

Macrophage activation by *Paepalanthus* spp. extracts

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RESUMO: Avaliou-se a atividade antiinflamatória do extrato etanólico de própolis – EEP, sobre o edema desencadeado por carragenina, dextrana e histamina. O EEP apresentou dose eficaz (DE_{50}) de 650 mg/kg (v.o), inibindo significativamente o processo inflamatório desencadeado pela carragenina, mas não inibiu o produzido por dextrana. O EEP antagonizou ainda o efeito edematogênico produzido por histamina. Nas úlceras produzidas por estresse, o EEP inibiu de forma significativa a geração dos diversos tipos classificados. Em todos os parâmetros analisados no estudo da toxicidade em fase de tratamento subcrônico (hematológicos, bioquímicos e histopatológicos), o grupo tratado com o EEP não apresentou diferença significativa em relação ao grupo controle. Desta forma, sugere-se que na dose de 650 mg/kg (dose eficaz) não existe a presença de efeitos tóxicos que possam comprometer a utilização deste extrato.

Unitermos: extrato etanólico de própolis, antiinflamatório, antiúlcera gástrica, toxicidade.

ABSTRACT: Macrophages are known to play an important role in host defense mechanisms when these cells are stimulated with natural and bacterial products (and others). A variety of cytokines and chemicals compounds are released to induce fundamental defense system. Among them hydrogen peroxide (H_2O_2) has been identified as molecules having multi-functions. Moreover it has been established that H_2O_2 is involved in a number physiological processes, e.g., neurotransmission, smooth muscle relaxation or immune regulation. By the determination of hydrogen peroxide (H_2O_2) in peritoneal macrophage cultures of mice, we determined the immunomodulatory action of two extracts (ethanolic and 70% ethanolic) obtained of the four species to the *Paepalanthus* genus (Eriocaulaceae) in the concentration of 10 mg/mL. The 70% ethanolic extracts from capitula of *P. hilairei*, *P. robustus*, *P. vellozioides* and *P. speciosus* presented higher liberation of H_2O_2 than the ethanolic extracts.

Key words: Eriocaulaceae; *Paepalanthus*, macrophages; hydrogen peroxide

INTRODUCTION

Eriocaulaceae is a pantropical, predominantly herbaceous monocotyledon family, comprising around 1200 species in 10-14 genera, occurring mainly in sandy soils (Giulietti et al., 1995). In Brazil they are found in the “Cadeia do Espinhaço”, Bahia State and Minas Gerais State. Some species are exported due to their ornamental value, as they are called “everlasting flowers”. The genus *Paepalanthus* is endemic to the Serra do Cipó - MG-Brazil (Hensold and Giulietti, 1991; Giulietti et al., 1995). The aerial parts of many plants from this genus have been reported to contain flavonoids and naphthopyranones (Bate-Smith and Harbone, 1969; Vilegas et al., 1990, 1999; Andrade et al., 1999). Some investigation concerning the biological activity of the paepalantine, an isocoumarin isolated of capitula of *Paepalanthus vellozioides* has been done, and showed significant mutagenic, cytotoxic and antibiotic activities (Varanda et al., 1997).

Currently, there is a strong tendency to study natural compounds that may be involved in the modulation of the immunological system. A variety of materials derived from plants have been reported to stimulate the immune system (Barsucov et al., 1990; Kreher et al., 1990; Hase et al., 1997; Marques et al., 1999). The macrophages, being the first cells to participate in the immunological response, can be activated by a variety of stimuli and their principal functions include the phagocytosis of foreign particles, show the presence of antigens and the production of cytokines and intermediary compounds of oxygen (H_2O_2) and nitrogen (NO) (Halliwell and Gutteridge, 1984; Janeway, 1997). The use of colorimetric methods "in vitro" allow the measurement of the liberation of hydrogen peroxide (H_2O_2) in peritoneal macrophage cultures of mice (Kreher et al., 1990; Marques et al., 1999). In the present study, we have investigated the immunomodulatory activity of eight extracts, obtained from four species of *Paepalanthus* (Eriocaulaceae).

