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Protective Effects of Cereal Grain Extracts on Alcohol-Induced Hepatocyte Damage

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ABSTRACT: This study investigated the protective effects of cereal grains on alcohol-induced hepatocyte damage. Cereal grains were extracted with methanol, and their radical scavenging properties and total phenolic contents were examined. Black rice extract exhibited the highest total polyphenol content and radical scavenging capacity. Treatment with sorghum extract increased the viability of cells exposed to alcohol by up to 81.6%. All cereal grain extracts decreased reactive oxygen species and malondialdehyde production and glutathione depletion in HepG2 cells exposed to ethanol. In particular, black rice and sorghum extracts exhibited greater antioxidant effects than other cereal grains. Treatment with black rice extract increased the levels of alanine aminotransferase and aspartate aminotransferase of alcohol-exposed cells to control levels. Overall, black rice extract showed a greater protective effect compared with other cereal grains against alcohol exposure in HepG2 cells and could improve alcohol-induced liver problems.

Keywords: cytoprotection, ethanol, grain, HepG2 cells, oxidative stress

INTRODUCTION

Reactive oxygen species (ROS) are highly reactive molecules, and high levels of ROS can damage complex cellular molecules (Cederbaum et al., 2009). Under acute or chronic alcohol exposure, ROS production is increased, and antioxidant enzyme levels are decreased. Alcohol consumption enhances oxidative stress in various organs, particularly the liver (Albano, 2002; Dey and Cederbaum, 2006). Lipid oxidation, which is facilitated by free radicals, is considered as a principal mechanism leading to cell membrane degradation and cellular injury (Mohan and Priya, 2010). Alcoholic liver disease (ALD) is a significant contributor to global illness and mortality; thus, the effects of ethanol on health have been extensively investigated (Calabrese et al., 1996). According to a previous study, ALD is related to excessive ROS generation from alcohol metabolism (Purohit et al., 2003).

Polyphenols are present in fruits, vegetables, and grains, and various factors, including genetics, environmental conditions, and processing methods, can affect their quantities and types (Kris-Etherton et al., 2002). Cereal grains are major sources of energy and nutrients worldwide (Liu, 2007). Phenolic acids, flavonoids, and tannins are the primary phenolic compounds found in cereal grains; these compounds regulate cellular oxidative balance and protect essential biological molecules such as DNA, proteins, and membrane lipids from oxidative damage (Yu et al., 2002). Previously, the consumption of whole cereal grains has been associated with reduced severity of metabolic syndrome and related chronic conditions including type 2 diabetes and cardiovascular disease (Rippe and Angelopoulos, 2016). The antioxidant effects of cereal grains, including black rice (Hu et al., 2003), oats (Handelman et al., 1999), sorghum (Awika et al., 2003), barley (Kim et al., 2012), and adlay (Kim et al., 2012), have been well documented. Among them, brown rice is rich in phenolic acids, which exhibit antioxidative properties that protect cells from oxidative stress (Ravichanthiran et al., 2018). With the increasing desire to maintain a nutritious diet, there is a growing demand for diverse varieties of whole grain rice, including brown rice, black rice, barley, oats, and beans (Han et al., 2012). HepG2 cells are considered as an effective model for investigating xenobiotic metabolism and liver toxicity *in vitro*. Moreover, they are suitable for studying the effects of compounds on cytoprotection, genotoxicity, and antigenotoxicity (Knasmüller et al., 2004). This characteristic is because of their ability to retain various

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antioxidant and phase II enzymes (Knasmüller et al., 2004). The present study aimed to explore the hepatoprotective properties of methanolic extracts derived from commonly consumed grains in Korea, including white rice, brown rice, black rice, barley, oats, adlay, and sorghum. In addition, the correlation between antioxidant activity and the protective effects of these extracts against alcohol-induced oxidative stress in HepG2 cells was examined.

MATERIALS AND METHODS

Chemicals

Gallic acid, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), glutathione (GSH) reductase, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2',7'- dichlorofluorescein diacetate, reduced GSH, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and absolute ethanol were purchased from Sigma-Aldrich. All other solvents and reagents used were of analytical grade.

