CHAPTER 11

Protective Effect of Polysaccharide from *Hizikia fusiformis* Against Ethanol-Induced Toxicity

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Contents

Abstract

Polysaccharide extracted from *Hizikia fusiformis* (Hf-PS-1) exhibited protective effects against ethanol-induced peptic injury. In *in vivo* assay, the ethanol group exhibited decrease of total glutathione (GSH) and increase of jun N-terminal kinase (JNK) phosphorylation relative to the control group, whereas levels were significantly increased and decreased, respectively, in the Hf-PS-1 group. Hf-PS-1

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reduced ethanol-induced gastric injury. In *in vitro* assay, ethanol induced IEC-6 cells' death in a dose-dependent manner. Ethanol decreased the phosphorylation of Shc and the binding of Grb2 to Shc, and Hf-PS-1 pretreatment increased them. Ethanol also induced the phosphorylation of JNK and extracellular signal-regulated kinase (ERK), whereas Hf-PS-1 pretreatment decreased JNK activation but not ERK. Co-treatment with JNK inhibitor and ethanol decreased GSH levels, indicating that JNK phosphorylation is a critical factor during ethanol-induced injury. Therefore, Hf-PS-1 may be useful to protect against ethanol-induced gastrointestinal injury.

I. INTRODUCTION

Marine algae have provided great biological diversity for sampling in the discovery phase of drug development (Munro *et al.*, 1987). Marine organisms in general have been an important source of compounds with potential antiviral and anticancer activities (Von Vaupel Klein, 1987). Certain seaweeds are not only significant sources of essential proteins, vitamins, and minerals, but several species of algae also produce or contain secondary metabolites, polysaccharides, and glycoproteins with antitumor, antiviral, or immunostimulatory activity (Sheu *et al.*, 1996; Shin *et al.*, 2006; Yamamoto *et al.*, 1987). Yuan and Walsh (2006) reported that extracts of a variety of edible seaweeds had antioxidative and antiproliferative activities, while Bae and Choi (2007) suggested that a methanol extract of the seaweed *Gloiopeltis furcata* induced G2/M arrest and inhibited cyclooxygenase-2 activity in HepG2 cells. Furthermore, Kang *et al.* (2005) and Ara *et al.* (2005) reported the biological activities of ethanol extracts from *Callophyllis japonica* and *Spatoglossum asperum*, respectively.

Gastrointestinal disorders such as gastric and peptic ulcers, inflammation of gastric mucosa, and gastritis are important causes of human morbidity in nonindustrialized countries. The pathophysiology of gastric ulcers is dependent on the balance between aggressive and protective factors in the stomach. When aggressive factors such as acid–pepsin, secretin, and mental stress predominate over protective factors like cellular regeneration, prostaglandins, and epidermal growth factors (EGFs), ulcers are likely to be present. Many pharmaceutical products have been developed for the treatment of gastrointestinal symptoms such as hemorrhages and perforations (Higham *et al.*, 2002). However, despite recent pharmaceutical advances, many pharmaceutical products are relatively expensive and associated with various medical problems. Drugs that relieve pain, heal ulcers, delay ulcer recurrences, and even cure disease have been developed, but they generally have important side effects.

Hence, in the search for effective treatments for gastrointestinal disease, many researchers have begun to investigate the natural products that are less likely to produce side effects.

Various botanical products such as *Laurus nobilis* seeds (Afifi *et al*., 1997), *Anchusa strigosa* roots (Disi *et al*., 1998), *Camellia sinensis* (Maity *et al*., 1995), and *Picrorhiza kurroa* (Banerjee *et al*., 2008) possess antiulcer properties. Borrelli and Izzo (2000) suggested that an extensive number of chemical compounds isolated from medicinal plants have such properties. *Hizikia fusiformis* is a widely consumed brown alga in the coastal regions of Korea, China, and Japan. This alga possesses a number of potential compounds, including antioxidants (Siriwardhana *et al*., 2004) and anticoagulants (Kim *et al*., 1998). It also contains inorganic arsenic, which is carcinogenic to humans (Nakamura *et al*., 2008; Watanabe *et al*., 1979). Furthermore, the extracts of *H. fusiformis* markedly stimulate the proliferation of human lymphocytes (Shan *et al*., 1999). Brown seaweeds also contain various soluble polysaccharides in the form of alginates, fucans, and laminarins (Lahaye and Kaeffer, 1997; Mabeau and Kloareg, 1987). For example, Li *et al*. (2006) verified the precise structure of a fucoidan obtained from the hot water extract of *H. fusiformis*. Although many studies have been conducted on this species, the health effects of dietary *H. fusiformis* remain scientifically unclear, especially with regard to its possible protective effects.

