INTRODUCTION

The liver is the primary site for ethanol metabolism (Lieber, 1997). Ethanol is metabolized by oxidative and non-oxidative pathways in the liver (Best et al., 2006). Alcohol dehydrogenase (ADH) with cytochrome P450 2E1 (CYP2E1) and catalase (CAT) is responsible for the oxidative metabolism of ethanol, and fatty acid ethyl esters (FAEE) synthase produces FAEE via non-oxidative metabolism (Wu et al., 2006). Treatment of 4-methylpyrazole (4-MP), which is an ADH inhibitor, has been known to cause the non-oxidative metabolism of ethanol with elevation of plasma FAEE level (Best et al., 2006) and 30-40% decrease in ethanol elimination rate by oxidative metabolism (Sarkola et al., 2002). Chronic and acute ethanol exposure are reported to generate excessive reactive oxygen species (ROS), including superoxide anion (O₂⁻), hydroxyl radical (·OH), and hydrogen peroxide (H₂O₂). Ethanol exposure also leads to oxidative stress, which results in decreased glutathione (GSH) levels and enhanced peroxidation of lipids, proteins and DNA (Lu and Cederbaum, 2008; Kahraman et al., 2012). In addition, there is growing evidence that intracellular ROS resulting from ethanol metabolism play an important role in ethanol-induced liver injury (Nordmann et al., 1992; Calabrese et al., 1996).

Phytochemicals are naturally occurring secondary metabolites that have antioxidant properties and are present in fruits and vegetables (Matés et al., 2011). They have been reported to prevent various diseases associated with oxidative stress, such as cancer, cardiovascular diseases and neurodegenerative diseases (Russo et al., 2012). Quercetin and catechin are known to have high free radical scavenging activity (Ross and Kasum, 2002), and they exhibit in vivo and in vitro hepatoprotective effects from ethanol-induced oxidative stress (Molina et al., 2003; Liu et al., 2010; Oliva et al., 2011) (Fig. 1). Caffeic acid is an antioxidant that is widely distributed in vegetables, fruits, coffee and tea (Rampart et al., 1986) and is known to...
and trypsin-EDTA were obtained from Gibco (Invitrogen, NY, USA). The cell culture medium, DMEM, fetal bovine serum (FBS), penicillin/streptomycin, molecular Probes (Eugene, OR, USA). The cell culture media—DCF-DA and rhodamine 123 (Rh-123) were purchased from BD Biosciences (San Jose, CA, USA). For cell proliferation experiments, after reaching 70-80% confluence, the cells were starved with serum-free DMEM for 12 h. Then, the cells were simultaneously treated with 5 μM of individual phytochemicals and 100 mM ethanol for 24 h. An adherent sealing membrane was overlaid onto each plate to minimize ethanol evaporation (Rampart et al., 1986).

**Cell proliferation**

The MTT assay was performed to measure the cell proliferation of cultured cells (Kang et al., 1996). Briefly, SK-Hep-1 cells were plated on 96-well culture plates at 1×10^4 cells in DMEM supplemented with 10% FBS. After incubation for 72 h, MTT was added at a final concentration of 0.5 mg/ml per well for the remaining 6 h of incubation time. The cell pellets were dissolved by adding 0.17 M acidified isopropl alcohol and 3% sodium dodecyl sulfate (SDS). The absorbance was measured at a wavelength of 590 nm using a plate reader (SpectraMax 190, Molecular Devices, Inc., CA, USA).

**Evaluation of cytotoxicity by a lactate dehydrogenase leakage assay**

SK-Hep-1 cells were seeded at 3.0×10^4 cells/well in 6-well plates. Briefly, culture medium was collected separately, and the cells were scraped. Cell suspensions were sonicated to ensure breakdown of the cell membrane to release the lactate dehydrogenase (LDH). After centrifugation to clear the cell lysate, a mixture of 5 mM pyruvate, 0.35 mM NADH, and 84 mM Tris was added to the cell lysate and the culture media. Immediately, the absorbance was read at 340 nm for 3 min in a microplate reader (BioTek, VT, USA). LDH leakage was estimated from the ratio of the LDH activity in the culture medium to that of the whole cell content (Granado-Serrano et al., 2006).

