Cytotoxic and Apoptotic-inducing Effects of Purple Rice Extracts and Chemotherapeutic Drugs on Human Cancer Cell Lines

Ratana Banjerdpongchai, Benjawan Wudtiwai, Korawan Sringarm

Abstract

Pigmented rice is mainly black, red, and dark purple, and contains a variety of flavones, tannin, polyphenols, sterols, tocopherols, γ-oryzanos, amino acids, and essential oils. The present study evaluated the cytotoxic effects of purple rice extracts (PREs) combined with chemotherapeutic drugs on human cancer cells and mechanisms of cell death. Methanolic (MeOH) and dichloromethane (DCM) extracts of three cultivars of purple rice in Thailand: Doisaket (DSK), Nan and Payao (PYO), were tested and compared with white rice (KK6). Cytotoxicity was determined by 3-(4, 5-dimethyl)-2, 5-diphenyltetrazolium bromide (MTT) assay in human hepatocellular carcinoma HepG2, prostate cancer LNCaP and murine normal fibroblast NIH3T3 cells. MeOH-PYO-PRE was the most cytotoxic and inhibited HepG2 cell growth more than that of LNCaP cells but was not toxic to NIH3T3 cells. When PREs were combined with paclitaxel or vinblastine, they showed additive cytotoxic effects on HepG2 and LNCaP cells, except for MeOH-PYO-PRE which showed synergistic effects on HepG2 cells when combined with vinblastine. MeOH-PYO-PRE plus vinblastine induced HepG2 cell apoptosis with loss of mitochondrial transmembrane potential (MTP) but no ROS production. MeOH-PYO-PRE-treated HepG2 cells underwent apoptosis via caspase-9 and -3 activation. The level of γ-oryzanol was highest in DCM-PYO-PRE (44.17 mg/g) whereas anthocyanin content was high in MeOH-PYO-PRE (5.80 mg/g). In conclusion, methanolic Payao purple rice extract was mostly toxic to human HepG2 cells and synergistically enhanced the cytotoxicity of vinblastine. Human HepG2 cell apoptosis induced by MeOH-PYO-PRE and vinblastine was mediated through a mitochondrial pathway.

Keywords: Purple rice extracts - cytotoxicity - human cancer cells - apoptosis - chemotherapeutic drugs
Materials and Methods

Chemicals

Dimethyl sulfoxide (DMSO), paclitaxel, vinblastine, 3, 3-diheptyloxycarbocyanine iodide (DiOC	extsubscript{5}), 2',7'-dichlorofluorescein diacetate (DCFH-DA) and 3-(4,5-dimethyl)-2, 5-diphenyltetrazolium bromide (MTT) were obtained from Sigma/Aldrich, St. Louis, MO, USA. RPMI-1640 medium, DMEM medium, Z-DEVD-AFC (Asp-Glu-Val-Asp-7-amino-4-trifluoromethylcoumarin), Z-LEHD-AFC (Leu-Glu-His-Asp-7-amino-4-trifluoromethylcoumarin), and Z-IETD-AFC (Ile-Glu-Thr-Asp-7-amino-4-trifluoromethylcoumarin) substrates were obtained from Invitrogen, USA. Annexin V-fluos staining kit was purchased from Roche, Indianapolis, IN, USA.

Rice materials and extraction

Purple glutinous rice (Oryza sativa var. indica) such as Nan, Doi Saket and Payao. The purple rice and white rice were separated within a rice kernel. Nitrogen and the minerals are found to be more abundant in the outer than in the inner portion, but amylose is rich in the inner portion. The color of flour samples become red rice or purple rice because only the surface of hulled rice contains pigments (Itani et al., 2002). There are several cultivars in Thai purple rice (Oryza sativa var. indica) such as Nan, Doi Saket and Payao. The aims of the study were to determine the cytotoxic effect of the methanolic (MeOH) and dichloromethane (DCM) extracts of these three purple rice cultivars compared to white rice (Kor Khor6) on human hepatocellular carcinoma HepG2 and prostate cancer LNCaP cells compared to murine normal fibroblast NIH3T3 cells. The combined effects with paclitaxel or vinblastine on growth inhibition of cancer cells were demonstrated. The mode and mechanisms of cell death were determined. Anthocyanin and γ-oryzanol amounts in PREs were measured.

