บทความวิจัย

Antiproliferative and cytotoxicity effects of grape products and resveratrol in human cancer cell lines

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Abstract

Red grape products have been known to be antioxidants and anticarcinogenic properties. The present study investigated the total phenolic compound (TPC) contents of Zinfandel grape products, wine, juice and pomace, from the Suranaree University of Technology farm. The effects of grape products and trans-resveratrol on cytotoxicity and apoptotic induction on human cancer cell lines were investigated. The TPC content of ethanolic grape pomace extract (4,407.33±13.65 mg/L) was significantly higher than those of red wine (3,613.00±15.13 mg/L) and grape juice (1,102.67±21.96 mg/L). Trans-resveratrol and ethanolic grape pomace extract exhibited cytotoxic effects on pancreatic cancer : Panc 2.03 cells and cholangiocarcinoma : SNU 1079 cells in a dose dependent manner assessed by MTS assay. The cytotoxic activity was mediated via apoptosis. These data suggest a possible mechanism of cytotoxicity in both cancer cell lines, at least in part, through the regulation of apoptosis-related proteins.

Introduction

The use of synthetic antioxidants in food industry is severely restricted to both application and level. Hence there is a wide interest to natural antioxidants extracted from plants. Several polyphenol compounds

extracted from plants possess antioxidant activity, and the research on polyphenols occurring in plants has attracted considerable interest due to the numerous and health-beneficial effects, such as antimutagenic, anticarcinogenic, antiatherogenic, etc. Recently, a wide variety of polyphenolic compounds and non-flavonoids have been found mainly in vegetables and fruits especially in grapes and their derivatives (Frankel et al., 1993). One of the mian phytochemicals which is found in red grapes (*Vitis vinifera*) is trans-resveratrol (Figure 1). The beneficial effects of trans-resveratrol consumption include suppression of lipid peroxidation and eicosanoid synthesis, inhibition of platelet aggregation, anti-inflammatory, and vasorelaxant activities. Trans-resveratrol also has anticancer activities by affecting cell signaling pathway, modulating transcription factors, gene induction, regulation of enzyme activities and protein interactions in several *in vivo* and *in vitro* study. Cholangiocarcinoma is the highest incident primary liver cancer in the Northeast of Thailand (Vatanasapt et al., 1993) and is still a major health problem of people in this area. Pancreatic cancer is known as a cancer with poor prognosis. This malignant tumor is highly fatal and poor prognosis because there is no method for early detection and lack of effective treatments. Failure to Surgical resection of pancreatic cancer is available only in 15-20 % of all patients, while medical approaches, such as chemotherapy or radiation, have no cure. Induce apoptosis is a major factor limiting the efficacy of common treatment for cancer: surgical treatment, chemotherapy and radiotherapy (Dive, 1997). The resistance of pancreatic cancer and cholangiocarcinoma to chemotherapeutic agents is one of the serious problems in clinical situations. Therefore, suppression of apoptosis may be a feature of tumor promotion by chemical carcinogens. Indeed, many chemopreventive agents may act through the induction of apoptosis as a mechanism of anticarcinogenic action. Though there is enormous amount of data supporting trans-resveratrol's and certain grape products such as wine and juice possess anticancer effects *in vitro* and *in vivo*, There is not much data on the chemopreventive and therapeutic effects of grape promace especially those that prepared from Zinfandel red grapes. In addition, to the best of the author's knowledge, no studies of cytotoxic acitivities of trans-resveratrol and

