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Functional properties of hot water extract of a fish, seaweed, and mushroom mixture

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Abstract−The purpose of this study was to evaluate the effect of hot water extracts of a fish, seaweed, and mushroom mixture (FSM extract) on the antioxidant and anticancer activities for use as health and functional food resources. DPPH radical scavenging activity increased from 13.4 to 93.5% when the FSM extract concentration ranged from 0.5 to 25 mg/mL. The reducing power increased from 0.04 to 1.06 OD 700 nm when the FSM extract concentration increased from 0.25 to 10 mg/mL. Nitrite scavenging activity increased from 10.3 to 96.9% when the FSM extract concentration increased from 1 to 25 mg/mL. The activities of alcohol dehydrogenase and acetaldehyde dehydrogenase in the FSM extract-fed group were 2.45 and 6.12 units/min, respectively. The activities of CAT, SOD, and GSH-Px in the FSM extract-fed group were 19.7 units/mg protein, 11.5 units/mg protein, and 16.9 units/mg protein/min, respectively. Cell viabilities of SNU213, SNU324, and SNU354 were 7.5, 9.4, and 8.9%, respectively. Cell viabilities of SNU719, SNU1, and SNU5 ranged from 14.6 to 16.8%. However, for SNU216, SNU484, SNU601, SNU638, SNU668, SNU16, and SNU520, they were below10%. These results demonstrate that the FSM extract can be used in the functional food, pharmaceutical, and cosmetic industries.

Key words: Antioxidant Activity, Anticancer Activity, Hot Water Extract, Functional Food

INTRODUCTION

Economic growth has been accompanied by dietary changes that have resulted in increased adult diseases, such as high blood pressure, arteriosclerosis, stroke, diabetes, cancer, and heart disease. Therefore, many people have begun to show interest in health and functional foods that may prevent or control these diseases. Consumer preference for natural substances, due to their safety and benefit to the human body as well as various demonstrated physiological effects, is a growing trend. With changes in modern life style, the first consideration of the consumer has been convenience, and a number of drink-type medical supplies or functional foods have been developed by extracting effective components from natural substances to use in daily life [1]. One of the various factors that can cause these adult diseases is oxidative damage caused by reactive oxygen species including hydroxyl radicals, superoxide, nitric oxide, and peroxyl radicals [2]. Antioxidants are free radical scavengers, which postpone oxidation and block the chain reaction initiated by high-energy molecules, thereby protecting the body against oxidative damage [3]. Although many synthetic antioxidants are promising for various human ailments, their pro-oxidant or cytotoxic nature at higher concentrations prevents their long-term use. These findings, together with consumer interest in natural foods, have increased the attention devoted to the development of alternative antioxidants from natural products [4].

Generally, from a nutritional point of view, fish including *Mugil cephalus*, *Muraenesox cinereus*, and *Paralichthys olivaceus* have been consumed as a health food for a long time. They also contain numerous components that prevent adult disease (e.g., high blood pressure, aging). In particular, *Mugil cephalus* prevents gastritis, nyctalopia, and arteriosclerosis, and *Muraenesox cinereus* skin contains chondroitin, which prevents skin aging [5]. *Paralichthys olivaceus* is rich in protein, amino acids, collagen, and chondroitin, and it also lowers cholesterol and possesses antitumor properties [6]. *Undaria pinnatifida* and *Laminaria japonica* are rich in protein, amino acids, alginate, fucoidan, and minerals. They also prevent aging, tumors, arteriosclerosis, and nephritis [7]. For a long time, mushrooms have been valued for food and medicinal purposes. The best known substances are polysaccharides with antitumor, antioxidant, and immunostimulating properties [8]. Previously in our laboratory, the nutritional value of *Cordyceps militaris* and *Lentinus edodes* was investigated by studying the extract compositions of the fruiting bodies and mycelia [9,10], and the biological activities of *Fomitopsis pinicola* and *Antrodia camphorata* extracts were investigated [11,12]. Recently, we investigated the effect of various extracts of fish and mushroom on biological activity. The hot water extracts of a fish and mushroom mixture showed high biological activity compared to sole one.

