Anti-oxidant, anti-proliferative and anti-inflammatory activities of the extracts from black raspberry fruits and wine

Ji-Hyun Jeong a, Hana Jung a, Sae-Rom Lee a, Hee-Jae Lee a, Keum Taek Hwang a,*, Tae-Young Kim b

a Department of Food and Nutrition, Research Institute of Human Ecology, Seoul National University, Seoul 151-742, Republic of Korea
b Rural Resource Development Institute, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-853, Republic of Korea

ARTICLE INFO

Article history:
Received 24 December 2009
Received in revised form 25 February 2010
Accepted 21 April 2010

Keywords:
Black raspberry
Anti-oxidation
Anti-proliferation
Anti-inflammatory activity
Anti-oxidant enzyme

ABSTRACT

The purpose of the study was to determine polyphenols, anthocyanins and ascorbic acid in the extracts of black raspberry fruits and wine, along with their anti-oxidant, anti-proliferative and anti-inflammatory activities. Black raspberry fruits without or with seeds crushed were blended in 60% ethanol (FE and FES, respectively) or in water (FW and FWS, respectively). Black raspberry wine without or with seeds crushed (W and WS, respectively) were prepared. Polyphenol content was the highest in the FES (8.25 mg/g fruit). Generally the ethanol extracts with seeds crushed showed higher anti-oxidant activities with the lowest DPPH IC 50 (130 μg/ml (freeze-dried extract/reaction solution)) for the FES and the lowest ABTS IC 50 (198 μg/ml) for the WS. Cell viabilities were reduced by 13–70% when treated with 100 μg/ml (freeze-dried extract/medium) for HT-29 cells and 1000 μg/ml for LNCaP cells. The FES most actively suppressed nitric oxide production in LPS-stimulated RAW264.7 cells (p < 0.05). Superoxide dismutase and glutathione peroxidase activities treated with the extracts were higher than the control (p < 0.05).

1. Introduction

Black raspberry (Rubus occidentalis) is widely cultivated in Korea. The fruits are not only available fresh but also generally consumed frozen and processed into juice, jam, ice cream and wine (Hager, Howard, Prior, & Brownmiller, 2008). They have a wide range of phytochemicals such as anthocyanins (cyanidin 3-sambubioside, cyanidin 3-glucoside, cyanidin 3-xyllosylrutinoside, cyanidin 3-rutinoside and pelargonidin 3-rutinoside), flavonoids (flavonols and flavanols), tannins (proanthocyanidins, ellagitannins and gallotannins), stilbenoids (resveratrol), phenolic acids and lignans (Seeram, 2008; Tulio et al., 2008). Black raspberry demonstrated high anti-oxidant capacity, which has been attributed to their high concentration of total anthocyanins and total phenolic compounds (Wang & Lin, 2000).

Recent studies have shown the anti-proliferative properties of black raspberry fruits in human colon (HT-29, HCT116), oral (KB, CAL-27), breast (MCF-7) and prostate (LNCaP) tumour cell lines (Seeram et al., 2006). Black raspberry extracts also down-regulated the expression of cyclooxygenase-2 and inducible nitric oxide synthase (Chen, Hwang, Rose, Nines, & Stoner, 2006). Black raspberry juice has strong or comparable scavenging activities against superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen (Wang & Jiao, 2000). Jung et al. (2009) reported that black raspberry wine showed anti-oxidant activity comparable to that of imported red wines and α-tocopherol, and had anticancer (human adenocarcinoma and hepatoblastoma) effect. Black raspberry seed powder contained significant levels of anti-oxidants and exhibited strong DPPH and ABTS radical scavenging activities (Parry & Yu, 2004; Parry et al., 2006). However, these positive functional properties of the black raspberry fruits, wine and seeds have been mostly reported individually. The functional properties on the black raspberry seeds have been studied only for the seed powder rather than juice or wine containing the seeds crushed in it. Thus, an integrated and comparable study enclosing extraction medium and inclusion of the seeds crushed in the fruit juice and wine is needed.

The objective of this study is to determine the contents of polyphenols, anthocyanins, ascorbic acid in black raspberry fruit and wine extracts without or with the seeds crushed, along with their anti-oxidant, anti-proliferative and anti-inflammatory activities measuring radical scavenging activities, growth inhibition of cancer cells, nitric oxide (NO) level and anti-oxidant enzymes in a normal cell.