grape products against human cholangiocarcinoma SNU 1079 and pancreactic Panc 2.03 cells are conducted. Since both cholangiocarcinoma and pancreatic cancers are very poor prognosis, resistant to the available chemotherapeutic agents and hence represent the serious problems in clinical treatment, the present study aimed to explore the therapeutic potential of trans-resveratrol and certain grape products against two human cholangiocarcinoma and pancreatic cancer cell lines, SNU 1079 and pancreactic Panc 2.03 cells, respectively. In initial phase of the study, the total phenolic contents of the products of red grapes grown at Suranaree University of Technology (SUT) farm and trans-resveratrol absorption in mice were determined. Then the chemopreventive effect of the products of red grapes on ultrastructural changes of liver tissue in mice was investigated. The last part of the study was to assess the cytotoxic and antiproliferative effects of trans-resveratrol and grape products on SNU 1079 and Panc 2.03 cells. The alteration of singaling protein factors in apoptotic pathway as the molecular mechanism of cytotoxicity on the cancer cell lines was also explored. Therefore, the objectives of the study were to investigate the total phenolic content of red grapes products (red win, grape juice and grape pomace) and to find cytotoxicity of commercial trans-resveratrol and red grapes products against selected human cancer cells *in vitro*.

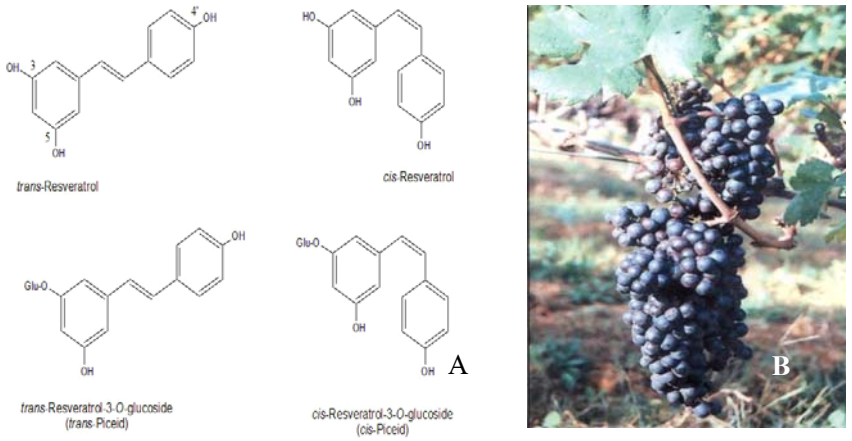

Figure 1. (A) Structure of trans-resveratrol and its glucosides (B) Zinfandel red grape

Source : (http://lpi.oregonstate.edu/infocenter/phytochemicals/trans-resveratrol/cistrans.html)

Materials

1. Plant

Zinfandel red grape (*Vitis vinifera*) was grown on Suranaree University of Technology (SUT) Farm, Nakhon Ratchasima province.

2. Human cancer cell lines

Two selected cancer cell lines: SNU 1079 (Seoul National University 1079) human cholangiocarcinoma and Panc 2.03 human pancreatic adenocarcinoma were used in cytotoxicity and apoptotic studies. These are from The Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University, Baltimore, MD, USA.

The two cell lines were cultured in RPMI 1640. media (Gibco, Grand Island, NY) supplemented with 10% heated-inactivated fetal bovine serum (FBS), 100 Unit/ml penicillin, 100 µg/ml streptomycin. All cell lines were maintained at 37°C with 5% CO_2 in humidified air and subculture weekly. In the apoptosis experiment low serum media (RPMI 1640 containing 1% FBS with similar supplements was used).

Methods

1. Preparation of Zinfandel red grape extracts

Zinfandel red grapes were harvested from farm. The preparation of grape juice and grape pomace were performed at SUT. Red wine was obtained from SUT farm and was used directly from the bottle. Total phenolic content and alcohol percentage were determined prior storage at 4°C until use.

2. Determination of total phenolic compound content (TPC)

Total phenolic compounds (TPC) of red wine, grape juice and pomace were determined by a modified Folin Ciocalteu's method (Swain and Hills, 1959; Matthaus, 2002) and results were expressed as as milligrams (mg) of gallic acid equivalents (GAE) per liter.

