

**PHARMACOLOGICAL ACTIVITY OF CAFFEINE ISOLATED FROM *Ilex paraguariensis* ON PEROXIDASE SECRETION IN RAT SUBMANDIBULAR GLANDS.**

**Anesini Claudia<sup>a</sup>, Turner Sebastian<sup>a</sup>, Manuele María Gabriela<sup>a</sup>, Ferraro Graciela<sup>ac</sup>, and Filip Rosana<sup>c</sup>.**

<sup>a</sup>IQUIMEFA (UBA-CONICET), <sup>c</sup>Pharmacognosy Unit, Faculty of Pharmacy and Biochemistry University Buenos Aires, Argentina. E-mail: [canesini@yahoo.com.ar](mailto:canesini@yahoo.com.ar)

**Summary**

Free radicals are involved in diseases such as tumoral, central nervous system alterations, immunological and inflammatory pathologies. Peroxidase is an oral enzyme implicated in the defense of oral cavity. In previous study the presence of caffeoyl derivatives and methylxantines such as caffeine in the aqueous extract of the *I. paraguariensis* was investigated by HPLC analysis. Caffeine, have been shown to modulate the cAMP pathway through inhibition of phosphodiesterases. In this study, we propose: 1-to investigate the influence of *I. paraguariensis* and the commercial product made with it "Yerba Mate", on peroxidase secretion in female rat submaxillary glands. 2- to isolate and quantify the caffeine present in both extracts. 3- to analyze the effect of the isolated caffeine on peroxidase secretion. 4- to investigate the mechanism of action evaluating the participation of cAMP. Peroxidase activity was determined by spectrophotometry in triplicate; caffeine was isolated and quantified by HPLC with Photodiode-Array Detector. *I. paraguariensis* and "Yerba mate" significantly increased the activity of secreted peroxidase, effective medium concentrations (EC<sub>50</sub>) (μg/ml) (*I. paraguariensis*: 841 ± 20; Yerba Mate: 148 ± 10). Caffeine was quantified in the aqueous extracts, results were expressed as g/100 g of dried plant material: *I. paraguariensis*: 1.06 ± 0.06; "Yerba Mate": 0.70 ± 0.06. The isolated caffeine increased peroxidase secretion in concentration-response relationship: EC<sub>50</sub> (μg/ml): 238 ± 15. This effect was blunted by imidazole a phosphodiesterase activator; stimulation index of secreted peroxidase with caffeine 1000 μg/ml: 5 ± 0,3; caffeine + imidazole (μg/ml): 2 ± 0,15 ). These results suggest that caffeine exerted an important role in the secretion of peroxidase induced by the aqueous extracts where cAMP could be one of the intracellular signals related in the caffeine action.

Key words: *Ilex paraguariensis*, Yerba Mate, rat submandibular peroxidase secretion

Reactive species of oxygen (ROS) are well known inducers of cellular and tissue pathogenesis leading to numerous disorders including periodontal disease (1) Peroxidase is one of the most important scavenger enzymes of the antioxidant system of submandibular glands. *Ilex paraguariensis*, fresh leaves and stems are industrialized and the commercial product obtained with it "Yerba Mate". is used in north-eastern Argentina, southern Brazil and eastern Paraguay to prepare a tea-like beverage named "Mate" (infusions and decoctions) that is consumed by 30 % of population at a rate of 1 L/day (2). Caffeine was detected in *Ilex paraguariensis* extracts. Caffeine has been shown to modulate the cAMP pathway through inhibition of phosphodiesterase and protein secretion.

Taking into account the widely popular consumption of "Yerba Mate" by South American population and the antioxidant activity previously reported, the aim of this study was: 1) to determine the effect of *I. paraguariensis* and "Yerba Mate" extracts on peroxidase secretion in submandibular glands. 2) to isolate and quantify caffeine from *Ilex* extracts and 3) to analyze the effect of the isolated caffeine on peroxidase secretion 4) to investigate the mechanism of action evaluating the participation of cAMP.

## Materials and Methods

### *Plant material, extract preparation and High Performance Liquid Chromatography.*

*Ilex paraguariensis* was collected from its original habitat (Corrientes, Argentina). Voucher specimen was deposited at the Museum of Pharmacobotany School of Pharmacy and Biochemistry, University of Buenos Aires under the BACP number: 502. Yerba Mate derived from a commercial sample. The material (10g) was boiled with water (200 ml) during 20 min and cooled at room temperature down to 40-45°C. After filtration, extracts were lyophilized yielding the following weights of aqueous crude extracts: *I. paraguariensis*, 1.25 g and “Yerba Mate” 1.30 g. Caffeine was quantified by HPLC methods using UV and photodiode array detector published by Filip et al (3,4).

