

## Evaluation of Antitumoral Activity of a Fraction of Water-Soluble Components of the Edible Mushroom *Pleurotus ostreato-roseus*

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**SUMMARY.** A water-soluble fraction was obtained from the pink oyster mushroom *Pleurotus ostreato-roseus* and tested on NCI-H292 cell culture and in vivo Sarcoma 180. Non apparent toxic effect was observed in cell culture. However, an increase on cell protein content was observed in treated cells indicating a possible mitogenic effect. The group of treated mice showed clinical signals suggesting toxicity and have mortality started earlier than non treated group. However, in the treated mice was verified a 41.96% reduction on tumor. These results that suggest this water soluble fraction has a pharmacological potential in tumor therapy and point to the necessity of further researches.

**RESUMEN.** "Evaluación de la actividad antitumoral de una fracción de componentes extraíbles en agua del hongo comestible *Pleurotus ostreato-roseus*". Una fracción extraíble con agua fue obtenida del hongo comestible *Pleurotus ostreato-roseus* y ensayada in vitro en cultivo de células NCI-H292 e in vivo contra el Sarcoma 180. No se verificaron efectos tóxicos en el cultivo celular. Se observó un aumento en la concentración de proteína indicando un posible efecto mitógeno. Los ratones tratados mostraron señales clínicas que indican toxicidad y tuvieron mortalidad antes que el grupo no tratado. Los ratones que recibieron la fracción tuvieron una reducción promedio de 41,96% en el crecimiento del tumor en comparación con los no tratados. Estos resultados califican la fracción estudiada como un fármaco potencial, señalando la necesidad de mayores investigaciones.

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### INTRODUCTION

The term mushroom refer to the macroscopic structure of some fungi, mainly those belonging to the division Basidiomycotina, class Hymenomycetes, order Agaricales <sup>1</sup>.

Since remote ages, plants and fungi traditionally has been used as source of treatment of a great number of diseases for their healthful and therapeutic properties <sup>2</sup>. The fruit bodies of the edible mushroom of genus *Pleurotus* has attracted attention of researchers for their nutritional and medicinal values. *Pleurotus* mushrooms are source of carbohydrates, fibers, essential amino acids, minerals and complex B vitamins, particularly folic acid <sup>3</sup>. In regard to medicinal properties, *Pleurotus* fruitbodies contain mevinolin (lovastatin), a substance of low molecular weight

the act as competitive inhibitor of 3-hydroxy-3-methyl-glutaryl-Coenzyme-A-reductase, decisive enzyme of cholesterol metabolism <sup>4,5</sup>. Besides their hypolipidemic activity, the species belonging this genus show antitumoral properties, attributed to structural polysaccharides of cell wall <sup>6-10</sup>, and antioxidants and cancer chemopreventive properties <sup>11</sup>. Mice treated with the carcinogen N-butyl-N'-butanolnitrosoamine and mushrooms (*Lentinus edodes*, *Grifola frondosa* or *Pleurotus ostreatus*) showed reduction in the formation of urinary bladder carcinoma <sup>12</sup>. The antitumoral activity of mushroom cell wall polysaccharides is related to the biomodulation of host immune system by macrophages and natural killer cells and cytokine liberation <sup>12-14</sup>.

In the present work a water-soluble fraction

**KEYWORDS:** Antitumoral activity, Edible mushroom, *Pleurotus ostreato-roseus*, Water-soluble fraction.

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of the edible mushroom *Pleurotus ostreato-roseus* Sing. was tested against culture of human neoplastic cell and laboratory tumor implanted in mice. This mushroom belong to pink mushroom complex of *Pleurotus djamor* (Fr.) Boedjin, found in tropical and subtropical regions of many countries<sup>15</sup> and is common in Brazil's rainforest. *P. ostreato-roseus* is easily cultivated in a large variety of lignocelulosic materials<sup>16</sup> and has a good acceptability in market, where is named salmon mushroom. However, little information about their medicinal properties have been reported. Recently, water-soluble fractions obtained from this mushroom have been described that altered eletrophoretic profile serum proteins of mice<sup>17</sup>.

## MATERIAL AND METHODS

### Fraction extraction

The mushrooms utilized for producing the fractions were purchased from a commercial producer located in Paudalho town, "zona da mata" of Pernambuco State, Brazil. The mushrooms were harvested and immediately dried at 60 °C during 24 h and extracted according a protocol of Zhuang *et al*<sup>6</sup>. For *in vitro* and *in vivo* tests, the low molecular weight fraction (FS-POR), non precipitable by ethanol, was chose. This fraction, when obtained from others *Pleurotus* species by the same protocol has not been tested to its bioactivity by others authors. The water-soluble fraction was analyzed in relation to total solids in digital balance coupled to an infrared device (Mod. GEHAKA 440) and spectrophotometrically to total carbohydrate<sup>18</sup> and protein<sup>19</sup> contents.

