THE INFLUENCE OF CULINARY-MEDICINAL MUSHROOMS: *AGARICUS BISPORUS, LENTINULA EDODES* AND *PLEUROTUS OSTREATUS* ON INJURIES OF GASTRIC MUCOSA IN RATS EVOKED BY STRESS.

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ABSTRACT

Agaricus bisporus, Lentinula edodes and *Pleurotus ostreatus*, are widely used culinary-medicinal mushrooms that have been traditionally consumed in many countries to prevent cancer, diabetes, hyperlipidemia, arteriosclerosis, and chronic hepatitis. Many components of these mushrooms have potentially significant biologic activity and perspective for development of new medicines. The aim of this study was to evaluate effects of edible mushrooms in model of stress-induced gastric ulcerogenesis in rats. Rats were randomly assigned to one of four groups; each group consisted of six animals. Jelly starch (control group) and jelly starch powder suspensions from dry fruiting body of *A. bisporus*, *L. edodes* and *P. ostreatus* (200 mg/kg, i.g.), were administered in 0.5ml volumes, daily, during a 7-day period to different groups. The animals were exposed to acute immobilization stress combined with water immersion on fifth day, and were sacrificed on eighth day after starting experiment. Gastric wounds were classified as erosions - small lesions on the sub-mucosa, and ulcers with hemorrhagic borders own areal and deep mucus invasion.

Our research has shown that treatment with *P. ostreatus* and *L. edodes* inhibited ulcer formation by 76.2% and 87.1% respectively, compared to the control group ($P<0.05$). Daily administration of powder suspensions of *L. edodes* inhibited erosion formation by 71.5% ($P<0.05$). *Pleurotus ostreatus* and *A.bisporus*, presented only unproven activity.

We conclude that using *P. ostreatus* and *L.s edodes* by gastric ulcer disease patients might help in the clinical as a prophylactic agent, and in healthy persons as stress-protective agents.

Keywords: *Agaricus bisporus* (brown strain), *Lentinula edodes, Pleurotus ostreatus*, stress, gastric ulcers.

INTRODUCTION

Higher Basidiomycetes represent an important group of fungi for industrial production of a nutritionally functional food and different kinds of new pharmaceutical products [1]. Healthy nutrition and diet are gaining importance, not only in the everyday life of human beings, but also in the treatment of chronic diseases. Mushrooms have recently become attractive as a functional food and source for the development of new drugs. Edible mushrooms are a valuable source of biologically active compounds. Some are used in the prophylaxis and therapy of such diseases as cancer and cardiovascular diseases. The biologically active substances in mushrooms decrease DNA damage, reduce carcinogen concentrations and their activation, inhibit the growth of cancer cells by scavenging free radicals, stimulate the immune system, and induce tumor cell apoptosis. The stimulation of the immune system by the biologically active compounds in edible mushrooms

protects against cold, flu, infections, well as AIDS by inhibition of viral replication. Mushrooms contain effective substances which decrease the LDL fraction of cholesterol in blood. They also prevent the accumulation of serum triacylglycerols, thus decreasing the risk of developing cardiovascular disease [2-4].

Stressful environment leads to development of many chronic and acute diseases globally in the modern world. Dietary mushrooms contain a diverse array of biologically active molecules rendering them potentially protective against stress. In fact, dietary mushrooms have been shown to improve cardiovascular health, stimulate immune function, contribute to glucose homeostasis, and to modulate detoxification, as well as exert anti-allergic, anti-tumor, anti-viral, antibacterial, antifungal, and anti-inflammatory activities [5, 6]. As a result, both cellular components and secondary metabolites of myriad dietary mushrooms have been used in treatment for a variety of diseases [7]. Some of the therapeutic properties of mushrooms result from the specific polysaccharides, such as beta-glucans and chitosans that are present in the fructification of fungi [8].