Several investigators have described the use of medicinal plants against ulcer diseases in traditional medicine (Afifi *et al*., 1997; Borrelli and Izzo, 2000; Disi *et al*., 1998; Maity *et al*., 1995; Schmeda-Hirschmann and Yesilada, 2005).

Seaweeds have been widely used as a food source and in medicine; their consumption has been strongly promoted for children and pregnant women as well-balanced, harmless, and natural sources of highly bioavailable trace elements (Booth, 1964). The reason is that marine organisms have proven to be rich sources of structurally novel and biologically active natural compounds. These compounds have served as important chemical compounds for the discovery of new drugs in the treatment of various human diseases (Usami, 2009; Zhang and Kim, 2009). Yang *et al*. (2010) suggested that the extracts from *Laurencia okamurae*, *Garrya elliptica*, *Spiraea thunbergii*, *G. furcata*, and *H. fusiformis* may be considered as possible candidates for anti-inflammatory agents. They revealed that extracts should inhibit the production of proinflammatory mediators such as nitric oxide, prostaglandin E2, interleukin-6, and tumor necrosis factor-α. Especially, Shan *et al*. (1999) determined the effect of eight seaweed extracts on human lymphocytes, among them, the activity of *H. fusiformis* associated with polysaccharides which were extracted with ethanol. In this chapter, potential effects of polysaccharide from *H. fusiformis*, Hf-PS-1, against ethanol-induced peptic injury in rats is discussed.

II. THE EFFECT OF HF-PS-1 AGAINST ETHANOL-INDUCED GASTRIC DAMAGE IN RATS

A. Preparation and effect of Hf-PS-1 in SD rats

The abuse of ethanol is associated with detrimental effects on several bodily organs and is one of several factors causing gastrointestinal disorders. For the treatment of gastrointestinal symptoms such as ulcerative hemorrhages and perforations, many pharmaceutical products (e.g., nonsteroidal anti-inflammatory drugs, NSAIDs) have been developed (Higham *et al*., 2002). However, NSAID group presents many important medical problems relating to their expense and side effects, despite recent pharmaceutical advances that have generally improved their therapeutic effects. Therefore, many researchers are investigating the natural materials that have pharmaceutical effects with fewer side effects. Among these natural materials, seaweed compounds, many of which are polysaccharides, have been studied for their various pharmaceutical effects and diverse biological activities. Sulfated galactans from the red marine alga *Champia feldmannii* show acute anti-inflammation, anticoagulation, and antinociceptive activities (Assreuy *et al*., 2008). Hong *et al*. (1997) reported that porphyran from *Porphyra yezoensis* decreases cholesterol levels and possesses antimicrobial and antitumor activities. Fucoidans from *Ecklonia kurome* have anticoagulation and antitumor properties (Nishino *et al*., 1991).

Brown seaweeds contain various soluble polysaccharides, including alginates, fucans, and laminarins, together with the insoluble fibers made of cellulose (Lahaye and Kaeffer, 1997; Mabeau and Kloareg, 1987). Recent studies have suggested that *H. fusiformis* contains a variety of biological benefits including antioxidative properties (Kim *et al*., 1998) and immune modulation (Okai *et al*., 1998). We hypothesized that the polysaccharides from *H. fusiformis* have some biological effects.

The powdered *H. fusiformis* sample consisted of 6.7 $\pm$ 0.0% moisture, 11.8 $\pm$ 0.0% protein, 1.8 $\pm$ 0.3% fat, 35.1 $\pm$ 0.1% ash, and 17.5 $\pm$ 0.0% salt. A 3.527 g of the crude polysaccharide extract, Hf-PS, which contained 0.073 g protein, was isolated. The protein level decreased to 0.011 g after pronase treatment to produce Hf-PS-1 (Fig. 11.1). The powdered Hf-PS-1 contained 7.34 $\pm$ 1.16% moisture and 76.01 $\pm$ 1.00% carbohydrate, but no detectable protein or fat (Table 11.1). The samples were fractionated by electrophoresis on agarose gels, and polysaccharide was visualized by toluidine blue staining (Fig. 11.2; upper band Hf-PS-1, lower band Hf-PS).

