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Cytotoxicity of Crude Proanthocyanidin Extract from Purple Glutinous Rice Bran (*Oryza sativa* L.) (Kum Doi Saket) Compared with Cyanidin 3-Glucoside on X63 Myeloma Cancer Cell Lines

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ABSTRACT

Proanthocyanidin and other phenolic compounds may potentially reduce the risk of cardiovascular diseases and cancer, as well as having antioxidant, anti-inflammatory and chemoprotective properties. Proanthocyanidin (PA) and cyanidin 3-glucoside (C3G), a polyphenolic compound of purple color found in purple glutinous rice (*Oryza sativa L.*), may also manifest these positive effects. This research evaluated the effect of PA and C3G on X63, a mouse-plasma cancer cell line of myeloma cells. PA and C3G were extracted from the purple rice bran of a local, Thailand, purple, rice genotype (Kum Doi Saket). The results showed that the amount of C3G extract from the rice genotype was 54.47 mg/100 g rice bran. The cytotoxicity of the crude PA extract was demonstrated by a dose-dependent decrease in the percentage cell viability of the control in the PA group. A significant difference ($p<0.05$) began at 100 μg/ml and IC_{50} occurred at 62.29 μg/ml. The C3G extract also exhibited a dose-dependent decrease, but the significant difference ($p<0.05$) began at 10 μM and IC_{50} occurred at 8.4 μM. This research demonstrated a dose-related cytotoxic effect on cancer cells by the crude PA and C3G extracts from purple glutinous rice. The results indicated the benefit of the purple rice genotypes as a functional food with potential anticancer properties.

Key words: cytotoxicity, proanthocyanidin, cyanidin 3-glucoside, purple glutinous rice

INTRODUCTION

Proanthocyanidins are naturally occurring plant metabolites widely available in fruits, vegetables, nuts, seeds, flowers and bark. Also known as procyanidins, these substances are the main precursors of the blue-violet and red pigments in plants (Bagchi *et al.*, 1997). Proanthocyanidins are high-molecular-weight polymers comprised of the monomeric unit flavan-3-ol. Proanthocyanidins have been reported to have antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic and vasodilatory actions (Anne, 2000). *In vivo* studies have shown grape seed proanthocyanidin extract is a better free-radical scavenger and inhibitor of oxidative

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tissue damage than vitamin C, E or beta carotene.

Anthocyanin is a subunit substance of proanthocyanidin. Anthocyanin and other phenolic compounds also have potentially beneficial effects, including reducing the risk of cardiovascular diseases and cancer as well as having antioxidant, anti-inflammatory and chemoprotective properties (Chen *et al*., 2006). Anthocyanins also showed inhibitory effects on the growth of some cancer cells such as human hepatoma cells (Yeh and Yen, 2005), human lung cancer cells and gastric cancer cells. Furthermore, the anthocyanins from the *Oryza sativa* L. *indica* type had inhibitory effects on the growth of Lewis lung carcinoma cells *in vivo* (Chen *et al*., 2005).

This study aimed to investigate the cytotoxicity effects of proanthocyanidin extracted from purple rice bran (Kum Doi Saket) and cyanidin 3-glucoside, with the screening done by the inhibition of the growth of myeloma cancer cells (X63) and determined using a flow cytometry technique.

MATERIALS AND METHODS

Extraction of proanthocyanidin

A sample of 40 gm of purple glutinous rice bran (Kum Doi Saket variety) was extracted by incubating and shaking with 0.8 g of ascorbic acid and 70% acetone/H_2O for 30 minutes. The solvent was filtered and evaporated at 40°C by a rotary evaporator. The solid was then dissolved in 40% methanol and filtered again. The extract solution was separated using a Sephadex LH 20 column and evaporated once more at 40°C. Finally, the extract solution was freeze-dried by a lyophilizer to obtain the powder of crude proanthocyanidin (PA) (Dalzell and Kervan, 1998).

Cyanidin 3-glucoside in purple glutinous rice bran was analyzed by an HPLC method (Ryu *et al*., 1998).