**Determination of reactive oxygen species**

The formation of intracellular ROS was evaluated using a DCF-DA probe (Wang and Joseph, 1999). Briefly, SK-Hep-1 cells were plated at 3.0×10^4 cells/well in 24-well plates. The cells were washed with PBS, and the media were replaced with 5 μM DCF-DA in DMEM. The cells were incubated at 37°C for 30 min and then washed three times with PBS. The fluorescence of the cells from each well was measured using 485 nm excitation and 535 nm emission with a Wallac Victor 1420 Multilabel Counter (Perkin Elmer, Inc., CA, USA).

**Determination of lipid hydroperoxide**

Lipid peroxidation was determined using a lipid hydroperoxide assay kit from Cayman Chemical Company (Ann Arbor, MI, USA). Briefly, cells were treated as indicated and then scraped. Immediately after sonication, lipid hydroperoxides were extracted using chloroform:methanol (2:1, v/v). Chromogenic reagent was mixed with the chloroform extract, and the mixture was transferred to a 96-well plate reader. The absorbance was measured at 500 nm using a plate reader. 13-Hydroperoxy octadecadienoic acid was used as the standard. The results are expressed as LOOH nmol/ml/mg total protein.

**Measurement of mitochondrial membrane potential (MMP)**

Mitochondrial membrane potential was monitored using rhodamine 123 (Rh-123) (Satoh et al., 1997). Briefly, cells were treated as indicated, washed with PBS, and resuspended in 10 μg/ml Rh123 in DMEM. The cells were incubated at 37°C for 30 min and washed three times with PBS. The fluo-

![Chemical structures of phytochemicals used in this study: quercetin, catechin, caffeic acid, and phytic acid.](image-url)

**Fig. 1.** Chemical structures of phytochemicals used in this study: quercetin, catechin, caffeic acid, and phytic acid.
rescence was measured using 485 nm excitation and 535 nm emission with a Wallac Victor 1420 Multilabel Counter (Perkin Elmer Inc., CA, USA).

**Determination of intracellular GSH content**

SK-Hep-1 cells were lysed by three cycles of freezing and thawing (-70°C to 37°C) and centrifuged at 10,000 rpm for 15 min to obtain clear supernatants. A mixture of 143 mM NaPO₄ (pH 7.5), 6.3 mM EDTA, 0.6 mM DTNB, 0.25 mM NADPH, and 0.25 U/ml glutathione reductase was added to the supernatants. After incubation at room temperature for 5 min, the absorbance was measured at 412 nm using a UV-2401 PC UV/Vis spectrophotometer (Shimadzu Corporation, Koyto, Japan). The results were calculated from a standard curve obtained with reduced glutathione and expressed as nmol per μg of protein.

**Measurement of protein oxidation**

Cell lysate obtained by the freezing and thawing method was mixed with 500 μl of 20 mM DNPH dissolved in 2 M HCl and incubated for 1 h at room temperature (with vortexing for 3 sec every 10 min). The mixture was added to 500 μl of 20% TCA and incubated for 20 min on ice. After incubation, the mixture was centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was discarded and the pellet was washed three times with an ethanol:ethyl acetate solution (1:1 v/v). The pellet was resuspended in 800 μl of 6 M guanidine HCl and 200 μl of phosphate buffer (pH 6.0) and incubated for 15 min at 37°C. After centrifugation at 12,000 rpm for 5 min, the absorbance of the supernatant was measured at 370 nm using a UV-2401 PC UV/Vis spectrophotometer (Shimadzu Corporation, Koyto, Japan). The amount of oxidized protein was calculated using a molar absorption coefficient of 22,000 M⁻¹ cm⁻¹ (Lenz et al., 1989).

**Fig. 2.** Protective effect of phytochemicals against ethanol-induced cell death in SK-Hep-1 cells. Two concentrations of Qu, Ct, Cf, or Py (1 and 5 μM) were simultaneously treated with ethanol for 72 h. (A) Cell viability was measured by MTT assays. (B) Cytotoxicity was examined by LDH assay. Each bar represents the mean ± SD of six independent experiments. Qu: quercetin; Ct: catechin; Cf: caffeic acid; Py: phytic acid; ++:100 mM ethanol; +++: 200 mM ethanol. *p<0.05 versus ethanol-untreated cells, **p<0.05 versus 100 mM ethanol-treated cells.