Ethanol is commonly used to study gastrointestinal damage (Birdane *et al*., 2007; Hernandez-Munoz *et al*., 2000). Therefore, gastrointestinal damage was induced using a simple ethanol treatment in SD rats. For the most part, no significant changes in total body and gastric weights among the three experimental groups of animals were observed.

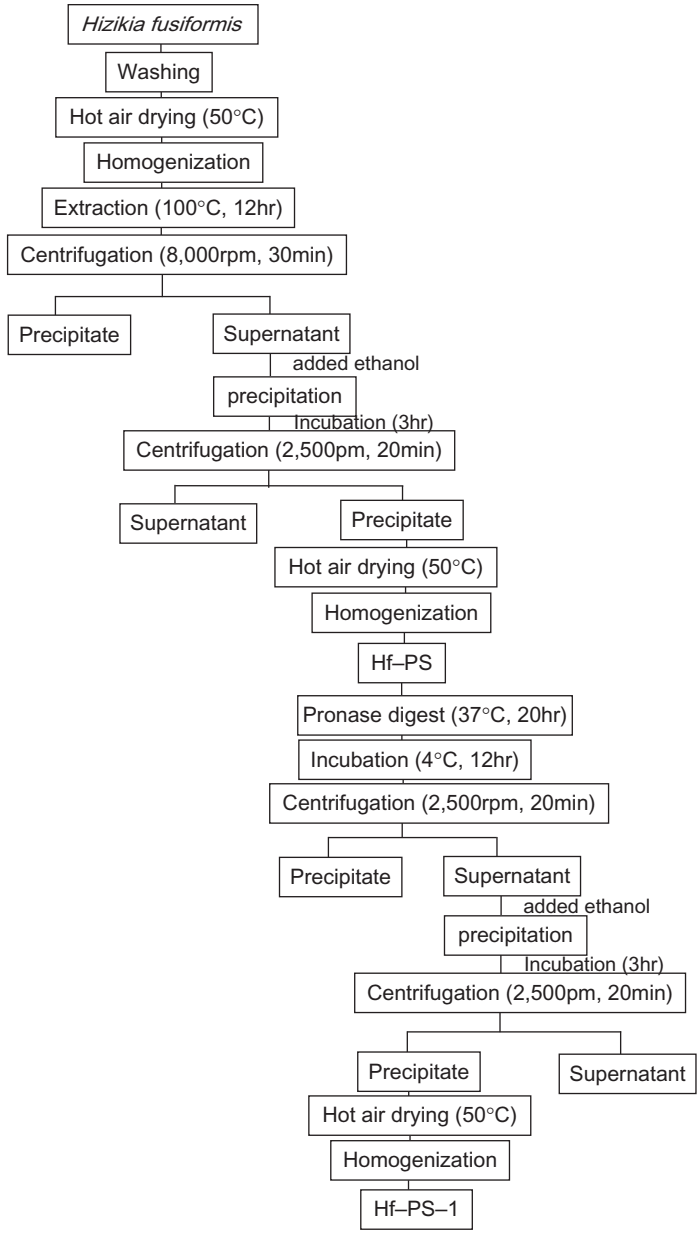

FIGURE 11.1 Purification procedures of Hf-PS-1 from *H. fusiformis*.

TABLE 11.1 Proximate composition of *H. fusiformis* powder and Hf-PS-1 (%)

	Moisture	Crude lipid	Crude protein	Crude ash	Carbohydrate[a]
H. fusiformis	4.27 ± 0.12	1.76 ± 0.07	12.94 ± 3.61	19.18 ± 0.09	61.85 ± 3.56
Hf-PS-1	7.34 ± 1.16*	Trace*	Trace*	16.65 ± 0.16*	76.01 ± 1.00*

Results are means ± SD ($n = 3$) on wet weight basis. Significant differences between the means were determined by an independent t-test.
* Means significant ($P < 0.05$).
[a] 100 − (moisture + crude lipid + crude protein + crude ash).

