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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Entomological research http://dx.doi.org/10.1016/j.apjtb.2015.12.011

Bee pollen extract of Malaysian stingless bee enhances the effect of cisplatin on breast cancer cell lines

Wan Adnan Wan Omar*, Nur Asna Azhar, Nurdianah Harif Fadzilah, Nik Nur Syazni Nik Mohamed Kamal

Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200, Penang, Malaysia

ARTICLE INFO

Article history:
Received 10 Nov 2015
Received in revised form 30 Nov 2015
Accepted 8 Dec 2015
Available online 31 Dec 2015

Keywords:
Bee pollen extract
Antioxidant
Antiproliferative activity
Synergistic effect
Malaysian stingless bee

ABSTRACT

Objective: To evaluate the antioxidant and antiproliferative effect of methanolic bee pollen extract (BPE) of Malaysian stingless bee [*Lepidotrigona terminata* (*L. terminata*)] and its synergistic effect with cisplatin (a chemotherapeutic drug) on MCF-7 cancer cell line.

Methods: The antioxidant activity of BPE from *L. terminata* was measured by using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay. Antiproliferative activity at different concentrations of BPE and cisplatin was determined through using MTT assay on MCF-7 and L929 cell lines. An interaction effect (synergistic, additive and antagonistic) between BPE and cisplatin was determined by CompuSyn software based on MTT assay data.

Results: The EC_{50} (50% decrement of DPPH inhibition) of BPE was 0.5 mg/mL. *L. terminata* BPE exhibited antiproliferative activity on both cancer and normal cell lines. The IC_{50} (concentration of drug that was required for 50% of cell inhibition *in vitro*) of BPE on MCF-7 was 15 mg/mL whereas in normal cell line L929 was 26 mg/mL. The IC_{50} for cisplatin on MCF-7 was 20 μmol/L. The combination effect of BPE and cisplatin on MCF-7 cells showed that BPE at 15 mg/mL was able to potentiate the inhibitory effect of cisplatin at all different concentrations (2.5–20.0 mg/mL). The average of cancer cells inhibition which was potentiated by BPE was around 50%. A combination index values of less than 1 reported in the CompuSyn software further proved the synergistic effect between BPE and cisplatin, suggesting that BPE was working synergistically with cisplatin.

Conclusions: Our study therefore suggested that BPE of Malaysian stingless bee, *L. terminata* is a potential chemopreventive agent and can be used as a supplementary treatment for chemotherapy drugs. BPE might be able to be used to potentiate the effect of chemotherapy drugs with the possibility to reduce the required dose of the drugs. The molecular mechanisms of how the BPE exerts antiproliferative activity will be a much interesting area to look for in future studies.

1. Introduction

Bee pollen is considered as a functional food due to its compositions of carbohydrates, proteins, amino acids, lipids, sugars, fibers, vitamins and mineral salts [1,2]. It is a collection of pollen grains collected by the bees from various botanical sources, mixed with nectar and secretion from the hypopharyngeal glands such as β-glycosidase enzymes. Its beneficial effect on health is due to the presence of phenolic compounds with high antioxidant activities [1,3].

Oxidative damage and carcinogenesis may gradually lead to the formation of tumors through several mechanisms. Polyphenols present in bee pollen and other bee products contain antioxidant and antiproliferative activities that can regulate cell proliferation and induce apoptosis [4]. Bee pollen has the antioxidative capacity and the ability to neutralize active oxygen species, extensively due to the action of polyphenols

*Corresponding author: Wan Adnan Wan Omar, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200, Penang, Malaysia.
Tel: +60 45622570
E-mail: wanadnan@usm.my

Foundation Project: Supported by Fundamental Research Grant Scheme, Ministry of Education Malaysia (Grant No: FRGS/2/2013/SKK01/USM/03/3).
Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

combination. Previous studies have demonstrated that bee pollen can inhibit tumor growth and alleviate the pain of chemotherapy in cancer patients [5,6].

In China, bee pollen of *Brassica campestris* L. (*B. campestris*) has been widely used as food supplement to strengthen body resistance against cancer [7]. A steroid fraction of bee pollen chloroform extract of *B. campestris* was shown to strongly induce apoptosis in human prostate cancer PC-3 cells [7]. A study by Izuta *et al*. showed that bee pollen was able to suppress cells proliferation in *in vitro* model of vascular endothelial growth factor-induced human umbilical vein endothelial cells proliferation and migration [8]. Constituents of bee pollen such as polysaccharides were also shown by Wang *et al*. to have significant antiproliferative activity in colon cancer cell lines [5]. Thus the aim of the study is to evaluate the antiproliferative activity of bee pollen extract (BPE) from Malaysian stingless bee, *Lepidotrigona terminata* (*L. terminata*) and its effect when combined with cisplatin on MCF-7 cancer cell lines.

