Supplementary Materials: Fraternine, a Novel Wasp Peptide, Protects against Motor Impairments in 6-OHDA Model of Parkinsonism

Andréia Mayer Biolchi, Danilo Gustavo Rodrigues de Oliveira, Henrique de Oliveira Amaral, Gabriel Avohay Alves Campos, Jacqueline Coimbra Gonçalves, Adolfo Carlos Barros de Souza, Marcos Robalinho Lima, Luciano Paulino Silva and Márcia Renata Mortari





Figure S1. Molecular mass determination of Fraternine. (a) Mass spectrometry analysis of peptide obtained in the reflected mode showing mass of $[M + H]^+ = 2748.5$. (b) Mass spectrometry analysis showed the adducts with Na⁺ and K⁺.



Figure S2. MS/MS sequencing of the ion $[M + H]^+ = 2478.5$. The y-ion and the b-ion series are shown. Sequencing de novo obtained from the peptide Fraternine. Performed by MALDI TOF/TOF in LIFT mode.



Figure S3. Mass analysis of the peptide after reduction and alkylation processes. (a) Mass spectrometry analysis of peptide obtained in the reflected mode showing mass of $[M + H]^+ = 2864.6$. (b) MS/MS sequencing of the ion $[M + H]^+ = 2864.6$. The y-ion and the b-ion series are shown.

Sequencing de novo obtained from the ion $[M + H]^+ = 2864.6$. Performed by MALDI TOF/TOF in LIFT mode.







Figure S4. Mass analysis of the peptide after reduction and alkylation processes and enzymatic digestion with GluC. (a) Mass spectrum after 1 h of addition of GluC. (b) Mass spectrum after 1.5 h of addition of GluC. (c) Mass spectrum after 3 h of addition of GluC. (d) Mass spectrum after 4.5 h of addition of GluC. (e) Mass spectrum after 5.5 h of addition of GluC.



Figure S5. MS/MS sequencing of the ion $[M + H]^+ = 978.6$. The y-ion and the b-ion series are shown. Sequencing de novo obtained from the fragment after GluC digestion. Performed by MALDI TOF/TOF in LIFT mode.





Figure S6. Mass analysis of the peptide after reduction and alkylation processes and enzymatic digestion with Trypsin. (a) Mass spectrum after 1 h of addition of Trypsin. (b) Mass spectrum after 3 h of addition of Trypsin. (c) Mass spectrum after 5.5 h of addition of Trypsin.









Figure S7. MS/MS sequencing of the ions after reduction and alkylation processes and enzymatic digestion with Trypsin. The y-ion and the b-ion series are shown. Sequencing de novo obtained from the fragment after GluC digestion. Performed by MALDI TOF/TOF in LIFT mode. (a) MS/MS sequencing of the ion $[M + H]^+ = 849.5$. (b) MS/MS sequencing of the ion $[M + H]^+ = 1106.6$. (c) MS/MS sequencing of the ion $[M + H]^+ = 1493.8$. (d) MS/MS sequencing of the ion $[M + H]^+ = 2219.2$. (e) MS/MS sequencing of the ion $[M + H]^+ = 2864.5$.



Figure S8. Toxicity test on the rota-rod R-TET assay. Latency to fall on the rota-rod apparatus was evaluated in SHAM animals (n = 9), 6-OHDA lesioned (n = 7) and animals receiving 10 µg of fraternine (n = 8) during a total of six hours to see if the peptide caused motor alterations in mice that prevented it to walk freely. Symbol # represents significative difference from the 6-OHDA group.