MATERIALS AND METHODS

Plant material

The plants utilized in this study were collected at the "Serra do Cipó"- Minas Gerais State-Brazil and were identified by the Professor Dr. Paulo Takeo Sano from Instituto de Biociências da Universidade de São Paulo. The voucher of each species was deposited in the Herbarium of the Instituto de Botânica, USP - São Paulo (*P. hilairi* Koern.-CFSC 13843, *P. robustus* Silveira - CFSC 13840, *P. vellozioides* - CFSC 13842, *P. speciosus* (Bong.) Koern.- CFSC 8486).

Preparation of the extracts

Capitula of each species were dried in oven at 60°C for 1 week and powdered. The resulting material was macerated at room temperature using ethanol and 70% ethanol for 1 week in each solvent. The solvents were evaporated under reduced pressure affording the corresponding extracts.

Determination of hydrogen peroxide (H_2O_2) liberation

Using cell culture experiments and plant extracts, the liberation of reactive compounds of oxygen (Pick and Keisari, 1980; Pick and Mizel, 1981) was determined. The method consists in the determination of liberation of hydrogen peroxide (H_2O_2) in the culture of peritoneal macrophages from Swiss mice. Suspensions of peritoneal cells were performed using a concentration of 2×10^6 cells/mL in a solution of phenol red, containing 140 mM NaCl, 10 mM potassium phosphate, pH 7.0; 5.5 mM dextrose; 0.56 mM phenol red and type II horseradish peroxidase 0.01 mg/mL (Sigma). Aliquots of 0.1 mL were transferred to culture plates, flat bottomed containing 96 wells (Corning). To each well was added either 50 μ L of the extract solution (10 mg/mL in dimethyl sulphoxide) or 50 μ L of the Zymosan solution (5 mg/mL, Sigma) or 50 μ L of dimethyl sulphoxide. The samples were incubated for one hour at 37 °C in a humid atmosphere. After the period of incubation, the reaction was interrupted by the addition of 10 μ L of NaOH 4N. Experiments were done in quadruplicate. Absorbance was determined in a ELISA automatic photometer, with a 620 nm filter. The results were expressed in nanomoles of H_2O_2 2×10^5 peritoneal cells, from a standard curve established in each test, constituted of known molar concentrations of H_2O_2 in buffered phenol red.

Statistical analysis

Data are expressed as mean \pm Standard Deviation, and Student's *t*-test was used to determine the significance of the differences between the control and experimental groups ($p < 0.05$).

RESULTS AND DISCUSSION

Reactive oxygen metabolites (H_2O_2) have been suggested as potentially important signaling

molecules in both intra-and intercellular reactions in a number different cell types (Dirsch et al., 1997).

There is an increasing evidence that H₂O₂ plays a complex role in the modulation of the inflammatory response (Dirsch et al., 1997). Inflammatory macrophages secrete large amount of mediators that control the initiating process of inflammation (Fushiya et al., 1998).

In this study, the macrophages stimulation was determined through the determination of H₂O₂ release after stimulation with Zymosan and plant extracts.

From the results presented in Figure 1, it can be observed that all tested extracts presented significative liberation of H₂O₂ in the concentration of 10 mg/mL. The activities were 1.2 - 2.4 times greater than that of Zymosan, strongest immunomodulator known. Flavonoids and naphthopyranones are present in all assayed extracts (Table 1).

