Luffa operculata infusion affects mucociliary function of the isolated frog palate

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Abstract

Background: *Luffa operculata* is a medicinal plant found in many European remedies used for treatment of allergic rhinitis and rhinosinusitis. In Latin America, a homemade Luffa *operculata* dry fruit infusion (*Lop*DFI) is popularly used into the nose, delivering profuse mucous secretion and relieving nasal symptoms. Nevertheless, this may cause nasal mucosa irritation, epistaxis or anosmia.

Methods: We evaluated the effects of *Lop*DFI on mucociliary transport velocity (MTV); ciliary beat frequency (CBF) and transepithelial potential difference (PD) in isolated frog palate preparation. We tested 46 palates immediately before, 5 and 20 minutes after immersion. Four groups (n=10) were tested into frog Ringer (=control) *Lop*DFI 60mg/L, 600mg/L and 1200mg/l, and also a group of *Lop*DFI in water (600mg/L H_2O, n=6). Epithelial samples were harvested for ultrastructural study.

Results: In treated palates, MTV significantly decreased ($p<0.001$), CBF was affected ($p<0.008$) and PD was altered in a dose-dependent manner ($p<0.007$). PD findings pointed towards ion-fluid transport abnormalities, which were confirmed by transmission electron microscopy that showed enlargement of interepithelial spaces.

Conclusions: *Lop*DFI promoted significant changes on mucociliary epithelium function of this *ex vivo* model, suggesting that is potentially noxious to human nasal mucosa.

Introduction

Nowadays, patients with all sorts of diseases search for cure in Complementary Medicine such as homeopathy, acupuncture or phytotherapy. Phytotherapy is the most ancient and spread among these practices. Mankind has been using medicinal plants for 60.000 years now (1). A recent survey demonstrated, for example, that about US$17.8 billion were spent on herbs, botanical remedies and dietary supplements in the United States in 2001 (2).

Many patients do not tell their doctors they are using medicinal plants (7,8). On the other hand, many doctors do not know the real impact of this practice on health, nor the possible interaction among herbs and allopathic drugs (9,10, 11, 12, 13, 14). Patients with chronic respiratory diseases - as allergic rhinitis, rhinosinusitis, asthma - or repeated upper airways infections are prone to resort to phytotherapy so as to avoid treatments with antibiotics or corticosteroids (3, 4, 5, 6). That is the reason why more research is needed on the effects of medicinal plants on the respiratory system.

Alternative medicine can no longer be ignored by academia (15).

Luffa operculata (*Lop*) is a very popular medicinal plant used for the treatment of rhinitis and rhinosinusitis. Linnaeus firstly described the plant in XVIII century as *Momordica operculata*, and Cogniaux moved it to the *Luffa* gender in 1978. In Europe, especially in Germany, there are many homeopathic medicines with this plant. In Latin America its dry fruit, which is available from informal sellers, is commonly used for preparing an infusion that is either inhaled or instilled into the nose, instantly delivering profuse mucous secretion and thus alleviating nasal symptoms.

Nevertheless, the "natural" treatment with *Luffa operculata* dry fruit infusion (*Lop*DFI) may cause severe nasal mucosa irritation, epistaxis or anosmia. Its chemical features include saponin, which breaks the superficial tension of the mucosa, and isocucurbitacin, which seems to be the most important active principle and is easily absorbed after saponin action (16). It has an irritating effect on every mucosae, so it is popularly used also as laxative and abortive. It shows high *in vitro* and *in vivo* toxicity: the LD_{50}, the lethal dosis for half of laboratory mice, is around 170 mg/kg, corresponding to 1g of *Lop* extract for a 70 kg adult human. (17, 18)

Considering the widespread usage and the lack of scientific data about the action of *Lop* on the nasal mucosa, we carried out a study to evaluate the effects of *Lop*DFI on the epithelium and mucociliary activity. We used the *ex vivo* model of the isolated frog palate, which is very useful and convenient, and whose pseudostratified columnar ciliated epithelium is virtually identical to human respiratory mucosa. In this model mucus secretion and ciliary beating are maintained until 14 days after animal death (19, 20, 21).

Material and Methods – The experiment was carried out with 46 palates, subdivided in five groups, as follows in Table 1.