Cell culture

X63, a mouse-plasma cancer cell line of myeloma cells, was cultured in IMDM (Iscove's modified Dulbecco's media) supplemented with 10% fetal calf serum, 0.2 U/ml of gentamycin and 100 μg/ml streptomycin sulfate. All cells were maintained at 37°C in a humidified atmosphere of 5% CO_2.

Cytotoxicity determination by Flow cytometry

To evaluate the cytotoxicity of the crude proanthocyanidin extract and cyanidin 3-glucoside, flow cytometry was performed to determine the cell viability. Cells were seeded in 24-well plates at a density of 5×10^4 cell/well and treated with crude 0-500 μg/ml proanthocyanidin extract and 0-500 μM pure cyanidin 3-glucoside (BioChemika 52976), incubated at 37°C in a humidified atmosphere of 5% CO_2 for 72 hours. After the exposure period, media were resuspended and the cells counted by flow cytometer at 20 μl, low speed and a 60 second time period/well.

Statistical analysis

The statistical significance of the differences was calculated by the Student's t-test.

RESULTS

Analysis of cyanidin 3-glucoside from purple glutinous rice by HPLC

The amount of cyanidin 3-glucoside from the purple glutinous rice bran was 54.47 mg/100 g rice bran.

The cytotoxicity of crude proanthocyanidin extract from purple glutinous rice and cyanidin 3-glucoside (C3G) standard

The decrease in the percentage cell viability of the control in the PA group was dose-dependent (Figure 1A). The significant difference ($p<0.05$) began at 100 μg/ml (or 11.23 μM of

cyanidin 3-glucoside) and IC_{50} occurred at 62.29 μg/ml (or 6.99 μM of cyanidin 3-glucoside) as shown in Figure 2A. The decrease for the cyanidin 3-glucoside group was also dose-dependent, but the significant difference ($p<0.05$) began at 10 μM (Figure 1B) and IC_{50} occurred at 8.4 μM (Figure 2B). Figure 3 shows the photographs of myeloma cells at 0 hour and 72 hours in both the control and PA groups. At 72 hours, more dead cells were found in the PA group than in the control group.

Comparison effect of the level of crude proanthocyanidin extract with pure cyanidin 3-glucoside

The effective level for PA was initiated at 100 μg/ml and for C3G at 10 μM. The comparative dose effect is shown in Figure 4, with the relationship of the effect of PA to C3G having an R^2 value of 0.9643. The effectiveness per dose rate was 10 times higher for C3G compared to PA.

DISCUSSION

Cyanidin 3-glucoside has been reported to have a cytotoxic effect on several cancer cells, such as: on human lung cancer cell lines (A549), being dose-dependent at 25-100 μM (Chen *et al*., 2006); or on human hepatoma cancer cells, being

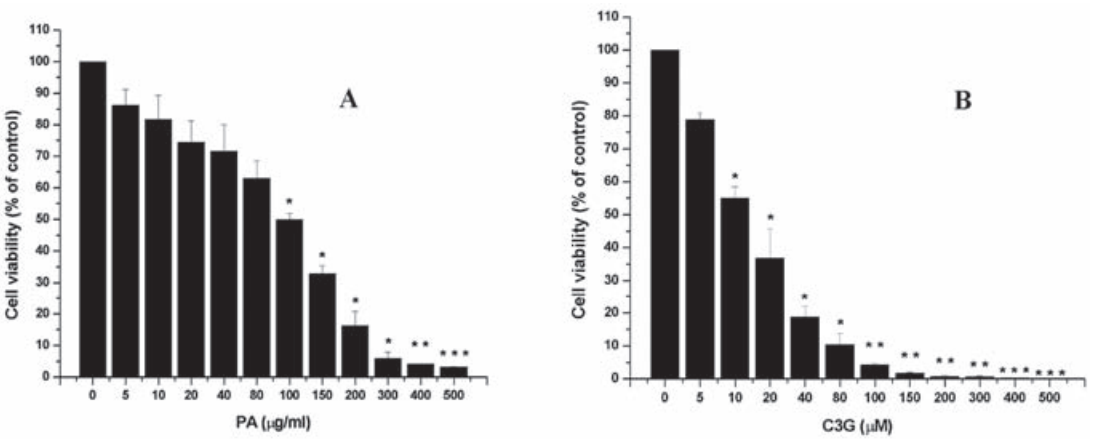

Figure 1 Cell viability of crude proanthocyanidin extract (PA) treated cells (A) and Cyanidin 3-glucoside (C3G) treated cells (B)
(* mean significant difference at $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