In this study, for the long-term goal of manufacturing health and functional foods using hot water extracts of a fish, seaweed, and mushroom mixture (FSM extract), the antioxidant activities including

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the DPPH scavenging activity, reducing power, and nitrite scavenging activity *in vitro* were investigated. We also studied the impact of FSM extract on glutathione peroxidase (GHS-Px), catalase (CAT), superoxide dismutase (SOD), alcohol dehydrogenase (ADH), and acetaldehyde dehydrogenase (ALDH) activities in rat livers. Finally, the antitumor activities of the FSM extract were examined in eight kinds of pancreatic cancer cells, six kinds of liver cancer cells, and eleven kinds of stomach cancer cells.

MATERIALS AND METHODS

1. Preparation of FSM Extract

Three kinds of fish (*Mugil cephalus*, *Muraenesox cinereus* skin, and *Paralichthys olivaceus*), two varieties of dried seaweed (*Undaria pinnatifida* and *Laminaria japonica*), and six kinds of mushroom (*Agaricus blazei*, *Mycoleptodonoides aitchisonii*, *Pleurotus eryngii*, *Grifola frondosa*, *Fomitella fraxinea*, and *Pleurotus florida*) were used for obtaining hot water extracts. A mixture of 10 kg of raw fish, 10 kg of raw seaweed, and 5 kg of raw mushroom was added to a 50 L vessel containing 5 L of distilled water (DW) and heated for 30 min at 100 ºC and for 1 hr at 80 ºC. The mixture was filtered and centrifuged at 600 ×g for 30 min. The supernatant was dried with a cool dryer. The dried powder (5 g) was added to 250 mL of DW and heated for 30 min at 80 ºC. The saturated solution was filtered by microfilter (0.2 μm) for antioxidant and anticancer activity assay.

2. Antioxidant Activity of FSM Extract *In Vitro*

DPPH radical scavenging activity: The DPPH (1, 1-diphenyl-2-picry-hydrazil) radical scavenging activity was determined by spectrophotometer. A sample (1 mg/mL) was added to 1 mL of DPPH (10 mM) in methanol. The mixture was shaken and maintained at room temperature for 10 min. The absorbance was measured at 517 nm. Reducing power: A sample (1 mL of extract) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium hexacyano-ferrate ($K_3Fe(CN)_6$, 1%). The mixture was placed in a 50 ºC water bath for 30 min. A sample (2.5 mL of extract) of trichloroacetic acid (10%) was added, and the mixture was centrifuged for 10 min at 800 ×g. The supernatant was recovered, and 2.5 mL of DW and 0.5 mL of ferric chloride (0.1%) were added. The absorbance of the sample was measured at 700 nm. Total phenolic content: the sample (0.5 mL of extract solution) was mixed with 0.5 ml of 1 N Folin-Ciocalteu reagent. The mixture was maintained for 2-5 min, followed by the addition of 1 mL of 20% Na_2CO_3. After 10 min of incubation at ambient temperature, the mixture was centrifuged for 8 min (12,000 g). The absorbance of the supernatant was measured at 700 nm. Nitrite-scavenging ability: a sample (1 mL of extract)) was added to 1 mL of sodium nitrite (1 mM) at pH 1.2, after which the reaction mixture was incubated in a water bath at 37 ºC for 1 h. Five milliliters of acetic acid (2%) and 0.4 mL of Griess reagent (a 1 : 1 ratio of 1% sulfanilic acid in acetic acid (30%) and naphthylamine (1%) in acetic acid (30%)) were added to the reaction mixture (1 mL) and incubated at room temperature for 15 min. Spectrophotometric analysis was performed at 520 nm to determine residual nitrite levels.

3. Test Animals

White male rats (Sprague-Dawley), weighing approximately 200 g, were raised under constant conditions for eight weeks for acclimatization to the lab environment. The temperature and relative humidity were maintained at 20 ºC and 50%, respectively. Over the course of the experiment, rats were provided solid feed from Sam Yang Ltd. (Korea) and water. After the rats were predomesticated for two weeks, oxidative stress was induced by administering 5.0 mL (2.5 mL at 10:00, and 2.5 mL at 18:00) of ethanol (20%) to the control group, silymarin-fed group, and FSM extract-fed group, respectively, for 1-4 weeks. To obtain the enzyme solution from liver, 5.0 ml (2.5 mL at 10:00, and 2.5 mL at 18:00) of ethanol (20%) and 0.5 mL of saline to the control group, 1 mL of silymarin (1 mg silymarin/100 g body weight/day; administered at 19:00) to the silymarin-fed group, and 1 mL of FSM extract (at 19:00) to the FSM extract-fed group were administered daily for 5-8 weeks.

