STUDY REPORT

Copy No. 1/2

Study Title

Evaluation of the test product for *in vitro* cytotoxicity activity on Human Dermal Fibroblast (HDF) cell line

Study Director

Dr. Ashok Godavarthi

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.

Date: 17/11/2023

Study Director
Dr. Ashok G

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 GERA-HAYAMI LLP which will not be disclosed to anyone without the expressed or written approval of authorized personnel.

Date: 17/11/2023

Management
Dr. Ashok G
C.E.O

DECLARATION

The Study No: RR231626/CB/CT/11-23, entitled "**Evaluation of test product for *in vitro* cytotoxicity activity on Human Dermal Fibroblast (HDF) cell line**" has been inspected regularly according to the Standard Operating Procedure of the test facility's Quality Assurance Unit. The report was audited against approved study plan and pertinent raw data and accurately reflects the raw data.

Date: 17/11/2023

QA Head
Gopi Mareedu

ABBREVIATIONS USED

CT : Cytotoxicity

CB : Cell Biology

FBS : Fetal Bovine Serum

CTC_{50} : Cytotoxicity Concentration

mg : Milli gram

mL : Milli litre

nm : Nano meter

µL : Micro litre

µg : Micro gram

EDTA : Ethylene diamine tetra acetic acid

MTT : 3-(4,5-Dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide

DMSO : Dimethyl Sulfoxide

DPBS : Dulbecco's Phosphate Buffered Saline

RT : Room Temperature

IU : International Unit

TPVG : Trypsin Phosphate Versene Glucose Solution

DMEM: Dulbecco's Modified Eagle Medium

LIST OF TABLE AND FIGURE

1. STUDY DETAILS

1.1.	Study title	: Evaluation of the test product for *in vitro* cytotoxicity activity on Human Dermal Fibroblast (HDF) cell line
1.2.	Study number	: RR231626/CB/CT/11-23
1.3.	Test Product	: Dish wash
1.4.	Sponsor details	: GERA-HAYAMI LLP
1.5.	Test Facility	: Radiant Research Services Pvt. Ltd. No.: 99/A, 8th Main, 3rd Phase, Peenya industrial area, Bengaluru-560 058

1.6. Test Schedule

Study Initiation Date	: 11/10/2023
Experimental Start Date	: 07/11/2023
Experimental Completion Date	: 17/11/2023
Study Completion Date	: 17/11/2023

1.7. Study Responsibilities

Study Director	: Dr. Ashok Godavarthi
Study Co-Ordinator	: Dr. Sahina. S

2. OBJECTIVE

The purpose of this study is to assess the test product for their *in vitro* cytotoxicity activity by MTT assay on Human Dermal Fibroblast (HDF) cell line.

3. AMENDMENT AND DEVIATION PROCEDURE

No deviation has been observed during the conduct of the experiment.

4. MATERIALS

4.1 Test Product information

Sample Code	Sample name	Batch number	Physical appearance	Storage condition
RR231626	Dish wash	-	Liquid	RT

4.2 Reference Material/Chemicals

Chemicals	Batch / Lot No.	Manufacturer	Expiry Date
MTT	0000454015	HiMedia, India	Oct-2024
DMEM-HG	2365585	Gibco, USA	Feb-2024
Fetal Bovine serum	2422662	Gibco, Brazil	Sep-2026
DPBS	0000474192	HiMedia, India	Mar-2024
Trypsin - EDTA	0000564136	HiMedia, India	Nov-2024
Antibiotics	0000493509	HiMedia, India	Apr-2024
DMSO	J19A/0416/0305/13	SDFCL, India	Sep-2024

4.3 Equipments

S. No.	Name of the Instrument	Make	Instrument ID
1.	Biosafety Cabinet	Ascension, India	RRS/INS/CB/01
2.	CO_2 Incubator	NuAire, USA	RRS/INS/CB/02
3.	Inverted tissue culture microscope	Nikon, Japan	RRS/INS/CB/08
4.	Automated micro plate reader	Biotek, USA	RRS/INS/MB/05

5. REFERENCE

Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH, Currens MJ, Seniff D, Boyd MR. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res. 1988 Sep 1;48 (17):4827-33.

6. METHOD

6.1 Outline of the method

The *in vitro* MTT assay was performed for the test product on Human Dermal Fibroblast (HDF) cell line to determine the level of cytotoxicity.

6.2 Preparation of test product for cytotoxicity screening

10 mg of test product was weighed separately and dissolved in DMEM-HG medium supplemented with 2% inactivated FBS to obtain a stock solution of 10 mg/mL. Furthermore, a serial two-fold dilution was prepared from the stock solution to prepare lower concentrations for cytotoxicity testing.

6.3 Cell line and culture medium

Human Dermal Fibroblast (HDF) cell line was procured from AddexBio, USA. Stock cells were cultured in DMEM-HG supplemented with 10% inactivated Fetal Bovine Serum (FBS),

penicillin (100 IU/mL), streptomycin (100 µg/mL) and amphotericin B (5 µg/mL) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% Trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and cytotoxicity studies were carried out in 96-well micro titre plate (Tarsons India Pvt. Ltd., Kolkata, India).

6.4 Determination of cell cytotoxicity by MTT Assay

The cell culture monolayer was trypsinized and the cell count was adjusted to 100,000 cells/mL using DMEM-HG containing 10% FBS. To each well of the 96-well micro titre plate, 0.1 mL of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with DPBS and different test concentrations were added in the micro titre plates. The untreated cells were maintained as cell control for comparison. The plate was then incubated at 37°C for 24 h in 5% CO_2 atmosphere. After 24 h, microscopic examination was carried out and observations were noted, the test solutions in the wells were discarded and 100µL of MTT diluted with DPBS was added to each well. The plate was gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100µL of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a micro plate reader at a wavelength of 570 nm.

7. RESULTS

Table 1: *In vitro* cytotoxicity of test product in terms of percentage cell viability against Human Dermal Fibroblast (HDF) cell line by MTT assay

Test Product	Concentration (µg/mL)	Percentage of cell viability after treatment
Dish wash	1000	51.11 ± 0.63
	500	84.38 ± 0.24
	250	92.74 ± 3.51
	125	95.14 ± 1.87
	62.5	96.59 ± 0.72
	31.25	97.88 ± 0.77
	15.625	98.56 ± 0.38
	7.8	99.09 ± 0.43

8. DISCUSSION AND CONCLUSION

Test product- Dish wash was assayed for *in vitro* cytotoxicity study against Human Dermal Fibroblast (HDF) cell line by exposing the cells to different concentrations ranging from 1000µg/mL to 7.8µg/mL. MTT assay was employed to test the cytotoxic effect of selected concentrations of given test product on Human Dermal Fibroblast (HDF) cell line by measuring the metabolic activity through a colorimetric determination. The MTT assay is usually carried out to detect the cells with constant mitochondrial activity, thereby an increase or decrease in the number of viable cells is linearly related to mitochondrial activity.

In the present study, the cytotoxicity of test product Dish wash was determined in terms of percentage cell viability and it was found to be 51.11 ± 0.63% at 1000µg/mL concentration on Human Dermal Fibroblast (HDF) cell line.

Conclusion: The result concluded that the test product **Dish wash** is considered to be **safe and non-toxic** in Human Dermal cells at below 1000µg/mL concentration.

9. ARCHIVING

- Test product will be stored for 3 months after the final report submission.
- Raw data, documents, report will be archived for 3 years.

10. REPORT DISTRIBUTION

- Sponsor : One signed final report (Copy no. 1/2) in original.
- Archives : One signed final report (Copy no. 2/2) in original along with raw data file.

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