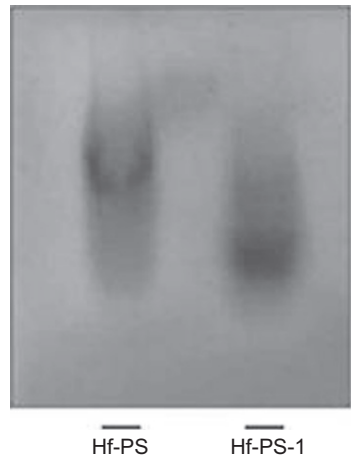

FIGURE 11.2 Electrophoresis bands of Hf-PS-1. Hf-PS-1 was separated by 0.6% agarose gel electrophoresis and analyzed by toluidine blue staining for polysaccharide.

In long-term experiments (3 weeks), the feed efficiency ratio decreased in ethanol-treated animals because digestion capacity, absorption capacity, and caloric efficiency declined (Ko *et al.*, 2002), but in short-term experiments like ours, body weight changes were not observed (Kim *et al.*, 2004; Park *et al.*, 2005). Ethanol intake produces pathological states such as infection of the gastric mucosa, gastritis, and gastric and acute peptic ulcers (Franke *et al.*, 2005; Hernandez-Munoz *et al.*, 2000; Lee *et al.*, 2000; Taylor and Rehm, 2005). The intestinal surface of stomach samples derived from rats in the ethanol-only group indicated hemorrhaging and other damage that was not evident in samples from the control group (Fig. 11.3). Comparable samples from the ethanol + Hf-PS-1 group exhibited the least signs of gastric damage.

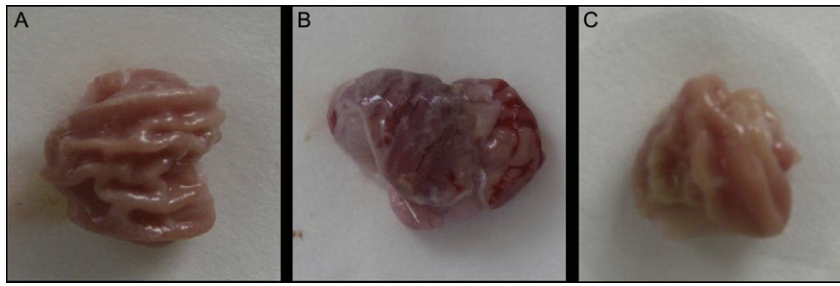

FIGURE 11.3 Photography of stomach. Hf-PS-1 protects against ethanol-induced hemorrhage on the inner surface of stomach. (A) Control group. (B) Treated with only alcohol (40%, 8.0 ml/kg BW). (C) Treated with alcohol (40%, 8.0 ml/kg BW) after Hf-PS-1 (300 mg/kg BW) pretreatment.

The epithelial cells of the gastric surface produce mucus that protects against gastric mucosal injury from ethanol, NSAIDs, and other substances (Allen *et al*., 1986; Hingson and Ito, 1971). Generally speaking, corrosion of the gastric mucosal membrane is not directly related to ulceration because of epithelial cell restoration of the necrotic state with mucus and fibrin (Lacy and Ito, 1984; Morris and Wallace, 1981). At the epithelial surface, mucus cannot effectively protect against harmful agents such as ethanol or aspirin (Allen *et al*., 1986). Ethanol induced surface epithelial cell destruction and loss of the surface mucosa layer compared to the control group, but co-treatment with Hf-PS-1 had protective effects against these damages (Fig. 11.4).

Caspases exist as inactive precursors known as procaspases. When procaspases are cleaved and activated, they induce DNA fragmentation and death receptor activation. Caspase-3 is an effector caspase that cleaves other protein substrates under apoptotic processes, and caspases 8 and 9 are the initiator caspases that cleave inactive proforms of effectors. Caspase-8 is activated in the formation of death-inducing signaling complex, and caspase-9 is activated within the apoptosome (Kaufmann and Earnshaw, 2000). Caspase cascades include death pathways and mitochondrial pathways. Caspase-9 is related to the mitochondria-dependent apoptosis and activates caspase-3. Furthermore, PARP is a primary substrate of caspase-3. In agreement with the apoptotic results described above, exposure to 40% ethanol at 80 ml/kg body weight resulted in significant activation of caspases 3, 8, and 9, and co-treatment with Hf-PS-1 inhibited this activation (Fig. 11.5). Additionally, ethanol administration produces reactive oxidants and oxidant-induced DNA fragmentation (Sehirli *et al*., 2008). In our experiment, ethanol treatment induced DNA fragmentation but co-treatment with Hf-PS-1 did not (Fig. 11.6).