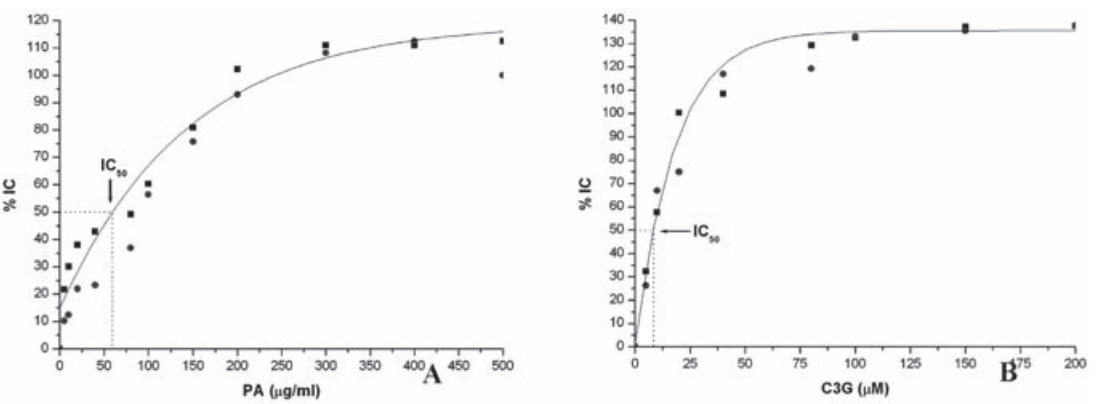

Figure 2 Inhibitory concentration percentages (% IC) of crude proanthocyanidin extract (PA) treated cells (A) and cyanidin 3-glucoside (C3G) treated cells (B) to myeloma cell (X63).

dose-dependent at 50-200 μM (Yeh and Yen, 2005). The sources of cyanidin were reported to be from berries and grapes (Zhao *et al*., 2004).

The results in this study indicated that proanthocyanidin and cyanidin 3-glucoside extracted from purple rice bran exhibited anticancer effects in inhibiting the viability of X63 cells. The cell viability inhibition by crude proanthocyanidin extract was found to be significant at 100 μg/mL (or 11.23 μM of cyanidin 3-glucoside) which was equal to the reported dose of the anthocyanidin fraction extracted from black rice which was significant at 100 μg/mL for LLC (mouse lung cancer cell lines) (Chen *et al*., 2005). The similarity between the pattern of viability screened from proanthocyanidin and the pattern screened from cyanidin 3-glucoside indicated that cyanidin 3-glucoside was a major source of anticancer activity. Cyanidin 3-glucoside was found to be a major compound in proanthocynadin

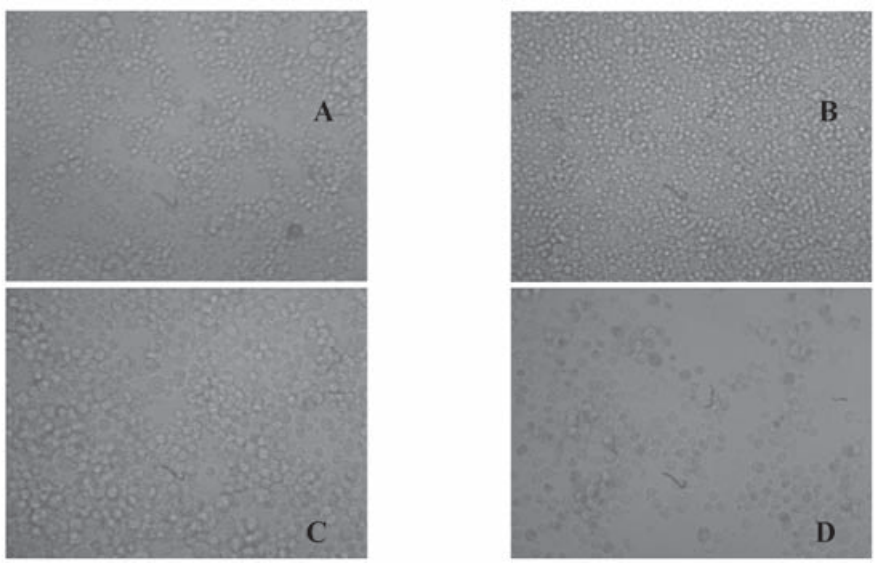

Figure 3 The photographs of myeloma cells (X63), control group at 0 h (A), control group at 72 h (B), proanthocyanidin group at 0 h (C), and proanthocyanidin group at 72 h (D).