**Fig. 3.** Protective effect of phytochemicals against ethanol-induced oxidative stress in SK-Hep-1 cells. Two concentrations (1 and 5 μM) of Qu, Ct, Cf, or Py were simultaneously treated with ethanol for 72 h. (A) Intracellular ROS levels were measured using DCFH-DA. (B) The mitochondrial membrane potential was examined using Rh123. Each bar represents the mean ± SD of five independent experiments. Qu: quercetin; Ct: catechin; Cf: caffeic acid; Py: phytic acid. *p<0.05 versus ethanol-untreated cells, **p<0.05 versus ethanol-treated cells.
Determination of antioxidant enzyme activities

The activities of antioxidant enzymes such as catalase (CAT) and glutathione peroxidase (GPX) were assayed in the supernatant of cell lysate. CAT activity was determined by monitoring the rate of H$_2$O$_2$ consumption in a mixture containing 50 mM potassium phosphate buffer (pH 7.0), 10 mM H$_2$O$_2$, and the supernatant for 3 min at 240 nm (Aebi, 1984). GPX activity was determined by following the enzymatic NADPH oxidation in a mixture containing 1 mM NaN$_3$, 1 mM reduced glutathione, 0.2 mM NADPH, 1 unit/ml glutathione reductase, 2 mM EDTA, 50 mM potassium phosphate buffer (pH 7.6) and the supernatant for 3 min at 340 nm (Flohé and Günzler, 1984).

Caspase-3 activity

Caspase-3 activity was evaluated using a Caspase-3 Fluorescent Assay Kit (Clontech, Palo Alto, CA, USA). Briefly, 50 μl of supernatant was mixed with an equal volume of reaction buffer containing 40 mM PIPES (pH 7.2), 150 mM NaCl, 5 mM EDTA, 0.2% CHAPS, 15% sucrose and 10 mM DTT and incubated for 30 min at 37°C. The fluorescence was measured using 360 nm excitation and 460 nm emission with a Wallac Victor 1420 Multilabel Counter (Perkin Elmer Inc., CA, USA). The data are expressed as a percentage relative to caspase-3 activity in a control group.

Statistical analysis

For statistical analysis of data, p values were analyzed using a paired Student t test software program (Startview 5.1; Abacus Concepts, Berkeley, CA, USA). The results were considered statistically significant when p values were <0.05.

RESULTS

Phytochemicals protect SK-Hep-1 cells from reduced cell viability due to oxidative ethanol metabolism

We assessed the effect of phytochemicals on decreased cell viability caused by exposing SK-Hep-1 cells to ethanol. We found that proliferation of SK-Hep-1 cells, measured by an MTT reduction assay, was reduced to 86.5 ± 4.4% and decreased by 14-18% with the addition of Qu, Ct, Cf and Py (Table 1). The protein oxidation level, which measures the extent of the oxidative damage to protein, was elevated by 10-14% (Table 1). The level of reduced glutathione (GSH) decreased proliferation of SK-Hep-1 cells to a range of 93-106% (Fig. 2A). The cytotoxicity of SK-Hep-1 cells, as measured by a LDH release assay, was elevated to 117.4 ± 4.3% with a 100 mM ethanol treatment, as compared to an ethanol-untreated control (100.0 ± 2.2%). Co-treatment of cells with ethanol and Qu, Ct, Cf and Py (1 or 5 μM) decreased cytotoxicity to 95-110% (Fig. 2B). These data indicate that ethanol-induced hepatotoxicity was suppressed by treatment of Qu, Ct, Cf and Py in SK-Hep-1 cells.

Table 1. Effect of phytochemicals on biomarkers for ethanol-induced oxidative stress in SK-Hep-1 cells

