Institute for Industrial Research & Toxicology
औद्योगिक अनुसंधान एवं विष विज्ञान संस्थान
Registration No. 1303/C/09/CPCSEA (Ministry of Environment & Forests, Government of India)
GLP Certified, NABL (ISO/IEC 17025) Accredited and FDA Approved (Drug & Cosmetics)
AN ISO 9001 : 2015, ISO 14001 : 2015, ISO 45001 : 2018 Certified Organization

CYTOTOXICITY OF "GEL - ARTICLE NO - 10252203174" BY MTTTETRAZOLIUM ASSAY METHOD

(ISO 10993-5, Test for In-vitro Cytotoxicity)

SPONSORED BY

NUTRIN GMBH & GALAXA PHARMA APS

CRO

MITTAL GLOBAL CLINICAL TRIAL SERVICES (MGCTS)

DATA REQUIREMENTS

ISO GUIDELINE 10993 PART 5 BIOLOGICAL EVALUATION OF MEDICAL DEVICES- TEST FOR IN VITRO CYTOTOXICITY

TESTING LABORATORY

INSTITUTE FOR INDUSTRIAL RESEARCH & TOXICOLOGY
F-209, PHASE -1, UPSIDC, MG ROAD, GHAZIABAD-201302

PROJECT NO. : **202010-052**

REPORT NO : **IIRT/MD/202010/516/CYT**

ULR NO : **TC661219000000516P**

DATE : **22-08-2020**

TEST COMPOUND : GEL - ARTICLE NO - 10252203174"
SPONSORED BY : NUTRIN GMBH & GALAXA PHARMA APS
CRO : MITTAL GLOBAL CLINICAL TRIAL SERVICES (MGCTS)
STUDY : IN VITRO CYTOTOXICITY
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1. GLP STATEMENT OF COMPLIANCE

I, undersigned hereby declare that Project No -202010-052/Report No IIRT/MD/202010/516/CYT; entitled *In-vitro* Cytotoxicity of **"GEL " ARTICLE NO - 10252203174"** was performed in accordance with the standard procedures of Pharmacology/Toxicology Department, Institute for Industrial Research & Toxicology, Ghaziabad, UP, India as well as the approved study plan.

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

The study was conducted in compliance with International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5 Tests for In-vitro Cytotoxicity following Good Laboratory Practice regulations (GLP), 21 CFR 58.

All original raw data including documentation, the draft report, a copy of the final report and the representative test item are archived in the archives at Pharmacology/Toxicology Department, Institute for Industrial Research & Toxicology, Ghaziabad, UP, India**.** There were no known circumstances that may have affected the quality or integrity of the study.

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

Anjali Sharma

Ms. Anjali Sharma **22-08-2020**

Study Director **Signature**
Date

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2. STATEMENT BY TEST FACILITY MANAGEMENT

Management of the test facility has made available all the resources to the Study Director 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 compliance of Good laboratory Practice.

22-08-2020

Laboratory In-charge **Date**

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QUALITY ASSURANCE STATEMENT

This **Project No -202010-052/Report No IIRT/MD/202010/516/CYT** entitled In-vitro Cytotoxicity of **"GEL-ARTICLE NO - 10252203174"** was carried out according to International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for *Invitro* Cytotoxicity following Good Laboratory Practice regulation, 21 CFR 58 was subjected to inspections by the Quality Assurance Unit.

This report has 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 QA evaluation is reported to the Study Director 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	:	01-08-2020
Test Material Preparation	:	04-08-2020
Date of Testing	:	04-08-2020
Draft Report Audit	:	08-08-2020
Final Report Date	:	22-08-2020

Ms. Shalini Mishra		**22-08-2020**
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Quality Assurance Head	**Signature**	**Date**

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4. STUDY INFORMATION

PROJECT NUMBER : 202010-052

REPORT NUMBER : IIRT/MD/202010/516/CYT

STUDY TITLE : In vitro Cytotoxicity of **"GEL- ARTICLE NO - 10252203174"** using MTT Tetrazolium assay (ISO 10993-5:2009 (E)) following Good Laboratory Practice regulation.