### Citotoxicity assay

The *in vitro* test of citotoxicity was carried out with cell culture with purpose to find the concentration to inhibit 50% of cell growth (CI50) to be employed as criteria for test *in vivo*<sup>20</sup>. This *in vitro* assay was done in cultures of NCI-H292 cells, originated from human lung carcinoma and maintained in The Cell Culture Laboratory of Department of Antibiotics of the Pernambuco Federal University. Cell culture maintenance and test assay were done in culture media (DMEM - Sigma) supplemented with 10% (v/v) bovine fetal serum (Gibco), 1% (v/v) 200 mM Glutamine and 1% (v/v) antibiotic solution, containing 100,000 IU/mL G-potassic penicillin (Purex) and 25 mg/mL Streptomycin sulfate (Purex).

Before test realization, cellular viability was

determined by Tripan Blue exclusion method<sup>21</sup> and a cell suspension of  $5 \times 10^4$  cells/mL was prepared to be used. The cell suspension was distributed into 24 wells cell culture plates (Nunclon®), and the FS-POR fraction, previously diluted in 0.85% NaCl (w/v) and added to cell culture until final concentrations of 5, 50 and 500 µg/mL. As negative control was used 0.85% NaCl (w/v). The plates were incubated at 37 °C under humid atmosphere with 5% CO<sub>2</sub> by 72 h. The plates were observed daily at optical inverse microscope to check cell development by evaluation of color changes in culture medium, cell adherence and formation of cell clones.

After incubation time the culture media was aspirated, cell washed and lysed as previously described by Sousa *et al.*<sup>22</sup>. Toxic effect of FS-POR was determined by the content of total protein of lysed cells according Bradford<sup>19</sup>, comparing values of absorbance (595 nm) against a standard curve of bovine serum albumin (Sigma).

### Antitumoral activity

After realization of *in vitro* tests, the dose 400mg/kg was chose for test *in vivo*, according protocols of Geran *et al.*<sup>20</sup> for tests of products which toxicity is unknown. The FS-POR fraction was tested in male swiss albino mice aging between 45 and 60 days, transplanted with solid sarcoma 180 (S-180) in right axillary region according Sigiura<sup>23</sup>. The animals were maintained under proper environmental conditions, *i.e.*, temperature  $25 \pm 2$  °C, and humidity  $50 \pm 5$  % with a 12 h light/dark period. Before starting treatment, the animals were weigh, identified and raffled into treated and control groups with 8 animals each one. The groups were kept in individual cages and supplied with water and feed *ad libitum*. The animals of treated group received two doses of 0.1 mL / 10g weight containing 400 mg/kg each dissolved in 0.85% NaCl (w/v) at intervals of 7 days. Animals of control group received equivalent volume of 0.85% NaCl solution. Both groups were observed daily for calculation of survival time and weighed and the tumors measured weekly with a calliper rule device until occurrence of first death. The approximate weight of tumors was obtained by means mensuration (length x width) and calculated by the equation 1, according Geran *et al.*<sup>20</sup> and tumoral inhibition calculated by the equation [2], according Machon *et al.*<sup>24</sup>, showed below.

$$TW = \frac{l \times w}{2} \quad [1]$$

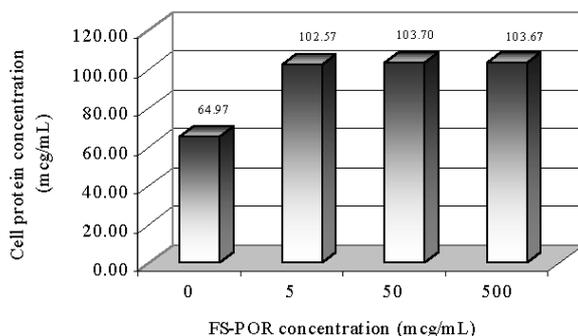
where: *TW* = tumor weight (mg); *l* = tumor length (mm); *w* = tumor width (mm<sup>2</sup>)

$$TWI(\%) = \frac{MCTW - MTTW}{MCTW} \times 100 \quad [2]$$

where: *TWI* = percent tumor weight inhibition; *MCTW* = mean control tumor weigh; *MTTW* = mean treated tumor weight.