| Group       | LPO (nmol/ml/mg protein) | GSH (nmol/μg protein) | Protein oxidation (nmol/mg protein) | CAT (mU/mg protein) | GPX (mU/mg protein) |
|-------------|--------------------------|-----------------------|-------------------------------------|---------------------|---------------------|
| Control     | 1.19 ± 0.10              | 6.96 ± 0.42           | 0.048 ± 0.003                       | 17.0 ± 1.8          | 11.0 ± 1.2          |
| 100 mM EtOH | 1.48 ± 0.21*             | 6.54 ± 0.50*          | 0.059 ± 0.003*                      | 20.0 ± 2.0*         | 14.0 ± 1.1*         |
| 100 mM EtOH+5 μM Qu | 1.27 ± 0.18             | 6.62 ± 0.47           | 0.051 ± 0.002**                     | 17.0 ± 1.6**        | 13.0 ± 1.5**        |
| 100 mM EtOH+5 μM Ct | 1.22 ± 0.09             | 6.65 ± 0.51           | 0.053 ± 0.002**                     | 18.0 ± 1.6**        | 11.0 ± 1.3**        |
| 100 mM EtOH+5 μM Cf | 1.28 ± 0.11**            | 6.71 ± 0.43**         | 0.052 ± 0.003**                     | 17.0 ± 1.7**        | 12.0 ± 1.3**        |
| 100 mM EtOH+5 μM Py | 1.25 ± 0.10**            | 6.61 ± 0.44           | 0.052 ± 0.002**                     | 18.0 ± 1.9**        | 12.0 ± 1.5**        |

SK-HepG-1 cells were incubated with 100 mM ethanol with or without Qu, Ct, Cf or Py. Values are mean ± SD of five independent experiments. LPO: lipid hydroperoxide; GSH: reduced glutathione; CAT: catalase; GPX: glutathione peroxidase; EtOH: ethanol; Qu: quercetin; Ct: catechin; Cf: caffeic acid; Py: phytic acid. *p<0.05 versus ethanol-untreated cells, **p<0.05 versus 100 mM ethanol-treated cells.

Phytochemicals protect SK-Hep-1 cells from oxidative stress induced by oxidative ethanol metabolism

We examined whether phytochemicals affect intracellular ROS production and mitochondrial membrane potential (MMP) in SK-Hep-1 cells. The intracellular ROS level, measured using a DCF-DA probe, was significantly increased by 100 mM ethanol treatment compared to untreated cells (100 ± 8.1% versus 133.5 ± 7.7%). However, co-treatment of Qu, Ct, Cf or Py (1 and 5 μM) with 100 mM ethanol restored intracellular ROS levels to a range of 98-123% (Fig. 3A). Moreover, the MMP of SK-Hep-1 cells, measured by Rh-123, was reduced by 100 mM ethanol (88.7 ± 4.2% versus 100 ± 5.4% in ethanol-untreated) and elevated to a range of 91-116% by the addition of Qu, Ct, Cf and Py (Fig. 3B). In particular, Ct was very effective at recovering levels of intracellular ROS and MMP altered by ethanol treatment.

Phytochemicals restored levels of oxidative stress markers altered by oxidative ethanol metabolism

In addition to acetaldehyde and acetate, ethanol metabolism generates ROS, which induces GSH depletion, damage to the cell membrane, protein oxidation, DNA damage, and alteration of antioxidant enzyme activity (Kurose et al., 1996; Navasumrit et al., 2000; Ogony et al., 2008). Lipid hydroperoxide (LPO) is one of many lipid peroxidation substances induced in the cell membrane by oxidative stress. In this study, LPO levels were increased by 100 mM ethanol (1.48 ± 0.21 versus 1.19 ± 0.1 nmol/ml/mg protein in ethanol-untreated), and decreased by 14-18% with the addition of Qu, Ct, Cf and Py (Table 1). The protein oxidation level, which measures the extent of the oxidative damage to protein, was elevated by ethanol (0.059 ± 0.003 versus 0.048 ± 0.003 nmol/ml/mg protein in ethanol-untreated). However, co-treatment of cells with ethanol and Qu, Ct, Cf and Py decreased the extent of protein oxidation by 10-14% (Table 1).
one (GSH) was decreased by ethanol treatment (6.54 ± 0.50 versus 6.96 ± 0.42 nmol/ml/mg protein in ethanol-untreated) and increased to a range of 6.61-6.71 nmole/μg protein with the addition of Qu, Ct, Cf and Py (Table 1). The activity of CAT and GPX, enzymatic antioxidants, was increased by 18 and 27%, respectively, with ethanol treatment, and the addition of Qu, Ct, Cf and Py restored CAT and GPX activities to control levels. These data indicate that Qu, Ct, Cf and Py protect SK-Hep-1 cells from oxidative damage by regulating markers of oxidative stress.