Preparation of cereal grain extracts

Cereal grains such as black rice, brown rice, adlay, sorghum, oats, barley, and white rice were obtained from a local market in Cheongju, Korea. Each cereal grain (30 g) was washed with distilled water, dried, and milled into flour. Subsequently, 5 g of cereal flour was combined with 100 mL of methanol and shaken at 23°C for 18 h. The obtained mixture was filtered using Whatman No. 2 filter paper (GE Healthcare). Subsequently, the extraction solvents were eliminated using a vacuum evaporator (EYELA N-1000, Rikakikai Co.). The remaining residues were dissolved in dimethyl sulfoxide (DMSO) and stored at −70°C until use.

Determination of antioxidant activity

The total polyphenol content was measured using the Folin-Ciocalteu method (Dewanto et al., 2002). The results were expressed as milligrams of gallic acid equivalent (GAE) per sample weight. The DPPH radical scavenging activity was determined in accordance with the method of Kim et al. (2002). The DPPH radical scavenging activity was quantified as Trolox equivalent antioxidant capacity, denoted in milligrams of Trolox equivalent per sample weight. The ABTS radical scavenging activity was measured in accordance with the method of Re et al. (1999). The absorbance was measured at 735 nm using a spectrophotometer.

Cell culture and cytotoxicity

HepG2 cells were obtained from the Korean Collection for Type Cultures and cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 5.5 mM D-glucose. Subsequently, the cells were maintained in a humidified incubator with 5% CO_2 at 37°C. The cells were then treated with cereal grain extracts (25 μg/mL) for 24 h, and MTT assay was conducted to evaluate the cytotoxicity of each sample. In addition, HepG2 cells were exposed to 3% alcohol and cereal grain extracts for 24 h to evaluate the protective effects of cereal grains (25 μg/mL) against alcohol-induced stress. After 24 h, MTT assay was conducted. The final DMSO concentration never exceeded 0.1% (v/v) in any treatment group.

Determination of intracellular ROS levels

Intracellular ROS levels were measured in accordance with the method of Wang and Joseph (1999). The fluorescence intensity, which indicates intracellular ROS production, was assessed using a fluorescence spectrophotometer (Perkin-Elmer) for 2 h, with an excitation wavelength of 485 nm and emission wavelength of 530 nm.

Determination of hepatic alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels

The ALT and AST levels in the culture medium were assessed using commercially available assay kits (BioVision), in accordance with the manufacturer's instructions.

Determination of GSH levels and lipid peroxidation

The intracellular GSH levels were assessed using the modified 5,5'-dithiobis(2-nitrobenzoic acid)-GSH disulfide reductase recycling method, as outlined in the study of Baker et al. (1990). The degree of lipid peroxidation was evaluated by quantifying malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances assay, as detailed in the study of Buege and Aust (1978).

Statistical analysis

The results were expressed as mean±standard error and derived from three or more independent experiments. Statistical analyses were performed using one-way analysis of variance with SAS version 9.0 (SAS Institute). Statistical significance was considered at $P<0.05$. The results of correlation analysis regarding the cytoprotective effects and antioxidant capacity of cereal grains were presented as Pearson correlation coefficients using IBM SPSS Statistics version 24.0 (IBM Corp.).

RESULTS AND DISCUSSION

Polyphenolic contents and antioxidant activities of cereal grains

Polyphenols have antioxidative properties that can inhib-

Table 1. Polyphenolic contents and antioxidant activities of cereal grains

	Total polyphenol (mg GAE/g sample)	ABTS (mg TEAC/g sample)	DPPH (mg TEAC/g sample)
White rice	0.56±0.02^{d}	15.88±0.61^{c}	0.77±0.12^{c}
Barley	0.80±0.02^{c}	7.76±0.02^{d}	1.54±0.10^{c}
Oat	0.19±0.02^{e}	3.22±0.51^{e}	0.37±0.10^{c}
Adlay	0.23±0.01^{e}	3.79±0.29^{e}	0.38±0.08^{c}
Brown rice	0.83±0.02^{c}	8.03±0.08^{d}	1.16±0.09^{c}
Black rice	5.44±0.17^{a}	79.37±0.46^{a}	10.83±1.56^{a}
Sorghum	4.73±0.16^{b}	52.81±1.26^{b}	7.49±1.65^{b}