The basidiomycete fungus *Pleurotus ostreatus, Agaricus bisporus* and *Lentinula edodes* are widely used culinary-medicinal mushroom that have been traditionally used as a health food source in many countries for the prevention of cancer, diabetes, hyperlipidemia, arteriosclerosis, and chronic hepatitis. Nowadays, these mushrooms are produced on an industrial scale. Many of the components of these mushrooms potentially act as biological response modifiers, meaning that they are able to affect physiological responses, thus attracting attention to the development of new medicines, as a cure or for prophylactic uses [9]. Some authors have also described activity in mushrooms and/or mushroom extracts as dietary supplements based on theories that they enhance immune function and promote health. To some extent, selected mushrooms have been shown to have stimulatory action on immune responsiveness, particularly when studied in vitro [6, 9-13]. However, despite their widespread use for potential health benefits, there is a surprising paucity of epidemiologic and experimental studies that address the biological activities of mushrooms after oral administration to animals or humans.

Considering all the effects found for the biological activity of commonly used culinary medicinal mushrooms and its relation to important physiological processes, the aim of this study was to evaluate protection effects of edible mushrooms in model of stress-induced gastric ulcerogenesis in rats.

MATERIALS AND METHODS

Air dry fruiting bodies of cultivated culinary-medicinal mushrooms *L. edodes* and *P. ostreatus* were obtained from a local market. Fruiting body samples of *A. bisporus* were collected for this study in a mushroom house during first flush and then air dried.

The crude dried fruiting body of *A. bisporus, L. edodes* and *P. ostreatus* was powdered and aqueous suspensions were prepared by mixing dry powder of mushrooms in starch jelly as vehicle (300 mg starch add to 30 ml of distilled water and hit at 90°C in a water bath for 3 minutes).

Male rats *(Rattus norvegicus,* albino, nonlinear), weighing 150-200 g, n=24 were acquired from the vivarium of the National Taras Shevchenko University of Kyiv, Ukraine. The animals were kept in small groups in polyethylene boxes in a standard vivarium environment and with food and water *ad libitum*, for at least 30 days before the experiments. The animals were provided only water *ad libitum* for the 24 hours before stress procedure. This study was conducted according to internationally accepted principles of laboratory animal use [14, 15].

Animals were exposed to acute immobilization stress combined with water immersion [16, 17] for 180 minutes. Restrained in metal perforated containers rats were immersed in the water bath (22° C) in such a way that the head of the animal was outside of water. After the exposure to stress the rats were dried and all rats were returned to the home cage.

Rats were randomly assigned to one of four groups, all groups were exposed to stress. Each group consisted of six animals. Jelly starch (control group) and jelly starch powder suspensions of *P. ostreatus*, *A. bisporus* and *L. edodes* (200 mg/kg, i.g.) were administered in 0.5-ml volumes, daily, during a 7-day period to different groups. The animals were stressed on fifth day, and they were sacrificed on eighth day after starting experiment. Stomachs were removed and opened at the small curvature for analysis. Gastric wounds were classified in different levels, as follows: Type I wounds present erosions, small lesions on the sub- mucosa, and Type II wounds present ulcers with hemorrhagic borders own areal and deep mucus invasion. Lesions were observed using an optical lens (X5) and quantified by its length for erosions and area for ulcers.

The statistical analyses were done using t-test for independent samples analysis followed by the Shapiro-Wilk's W tests of normality. Results with $P < 0.05$ were considered to be significant. Data are expressed as Mean ± SD values.

RESULTS AND DISCUSSION

It was observed that stress evokes wide-spread effect and leads to various injuries on gastric mucosa: ulcers, erosions. The inflammatory reaction in the non-treated group (control) was more intense than in the treated groups. The control group presented an ulcer area of 22.6 mm^2 per animal. These values were defined as 100%. The treatment with *P. ostreatus* and *L. edodes* inhibited ulcers formation by 76.2% and 87.1%, respectively, compared to the control group ($P < 0.05$). The other treatment with *A. bisporus* did not show inhibition of ulcer formation. *Lentinula edodes*, commonly known as Shiitake mushroom has been used as medicinal food in Asian countries, especially in China and Japan and is believed to possess strong immunomodulatory properties [18], showed the best performance, inhibiting ulcers formation in rats after treatment. These results are shown in Fig. 1.