4. Preparation of Enzyme Solution

A tissue sample (1 g) was taken from the rat liver and added to 10 mL of sucrose (0.25 M). The sample was homogenized using a glass teflon homogenizer under cool conditions at 800 rpm, and the mixture was centrifuged at 600 ×g for 15 min. The supernatant (9 mL) was recentrifuged at 10,000 ×g for 20 min, and the precipitate was added to 1.0 mL of phosphate buffered saline (50 mM, pH 7.0) for use in the catalase (CAT) assays and alcohol dehydrogenase (ADH) assay. The second supernatant was recentrifuged at 105,000 ×g for 1 hr and used in the glutathione peroxidase (GHS-Px), acetaldehyde dehydrogenase (ALDH), and superoxide dismutase (SOD) assays.

5. Antioxidant Enzyme Activity *In Vivo*

GSH-Px: A 500 μL mixture of potassium phosphate buffer (0.1 M, pH 7.0) containing 1×10^{-3} M sodium azide, 1 mM EDTA, 10 μL of enzyme solution, 100 μL of glutathione reductase (2.768 U/mL), and 100 μL of glutathione (1×10^{-2} M) was mixed and precultured for 10 min at 37 ºC. Next, an aliquot of $NaHCO_3$ (0.1%) containing 100 μL of NADPH (1.5×10^{-3} M) and 100 μL of H_2O_2 (1.5×10^{-3} M) was added to the reaction mixture. The absorbance was measured for 1 min at 340 nm. CAT: A 0.1 mL aliquot of surfactant was added to 50 mM potassium phosphate buffer at pH 7.0 and 10.5 mM H_2O_2. The reaction took place for 30 sec at 25 ºC. The amount of enzyme activity required to decompose 1 μmole H_2O_2/sec via this reaction was defined as one unit of activity. SOD: SOD activity was determined by recording the inhibition of ferricytochrome C reduction with EDTA. In each sample the amount of enzyme sufficient to inhibit the rate of cytochrome C reduction by 50% was determined.

6. Alcohol Dehydrogenase and Acetaldehyde Dehydrogenase Activity

ADH activity: 0.1 mL of ethanol (0.2 M), 0.02 mL of semicarbazide (0.5 M), 0.02 mL of NAD (0.1 M in 0.01 M HCl), and 2.0 mL of Tris buffer (0.1 M, pH 8.5) were mixed at 30 ºC, and 0.1 mL of enzyme was added to the mixture; absorbance was measured for 1 min at 340 nm. ALDH activity: 0.4 mL of phosphate buffer (75 mM, pH 8.8), 70 mL of DW, 70 mL of NAD (12 mM), 70 mL of magnesium chloride (12 mM), 70 mL of 4-methyl pyrazole 92.4 mM), and 2 mL of rotenone (8 mM in MeOH) were mixed at 30 ºC. Enzyme (0.1 mL) was added to the mixture, and absorbance was measured for 1 min at 340 nm.

7. Cancer Cell Culture

The eight kinds of pancreatic cell lines (miapaca, PanC1, capan1, capan2, AsPC-1, SNU213, SNU324, and SNU410), six kinds of liver cancer cells (HEP-G2, HEP-3B, SNU182, SNU354, SK-HEP1,

and PLC-RF5), and 11 kinds of stomach cell lines (SNU1, SNU5, SNU16, SNU484, SNU520, SNU601, SNU216, SNU638, SNU668, SNU719, and SNU620) used in this study were obtained from the Korean Cell Culture Bank. HepG2, Hep3G, SK-Hep1, and PLC-RF5 were cultured in DMEM (supplemented with 10% FCS and penicillin/streptomycin, 100 U/100 mg/mL), and the other cancer cells were cultured in RPMI-1640 medium (GIBCO RBL) supplemented with 10% (v/v) fetal bovine serum, 100 U/mL of streptomycin, and 100 U/mL of penicillin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2, and were subcultured twice a week.