Values are presented as the mean±SD.
Different letters in the same column (a-e) indicate a significant difference (ANOVA and Duncan's test, P<0.05).
ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalent; TEAC, Trolox equivalent antioxidant capacity.

it free-radical-induced tissue damage by preventing radical formation, scavenging, or promoting decomposition (Ratnam et al., 2006). Hence, polyphenol levels need to be quantified, and their impact on antioxidant capabilities need to be evaluated. The polyphenol content in the methanolic extracts of seven grains was measured as milligrams of GAE per gram of sample (Table 1). The polyphenolic contents of black rice (5.44±0.17 mg GAE/g) and sorghum (4.73±0.16 mg GAE/g) were remarkably higher compared with those of other cereal grains. These results suggest that compounds that contribute to pigmentation, including anthocyanins, can enhance polyphenol levels and antioxidant activity (Dykes and Rooney, 2007). In methanolic extracts, brown rice exhibited higher polyphenolic levels compared with white rice. According to a previous study, the milling process can eliminate a substantial portion of polyphenolics (Choi et al., 2007). The antioxidant activity of cereal grains was evaluated using DPPH and ABTS radical scavenging activity (Table 1). Black rice extract showed the highest antioxidant capacity, followed by sorghum extract. Phenolic compounds possess potent antioxidant capabilities because of the hydroxyl substituents on their aromatic rings, which enable them to effectively neutralize free radicals and prevent the formation of superoxide anion radicals (Tsao, 2010). Total phenols are strongly correlated with antioxidant activity, indicating that phenolic compounds contribute significantly to antioxidant activity (Turumtay et al., 2014). Notably, pigmented black rice and sorghum exhibited notably higher antioxidant activities and contained higher levels of polyphenolic compounds compared with other cereal grains. These findings are consistent with the results of Choi et al. (2007), who also observed relatively higher antioxidant activity levels in black rice and red sorghum.

Protective effects of cereal grains against alcohol-induced oxidative stress

None of the seven grain extracts affected the cytotoxicity of HepG2 cells at the specified concentration (25 μg/mL) for 24 h (Fig. 1A). However, ethanol exposure reduced cell viability. Cells exposed to ethanol concentrations exceeding 3% exhibited greatly reduced cell viability (Fig. 1B). Consequently, the noncytotoxic concentrations of grains (25 μg/mL) and cytotoxic concentration of 3% ethanol were selected for further evaluation. MTT assay was conducted to assess the protective effects of cereal grains against ethanol-induced toxicity in HepG2 cells. As shown in Fig. 1C, alcohol exposure significantly decreased HepG2