Cell culture

Human prostate cancer LNCaP cells were purchased from American Type Culture Collection (ATCC), Manassas, VA, USA. Human hepatocellular carcinoma HepG2 cells and murine fibroblast NIH3T3 cells were gifts from Associate Professor Prachya Kongtawelert (Faculty of Medicine, Chiang Mai University). The prostate cancer LNCaP cells were cultured in RPMI-1640 medium with 25 mM NaHCO	extsubscript{3}, 20 mM HEPES, 100 units/mL penicillin, 100 µg/mL streptomycin and supplemented with 10% fetal bovine serum. Human hepatocellular carcinoma HepG2 and normal mouse fibroblast NIH3T3 cells were cultured in DMEM medium. The dry powder extract was dissolved in dimethyl sulfoxide (DMSO) as a vehicle and the maximal volume used did not exceed 10 µl/mL of media. The cell lines were grown at 37°C in a 5% CO	extsubscript{2} atmosphere. The murine normal fibroblast cells and human cancer cells (1X10	extsuperscript{5}) were treated with methanolic and dichloromethane purple rice extracts at indicated concentrations and durations.

Cytotoxicity by MTT assay

HepG2 and LNCaP cells (3X10	extsuperscript{5} cells/ml) and NIH3T3 (1X10	extsuperscript{4} cells/ml) were cultured and incubated with methanolic and dichloromethane PREs (0, 50, 100, 150 and 200 µg/ml) at 37°C in 5%CO	extsubscript{2} atmosphere for 24 or 48 h. The cell viability was determined by using MTT assay (Wudtiwai et al., 2011). Briefly, MTT solution (sterile stock solution of 5 mg/ml) was added to cell media at the final concentration of 100 µg/ml and the solution incubated for 4 h at 37°C in a humidified 5%CO	extsubscript{2} atmosphere. The medium was then removed and cells were treated with DMSO for 30 min. The optical density of the cell lysate was measured at 540 nm with reference wavelength of 630 nm using microtiter plate reader (Biotek, USA) at 570 nm. The percentage of cell viability was calculated and 10, 20 and 50% inhibitory concentrations (IC	extsubscript{10}, IC	extsubscript{20} and IC	extsubscript{50}) were determined and used for further experiments. The cytotoxic effect of the combined treatment was determined by MTT assay. The combined effects of the extracts (at IC	extsubscript{50} level) with the chemotherapeutic drugs (paclitaxel or vinblastine, at IC	extsubscript{50} level) were incubated for 48 h and determined by MTT assay in human HepG2 and LNCaP cells.

Determination of phosphatidylserine externalization in apoptotic cells

PRE-treated HepG2 cells were washed once in phosphate-buffered saline solution, centrifuged at 200X g and the cell pellet was suspended in 100 µl of binding buffer provided by the annexin V-fluos staining kit. Annexin V-FITC (20 µl) and propidium iodide (PI, 10 µl) were added and the cell suspension was left at room temperature for 15 min in the dark. Finally, 970 µl of binding buffer were added. Analysis was conducted using FACScan (Becton Dickinson, USA). Cells that were stained with annexin V-FITC, and annexin V-FITC together with PI, were designated as early and late apoptotic cells, respectively (Prommaban et al., 2012).
Either 40 nM 3, 3′-dihexyloxocarbocyanine iodide (DiOC 6) for mitochondrial transmembrane potential (MTP) determination or 5 μM 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) for ROS detection were added for 15 min at 37°C before cells were subjected to flow cytometry. Cells were analyzed by a FACScan equipped with a 488 nm argon laser using CellQuest software (Becton-Dickinson, USA). Data were depicted as histograms and percentage of cells displaying loss of MTP or increase of ROS production.

**Assay of caspase-3,-8 and -9 activities**

Cleavage of the fluorogenic peptide substrates DEVD-AFC, IETD-AFC and LEHD-AFC indicative of caspase-3-, caspase-8- and caspase-9-like enzyme activity, was estimated. Cell lysates (1×10⁶ cells) and substrate (50 μM) were combined in a standard reaction buffer and added to a 96-well plate. Enzyme-catalyzed release of AFC was measured by a fluorescence plate reader (Biotek, USA) using 355 nm excitation and 460 nm emission wavelengths.