8. Anticancer Activity

Anticancer activity was determined by the viability of cells using an MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay. The human cancer cells were cultured in RPMI-1640 containing 10% FBS and were added to 96-well microtiter plates at a concentration of 2×104 cells/well. After the FSM extract was added to each well, the 96-well plates were maintained in a CO_2 incubator at 37 °C for two days. Once the cultivation was complete, the RPMI-1640 was removed, and 50 μL of MTT and 200 μL of fresh RPMI-1640 were added to the 96-well plates. Once again, the plates were maintained in a CO_2 incubator for 4 hr to allow for formazan formation. Once formed, the formazan was dissolved in 150 μL of DMSO (dimethyl sulfoxide), and the absorbance was measured at 540 nm. The control values were determined from the cancer cell cultures grown in medium without addition of FSM extract and were considered to represent 100%.

9. Statistical Analysis

All the tests were conducted in triplicate, and the data were averaged. Standard deviations also were calculated. Student's t-test was used to evaluate significant differences ($P<0.05$) between the means of each sample.

RESULTS AND DISCUSSION

1. DPPH Radical Scavenging Activity

Free radicals have a significant effect on the oxidation of unsaturated lipids. Antioxidants are believed to intercept the free radical chain of oxidation by donating hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate oxidation of the lipids [13]. DPPH has been widely used to evaluate the free radical scavenging activity of natural antioxidants. DPPH is a stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The scavenging activity of antioxidants by hydrogen donation decreases absorbance and the color changes from purple to yellow at 517 nm [14]. To evaluate the scavenging effects of free radicals related to FSM extract concentration, DPPH radical scavenging activity was investigated. The results are displayed in Fig. 1. DPPH radical scavenging activity increased with an increase of FSM extract. When FSM extract concentration ranged from 0 to 0.25 mg/mL, the DPPH radical scavenging activity was less than 3.1%. However, when the FSM extract concentration ranged from 0.5 to 25 mg/mL, it increased from 13.4 to 93.5%. For concentrations greater than 50 mg/mL of FSM extract, it did not increase. With BHA, when DHA concentration increased from 0.05 to 0.1 mg/mL, it increased from 85.3 to 95.2% but did not increase for concentrations greater than 0.25 mg/mL.

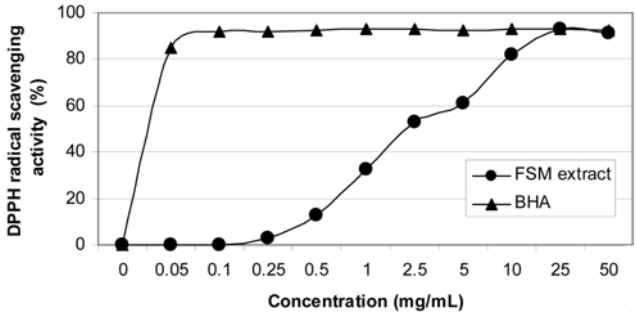

Fig. 1. Effect of FSM extract concentration on DPPH radical scavenging activity.

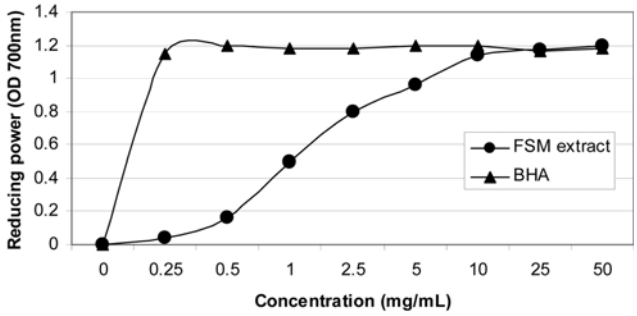

Fig. 2. Effect of FSM extract concentration on reducing power.