2. Materials and methods

2.1. Materials and reagents

Black raspberry (R. occidentalis) fruits, harvested in June 2008, were obtained from Gochang (Korea). The fruits were stored at...
–25 °C for further studies. Black raspberry fruits (20 g) were blended without or with seeds crushed using a mixer and immersed in 100 ml 60% (v/v) ethanol (FE and FES, respectively) or in water (FW and FWS, respectively) for 1 h. Black raspberry wine without or with the seeds crushed (W and WS, respectively) were prepared. The extracts were filtered through a Whatman No. 1 filter paper. The filtrate was used for determination of the anti-oxidant compounds. The fruit and wine extract filtrates were concentrated using a vacuum rotary evaporator (Eyela Co., Tokyo, Japan) and freeze-dried using a freeze dryer (Ishin Lab Co., Seoul, Korea). The equal weights of the freeze-dried extracts were dissolved in reaction solution or media to be used for anti-oxidant activities and cell cultures.

Reagents used in the study were as follows: ethanol and methanol were purchased from Ducksan Pure Chemicals (Seoul, Korea) or Samchun Pure Chemicals (Pyungtaek, Korea); potassium chloride, sodium acetate buffer, hydrochloric acid (1 N), trichloroacetic acid and dimethyl sulfoxide (DMSO) from Samchun Pure Chemicals; Folin–Ciocalteu’s phenol reagent (2 N), gallic acid, L-ascorbic acid and dimethyl sulfoxide (DMSO) from Samchun Pure Chemicals (Pyungtaek, Korea); potassium chloride, sodium acetate buffer, hydrochloric acid (1 N), trichloroacetic acid and dimethyl sulfoxide (DMSO) from Samchun Pure Chemicals; Folin–Ciocalteu’s phenol reagent (2 N), gallic acid, L-ascorbic acid and dimethyl sulfoxide (DMSO) from Samchun Pure Chemicals; potassium chloride, sodium acetate buffer, hydrochloric acid (1 N), trichloroacetic acid and dimethyl sulfoxide (DMSO) from Samchun Pure Chemicals; potassium chloride, sodium acetate buffer, hydrochloric acid (1 N), trichloroacetic acid and dimethyl sulfoxide (DMSO) from Samchun Pure Chemicals; potassium chloride, sodium acetate buffer, hydrochloric acid (1 N), trichloroacetic acid and dimethyl sulfoxide (DMSO) from Samchun Pure Chemicals; potassium chloride, sodium acetate buffer, hydrochloric acid (1 N), trichloroacetic acid and dimethyl sulfoxide (DMSO) from Samchun Pure Chemicals.

Monomeric anthocyanin (mg/l) = ([A × MW × DF × 1000]/(ε × C)); A: absorbance of the diluted sample; ε: molar absorptivity; MW: expressed as cyanidin 3-glucoside (450); DF: the dilution factor; and C: the equal weights of the freeze-dried extracts were dissolved in reaction solution or media to be used for anti-oxidant activities and cell cultures.

2.2. Determination of total polyphenols, monomeric anthocyanins and ascorbic acid

Total polyphenol contents in the extracts were determined by Folin–Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999). Twenty μl of the extract filtrates were mixed with 1.58 ml water. Folin–Ciocalteu’s phenol reagent (100 μl) was added. After 3 min, 300 μl of 20% (w/v) sodium carbonate solution was added, and the mixture was incubated for 30 min at 40 °C. Absorbance of each sample was measured at 765 nm using a spectrophotometer (Beckman DU® 530, Bechem Coulter Inc., Fullerton, CA, USA). Total polyphenol content was expressed as gallic acid equivalent (GAE) calibrated.

Monomeric anthocyanin contents in the extracts were determined by the modified pH differential method (Giusti & Wrolstad, 2000). Appropriate dilution factors for the extract filtrates were determined diluting it with 0.025 M potassium chloride buffer (pH 1.0), until the absorbance of the sample ranged from 0.7 to 1.0. Each of the extract samples was diluted with potassium chloride buffer (pH 1.0) or sodium acetate buffer (pH 4.5) by the determined dilution factor. After 15 min, absorbance of each diluted sample was measured at 700 nm. Monomeric anthocyanin content was calculated as follows:

\[
\text{Cell viability} = \frac{\text{sample absorbance with cells} - \text{sample absorbance without cells}}{\text{control absorbance with cells} - \text{control absorbance without cells}} \times 100
\]
were plated at a density of $1 \times 10^6$ cells in a 96-well, flat-bottom plate with 200 μl of culture medium in each well. After 24 h incubation at 37 °C in 5% CO₂, different concentrations of the freeze-dried extracts were added in the presence or absence of 10 ng lipopolysaccharides/ml. After 24 h incubation, 100 μl of the macrophage culture supernatants were mixed with 100 μl Griess reagent (made with 1% (w/v) sulfanilamide and 0.1% (w/v) naphthylethlene diaminediaryl hydrochloride in 2.5% (v/v) phosphoric acid, 1:1). After 20 min at room temperature, absorbances of the plates were measured at 540 nm using the ELISA microplate reader. NO levels were calculated from standard curve prepared with sodium nitrite. For anti-oxidant enzyme assay, RAW264.7 cells were plated at a density of 2 × 10⁵ cells in 6 × 15 dish with 2 ml of the DMEM. After 24 h incubation at 37 °C in 5% CO₂, the medium was removed from the dish leaving cells on the bottom. New media (2 ml) containing different concentrations of the freeze-dried extracts were added to the dish. After 24 h incubation, 1 ml of ice cold lysis buffer (containing 50 mM Tris–HCl (pH 7.4) and 10 mM EDTA) was added and cells were collected. The cell pellet was homogenised using a homogeniser (Daian Scientific, Seoul, Korea) for 1 min and centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was collected for assay and stored at −80 °C. Total protein contents were quantified using Lowry’s method using BSA standard (Lowry, Rosebrough, Farr, & Randall, 1951).

SOD and GPx activities were measured according to each manufacturer’s instructions of the kits. Enzyme activity was expressed as unit/mg proteins. One unit for SOD was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. One unit for GPx activity was defined as the amount of the enzyme oxidising 1.0 nmol of NAPDH to NADP⁺ per minute.

2.5. Statistical analysis

All experiments were carried out in triplicate or quadruplicate and expressed as mean ± standard error. Statistical analyses were performed with SPSS programme (SPSS version 12.0, SPSS Chicago, IL, USA) using unpaired t-tests or one-way repeated measures ANOVA when appropriate. If significant by ANOVA, differences in the means were determined using Duncan’s multiple range tests ($p < 0.05$). The Pearson correlation test was conducted to determine the correlations among the means.

3. Results and discussion

3.1. Determination of anti-oxidant compounds in black raspberry extracts

The anti-oxidant compounds in black raspberry extracts were expressed on the basis of the fruit or wine weight. Total polyphenol contents in the black raspberry extracts ranged from 3.62 to 8.25 mg GAE/g fruit or wine (Table 1). The FES had the highest polyphenol contents among the tested extracts. The WS had the next highest value with 6.08 mg GAE/g, followed by W, FWS, FE and FW. Similar results were reported in the previous papers for raspberry and black currant fruits (1.77 and 6.39 mg GAE/g, respectively; Benvenuti, Pellati, Melegari, & Bertelli, 2004), strawberry (2.64 mg GAE/g; Brat et al., 2006) and Andes berry (2.94 mg GAE/g; Garzon, Riedl, & Schwartz, 2009). Furthermore, total polyphenol contents of black raspberry extracts in this study were higher than those in cherry (0.94 mg GAE/g) and grapefruit (0.39 and 0.47 mg GAE/g) as reported by Brat et al. (2006). However, total polyphenol contents in black raspberry extracts were lower than that reported by Parry et al. (2006) for black raspberry seeds (41.2 mg GAE/g).

### Table 1

<table>
<thead>
<tr>
<th>Samples</th>
<th>Polyphenols (mg GAE/g)</th>
<th>Anthocyanins (mg CGE/g)</th>
<th>Ascorbic acid (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE</td>
<td>5.6 ± 1.64&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>2.64 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.42 ± 0.18</td>
</tr>
<tr>
<td>FW</td>
<td>3.62 ± 0.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.54 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 ± 0.16</td>
</tr>
<tr>
<td>FES</td>
<td>8.25 ± 0.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.91 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.59 ± 0.22</td>
</tr>
<tr>
<td>FWS</td>
<td>5.36 ± 0.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.77 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.47 ± 0.24</td>
</tr>
<tr>
<td>W</td>
<td>6.08 ± 1.79&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.43 ± 0.11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.41 ± 0.06</td>
</tr>
<tr>
<td>WS</td>
<td>6.26 ± 1.74&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.23 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.47 ± 0.02</td>
</tr>
</tbody>
</table>

* FE: 60% ethanol extract of the fruits; FW: water extract of the fruits; FES: 60% ethanol extract of the fruits with seeds crushed; FWS: water extract of the fruits with seeds crushed; W: black raspberry wine and WS: black raspberry wine produced with seeds crushed.