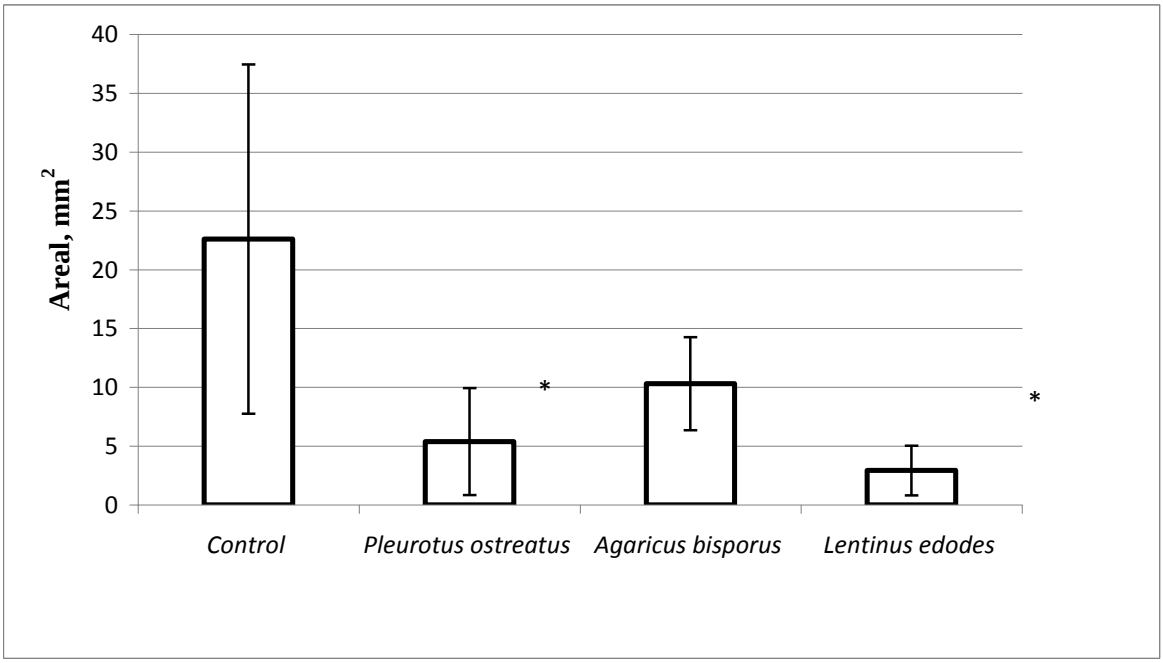

Figure 1: Stress-induced gastric ulcers in rats treated with powder suspensions of *Pleurotus ostreatus*, *Agaricus bisporus* and *Lentinula edodes* (200 mg/kg, i.g.). Data are mean ± SD
* - $P < 0.05$, compared to control group of animals.

Daily administration of powder suspensions of *L. edodes* (200 mg/kg, i.g.) for a 7-day period inhibited erosion formation by 71.5% ($P < 0.05$). The *P. ostreatus* and *A. bisporus* presented only unproven activity, inhibiting this formation, as shown in Fig. 2.