**Determination of anthocyanin and γ-oryzanol contents in purple rice extracts**

Anthocyanin content was determined by Ryo method (Ryu et al., 1998). Briefly, each PRE (1 g) was dissolved in 0.5% trifluoroacetic acid (TFA) in 95% ethanol (20 ml), mixed and stirred for 9 h at room temperature, then filtered with filter paper No. 4, C18 cartridge and finally 0.45 μm filter before processing through HPLC-photodiode array (PDA) (Shimadzu, Japan). Detector system is photodiode array detector (520 nm), column 25 cm ×4.6 mm diameter column Allure C18 (Restek, USA), mobile phase A is 0.1% TFA in H₂O whereas mobile phase B is 0.1% TFA in methanol, flow rate 1.0 ml/minute. The chromatogram was compared to the standard anthocyanins, viz., C3G and P3G.

The γ-oryzanol content was determined by HPLC method. Briefly, PRE (1 g) was dissolved in 0.5% TFA in 95% ethanol and stirred well at room temperature. HPLC profiles are as follows: UV-vis diode array detector (set 330 and 450 nm), column 25 cm ×4.6 cm of microsorb-MV C18, isocratic mobile phase with methanol: acetonitrile: dichloromethane: acetic acid (50:44:3:3) and flow rate of 1.4 ml/minute. The chromatogram was obtained and compared to standard γ-oryzanol.

**Statistical analysis**

Results are expressed as mean±SD. Statistical difference between control and treated group was determined by one-way ANOVA (Kruskal Wallis analysis) at limit of p<0.05 from 3 independent experiments conducted in triplicate. For comparison between two groups, data were analyzed using Mann-Whitney U test.

**Results**

**Cytotoxicity of PREs and/or chemotherapeutic drugs**

Methanolic Payao purple rice extract (MeOH-PYO-PRE) was the most cytotoxic to the HepG2 cells compared to other extracts. Percent cell viability of cancer cells decreased dose- and time- dependently in both methanolic (MeOH) and dichloromethane (DCM) extracts of all purple rice cultivars. The sensitivity of the cells towards the extracts was as follows, viz., HepG2>LNCaP>NIH3T3 cells as shown in Figure 1 and 2. Murine normal fibroblast NIH3T3 cells were most resistant to the eight rice extracts (Figure 2). The 50% inhibitory growth concentration was detectable at 48 h to be 175.95±8.02 μg/ml MeOH-PYO-PRE affecting on HepG2 cells, whereas those of the other PREs were more than 200 μg/ml for both cancer cell lines. Paclitaxel or vinblastine was incubated with human HepG2 liver cancer cells and IC₅₀ level was calculated to be 7.8±0.42 pM (picomolar) and 40.95±0.02 nM from inhibitory growth curve at 48 h treatment (Figure 3) whereas the IC₅₀ levels of paclitaxel or vinblastine towards LNCaP cells were more than 2 μM (Table 1 and 2).

The cell viability of LNCaP and HepG2 cancer cells (when combined with MeOH- or DCM-PREs at IC₅₀ levels
plus paclitaxel or vinblastine at 20% inhibitory growth concentration) decreased as an additive effect (Figure 4A-4D and Figure 5A, 5B and 5D). However, 200 µg/ml MeOH-PYO-PRE and 41 nM vinblastine synergistically and significantly reduced cell viability of HepG2 cells as shown in Figure 5C.

**Table 2. Cytotoxic Effect of Vinblastine on Human Hepatocellular HepG2 and Prostate Cancer LNCaP Cells with IC$_{10}$, IC$_{20}$ and IC$_{50}$ Values at 24 and 48 h**

<table>
<thead>
<tr>
<th>Human cancer cell lines and incubation time</th>
<th>Vinblastine (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HepG2 24 h (µM)</td>
<td>IC$_{10}$ 80.18±0.09 &lt;2000</td>
</tr>
<tr>
<td>HepG2 48 h (µM)</td>
<td>IC$_{20}$ 40.95±0.02 561.92±0.02</td>
</tr>
<tr>
<td>LNCaP 24 h (µM)</td>
<td>288.65±0.01 717.39±0.01 &gt;2000</td>
</tr>
<tr>
<td>LNCaP 48 h (µM)</td>
<td>IC$_{50}$ 20.0±0.02 &gt;2000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Human cancer cell lines and incubation time</th>
<th>Paclitaxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>HepG2 24 h (µM)</td>
<td>IC$_{10}$ 0.0374±0.0305 0.105±0.09048 &gt;2</td>
</tr>
<tr>
<td>HepG2 48 h (µM)</td>
<td>IC$_{20}$ 3.4±0.1 7.8±0.42 36.6±1.93</td>
</tr>
<tr>
<td>LNCaP 24 h (µM)</td>
<td>1.486±0.758 1.891±0.479 &gt;2</td>
</tr>
<tr>
<td>LNCaP 48 h (µM)</td>
<td>0.215±0.143 0.747±0.448 &gt;2</td>
</tr>
</tbody>
</table>