### *Animals and submandibular gland preparation*

Female albino rats of the Wistar strain, weighing between 150-200 grams were used. Animals were used according to the Guide to the Care and Use of Experimental Animals (DHEW Publication, NIH 80-23). All experiments were performed on submandibular gland removed from female rats. Peroxidase was determined in supernatant of gland incubation medium and in the gland homogenate, spectrophotometrically (5).

### *Statistical analysis*

The Student “t” test for unpaired values was used to determine the levels of significance. When multiple comparisons were necessary, Dunnett’s test (6) was applied after ANOVA. Differences between means were considered significant if  $P < 0.05$ .

## Results

*Ilex paraguariensis* and “Yerba Mate” exerted peroxidase secretion on rat submandibular glands. “Yerba Mate” was more active in peroxidase secretion as is shown by its  $EC_{50}$  value in comparison with that of *I. paraguariensis* (Figure 1A and Table 1). A concentration – respond relationship was observed on caffeine action (Figure 1B). Caffeine was identified, quantified and isolated from *I. paraguariensis* extract and Yerba Mate (Table 2). In order to analyze the participation of cAMP in the effect of caffeine, the effect of imidazole, a phosphodiesterase activator on caffeine action was analyzed. The inhibitor blunted the effect of caffeine (Figure 1B).

Table 1.  $CE_{50}$  of *I. paraguariensis* and caffeine tested on rat submandibular gland peroxidase secretion.

Treatment	$CE_{50}$ ( $\mu\text{g/ml}$ )
<i>Ilex paraguariensis</i>	<b>841 <math>\pm</math> 20</b>
<i>Yerba Mate</i>	<b>148 <math>\pm</math> 10</b>
<i>Caffeine</i>	<b>238 <math>\pm</math> 15</b>

The results represent the media  $\pm$  SEM of three experiments performed by triplicate.

