Effects of Puffer (Sphoeroides rubripes) Supplementation on Disruption of Antioxidant Defense Systems in Ethanol-Treated Rats

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Summary We investigated the effects of puffer (Sphoeroides rubripes) supplementation on antioxidant metabolism in ethanol-treated rats. Sprague-Dawley rats were randomly assigned into 4 groups of 7 rats each and fed (1) an AIN-93G diet (NC), (2) 25% ethanol (E), (3) 25% ethanol and an AIN-93G diet containing 1% puffer flesh (E+F), or (4) 25% ethanol and an AIN-93G diet containing 1% puffer skin (E+S) for 5 wk. At the end of the experimental period, the rats were sacrificed and their blood and organs were collected. To evaluate the effect of puffer supplementation, lipid-soluble antioxidant vitamin and conjugated diene (CD) levels, DNA damage, and mRNA expression of heme oxygenase-1 (HO-1) were assessed. Animals that were fed ethanol showed reduced plasma levels of lipid-soluble antioxidant vitamin and significantly increased levels of lipid peroxides, DNA damage, and HO-1 expression. Dietary supplementation with puffer conferred an antioxidant effect by significantly increasing the levels of γ-tocopherol, a lipid-soluble antioxidant vitamin, and by significantly decreasing the plasma levels of CD, DNA damage, and HO-1 expression. These results suggest that consumption of puffer improves the antioxidant status of ethanol-treated rats.

Key Words puffer, ethanol intake, antioxidant metabolism, comet assay, heme oxygenase-1

Alcohol consumption may increase oxidative stress by inducing the formation of lipid peroxides and free radicals (1). Ethanol-induced oxidative stress is not restricted to the liver, where ethanol is actively oxidized, but can affect various extra hepatic tissues, as shown by experimental data obtained in rat models of acute or chronic ethanol intoxication (2). Reactive oxygen species (ROSs) are generated during ethanol metabolism, mainly by the catalytic action of ethanol-inducible cytochrome P450 2E1 in microsomes (3). Chronic and excessive alcohol consumption may accelerate an oxidative mechanism directly or indirectly, eventually leading to cell death and tissue damage (4). Many in vivo or in vitro studies have confirmed the toxicity, specifically the genotoxicity, of ethanol (5, 6).

Seafood is considered a competent antioxidant source (7) because of its relatively low content of saturated fat and high content of antioxidant compounds and certain amino acids (8). In Korea, puffer (Sphoeroides rubripes) has been traditionally used to alleviate hangover symptoms. Puffer skin extract is effective in the management of acetaldehyde intoxication (9). Puffer flesh is a rich source of fish protein, which contains glutamic acid, aspartic acid, lysine, leucine, glycine, alanine, and arginine (10), and possesses liver detoxification, anticancer, and anti-inflammatory activity (11–14). Nonedible tissues of puffer contain valuable oils with long-chain n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid and docosahexaenoic acid (15, 16). Fish oil containing PUFAs has been reported to have a positive effect on lipoprotein metabolism, coagulation, and platelet function, and to lower the risk of cardiovascular diseases (17). Additionally, puffer skin is abundant in collagen and taurine, and improves blood circulation (18). Although several studies have assessed puffer composition and toxicity and its effect on acetaldehyde metabolism (9, 19–21), very little is known regarding its antioxidant activity. In addition, most of the puffer skin is discarded during cooking, and only 15–20% of its mass is finally used (15, 18, 22). Thus, it would be preferable to investigate the effect of puffer supplementation on the basis of the part used, namely, the edible flesh and the PUFA-containing nonedible skin.

In this study, we investigated how dietary supplementation with dried powders of puffer flesh and skin affects the antioxidant status of ethanol-treated rats, and whether value addition of puffer as a component in functional food is feasible.

