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Anticancer potential of *Hericium erinaceus* extracts against human gastrointestinal cancers

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ABSTRACT

Ethnopharmacological relevance: Hericium is a genus of mushrooms (fungus) in the *Hericiaceae* family. *Hericium erinaceus* (HE) has been used for the treatment of digestive diseases for over 2000 years in China. HE possesses many beneficial functions such as anticancer, antiulcer, antiinflammation and antimicrobial effects, immunomodulation and other activities. The aim of the studies was to evaluate the anticancer efficacy of two extracts (HTJ5 and HTJ5A) from the culture broth of HE against three gastrointestinal cancers such as liver, colorectal and gastric cancers in both of *in vitro* of cancer cell lines and *in vivo* of tumor xenografts and discover the active compounds.

Materials and methods: Two HE extracts (HTJ5 and HTJ5A) were used for the studies. For the study of chemical constituents, the HTJ5 and HTJ5A were separated using a combination of macroporous resin with silica gel, HW-40 and LH-20 chromatography then purified by semipreparative high-performance liquid chromatography (HPLC) and determined by nuclear magnetic resonance (NMR) spectra. For the *in vitro* cytotoxicity studies, HepG2 and Huh-7 liver, HT-29 colon, and NCI-87 gastric cancer cell lines were used and MTT assay was performed to determine the *in vitro* cytotoxicity. For *in vivo* antitumor efficacy and toxicity studies, tumor xenograft models of SCID mice bearing liver cancer HepG2 and Huh-7, colon cancer HT-29 and gastric cancer NCI-87 subcutaneously were used and the mice were treated with the vehicle control, HTJ5 and HTJ5A orally (500 and 1000 mg/kg/day) and compared to 5-fluorouraci (5-FU) at the maximum tolerated dose (MTD, 25–30 mg/kg/day) intraperitoneally daily for 5 days when the tumors reached about 180–200 mg (mm^3). Tumor volumes and body weight were measured daily during the first 10 days and 2–3 times a week thereafter to assess the tumor growth inhibition, tumor doubling time, partial and complete tumor response and toxicity.

Results: Twenty-two compounds were obtained from the fractions of HTJ5/HTJ5A including seven cycli dipeptides, five indole, pyrimidines, amino acids and derivative, three flavones, one anthraquinone, and six small aromatic compounds. HTJ5 and HTJ5A exhibited concentration-dependent cytotoxicity *in vitro* against liver cancer HepG2 and Huh-7, colon cancer HT-29, and gastric cancer NCI-87 cells with the IC_{50} in 2.50 ± 0.25 and 2.00 ± 0.25, 0.80 ± 0.08 and 1.50 ± 0.28, 1.25 ± 0.06 and 1.25 ± 0.05, and 5.00 ± 0.22 and 4.50 ± 0.14 mg/ml; respectively. For *in vivo* tumor xenograft studies, HTJ5 and HTJ5A showed significantly antitumor efficacy against all four xenograft models of HepG2, Huh-7, HT-29 and NCI-87 without toxicity to the host. Furthermore, HTJ5 and HTJ5A are more effective than that of 5-FU against the four tumors with less toxicity.

Conclusion: HE extracts (HTJ5 and HTJ5A) are active against liver cancer HepG2 and Huh-7, colon cancer HT-29 and gastric cancer NCI-87 cells *in vitro* and tumor xenografts bearing in SCID mice *in vivo*. They are more effective and less toxic compared to 5-FU in all four *in vivo* tumor models. The compounds have the potential for development into anticancer agents for the treatment of gastrointestinal cancer used alone and/or in combination with clinical used chemotherapeutic drugs. However, further studies are required to

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find out the active chemical constituents and understand the mechanism of action associated with the super *in vivo* anticancer efficacy. In addition, future studies are needed to confirm our preliminary results of *in vivo* synergistic antitumor efficacy in animal models of tumor xenografts with the combination of HE extracts and clinical used anticancer drugs such as 5-FU, cisplatin and doxurubicin for the treatment of gastrointestinal cancers.

1. Introduction

Gastrointestinal (GI) cancers such as liver, gastric and colorectal cancers are one of the most common forms of cancers and accounted for about 25% of all cancers from the estimate by the United States (US) National Cancer Institute (Chuang et al., 2009). Liver cancer is the fifth most common cancer and the third most common cause of cancer-related deaths in the world. China has the highest rate of liver cancer in the world and accounts for more than 55% of all cases of primary liver cancer (Chuang et al., 2009). Although liver cancer is uncommon in the US, it is the fastest increasing cause of cancer related deaths and has more than tripled during the past two decades. Gastric (stomach) cancer is the fourth common cancer and the second most common cause of cancer-related deaths in the world (Siegel et al., 2011). Similar to liver cancer, gastric cancer has a considerable high incidence and mortality rates in China (~50% death in the world from China) (Siegel et al., 2011). Colorectal cancer (CRC) is one of the most common and leading cause of cancer-related mortality in the Western world, ranked third in prevalence and lethality (Siegel et al., 2011). CRC is usually diagnosed later in life with most patients presenting after the age of 50. The incidence of CRC in China is lower than that in the Western countries, but has significantly increased in recent years, particularly in the more developed areas. The treatment plan for patients with GI cancers may include surgery, chemotherapy, radiation therapy and immunotherapy. Chemotherapy is the mainstay of treatment for the patients with GI cancers although the treatment remains mainly palliative. However, drug resistance and dose-limiting toxicity limit the success. Therefore, discovery and development of novel anticancer drugs with more efficacy and/or less toxicity are urgently needed.