**Phytochemicals protect SK-Hep-1 cells from cell death induced by non-oxidative ethanol metabolism**

As shown in Fig. 4A, non-oxidative ethanol metabolism stimulated cytotoxicity of SK-Hep-1 cells in 2 mM 4-MP plus 200 mM ethanol (124.3 ± 3.8% versus 100.0 ± 2.8% in untreated cells) without any effect of 4-MP alone. Non-oxidative ethanol metabolism also showed increased cytotoxicity as compared to oxidative ethanol metabolism (124.3 ± 3.8% versus 116.3 ± 4.5%). However, the addition of Qu, Ct, Cf and Py lowered cytotoxicity to a range of 101-112%. Furthermore, caspase-3 activity showed an increase in non-oxidative ethanol metabolism (120.0 ± 2.5% versus 100.0 ± 4.4% in untreated cells) without any effect of 4-MP alone, and this activity was higher than in cells treated with 200 mM ethanol alone. The addition of Qu, Ct, Cf and Py decreased caspase-3 activity to a range of 90-100% (Fig. 4B).

**Phytochemicals protect SK-Hep-1 cells from oxidative stress induced by non-oxidative ethanol metabolism**

We investigated the effect of phytochemicals on ROS production and MMP in non-oxidative ethanol metabolism-in-
markers increased by non-oxidative ethanol metabolism. Phytochemicals attenuate levels of oxidative stress bio-
duction that was induced by non-oxidative ethanol metabolism (Table 2). Treatment of cells with Qu, Ct, Cf and Py showed a decrease in LPO levels to 62-

**DISCUSSION**

The liver is not the only organ responsible for metabolizing ingested ethanol, but it is the primary location for the metabolism of various xenobiotics (Lieber, 1997). Acute or chronic consumption of ethanol is known to be one of the most important factors that cause liver damage (Lu and Cederbaum, 2008). It is caused by deleterious metabolites including acetaldehyde, FAEE and ROS generated by ethanol metabolism (Best et al., 2006; Lu and Cederbaum, 2008). This study focused on the effects of phytochemicals on the alteration of the antioxidative defense system against oxidative stress in a SK-

**Table 2. Effect of phytochemicals on biomarkers for oxidative stress induced by non-oxidative metabolism of ethanol in SK-Hep-1 cells**

| Group | LPO (nmol/ml/mg protein) | Protein oxidation (nmol/mg protein) | CAT (mU/mg protein) | GPX (mU/mg protein) |
|-------|--------------------------|-----------------------------------|---------------------|--------------------|
| Control | 1.16 ± 0.08               | 0.094 ± 0.004                     | 21.3 ± 1.6          | 15.2 ± 0.6         |
| 200 mM EtOH | 2.13 ± 0.10*             | 0.104 ± 0.001*                    | 25.2 ± 0.8*         | 17.4 ± 0.6*        |
| 200 mM EtOH+4-MP | 3.30 ± 0.14*             | 0.126 ± 0.006*                    | 26.5 ± 0.8*         | 18.9 ± 0.8*        |
| 200 mM EtOH+4-MP+5 μM Qu | 2.57 ± 0.10**            | 0.107 ± 0.008**                    | 23.1 ± 0.8**        | 17.5 ± 1.2        |
| 200 mM EtOH+4-MP+5 μM Ct | 2.37 ± 0.09**            | 0.104 ± 0.007**                    | 22.7 ± 1.9**        | 16.2 ± 0.7**       |
| 200 mM EtOH+4-MP+5 μM Cf | 2.58 ± 0.17**            | 0.099 ± 0.008**                    | 24.7 ± 0.7**        | 16.7 ± 0.9**       |
| 200 mM EtOH+4-MP+5 μM Py | 2.59 ± 0.22**            | 0.107 ± 0.008**                    | 24.1 ± 1.0**        | 17.2 ± 0.4**       |

Values are mean ± SD of five independent experiments. LPO: lipid hydroperoxide; CAT: catalase; GPX: glutathione peroxidase. EtOH: ethanol; 4-MP: 4-methylpyrazol; Qu: quercetin; Ct: catechin; Cf: caffeic acid; Py: phytic acid. *p<0.05 versus ethanol-untreated cells, **p<0.05 versus 2 mM 4-MP plus 200 mM ethanol-treated cells.