* Based on the fruit or wine weight; values are means ± SE ($n = 3$).

* GAE: gallic acid equivalent.

* CGE: cyanidin 3-glucoside equivalent.

<sup>a</sup>,<sup>b</sup>,<sup>c</sup>,<sup>d</sup>,<sup>e</sup> Values with different superscripts within the same column are significantly different ($p < 0.05$; one-way repeated ANOVA and Duncan’s multiple range test).

In this study, the 60% ethanol extracts of black raspberry had more polyphenols than water extracts, comparing the FE against the FW and the FES against the FWS, although not significantly different ($p > 0.05$) (Table 1). Cho et al. (2005) also reported that 60% ethanol was the optimal solution for extracting phenolic compounds in *Rubus coreanus*. The extracts with the seeds crushed contained significantly more polyphenols than those without the seeds, comparing the FES against the FE and the FWS against the FW ($p < 0.05$) (Table 1). Black raspberry fruit seeds contained significant levels of anti-oxidants (Parry et al., 2006). Juranic et al. (2005) reported that the ellagic acid in seeds (4–14 μM) was higher than in pulps (1.8–4.0 μM). The WS had more polyphenols than the W without significance ($p > 0.05$) (Table 1). These results imply that ethanol extracts more polyphenols than water and that inclusion of the seeds in the extracts increases their polyphenol content.

Monomeric anthocyanins in black raspberry extracts ranged from 1.54 to 2.91 mg cyanidin 3-glucoside equivalents (CGE/g) (Table 1). The FES had the highest anthocyanins (2.91 mg CGE/g) among the tested extracts, followed by FE, W, WS, FWS and FW. The 60% ethanol (FE and FES) extracted significantly more anthocyanins than water (FW and FWS) ($p < 0.05$), suggesting that ethanol is a more effective medium to extract anthocyanins from black raspberry than water. Monomeric anthocyanins in black raspberry extracts in this study were greater than those found in fresh Heritage, Kiwigold, Goldie and Anne raspberry fruit extracts (0.17–57.6 mg CGE/100 g) (Liu et al., 2002) and similar to blackberry fruits (0.8–2.3 mg CGE/g) (Moyer, Hummer, Finn, Frei, & Wrolstad, 2002).

The weights of the freeze-dried extracts from 100 g samples were 5.74 ± 0.09 g for FE, 5.71 ± 0.06 g for FW, 6.90 ± 0.01 g for FES, 6.46 ± 0.03 g for FWS, 3.00 ± 0.11 g for W and 2.55 ± 0.07 g for WS. The equal weights of the freeze-dried extracts were used for the anti-oxidant activities and cell culture experiments. The FES showed the lowest DPPH IC₅₀ (130 μg/ml (freeze-dried extract/reaction solution)), followed by WS, W, FE, FWS and FW (Table 2). The WS had the lowest ABTS IC₅₀ (198 μg/ml), followed by FES, FE, W, FWS and FW. Although the DPPH IC₅₀ and ABTS
IC₅₀ of the black raspberry extracts were much higher than those for L-ascorbic acid and butylated hydroxyanisole (BHA), considering that the extracts simply included all the materials with sugars dissolved in the ethanol and water, the anti-oxidant activities of the extracts could not be underestimated.

Inclusion of the seeds in the extracts somewhat raised the anti-oxidant activities of the extracts with ($p < 0.05$) or without statistical significances ($p > 0.05$) (Table 2), implying that inclusion of the seeds in black raspberry products would be recommendable to increase their anti-oxidant activities. The anti-oxidant activities in the 60% ethanol extracts of the black raspberry fruits were significantly higher than those of the sample with the water extracts, comparing the FE against the FW and the FES against the FWS ($p < 0.05$), suggesting that alcohol fermentation of black raspberry may increase anti-oxidant activities.

The DPPH IC₅₀ values of the black raspberry extracts in this study were lower than those of *R. coreanum* Miquel extract (1600 l g/ml) as reported by Cha, Youn, Park, Choi, and Kim (2007), but similar to those of *Syzygium cumini* fruit extract (168 l g/ml) and black raspberry wines (275 l g/ml) as reported by Banerjee, Dasgupta, and De (2005) and Lee (2009), respectively. The anti-oxidant activity of the black raspberry wine extract was similar to that of black raspberry wines (ABTS IC₅₀ 374–407 l g/ml) as reported by Lee (2009).

