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Original Article

Effects of vinegar–egg on growth inhibition, differentiation human leukemic U937 cells and its immunomodulatory activity

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ABSTRACT

Vinegar and eggs have rich nutrients. In this study, the mixed form of both derived products, vinegar–egg solution and its products (vinegar–egg concentrate and vinegar–egg condensate) were chosen for an assessment of their biological activity. To further our understanding regarding the anticancer and immunomodulatory effects of vinegar–egg, we investigated its effects on the proliferation and differentiation of U937 cells. Vinegar–egg was treated using spray drying, freeze drying and vacuum concentration and used to stimulate human mononuclear cells. The conditioned media obtained from these cultures by filtration were used to treat U937 cells. Three conditioned media inhibited U937 cell growth by 22.1–67.25% more effectively than PHA-treated control (22.53%). CD11b and CD14 expression on the treated U937 cells were 29.1–45.4% and 31.6–47.2%, respectively. High levels of cytokines IL-1β, IFN-γ and TNF-α were detected in the three conditioned media. Vinegar–egg stimulates human mononuclear cells to secrete cytokines, which inhibit the growth of U937 cells and induce their differentiation.

1. Introduction

The egg is one of the few foods that is shared worldwide. Eggs have rich nutrients, high protein content and amino acid compositions that are similar to human proteins. Eggs have high quality (complete protein) protein and contain a large number of biologically active components. Many studies show that eggs have many biological activities, such as antifungal, antihypertensive, antitumor, antioxidant and immune regulation [1–5]. Vinegar is also an important condiment internationally. In the early stage, people used vinegar only as a condiment. As vinegar research increased, the use of vinegar has diversified from a condiment to many food types, such as meal, beverages and various healthcare products. Many studies have reported that vinegar has a variety of nutritional and healthcare functions and medical value; for example, vinegar contains more than 2% protein, at least 18 kinds of

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amino acids and 8 essential amino acids. Vinegar contains many energetic sugars, such as glucose, fructose and maltose. In addition, vinegar contains vitamins B1, B2 and C, and mineral salts, such as K, Ca, Fe, Zn, Cu and P, which are the essential for human body development, reproductive and metabolic processes [6–11]. Leukemia is the most common hematological malignancy, particularly in children. There are five major approaches to the treatment of leukemia include cytotoxic chemotherapy, interferon therapy, radiation therapy, the induction of differentiation and stem cell transplantation (SCT) [12]. Several studies were also show that there are less toxicity and more safety by using inducing leukemic cell differentiation [13,14]. Therefore, attempting to activate human mononuclear cells to induce U937 cells differentiation through cytokine secretion might be a natural and alternative way to treat leukemia [15]. Several studies have pointed out that dietary black soybean [16], bovine colostrums and their protein hydrolyzates [12,17] mushroom [18] and rice [15] are able to inhibit the human leukemic U937 cells growth and induced their differentiation via stimulation of cytokine secretion by human mononuclear cells. In the human diet, the egg and vinegar are common, and their nutrition has also been widely discussed and studied. But the egg treated with vinegar is seldom discussed. Based on "natural" and "cost" considerations, this study used the acidity of vinegar to replace commercial enzymes and mineral acids to hydrolyze the egg protein and to evaluate.

2. Materials and methods

2.1. Preparation of vinegar–egg

Fresh eggs and 9° rice vinegar were obtained from the traditional market. The eggs were washed with water, disinfected with 95% ethanol and then air dried. We added 9° rice vinegar to the egg sample (the ratio of eggs/vinegar were 1 g:3 mL) and placed them at room temperature for 72 h. After the egg shell was dissolved, the solution was filtered with sterile gauze. The filtrate was again incubated at room temperature for 72 h. The final vinegar–egg solutions were treated in three manners; spray drying (SVE) (Spray dryer system (CR22E, Eyela, Co. Ltd., Tokyo, Japan) was used at 50–55 °C for inlet temperature; 60–65 °C for outlet temperature and the flow was 1 mL/min), freeze drying (FVE) (Freeze-drier (EYELA FDV-2100, Rikakikai Co. Ltd., Tokyo, Japan) was used at −53 °C, 553 Pa for 72 h to dry) and vacuum concentration (VVE). The obtained samples were stored at −20 °C until use.

2.2. Antioxidation activities

2.2.1. Reducing power

A method developed by Liao et al. [19] for a reducing power test was used. In brief, sample solutions (the concentration of vinegar–egg concentrate was 0.1–2.0 mg/mL; the vinegar–egg solution and vinegar–egg condensate were 10–100%), α-tocopherol, and butylated hydroxyanisole methanolic solutions (positive control group) were spiked with 2.5 mL of phosphate buffer and 2.5 mL of 1% potassium ferricyanide. Mixtures were kept in a 50 °C water bath for 20 min, cooled by placing it in 20 °C water bath for 5 min, spiked with 2.5 mL of 10% trichloroacetic acid, and then centrifuged at 800 × *g* for 10 min. The supernatant (5 mL) was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride. The absorbance at 700 nm was then detected with a spectrophotometer after reaction for 10 min; higher absorbance (A_{700}) represents stronger reducing power.

2.2.2. DPPH free radical scavenging activity

The scavenging effect on DPPH free radical was measured following the method described by Shimada et al. [20] with some modifications. Sample solutions (5 mL) and α-tocopherolmethanolic solutions (positive control group) were added to 1 mL of 1 mM DPPH in methanolic solution. The mixture was shaken and left to stand for 30 min at room temperature. The absorbance rates of the resulting solution and the positive control group was measured at 517 nm. The DPPH scavenging activity percentage was expressed as [1 − (Abs sample/Abs blank)] × 100%.

2.2.3. Ferrous ion chelating ability

The method described by Decker and Welch [21] was adopted. Five milliliters of the test solutions, including sample and EDTA solutions, was spiked with 0.1 mL of 2 mM $FeCl_2$ and 0.2 mL of 5 mM ferrozine solutions. After reaction for 10 min, the absorbance at 562 nm of the resulting solutions was recorded. The higher ferrous ion chelating ability of the test sample gave a lower absorbance (A_{562}). The ferrous ion chelating ability percentage was expressed as [1 − (Abs sample/Abs blank)] × 100%.

2.2.4. Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of vinegar–egg solution and its products based on the method described by Liu et al. [22] Superoxide radicals are generated in PMS–NADH systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In this experiments, the superoxide radicals were generated in 3 mL of Tris–HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 mM) solution, 1 mL NADH (78 mM) solution and sample solution in water were mixed. The reaction was started by adding 1 mL of phenazine methosulphate (PMS) solution (10 mM) to the mixture. After reaction for 5 min at 25 °C, the absorbance at 560 nm of the resulting solutions was recorded. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was expressed as [1 − (Abs sample/Abs blank)] × 100%.

2.3. In vitro assay of ACE inhibition

The ACE inhibitory activity was measured using the spectrophotometric method and modification by Muguruma et al. [23]. The reaction mixture contained 5 mM Hip-His-Leu as a substrate, 300 mM NaCl and 8 mU enzymes in 100 mM sodium borate buffer (pH 8.3). A sample (150 μL, 150 mg/mL) was added to the reaction mixture referred to previously (150 μL) and mixed with 150 μL of 15 mM Hip-His-Leu containing 1 M NaCl. After incubation at 37 °C for 60 min, the reaction was stopped by adding 0.5 mL of 1 N HCl. The resultant hippuric acid was

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