MATERIALS AND METHODS

Diet preparation. Puffer (about 70 cm of body length and 800–900 g of weight) included in the family Tetraodontidae was purchased in a market in Masan, Korea, in 2008. After being thawed, the muscle and skin of the frozen puffer were separated. Each of them was homogenized (BM-1, Japan), supplemental with a triple vol-
ume of water, concentrated with a stirrer in a bain-marie at 98˚C until 40˚ Brix and dried with a freeze drier (Freeze Dryer Alpha 1–2 LDplus, Martin Christ, Germany). The dried puffer was ground with a mill in order to pass through a 0.053 mm sieve and it was used for analysis and animal diet. The yield of sample was 7.6% in muscle and 7.9% in skin.

Animals and diets. Twenty-eight male Sprague-Dawley rats aged 7 wk and weighing 260–270 g were obtained from the Central Lab Animal Inc. (Seoul, Korea). Animals were housed and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (23). The animals were kept in individual stainless steel wire cages kept in an isolated room at a controlled temperature (20–22˚C) and ambient humidity (60–65%). Lights were maintained on an artificial 12 h light/12 h dark cycle and animals were fed as described below. Following an adaptation period of 7 d, the animals were randomly assigned into four groups of 7 rats each and received the following treatments: The normal control was fed with AIN-93G based standard diet and tap water ad libitum for 5 wk. Experimental groups received 25% of ethanol in tap water in place of normal drinking water ad libitum and the standard diet without/with either 1% puffer flesh or puffer skin for 5 wk (Table 1). The alcohol intake volume was recorded daily.

Animals were monitored daily for general health, and body weights were recorded every week for the duration of the study. At the end of the experimental period, the rats were anesthetized with ethyl ether, and blood was collected from the abdominal artery in a heparinized sterile tube. Plasma was obtained from the blood samples by centrifugation (1,500 rpm for 30 min) and stored at −80˚C until required for further analysis. After blood collecting, liver, heart, kidney and spleen from each animal were dissected out and washed with 0.9% of NaCl. One gram of the liver was homogenized, put in 20 mL of cold PBS and freeze-stored at −80˚C until used.

Plasma lipid profile. Plasma lipid profiles [total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol] were measured using enzymatic colorimetry methods with a commercial kit (Bionical System Corporation, Kyungki-do, Korea) and a photometric autoanalyzer (SEAC, CH-100 plus, Italy). Plasma LDL cholesterol levels were calculated using the formula developed by Friedewald et al. (24).

Plasma lipid soluble vitamins. Plasma concentrations of retinol and tocopherols were determined simultaneously by RP-HPLC (Reversed Phase High Pressure Liquid Chromatography) according to the method of Jakob and Elmadfa (25). Briefly, plasma proteins were precipitated with ethanol and lipids were extracted with n-hexane. After evaporation, dry residue was resuspended with 150 μL of methanol-dichloromethane (85 : 15, v/v) and mixed, and then 100 μL of this solution was injected into a guard-column (Merck LiChrospher 100 RP18 (10 μm), 250 × 4 mm). Samples were run at a flow rate of 1.0 mL/min on a Dionex HPLC system (Summit™ HPLC, USA). Absorption was monitored at 325 nm for retinol and at 295 nm for tocopherols. Concentrations were calculated from areas under the curve using an external calibration curve and then normalized to plasma total cholesterol (mg/dL). Plasma cholesterol was measured using enzymatic colorimetry methods with a commercial kit (Sigma Chemical Co.) and a photometric autoanalyzer (SEAC, CH-100 plus).

