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Screening for Anti-angiogenic Activity in Shiitake Mushroom (*Lentinus edodes* Berk) Extracts

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The formation of new blood vessel from pre-existing vessels or angiogenesis is an important phenomenon in the pathology of solid tumor growth and metastasis. We found that chloroform extracts from *Lentinus edodes* (Berk.) Sing., an edible mushroom reported to demonstrate anti-tumor and immunomodulatory activities inhibited heparin-induced angiogenesis on chick chorioallantoic membrane. Methanol and hexane fractions appeared to have no anti-angiogenic activity. This study holds promise for the potential use of this mushroom as a dietary supplement for the management of various angiogenic-related diseases, especially cancer.

Key words: Shiitake mushroom, anti-angiogenic, *Lentinus edodes*, chorioallantoic membrane assay

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INTRODUCTION

Cancer cells, like normal cells, need a sufficient supply of nutrients and a mechanism for the removal of toxins. To fill this need, as tumor cells proliferate, they secrete substances that promote angiogenesis, or the growth of tiny blood vessels from pre-existing ones. Because these capillaries adequately feed and oxygenate the tumor bed, malignant cells present are stimulated to grow, penetrate into the lymphatic and blood vessels, circulate into the bloodstream, invade nearby tissue and finally spread to other parts of the body or metastasize. Dr. Judah Folkman in 1971 first proposed the idea that cancer growth can be controlled through the process of angiogenesis[1-3]. Isolation of natural substances that inhibit tumor-induced angiogenesis has been pursued. Among these potential drugs are angiostatin[4,5] and endostatin[6], both of which have successfully blocked angiogenesis and metastatic growth in mice and are presently undergoing phase-3 clinical trials[7].

Dietary approaches in cancer management is also gaining popularity as new *in vitro* and *in vivo* research results indicate that some of these food supplements do indeed show anti-cancer activities. Natural products capable of suppressing angiogenesis were reported in some medicinal supplements including epigallo catechin-O-gallate (EGCG) in *Camelia sinensis* (green tea)[8], genistein in *Glycine max* (soya beans)[9], diallyl sulfide in *Alium sativum* (garlic)[10], ginsenoside from *Panax ginseng* roots (ginseng)[11] and aminosterols and matrix metalloproteinase-inhibitors from shark cartilage[12].

Shiitake mushroom, *Lentinus edodes* (Berk), is a mushroom used commonly in Japanese and Chinese cuisine. KS-2 and a polysaccharide fraction from its adult fruiting bodies possesses anti-tumor activity against mouse implanted S-180 tumors and human hepatoma SMMC-7721 cells *in vitro*[13-18]. The bioactive polysaccharide fraction is believed to consist mainly of sulfated alpha (1→3)-D-glucan, a sulfated glycan derivative from the Shiitake mushroom, based on cytotoxicity assays with four tumor cell lines. From this experiment, human MCF-7 breast carcinoma cells was most responsive[19].

Another popular anti-carcinogenic agent from the Shiitake mushroom is lentinan, an immunopotentiator of T-cell and macrophage-mediated immunity[20]. Lentinan was able to suppress growth of human colon carcinoma cells in nude mice[21]. Clinical trials patients with gastric cancers reported significant improvements in survival rates possibly through improvement in immune functions. Complementarily, intake of lentinan has also helped AIDS-infected individuals experience a improved immunity against opportunistic infections[22]. With the outpouring of anti-cancer/carcinogenic properties attributed to intake of natural products from the Shiitake mushroom, it is of interest for this study to demonstrate anti-angiogenic properties that would highlight the importance of dietary interventions as adjunct to long-term cancer management.

MATERIALS AND METHODS

Solvent extraction: Initial methanolic crude extracts were obtained from dried fruiting bodies of shitake mushroom *Lentinus edodes* (Berk) (200 g) soaked in 1.5 L of methanol for three days. The extract was then subjected to solvent extractions: first, with hexane and followed by chloroform. Extraction with these solvents was done twice and both fractions were collected. Excess solvents were removed by rotary evaporation at 40°C under vacuum.

Chorioallantoic membrane (cam)assay: Two doses of the shitake mushroom extracts were used, 500 and 100 μg/egg. An angiogenic-inducer, heparin (10 μg/egg)[23] and an angiogenic-inhibitor, spironolactone (200 μg/egg)[24] were used as controls. These test substances were mixed together with 40% gelatin. After solidification, the gel sponge (total volume of 100 μL, diameter of 0.5 cm) was aseptically placed onto the CAM of ten-day old duck *Anas luzonica* eggs. The eggs were sealed with paraffin and incubated for 3 days at 37°C[25]. Examination of the blood vessels on the chorioallantoic membrane was done for angiogenetic inhibition.

RESULTS AND DISCUSSION

The angiogenic effect of the different solvent fractions of Shiitake mushroom *Lentinus edodes* (Berk) extract was determined using the chorioallantoic membrane (CAM) assay with duck *Anas luzonica* (Fraser 1839) embryo. CAM assay is considered as one of the most common methods for screening angiogenic substances[25, 26]. Some of the recent angiogenic inhibitors screened using CAM assay include zoledronic acid[27], anginex[28], 2-nitroimidazole KIN-841[29] and endorepellin[30].

Results from the different treatments showed that only high dosage chloroform fraction showed angiogenic inhibition. In addition, natural products that fractionated in the chloroform solvent were able to overcome angiogenic-induction with heparin. There is lesser branching of blood vessels on the chorioallantoic membrane similar to anti-angiogenesis activity of spironolactone with and without heparin on the duck

CAM. A dose response was evident as the lower dosage of the chloroform fraction displayed lesser degree of angiogenic inhibition.

The activity of the chloroform extract could be attributed to lentinan because of its semi-polar nature[13]. In comparison, methanolic and hexane fractions contain the polar and non-polar components, respectively. Other substances that fractionated in chloroform may be responsible for the anti-angiogenic activity. A semi-polar compound distinct from lentinan was found to increase IL-1 production and apoptosis in the U-937 monocytic cell line[31]. Another candidate anti-angiogenic agent is lenthionine, an antibacterial and antifungal sulfur-containing compound, that can be extracted by chloroform[32]. Further validation of these isolated natural products from shiitake mushroom is highly warranted.

Chloroform fraction from shiitake mushroom *Lentinus edodes* (Berk) produces an anti-angiogenic effect on the chorioallantoic membrane of the duck *Anas luzonica* (Fraser, 1839) embryo in the presence or absence of an angiogenic-inducer, heparin. The other fractions, hexane and methanol fractions neither stimulated nor inhibited angiogenesis.

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