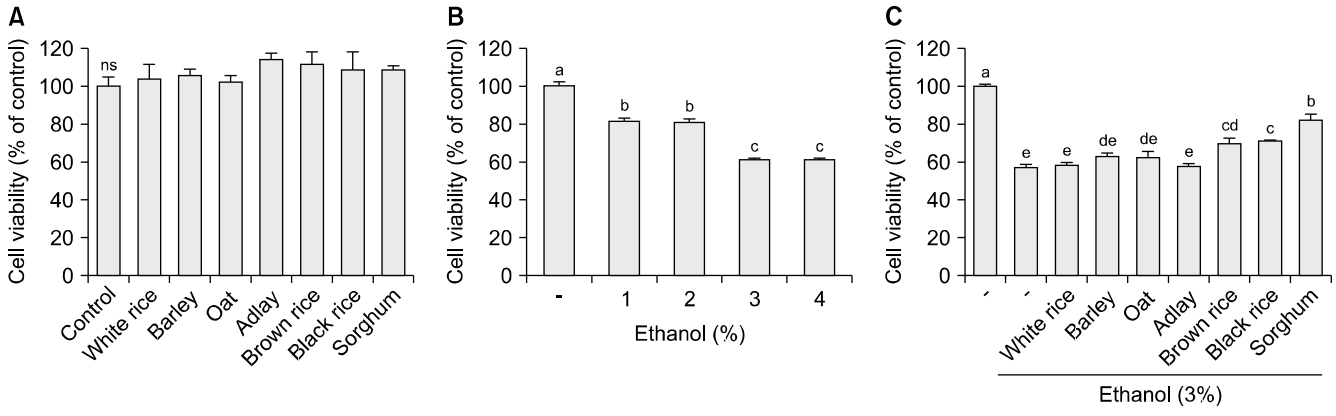

Fig. 1. Cytotoxic effect of cereal grain extracts (A) and ethanol (B) on cell viability. Protective effect of cereal grain extracts on the cell viability of HepG2 cells exposed to alcohol (C). Values are the mean±SD. Different letters (a-e) indicate a significant difference (ANOVA and Duncan's test, P<0.05). ns, not significant.

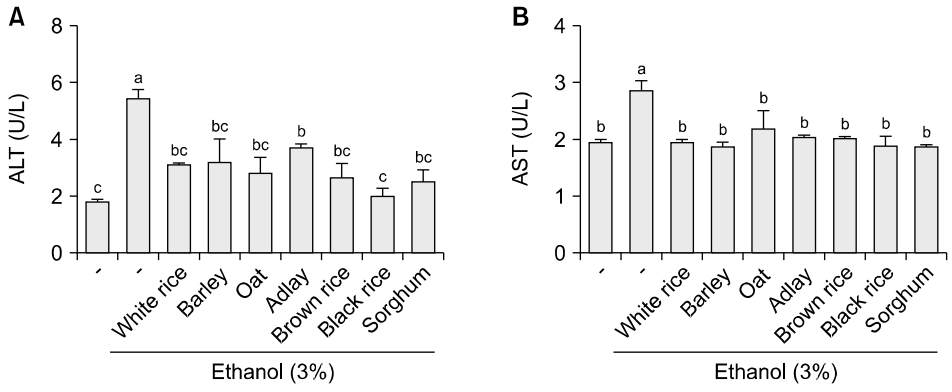

Fig. 2. Effects of cereal grains (25 μg/mL) on alcohol-induced alanine aminotransferase (ALT) (A) and aspartate aminotransferase (AST) (B) production in HepG2 cells. Values are the mean±SD. Different letters (a-c) indicate a significant difference (ANOVA and Duncan's test, $P<0.05$).

cell viability by 57.1% compared with the control group. However, treatment with sorghum, black rice, and brown rice extracts significantly increased cell viability, with sorghum extracts showing the highest cell viability (81.6%). These results suggest that brown rice, black rice, and sorghum can efficiently protect hepatocytes from damage mediated by alcohol-induced oxidative stress. The release of hepatic transaminase is the main marker of hepatic damage (Dufour et al., 2000). Hepatotoxicity can be assessed by directly measuring the levels of hepatic transaminases released into the culture medium (Liu et al., 2005). In Fig. 2, ethanol treatment increased ALT and AST levels compared with the control cells, and this elevation was effectively mitigated by treatment with cereal grains. Ethanol exposure significantly augmented the release of ALT and AST from the cytoplasm into the culture medium, which is consistent with previous reports *in vivo* and *in vitro* (Yao et al., 2007). A previous study showed that polyphenol compounds, such as rutin, have hepatoprotective effects (Pan et al., 2014). Therefore, the cytoprotective effects of cereal grains might be associated with their phenolic compounds, and black rice and red sorghum could exhibit therapeutic potential in the treatment of liver damage.