Baseline conjugated dienes in LDL. The baseline low density lipoprotein (LDL) conjugated diene levels were determined according to the methods outlined by Ahotupa and Vasankari (26), with slight modifications. Plasma (100 μL) was added to 700 μL of heparin citrate buffer (0.064 M trisodium citrate, 50,000 UI/L heparin, pH 5.05), and this suspension was incubated for 10 min at room temperature. The insoluble lipopro-

**Table 1. Composition of diets.**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>NC1</th>
<th>E</th>
<th>E+F</th>
<th>E+S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>55.949</td>
<td>55.949</td>
<td>55.949</td>
<td>55.949</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Mineral mix.</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mix.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Butylated hydroxyl toluene</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Puffer flesh powder</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Puffer skin powder</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

NC: normal control, E: 25% ethanol intake, E+F: 25% ethanol intake+1% flesh powder, E+S: 25% ethanol intake+1% skin powder.
teins were then sedimented by centrifugation at 1,000 ×g rpm for 10 min. The pellet was resuspended in 100 μL of 0.1 M Na-phosphate buffer containing 0.9% NaCl (pH 7.4). Lipids were extracted from 100 μL of the LDL suspension with chloroform-methanol (2:1), dried under nitrogen, redissolved in cyclohexane, and analyzed at 234 nm using a spectrophotometer (Shimadzu, Tokyo, Japan). EDTA was added to the sample to prevent oxidation during sample preparation.

**DNA damage determination by alkaline comet assay.** The alkali comet assay was conducted according to Singh et al. (27) with little modification. The isolated leukocytes were suspended in PBS with 100 μM H2O2 for 5 min on ice for oxidative stress. Frozen slides (Fisher Scientific) were prepared with a basal layer of 0.5% normal melting agarose and lymphocytes mixed with 75 μL of 0.7% low melting agarose (LMA) were added to the slides. The slides were covered again with coverslips and kept in the refrigerator for 10 min. Then coverslips were removed and a top layer of 75 μL of 0.7% LMA was added and the slides were again kept cold for 10 min. After removal of the coverslips, the slides were immersed in a jar containing cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium laurylsarcosine, pH 10; 1% Triton X-100 and 10% DMSO were added freshly) and stored in refrigerator for 1 h. After the lysis, the slides were placed in a horizontal electrophoresis tank (Threeshine Co., Ltd., Korea). The slides were covered with fresh alkaline buffer (300 mM NaOH, 10 mM Na₂EDTA, pH 13.0) at 4˚C for 40 min. To electrophores the DNA, an electric current of 25 V/300 mA was applied for 20 min at 4˚C. The slides were washed three times with neutralizing buffer (0.4 M Tris, pH 7.5) for 5 min at 4˚C and then treated with ethanol for another 5 min. All of these steps from the lysis treatment were processed under dark conditions to prevent additional DNA damage. Fifty microliters of ethidium bromide (20 μL/mL) was added to each slide and analyzed using a fluorescence microscope (LEICA DMLB, Germany). Images of 100 randomly selected cells (50 cells from each of two replicate slides) were analyzed from each subject. Measurements were made by image analysis (Kinetic Imaging, Komet 5.0, UK), determining the percentage of DNA in the tail.

**Real-time quantitative PCR.** Total RNA was isolated from the mucosa in the distal part of the liver using the Trizol protocol (Invitrogen, Carlsbad, CA, USA), and reverse transcribed using the Superscript First-Strand cDNA Synthesis kit (Invitrogen). Primer sequences and sizes of PCR amplification products are described in Table 2. Thermal cycling conditions for the PCR reactions were 95˚C for 10 min followed by 40 cycles of 94˚C for 15 s, 55˚C for 15 s, and 72˚C for 30 s. Real-time PCR reactions were carried out with an iCycler real-time machine (Bio-rad, Hercules, CA, USA) using the 2x QuantiTect SYBR Green PCR Master Mix kit (Bio-rad). The levels of mRNA for each gene were normalized against GAPDH.

**Statistical analysis.** Data were analyzed using the SPSS package for Windows (Version 12). Values were expressed as mean±standard error (SE). The data was evaluated by one-way ANOVA and the differences between the means were assessed using Duncan’s multiple-range test. The differences were considered significant at p<0.05. Evaluation of the associations between parameters was carried out using Pearson’s correlation.