The total polyphenol contents in the black raspberry extracts were significantly correlated with their DPPH values ($r² = 0.86$) and ABTS values ($0.74$). Monomeric anthocyanins and ascorbic acid were also positively correlated with anti-oxidant activities, mea-

### Table 2

DPPH and ABTS radical scavenging activities of black raspberry extractsa.

<table>
<thead>
<tr>
<th>Samples</th>
<th>DPPH IC₅₀b (µg/ml)</th>
<th>ABTS IC₅₀c (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE</td>
<td>241 ± 15.8e,f</td>
<td>251 ± 42.8f</td>
</tr>
<tr>
<td>FW</td>
<td>439 ± 37.8d</td>
<td>609 ± 35.8d</td>
</tr>
<tr>
<td>FES</td>
<td>130 ± 17.0f</td>
<td>203 ± 41.5f</td>
</tr>
<tr>
<td>FWS</td>
<td>290 ± 41.6e</td>
<td>397 ± 22.9g</td>
</tr>
<tr>
<td>W</td>
<td>221 ± 82.9f</td>
<td>295 ± 11.7f</td>
</tr>
<tr>
<td>WS</td>
<td>187 ± 62.2d,f</td>
<td>198 ± 35.1e</td>
</tr>
<tr>
<td>L-ascorbic acid</td>
<td>10.4 ± 1.4e</td>
<td>17.8 ± 0.9f</td>
</tr>
<tr>
<td>BHA</td>
<td>11.9 ± 2.6e</td>
<td>17.1 ± 3.8e</td>
</tr>
</tbody>
</table>

FE, FW, FES, FWS, W and WS: see Table 1.

b DPPH IC₅₀ 50% DPPH radical scavenging activity.

c ABTS IC₅₀ 50% ABTS radical scavenging activity.

d,e,f,g Values with different superscripts within the same columns are significantly different ($p < 0.05$; one-way repeated ANOVA and Duncan’s multiple range test).

Fig. 1. Anti-proliferation activities of black raspberry extracts against human HT-29 colon cancer cells (a) and human LNCaP prostate cancer cells (b). µg/ml: freeze-dried extract/medium. Expressed as % of cell viability to the control. Values are means ± SE ($n = 4$). FE, FW, FES, FWS, W and WS: see Table 1. Statistical significance is based on the difference when compared with the cells without treating extracts (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).
sured as DPPH (0.89 and 0.65, respectively) and ABTS (0.85 and 0.48, respectively), implying that polyphenols, monomeric anthocyanins and ascorbic acid all contributed to the anti-oxidant activities of black raspberry extracts. Studying 17 fruits from Ecuador, positive correlations were obtained between phenolic contents and anti-oxidant capacities reported as DPPH and ABTS, of 0.66 and 0.56, respectively, by Vasco, Ruales, and Kamal-Eldin (2008).

3.3. Inhibition of cancer cell proliferation

The black raspberry extracts suppressed the proliferation of both HT-29 colon cancer cells and LNCaP prostate cancer cells (Fig. 1). Cell viability decreased in a dose-dependent manner (17–63% reduction) when treated with 100 µg/ml (freeze-dried extract/medium) or more for HT-29 cells and 1000 µg/ml or more for LNCaP cells. Generally the HT-29 cells were inhibited by less amounts of the extracts than the LNCaP cells. The FES had the highest inhibition effect on the proliferation of HT-29 and LNCaP cells (IC$_{50}$ 89.9 and 1420 µg/ml (freeze-dried extract/medium), respectively). Anti-proliferation activity of the FES on the HT-29 cells was significantly higher than that of the FE (IC$_{50}$ 334 µg/ml), and FWS (IC$_{50}$ 103 µg/ml) than FW (IC$_{50}$ 290 µg/ml) (p < 0.05). Anti-proliferation effects of the two wine samples were not significantly different (p > 0.05). The IC$_{50}$ value for anti-proliferation of the LNCaP cells treated with the FES was significantly lower than that with the FE (2157 µg/ml) (p < 0.05). These results imply that ethanol extraction and/or inclusion of the seeds in the black raspberry juice may increase its anti-proliferation effect on the cancer cells. Seeram et al. (2006) reported that the black raspberry fruit extracts inhibited the proliferation of human colon (HT-29) and breast (MCF-7) tumour cells at IC$_{50}$ values of 92.6 and 145 µg/ml, respectively, after 48 h of incubation. Sun, Chu, Wu, and Liu (2002) reported that cranberry showed the highest inhibitory effect against HepG2 human liver cancer cells with an IC$_{50}$ value of