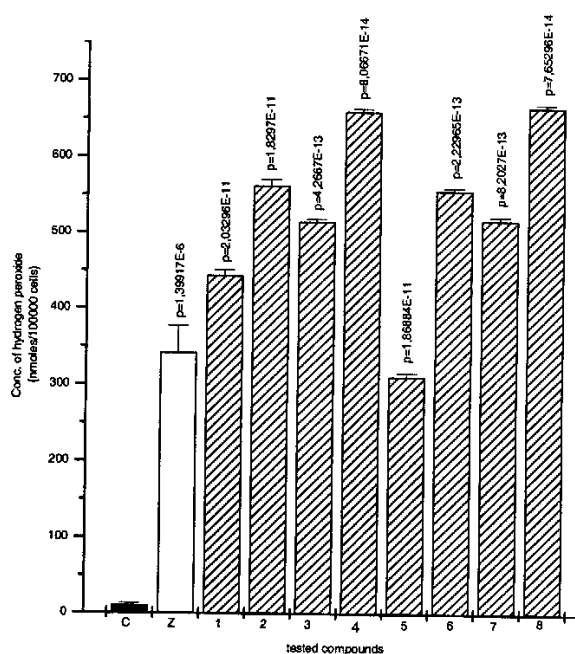


FIGURE 1. Production of H₂O₂ by peritoneal macrophage cell culture with control DMSO (dimethyl sulphoxide) (C), Zymosan (250 mg / well) (Z) and plant extracts (500 mg/well):

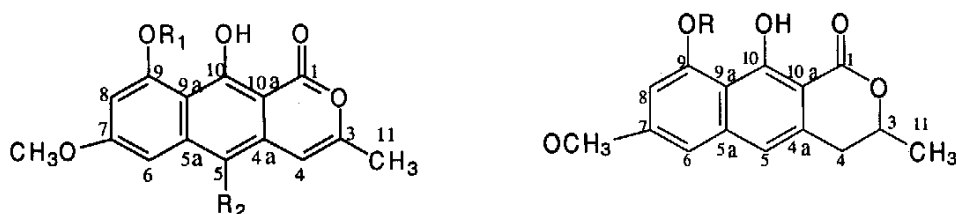
1. *P. hilairei* (ethanol); 2. *P. hilairei* (70% ethanol); 3. *P. robustus* (ethanol); 4. *P. robustus* (70% ethanol);
5. *P. vellozioides* (ethanol); 6. *P. vellozioides* (70% ethanol); 7. *P. speciosus* (ethanol); 8. *P. speciosus* (70% ethanol).

Each bar represent the mean ± SD of four animals. All the results were statistically significant when compared with the control group and Zymosan (p < 0,05).

TABLE 1. Distribution of compounds in *Paepalanthus* species. For numbers, see chemical structures of Figure 2.

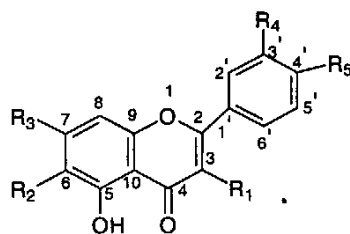
Plant	Flavonoids	Naphthopyranones	References
<i>P. hilarei</i>	6, 7, 9, 10	1, 2	Andrade et al. (1999)
<i>P. vellozioides</i>	7, 8, 10, 11, 12	1, 2	Andrade et al. (1999)
<i>P. robustus</i>	6, 13, 14, 15, 16	1, 2	Vilegas et al. (1999)
<i>P. speciosus</i>	7	3, 4, 5	Study in progress

1. Paepalantine-9-O- β -D-glucopyranoside; 2. Paepalantine-9-O- β -D-allopyranosylglucopyranoside; 3. 9-O- β -D-glucopyranosyl-5-desmethoxy-paepalantine; 4. Paepalantine; 5. 9-O- β -D-glucopyranosyl-5-desmethoxy-3,4-dihydropaepalantine; 6. 6-methoxykaempferol; 7. 6-methoxykaempferol-3-O- β -D-glucopyranoside; 8. Patuletin; 9. Patuletin-3-O- β -D-rutinoside; 10. Quercetagetin-7-O- β -D-glucopyranoside; 11. 5,7,4'-trihydroxy-6,3'-dimethoxyflavone; 12. 5,7,4'-trihydroxy-6,3'-dimethoxyflavonol; 13. 6-methoxykaempferol-3-O- β -D-6'-(*p*-coumaroyl)glucopyranoside; 14. 6-methoxyquercetin-3-O- β -D-6'-(*p*-coumaroyl)glucopyranoside; 15. Quercetagetin; 16. Patuletin-3-O- β -D-glucopyranoside