### RESULTS

**Change of weight gain, food intake, ethanol intake and organ weight**

Table 3 summarizes the effect of dietary supplementation with dried powders of puffer flesh and skin on weight, food intake, food efficiency ratio (FER), and ethanol intake. The mean weight gain, food intake, FER, and ethanol intake did not differ significantly among the groups. In addition, no significant difference was observed in organ weight among the groups (Table 4).

<table>
<thead>
<tr>
<th>Primers</th>
<th>Primer sequences (in 5–3 direction)</th>
<th>Base pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO-1</td>
<td>sense: GAAGGCTAGGTGTCCAGGC</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>antisense: GAGTGGGGCATAGACTGGGT</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>sense: TCCARTCCCGRAGATCAGAC</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>antisense: CCTTCTGGGGTGTGGAGGAGG</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>NC1</th>
<th>E</th>
<th>E+F</th>
<th>E+S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (g/d)</td>
<td>2.9±0.1</td>
<td>2.8±0.2</td>
<td>2.8±0.2</td>
<td>2.8±0.2</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>20.3±0.9</td>
<td>20.3±0.9</td>
<td>20.2±0.9</td>
<td>20.4±0.9</td>
</tr>
<tr>
<td>FER1</td>
<td>0.1±0.2</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
<td>0.1±0.2</td>
</tr>
<tr>
<td>Ethanol intake (g/d)</td>
<td>4.0±0.3</td>
<td>4.2±0.3</td>
<td>4.2±0.2</td>
<td></td>
</tr>
</tbody>
</table>

1NC: normal control, E: 25% ethanol intake, E+F: 25% ethanol intake+1% flesh powder, E+S: 25% ethanol intake+1% skin powder.
2Values are means±SE for 7 animals in each group.
3NS: not significant.
4FER: food efficiency ratio.
Table 4. Effects of dried puffer flesh and skin powders on relative organ weight in ethanol-administered rats.

<table>
<thead>
<tr>
<th></th>
<th>NC1</th>
<th>E</th>
<th>E+F</th>
<th>E+S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2.8±0.036NS1</td>
<td>2.8±0.23</td>
<td>2.9±0.32</td>
<td>2.9±0.46</td>
</tr>
<tr>
<td>Heart</td>
<td>0.3±0.032NS</td>
<td>0.3±0.01</td>
<td>0.3±0.01</td>
<td>0.3±0.03</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.8±0.077NS</td>
<td>0.8±0.07</td>
<td>0.8±0.06</td>
<td>0.7±0.06</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.2±0.04NS</td>
<td>0.2±0.01</td>
<td>0.2±0.06</td>
<td>0.2±0.01</td>
</tr>
</tbody>
</table>

1 NC: normal control, E: 25% ethanol intake, E+F: 25% ethanol intake+1% flesh powder, E+S: 25% ethanol intake+1% skin powder.
2 Values are means±SE for 7 animals in each group.
3 NS: not significant.

Table 5. Effects of dried puffer muscle and skin powders on plasma lipid profiles in ethanol-administered rats.

<table>
<thead>
<tr>
<th></th>
<th>NC1</th>
<th>E</th>
<th>E+F</th>
<th>E+S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>48.9±1.623</td>
<td>58.6±0.98</td>
<td>50.9±0.8bc</td>
<td>53.2±0.8b</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>49.7±1.1b</td>
<td>60.0±2.8a</td>
<td>59.7±1.1a</td>
<td>54.4±2.5ab</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>29.0±1.3a</td>
<td>20.2±0.8a</td>
<td>24.4±1.3b</td>
<td>24.1±1.5b</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>29.9±2.4c</td>
<td>46.3±0.5a</td>
<td>42.7±1.3ab</td>
<td>40.4±1.7b</td>
</tr>
</tbody>
</table>

1 NC: normal control, E: 25% ethanol intake, E+F: 25% ethanol intake+1% flesh powder, E+S: 25% ethanol intake+1% skin powder.
2 Values are means±SE for 7 animals in each group.
3 Means with the same superscripts in each row are not significantly different (p<0.05).