Mushrooms have been used as edible and medicinal resources for thousands of years and antitumor substances such as polysaccharides have been identified in many mushroom species (Ikekawa et al., 1969; Mizuno et al., 1995; deVere White et al., 2002; Wasser, 2002; Zhang et al., 2007; Ferreira et al., 2010). *Hericium erinaceus* (HE) is an edible mushroom which has been used as a traditional Chinese medicine (TCM) for the treatment of digestive diseases for over 2000 years in China. HE polysaccharides have been widely studied and exhibited anticancer, immune stimulation, lowering cholesterol, and stimulating neurite outgrowth activities (Mizuno, 1995; Park et al., 2002; Zhang et al., 2007; Choi et al., 2010). Besides HE polysaccharides, a series of *erinacines* are regarded to have nerve regenerating property and able to pass through the blood brain barrier to heal on myelin or nerve tissue (Mori et al., 2008; Ma et al., 2010). HE also possesses many other beneficial functions such as anticancer and anti-metastasis (Mizuno et al., 1992; Kim et al., 2011; Kim et al., 2013), anti-ulcer (Abdulla et al., 2008), anti-inflammation and antimicrobial (Okamoto et al., 1993; Okwulehie and Odunze, 2004; Kim et al., 2012), immunomodulation (Xu et al., 1994), improving liver function (Lindequist et al., 2005; Zhang et al., 2012a, 2012b), anti-aging (Zhang et al., 2012a, 2012b), lower blood sugar and lipids (Yang et al., 2003; Wang et al., 2005; Hiwatashi et al., 2010), and improving the body hypoxia tolerance, increasing cardiac blood output and improving the body's blood circulation (Chen et al., 1996).

In the present studies, we investigated the chemical constituents of HTJ5/HTJ5A by separating them with a combination of macroporous resin consisting of silica gel, HW-40 and LH-20 chromatography and purified by semipreparative high-performance liquid chromatography (HPLC) and determined by nuclear magnetic resonance (NMR) spectra. We further evaluated the *in vitro* cytotoxic effect of HTJ5 and HTJ5A on HepG2 and Huh-7 liver, HT-29 colon, and NCI-87 gastric cancer cell lines by MTT assay and *in vivo* antitumor efficacy and toxicity in animal models of SCID mice bearing liver HepG2 and Huh-7, colon cancer HT-29 and gastric cancer NCI-87 tumor xenografts subcutaneously and compared to the effect of 5-FU.

2. Materials and methods

2.1. Collection, extraction and isolation of extracts of HE: HTH5 and HTJ5A

The solid cultures of HE (200 g) were obtained from Hunan Xinhui Pharmaceutical Co., Ltd. (Changsha, China) and dispersed in 1000 ml water, extracted with Herbal Blitzkrieg Extractor under 30°C for 20 min and centrifugalized at 3000 rpm/min for 10 min. The precipitations were extracted and centrifugalized for 10 min. The supernatants were concentrated under reduced pressure and lyophilized to obtain HTJ5 (54 g).

The HTJ5 was dissolved in 200 ml water, the polysaccharides and proteins were separated from HTJ5 by adding 600 ml ethanol, the ethanol solution was filtered and concentrated under reduced pressure to obtain a brown crude extract HTJ5A (20 g).

2.2. Chemical constituent study

Thin layer chromatography (TLC) and column chromatography (CC) were performed on plates precoated with silica gel GF254, silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (General Electric Healthcare Life Sciences, Piscataway, NJ, USA) and Toyopearl HW-40 (Tosoh Corporation, Tokyo, Japan), respectively. Nuclear magnetic resonance (NMR) spectra were taken on a BRUKER AV-400 and a BRUKER AV-500 spectrometers (Bruker Corporation, Billerica, MA, USA) using Dimethyl sulfoxide-d_6 (DMSO-d_6) as solvent and tetramethylsilane (TMS) as internal standard. Semipreparative high-performance liquid chromatography (HPLC) was performed using an ODS column (YMC Triart C_{18}, 5 μm, 20 mm × 250 mm, YMC America Inc., Allentown, PA, USA) and an Agilent 1200 series system consisting of degasser, quad pump, and variable wavelength detector.

The HTJ5A gradient elution on macroporous resin with water, 20%, 40%, and 95% alcohol, obtained the fractions A, B, C, and D, respectively. Each fraction was separated by the combination of silica gel column chromatography, gel HW-40, and LH-20 column, and purified by semi-preparation.

For determination of the contents of HTJ5 and HTJ5A, the polysaccharides were determined by the modified phenol–sulfuric acid method (Dong et al., 1996), while the nitrogenous compounds were determined by Kjeldahl method (Castillo et al., 1962).