**Figure 3. Cell Cytotoxicity of Human Cancer Cells after Treatment with Paclitaxel for 24 or 48 h by MTT Assay.** Percent cell viability of HepG2 cells treated with paclitaxel for A) 24 h; B) 48 h and LNCaP cells treated with paclitaxel for C) 24 h; and D) 48 h. Shown as mean±SD. The data were obtained from triplicate of 3 independent experiments. *p<0.05 compared to control

Human hepatocellular carcinoma HepG2 cells treated with MeOH-PYO-PRE combined with vinblastine depolarized the mitochondrial transmembrane potential (MTP) with the reduction of DiOC$_6$ fluorescence determined by flow cytometry. Percent MeOH-PYO-PRE-treated HepG2 with loss of MTP increased significantly at the dose of 200 µg/ml. The combined MeOH-PYO-PRE plus vinblastine-treated cells also reduced MTP when...
Cytotoxic and Apoptotic-inducing Effects of Purple Rice Extracts and Chemotherapeutic Drugs on Human Cancer Cell Lines

Figure 5. Cell Cytotoxicity of Human Cancer Cells after Combined Treatment with Purple Rice Extracts and Vinblastine for 48 h by MTT Assay. Percent cell viability of LNCaP cells when treated with A) MeOH-PRE; B) DCM-PRE; HepG2 cells when treated with C) MeOH-PRE; and D) DCM-PRE, in the presence or absence of vinblastine, is shown as mean±SD. The data were obtained from triplicate of 3 independent experiments.*p<0.05 compared to control, MeOH, methanol; DCM, dichloromethane; Vin, vinblastine

Figure 6. Apoptosis Cell Induction and Loss of Mitochondrial Transmembrane Potential of HepG2 Cells. A) MeOH-PYO-PRE-treated cells in the presence or absence of vinblastine at IC_{50} levels were examined by staining with annexin V-FITC/propidium iodide and using flow cytometry. Percentage of early apoptotic cells is shown. B) MTP was measured by staining the cells with DiOC_{6} and proceeding through flow cytometer. Percentage of cells with loss of MTP is shown.*p<0.05 compared to control (without treatment) or vinblastine alone as shown in Figure 6B. However, there was no ROS production as demonstrated by DCFH-DA staining and FACscan (data not shown).

Induction of caspases-3, -8 and -9 activities

To confirm the pathway of apoptosis, caspase-3, -8 and -9 activities were measured by using fluorogenic substrates and the fluorescence microplate reader. The caspase-9 and -3 activities increased significantly in HepG2 cells treated with MeOH-PYO-PRE plus vinblastine at IC_{50} levels compared to control as shown in Figure 7A and 7C, whereas caspase-8 activity did not significantly alter (Figure 7B). This indicated the involvement of intrinsic or mitochondrial pathway of apoptosis which is consistent with the reduction of mitochondrial transmembrane potential.

Anthocyanin and γ-oryzanol contents

Purple rice contains the dark purple pigments in its bran (outer coat of the rice), which are mainly anthocyanins (Abdel-Aal et al., 2006). The content of anthocyanins was then measured to evaluate the amounts of pigments whether they were related to their cytotoxicity. Cyanin 3-glucoside (C3G) was the main component of anthocyanins in purple rice MeOH extracts. In MeOH-Nan-PRE, the total anthocyanins including C3G and peonidin-3-glucoside (P3G) were 7.75 mg/g, which were highest among the three cultivars tested. Total anthocyanins of DSK-PRE were 4.47 mg/g and those of PYO-PRE were 5.80 mg/g whereas there was no anthocyanin detected in KK6 rice extract as shown in Table 3. The anthocyanins could not be detected in dichloromethane extracts since anthocyanins are hydrophilic compared to dichloromethane, which are more hydrophobic.