Intracellular antioxidant activity of cereal grains

Intracellular ROS production was measured to assess whether cereal grains can attenuate alcohol-induced oxidative stress in HepG2 cells. As shown in Fig. 3A, all grains inhibited ROS generation compared with alcohol treatment alone. Remarkably, treatment with black rice and sorghum significantly reduced ROS generation. This finding indicates that cereal grains have a hepatoprotective effect against ethanol-induced liver damage. In the progression of liver diseases associated with alcohol consumption, the harmful effects of ethanol primarily stem from oxidative stress, marked by the overproduction of ROS and MDA, along with the reduction of GSH levels (Senthil Kumar et al., 2012). Cellular ROS accumulation is a key indicator of oxidative damage to living cells. Moreover, elevated ROS levels resulting from alcohol metabolism are linked to decreased hepatocyte viability (Lu and Cederbaum, 2008).

MDA levels were measured to examine the effect of cereal grains on alcohol-induced lipid peroxidation (Fig. 3B). Treatment with cereal grain extracts prevented the elevation of MDA levels against alcohol damage. In particular, treatment with black rice, sorghum, and brown rice completely inhibited the elevation of MDA to levels near those of the control. The excessive ROS produced dur-

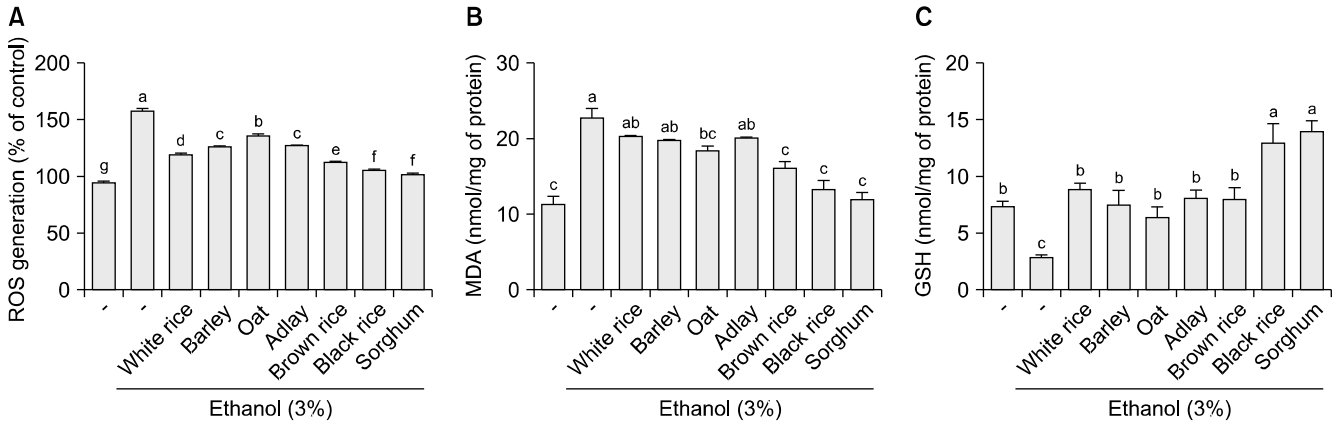

Fig. 3. Effect of cereal grain extracts (25 μg/mL) on reactive oxygen species (ROS) generation (A), lipid peroxidation (B), and glutathione (GSH) depletion (C) in HepG2 cells exposed to alcohol. Values are the mean±SD. Different letters (a-g) indicate a significant difference (ANOVA and Duncan's test, $P<0.05$). MDA, malondialdehyde.

