PHARMACEUTICAL BIOTECHNOLOGY DEPARTMENT
Maltepe, Istanbul/Turkey

IN VITRO CYTOTOXICITY TEST REPORT

Report No: SA_2025-1_4

Report Date: 17.01.2025

Client Name: Apron İlaç Med İth. İhr. San. ve Tic. Ltd. Şti.

Client Address: Kozyatağı Mah. Saniye Ermutlu Sok. By Otel No:3/1 Kadıköy/Istanbul

Subject: In Vitro Cytotoxicity Report for Asthins Product

These results are valid only for the analyzed samples.

Test Conducted by: Dr. Ceyda EKENTOK ATICI - Research Assistant Işınsu MUTLU

Approved by: Prof. Dr. Ali Demir SEZER

Unsigned reports are invalid.

This report has been prepared in two copies, one for the client and one for the institution's archive, totaling 6 pages.

MARMARA UNIVERSITY FACULTY OF PHARMACY
PHARMACEUTICAL BIOTECHNOLOGY DEPARTMENT
Maltepe, Istanbul/Turkey

CONTENTS

Page

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1. Title

In Vitro Cytotoxicity Study for Asthins Product

The purpose of this report is to present the results obtained in the study, explain how the study was conducted, and describe how the data were collected.

2. Product Supplier

Apron İlaç Med İth. İhr. San. ve Tic. Ltd. Şti.
Kozyatağı Mah. Saniye Ermutlu Sok. By Otel No:3/1 Kadıköy/Istanbul

3. Test Location

Marmara University Faculty of Pharmacy, Pharmaceutical Biotechnology Department
Maltepe, Istanbul/Turkey

4. Guidelines

This study was conducted in accordance with the following internationally accepted guidelines:

EN ISO 10993-5 “Tests of cytotoxicity: in vitro methods”
EN ISO 10993-12 “Sample preparation and reference materials”

5. Product

Product	Asthins
Lot	-
Material	Mixture of plant extracts
Formulation	Oral suspension
Number of Samples Sent	2
Manufacturer/License Holder	Apron İlaç Med İth. İhr. San. ve Tic. Ltd. Şti.
Storage Conditions	Room temperature

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6. Study Dates

Sample Arrival Date: 30.12.2024
Study Start Date: 06.01.2025
Study End Date: 10.01.2025
Report Date: 17.01.2025

7. Cytotoxicity Study

The purpose of this study is to determine the in vitro cytotoxicity of the tested product on mammalian cells. Cell viability was quantitatively determined using a colorimetric method.

The test principle is based on the metabolic reduction of the soluble dye [MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide)] by mitochondrial succinate dehydrogenase in metabolically active cells, converting it into insoluble formazan crystals. This allows the determination of mitochondrial function in the cultured cells. The number of viable cells is proportional to the amount of formazan produced.

The study was conducted according to the specifications of the EN-ISO10993-5:2009 standard.

7.1. Material, Solution, Equipment

Material:

- 96-well cell culture plates
- T-25 cell culture flasks
- Sterile pipettes and pipette tips
- 1.5 ml centrifuge tubes

Equipment:

- Laminar flow cabinet
- CO2 incubator
- Inverted microscope
- Incubator
- ELISA Reader

Solutions and Reagents:

- Cell line L929 (mammalian fibroblast)
- Fetal bovine serum
- DMEM medium
- Sterile PBS (pH: 7.4) buffer
- DMSO (dimethyl sulfoxide)
- Absolute ethanol
- Cell Proliferation Kit I (MTT kit) (Roche)

7.2. Experimental Procedure

- L929 cells were cultured in DMEM medium containing 10% FBS at 37°C in 5% CO2 and seeded into 96-well culture plates at 5x10^3 cells/well, followed by 18-24 hours of incubation.

- The next day, the medium in the wells was removed, fresh medium was added, and the samples were applied to the wells.

- Study groups:

  Product: final concentration 5% (v/v), 5 µl sample in 100 µl medium
  Product: final concentration 10% (v/v), 10 µl sample in 100 µl medium
  Control: cells only (L929)
  Positive control: 20% (v/v) DMSO
  Negative control: PBS

Each group was studied with n=6.

- After 48 hours of incubation, the medium in the wells was removed, the wells were washed with 100 µl PBS, and 100 µl of fresh medium was added to each well. Then, 10 µl of MTT solution was added to each well, and the plates were incubated for 4 hours to allow crystal formation.

- 100 µl of 10% SDS was added to each well, mixed, and incubated at 37°C to dissolve the crystals.

- Absorbance was then measured at 550 and 690 nm using an ELISA Reader, and calculations were performed.

- For calculations, the difference between the absorbances measured at 550 and 690 nm was taken, and the percentage viability was calculated using the following formula:
  % viability = (absorbance x 100) / control group average absorbance

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8. Results

- At both concentrations studied, cell viability was observed to be above 70% compared to the control group.

- According to the obtained data, the product is not cytotoxic.

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