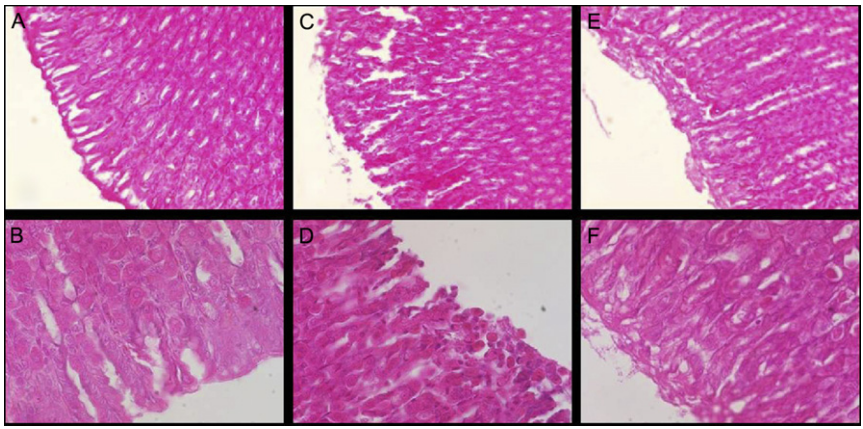

FIGURE 11.4 Photographs of epithelium of stomach by H&E staining. (A, B) Control group (200×, 400×); (C, D) ethanol treatment group (40%, 8.0 ml/kg BW; 200×, 400×) induced some loss of surface mucosa layer; and (E, F) ethanol (40%, 8.0 ml/kg BW) and Hf-PS-1 co-treatment group (300 mg/kg, BW; 200×, 400×) appeared a complete surface mucosa layer.

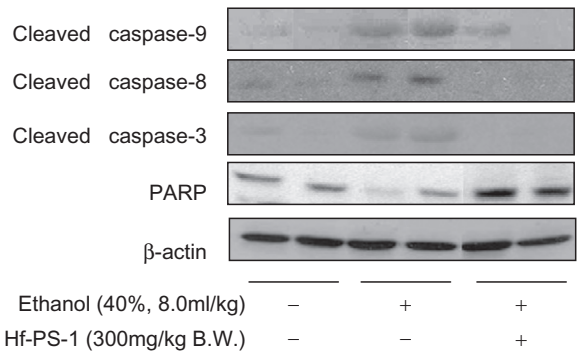

FIGURE 11.5 Effects of Hf-PS-1 on ethanol-induced gastric damage. Animals were treated and gastric tissues were collected. Protein of tissues was extracted with lysis buffer and the expression of caspases, PARP, and β-actin proteins were analyzed by Western blot analysis. β-Actin was the loading control. Each lane represents a sample from an individual rat.

B. Protective effect of Hf-PS-1 against ethanol-induced oxidative stress

Glutathione (GSH), a tripeptide found in many mammalian tissues, plays a major protective role as a scavenger of free radicals that combine with nonprotein thiols at the GSH reactive center to abolish free radical toxicity

(Swierkosz *et al.*, 1995; Vane *et al.*, 1994). Antioxidation by GSH protects the body from many diseases and conditions such as damage by H_2O_2, ethanol, and numerous other insults and toxins. GSH levels decreased by 34% in the ethanol-only group relative to the control group (100%), whereas they increased to 103.9% in the ethanol + Hf-PS-1 group (Fig. 11.7).

Mitogen-activated protein kinases (MAPKs) mediate apoptosis and cell growth, and jun N-terminal kinase (JNK) in particular is activated by oxidative stress. Yang *et al.* (2008) suggested that JNKs/SAPKs and p38 MAPK are classic oxidative stress-activated protein kinases, and Kim *et al.* (2004) observed that treatment with selenite increased intracellular reactive oxygen species (ROS) levels and JNK1 phosphorylation in Chang liver cells.

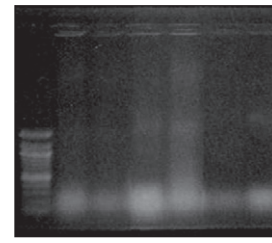

FIGURE 11.6 Effects of Hf-PS-1 on ethanol-induced gastric damage by DNA fragmentation. Animals were treated and gastric tissues were collected. DNA of tissues was extracted with 10 mM Tris–HCl (pH 7.4), 10 mM EDTA, and 1% Triton X-100 and electrophoresis in 1% agarose gels and visualized by ethidium bromide staining with a UV transilluminator. Each lane represents a sample from an individual rat.