Table 6. Effect of dried puffer flesh and skin powder supplementation on plasma retinol and tocopherols in ethanol-administered rats.

<table>
<thead>
<tr>
<th></th>
<th>NC1</th>
<th>E</th>
<th>E+F</th>
<th>E+S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol/T-Chol2</td>
<td>19.9±2.13NS4</td>
<td>17.0±2.8</td>
<td>22.1±2.7</td>
<td>23.8±2.6</td>
</tr>
<tr>
<td>γ-tocopherol/T-Chol</td>
<td>6.1±0.4ab5</td>
<td>3.9±1.0b</td>
<td>4.4±1.0ab</td>
<td>6.9±1.0a</td>
</tr>
<tr>
<td>α-tocopherol/T-Chol</td>
<td>198.1±24.77NS</td>
<td>159.4±22.5</td>
<td>179.1±23.5</td>
<td>224.5±29.1</td>
</tr>
</tbody>
</table>

1 NC: normal control, E: 25% ethanol intake, E+F: 25% ethanol intake+1% flesh powder, E+S: 25% ethanol intake+1% skin powder.
2 Corrected by plasma total cholesterol.
3 Values are means±SE for 7 animals in each group.
4 NS: not significant.
5 Means with the same superscripts in each row are not significantly different (p<0.05).

Effect of puffer on plasma lipid concentration after alcohol intake

Total cholesterol, triglycerides, and LDL-cholesterol significantly increased in the E group when compared to the NC group (Table 5). These lipid profiles were significantly lower because of supplementation of puffer than in the E group and the effect was evident in total cholesterol. The E+S group was more effective in triglycerides and LDL-cholesterol than the E+F group. Although HDL-cholesterol significantly decreased in the E group, compared with the NC group, its level significantly increased in the puffer supplementation groups.

Effect of puffer on plasma lipid-soluble vitamin concentration after alcohol intake

The plasma γ-tocopherol concentration was lower in the E group than in the NC group and significantly higher in the E+S group than in the E group (Table 6). Plasma retinol and plasma α-tocopherol concentrations decreased in the E group and increased in the E+F and E+S groups; however, the difference between groups was not statistically significant.

Effect of puffer on the alcohol induced lipid peroxidation

Plasma levels of conjugated diene (CD), a biomarker of lipid peroxidation, significantly increased in the E group and significantly decreased in the E+F and E+S groups (Fig. 1); they were significantly higher in the E+S group than in the E+F group. Correlation analysis revealed that the increase in the plasma γ-tocopherol level correlated with the decrease in CD level (r=−0.399, p=0.035).

Effect of puffer on the alcohol induced DNA damage

We used alkaline single-cell gel electrophoresis to detect ethanol-induced DNA damage in rat peripheral
leukocytes in vivo. Compared to the NC group, the percent tail DNA in the E group was significantly higher while the percent tail DNA in the E+F and E+S groups was recovered to that in the NC group (Fig. 2).

Effect of puffer on heme oxyenase-1 (HO-1) mRNA expression level after alcohol intake

The HO-1 mRNA expression level in the E group was 4 times that in the NC group; HO-1 mRNA expression in the E+F and E+S groups was significantly lower than that in the E group.

DISCUSSION

ROSs are released as byproducts of ethanol metabolism (2). Elevated ROSs production, together with ethanol-induced inhibition of the antioxidative system, generates a state of oxidative stress and finally leads to cell damage through various mechanisms (28). Our in vivo study confirmed that alcohol consumption results in elevated levels of lipid peroxidation, DNA damage, and oxidative stress.

Ethanol-treated rats are reported to have higher levels of lipid hydroperoxides than control rats (29); our results were in accordance with this fact. During ethanol metabolism, potentially dangerous byproducts, including ROSs, are generated (30, 31); these byproducts react with membrane lipids leading to lipid peroxidation and consequently cell death (32).

