Inhibitory action of hesperetin on a venom metalloprotease from the Bothrops asper snake.

Henrique F. M de Oliveira*, Roney Vander dos Santos, Ljubica Tasic.

Abstract
A large portion of ophidian accidents in South America are provoked by snakes of the genera Bothrops and Crotalus, which have proteases that are zinc(II) dependent. Little is known in regard of flavonoids as inhibitors of metalloproteases, nevertheless, these secondary plant metabolites, such as hesperetin, can effectively bind with zinc(II). After obtaining the pure enzyme (BaP1) and tests that demonstrated the chelating effect of hesperetin with zinc(II) ions, we can propose that hesperetin can act as a possible inhibitor for metalloproteases that are zinc(II) dependent.

Key words: Metalloprotease, Snake venom, Hesperetin

Introduction
In Latin America, a large proportion of ophidian accidents is due to snakes of the Viperidae family, more specifically the genera Bothrops and Crotalus, which have zinc(II)-dependent metalloproteases in their venoms, among other enzymes and compounds.

Hesperetin is a bioflavonoid belonging to the class of flavonones. There are studies that report on the chelating effect of flavonoids on metal ions and inhibitory action on enzymes, as recently tested by the use of hesperetin and naringin as inhibitors of Chikungunya virus replication.

With this in mind, this research project aimed to understand the inhibitory potential of hesperetin with the BaP1 metalloprotease, present in the venom of the Bothrops asper (B. asper) snake.

Results and Discussion
Purification of the B. asper venom proteins included two liquid chromatography steps, one gel filtration (FPLC-DEAD) and the other by affinity (RP-HPLC), resulting in a pure protein named BaP1.

The purity of the protein was verified by gel electrophoresis, showing that BaP1 has a molar mass of around 22.8 kDa. Activity of this protein was verified using azocasein, a substrate which upon cleaving releases the azo group, a chromophore which can be analysed by UV-Vis at 595 nm.

Biophysical analyses were initiated, and spectra of circular dichroism and fluorescence were indicative for folded proteins.

Results obtained by UV-Vis show that there is a displacement of the absorbance of hesperetin (Hst) when mixed with a saturated solution of ZnCl₂ in organic medium (Figure 1), which may indicate that the hydroxyl groups at positions 5 and 7 of ring A of hesperetin chelate with the Zn²⁺ ions, contributing to the idea that it would be possible to delay the action of the metalloprotease by this Hst chelating effect.

Conclusions
With the present study and with the results of the hesperetin complexation test with the ZnCl₂ solution, it is possible to state that hesperetin has shown chelator properties in regard to Zn²⁺ ion, important for the BaP1 activity.


DOI: 10.19146/pibic-2017-78177

XXV Congresso de Iniciação Científica da UNICAMP