duced SK-Hep-1 cells. As shown in Fig. 5A, cells treated with 2 mM 4-MP plus 200 mM ethanol showed increased intracellular ROS production (132.6 ± 7.7%) compared to untreated cells (100.0 ± 8.2%), as well as cells treated with 200 mM ethanol alone (112.0 ± 7.7%). The 4-MP alone did not show any effects on intracellular ROS production. However, the addition of Qu, Ct, Cf and Py decreased intracellular ROS production that was induced by non-oxidative ethanol metabolism to a range of 106-113%. In addition, MMP was decreased in 2 mM 4-MP plus 200 mM ethanol-treated cells. In addition, MMP was decreased in 2 mM 4-MP plus 200 mM ethanol-treated cells. In addition, the concentration of 5 μM could be safely used when phytochemicals were tested for their potential cytotoxicity on SK-Hep-1 cells (data not shown). Therefore, a dosage range between 1 and 5 μM was selected for all experimental conditions.

Exposure of SK-Hep-1 cells to ethanol resulted in decreased cell viability in oxidative ethanol metabolism. Furthermore, there was elevated ROS production, which led to lipid peroxidation, protein oxidation, loss of MMP, reduction of GSH, and an increase of antioxidant enzyme activity. However, co-treatment of cells with quercetin, catechin, caffeic acid and phytic acid and ethanol significantly recovered cell viability initially reduced by ethanol. To explore whether the cytoprotection of these phytochemicals is due to ROS scavenging activity, we investigated intracellular ROS levels by co-treatment of the phytochemicals with ethanol in SK-Hep-1 cells. All phytochemicals decreased ROS production elevated by ethanol and restored increased LPO levels and protein oxidation, loss of MMP, and alteration of antioxidant system activity. The liver has a minor non-oxidative metabolism pathway for ethanol, besides the major oxidative metabolism pathway of ethanol oxidized by ADH, CYP2E1, and catalase. The non-

lecular ROS production (132.6 ± 7.7%) compared to untreated control cells (100.0 ± 8.1%) and cells treated with 200 mM ethanol alone (112.0 ± 7.7%). In addition, MMP was decreased in 2 mM 4-MP plus 200 mM ethanol showed increased intracellular ROS production (132.6 ± 7.7%) compared to untreated cells (100.0 ± 8.2%), as well as cells treated with 200 mM ethanol alone (112.0 ± 7.7%). The 4-MP alone did not show any effects on intracellular ROS production. However, the addition of Qu, Ct, Cf and Py decreased intracellular ROS production that was induced by non-oxidative ethanol metabolism to a range of 106-113%. In addition, MMP was decreased in 2 mM 4-MP plus 200 mM ethanol-treated cells. In addition, MMP was decreased in 2 mM 4-MP plus 200 mM ethanol-treated cells. Co-treatment of cells with quercetin, catechin, caffeic acid and phytic acid and ethanol significantly recovered cell viability initially reduced by ethanol. To explore whether the cytoprotection of these phytochemicals is due to ROS scavenging activity, we investigated intracellular ROS levels by co-treatment of the phytochemicals with ethanol in SK-Hep-1 cells. All phytochemicals decreased ROS production elevated by ethanol and restored increased LPO levels and protein oxidation, loss of MMP, and alteration of antioxidant system activity.

The liver has a minor non-oxidative metabolism pathway for ethanol, besides the major oxidative metabolism pathway of ethanol oxidized by ADH, CYP2E1, and catalase. The non-oxidative metabolism pathway of ethanol synthesizes FAEE, which is a toxic metabolite (Laposata, 1998). Recent studies reported that inhibition of the oxidative metabolism pathway of ethanol induced by 4-MP caused a 2- to 3-fold increase in the production of FAEE in the liver and pancreas (Best et al., 2006), and FAEE induced apoptosis in lymphocytes and
HepG2 cells (Aydin et al., 2005).

