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DIPARTIMENTO DI
FARMACIA E SCIENZE
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REPORT

N° MUCD041-0503F FTP 82 GEL MUCOSITI

MUCOADHESIVITY IN VITRO EVALUATION

SAMPLE	***FTP 82 GEL MUCOSITI LOTTO H2543M***

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Aims

The main purpose of this research is the evaluation, in a first phase of the experimentation, the in vitro mucoadhesion of the **FTP 82 GEL MUCOSITI** product towards human buccal cells, as a model of oral tract and in particular esophageal cells.

Information about the performed mucoadhesivity model

One of the substantial differences between the structure of the skin and the mucous membranes is represented by the absence, in the second one, of a selective barrier such as the stratum corneum. Contact of the oral mucous membranes (esophageal gold tract) with harmful or irritating substances (acid solutions) can cause a high penetration of these into the mucous membranes with serious degenerative effects.

The possibility of creating, through the administration of appropriate natural substances with mucoadhesive properties, an additional barrier on the buccal mucous membranes can highly contribute to protect the mucous membranes from the risk of aggression by harmful and / or irritating substances.

The mucoadhesive effect of a product, which contributes to the formation of the protective barrier on the mucous membranes, can be evaluated using appropriate in vitro models.

In the model proposed by us, which derives from the optimization of previous experimental protocols and scientific works on the subject (1-16), the mucoadhesiveness of products intended for the treatment of mucous membranes can be determined by evaluating the percentage of inhibition of the lectin-glycoprotein bond. Buccal or vaginal mucosal cells can be used in this model. Initially, the cells are treated with biotinylated lectin (Con-A), a protein contained in some leguminaceae (Canavalia ensiformis) which has a high affinity for the glucosidic and mannosidic residues present in the membrane glycoproteins. In this way the glycoprotein sites of the mucosal membranes will be engaged with the biotinylated lectin. The presence of biotin (vitamin H) in the lectin is essential for the next stage. Streptavidin peroxidase is added to the cells, already treated with biotinylated lectin, allowing, to form the protein-glucoseolectin-biotin-streptavidin peroxidase complex, thanks to the high affinity between biotin and spreptavidin.

At this point the cells are washed and the protein-glucose-lectin-biotinastreptavidin peroxidase complex is quantified, thanks to the presence of peroxidase, through the oxidation reaction of ortho-phenylenediamine.

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The intensity of the yellow-orange color of the solution (measured with a spectrophotometer at λ = 450 nm) is proportional to the amount of glycoprotein-lectin bonds and, therefore, to the amounts of sites available for mucoadhesion (glycoproteins).

The determined absorbance value constitutes the "control". In determining the muco-adhesiveness of a product, the cells are previously treated with this (incubation at 30 ° C for 15 minutes before treatment with lectin) and, if the product under examination contains mucoadhesive substances, these will bind to the glucosidic and mannosidic sites present in membrane glycoproteins.

In the next phase, adding the sequence of biotinylated lectin, streptavidin peroxidase and ortho-phenylenediamine, a less intense staining will be obtained compared to the control, because part of the glucosidic sites available for binding with Con-A have already been engaged by the mucoadhesive substances present in the product to be tested. In fact, the initial bond between the muco-adhesive substances, contained in the product to be tested, and the glucoside sites partly affects the subsequent conjugation of Con-A with the streptavidin peroxidase complex and the consequent development of color after the addition of hydrogen peroxide.

The decrease in the absorbance value is proportional to the ability of the substances under examination to “mucoadire” to the mucosal cells.

The mucoadhesive capacity is expressed as a percentage of inhibition of the glycoproteinalectin bond or better as a percentage of mucoadhesion of the product according to the equation:

Percentage of mucoadhesion of the product = (1 – sample abs/control abs) x 10

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START TEST: 04-03-2019

END TEST: 05-03-2019

Experimental protocol

Mucoadhesivity evaluation

The oral cavity of 10 donors 4 male and 6 female (age 25-37 years), who had neither eaten nor drunk for at least 60 minutes, was gently scraped with a wooden spatula and the cells dipped in TBS (Tris Buffer Saline) 0.05 M pH 7.6.

After counting with 0.5% Trypan blue, the cells were diluted with 0.9% NaCl until a final concentration of 180,000 cells was obtained for each sample to be used in the experiment. The cells were subsequently left at a temperature of 4 ° C.