2. Reducing Power

The transformation of Fe^{3+} into Fe^{2+} in the presence of various concentrations was measured to determine the reducing power. The reducing capacity of a compound serves as a significant indicator of its potential antioxidant activity. The reducing ability of a compound generally depends on the presence of reductones (antioxidants), which exert antioxidant activity by breaking the free radical chain by donating a hydrogen atom [15]. Fig. 2 shows the reducing power of different concentrations of FSM extract (0-50 mg/mL) compared to that of standard BHA. When FSM extract concentration increased from 0.5 to 10 mg/mL, the reducing power increased from 0.18 to 1.16 OD 700 nm. However, it did not increase when the FSM extract concentration was greater than 25 mg/mL. When BHA concentration was 0.5 mg/mL, reducing power was highest, with 1.2 OD 700 nm. The antioxidant components present in the FSM extract caused the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form, thus, demonstrating the reducing power. These results indicate that FSM extract acts as an antioxidant.

3. Total Phenolic Content

Phenolic compounds are one of the most widely distributed plant secondary products. The ability of these compounds to act as antioxidants has been established. Polyphenols behave as multifunctional antioxidants by acting as reducing agents, hydrogen donating antioxidants, and singlet oxygen quenchers. It has been reported that the antioxidant activity of plant materials correlated with the content of their phenolic compounds [16]. Thus, it is important to consider the effects of FSM extracts on phenolic content. As shown in Fig. 3, the total phenol concentration increased with an increase of FSM extract concentration. In particular, when FSM extract concentration increased from 0 to 2.5 mg/mL, total phenol concentra-

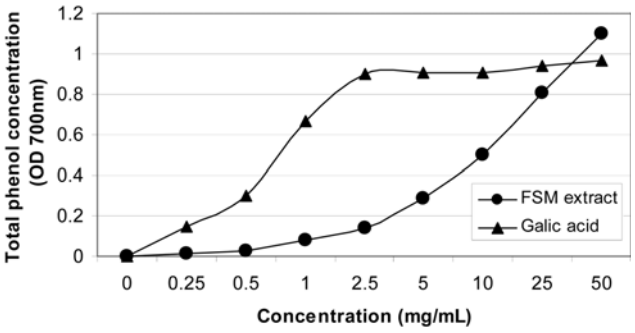

Fig. 3. Effect of FSM extract concentration on total phenol concentration.

tion was below 0.2 OD 700 nm. However, it increased from 0.29 to 1.10 OD 700 nm when FSM concentration increased from 5 to 50 mg/mL. In the standard, it sharply increased from 0.3 to 0.9 OD 700 nm when the garlic acid concentration increased from 0.5 to 2.5 mg/mL, and it ranged from 0.91 to 0.97 OD 700 nm for concentrations over 5.0 mg/mL.

4. Nitrite Scavenging Activity

N-nitrosamines (NA) are found in various foods, and thiocyanate catalyzes their formation, especially under acidic conditions. However, the level of nitrite in food or drinking water is generally very low. Nitrate itself is relatively nontoxic, but above 5% of all ingested nitrate is converted to the more toxic nitrite in the oral cavity [17]. There, nitrite is converted to nitrous acid and subsequently to N_2O_3 at low pH; in its deprotonated state, it reacts with secondary amines to form NAs. The formation of NAs may be inhibited by dietary antioxidants [18]. Consumption of whole strawberries, kale juice, garlic juice, Korean green tea, and Maesil, which contain antioxidants, has been reported to be effective in inhibiting the formation of *N*-nitrosodimethylamine [19]. As shown in Fig. 4, nitrite scavenging activity increased with the increase of FSM extract concentration. When FSM extract concentration increased from 1 to 25 mg/mL, the nitrite scavenging activity increased from 10.3 to 96.9%. However, it ranged from 96.9 to 97.5% at concentrations over 50 mg/mL. In the standard, it was 97.3% at 0.25 mg/mL of BHA and did not increase for BHA concentrations greater than 0.5 mg/mL. These results suggest that the FSM extract could be useful for preventing nitrosamine formation in foods.