Figure 7. Effects of Methanolic Payao Purple Rice Extract and/or Vinblastine on Caspase-3,-8 and -9 Activities in HepG2 Cells. MeOH-PYO-PRE with or without vinblastine-treated HepG2 cells were determined for A) Caspase-3; B) Caspase-8; and C) Caspase-9 activities as described in the Materials and Methods.*p<0.05 compared to control (without treatment)
The dietary polyphenol cyanidin, but not its glycosides, is a potent inhibitor of neurotensin- and epidermal growth factor-induced apoptosis in human SH-SY5Y neuronal cells. The protection of the cells by the GBR extract is linked to its ability to induce transcriptional changes in antioxidant (SOD 1, SOD 2 and catalase) and growth factor-induced metabolic activity. It increases the free intracellular Ca$^{2+}$ and cellular growth of cultured colon carcinoma cells in vitro (Briviba et al., 2001). The anthocyanins also inhibit tumor development in vivo (Kang et al., 2003). The aglycones of the most abundant anthocyanins in food, anthocyanidins and delphinidin are potent inhibitors of the EGFR, shutting off downstream MAPK and Elk-1 signaling cascades, contributing substantially to the growth-inhibitory effects on cancer cells (Meiers et al., 2001). Anthocyanidins/anthocyanins and anthocyanin-rich extracts induce TNF-alpha production and act as modulators of the immune response in activated macrophages (Wang and Mazza, 2002).

A standardized extract of black rice (*Oryza sativa* L. indica) pigmented fraction (BRE) containing known proportions of cyanidin 3-glucoside and peonidin 3-glucoside exhibits marked antioxidant activities and free radical scavenging capacities in an in vitro model system (Hu et al., 2003).

*Oryza sativa* cv. Heugjinjubyeo (Gramineae), anthocyanin-pigmented rice, having dark purple grains, is well known as enriched rice with an improved taste. From spectral analysis, the cytotoxic components from this rice trait are the anthocyanidins: cyanidin and malvidin. The 50% growth inhibitory concentrations (IC$_{50}$) of cyanidin and malvidin on U937, human monocytic leukemia cells, are 60 and 40 µg/mL, respectively. These compounds shows cytotoxicity through the arrest of the G(2)/M phase of cell cycle and induction of apoptosis (Hyun and Chung, 2004).

Two bioactive compounds, peonidin 3-glucoside and cyanidin 3-glucoside, from *Oryza sativa* L. indica are isolated and tested with many cancer cell lines. Human ductal breast carcinoma HS578T cells line is sensitive to peonidin 3-glucoside and cyanidin 3-glucoside. Peonidin 3-glucoside or cyanidin 3-glucoside treatment strongly inhibits cell growth via G2/M arrest. Regarding cell cycle-related proteins, peonidin 3-glucoside treatment results in down-regulation of protein levels of cyclin-dependent kinase (CDK)-1, CDK-2, cyclin B1, and cyclin E, whereas cyanidin 3-glucoside decreases the protein levels of CDK-1, CDK-2, cyclin B1, and cyclin D1. In addition, cyanidin 3-glucoside or peonidin 3-glucoside also induces caspase-3 activation, chromatin condensation, and cell death. Furthermore, anthocyanins from *O. sativa* L. indica are evidenced by their inhibition on the growth of Lewis lung carcinoma cells in vivo (Chen et al., 2005).

Rice bran exerts beneficial effects towards several types of cancer, such as breast, lung, liver and colorectal cancer. The chemopreventive potential has been related to the bioactive phytochemicals present in the bran portion of the rice, viz., ferulic acid, tricin, β-sitosterol, γ-oryzanol, tocotrienols/tocopherols and phytic acid. These bioactive compounds contain scavenging activity of free radicals and block chronic inflammatory response. The anticancer effects of the rice bran-derived bioactive components are mediated through apoptosis induction, inhibition of cell proliferation and cell cycle progression alteration in malignant cells (Henderson et al., 2012).

The ethyl acetate extract of germinated brown rice (GBR) has higher total phenolic content and antioxidant capacity compared to brown rice. The GBR extract (up to 10 ppm) prevents H$_2$O$_2$-induced apoptosis in human SH-SY5Y neuronal cells. The protection of the cells by the GBR extract is linked to its ability to induce transcriptional changes in antioxidant (SOD 1, SOD 2 and catalase) and the most abundant apoptotic (AKT, NF-Kβ, ERK1/2, JNK, p53 and p38 MAPK) genes that tends towards survival (Azmì et al., 2013). Black rice pericarp extract can inhibit proliferation, change the cell cycle distributions and induce apoptosis in human prostatic cancer cell PC-3. Its inhibitory effect is through promoting activation of the JNK and p38 signaling pathway (Jiang et al., 2013).