ing ethanol metabolism swiftly interact with lipid membranes. The intracellular concentration of MDA directly reflects the peroxidation of membrane unsaturated fatty acids and has been assessed as a biomarker for ethanol-induced oxidative damage in hepatocytes (Shah et al., 2014). To prevent damage from ROS, cells possess an antioxidant defense mechanism that encompasses nonenzymatic antioxidants such as GSH, which operates within the cellular thiol/disulfide system (Barrera, 2012). Intracellular GSH levels were significantly decreased by ethanol exposure, whereas treatment with cereal grains prevented hepatic GSH depletion induced by ethanol (Fig. 3C). In particular, treatment with black rice and sorghum significantly increased GSH generation, exceeding the control values. During periods of oxidative stress, endogenous GSH is utilized, leading to rapid depletion of GSH at the cellular level (Eklöw-Låstbom et al., 1986). Alcohol-induced ROS generation plays a crucial role in the onset of ALDs and in suppressing the expression of cytoprotective genes (Das and Vasudevan, 2007; Motohashi et al., 2010). Therefore, reducing ROS accumulation through the cellular antioxidant defense system could aid in maintaining intracellular redox homeostasis. These findings indicate that treatment with cereal grains significantly mitigated the ethanol-induced depletion of GSH, lipid peroxidation, and ROS generation in HepG2 cells.

Correlation between hepatoprotective potential and antioxidant activities

The relationship between hepatoprotective potential and antioxidant activities is shown in Table 2. Overall, antioxidant activities showed a positive correlation with GSH levels and protective effects, whereas they showed a negative correlation with oxidative stress biomarkers, including MDA, ROS, ALT, and AST levels in HepG2 cells. In particular, a strong correlation was observed between total polyphenol content and hepatoprotection. These results suggest that polyphenol compounds play a major role in hepatoprotection against ethanol-induced oxidative stress. The relationship between antioxidant activities and hepatoprotective potential of methanolic plant extracts has been explored (Gutierrez and Navarro, 2010; Srirama et al., 2012). Previous studies have demonstrated the presence of anthocyanins, particularly cyanidin-3-O-glucoside and peonidin-3-O-glycoside, in the aleurone layer of black rice, which enhance cell viability (Ryu et al., 1998; Hu et al., 2003; Lee, 2010). Anthocyanins contribute to antioxidant activities, which are associated with the alleviation of oxidative stress (Hu et al., 2003). Park et al. (2008) observed that the anthocyanins found in black rice, including cyanidin-3-O-glucoside and peonidin-3-O-glycoside, exhibited potent antioxidant properties *in vitro*. Furthermore, cyanidin-3-O-glucoside, isolated from black rice, demonstrated hepatoprotective effects by scavenging superoxide anion radicals in HepG2 cells (Shim et al., 2006). A previous study has also shown that certain cereals such as sorghum contain antioxidants comparable to those found in fruits and vegetables (Awika and Rooney, 2004).

In conclusion, excessive alcohol consumption was closely linked to irreversible liver damage, largely because of the heightened oxidative stress characterized by increased lipid peroxidation and elevated ROS production. The methanolic extracts of cereal grains could decrease ROS and MDA levels and GSH depletion induced by alcohol exposure in HepG2 cells. In particular, the antioxidant activity of extracts from black rice and sorghum could alleviate oxidative stress induced by alcohol. Therefore, the development of dietary supplements, including colored grains such as black rice and red sorghum, holds promise for protection against alcoholic liver damage.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: HL, HSJ, Junsoo L. Analysis and interpretation: JS. Data collection: Jaemin L. Writing the ar-

Table 2. Correlation analysis between the cytoprotective effect and antioxidant activity of cereal grain extracts

	ALT	AST	GSH	MDA	ROS	Cytoprotection
Total polyphenol	−0.793*	−0.614	0.953**	−0.899**	−0.852**	0.798*
ABTS	−0.797*	−0.595	0.920**	−0.821*	−0.810*	0.672*
DPPH	−0.805*	−0.595	0.912**	−0.856**	−0.803*	0.723*

Values are presented as r (*P*-value) unless otherwise indicated. *P*-values were calculated by partial correlation analysis. *P=0.05, **P=0.01.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GSH, glutathione; MDA, malondialdehyde; ROS, reactive oxygen species; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl.

ticle: JS. Critical revision of the article: YK. Final approval of the article: all authors. Statistical analysis: Jaemin L. Obtained funding: YK. Overall responsibility: YK.

REFERENCES

Albano E. Free radical mechanisms in immune reactions associated with alcoholic liver disease. Free Radic Biol Med. 2002. 32:110-114.