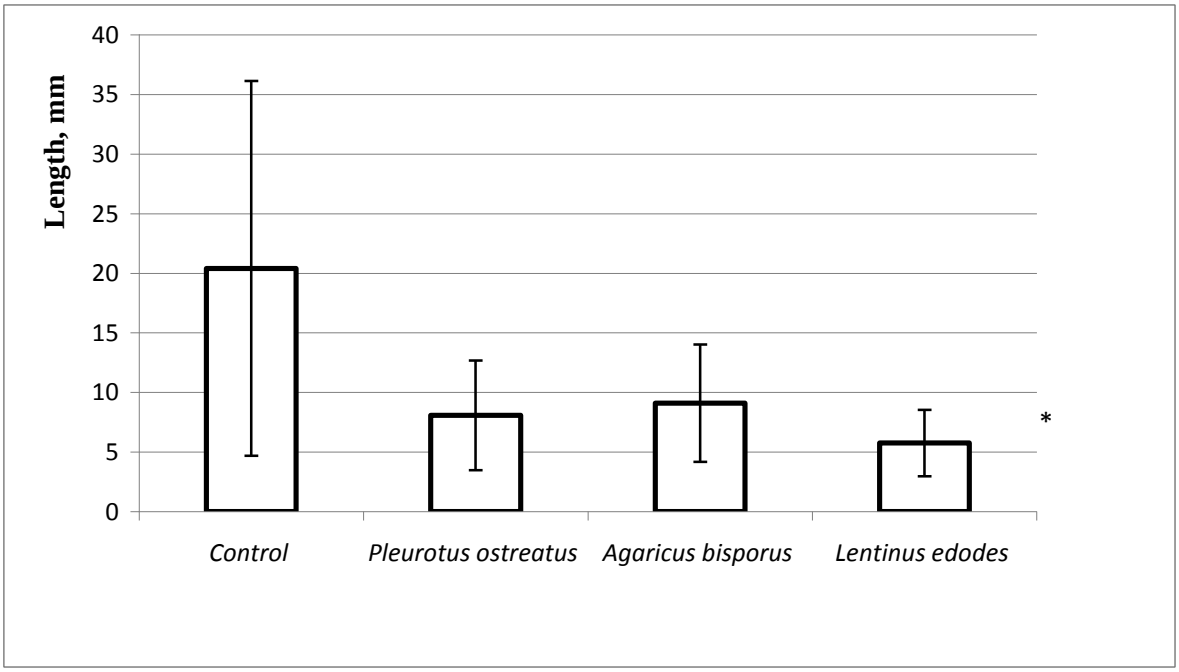

Figure 2: Stress-induced gastric erosions in rats treated with powder suspensions of *Pleurotus ostreatus*, *Agaricus bisporus* and *Lentinula edodes* (200 mg/kg, i.g.). Data are Mean ± SD
* - $P < 0.05$, compared to control group of animals.

The suspension crude powders of *P. ostreatus* and *L. edodes* were able to strongly inhibit the gastric wounds induced by combined stress, on day 3 after stress implying ($P < 0.05$).

Also it is possible to suggest a trend of protective effect of *A. bisporus* on the gastric damages induced by stress, unproven in this study possibly due to high variation. The treatment with suspension crude powder of *P. ostreatus* and *L. edodes* effectively protected stomach from more complicated lesions, such as ulcers when compared to the control group.

In the present study, our group evaluated the stress-protective activity of powder suspension of *P. ostreatus*, *A. bisporus* (brown strain) and *L. edodes*, in model of acute stress in rats.The investigated mushrooms are cultivated worldwide. The special importance of these mushrooms is determined not only by their high gastronomic value but also by the fact that they represent a source of valuable pharmaceuticals [19]. In our study it is generally considered that administration of mushrooms powder suspension for a 7-day period had a noticeable effect in inhibiting ulcers and erosions formation, probably due to immunomodulatory activity on the cell-mediated immunity.

The peripheral inflammation induced by stress might cause enhancing proinflammatory cytokine expression in the tissues. One possible mechanism for gastroprotection is through the release of pro-inflammatory cytokines, such as interleukin-1, tumor necrosis factor-α, and interleukin-6. Administration of *P. ostreatus* and *L. edodes* may inhibit this process, probably regulating cell migration and release of chemotaxic factors. Other studies suggest an immune modulatory action of the mushrooms extracts, it may inhibit the effect of stress by enhancing the anti-inflammatory activity in stress model [8, 10, 11, 13].

Anti-inflammatory drugs used in chronic diseases usually lead to gastric ulceration as a side effect. The development of new medicines that do not have this side effect would be a major aid to

patients with chronic inflammatory ailments, such as arthritis. *Pleurotus ostreatus*, and *L. edodes* were able to inhibit the ulceration process, protecting the gastric mucous wall.