2. Materials and methods

2.1. Samples and reagents

Bee pollen or bee bread sample was acquired from a local stingless bee company, K.B meliponini. The source of bee pollen was from the species of *L. terminata*. The species was identified by the Entomology Section, Malaysian Agriculture and Research Development Institute, Serdang, Malaysia. Bee pollen sample was dried at 37 °C for two consecutive days and once dried, was kept at 4 °C until further use.

2.2. Bee pollen crude extract

The crude BPE was prepared by using modified method from LeBlanc *et al*. by extracting 10 g of bee pollen in 25 mL of 100% methanol [9]. The mixture was vortexed for 10 min and sonicated for 1 h at 41 °C before kept overnight at 4 °C. The mixture was then centrifuged at 6000 r/min for 10 min and the precipitation was discarded. The supernatant was filtered by using a 0.2 μm sterile filter unit (Millipore, USA). Once filtered, it was dried in the rotary evaporator at 41 °C and freeze dried. The freeze dried BPE was kept at 4 °C until further analysis.

2.3. Antioxidant activity

The free radical-scavenging capacity of bee pollen methanolic extract was determined by its ability to bleach the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and was expressed as the percentage of its neutralization. A modification of a method reported by Baharum *et al*. was employed [10]. The reaction mixture contained 150 μL of DPPH (600 μmol/L) with 7.5 μL of different concentrations of BPEs (3.125, 6.250, 12.500, 25.000, 50.000 and 100.000 mg/mL). The final concentrations of bee pollen were 0.15, 0.30, 0.60, 1.20, 2.40 and 4.80 mg/mL. After 30 min at room temperature, the reaction mixture was measured by the absorbance at λ = 517 nm. Trolox, a water-soluble derivative of vitamin E at concentration of 25–600 μmol/L, was used as standard. DPPH free radical scavenging activity was calculated through using the following formula:

$$\%\text{Scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

2.4. Cell culture

MCF-7 and L929 cell lines used in this study were obtained from the Advanced Medical and Dental Institute, Universiti Sains Malaysia. The cell lines were cultured in a complete Dulbecco's modified Eagle's medium (DMEM) containing 4 mmol/L L-alanyl-glutamine (GlutaMAX™) and supplemented with 10% (v/v) fetal bovine serum, 100 IU/mL of penicillin and 0.1 mg/mL of streptomycin. The cells were grown in humidified atmosphere under the conditions of 37 °C with 5% (v/v) CO_2.

2.5. Cell counts

MCF-7 and L929 cells were harvested by removing the medium, washed with phosphate buffer saline (pH 7.45 without Ca^{2+} and Mg^{2+}) and then incubated with 1 mL of 0.25% trypsin for 2 min. The flask was gently tapped to detach them from the plastic surface. Five milliliters of medium was added to the cell suspension while the remaining cells were vigorously washed from the bottom of the culture vessel. The suspended cells were then collected in a 15 mL centrifuge tube and an aliquot of 10 μL was taken out prior centrifugation for cell count by using hemocytometer. The number of cells per milliliter and the total cell number were calculated. Subsequently after centrifugation, an appropriate volume of complete medium was added to the cell pellet.

2.6. Preparation of compound treatments

BPE stock solution (100 mg/mL) was prepared by diluting 0.1 g of crude extracts in 1 mL DMEM. The solution was then filtered by using a 0.2 μL sterile filter unit (Millipore, USA) and diluted with culture medium to the final concentration of 2.5 mg/mL, 5 mg/mL, 10 mg/mL, 20 mg/mL and 40 mg/mL for treatments.

Cisplatin stock solution (10 mol/L) was prepared by dissolving 6 mg of cisplatin (molecular weight = 300.01 g/moL) in 2 mL DMEM. The stock solution was then diluted with culture medium to the final concentration of 2.5 μmol/L, 5 μmol/L, 10 μmol/L, 20 μmol/L and 40 μmol/L for treatments. The working solutions of the drug were freshly prepared prior usage.

2.7. Cytotoxicity determination by using MTT assay

Briefly, the cells were seeded at density of 1×10^4 cells per well of flat-bottomed 96-well microplate. The cells were incubated at 37 °C in a humidified 5% (v/v) CO_2 for 24 h to let the cells attach to the bottom of each well. The cultured cells were then treated with different concentrations of BPE and cisplatin, alone and with combination when the cells reached 70% confluence.