SPONSOR	:	**NUTRIN GMBH & GALAXA PHARMA APS**
CRO	:	**MITTAL GLOBAL CLINICAL TRIAL SERVICES (MGCTS)**
TESTING LABORATORY	:	**INSTITUTE FOR INDUSTRIAL RESEARCH & TOXICOLOGY** **F-209, PHASE I, UPSIDC, MG ROAD, GHAZIABAD** **E-MAIL: INFO@TOXICITYINDIA.ORG** **WEBSITE: www.toxicityindia.org** **TEL No.: +91 9711623080** **FAX: +91 11 22235111**

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STUDY PERSONNEL

Study Director	:	Ms. Anjali Sharma M. Pharm. Pharmacology
Study Personnel	:	Mr. Shahnawaz Ahmed, M. Sc. Toxicology
Histopathology & Veterinarian	:	Dr. Naresh Chandra, M. V. Sc. Pathology

INTRODUCTION

Purpose:

The purpose of this study was to evaluate the Cytotoxicity of **"GEL - ARTICLE NO - 10252203174"** using the MTT Tetrazolium assay.

Testing Guideline

The study was conducted based on the International Organization for Standardization 10993, Biological Evaluation of Medical Devices, Part 5-Test for Invitro Cytotoxicity &International organization for standardization (ISO) 10993, Biological evaluation of Medical Devices Part-12, Sample Preparation and Reference Materials (2004)

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SUMMARY

The medical device (Topical Application Gel) was prepared according to International organization for standardization (ISO) 10993, Biological evaluation of Medical Devices Part-12, Sample Preparation and Reference Materials (2004). HeLa cells were seeded at a concentration of 0.35×10^6*cells*/ml in 96 well plate with DMEM media containing 10% FBS(Fetal Bovine Serum) and 1% Penicillin-Streptomycin incubated at 37℃ in 5% CO_2 incubator. The test materials were taken out from the tube in asceptical manner under hood for extract preparation. Cells were treated with medical device (GEL) extract prepared as per International organization for standardization (ISO) 10993 guidelines and was incubated for 24, 48, 72 hours at 37℃, further 10µl MTT was added (5mg/ml stock in 1× PBS pH 7.4), to make final concentration to 0.5mg/ml. After incubation at 37℃ in CO_2 incubator for 4h in dark, the media was removed and formazan crystals were dissolved in 100µl of DMSO. The crystals were then measured by BioRAD ELISA reader at 570 nm and 690 nm reference wavelength respectively.

Results clearly indicated that there was no significant difference observed in the OD of control treated and test treated samples and the test and control sample was not toxic for cell growth.

MATERIALS AND METHOD

8.1 Sample description

Test Article Name	**:**	**GEL "Article No - 10252203174"**
Batch No	**:**	**rd20014**
Mfg Date	**:**	**-**
Exp Date	**:**	**-**

8.2 Materials Required

HeLa cells: Human cervix carcinoma cells

DMEM Media: Dulbecco's Modified Eagle Medium (Hi-Media)

Penicillin-streptomycin antibiotic

MTT: (3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium assay (yellow tetrazole dye)

PBS: Phosphate Buffered Saline

FBS: Fetal Bovine Serum

DMSO: Dimethylsulphoxide AR

MicrotitreNails: 96 well

Bio-RAD ELISA reader

8.3 Method

- HeLa cells were seeded at a concentration of 0.35×106*cells*/ml in 96 well plate with DMEM media containing 10% FBS and 1% Pen-Strep incubated at 37℃ in 5% CO_2 incubator.
- The cells were treated with medical device extract prepared according to International organization for standardization (ISO) 10993, Biological evaluation of Medical Devices Part -12, Sample Preparation and Reference Materials (2004) and was incubated for 24, 48, 72h at 37℃.
- After this, 10µl MTT was added (5mg/ml stock in 1× PBS pH 7.4), to made final concentration 0.5mg/ml.
- The plate was then incubated at 37℃ in CO_2 incubator for 4h in dark.