GROUP	n	INFUSION	CONCENTRATION	SOLVENT
1	10	Control	frog Ringer	frog Ringer
2	10	Diluted	60mg/L = 0.3g/5000mL	frog Ringer
3	10	Basic	600mg/L = 0.3g/500mL	frog Ringer
4	10	Concentrated	1200mg/L = 0.3g/250mL	frog Ringer
5	6	Watery	600mg/L H_2O = 0.3g/500mL H_2O	mineral water

We weighted ten dry fruits that ranged from 1.0 to 1.5g. The average was 1.2g, which we considered the standard fruit weight. Therefore, 1/4 of the fruit is 0.3g, diluted in 500mL of frog Ringer (group 3) and in water (group 5), which is the empirical recommended solution by hawkers (herbal medicine sellers). Group 2 was supposed to be similar to diluted medications and group 4, to repeat a double dosis medication. We used parts of the peel, the inner fiber and seeds to prepare the infusion.

Three measures were performed for each palate: immediately before, 5 minutes after and 20 minutes after immersion into the infusion, simulating the effects of direct instillation of medications into the nose.

We studied the mucociliary function with three different parameters: the mucociliary transport velocity (MTV)(19, 20, 21), the ciliary beat frequency (CBF)(19, 20, 21) and the transepithelial potential difference (PD)(20, 22), plus the qualitative histological aspects of epithelial samples in the tested palates. In addition, histological samples were drawn for ultrastructural study.

MTV was measured in millimeters per second (mm/s) and assessed by measuring the displacement of an autologous mucus sample through the frog palate with a charcoal particle placed on it, under a microscope (ZEISS). CBF was determined by observing ciliary movement of the epithelium under stroboscopic flashlight in a light microscope (Olympus BX 50), and measured in Hertz (Hz) in a computerized system, in which the images were captured and recorded. TPD was measured in volts (V) and assessed by positioning agar bridges microelectrodes in the epithelium and the lamina propria of the palate (PROCYON voltmeter). Two samples of each group were collected to study histological features of the epithelium under electron transmission microscopy.

Statistical analysis was performed using generalized linear models for repeated measures with the aid of the program SPSS version 10 for Windows, adopting the level of significance of 5% ($p<0,05$).

Results – After 5 and 20 minutes, *Luffa operculata* dry fruit infusion (*Lop*DFI) at 600mg/L and 1200 mg/L in frog Ringer, plus 600mg/L in water significantly decreased MTV ($p<0.001$)(Figure 1).

CBF suffered interaction time/treatment ($p<0.008$), that is, the time effect was not similar to all of the treatments. *Lop*DFI inhibited the increase of CBF observed in the control group, which was tested in isotonic solution (Figure 2).

PD alterations were observed at the higher concentrations (600 mg/l and 1200 mg/l) and in watery infusion, after 20 minutes of immersion ($p<0.007$). This points to augmented permeability of the frog palate mucosal epithelium, due to epithelial damage, thus results in lower resistance to fluid transport (Figure 3).

This finding was confirmed by the electron transmission microscopy, in which we found intercellular spaces frequently widened in the epithelium of the treated frog palates, related to tight junctions disruption due to epithelial damage. (Figure 1)

Discussion - Regarding the widespread usage of *Lo*DFI and the frequent informal referrals of its side effects, it is alarming the lack of studies about it. The observed profuse mucous releasing is a sign of its activity on mucociliary function, and therefore was the central aim of this study. We observed consistent alterations in the three tested parameters of mucociliary activity: 1- MTV, that indicates the integrity of the mucus, cilia and their interaction; 2 - CBF, that evaluates the motor of the system, and 3 - PD, which reflects the integrity of ions and fluids transport. These alterations were dose-dependent and further corroborated by the ultrastructural findings of the tested palates.

Epithelium electrical gradient tends to negative. The observed positivation of voltage in PD (figure 3) is due to epithelial damage and results in disruption of tight junctions, protein structures that serve as a barrier between the lumen and the interstitium. Tight junctions encircle the lateral portion of each cell just beneath the apical surface. They are recognized functionally by inhibiting the movement of solute (ion) and water across the epithelium (23), even though recent studies attribute them other possible functions (24). Therefore, the findings of enlargement of intercellular spaces, as in figure 4, are caused by epithelial leakage and can be attributed to changes in ions and fluid transport, because of tight junctions damage.

The ciliary beat has two components: the effective and the recovery stroke (25). If there is an inadequate quantity or quality of mucus, mucociliary transport decreases or even ceases. Macroscopically, we observed increased mucus production after the immersion of treated palates into *Lop*DFI. We suppose the enhance of periciliary fluid due to epithelial leakage, which was observed in this experiment, leaded to decreased MTV (figure 1) because of an uncoupling between the top of the cilia and the gel upper layer of the mucus.

