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Short Communication

Concerns Regarding the Toxicity of *Mentha x piperita*

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Abstract

While *Mentha x piperita L.* is a popular herb used in the northern region of Portugal to prepare tisanes the information about its possible toxicity is scarce. This Study was designed to assess the toxicity of the aqueous extract on the physiology, morphology and biochemistry of *Tetrahymena pyriformis*. Mint extract affected the generation time and growth in a dose-dependent manner. Also morphometric features of the cells of *T. pyriformis* are affected. The MTT assay showed a dose-dependent response of mint extracts.

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INTRODUCTION

The use of herbal medicines as alternative treatments has been increasing worldwide and gaining popularity in developing countries [1]. Following the popular believe that plants are less toxic and safer than manufactured drugs, *Mentha x piperita L.*, a hybrid of spearmint (*M. spicata L.*) and water mint (*M. aquatica L.*), is widely used in the northern region of Portugal, to prepare tisanes. Despite that most of the information available to the consumer with regard to the medicinal herbs is not backed by credible scientific data. Actually, the increase of the number of users as opposing to the scarcity of scientific evidences on the safety of the medicinal plants have raised concerns regarding toxicity and detrimental effects of these remedies [2]. *Tetrahymena pyriformis* is a protozoan which was confirmed to be a convenient model for the toxicological evaluation of various substances, such as carcinogens [3], inorganic and organic chemicals [4], bioflavonoids [5], phenol derivatives [6], pharmaceutical drugs, phototoxicity and environmental radiation evaluation [7]. Considering the significant use of *Mentha x piperita L.*, the present study aims to assess the toxic effects of the aqueous extract of this medicinal plant on the physiology, morphology and biochemistry of *T. pyriformis.*

MATERIALS AND METHODS

Plant extracts

Dried and powered plant material was submitted to maceration for 48h at room temperature in the dark. The aqueous extract was filtered through Whatman filter paper and then through 450 m carbonates filters, lyophilized and stored at 4º C until used.

Toxicological tests

All toxicological assays that used axenic *T. pyriformis* followed the culture conditions described elsewhere [9]. Exponentially growing cells (104 cells/ml) were used for inoculation. *M. piperita* was added to the cells at 53,8 mg/ml, 5,4 mg/ml; 0,54 mg/ml; 0,27 mg/ml; 0,054 mg/ml. Non-exposed cells to the extract were used as control. *T. pyriformis* cells were incubated with the extracts in 96-well h of treatment to check for the toxicity effect. The population growth impairment and generation time determination as well as the morphometric analysis (cell area width/length) were all performed following the procedure described by Dias et al., [9]. The MTT test was performed following the procedure described by Dias et al., [10].

RESULTS AND DISCUSSION

The results of toxicological tests using 24-h exposure time are summarized in Table (1). Addition of mint extract affected generation time and growth in a dose-dependent manner since an increase of extract concentration led to a decrease in *T. pyriformis* growth and an increase in generation time. A 24 h exposure to the lowest mint extract concentration did not significantly alter cell viability. However, when the concentration was raised to 0.54 mg/ml and to 5,4 mg/ml of mint extract highly significant decrease ($P < 0.01$) of *T. pyriformis* viability was observed. At the highest mint extract concentration, 53,8 mg/

Table 1: Effect of extract toxicity on generation time and viability in *Tetrahymena pyriformis* using 24-h exposure.

Concentration (mg/ml)	Generation time (h)	Viability (% of control)
Control	6,83±1,23	100±0,0
53,8	-	-
5,38	-	-
0,538	14,87±1,45	66,6±4,54
0,27	11,63±2,05	71,1±1,52
0,0538	7,43±1,19	96,5±,89
Each value is the mean of two independent assays ± standard deviation		

ml, there is no growth. Mint extracts effects were also observed in the morphometric features of the cells. The results for two independent assays on cell area and W/L ratio are shown in Figure (1). As seen, the lowest mint extract concentration did not cause significant differences in cell area. Instead, concentrations above 0,27 mg/ml of mint extract significantly influenced both area and W/L ratio during the complete experiment period: the cell size decrease and W/L ratio increase suggesting rounding off of the cells. According Kovács et al., [8], the rounding off of *T. pyriformis* is a sign of prospective death resulting from the changes occurred in microfilament architecture. Analysis of cytotoxicity by MTT assay showed a dose-dependent response of mint extracts. The experiments pointed for a decrease of dehydrogenate activity when the extract concentration raised up to 53,8 mg/ml and, at the lower concentration of 5,38 mg/mL the production of formazan as reductive metabolite of MTT (3-diphenyltetrazolium bromide) was suppressed.

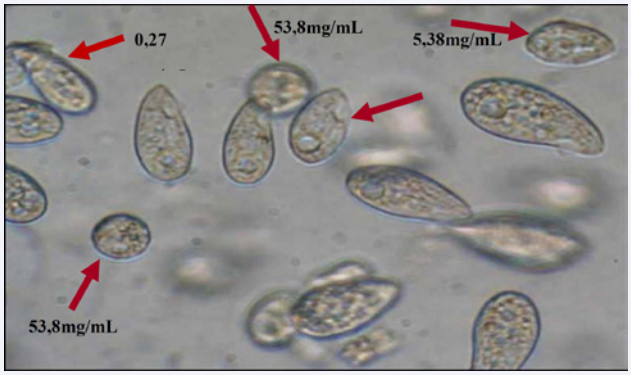

Figure 1 Effect of mint extracts on *T. pyriformis* morphology24h-experiment.

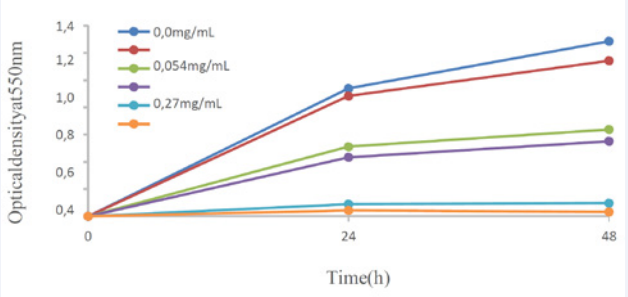

Figure 2 Effect of mint extracts on the MTT reduction.

CONCLUSION

Although medicinal plants are widely used and assumed to be safe they can potentially be toxic so the toxicity study of medicinal plants is essential. In the present study a battery of representative toxicological tests (physiological, morphological and biochemical), were used to provide global information for cytotoxicity of plant extract concentrations on the ciliate *T. pyriformis*. According to the results people can use this plant but they must use it in a moderate way.

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