Characterization of fractions of aqueous-soluble constituents of the mushroom *Pleurotus ostreato-roseus* Sing. and its effects on the serum protein profile of mice

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Abstract

Various species of mushrooms belonging to genus *Pleurotus* (Fr.) Kumm. are known by its nutritional value and medicinal properties, particularly anti-tumor activity and immunomodulation, related to structural polysaccharides. In order to study the composition and the effects of aqueous-soluble constituents from *Pleurotus ostreato-roseus* Sing. on mice serum proteins, the *P. ostreato-roseus* strain POR-020 was cultured on powered grass-sugarcane bagasse-sawdust-wheat bran-substrate, and fruiting bodies were produced, milled, extracted with ethanol and hot water, and fractional precipitated with ethanol producing two fractions, FI-1 and FI-2. These fractions showed, respectively, 47.87 and 26.45% (d. m.) carbohydrate, and 2.07 and 1.97% (d.m.) protein composition. The fractions were administered i.p. in AJ male mice in a single-dose of 257 mg/kg (FI-1) and 31.5 mg/kg (FI-2). After 15 days administration, electrophoretic analysis of serum proteins detected that FI-1 increased 50, 25, 0.35, and 28.6% in b-, a1-, a2- and g-globulin regions, and FI-2 increased 4.4 and 21 % in b- and a1-globulin, respectively.

In the last three decades, numerous researches points polysaccharides and polysaccharide-protein complexes isolated from mushrooms as source of therapeutic agents against cancer. These compounds are suggested to enhance cell-mediated immune response, increasing the host defense by activating of many kind of immune cells so important for natural anti-tumor mechanisms[1]. *Pleurotus ostreato-roseus* Sing. is a tasty edible fungus belonging to genus *Pleurotus*, occurring in pan tropical regions[2]. In spite of its current use in cooking, there are almost no studies on food and medicinal effects of its components. In order to characterize the chemical composition and bioactive properties of this species, a strain of *P. ostreato-roseus* POR 020 was cultured, and fruiting bodies fractions obtained and administered in AJ male mice to evaluated the effect on serum protein profile.

Two fractions, FI-1 corresponding to a supernatant after ethanol precipitation, and FI-2 corresponding to precipitate were both obtained from aqueous soluble component extraction. These fractions showed, respectively, total carbohydrate and protein contents 47.87 and 2.07 % (FI-1) and 25.45 and 1.97 % (FI-2). These values suggested a higher rate of carbohydrate: protein when in comparison to values found by Zhang and cols.[3] and Zhuang et al.[4] to the same fractions obtained from *P. citrinopileatus* and *P. sajor-caju*, respectively. In Polyacrilamide Gel Electrophoresis (PAGE) analysis, the fractions showed two kinds of bands colored with Coomassie Brilliant Blue R (Sigma). The first kind of band, common to two fractions, appeared as a unique and well-defined band with highest relative mobility. The second kind, was a spotted and slightly colored band, with low relative mobility in fraction FI-2 and intermediate relative mobility in fraction FI-1.

The administration of a single-dose of fraction FI-1 via i.p. in AJ male mice increased after 15 days the percent values of electrophoretic regions b-, a1-, a2- and g-globulin in 50.0, 25.0, 0.35 and 28.6%, respectively, whereas FI-2 increased 4.4 and 21.0 of b- and a1-globulin, respectively. Maeda et al.[4], utilizing lentinan, a polysaccharide extracted from shiitake mushroom, reported an increasing in electrophoretic regions of a- and b-globulin, namely, caused by increasing of complement C3, hemopexin, and ceruloplasmin. In order to understand the immune mechanism triggered by its constituents, further experiments will be done to obtain and characterize fine fractions of *P. ostreato-roseus* POR020 and verify the possible action in macrophage activation.