It is noteworthy that mushrooms also contain high levels of bioactive agents including polyphenols and the novel antioxidant ergothioneine, which is produced exclusively in mushrooms and some bacteria [20]. It is possible that varying levels of these bioactive agents alone or in combination contributed to the beneficial effects observed.

CONCLUSIONS

Therefore, the use by gastric ulcer disease patients of *P. ostreatus*, and *L. edodes* as a health food may help in the clinical situation as a prophylactic agent, and in healthy persons as stress-protective agents.

REFERENCES

[1] Wasser SP, Weis L. (1999). Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives. *Int J Med Mushr*. 1:31-62.
[2] Beelman R, editor. (2003). Executive summary. Nutritional Research Advisory Panel Meeting; 2003 September 17. *Mushroom Council*, American Mushroom Institute, Washington, D. C.
[3] Mizuno T. (1999). The extraction and development of antitumor-active polysaccharides from medicinal mushrooms in Japan (review). *Int J Med Mushr*.;1:9-29.
[4] Wasser SP. (2010). Medicinal Mushroom Science: History, Current Status, Future Trends, and Unsolved Problems. *Int J Med Mushr*. 1:1-16.
[5] Borchers AT. *et al*. (2008). The immunobiology of mushrooms. *Exp Biol Med (Maywood)*. 233(3):259-76.
[6] de Kok TM, van Breda SG, Manson MM. (2008). Mechanisms of combined action of different chemopreventive dietary compounds: a review. *Eur J Nutr*. May;47 Suppl 2:51-9.
[7] Lindequist U. *et al*. (2010) [Higher fungi in traditional and modern medicine]. *Med Monatsschr Pharm*. 33(2):40-8.
[8] Rajewska J, Balasinska B. (2004). [Biologically active compounds of edible mushrooms and their beneficial impact on health]. *Postepy Hig Med Dosw (Online)*. 58:352-7.
[9] Borchers AT, Keen CL, Gershwin ME. (2004). Mushrooms, tumors, and immunity: an update. *Exp Biol Med (Maywood)*. 229(5):393-406.
[10] Ferreira IC, Barros L, Abreu RM. (2009) Antioxidants in wild mushrooms. *Curr Med Chem*.;16(12):1543-60.
[11] Lull C, Wichers HJ, Savelkoul HF. (2005). Antiinflammatory and immunomodulating properties of fungal metabolites. *Mediators Inflamm*. 9;2005(2):63-80.
[12] Martin KR. (2010) Both common and specialty mushrooms inhibit adhesion molecule expression and in vitro binding of monocytes to human aortic endothelial cells in a pro-inflammatory environment. *Nutr J*.; 9:29.
[13] Padilha MM. *et. al*. (2009). Anti-inflammatory activity of aqueous and alkaline extracts from mushrooms (*Agaricus blazei* Murill*). J Med Food*. 12(2):359-64.
[14] Commission Recommendation of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes. (2007). *Official Journal of the European Union*. 50(L197):1-89.
[15] Hollands C. (2007). The Animals (scientific procedures) Act 1986. Lancet. 1986. 5; 2(8497):32-3.
[16] Klenerova V. *et al.* (2007). Effects of two types of restraint stress on spontaneous behavior of Sprague-Dawley and Lewis rats. *J Physiol Pharmacol*. 58(1):83-94.
[17] Landeira-Fernandez J. (2004) Analysis of the cold-water restraint procedure in gastric ulceration and body temperature. *Physiol Behav*. 15;82(5):827-33.

[18] Kuppusamy UR. *et. al.* (2009) Lentinula edodes (Shiitake) mushroom extract protects against hydrogen peroxide induced cytotoxicity in peripheral blood mononuclear cells. *Indian J Biochem Biophys*. 46(2):161-5.
[19] Perera CO. *et. al*. (2003) The Effect of Moisture Content on the Conversion of Ergosterol to Vitamin D in Shiitake Mushrooms. Drying Technology: *An Int. J*.. 21(6):1091 - 9.
[20] Mau JL, Chao GR, Wu KT. (2001). Antioxidant properties of methanolic extracts from several ear mushrooms. *J Agric Food Chem* 49(11):5461-7.