To determine whether the non-oxidative metabolism of ethanol affects cytotoxicity in SK-Hep-1 cells, we examined cell viability when oxidative metabolism of ethanol was inhibited by 4-MP. We found that the non-oxidative metabolism of ethanol encouraged cytotoxicity and apoptosis more than the oxidative metabolism of ethanol and was followed by augmentation of ROS production, lipid peroxidation, protein oxidation, antioxidant enzyme activity, and loss of MMP. These results indicate that non-oxidative ethanol metabolism induced oxidative insult in SK-Hep-1 cells more than oxidative ethanol metabolism.

Nevertheless, treatment with quercetin, catechin, caffeic acid and phytic acid in the non-oxidative metabolism of ethanol significantly restored cytotoxicity in SK-Hep-1 cells. Furthermore, these phytochemicals suppressed ROS and LPO production, protein oxidation, and loss of MMP and increased CAT and GPX activities during 4-MP-induced non-oxidative metabolism of ethanol in SK-Hep-1 cells.

In conclusion, this study demonstrates that quercetin, catechin, caffeic acid and phytic acid have protective roles against oxidative damage in SK-Hep-1 cells during the oxidative and non-oxidative metabolism of ethanol. The protective effects appear to be a result of the reduction of cytotoxicity via the inhibition of ROS production and restoration of the antioxidant system.