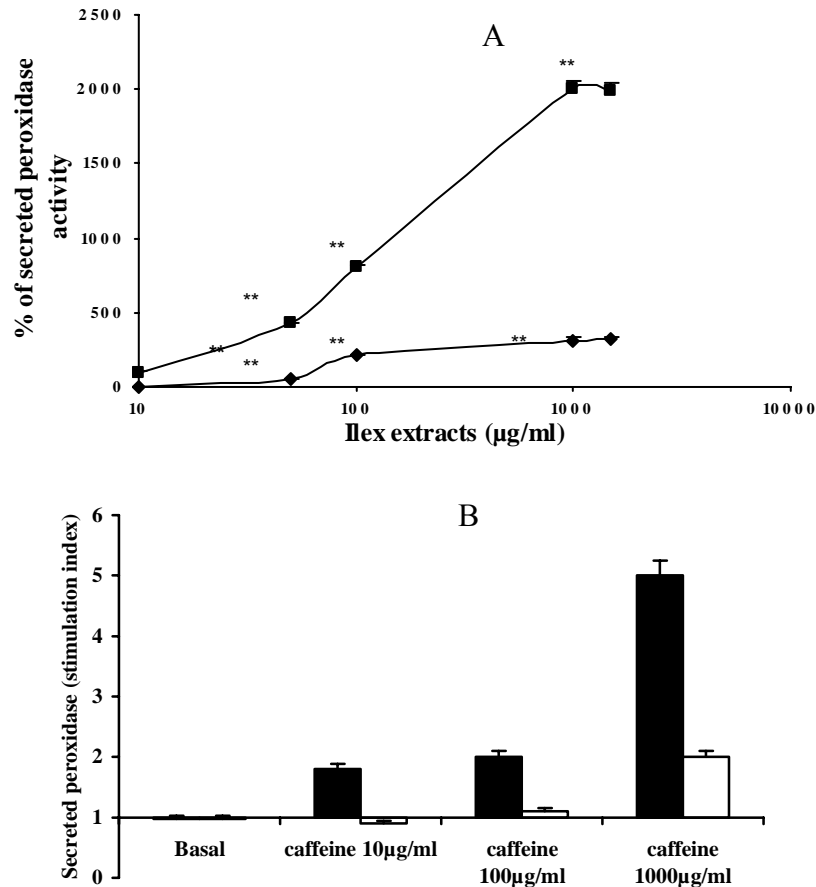


Figure 1 A and B: Effect of aqueous extract of *Ilex paraguariensis* and “Yerba Mate” (A) and caffeine (B) on peroxidase secretion in rat submandibular glands. In A Glands were incubated in presence of different concentrations of *I. paraguariensis* extract (◆), different concentrations of “Yerba Mate” (■) extracts. In B Glands were incubated in presence of different concentrations of caffeine alone (■) or in presence of imidazole (□) (1-1000 µg/ml). Results are expressed as % of secreted peroxidase or as stimulation index and represent the Media ± SEM of three experiments done by triplicate. \* p< 0.05; \*\* p< 0,01 with respect to basal values according to Dunnett test.

Table 2. Quantification of chlorogenic, caffeic acid and caffeine in *I. paraguariensis* and “Yerba Mate” extracts by HPLC.

Compound	<i>Ilex paraguariensis</i>	“Yerba Mate”
Caffeine	1.06 ± 0.06	0.70 ± 0.06*

The values are expressed in (g / 100 g dried plant material). The identification and quantification of the compounds, were carried on by HPLC, confronting the retention time and UV spectrum, obtained from diode array detector, with the retention times of standards substances. \* significantly differences of caffeine contents of “Yerba Mate” respect to caffeine content of *I. paraguariensis* (p< 0.05). Significantly differences accordingly to Dunnett’s test.

### Discussion

In this work, the effect of aqueous extracts of *Ilex paraguariensis* and "Yerba Mate" on the secretion of peroxidase of rat submandibular gland was demonstrated. The participation of caffeine in this action was also proved. The increase in peroxidase secretion was significantly concentration-dependent in both cases. The effect on peroxidase secretion with "Yerba Mate" was higher than that obtained with *I. paraguariensis*, as it can be concluded by the values of the maximum response and  $CE_{50}$  obtained. The power of induction of peroxidase secretion was 5.69 times higher for "Yerba Mate" than *I. paraguariensis*. The enhancement of supernatant peroxidase activity exerted by the extracts could be due, in principle, to an increase of secretion, enzyme activation or both processes.

The activity on peroxidase secretion exerted by *Ilex* extracts appeared to be related to the presence of caffeine. In the case of "Yerba Mate" activity, caffeine also played an important role in peroxidase secretion, but, other compounds present and formed during "Yerba Mate" industrial processing could also contribute to the high activity displayed by the extract. The mechanism of action of caffeine appeared to be related with the increase in cAMP levels as it has been shown to modulate the cAMP pathway through inhibition of phosphodiesterases. Imidazole a phosphodiesterase activator blunted caffeine activity.

Our results suggest that the extracts of *I. paraguariensis* and commercial "Yerba Mate" could be useful in the prevention of oral pathologies and are a promising source of natural antioxidants which will have potential chemoprotective action on oral tissues.

### Acknowledgement

This work was supported by funds provided by UBACYT B090 (Buenos Aires University).

### References

1. Shapira L, Borinski R, Sela M N, Soskolne A. Superoxide formation and chemiluminescence of peripheral polymorphonuclear leukocytes in rapidly progressive periodontitis patients. *Journal of Clinical Periodontology* 1991;18: 44-48.
2. Rosovsky, R. Contribución a la bibliografía de la yerba mate. Subsecretaría de Ciencia y Tecnología (SUBCYT) (Ed.), Buenos Aires 1983: 40.
3. Filip R, López P, Coussio J, Ferraro G. Mate Substitutes or Adulterants : Study of Xanthine Content. *Phytotherapy Research* 1983; 12: 129-131.
4. Filip R, López P, Giberti G, Coussio J, Ferraro G. Phenolic compounds in seven Southamerican *Ilex* species. *Fitoterapia* 2001; 72: 774-778.
5. Anesini C, Turner S, Borda E, Ferraro G, Coussio J. Effect of *Larrea divaricata* Cav. extract and nordihydroguaiaretic acid upon peroxidase secretion in rat submandibular glands. *Pharmacological Research* 2004; 49: 441-448.
6. Dunnett C. W. New table of multiple comparisons with a control. *Biometrics* 1964; 20: 482-491.