TECHNICAL REPORT

XemaTop™

Evaluation of the Safety and Toxicological Profile of XemaTop™ on the Vaginal Mucosa

Abstract: The vaginal mucosa is a common site for local and systemic delivery of medication. This study aims to compare the safety and toxicological profile of XemaTop, a proprietary topical compounding base, to that of purified water (negative control) and Gynoll II (positive control), using a 3D model of the human vaginal mucosa. Results have demonstrated that XemaTop presented an ET_{50} superior to 24 hr, as opposed to an ET_{50} of approximately 4hr for the Gynol II. Therefore, it may be concluded that XemaTop does not cause toxicity to the vaginal tissues. Compounded medicines including this proprietary topical compounding base may then be considered safe to be applied to the vaginal mucosa for over 24 hr.

Introduction:

Vaginal delivery of medication is advantageous in allowing for the medication to avoid first-pass metabolism and gastrointestinal degradation [1]. Lined with non-cornified, stratified squamous epithelium, the vaginal mucosa offers a large surface area and rich blood supply, making it a promising site for delivery of medication in the treatment of several conditions and also in hormone replacement therapy [2].

The aim of this study was to evaluate the safety and toxicological profile of XemaTop, a proprietary topical compounding base, in comparison to purified water (negative control) and the positive control Gynol II [nonoxynol-9 (3%)], an irritant of the vaginal mucosa, using a 3-dimensional (3D) *in vitro* model of the human vaginal mucosa.

Vaginal tissue model

The EpiVaginal™ tissue model by MatTek Corporation (Ashland, MA) is a highly differentiated tissue cultured from normal, human-derived vaginal epithelial and dendritic cells. Its tissue structure and cellular physiology closely parallels *in vivo* vaginal epithelial tissue. It is therefore an ideal *in vitro* research tool for safety and toxicological testing of feminine products. The EpiVaginal™ tissue containing epithelial VEC-100 cells was the model used in this study (Figure 1) [3].

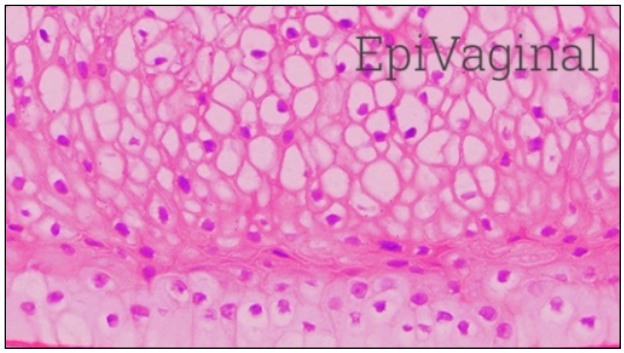

Figure 1. Illustration of the EpiVaginal™ tissue model. (*adapted from* MatTek Corporation, 2016)

Methodology:

Upon receipt of the EpiVaginal™ tissue model, the VEC-100 cells were maintained in the supplied culture media and stored in accordance to the manufacturer's protocol until the initiation of the study. Following preparation of the cells, the EpiVaginal™ tissues were treated in duplicate with 100 µL of the test product (XemaTop lot #7140425 or Gynol II) for 1, 4 and 24 hr. A set of EpiVaginal™ tissues was also treated (in duplicate) with 100 µL of purified water to serve as a negative control. Following the exposure period, the dosing solutions were removed and the cells were analyzed for cell viability by the MTT Effective Time 50 (ET_{50}) assay.

The MTT ET_{50} assay consists of measuring the reduction of MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide) by the cells. Succinate dehydrogenase enzymes within the mitochondria of viable cells have the ability to reduce soluble yellow tetrazonium salt of MTT to an insoluble purple formazan derivative. MTT is therefore an indicator of cell viability as the tissues are evaluated for their ability to reduce soluble-MTT (yellow) to formazan-MTT (purple) [4].

The MTT solution was prepared in the provided medium and added to the basal side of each tissue, followed by an incubation period of the tissues for 3 hr at 37°C. The purple formazan product was then extracted using the provided extractant, which was previously applied to both the apical and basal side of the tissues. Sample absorbance was read at 570 nm and reference absorbance at 650 nm with CLARIOstar – BMG Labtech Plate reader.

Results and Discussion:

Viability of the vaginal cells following exposure to the test products is represented by the absorbance of the respective extracts and expressed in percentage relative to the negative control (tissues treated with purified water), as follows:

% Cell Viability=100 x [OD(test product) / OD(negative control)

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The greater the absorbency of the extracts, the greater the amount of MTT reduced by succinate dehydrogenase and, as a result, the higher the percent cell viability within the tissue [3].

At the start of the study (t=0 hr), the viability of the cells was 100% for both the tissues exposed to XemaTop and the tissues exposed to the positive control Gynol II. Following 24 hr, the viability of the cells exposed to the positive control was less than 5%, which means that the vaginal tissue was no longer functional and thus confirms the toxicity of Gynol II. On the contrary, the viability of the cells exposed to XemaTop for 24 hr was superior to 80%, as shown in Table 1 and Figure 2.

Table 1. *Safety and toxicological profiles of XemaTop and Gynol II.*

Exposure time (hours)	Cell viability (%)	
	XemaTop (mean ± SD)	Gynol II (mean ± SD)
0	100.02±3.04	100.00±5.90
1	107.45±6.34	84.75±3.21
4	98.63±5.40	51.11±12.30
24	83.56±2.95	4.93±0.74

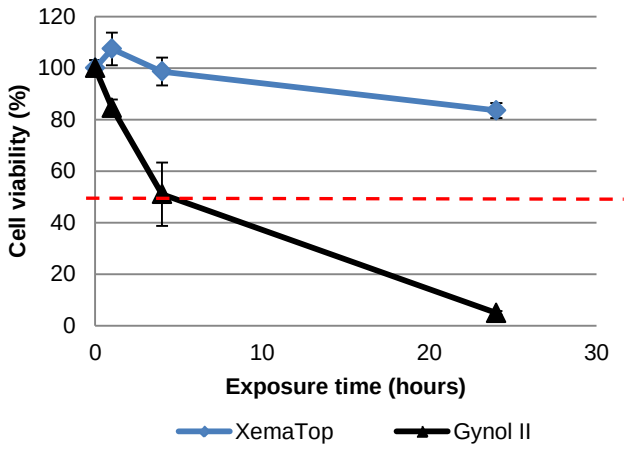

Figure 2. *Safety and toxicological profiles of XemaTop and Gynol II.*

The toxic exposure time (ET_{50}) is the time when cell viability is reduced to 50% [3]. The ET_{50} is represented by a red dashed line in Figure 2. According to the results obtained, the ET_{50} of the positive control is approximately 4 hr, as opposed to the ET_{50} of XemaTop which is superior to 24 hr. The proprietary compounding base XemaTop does not cause toxicity to the vaginal tissues and may then be considered safe to the vaginal mucosa for over 24 hr.

Conclusions:

Compounded medicines applied to the vaginal mucosa must be safe and non-toxic as vaginal toxicity can cause irritation and tissue damage, which weakens the natural defenses of the mucosa and increases the risk of infections such as HIV and herpes simplex [5].

This study has demonstrated that XemaTop presented an ET_{50} superior to 24 hr and, therefore, has a good safety and toxicological profile on the vaginal mucosa. As a result, compounded medicines including this proprietary topical compounding base may be applied to the mucosa without causing any toxicity to the vaginal tissues.

References:

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