We found important features related to the direct irritating effects of *Lop*DFI *on* the mucosa. Although the most important described active principium are cucurbitacins, the observed irritant reactions may be due to saponin, which acts on the superficial tension of the epithelial mucosa (18). Even though, this *ex vivo* model did not allow us to assess the effects of cholinergic system nor the inflammatory process.

We could not infer the toxicity or the therapeutical effects of the European medicines with *Luffa operculata,* because most of them are homeopathic dilutions (26,27,28,29). Nevertheless, we remind that *Luffa operculata* is not even included in Commission E German monographs, the official therapeutic guide to herbal medicines which describes the indications and risks of the available medicinal plants in that Country (30).

In this study, *Lop*DFI was prepared in different concentrations with frog Ringer, the isotonic solution for the frog. One group was studied with watery infusion, exactly as the homemade infusion, and its behavior was as noxious as the observed in the more concentrated infusion group. We observed significant changes induced by *Lop*DFI in a virtually normal respiratory epithelium model. Considering *Lop*DFI is intended to be used in pathological epithelium damaged by the illness condition itself (31), further by the ethiologic agents toxins (32) or medication compounds as vasoconstrictors and topical conservatives (33), we may conclude that the homemade preparation may cause adverse reactions by aggravating the pre-existing disease in the nasal mucosa.

This study explains the profuse mucus release observed for decades with this *Lop*DFI "natural treatment" for rhinitis and rhinosinusitis. *Luffa operculata* need further studies in order to be better elucidated, and physicians should be aware of the possible negative effects of *Lop*DFI to the mucociliary function, in order to orientate their patients.

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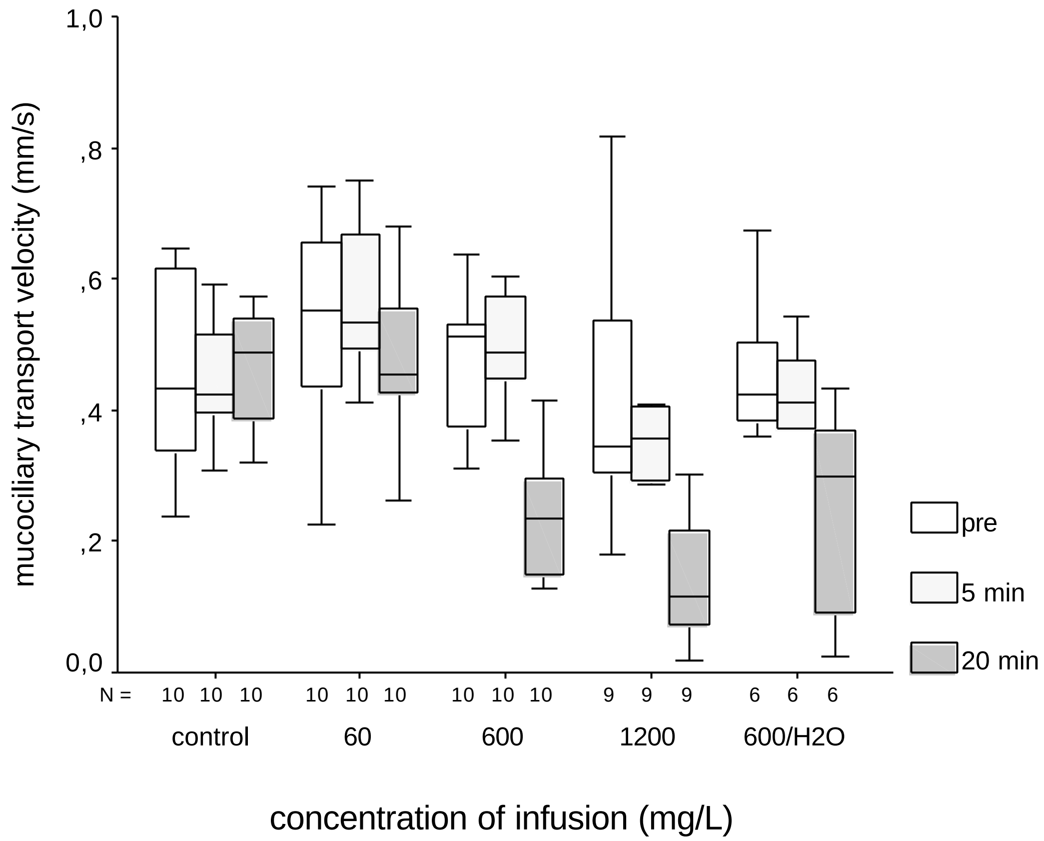

