Partially Purified *Gloriosa superba* Peptides Inhibit Colon Cancer Cell Viability by Inducing Apoptosis Through p53 Upregulation

Prapaphan Budchart, MS, Ariya Khamwut, MS,
Chomdao Sinthuvanich, PhD, Sunanta Ratanapo, PhD,
Yong Poovorawan, MD and Nattanan Panjaworayan T-Thienprasert, PhD

ABSTRACT

Background: Colon cancer is a major health problem worldwide. Available treatments such as surgery, chemotherapy, radiation and anticancer drugs are limited due to stage of cancer, side effects and altered biodistribution. The use of peptides extracted from natural products has appeared as a potential therapy. *Gloriosa superba* is known to contain colchicine and other alkaloids with anticancer activity. However, these peptides contained within the extracts have not been studied. This study, therefore, focuses on an investigation of anti–colon cancer activity from a partially purified protein hydrolysate of *G superba* rhizome.

Methods: Dried *G superba* rhizome was extracted using 0.5% sodium dodecyl sulfate and digested with pepsin. The protein hydrolysates with molecular weight lesser than 3 kDa were collected and subjected for cell viability assay. Then, the partial purification of the protein hydrolysate was performed using reverse-phase high-performance liquid chromatography. Fractions containing anticancer peptides were investigated, and their effects on apoptosis and protein expression using apoptosis test and Western blot, respectively.

Results: Partially purified peptides of *G superba* rhizome demonstrated anticolon activity in SW620 cells by inducing apoptosis through upregulation of p53 and downregulation of nuclear factor kappa B (NF-κB).

Conclusions: Consequently, *G superba* peptides showed high potential for further purification and development of anticolon therapeutics.

Key Indexing Terms: *Gloriosa superba*; Partially purified peptide; Colon cancer; Apoptosis; p53. **[Am J Med Sci 2017;354 (4):423–429.]**

INTRODUCTION

Colon cancer is one of the top 3 cancers found worldwide and is also considered the second most deadly.[1] Treatment for colon cancer is generally based on the stage of the cancer. Surgery is the gold standard for treatment, especially during the early stages of cancer. On the contrary, chemotherapy, radiation therapy and the combination of surgery and anticancer drugs are often recommended for the later stages.[2,3] In addition, approved anticancer drugs are usually associated with severe side effects. For example, high doses of 5-fluorouracil result in severe toxic effects in hematological, neural, cardiac and gastrointestinal systems,[4,5] and the long-term use of oxaliplatin has been reported to induce peripheral neuropathy such as numbness, pain and dysesthesias.[6] The development of alternative treatments or novel anti–colon cancer drugs with low toxicity is therefore required. Increasing interest has been focused on anticancer peptides because peptides offer unique advantages such as low molecular weight, target specificity and low cytotoxicity in normal cells. Moreover, they are modifiable and can be readily synthesized.[7]

Peptides are small protein molecules usually less than 10 kDa. They may be released from food (both animal or plant proteins) during gastrointestinal digestion or food processing.[8,9] In recent years, several plant bioactive peptides have been reported to show potential as anticancer therapies. For example, the soybean peptide lunasin demonstrated antineoplastic effects in colon, prostate, breast and leukemia cell lines by different modes of action depending on the tissue cells targeted.[10] Oral lactoferrin from whey has also been reported to significantly inhibit the growth of squamous cell carcinoma tumors in T-cell–immunocompromised mice.[11] Furthermore, Ting et al[12] reported that a cationic peptide, Pardaxin, derived from Red Sea Moses, induced significant cell death in various cancer cell lines. Finally, partially purified protein hydrolysates containing a mixture of peptides have also been studied[13-15] and their anticancer properties may be due to synergistic effects between complex peptides within the purified hydrolysates.

The focus of this study is to analyze the anti–colon cancer activity of partially purified peptides from the rhizome *Gloriosa superba* Linn, which is commonly consumed in Thai traditional medicine treatments for skin diseases and cancer.[16] The rhizome *G superba* is known to contain colchicine and related alkaloids such as 3-O-demethylcolchicine and its associated glycoside colchicoside.[17-19] Although colchicine can significantly inhibit growth of various cancer cells, its clinical use has been limited owing to its high toxicity.[20-22] On the contrary, a methanol extract of *G superba* root was reported to exhibit potent antiproliferative activity against human skin fibroblasts and colon (HT29) cell lines.[23] However, there is no reported anticancer activity studies on using protein hydrolysates or peptide extracts from *G superba*. This study, therefore, aims to investigate anti–colon cancer activity of partially purified protein hydrolysates of *G superba* root as well as examine its effects on apoptosis pathway and the expression of key proteins, p53 and NF-κB, that are involved in apoptosis and cellular proliferation, respectively.

