evaluation of Medical Devices Part -12, Sample Preparation and Reference Materials (2012)
- International Organization for Standardization (ISO) 10993, Biological evaluation of Medical Devices Part – 11, Test for Systemic Toxicity (2017)
- OECD Guidelines 423