3. *In vitro* cytotoxicity studies

The normal human fibroblast, SNU 1079 and Panc 2.03 cell were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 Unit/ml penicillin and 100 µg/ml streptomycin. All cell lines were maintained at 37°C in 5% CO_2 humidified incubator and

were subcultured weekly. The MTS assay was used in this study to indirectly determine cytotoxic effects of ethanolic grape pomace extract and trans-resveratrol on normal human fibroblast, SNU 1079 and Panc 2.03 cells. Cells ($1x10^4$ cells/ml) were diluted with RPMI 1640 complete medium and seeded in a 96-well microtiter plates in the volume of 200 µl/well and incubated at 37°C in a 5% CO_2 humidified incubator. After incubation, media was removed, 100 µl of trans-resveratrol or ethanolic grape pomace extract was added to each well in triplicates to obtain the final concentrations of 2.5, 5, 10 or 20 µg/ml of trans-resveratrol or 50, 100, 200 or 400 µg/ml of ethanolic grape pomace extract. Cells treated with final concentration of 0.1% DMSO in complete RPMI 1640 media containing 1% FBS were used as a vehicle control. After 48 hour incubation, 20 µl of 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTS reagent) was added to each well. The absorbance (OD) of each well was measured at 490 nm using ELISA plate reader (Wallac Model 1420 Multilabel counter, michigan, USA). Percentage of cell viability was calculated using a formula below. IC_{50} value was expressed as concentration of extract in microgram per milliter that caused a 50% growth inhibition comparing with controls.

$$\% \text{ cell viability} = \frac{\text{OD (test sample)-OD(medium)}}{\text{OD (DMSO control)–OD (medium)}} \times 100$$

Statistic analysis

Statistic analysis was performed on commercial computerized statistic software, Sigma Stat® 2.0 and Sigma plot® 5.0, and data were expressesed as mean ± SD. Student's test (unpaired) and Duncan's Multiple Range Test (DMRT) were used to compare the total phenolic compound contents between grape products and treated control.

Results and discussion

1. The total phenolic content (TPC)

The determination of TPC content from grape products was measured by Folin-Ciocalteau's phenol reagent as modified from the method of Matthaus (2002). The amount of TPC in ethanolic grape pomace extract (4,407.33±13.65 mg/L) was higher than red wine (3,613.00±15.13 mg/L) and juice (1,102.67±21.96 mg/L). They were significant differences ($p < 0.01$). The results were shown as in Figure 2. These results were similar to those

presented by Teissedre and Landrault (1996) who reported the variability in the levels of TPC ranged from 1,847 – 2,600 mg/L for red wine. Jang et al. (1997) reported about 50-100 μg of trans-resveratrol per gram in grape skin. This finding was similar to the result of Jeandet, et al. (1991) and Okuda et al. (1977) who reported that trans-resveratrol, one of the important polyphenol, was found only in grape skin.

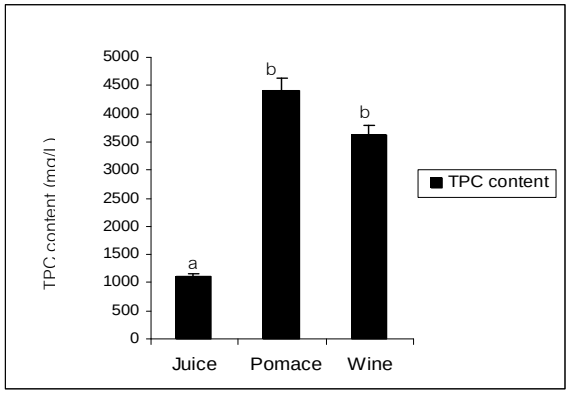

Figure 2. Comparison of TPC contents of Zinfandel grape products: juice, ethanolic grape pomace extract and wine expressed as mg/L GAE. Each sample was measured in triplicates. All values are mean±S.D. Values with differing alphabets are significantly different from each other at $p \leq 0.01$.