Figure 1 – Measures of mucociliary transport velocity (MTV)

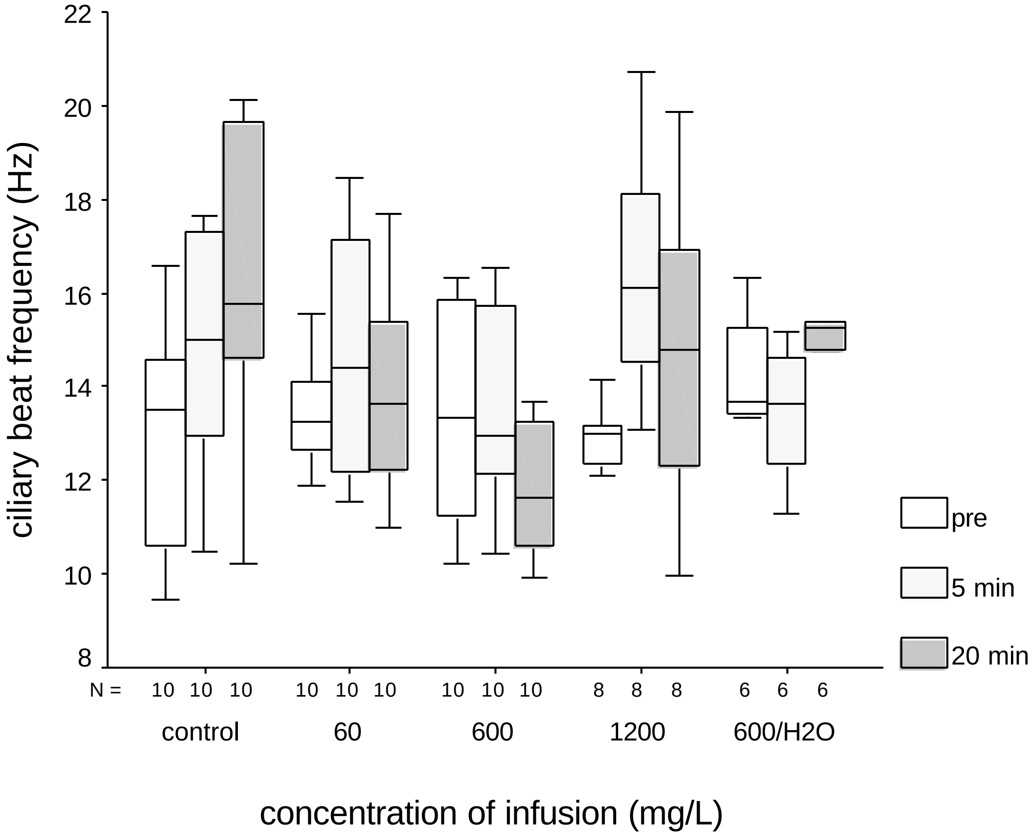

Figure 2 – Measures of ciliary beat frequency (CBF)