Puffer showed a remarkable hypocholesterolemic effect, with a partial reduction of plasma total and LDL cholesterol concentrations, with increasing effect on HDL cholesterol. Rats supplemented with puffer for a period of 5 wk showed a significant reduction in plasma CD levels, which can be attributed to the increase in plasma retinol, γ-tocopherol, and α-tocopherol levels. However, plasma retinol and α-tocopherol concentration showed an increased trend with puffer supplementation, but these were not statistically different. We could confirm negative correlation between plasma CD level and γ-tocopherol. The effect of γ-tocopherol on inhibition of lipid peroxidation was significantly stronger in the E+S group than in the E+F group. γ-Tocopherol, an isoform of vitamin E found in food items, significantly reduces the levels of oxidative stress biomarkers, plasma malondialdehyde, and lipid peroxides (33). γ-Tocopherol is also shown to have potent antioxidant effects (34). This experiment established that puffer supplementation reduces ethanol-induced oxidative stress. However, the amount of γ-tocopherol in puffer has not yet been reported. Therefore, further analysis is required to determine whether the increase in the plasma γ-tocopherol levels in the puffer-supplemented group was due to the presence of γ-tocopherol or other antioxidant compounds in puffer.

In our study, chronic ethanol intake was found to significantly increase DNA damage in rat peripheral leukocytes. Ethanol genotoxicity is considered to be mediated by free radical generated during ethanol metabolism (35, 36). Long-term ethanol administration results in a significant elevation in the ROSs levels in whole blood cells (37). Acetaldehyde is a well-known mutagenic substance that attacks DNA, proteins, and other cell molecules (38). We found that dietary puffer supplementation could restore damaged DNA in ethanol-treated rats. Vitamin C and E supplementation significantly decreases ethanol-induced DNA damage (39). We also confirmed that dietary supplementation with puffer flesh and skin increased antioxidant activity and inhibited DNA damage caused by ethanol-induced lipid peroxidation.

HO-1 is a 32 kD enzyme that is expressed under various pathological conditions. It has recently become the focus of considerable medical interest owing to its ability to metabolize large amounts of free heme to produce...
high concentrations of its enzymatic byproducts, and can consequently influence various biological events (40). HO-1 is also known to confer tolerance against oxidative stress, and its expression is upregulated following exposure to oxidants, xenobiotics, and ROSs (41). We examined HO-1 mRNA expression in the liver of rats fed either ethanol alone or ethanol with a puffer diet and found significantly higher hepatic HO-1 expression in the former group of rats. Thus, puffer supplementation inhibited induction of HO-1 by ethanol. This observation is consistent with that of Peng et al. (42), who reported that HO-1 levels markedly increased in the presence of 2,2'-azobis-(2-amidinopropane)-dihydrochloride (AAPH), a free radical generator, but decreased following antioxidant supplementation. This finding supports the idea that the antioxidant status modulates the synthesis of stress proteins by downregulation of HO-1.

Puffer skin is more effective than puffer flesh in lowering the plasma γ-tocopherol concentration and inhibiting lipid peroxidation. However, both puffer components significantly inhibited DNA damage and HO-1 mRNA expression.

In conclusion, puffer supplementation may prevent ethanol-induced oxidative damage in ethanol-fed rats by inhibiting lipid peroxidation and may exert antioxidative effects by inhibiting DNA damage and modulating the expression of oxidative stress-related genes. These protective effects of puffer may be mainly attributed to its antioxidant compounds, which may possess detoxifying effects. Therefore, the findings of the present study suggest that puffer possesses significant antioxidant activity, which confers protection against ethanol-induced toxicity. Thus, apart from puffer flesh, puffer skin—a byproduct obtained when puffer is cooked—also shows potential value as a functional food.

Acknowledgments

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REFERENCES

24) Friedewald WT, Levey RI, Fredrickson DS. 1972. Esti-