- (1) $R_1 = \text{glc}$, $R_2 = \text{OCH}_3$
(2) $R_1 = \text{glc (1} \rightarrow 6\text{)allo}$, $R_2 = \text{OCH}_3$
(3) $R_1 = \text{glc}$, $R_2 = \text{H}$
(4) $R_1 = \text{H}$, $R_2 = \text{OCH}_3$
(5) $R = \text{glc}$

gluc: glucose; allo: allose; rha: rhamnose; coum: coumaroyl



- (6) $R_1 = \text{OH}$, $R_2 = \text{OCH}_3$, $R_3 = \text{OH}$, $R_4 = \text{H}$, $R_5 = \text{OH}$
(7) $R_1 = \text{Oglc}$, $R_2 = \text{OCH}_3$, $R_3 = \text{OH}$, $R_4 = \text{H}$, $R_5 = \text{OH}$
(8) $R_1 = \text{OH}$, $R_2 = \text{OCH}_3$, $R_3 = \text{OH}$, $R_4 = \text{OH}$, $R_5 = \text{OH}$
(9) $R_1 = \text{Oglc (1} \rightarrow 6\text{)rha}$, $R_2 = \text{OCH}_3$, $R_3 = \text{OH}$, $R_4 = \text{OH}$, $R_5 = \text{OH}$
(10) $R_1 = \text{OH}$, $R_2 = \text{OH}$, $R_3 = \text{glc}$, $R_4 = \text{OH}$, $R_5 = \text{OH}$
(11) $R_1 = \text{H}$, $R_2 = \text{OCH}_3$, $R_3 = \text{OH}$, $R_4 = \text{OCH}_3$, $R_5 = \text{OH}$
(12) $R_1 = \text{OH}$, $R_2 = \text{OCH}_3$, $R_3 = \text{OH}$, $R_4 = \text{OCH}_3$, $R_5 = \text{OH}$
(13) $R_1 = p\text{-coum (1} \rightarrow 6\text{)glc}$, $R_2 = \text{OCH}_3$, $R_3 = \text{OH}$, $R_4 = \text{H}$, $R_5 = \text{OH}$
(14) $R_1 = p\text{-coum (1} \rightarrow 6\text{)glc}$, $R_2 = \text{OCH}_3$, $R_3 = \text{OH}$, $R_4 = \text{OH}$, $R_5 = \text{OH}$
(15) $R_1 = \text{OH}$, $R_2 = \text{OCH}_3$, $R_3 = \text{OH}$, $R_4 = \text{OH}$, $R_5 = \text{OH}$
(16) $R_1 = \text{Oglc}$, $R_2 = \text{OCH}_3$, $R_3 = \text{OH}$, $R_4 = \text{OH}$, $R_5 = \text{OH}$

FIGURE 2 - Chemical structures of compounds isolated from *Paepalanthus* species.

Our results do not allow us to assign the observed activities to a specific compound or class of compounds. However, we could observe that the 70% ethanol extracts of the capitula from all species presented higher activities than those of the ethanol extracts, with maximum activity for *P. speciosus*. Even though, regarding *Paepalanthus* extracts, previous experiment indicated that molecules with two units of sugars are predominant in the 70% ethanol extracts, whereas in the ethanol extracts molecules with one sugar unit, or their aglicones, are the major compounds found (Andrade et al., 1999; Villegas et al., 1999). Further studies with the isolated compounds are in progress in our laboratory.

