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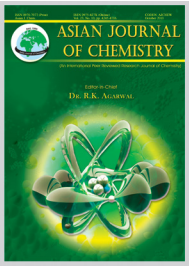

Antineoplastic Effect of Hydrolyzate from Oyster on Human Nasopharyngeal Carcinoma Cells

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To investigate the effects of hydrolyzate from oyster on antineoplastic of human nasopharyngeal carcinoma cell line CNE-1. The inhibited effects of the bioactive peptide, ploysaccharides and small molecule compounds from Guangxi oyster on the growth of nasopharyngeal carcinoma CNE-1 were tested by MTT. To observe the effect of oyster ploysaccharides on the change of morphocytology by means of HE staining method. The bioactive peptide with molecular weight under 3000Da of oyster can inhibit human CNE-1 cell culture, which IC_{50} is 120.00 mg/L. The inhibiting effect of bioactive peptide with molecular weight under 3000 Da after 250 mg/L treatment, the inhibitory rate of cellular growth was 48.26 %. The ploysaccharides of oyster can inhibit human CNE-1 cell culture, which IC_{50} is 138.25 mg/L.The inhibiting effect of ploysaccharides after 250 mg/L treatment, the inhibitory rate of cellular growth was 61.78 %. Scalaradial,the small molecule compound of oyster can inhibit human CNE-1 cell culture, which IC_{50} is 138.29 mg/L. The inhibiting effect of Scalaradial after 250 mg/L treatment, the inhibitory rate of cellular growth was 64.02 %. Under a microscope, the cells showed morphological changes significantly such as the cell nucleus shrinking, syrup diminishing, karyopyknosis occurring, cytoplasm concentrating and the number of cells reducing. Bioactive peptide, ploysaccharides and small molecule compounds from Guangxi oyster have a growing inhibiting effect on CNE-1 cells at a certain consistency. It also has a synergistic effect on differentiation of tumor cells.

Key Words: Oysters, Bioactive peptide, Ploysaccharides, Small molecule compounds, CNE-1.

INTRODUCTION

Oyster is an abundant resource from ocean, which contains 23.3 % proteins on a dry weight basis[1]. The extracts of oyster can keep human immune system function in a good conditions[2,3]. Proteins have diverse biological functions[4-7], the life movement of intravital cells is presented through that of proteies *in vivo*. Bioactive compound from the marine is an activated substance with special physiological functions, such as reducing blood fat, antioxidization resistance to free-radical and tumor development, generating immunity *etc*. The bioactive peptide, polysaccharides and small molecule compounds from Guangxi oyster have inhibited effects the growth of nasopharyngeal carcinoma CNE-1[8].

In our previous studies, the extract was found to exhibit the highest antineoplastic activity. In this study, we examined the antineoplastic effect of hydrolyzate from oyster, which might be a potential antineoplastic for application in pharmaceutical. The object of present study is to isolate and identify the antneoplastic components in the extracts of oyster.

EXPERIMENTAL

Oyster, Crassostrea talienwhanensis crosse, was purchased from a local shellfish market of Guangxi, China. Animal albumen proteolysis (200 U/mg, which contains endoproteinase, exonproteinase and flavourzyme) and CNE-1 cells were purchased from Center for Experimental, Guangxi Medical University. All other reagents used in this study were reagent grade chemicals.

Animal albumen proteolysis treatment of oyster proteins: Fresh oyster was treated with manual decladding and the remanent component was homogenized using a meat chopper. The admixture was stored at -20 ºC. Oyster protein solution was digested with animal albumen proteolysis at a protein substrate to enzyme ratio of 500:1 (w/w) at pH 5.5 and a temperature of 50 ºC for 5 h. The hydrolyzate was subsequently heated at 95 ºC for 10 min to inactive animal albumen proteolysis.

After removal of precipitate by centrifugation (25 min, 5000 g, 4 ºC) the supernatant fluid was adjusted to pH 7.0 by the addition of 1 mol/L of NaOH. The hydrolyzate passed through a 10,000 Da molecular weight membrane and the portion with molecular weight less than 10,000 Da was lyophilized and stored at 20 ºC until used.

Bioactive peptide, polysaccharides and small molecule compounds: The bioactive peptide from oyster was extracted according to the method of Chen *et al.*[9]. After removal of supernatant fluid by centrifugation (25 min, 5000 g, 4 ºC) the protein precipitate was dissolved in the same volume of PBS

as protein precipitate and subsequently dialyzed with a 10,000 Da molecular weight membrane over 48 h against distilled water.