5. Body and Liver Weights and Feed Efficiency Ratio in Rats

Table 1 shows the body and liver weights and feed efficiency ratios for eight weeks. Rats in the control group, in which only ethanol (20%, 5 mL/day) was administered, gained significantly less body weight than the normal group. In particular, the increased body weight of the control group was approximately 86.7% that of the normal group. However, compared to the control group, the increased body weight of rats administered ethanol combined with FSM extract was not significantly different from that of the control group. For the silymarin-fed group, it was 174.5 g, which was approximately 93.8% that of the control group. Average liver weight of the control group was 16.5 g, which was approximately 91% that of the normal group. In the silymarin and FSM extract-fed groups, liver weights were 15 and 15.2 g, respectively, which was 84% that of the normal group. The liver weight/body weight ratios in the normal and control groups were 3.55 and 3.4%, respectively. However, in the silymarin and FSM extract-fed groups, they were 3.20 and 3.15%, respectively. Feed efficiency ratios of the control, silymarin, and FSM extract-fed groups were 1.393, 1.214, and 1.291, respectively. These results indicate that ethanol administration significantly decreased the body and liver weights and feed efficiency ratio.

Table 1. Body weight, liver weight, and feed efficiency ratio of rat

Groups	Body weight (g)	Liver weight (g)	Liver/Body weight (%)	Feed efficiency ratio
Normal	214.5±8.0	18.2±0.6	3.55±0.02	1.935±0.01
Control	186.0±9.2†	16.5±0.6	3.48±0.03	1.393±0.02
Silymarin	174.5±9.1	15.0±0.6	3.20±0.04	1.214±0.03
FSM extract	187.2±6.8	15.2±0.4	3.15±0.02	1.291±0.02

Normal group: without ethanol, silymarin, or FSM extract
Control group: only ethanol 20% (5 mL/day)
Silymarin group: ethanol 20% (5 mL/day) and silymarin (1.0 mL/day)
FSM extract group: ethanol 20% (5 mL/day) and FSM extract (1 mL/day)

6. Activities of Alcohol Dehydrogenase and Acetaldehyde Dehydrogenase in Rat Liver

Table 2 shows alcohol dehydrogenase and acetaldehyde dehydrogenase activities in rat liver. The alcohol dehydrogenase in the normal group was 2.31 units/min. However, it was 2.96 units/min in the control group. When FSM extract was administered, it was 6.12 units/min, which is similar to that of the silymarin-fed group. Acetaldehyde dehydrogenase activity in the normal group was 6.67 units/min. However, it was 5.53 units/min in the control group. When FSM extract was administered, it was 2.45 units/min, which was

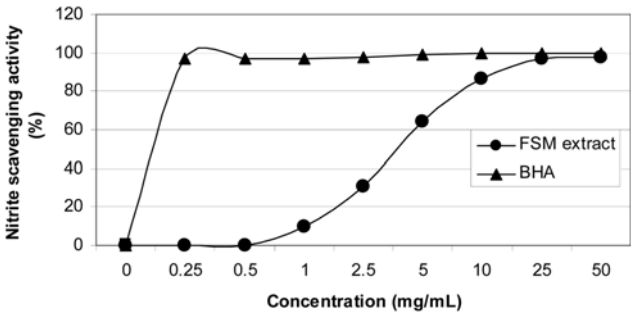

Fig. 4. Effect of FSM extract concentration on nitrite scavenging activity.

Table 2. Activities of alcohol dehydrogenase and acetaldehyde dehydrogenase in the liver of rat

Groups	Alcohol dehydrogenase (units/min)	Acetaldehyde dehydrogenase (units/min)
Normal	2.21±0.01	6.67±0.03
Control	2.96±0.01	5.53±0.02
Silymarin	2.47±0.01	6.15±0.025
FSM extract	2.45±0.02	6.12±0.02

Table 3. CAT, SOD, and GSH-Px activities in the liver of rat

Groups	CAT (units/ mg protein)	SOD (units/ mg protein)	GSH-Px (units/ mg protein/min)
Normal	26.1±0.4	11.8±0.2	16.7±0.3
Control	14.2±0.5	9.5±0.1	14.1±0.5
Silymarin	19.4±0.3	11.7±0.2	16.8±0.5
FSM extract	19.7±0.4	11.5±0.2	16.9±0.5

similar to that of the silymarin-fed group.