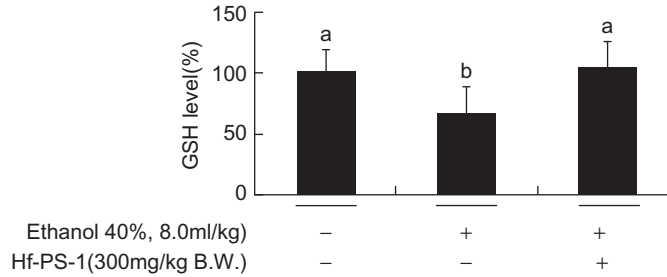

FIGURE 11.7 Effects of Hf-PS-1 on ethanol-induced oxidative damage. Animals were treated and gastric tissues were collected. One gram of tissue was used to measure the GSH levels. The GSH level was analyzed as per manufacturer's instruction. Each value represents the mean ± SD of 10 rats. Different alphabets are significant values among the group by Duncan's multiple range test.

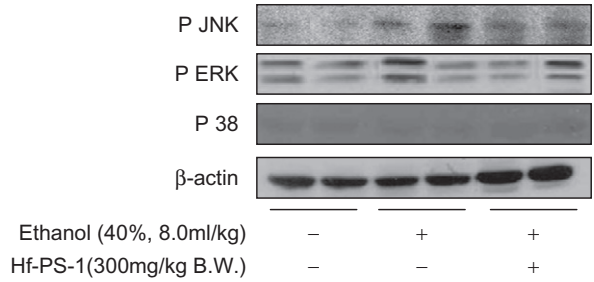

FIGURE 11.8 Effects of Hf-PS-1 on MAPK protein expression. Animals were treated and gastric tissues were collected. Protein of tissues was extracted with lysis buffer and MAPK protein expression was analyzed by Western blot analysis. β-Actin was the loading control. Each lane represents a sample from an individual rat.

Zhang *et al*. (2007) reported the ethanol-induced oxidative stress and further activation of the MAPKs, JNK, extracellular signal-regulated kinase (ERK), and p38 kinase. In agreement with these results and others (e.g., Villegas *et al*., 2006), JNK was activated in the ethanol-only treated group. Co-treatment with Hf-PS-1 and ethanol decreased JNK activation, but phospho-ERK levels were not significantly different among the three groups. These results suggested that the protective effect of Hf-PS-1 was primarily associated with the inhibition of JNK phosphorylation (Fig. 11.8).

III. EFFECTS OF HF-PS-1 AGAINST ETHANOL-INDUCED INJURY TO IEC-6 CELLS

A. Hf-PS-1 protects the ethanol-induced injury to IEC-6 cells

MTS assays of IEC-6 cells after exposure to 100–1000 μg/ml Hf-PS or Hf-PS-1 showed no cytotoxicity (Fig. 11.9). Furthermore, Hf-PS-1, but not Hf-PS, appeared to protect against ethanol-induced cytotoxicity (Fig. 11.10A). When cell viability assays were performed on cells after exposure to 0–500 μg/ml Hf-PS-1 for 3–24 h followed by exposure to 5% ethanol for 1 h, Hf-PS-1 was found to have a concentration- and time-dependent protective effect (Fig. 11.10B). Results of MTS assays showed that ethanol treatment induced the death of the IEC-6 cells and that pretreatment with Hf-PS-1 for 6–24 h counteracted this effect. Morphological studies also confirmed protection against ethanol toxicity by Hf-PS-1. Ethanol treatment induced cell shrinkage and decreased the number of viable cells (Fig. 11.11), but Hf-PS-1 pretreatment inhibited this damage. These results indicate that Hf-PS-1 pretreatment is protective against ethanol-induced cell death.