1000 mL of hydrolyzate was dissolved in 2333.3 mL of anhydrous ethyl alcohol at at 4 ºC for 12 h. After centrifugation (10 min, 5000 g, 4 ºC), the precipitate was obtained. The purified polysaccharides was further isolated by toyopearl HW-75 gel filtration column chromatography and one fraction was obtained successfully.

After removal of precipitate by centrifugation (25 min, 5000 g, 4 ºC) the supernatant fluid was dissolved in an equal volume of ethyl acetate at 40 ºC. The mixed solution samples was concentrated on a revolving evaporator. Silica gel column chromatography separation was performed with petroleum ether and ethyl acetate as extracting solvent.

Determination of cell growth curve: CNE-1 cells were cultured with 20 % heat-inactivated fetal calf serum, at 37 ºC in atmosphere containing 50 mL/L CO_2. CNE-1 cells were collected in logarithmic phase, then suspension of CNE-1 cells by 0.5 %. Trypsin was made in 1.0×10^5 cells/mL. The cells (100 µL) were seeded into polystyrene 96-well microtiter plates for 24 h. The experimental groups were treated with the reagents containing different kinds of differentiation-induced gradients while the control group was cultured continuously in fresh culture medium. The control group consisted of 3 samples was treat with 0.5 % alcohol-PBS solution.

CNE-1 cells were treated with culture medium containing bioactive peptide, polysaccharides or small molecule compounds after seeded for 24 h. CNE-1 cells were treated with 20 µL, 5 mg/mL MTT, at 37 ºC in atmosphere containing 50 mL/L CO_2 for 4 h, then pour off the remaining liquid and dissolved with 150 µL DMSO after CNE-1 cell was dyed, detect inhaling light degree (a numerical value) of 490 nm in detection instrument, calculate proliferation inhibitory rate of CNE-1 cell.

RESULTS AND DISCUSSION

Purification and identification of the small molecular compound: The extracts of oyster were subjected to repeated silica gel column chromatography affording one pure compounds, the structure of which was elucidated on the basis of spectroscopic analysis and comparison with the literature data. One known compound was isolated and identified as scalaradial[10] (Fig. 1).

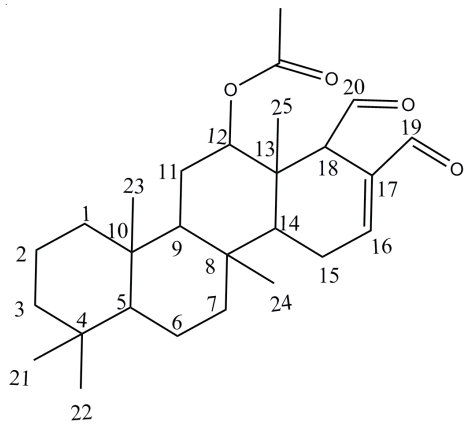

Fig. 1. Structure of scalaradial

Inhibited effects of bioactive peptide on the growth of nasopharyngeal carcinoma CNE-1: Fig. 2A presents inhibited effects of bioactive peptide on the growth of nasopharyngeal carcinoma CNE-1. As results from Fig. 2A, the bioactive peptide with molecular weight under 3000 Da of oyster can inhibit human CNE-1 cell culture, which IC_{50} is 120.00 mg/L.The inhibiting effect of bioactive peptide with molecular weight under 3000 Da after 250 mg/L treatment, the inhibitory rate of cellular growth was 48.26 %. The add of bioactive peptide strongly affects cellular reproduction. In the studied concentration range, the increase in the bioactive peptide with molecular weight under 3000 Da of oyster concentration interfere with cellular reproduction.