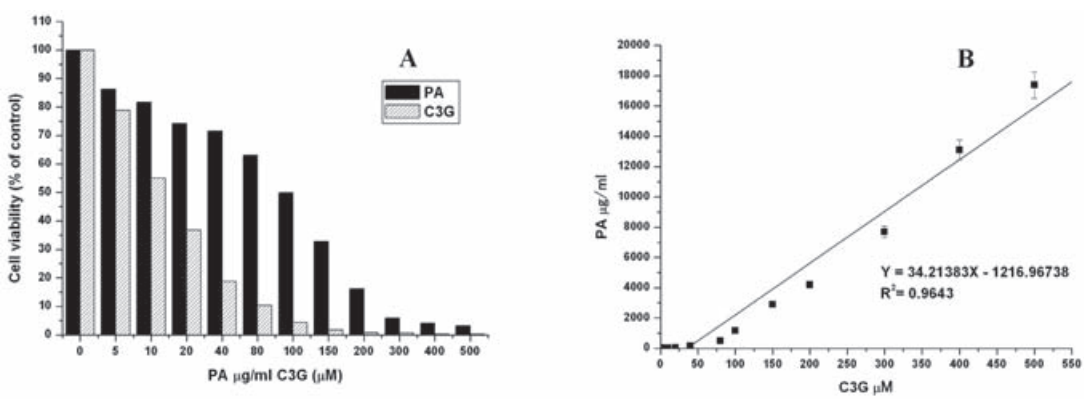

Figure 4 A comparison of percentage cell viability between crude proanthocyanidin extract (PA) treated cells with cyanidin 3-glucoside (C3G) treated cells (A), Relationship between concentrations of PA and C3G (B).

(Chen *et al*., 2005) and in this study 54.47 mg of this ingredient was found in purple glutinous rice. Another active ingredient also found in purple glutinous rice is gamma-oryzanol. Both these ingredients have pharmacological effects including antioxidant, anticancer and other effects which are considered beneficial for good health. For this reason, purple glutinous rice may be valuable for its anticancer properties as well as an agricultural source of a functional food type.

The crude proanthocyanidin extract (PA) of purple glutinous rice bran showed an anticancer effect by inhibiting the cell viability of myeloma cells (X63). The IC_{50} of PA occurred at 62.29 μg/mL which was equivalent to 6.99 μM of cyanidin 3-glucoside (C3G) when calculated at the C3G concentration and the IC_{50} of the C3G standard was 8.4 μM which was close to the calculated C3G level of PA (8.4 vs 6.99 μM). However, the IC_{50} level calculated for C3G from the crude proanthocyanidin extract was lower than for pure cyanidin 3-glucoside, because the crude proanthocyanidin extract has been reported to have other effective anthocyanins, such as delphinidin 3-glucoside, peonidin 3-glucoside and malvidin 3-glucoside which also had anticancer effects on cancer cells (Zhao *et al*., 2004), so that crude or natural forms of the antioxidant substances may have a greater effect than shown by the pure substance. The amount of purple glutinous rice bran at an effective level (IC_{50}) in this study was 6.106 mg which was calculated from purple glutinous rice having 1.02% proanthocyanidin in the compound (Wilaiwan, 2007). Thus a daily intake of a little purple glutinous rice bran or brown rice may have potential advantageous effects on health.

In conclusion, the crude proanthocyanidin extract of purple glutinous rice (Doi Saket variety, as approved by Department of Agronomy, Faculty of Agriculture, Chiang Mai University) was found to have an anticancer effect on plasma cancer cells of mice (X63) and to have a higher effect than pure cyanidin 3- glucoside.

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