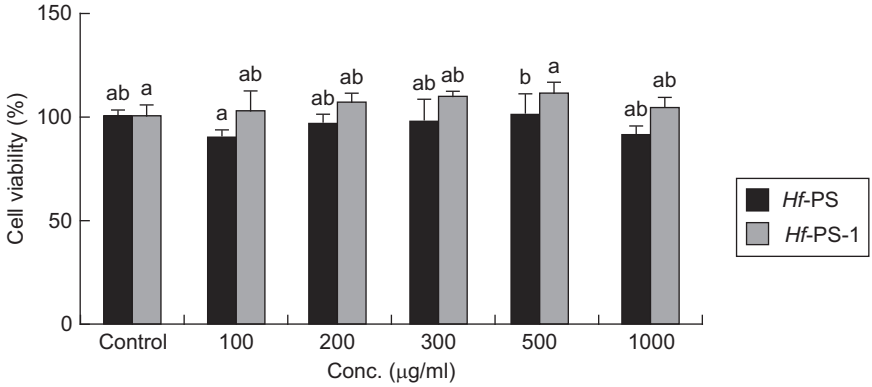

FIGURE 11.9 Effect of Hf-PS and Hf-PS-1 on cell viability. IEC-6 cells were incubated with Hf-PS or Hf-PS-1 at the indicated concentrations for 24 h. The results indicated mean ± SD in three independent experiments. Different alphabets are significant values among the group by Duncan's multiple range test.

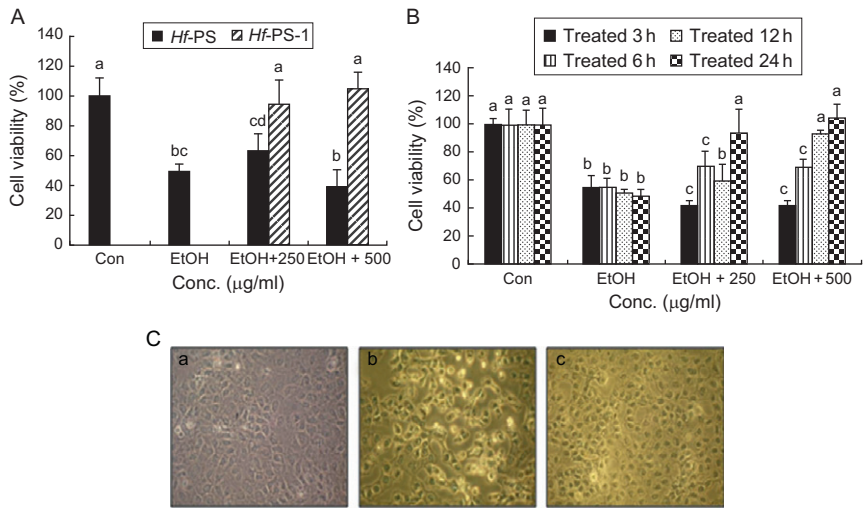

FIGURE 11.10 Protective effect of Hf-PS-1 on ethanol-induced damage. (A, B) Cells were incubated with SFM or Hf-PS-1 at the indicated concentrations and times and treated ethanol for 1 h. Cell viability was measured with MTS assay kit as manufacturer's instruction. Data were represented % of control (100%). Values are the mean ± SD. Different alphabets are significant values among the group by Duncan's multiple range test. (C) Cell morphology (400×). 'a' control; 'b' treated only ethanol; 'c' treated ethanol (5%, 1 h) after Hf-PS-1 (500 μg/ml) pretreatment.