![Fig. 2. Effect of black raspberry extracts on the viability of RAW264.7 cells. µg/ml: freeze-dried extract/medium. Expressed as % of cell viability to the control. Values are means ± SE (n = 4). FE, FW, FES, FWS, W and WS: see Table 1. Statistical significance is based on the difference when compared with the cells without treating extracts (*p < 0.05, **p < 0.01, ***p < 0.001).](image)

![Fig. 3. NO production in RAW264.7 cells treated with the black raspberry extracts. µg/ml: freeze-dried extract/medium. Values are means ± SE (n = 3). FE, FW, FES, FWS, W and WS: see Table 1. Statistical significance is based on the difference when compared with the cells without treating extracts (*p < 0.05, **p < 0.01, ***p < 0.001). LPS: lipopolysaccharides from Escherichia coli.](image)
14.5 mg/ml after 96 h incubation, followed by lemon, apple, strawberry, red grape, banana, grapefruit and peach.

3.4. Cytotoxicity of black raspberry extracts against RAW264.7 cell lines

Murine RAW264.7 macrophages were used to determine cytotoxicity and anti-inflammatory effects of the black raspberry extracts. Examining cytotoxicity of the extracts in RAW264.7 cells, no notable cytotoxicity (cell viability > 84%) was observed when the cells were exposed to the black raspberry extracts up to the level of 500 µg/ml (freeze-dried extract/medium) for 24 h (Fig. 2). In this study, the black raspberry extracts at the level of 250 µg/ml did not have cytotoxicity in RAW264.7 cells with inhibition of human cancer cell (HT-29 and LNCaP) growth.

3.5. Inhibition of LPS-induced production of NO

In order to assess the anti-inflammatory activities of the black raspberry extracts, RAW264.7 cells were stimulated with LPS in the presence or absence of the extracts, and the level of NO in the medium was measured. The inhibition of NO production was observed without noticeable cytotoxicity in the RAW264.7 cells with the addition of the black raspberry extracts (250 and 500 µg/ml (freeze-dried extract/medium)) (Fig. 3). The FES had the highest inhibition effect on the NO production. The NO production inhibition rates of the FES, WS and W (500 µg/ml) were more than 25% compared to the control when the RAW264.7 cells were treated with 100 ng/ml LPS. The extracts with seeds crushed showed significantly higher anti-inflammatory activities than those without seeds, comparing NO production of the FES against FE and FWS against FW (p < 0.05), suggesting the inclusion of the seeds in the black raspberry extract may reduce inflammation in the cells. The wine samples did not show significant difference in the NO production (p > 0.05). The FES inhibited significantly more NO production than the FWS (p < 0.05).

3.6. Effect of black raspberry extracts on SOD and GPx

In order to investigate whether the anti-oxidant activities of black raspberry extracts were mediated by an increase in anti-oxidant enzymes, SOD and GPx activities in RAW264.7 cells were measured only for FES, FE and WS with higher anti-oxidant, anti-dietary enzymes, SOD and GPx activities in RAW264.7 cells measured for black raspberry extracts were mediated by an increase in anti-oxidant, anti-proliferation and anti-inflammatory activities of the fruit extract, and that inclusion of the seeds crushed in the fruit extract and wine could also increase the functional activities of the juice and wine.

Acknowledgments

This study was carried out with the support of Cooperative Research Program for Agricultural Science and Technology Development (Project No. 200804AO1036095), RDA, Republic of Korea.

References


Table 3

<table>
<thead>
<tr>
<th>Samples</th>
<th>SOD (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.10 ± 0.05d</td>
<td>3.50 ± 0.89d</td>
</tr>
<tr>
<td>FE</td>
<td>2.61 ± 0.23***</td>
<td>4.22 ± 0.08**NS</td>
</tr>
<tr>
<td>FES</td>
<td>2.84 ± 0.31***</td>
<td>5.52 ± 0.37**</td>
</tr>
<tr>
<td>WS</td>
<td>1.98 ± 0.23***</td>
<td>4.91 ± 0.17**</td>
</tr>
</tbody>
</table>

*Significant as compared with the control group (p < 0.05, **p < 0.01, ***p < 0.001); NS: not significant as compared with the control group (p > 0.05).

4. Conclusion

It is concluded that ethanol (60%) could extract more polyphenols and anthocyanins than water from black raspberry and raised anti-oxidant, anti-proliferation and anti-inflammatory activities of the fruit extract, and that inclusion of the seeds crushed in the fruit extract and wine could also increase the functional activities of the juice and wine.