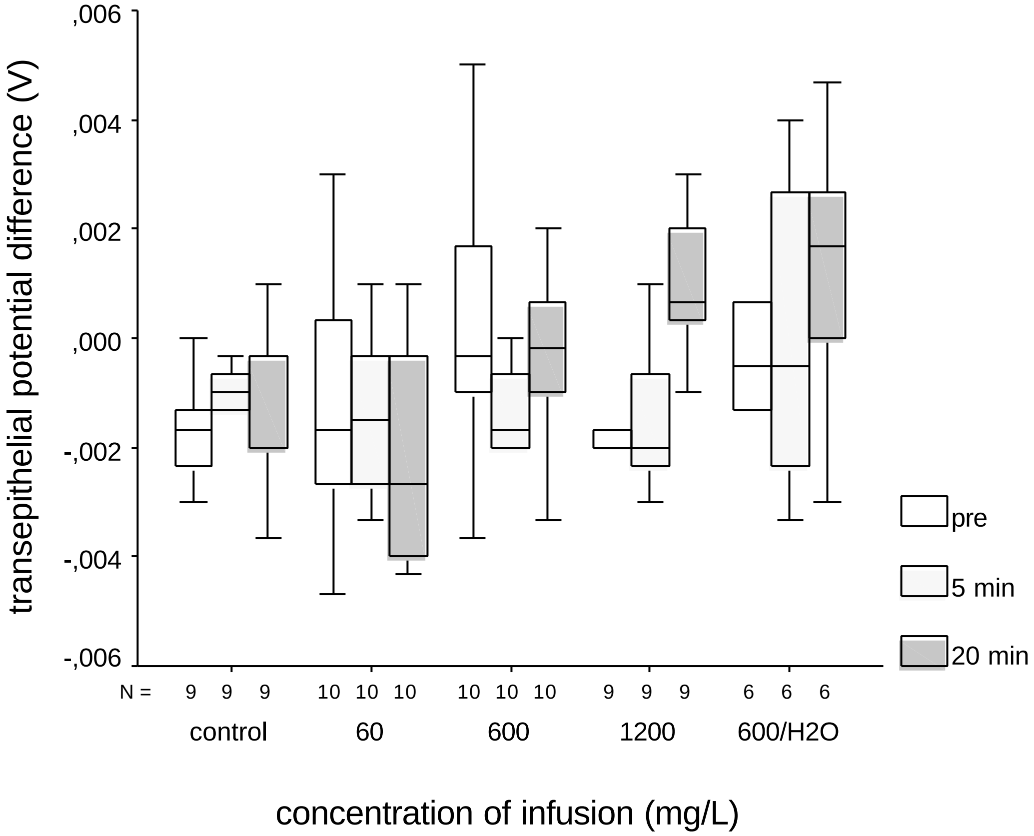

Figure 3 – Measures of transepithelial potential difference (PD)

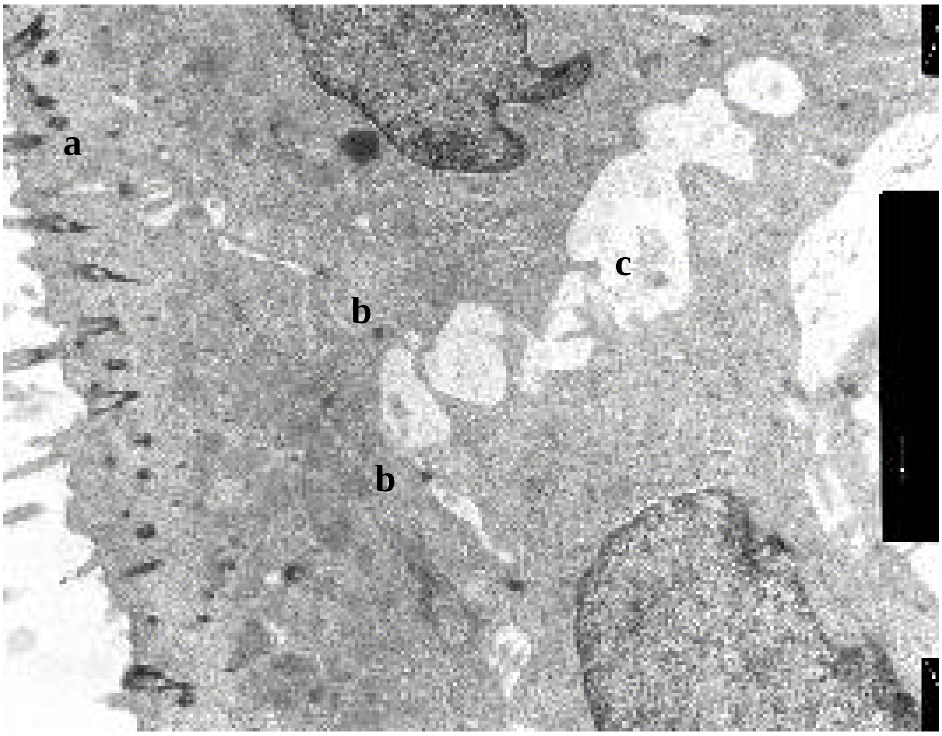

Figure 4 – Frog palate epithelium (group 5) showing tight juntion (a), desmossomes (b) and widened intercellular spaces (c). (Electron transmission microscopy, 40.000 X)

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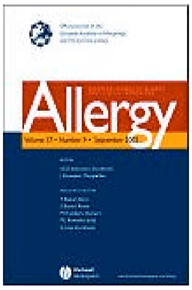

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