The water-soluble enzymatic extract from rice bran (EERB) induces MOLT-4 cell (human T cell acute model) pigmented fraction (BRE) containing known proportions of cyanidin 3-glucoside and peonidin 3-glucoside exhibits marked antioxidant activities and free radical scavenging capacities in an in vitro model system (Hu et al., 2003).

**Table 3. Concentrations of Anthocyanins in the Methanolic Extracts of Three Cultivars of Purple Rice and White Rice**

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Concentrations (mg/g)</th>
<th>C3G</th>
<th>P3G</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doi Saket (DSK)</td>
<td>2.5</td>
<td>1.97</td>
<td>4.47</td>
<td></td>
</tr>
<tr>
<td>Payao (PYO)</td>
<td>3.19</td>
<td>2.61</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>Nan</td>
<td>4.4</td>
<td>3.35</td>
<td>7.75</td>
<td></td>
</tr>
<tr>
<td>Kor Khor6 (KK6)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td></td>
</tr>
</tbody>
</table>

* N.D., not detectable; C3G: cyanidin 3-glucoside; P3G: peonidin 3-glucoside

**Table 4. Concentrations of Gamma-oryzanol in the Dichloromethane and Methanolic Extracts of Three Cultivars of Purple Rice**

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Concentrations (mg/g)</th>
<th>DCM</th>
<th>MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doi Saket (DSK)</td>
<td>36</td>
<td>2.18</td>
<td></td>
</tr>
<tr>
<td>Payao (PYO)</td>
<td>44.17</td>
<td>1.62</td>
<td></td>
</tr>
<tr>
<td>Nan</td>
<td>42.88</td>
<td>2.98</td>
<td></td>
</tr>
<tr>
<td>Kor Khor6 (KK6)</td>
<td>10.94</td>
<td>3.03</td>
<td></td>
</tr>
</tbody>
</table>

*DCM, dichloromethane; MeOH, methanol

The content of γ-oryzanol was detectable in both methanolic and dichloromethane extracts, but it was higher in dichloromethane extracts. The highest amount was found in DCM-PYO-PRE (44.17 mg/g) and the lowest level was MeOH-PYO-PRE (1.62 mg/g) as shown in Table 4.

**Discussion**

The grain anthocyanin content is easily influenced by the environment. The grain anthocyanin content of the P lines is much greater due to the reduced-grain weight, yield per plot and grain/brown rice thickness compared to the W lines. The small sink size is a key reason behind yield reduction of purple pericarp rice anthocyanin (Ji et al., 2012). C3G and P3G contents in PYO purple rice brans are more than those of DSK cultivar 1.2 folds (data not shown). But purple rice grains (DSK cultivar) contained C3G and P3G 1.8-fold more than Nan and PYO (unpublished data). The purple pigment in the grains of *Oryza sativa* is from the anthocyanin contents (Ryu et al., 1998).

The dietary polyphenol cyanidin, but not its glycosides, is a potent inhibitor of neurotensin- and epidermal growth factor-induced metabolic activity. It increases the free intracellular Ca$^{2+}$ and cellular growth of cultured colon carcinoma cells in vitro (Briviba et al., 2001). The anthocyanins also inhibit tumor development in vivo (Kang et al., 2003). The aglycones of the most abundant anthocyanins in food, anthocyanidins and delphinidin are potent inhibitors of the EGFR, shutting off downstream MAPK and Elk-1 signaling cascades, contributing substantially to the growth-inhibitory effects on cancer cells (Meiers et al., 2001). Anthocyanidins/anthocyanins and anthocyanin-rich extracts induce TNF-alpha production and act as modulators of the immune response in activated macrophages (Wang and Mazza, 2002).

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lymphoblastic leukemia) apoptosis in a dose-dependent way. Additionally, EERB exerts an immunomodulatory effect on N13 microglia cells, by inducing TNF-alpha (tumour necrosis factor-alpha) expression, which plays a key role in the innate immune response to infection (Revilla et al., 2013).

Brown rice fermented with Aspergillus oryzae, designated as FBRA, is a dietary fiber-rich food, and fully appreciated as one of the prebiotics, which are generally considered to be beneficial to the health of the body, because of stimulating the growth and/or the activity of bacteria in the digestive system. The exposure of human colorectal cancer HCT116 cells to FBRA extract reduces their viabilities in a concentration-dependent manner, and the cytotoxicity is attributed to the induction of apoptosis through the cellular oxidative stress. FBRA extract causes a significant elevation of Bax protein and a slight reduction of Bcl2 protein levels, and activates caspase-3 activity. Thus, FBRA extract can exert oxidative damage to the cells, resulting in apoptotic cell death by activating the mitochondrial pathway in human colorectal tumor HCT116 cells (Itoh et al., 2012).