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REFERENCES

Aebi, H. (1984) Catalase in vitro. Methods Enzymol. 105, 121-126. Aydin, H., Celik, H. A., Deveci, R., Karacali, S., Saydam, G., Bedii, Omay, S. and Batur, Y. (2005) Induction of apoptosis by fatty acid ethyl esters in HepG2 cells. Food Chem. Toxicol. 43, 139-145. Best, C. A., Sarkola, T., Eriksson, C. J., Cluette-Brown, J. E. and Laposa, T. (2006) Increased plasma fatty acid ethyl ester levels following inhibition of oxidative metabolism of ethanol by 4-methylpyrazole treatment in human subjects. Alcohol Clin. Exp. Res. 30, 1126-1131. Calabrese, V., Renis, M., Calderone, A., Russo, A., Barcellona, M. L. and Rizza, V. (1996) Stress proteins and SH-groups in oxidant-induced cell damage after acute ethanol administration in rat. Free Radic. Biol. Med. 20, 391-397. Flohé, L. and Günztler, W. A. (1984) Assays of glutathione peroxidase. Methods Enzymol. 105, 114-121. Granado-Serrano, A. B., Martín, M. A., Bravo, L., Goya, L. and Ramos, S. (2006) Quercetin induces apoptosis via caspase activation, regulation of Bcl-2, and inhibition of PI-3-kinase/Akt and ERK pathways in a human hepatoma cell line (HepG2). J. Nutr. 136, 2715-2721. Kahraman, A., Çakar, H. and Köken, T. (2012) The protective effect of quercetin on long-term alcohol consumption-induced oxidative stress. Mol. Biol. Rep. 39, 2789-2794. Kang, H. S., Kim, Y. H., Lee, C. S., Lee, J. J., Choi, I. and Pyun, K. H. (1996) Suppression of interleukin-1 and tumor necrosis factor-alpha production by acanthoic acid, (-)-pimara-9(11),15-dien-19-oic acid, and its antioxidant effects in vivo. Cell Immunol. 170, 212-221. Kurose, I., Hijichi, H., Kato, S., Miura, S. and Ishii, H. (1996) Ethanol-induced oxidative stress in the liver. Alcohol Clin. Exp. Res. 20(1 Suppl), 77A-85A. Laposata, M. (1998) Fatty acid ethyl esters: ethanol metabolites which mediate ethanol-induced organ damage and serve as markers of ethanol intake. Prog. Lipid Res. 37, 307-316. Lenz, A. G., Costabel, U., Shaltiel, S. and Levine, R. L. (1989) Determination of carboxyl groups in oxidatively modified proteins by reduction with tritiated sodium borohydride. Anal. Biochem. 177, 419-425. Lieber, C. S. (1997) Ethanol metabolism, cirrhosis and alcoholism. Clin. Chim. Acta 257, 59-84. Liu, S., Hou, W., Yao, P., Zhang, B., Sun, S., Nüessler, A. K. and Liu, L. (2010) Quercetin protects against ethanol-induced oxidative damage in rat primary hepatocytes. Toxicol. In Vitro 24, 516-522. Lu, Y. and Cederbaum, A. I. (2008) CYP2E1 and oxidative liver injury by alcohol. Free Radiol. Biol. Med. 44, 723-738. Matés, J. M., Segura, J. A., Alonso, F. J. and Márquez, J. (2011) Anticancer antioxidant regulatory functions of phytochemicals. Curr. Med. Chem. 18, 2315-2338. Molina, M. F., Sanchez-Reus, I., Iglesias, I. and Benedí, J. (2003) Quercetin, a flavonoid antioxidant, prevents and protects against ethanol-induced oxidative stress in mouse liver. Biol. Pharm. Bull. 26, 1398-1402. Navasumrit, P., Ward, T. H., Dodd, N. J. and O’Connor, P. J. (2000) Ethanol-induced free radicals and hepatic DNA strand breaks are prevented in vivo by antioxidants: effects of acute and chronic ethanol exposure. Carcinogenesis 21, 89-99. Nordmann, R., Ribiere, C. and Rouach, H. (1992) Implication of free radical mechanisms in ethanol-induced cellular injury. Free Radical Biol. Med. 12, 219-240. Ogony, J., Matthews, R., Anni, H., Shannon, K. and Ercal, N. (2008) The mechanism of elevated toxicity in HepG2 cells due to combined exposure to ethanol and ionizing radiation. J. Appl. Toxicol. 28, 345-355. Oliva, J., Bardag-Gorce, F., Tillman, B. and French, S. W. (2011) Protective effect of quercetin, EGCG, catechin and betaine against oxidative stress induced by ethanol in vitro. Exp. Mol. Pathol. 90, 295-299. Prasad, N. R., Jeyanthimala, K. and Ramachandran, S. (2009) Caffeic acid modulates ultraviolet radiation-B induced oxidative damage in human blood lymphocytes. J. Photochem. Photobiol. Photot. 95, 196-203. Rampart, M., Beetsens, J. R., Bult, H., Herman, A. G., Pamh, M. J. and Winkelmann, J. (1986) Complement-dependent stimulation of prostacyclin biosynthesis: inhibition by rosmarinic acid. Biochem. Pharmacol. 35, 1397-1400. Reddy, M. B., Hurrell, R. F., Juillierat, M. A. and Cook, J. D. (1996) The influence of different protein sources on phytate inhibition of non-heme-iron absorption in humans. Am. J. Clin. Nutr. 63, 203-207. Ross, J. A. and Kasum, C. M. (2002) Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu. Rev. Nutr. 22, 19-34. Russo, M., Spagnuolo, C., Tedesco, I., Bilotto, S. and Russo, G. L. (2012) The flavonoid quercetin in disease prevention and therapy: facts and fancies. Biochem. Pharmacol. 83, 8-15. Sarkola, T., Iles, M. R., Kohlenberg-Mueller, K. and Eriksson, C. J. (2002) Ethanol, acetate and acetate, and lactate levels after alcohol intake in white men and women: effect of 4-methylpyrazole. Alcohol. Clin. Exp. Res. 26, 239-245. Satoh, T., Enokido, Y., Aoshima, Y., Uchiyama, Y. and Hatanaka, H. (1997) Changes in mitochondrial membrane potential during oxidative stress-induced apoptosis in PC12 cells. J. Neurosci. Res. 50, 413-420. Shamsuddin, A. M., Vucenic, I. and Cole, K. E. (1997) IP6: a novel anti-cancer agent. Life Sci. 61, 343-354. Vucenic, I. and Shamsuddin, A. M. (2006) Protection against cancer by dietary IP6 and inositol. Nutr. Cancer 55, 109-125. Vucenic, I., Tomazic, V. J., Fabian, D. and Shamsuddin, A. M. (1992) Antitumor activity of phytic acid (inositol hexaphosphate) in murine transplanted and metastatic fibrosarcoma, a pilot study. Cancer Lett. 65, 9-13. Wang, H. and Joseph, J. A. (1999) Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. Free Radic. Biol. Med. 27, 612-616. Wu, H., Cai, P., Clemens, D. L., Jerrells, T. R., Ansari, G. A. and Kaphalia, B. S. (2006) Metabolic basis of ethanol-induced cytotoxicity in recombinant HepG2 cells: role of nonoxidative metabolism. Toxicol. Appl. Pharmacol. 216, 238-247.