7. Activities of CAT, SOD, and GSH-Px in Rat Liver

The damaging effects of oxidative stress appear to be related to the inability of cellular defense enzymes, such as CAT, SOD, and GSH-Px, to adequately reduce reactive oxygen species and oxygen-centered free radicals to levels that prevent their destructive effects. Table 3 shows the effects of FSM extract on CAT, SOD, and GSH-Px activities in liver. CAT, an enzyme rich in hepatocytes and erythrocytes, which is predominantly located in peroxisomes, catalyzes dismutation of toxic hydrogen peroxide. It is a major determinant of cellular resistance to hydrogen peroxide toxicity [20]. The CAT activity in the normal group was 26.1 units/mg protein. However, it was 14.2 units/mg protein in the control group. When FSM extract was administered, it was 19.7 units/min, which was similar to that of the silymarin-fed group. These results indicate that a high level of CAT activity induced by the FSM extract can protect cells from the damaging effects of hydrogen peroxide. SOD is a metalloenzyme that catalyzes the dismutation of superoxide, the product of univalent reduction of molecular oxygen, to molecular oxygen and hydrogen peroxide. Since the highly reactive superoxide and hydroxyl radicals can cause lipid peroxidation, denaturation of proteins, and structural damage of DNA, SOD plays an important role in enzymatic protection against oxygen toxicity in oxygen-metabolizing organisms [21]. The SOD activity in the normal group was 11.8 units/mg protein. However, it was 9.5 units/mg protein in the control group. When FSM extract was administered, it was 11.5 units/min, which was similar to that of the silymarin-fed group. These results indicate that FSM extract produced significant levels of SOD activity in the liver, which could reduce potential damage caused by free radicals. GSH-Px, a selenoenzyme located in the cytoplasm and mitochondria that deactivates hydrogen peroxide as well as a wide range of lipid hydroperoxides, works in tandem with CAT to scavenge endogenous peroxides [22]. The GSH-Px activity in the normal group was 16.7 units/mg protein/min. However, it was 14.1 units/mg protein/min in the control group. When FSM extract was administered, it was 16.9 units/mg protein/min, which was similar to that of the silymarin-fed group. GSH-Px activity due to the FSM extract suggests that such a treatment could protect cells or tissues against the cytotoxic or genotoxic effects of peroxide and hydroxyl radicals.

8. Anticancer Activity

Biological response modifiers that modulate natural biological responses against tumors have been developed for cancer therapy. A number of such anticancer agents have been isolated from natural products and used clinically as cancer treatments. To investigate the effects of FSM extract (2 mg/mL) on the viability of cancer cells *in vitro*, eight kinds of pancreatic cancer cell lines (Miapaca, PanC1, AsPC-1, Capan1, Capan2, SNU213, SNU324, and SNU410)), six varieties of liver cancer cell lines (HEP-G2, HEP-3B, SK-HEP1, PLC-RF5, SNU182, and SNU354), and 11 kinds of stomach cancer cell lines (SNU216, SNU484, SNU601, SNU638, SNU668, SNU719, SNU1, SNU5, SNU16, SNU52, and SNU520) were used. Fig. 5 demonstrates the effect of FSM extract on the viability of pancreatic cancer cell over three days. The viabilities of pancreatic cancer cells decreased with an increase of incubation time. When cells were incubated in a culture medium, cell viabilities ranged from 41.3 to 51.6%, except for AsPC-1, Capan1, Capan2, SNU213, SNU324, and SNU410. In particular, when Capan1 and Capan2 were incubated, the values were 21.6 and 21.7%, respectively. For SNU213 and SNU324, they were 7.5 and 9.4%. These results suggest that the FSM extract has a significant toxic effect on pancreatic cancer cells. Fig. 6 illustrates the effect of FSM extract on the cell viability of liver cancer over three days. The viabilities of liver cancer cells decreased with an increase of incubation time. The cell viability of PLC-RF5 was 42.6%. When HEP-G2, HEP-3B, SK-HEP1, and SNU182 were incubated, they ranged from 28.6 to 35.7%. However, for SNU354, it was below 10%. These results suggest that the FSM extract has a significant toxic effect on liver cancer cells. Fig. 7 displays the effect of FSM extract on the cell viability of stomach cancer over three days. The viabilities of stomach cells decreased with an increase of incubation time. The cell viability of SNU620 was 43.9%. When SNU719, SNU1, and SNU5 were incubated, they ranged from 14.6 to 16.8%. However, in the case of SNU216, SNU484, SNU601, SNU638, SNU668, SNU16, and SNU520, they were below10%. These results suggest that the FSM extract had a