The anthocyanin-rich extract from black rice (AEBR) reduces the viability of breast cancer cell lines MCF-7 (ER(+), HER2/neu(-)), MDA-MB-231 (ER(-), HER2/neu(-)), and MDA-MB-453 (ER(-), HER2/neu(+)) and induces apoptosis in MDA-MB-453 cells via the intrinsic pathway in vitro by activating caspase cascade, cleaving poly (ADP-ribose) polymerase (PARP), depolarizing mitochondrial membrane potential, and releasing cytochrome c. Oral administration of AEBR (100 mg/kg/day) to BALB/c nude mice bearing MDA-MB-453 cell xenografts significantly suppresses tumor growth and angiogenesis by inhibiting the expression of angiogenesis factors MMP-9, MMP-2, and uPA in tumor tissue (Hui et al., 2010).

The ethyl acetate extract of “Kurosu” (EK), Japanese traditional vinegar from unpolished rice, inhibits cell proliferation of human cancer cell lines, viz., colon adenocarcinoma (Caco-2), lung carcinoma (A549), breast adenocarcinoma (MCF-7), bladder carcinoma (5637), and prostate carcinoma (LNCaP) cells. Flow cytometry of EK-treated Caco-2 cells demonstrates a decrease of cell number in the G2/M phase and an increase in the sub-G1 phase (apoptotic). p21 mRNA expression is induced in EK-treated Caco-2 cells. Thus, EK causes G0/G1 arrest through p21 induction in Caco-2 cells (Nanda et al., 2004).

Paclitaxel enhances the polymerization of tubulins to stabilize microtubules. The drug binds specifically to the microtubule polymer and polymerizes tubulin even in the absence of cofactors such as guanosine triphosphate and microtubule-associated proteins. Paclitaxel blocks cell proliferation at the G2/M phase and such cells are unable to form a normal mitotic apparatus (Horwitz, 1994). The combined effect of all purple rice extracts on paclitaxel-induced cell cytotoxicity is not synergistic or antagonistic but additive (Figure 4A-D, 5A, 5B, 5D).

Significant prevention of supercoiled DNA strand scission induced by reactive oxygen species (viz., peroxyl radicals and hydroxyl radicals) and suppression of the oxidative modification of human low-density lipoprotein are obtained when incubated with the rice extracts (Hu et al., 2003). Vinblastine rapidly induces Noxa and acutely sensitizes primary chronic lymphocytic leukemia cells to ABT-737 (a Bcl-2 inhibitor), which therefore enhances CLL cells to undergo apoptosis (Bates et al., 2013). The synergistic effect of MeOH-PYO-PRE on vinblastine-induced HepG2 cytotoxicity was found in HepG2 cells (Figure 5C) and such extract plus vinblastine induced apoptotic cell death. It has been reported that γ-oryzanol reduces plasma cholesterol in hypercholesterolemic hamsters (Wilson et al., 2007) and also suppresses the accumulation of cholesterol in arterial endothelium (atheroma) in hypercholesterolemic rabbits, which is its metabolic effect (Hiramatsu et al., 1990).

Human prostate cancer LNCaP cells are more resistant to PREs, paclitaxel and vinblastine than human hepatocellular carcinoma HepG2 cells with higher IC50 levels in LNCaP cells than those of HepG2 cells (Figure 1-3, Table 1 and Table 2). The mechanism of resistance in prostate cancer cells remains elusive and needs further investigation. The synergistic effect of MeOH-PYO-PRE may be of clinical use in reducing the dose of vinblastine in hepatocellular cancer treatment.

In conclusion, methanolic purple rice extract of Payao (MeOH-PYO-PRE) cultivar contained the highest inhibitory growth effect on human hepatocellular carcinoma HepG2 cells, which related to the high anthocyanin contents, i.e., cyanin 3-glucosides and peonidin-3-glucosides rather than to γ-oryzanol amounts. MeOH-PYO-PRE induced human HepG2 cell apoptosis via the mitochondrial pathway with the loss of MTP and activation of caspase-3 and -9.

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References