Material and Methods

P. ostreato-roseus strain POR020, originally isolated from Atlantic Forest in Dois Irmãos Zoobotanical Reservation, Recife, Pernambuco State, Brazil, was cultured for fruiting bodies production in high density polyethylene bags containing a medium composed by powered grass, sugarcane bagasse, sawdust, and wheat bran. After sterilization, the medium was inoculated with mycelia and kept on suitable conditions of humidity and temperature for mushroom fructification. The fruiting bodies, obtained in rate of 50 to 120 g per bags, were dried at 50 °C, powered and extracted with 85% ethanol at 80 °C, filtered[3], and supernatant reserved for further assays. The remained solid residue was then extracted with distilled water at 95 °C, filtered and centrifuged. The supernatant was precipitated with absolute ethanol and centrifuged. The solid precipitate (fraction FI-2) was redissolved in saline solution (0.85% NaCl) and the supernatant (fraction FI-1) concentrated at vacuum until a viscous and slightly cloudy solution was obtained[6]. The fractions FI-1 and FI-2 were analyzed in regard to total solids by means of infra-red Gehaka-balance device; total protein was

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determined by the method of Coomassie Brilliant Blue R-protein binding reaction[7] and total carbohydrate by method of phenol-sulfuric reagent[8]. The protein and polysaccharide peptide complex profile of fractions FI-1 and –2 was done by PAGE [9]. For *in vivo* assays, fractions diluted in saline solution (0.85% NaCl) were administered i.p. in two groups of 5 AJ male mice with 16.4±1.4 g average weight, in single-doses of 257 mg/kg (FI-1) and 31.5 mg/kg (FI-2). For a control group, 10 mice were injected with equal volume of saline solution. After 15 days from dose administration, the mice were sacrificed by decapitation and blood samples collected and serum proteins analyzed by PAGE (9).

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Efeito da quercetina sobre o extravasamento plasmático no sistema nervoso dura-mater de ratos

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Resumo

Estudos recentes têm demonstrado que os bioflavonóides afetam vários sistemas enzimáticos nos mamíferos. A dura-máter é um tecido que recebe inervação peptidérgica. No cérebro, a inflamação neurogênica envolve a liberação da substância P (SP) por terminais nervosos sensoriais e é modulada pela endopeptidase neutra (NEP) e pela enzima conversora da angiotensina (ECA). Neste estudo, avaliamos o efeito da quercetina, que representa mais de 50% dos bioflavonóides, sobre o extravasamento plasmático induzido pela SP em tecidos selecionados no sistema nervoso central de ratos e dura-máter. Também examinamos o efeito da inibição seletiva das enzimas metabolizadoras da SP (NEP e ECA). A administração de SP (10 nmol/kg e 30 nmol/kg, i.v.) aumentou o extravasamento plasmático de maneira dose dependente na dura-máter, não apresentando nenhum efeito nos outros tecidos; este efeito foi potencializado por inibidores seletivos da NEP e da ECA. A quercetina (30 mg/kg v.o.) aumentou o extravasamento plasmático em relação ao controle em todos os tecidos. O pré-tratamento com quercetina potenciou significativamente o extravasamento plasmático induzido pela SP (10 nmol/kg) na dura-máter. Resultados obtidos com o pré-tratamento com antagonistas específicos para receptores da substância P e bradicinina (NK-1 e B2) sugerem que o aumento do extravasamento plasmático induzido pela quercetina e a potenciação da resposta à SP foram devidos ao acúmulo deste neuropeptídeo na dura-máter.

Estudos recentes têm demonstrados que os bioflavonóides, compostos polifenólicos encontrados em vários produtos alimentares e plantas, afetam vários sistemas biológicos nos mamíferos. Eles inibem a síntese e a liberação de enzimas, tem características imunossupressoras e podem funcionar como poderosos antioxidantes, sendo protetores cardiovasculares[1,2]. Na dura-máter, um tecido que recebe inervação peptidérgica, a liberação da substância P (SP) destas terminações nervosas sensoriais tem papel importante na inflamação neurogênica[3]. Esta resposta inflamatória é modulada