2. *In vitro* Cytotoxicity studies

Cytotoxic effect of trans-resveratrol and ethanolic grape pomace on SNU 1079 and Panc 2.03 cell lines was measured by MTS cell proliferation assay. Cells were treated with different concentrations of trans-resveratrol or ethanolic grape pomace for 48 hours**.** The results were shown as in Figure 3. The present study demonstrated that trans-resveratrol exerted cytotoxic activity in normal human fibroblast, Panc 2.03 and SNU 1079 cells. The data revealed that trans-resveratrol exerted a greater cytotoxic effect on Panc 2.03 cells than normal human fibroblast and SNU 1079 cells. Joe et al. (2002) reported that there is limited information on the toxicity of resveratrol in experimental animals, and there are, apparently, no clinical toxicity data on the use of pure resveratrol in human. Clement et al. (1998) demonstrated that resveratrol is minimally toxic to human peripheral blood cells. The different susceptibility of these cell lines are likely due to the different in genetic background. The results in this study agree with several previous reports which showed the growth inhibitory activity of resveratrol in various human cancer cell lines including epidermoid carcinoma A 431 cells (Ahmad et

al., 2001), human SW480 colorectal tumor cells (Delmas et al., 2002), melanoma cells (Niles et al., 2003), Seg-1 esophageal adenocarcinoma cells, MCF7 breast carcinoma cells, HL60 promyelocytic leukemia cells (Joe et al., 2002). These results supported our hypothesis that trans-resveratrol or ethanolic grape pomace extract could inhibit cancer cell growth or enhance the molecular mechanism of the chemopreventive effects on cancer.

Conclusion

This study demonstrates that the use of red grape products and trans-resveratrol possess anticancer and antiproliferative activities. The characterization of grape products is depended on the concentrations of total phenolic compound (TPC) contents and activities. The TPC contents of ethanolic grape pomace extract, red wine and juice measured by Folin Ciocalteu's method are 4,407.33±13.65, 3,613.00±15.13 and 1,102.67±21.96 mg/L, respectively. The study also suggests that trans-resveratrol and ethanolic grape pomace extract exhibit the potent cytotoxic and apoptotic activities towards cancer cells. This study demonstrated that trans-resveratrol and ethanolic grape pomace extract exhibited cytotoxic effect on Panc 2.03 and SNU 1079 cell lines in a dose dependent manner as assessed by MTS assay. These properties of trans-resveratrol and ethanolic grape pomace extract suggest that it could have a possible therapeutic in pancreatic and cholangiocarcinoma patients. Therefore, trans-resveratrol and ethanolic grape pomace extract possess antiproliferative properties towards cancer cells and could be promising anticarcinogens. Further explanation in the development of both compounds as chemopreventive agents should be highly warranty.

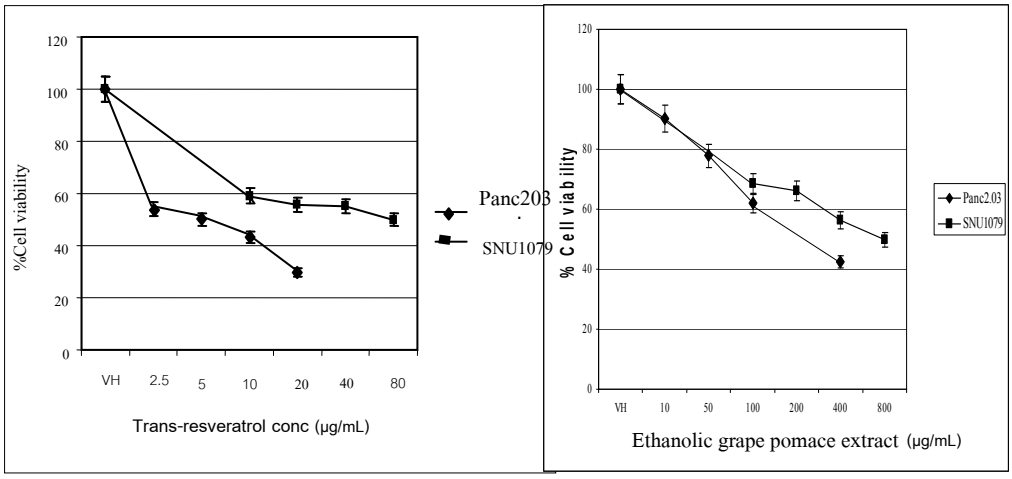

Figure 3. Cytotoxic effect of trans-resveratrol and ethanolic grape pomace extract on SNU 1079 and Panc 2.03 cell lines. Each value represents the means±S.E. of three independent experiments.

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