Awika JM, Rooney LW. Sorghum phytochemicals and their po-*Tential impact* on human health. Phytochemistry. 2004. 65: 1199-1221.

Awika JM, Rooney LW, Wu X, Prior RL, Cisneros-Zevallos L. Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. J Agric Food Chem. 2003. 51:6657-6662.

Baker MA, Cerniglia GJ, Zaman A. Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. Anal Biochem. 1990. 190:360-365.

Barrera G. Oxidative stress and lipid peroxidation products in cancer progression and therapy. ISRN Oncol. 2012. 2012: 137289. https://doi.org/10.5402/2012/137289

Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978. 52:302-310.

Calabrese V, Renis M, Calderone A, Russo A, Barcellona ML, Rizza V. Stress proteins and SH-groups in oxidant-induced cell damage after acute ethanol administration in rat. Free Radic Biol Med. 1996. 20:391-397.

Cederbaum AI, Lu Y, Wu D. Role of oxidative stress in alcohol-induced liver injury. Arch Toxicol. 2009. 83:519-548.

Choi Y, Jeong HS, Lee J. Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chem. 2007. 103: 130-138.

Das SK, Vasudevan DM. Alcohol-induced oxidative stress. Life Sci. 2007. 81:177-187.

Dewanto V, Wu X, Liu RH. Processed sweet corn has higher antioxidant activity. J Agric Food Chem. 2002. 50:4959-4964.

Dey A, Cederbaum AI. Alcohol and oxidative liver injury. Hepatology. 2006. 43(2 Suppl 1):S63-S74.

Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. Clin Chem. 2000. 46:2050-2068.

Dykes L, Rooney LW. Phenolic compounds in cereal grains and their health benefits. Cereal Foods World. 2007. 52:105-111.

Eklöw-Låstbom L, Moldéus P, Orrenius S. On the mechanisms of glutathione depletion in hepatocytes exposed to morphine and ethylmorphine. Toxicology. 1986. 42:13-21.

Gutierrez RM, Navarro YT. Antioxidant and hepatoprotective effects of the methanol extract of the leaves of *Satureja macrostema*. Pharmacogn Mag. 2010. 6:125-131.

Han G, Chung HJ, Lee Y, Yoon J. Quality characteristics of cooked rice with mixed cereals by blending ratio of the cereals frequently consumed in Korea. Korean J Community Living Sci. 2012. 23:537-552.

Handelman GJ, Cao G, Walter MF, Nightingale ZD, Paul GL, Prior RL, et al. Antioxidant capacity of oat (*Avena sativa* L.) extracts. 1. Inhibition of low-density lipoprotein oxidation and oxygen radical absorbance capacity. J Agric Food Chem. 1999. 47:4888-4893.

Hu C, Zawistowski J, Ling W, Kitts DD. Black rice (*Oryza sativa* L. *indica*) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. J Agric Food Chem. 2003. 51:5271-5277.

Kim DO, Lee KW, Lee HJ, Lee CY. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. J Agric Food Chem. 2002. 50:3713-3717.

Kim JY, Son BK, Lee SS. Effects of adlay, buckwheat, and barley on transit time and the antioxidative system in obesity induced rats. Nutr Res Pract. 2012. 6:208-212.

Knasmüller S, Mersch-Sundermann V, Kevekordes S, Darroudi F, Huber WW, Hoelzl C, et al. Use of human-derived liver cell lines for the detection of environmental and dietary genotoxicants; current state of knowledge. Toxicology. 2004. 198:315-328.

Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med. 2002. 113 Suppl 9B:71S-88S.

Lee JH. Identification and quantification of anthocyanins from the grains of black rice (*Oryza sativa* L.) varieties. Food Sci Biotechnol. 2010. 19:391-397.

Liu LG, Yan H, Yao P, Zhang W, Zou LJ, Song FF, et al. CYP2E1-dependent hepatotoxicity and oxidative damage after ethanol administration in human primary hepatocytes. World J Gastroenterol. 2005. 11:4530-4535.