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REFERENCES

- ANDRADE, F.D.P.; SANTOS, L.C.; DOKKEDAL, A.L.; VILEGAS, W. Acyl glucosylated flavonols from *Paepalanthus* species. **Phytochemistry**, 51: 411-415, 1999.
- BARSUCOV, A.; VETOSHKIN, A.; VORONTOV, E.; DUBICHEV, A. Macrophage activation by sanquiritrine. **Planta Med.**, 56: 696- 697, 1990.
- BATE-SMITH, E.C.; HARBONE, J.B. Quercetagenin and patuletin in *Eriocaulon*. **Phytochemistry**, 8: 1035-1037, 1969.
- DIRSCH, V. M.; KIEMER, A. K.; WAGNER, H.; VOLLMAR, A. M. The triterpenoid quinonemethide pristimerin inhibits induction of inducible nitric oxide synthase in murine macrophages. **Eur. J. Pharmacol.**, 336: 211-217, 1997.
- FUSHIYA, S.; KABE, Y.; IKEGAYA Y.; TAKANO, F. (+)-Rhododendrol and *epi*-Rhododendrin suppress the NO production by activated macrophages *in vivo*. **Planta Med.**, 64: 598-602, 1998
- GIULIETTI, A.M.; AMARAL, M.C.E.; BITTRICH, V. Phylogenetic analysis of inter-and infrageneric suppress the NO production by activated macrophages *in vivo*. **Planta Med.**, 64: 598-602, relationships of *Leiotrix* Ruhl. (Eriocaulaceae). **Kew Bulletin**, 50: 55-60, 1995. **Biological Abstracts**, 100 (9), 129831 UD 9504.
- HALLIWELL, B.; GUTTERIDGE, J.M.C. Oxygen toxicity, oxygen radicals, transition metals and disease. **Biochem. J.**, 219: 1-14, 1984.
- HASE, K.; BASNET, P.; KADOTA, S.; NAMBA, T. Immunostimulating activity of Celosian, na antihepatotoxic polysaccharide from *Celosia argentea*. **Planta Med.**, 63: 216-219, 1997.
- HENSOLD, N.C.; GIULIETTI, A.M. Revision and redefinition of the genus *Rondonanthus* Herzog (Eriocaulaceae). **Miss. Bot. Gard**, 78: 441-451, 1991. **Biological Abstracts**, 92 (2)1335 UD 9103.
- JANEWAY, C.J. **Immunobiology: the immune system in health and disease**. In: Travers P Editor. Garland Publishing Inc., New York, 1997.
- KREHER, B.; NESZMÉLYI, A.; JURCIC, K.; WAGNER, H. New phytochemical and immunological investigations of *Euphorbia resinifera* and *Daphne mezereum*. **Planta Med.**, 56: 572-573, 1990.
- MARQUES, M.F.; ARAUJO, A.R.; BOLZANI, V.A.R.; YOUNG, M.C.M.; PLACERES, M.C.P. Determinação dos compostos reativos do hidrogênio e do nitrogênio frente aos constituintes químicos de *Styrax ferrugineus*. Jornada Farmacêutica da UNESP. **ANAIS**: resumo 65, 1999.
- PICK, E.; KEISARI, Y. A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. **J. Immunol. Met.**, 38: 161-170, 1980.
- PICK, E.; MIZEL, D. Rapid microassays for the measurement of superoxide and hydrogen peroxide production by macrophages in culture using an automatic enzyme immunoassay reader. **J.**

Immunol. Met., 46: 211-226, 1981.

VARANDA, E.A.; RADDI, M.S.G.; DIAS, F.L.; ARAUJO, M.C.P.; GIBRAN, S.C.A.; TAKAHASHI, C.S.; VILEGAS, W. Mutagenic and cytotoxic activity of one isocoumarin (paepalantine isolated from *Paepalanthus vellozioides*. **Terat. Carcin. Mutag.**, 17: 85-95, 1997.

VILEGAS, W.; DOKKEDAL, A.L.; PIACENTE, S.; RASTRELLI, L.; PIZZA, C. New naphthopyranone glycosides from *Paepalanthus vellozioides* and *Paepalanthus latipes*. **J. Nat. Prod.**, 62: 5: 746-749, 1999.

VILEGAS, W.; ROQUE, N.F.; SALATINO, A.; GIESBRECHT, A.M.; DAVINO, S. Isocoumarin from *Paepalanthus bromelioides*. **Phytochemistry**, 29: 2299-2301, 1990.

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