METHODS

Plant Sample

Dried rhizome *G superba* was purchased from a traditional Thai pharmacy (Chao Khun Por, Bangkok, Thailand).

Preparation of Protein Hydrolysate Extract

The protein hydrolysate extract was prepared from the rhizome *G superba*. The sample was blended into a powder and then extracted using 0.5% sodium dodecyl sulfate (Univar, Australia). The sample was mixed and incubated for 24 hours at 37°C using an incubator shaker (Gallenkamp, China). The sample was then filtered using a cloth filter followed by centrifugation at 9,100 *g* for 30 minutes at 4°C. The supernatant was then further incubated with 80% acetone for 16 hours at −20°C. The supernatant was removed by centrifugation at 5,800 *g* for 30 minutes at 4°C. The resulting pellet was dissolved with 0.2 M sodium acetate buffer (pH = 4.5), and pepsin (Sigma-Aldrich, USA) was added using a ratio of 1 mg enzyme to 25 mg protein (1:25 weight-to-weight ratios). Next, the protein hydrolysate was mixed and incubated for 24 hours at 37°C using the incubator shaker to simulate digestion. The protein hydrolysate was then heated at 60-70°C for 10 minutes to stop the reaction, followed by centrifugation at 5,800 *g* for 30 minutes at 4°C. The protein hydrolysate was then collected using a vivaspin centrifugal concentrator with a 3-kDa molecular-weight cutoff (GE Healthcare) by centrifugation at 5,800 *g* for 30 minutes at 4°C. The total protein was then determined by the Bradford assay (Calbiochem, USA). The protein hydrolysate was lysophilized using a freeze dryer (LaboGeneTM, Denmark) and stored at −80°C until required for analysis.

Total Protein Determination

Protein concentration was determined by the Bradford assay (Calbiochem, USA) following the manufacturer's instruction. Bovine serum albumin (BSA) was used as a protein standard.

Partial Purification of Protein Hydrolysate Using Reverse-Phase HPLC

The partial purification of the protein hydrolysate was performed using reverse-phase high-performance liquid chromatography (RP-HPLC) with VYDAC 218TP C18 columns (250 × 10 mm^2 i.d., 10 μm particle size, 300 Å pore size) from Grace Davison Discovery Sciences. All preparative runs were carried out with a linear gradient of 0%-100% B over 100 minutes at a flow rate of 3 mL/minute, where eluent A was 0.1% aqueous TFA and eluent B was 0.1% TFA in acetonitrile (Fisher Scientific, England). The fractions were collected using a fraction collector (Amersham Pharmacia Biotech, UK), and then lyophilized into a powder and stored at −80°C until analysis.

Tissue Cell Culture

Vero cells (ATCC CCL-81) are derived from normal kidney epithelial cells extracted from an African green monkey. Chang (ATCC CCL-13) is a human hepatocellular carcinoma cell line with low-invasive potential. Vero and Chang were cultured in Dulbecco's Modified Eagle's medium (Gibco, Invitrogen, USA) and supplemented with 10% (v/v) heat-inactivated fetal bovine serum and 1% (v/v) antibiotic-antimycotic solution (Gibco, Invitrogen, USA). Human colon cancer cells, SW620 (ATCC CCL-227), were cultured using RPMI-1640 supplemented with 10% (v/v) heat-inactivated fetal bovine serum and 1% (v/v) antibiotic-antimycotic solution (Gibco, Invitrogen, USA). Cells were cultured in a 75 cm^3 sterile flask (NEST Biotech, England) at 37°C under 5% CO_2 atmosphere.

Cell Viability Assay

Cell lines (1.5 × 10^3) were seeded into 96-wells plate for 24 hours. Seeded cells were treated with either water (at 0 concentration) or various final concentrations of protein hydrolysate from 10-30 ng of protein/mL. For identifying partially purified fractions with anticancer activity, cells were treated with 30 ng of protein/mL of each fraction in quadruplicate. Cell viability was then performed using an MTT [(3-(4–5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay after 5 days of incubation as described previously.[24] In brief, after the incubation, the culture medium was removed from each well by aspiration, and 4 μg of MTT (Invitrogen, USA) was added into each well. After 3 hours of incubation, DMSO (Amresco, USA) was added to dissolve the purple formazan of MTT. The UV absorbance at 570 nm was then measured by a microplate reader (Tecan, Switzerland).