### RESULTS AND DISCUSSION

The FS-POR fraction showed 27 mg/mL total solid dissolved, with 47.67% carbohydrates and 2.07% total protein. Throughout the citotoxicity assay non culture alterations, suggesting toxicity were observed. The fraction did not interfere on the cell adherence. The culture features were identical both treated and non treated cells. However, an increase of number of clones was observed in the treated cells in comparison to non treated. The average protein contents of treated and control cells are showed in Fig. 1.

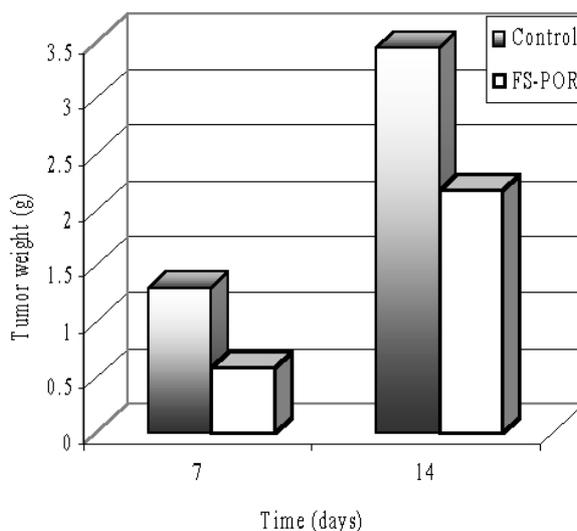


**Figure 1.** Cell protein concentration of NCI-H292 cell culture incubated with 0, 5, 50 and 500 µg/mL FS-POR.

In all FS-POR concentrations tested was verified a statistically significant increase ( $p < 0.001$ ) in the cell protein concentration in comparison to control cells. In comparing the FS-POR concentrations itself, non statistical significance ( $p > 0.05$ ) was observed, suggesting that FS-POR effect in non concentration dependent. These results associate to culture characteristics, suggest the FS-POR played a stimulative effect on cell proliferation. Mytogenic activity also observed in other mushroom species. Mizuno *et al.*<sup>25</sup>, analyzing splenic lymphocytes of mice treated with water soluble fraction of *Agaricus blazei*, veri-

fied an increase in populations of CD<sup>4</sup>, helper-T, CD<sup>8</sup> and T-citotoxic cells. Similar results were found by Song *et al.*<sup>26</sup> in observing a proliferative effect of polysaccharides extracted from the mushroom *Plellinus linteus* on B-lymphocytes *in vitro*. Although non toxic effect on the neoplastic cells has been observed in this work, this results were considered promising, since the antitumoral activity of mushroom derivatives do not occurs directly on the tumoral tissue, but by stimulating the host immune response<sup>27</sup>.

The animals treated with FS-POR showed a slight apathy, bristly hair, less weight gain, and mortality started earlier than control group. There was not statistically significant difference ( $p > 0.05$ ) between the average survival time of treated ( $22.64 \pm 1.6$  days) and non-treated ( $24.10 \pm 1.4$  days) groups. The clinic signals of suggestive toxicity, which were not observed *in vitro*, may be possible due a systemic effect of the FS-POR components or its metabolites as consequence of the via of administration. In regards to tumor inhibition, it verified a reduction of 41.96% on tumor growth treated with FS-POR in comparison to control group (Fig. 2). This difference was considered statistically significant ( $p < 0.01$ ). Lima *et al.*<sup>17</sup> observed that the same fraction, when administrated in unique dose to mice, caused an increase of 50, 25, 0.35 and 28.6% in eletrophoretic bands corresponding to  $\beta$ -,  $\alpha$ -1-,  $\alpha$ -2- and  $\gamma$ -globulin serum, respectively, suggesting that tumor inhibition resulted from the effect of the fraction on immune system. However, complementary studies will be neces-



**Figure 2.** Tumor weight 7 and 14 days after injection of two doses of 400 mg/kg FS-POR in mice.

sary to ensure it. Mizuno *et al.* <sup>28</sup> observed 24.5% inhibition on S-180 and only 16.5% mortality rate after six week implantation with administration of equivalent fraction obtained from the mushroom *Polyporus confluens*.

## CONCLUSIONS

In spite of the promising results obtained in this work, it is necessary further researches in order to characterize the chemical constituents of *P. ostreato-roseus* and its bioactivity, once this mushroom is an easily cultivable mushroom that may be used as an alternative source of nutritional supplement, functional food and pharmaceuticals.

