or Lucifer yellow leakage (2 fold) for 6 of 8 test compounds was observed. Furthermore, drug efflux transporter inhibitors increased drug absorption while decreasing the efflux ratio. Efflux ratios for talinolol, digoxin, and loperamide (Pgp substrates) were reduced by 45%, 40%, and 60%, respectively, in the presence of the Pgp inhibitor verapamil. Efflux ratio of the BCRP substrate nitrofurantoin was reduced by 63% in the presence of novobiocin, a known BCRP inhibitor. In conclusion, the newly developed SMI tissue models appear to be promising new tools for drug safety and permeation studies.

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Evaluation of inflammatory and genotoxic effects of smokeless tobacco using an organotypic in vitro human model of oral epithelium

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In addition to the well-known effects of tobacco use on the causation of lung and cardiovascular disease, tobacco use is also implicated as a major cause of oral cavity disease that leads to thousands of deaths per year. Snus, a smokeless tobacco applied to the oral cavity, has been proposed as a less harmful alternative to smoking although its safety has not been adequately evaluated. The objective of this study was to evaluate the cytotoxic, genotoxic and inflammatory effects of snus using an in vitro model of human oral mucosa (EpiOral). EpiOral tissues were treated topically with 5 or 25 mg of snus for 24–48 h and evaluated for cytotoxicity by MTT. Tissues treated with 5 mg of snus had comparable viability to vehicle treated controls while those treated with 25 mg displayed approximately a 20% decrease in viability after 24 and 48 h of exposure. Histological analysis revealed hyperchromic staining in tissues treated with 5 mg of snus at 24 h post-treatment whereas tissues treated with 25 mg of snus displayed a significant amount of sloughing of the apical layers. Following treatment, an inflammation-specific cytokine panel was used to analyze markers of inflammation at 24 and 48 h post treatment. Of the cytokines analyzed, significant increases (1.5–2 fold) in IP-10, GM-CSF and RANTES were observed at both 24 and 48 h post treatment in tissues treated with 25 mg of snus. As a measure of genotoxicity, the presence of γ-H2AX foci (specifically, phosphorlation at Serine 139) was evaluated in treated tissues. γ-H2AX is a phosphorylated derivative of the H2AX histone and is tightly bound to double strand DNA break sites, therefore serving as a biomarker of genotoxic insult. γ-H2AX foci were readily detected in the apical layer of tissues treated with 25 mg of snus at 24 and 48 h post treatment. These results demonstrate the utility of this organotypic oral tissue model to evaluate smokeless tobacco product safety.

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Lethal dosage and minimum inhibitory concentration of the itraconazole for oomycete *Saprolegnia* sp. in conditions in vitro

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Saprolegnia sp. is an oomycete of easy isolation and maintenance in laboratory, fast and uniform growth. Thus, it was used Saprolegnia sp as bioindicador in the lethal dosage evaluation of itraconazole (ITR) through of the inhibition concentration of growth (IC50%) and of the minimum inhibitory concentration 100% (MIC100) in vitro conditions. *Saprolegnia* sp. strain was isolated from fish farming water tanks of Oreochromis niloticus. The water was collected and were added hemp seeds, as baits/substratum for the fungus growth and kept at 25 °C. The parasitized baits were transferred for petri dishes with PDA medium (potato dextrose agar). After the growth of white colonies was observed the morphology of hyphae, induced the zoosporogenesis and did the PCR for molecular identification (KP941579 Genbank). For in vitro essays, *Saprolegnia* sp. strain was peaked in petri dishes with PDA, kept during three days at 25 °C, and used as source of fungus samples. First of all, it was evaluated the sensitivity strain with reference substance potassium dichromate being it IC50;72 h 136.28 mg L^{-1} (121.4–153.11 mg L^{-1}). After, from ITR stock solution were diluted the concentrations used in the IC50% essays: 50.0; 87.5; 153.2; 267.9; 468.9 and 820.7 mg L^{-1} and in the MIC essays: 650.0; 680.0, 710.0; 740.0 and 770.0 mg L^{-1} and a control with PDA medium. A fungus piece of 6 mm was disposed in the central position and kept at 25 0C by 72 h, with five repetitions, evaluation of diameter growth halo daily and in the end of 72 h was calculated IC50% using software Trimmed Spearman Karber and determinate MIC100%. IC50;72 h of ITR was 299.7 mg L^{-1}, with lower 273.13 and upper limit 328.86 mg L^{-1}. In 50.0 mg L^{-1} the mycelia growth was like the controls, in 87.5 mg L^{-1} occurred 9% of inhibition; 153.2, 17%; 267.9; 39%; 468.9, 65% and 820.7, 100%. In MIC essay, concentrations until 710.0 mg L^{-1} occurred an average 85% of inhibition and from 740.0 mg L^{-1} there was total inhibition (100%) in 72 h. *Saprolegnia* sp. showed to be resistant at this fungicide class, possibly not presents ergosterol, because the action mode of azoles is inhibits its synthesis. Thus, this fungicide is not indicating in the evaluation of azoles fungicides toxicity.

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