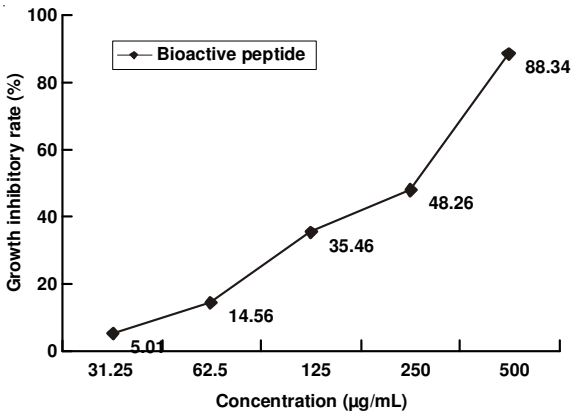

Fig. 2A

Inhibited Effects of polysaccharides on the growth of nasopharyngeal carcinoma CNE-1: Fig. 2B presents inhibited effects of polysaccharides on the growth of nasopharyngeal carcinoma CNE-1. As results from Fig. 2B, the polysaccharides of oyster can inhibit human CNE-1 cell culture, which IC_{50} is 138.25 mg/L. The inhibiting effect of polysaccharides after 250 mg/L treatment, the inhibitory rate of cellular growth was 61.78 %. The addition of polysaccharides strongly affects cellular reproduction. In the studied concentration range, the increase in polysaccharides of oyster concentration interfere with cellular reproduction. Under a microscope, the cells showed morphological changes significantly such as the cell nucleus shrinking, syrup diminishing, karyopyknosis occurring, cytoplasm concentrating and the number of cells reducing.

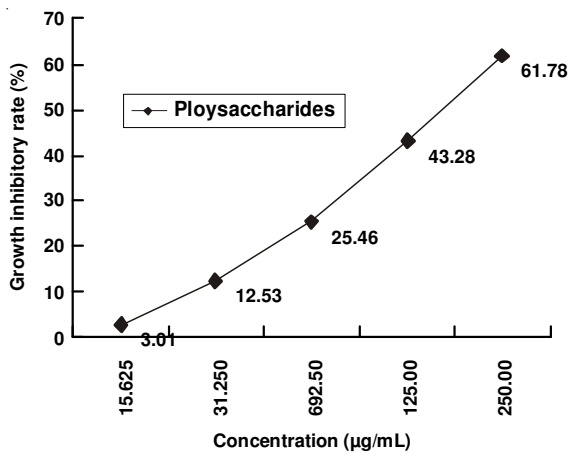

Fig. 2B

Inhibited effects of small molecule compounds on the growth of nasopharyngeal carcinoma CNE-1: Fig. 2C presents inhibited effects of scalaradial on the growth of nasopharyngeal carcinoma CNE-1. As results from Fig. 2C, scalaradial, one small molecule compound extracted from oyster can inhibit human CNE-1 cell from culture, which IC_{50} is 138.29 mg/L .The inhibiting effect of Scalaradial after 250 mg/L treatment, the inhibitory rate of cellular growth was 64.02 %. The add of scalaradial strongly affects cellular reproduction. In the studied concentration range, the increase in the scalaradial of oyster concentration interfere with cellular reproduction scalaradial.

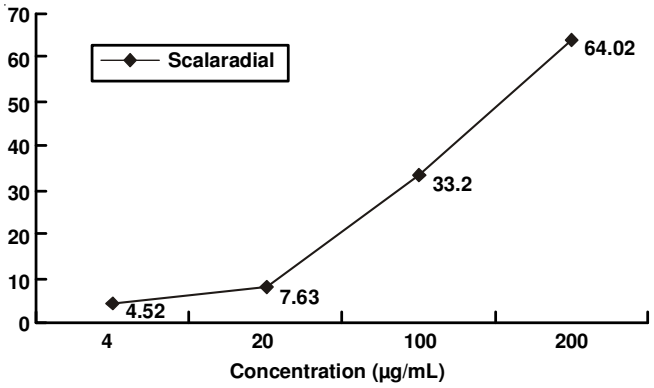

Fig. 2C

The extracts of oyster can keep human immune system function in a good conditions. Extracts can differ not only qualitative and quantitative composition of the antineoplastic, but also in the presence and concentration of natural acids, which makes their pH different. The presence of natural acids in extracts is rarely taken into consideration when comparing their antineoplastic properties. Moreover, to prepare extracts from matrices, investigators frequently apply extrahents of different pH. The reaction occuring between the examined antineoplastic and the cation radical is pH dependent

Conclusion

The antineoplastic potential of an examined material is usually described by qualitative and quantitative specification of its antineoplastic components and/or by the estimation of their antineoplastic properties. Recently, MTT assay has become one of the most widely employed methods for estimating antineoplastic activity[11]. The presented results show that such factors as the bioactive peptide, polysaccharides and small molecule compounds influence the estimation of antioxidant activity in MTT assay and create the difficulty in the estimation of exact and correct antineoplastic properties of food extracts. In fact, the antineoplastic activity depends on the presence of other components occur.

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