Liu RH. Whole grain phytochemicals and health. J Cereal Sci. 2007. 46:207-219.

Lu Y, Cederbaum AI. CYP2E1 and oxidative liver injury by alcohol. Free Radic Biol Med. 2008. 44:723-738.

Mohan SK, Priya V. Serum total sialic acid, lipid peroxidation, and glutathione reductase levels in patients with rheumatoid arthritis. Turk J Med Sci. 2010. 40:537-540.

Motohashi H, Kimura M, Fujita R, Inoue A, Pan X, Takayama M, et al. NF-E2 domination over Nrf2 promotes ROS accumulation and megakaryocytic maturation. Blood. 2010. 115:677-686.

Pan PH, Lin SY, Wang YY, Chen WY, Chuang YH, Wu CC, et al. Protective effects of rutin on liver injury induced by biliary obstruction in rats. Free Radic Biol Med. 2014. 73:106-116.

Park YS, Kim SJ, Chang HI. Isolation of anthocyanin from black rice (Heugjinjubyeo) and screening of its antioxidant activities. Korean J Microbiol Biotechnol. 2008. 36:55-60.

Purohit V, Russo D, Salin M. Role of iron in alcoholic liver disease: introduction and summary of the symposium. Alcohol. 2003. 30:93-97.

Ratnam DV, Ankola DD, Bhardwaj V, Sahana DK, Kumar MN. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. J Control Release. 2006. 113:189-207.

Ravichanthiran K, Ma ZF, Zhang H, Cao Y, Wang CW, Muhammad S, et al. Phytochemical profile of brown rice and its nutrigenomic implications. Antioxidants. 2018. 7:71. https://doi.org/10.3390/antiox7060071

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999. 26: 1231-1237.

Rippe JM, Angelopoulos TJ. Relationship between added sugars consumption and chronic disease risk factors: current understanding. Nutrients. 2016. 8:697. https://doi.org/10.3390/nu8110697

Ryu SN, Park SZ, Ho CT. High performance liquid chromatographic determination of anthocyanin pigments in some varieties of black rice. J Food Drug Anal. 1998. 6:729-736.

Senthil Kumar KJ, Liao JW, Xiao JH, Gokila Vani M, Wang SY. Hepatoprotective effect of lucidone against alcohol-induced oxidative stress in human hepatic HepG2 cells through the up-regulation of HO-1/Nrf-2 antioxidant genes. Toxicol *In Vitro*. 2012. 26:700-708.

Shah D, Mahajan N, Sah S, Nath SK, Paudyal B. Oxidative stress and its biomarkers in systemic lupus erythematosus. J Biomed Sci. 2014. 21:23. https://doi.org/10.1186/1423-0127-21-23

Shim S, Chung J, Lee J, Hwang K, Sone J, Hong B, et al. Hepatopotective effects of black rice on superoxide anion radicals in HepG2 cells. Food Sci Biotechnol. 2006. 15:993-996.

Srirama R, Deepak HB, Senthilkumar U, Ravikanth G, Gurumurthy BR, Shivanna MB, et al. Hepatoprotective activity of Indian *Phyllanthus*. Pharm Biol. 2012. 50:948-953.

Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients. 2010. 2:1231-1246.

Turumtay EA, İslamoğlu F, Çavuş D, Şahin H, Turumtay H, Vanholme B. Correlation between phenolic compounds and antioxidant activity of Anzer tea (*Thymus praecox* Opiz subsp. *caucasicus* var. *caucasicus*). Ind Crops Prod. 2014. 52:687-694.

Wang H, Joseph JA. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. Free Radic Biol Med. 1999. 27:612-616.

Yao P, Nussler A, Liu L, Hao L, Song F, Schirmeier A, et al. Quercetin protects human hepatocytes from ethanol-derived oxidative stress by inducing heme oxygenase-1 via the MAPK/Nrf2 pathways. J Hepatol. 2007. 47:253-261.

Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M. Free radical scavenging properties of wheat extracts. J Agric Food Chem. 2002. 50:1619-1624.