The cell suspensions were then centrifuged at 2000 rpm for 5 minutes and incubated with 10 ml of a solution obtained by dissolving 5 ml **FTP 82 MUCOSITE GEL** in a final volume of 50 ml of physiological solution in order to obtain a 1:10 dilution. For the control, the cell suspensions were instead incubated with 5 ml of 0.9% NaCl.

The incubation was continued for 15 minutes at a temperature of 30 ° C, shaking slightly.

After 3 washes with TBS, the buccal cells were incubated with 5 ml of 10 mg L-1 of Con-A at 30 ° C for 30 minutes, washed 3 times with TBS and then incubated at 30 ° C for 60 minutes with 5 ml of 5 mg L-1 of streptavidin peroxidase.

After 3 washes with TBS, 200,000 cells per sample were added to 2.5 ml of ophenylenediamine (o-pd) in 0.05M phosphate citrate and H2O2 and, after 5 minutes, the reaction was stopped with 1M H2SO4.

The absorbance values for the individual determinations were then read using a spectrophotometer

The single samples were repeated in triplicate and the reported results represent the means ± SEM of all the experiments performed

% of mucoadhesivity	Evaluation
0-20%	***SATISFACTORY***
20-40%	***DISCRETE***
40-80%	***GOOD***
80-100%	***EXCELLENT***

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Results

The obtained results (Table 1 and Figure 1) show that diluted 1:10 **FTP 82 GEL MUCOSITI** sample has a **EXCELLENT** mucoadhesivity activity.

In order to mime the real conditions of use, FTP 82 GEL MUCOSITI sample was diluted in a 0.9% NaCl solution.

TABLE 1.

Sample	Absorbance λ=450nm				Mucoadhesivity (%)	DEV. ST.	SEM
	I	II	III	MEDIA			
CONTROL (NaCl 0.9%)	0,95	0,96	0,91	0,940	0,00%	0,0265	0,027289
FTP 82 GEL MUCOSITI H2543 M	0,17	0,19	0,18	0,180	80,85%	0,0100	0,02357

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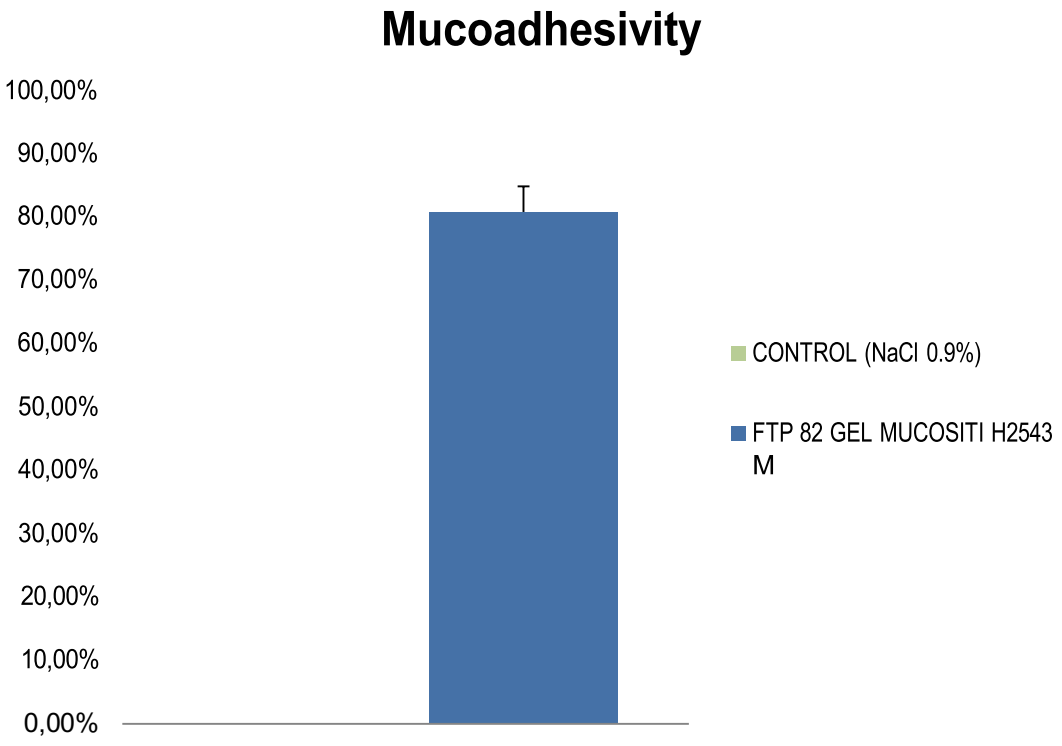

Figure 1

Date: 05-03-2019

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