- The media was removed and formazan crystals were made to dissolve in 100µl of DMSO and the amount of formazan crystal formed was measured by BioRAD ELISA reader at 570nm and 690 nm reference wavelength respectively.

8.4 <u>Supplementary Data/Confirmatory</u>

Acridine orange/Ethidium bromide Double staining method

For confirmation of toxicity impact of device extract, HeLa ($1x10^5$ cells /well) cells were grown on cover slip and treated with medical device extract. Cells were incubated in CO_2 incubator at 37℃ for 48 hours. Cells were washed with 1×PBS buffer, fixed with absolute methanol for 10 min, again wash with 1× PBS buffer.

After that the cells were stained with 1µl of AO/EB cocktail (AO/EB 100µg/ml) for 10-15 min and immediately washed by phosphate buffer finally and image captured by florescence microscope.

RESULT AND DISCUSSION

Generally MTT (3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium) assay is used for cell viability analysis. The assay is based on reduction of soluble yellow Tetrazolium into insoluble purple formazan crystals; the reduction emerged by mitochondrial dehydrogenase in metabolic active cells. Therefore, the rate of formazan crystal formation is directly proportional to number of viable cells. The absorbance of sample is directly proportion to cell viability.

Results clearly indicate that there is no significant different observed in the OD of control treated and test treated samples. Thus findings suggest that the test sample and control sample is not toxic for cell growth and the data is presented in Table-I.

Table –I Cytotoxicity of Medical Device, evaluated using MTT Tetrazolium assay.

Column **HeLa**	**Mean OD value 24 Hr**	**Mean OD value 48 Hr**	**Mean OD value 72 Hr**	**Standard Deviation for Ist**	**Standard Deviation for IInd**	**Standard Deviation for IIIrd**
Control	2.25	2.72	2.94	0.10	0.08	0.11
Control	2.54	2.8	3.08	0.12	0.11	0.13
Control	2.8	2.81	3.42	0.11	0.13	0.10
Extract	2.52	2.81	3.11	0.12	0.10	0.08
Extract	2.58	2.85	3.15	0.10	0.12	0.12
Extract	2.8	3.04	3.23	0.11	0.12	0.09

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CONCLUSION

Under the conditions of this study, it has been concluded that medical device extract did not created any significant toxicity toward human cells. Results clearly indicated that the **"GEL - ARTICLE NO - 10252203174"** was **non-cytotoxic** on HeLa cells and all procedures were conducted in conformance with good laboratory practices Results and conclusions apply only to the test article tested.

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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 nine years at Institute for Industrial Research and Toxicology, Ghaziabad, U.P India.

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CERTIFICATE

This is to certify that the In vitro Cytotoxicity of the Sodium Chloride extract of **"GEL-ARTICLE NO - 10252203174"** supplied by **NUTRIN GMBH & GALAXA PHARMA APS** with CRO **MITTAL GLOBAL CLINICAL TRIAL SERVICES (MGCTS)** was performed according to the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 5: Tests for In vitro Cytotoxicity using MTT (3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium) assay of the test sample and control sample is **not toxic** for cell growth.

The report of the In vitro Cytotoxicity test conducted has been submitted through **Study no. 202010-052** which can be concluded that the material **"GEL - ARTICLE NO - 10252203174" is non-Cytotoxic** and **safe** for use.

Note: Results and conclusions apply only to the test article tested.

Any extrapolation of these data to other samples is the sponsor's responsibility.

Study Director

3. Annexure-I: References

- International organization for standardization (ISO) 10993, Biological evaluation of Medical Devices Part-12, Sample Preparation and Reference Materials (2004)
- International organization for standardization (ISO) 10993, Biological evaluation of Medical Devices Part-5, Test for in-vitro Cytotoxicity (2009)