## REFERENCES

1. Miles, P.G & S.-T. Chang (1997) "*Mushroom biology: concise basics and current development*", World Scientific, Singapore, pp. 4-5.
2. Hayes, W.A. & S.H. Wright (1979) "*Edible mushrooms*", in "*Economic microbiology: microbial biomass*" (A.H. Rose, ed.), Academic Press, London, Vol. 4, pp. 168-9.
3. Chang, S.T. & J.A. Buswell (1996) *World J. Microbiol. Biotechnol.* **12**: 473-6.
4. Bobek, P. & L. Ozdin (1994) *Physiol. Res.* **43**: 205-6.
5. Gunde-Cimerman, M. & A. Cimerman (1995) *Exper. Mycol.* **19**: 1-6.
6. Zhuang, C., T. Mizuno, A. Shimada, H. Ito, C. Suzuki, Y. Mayuzumi, H. Okamoto, Y. Ma & J. Li (1993) *Biosci. Biotech. Biochem.* **57**: 901-6.
7. Li, H., L. Zang, N. Zhang, J. Cao & Y. Zhang (1994) *Yaowen. Shengwu. Jishu.* **1**: 35-8
8. Zhang, J., G. Wang, H. Li, C. Zhuang, T. Mizuno, H. Ito, C. Suzuki, H. Okamoto & J. Li (1994) *Biosci. Biotech. Biochem.* **58**: 1195-1201.
9. Wang, H., J. Gao & T.B. Ng (2000) *Biochem. Biophys Res. Commun.* **275**: 810-6.
10. Zhang, M., P.C. Cheung & L. Zhang (2001) *J. Agric. Food. Chem.* **49**: 5059-62
11. Bobek, P. & S. Galbavy (2001) *Br. J. Biomed. Sci.* **58**: 164-8.
12. Kurashige, S., Y. Akuzawa & F. Endo (1997) *Immunopharmacol. Immunotoxicol.* **19**: 175-83.
13. Hersh, E.M. & C.W. Taylor (1991) "Immunotherapy by active immunization: use of nonspecific stimulants and immunomodulators" en "Biology therapy of cancer" (V.T. De Vita, S., Hellmann & S.A. Rosemberg, eds.). J.B. Lippincott, Philadelphia, pp. 111-6.
14. Chihara, G. (1993) "Medicinal aspects of lentinan isolated from *Lentinus edodes* (Berk.) Sing", in "*Mushroom Biology and Mushroom Products*" (S.T. Chang, J.A. Buswell & S.W. Chiu, eds.), Chinese University Press, Hong Kong, pp. 261-6.
15. Stamets, P. (1993) "*Growing gourmet and medicinal mushrooms*", Tem Speed Press, Berkeley, pp. 297-303.
16. Correia, M.J. (1998) "Caracterização de *Pleurotus Kummer* quanto à produção de celulasas e cogumelos comestíveis". Tese de Doutorado. Universidade Federal de Pernambuco, Recife-PE, pp. 23-5.
17. Lima, N.B., M.R.Q. Sousa, O.G. Rodrigues & M.J. Correia (2002) *Rev. Bras. Farmacogn.* **12**: 36-7.
18. Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers & F. Smith (1956) *Anal. Chem.* **28**: 350-6.
19. Bradford, M.M. (1976) *Anal. Biochem.* **72**: 248-254
20. Geran, R.I., N.H. Greenberg, M.M. Macdonald, A.M. Schumacher & B.J. Abbott (1972) *Cancer Chemoth. Rep.* **3**: 17-56
21. Weisenthal, L.M., Marsden, J.A., Dill, P.L. & C.K. Macaluso (1983) *Cancer Res.* **43**: 749-57
22. Sousa, M.R.Q., Nascimento, S.C., Rodrigues, O.G., Medeiros, P.L. & J.M. Araújo (1998) *Genet. Mol. Biol.* **21**: 119.
23. Sigiura, K. (1965) "*Tumor Transplantation*", in "*Methods of Animal Experimentation*" (W. Gay, ed.), Academic Press, New York, pp. 177-222
24. Machon, Z., Kuczynski, L., Gieldanowski, J. Wiczorek, Z., Zimecki, M., Blaszyk, B., Mordarski, M., Wiczorek, J. & L. Fiszler-Maliszewska (1981) *Arch. Immunol. Ther. Exp.* **29**: 217-33
25. Mizuno, M., Morimoto, M., Minato, K. & H. Tsuchida (1998) *Biosci. Biotechnol. Biochem.* **62**: 434-7
26. Song, K.S., Cho, S.M., See, J.H., Kim, H.M., Ko, K.S. & I.D. Yoo (1995) *Chem. Pharm. Bull.* **43**: 2105-8
27. Wasser, S.P. & A.L. Weis (1999) *Crit. Rev. Immunol.* **19**: 65-96
28. Mizuno, T., Ando, M., Sugie, R., Ito, H., Shimura, K., Sumiya, T. & A. Matsuura (1992) *Biosci. Biotechnol. Biochem.* **56**: 34-41