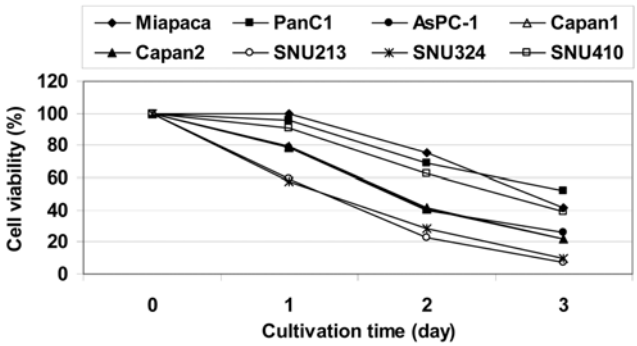

Fig. 5. Effect of FSM extract on the viability of pancreatic cancer cells.

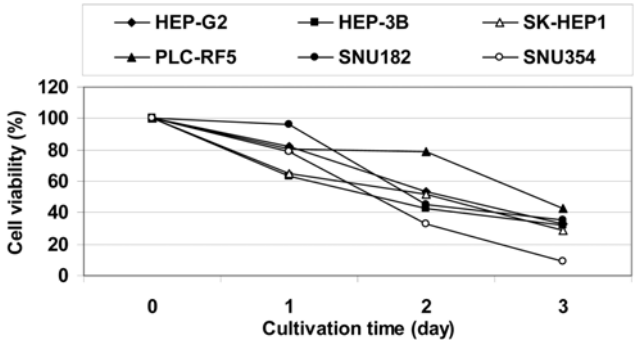

Fig. 6. Effect of FSM extract on the viability of liver cancer cells.

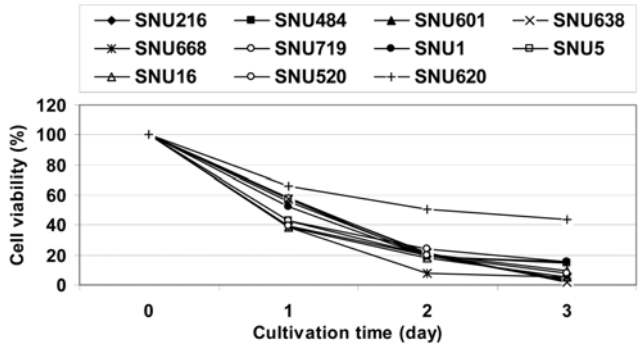

Fig. 7. Effect of FSM extract on the viability of stomach cancer cells.

significant toxic effect on stomach cancer cells. Thus, the FSM extract should be further characterized and identified for development of anticancer agents.

CONCLUSION

To manufacture functional foods using FSM extracts, we investigated their physiological activities including antioxidant and anticancer activity. Antioxidant activity increased with an increase of FSM extract concentration. The FSM extract showed the highest antioxidant capacity in the DPPH free radical activity (93.5%) at 25 mg/mL, reducing power (1.10 OD 700 nm) at 50 mg/mL, and nitrite scavenging activity (96.9%) at 25 mg/mL. The activities of alcohol dehydrogenase and acetaldehyde dehydrogenase in the FSM extract-fed group were similar to those of the silymarin-fed group. The activities of CAT, SOD, and GSH-Px in the FSM extract group were similar to that of the silymarin group. Most of the cancer cell lines containing SNU213, SNU324, SNU354, SNU216, SNU484, SNU601, SNU638, SNU668, SNU16, and SNU52 have greater than 90% effective growth inhibition. These results suggest that the hot water extract from a fish, seaweed, and mushroom mixture shows good potential as a source for functional food due to its antioxidant and cancer cell proliferation inhibition activities. However, further studies are necessary to elucidate the relationship between antioxidant and antitumor activities and the pharmacological activity of the FSM extract.

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