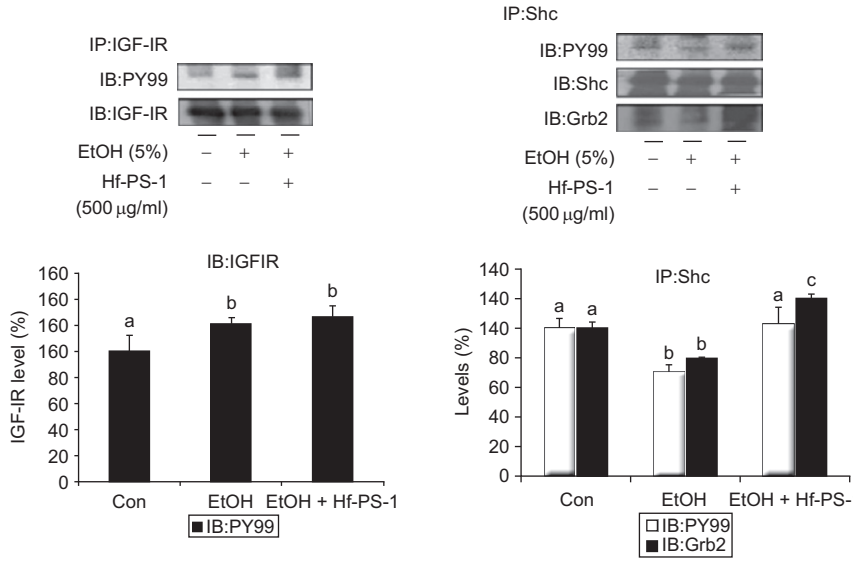

FIGURE 11.11 Effect of ethanol and Hf-PS-1 on IGF-IR and Shc phosphorylation in IEC-6 cells. Cells were pretreated with Hf-PS-1 (500 μg/ml) and ethanol (5%) or with ethanol only (5%) after incubation with SFM for 12 h. Cell lysates were immunoprecipitated with anti-IGF-IR, Shc antibody and the immune complexes were then analyzed by electrophoresis and immunoblotting with anti-phosphotyrosine, or anti-IGF-IR, Shc, Grb2 antibody. This gel photograph is representative of three experiments. Values are the mean ± SD. Different alphabets are significant values among the group by Duncan's multiple range test.

B. Hf-PS-1 inhibits ethanol-induced damage by downregulating JNK

Since binding of insulin-like growth factor-I (IGF-I) to its receptor, IGF-IR, activates multiple signal transduction cascades by activating tyrosine phosphorylation, whether IGF-IR was phosphorylated in response to ethanol and Hf-PS-1 was examined. Oh *et al*. (2008) recently demonstrated that ethanol treatment causes IGF-IR activation by inducing the expression and secretion of IGF-1. When IGF-1 binds to IGF-IR, the latter becomes phosphorylated on tyrosine residues, with effects on Shc and MAPKs. In studying the mechanism of ethanol-induced damage, the MAPK signaling pathway was focused, which has been proposed as an important part of the mechanism (Lee *et al*., 2007; Zhou *et al*., 2005). The oxidative metabolism of ethanol elicits the production of ROS and other oxidative mediators associated with MAPK signaling. For example, ethanol-induced generation of ROS affects MAPKs in hepatocytes (Aroor and Shukla, 2004; Lee and Shukla, 2005; Lee *et al*., 2006; Lieber, 2000;

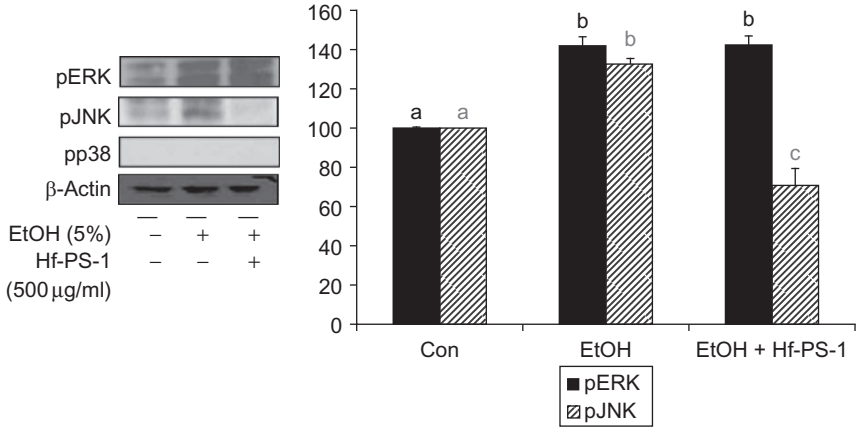

FIGURE 11.12 Effects of Hf-PS-1 on MAPK signaling pathway in IEC-6 cells. Cells were pretreated with Hf-PS-1 (500 μg/ml) and ethanol (5%) or with ethanol only (5%) after incubation with SFM for 12 h. Whole-cell extracts were prepared and analyzed by Western blotting using anti-phospho-ERK1/2, anti-phospho-JNK, anti-phospho p38, and anti-β-actin antibodies. This gel photograph is representative of three experiments. Values are the mean ± SD. Different alphabets are significant values among the group by Duncan's multiple range test.

Venugopal *et al.*, 2007; Wang *et al.*, 2008; Zhang *et al.*, 2007). Although ethanol pretreatment induced IGF-IR activation, Hf-PS-1 pretreatment enhanced this activation. In contrast, Shc phosphorylation and the association between Shc and Grb2 were decreased by ethanol treatment, but increased by Hf-PS-1 pretreatment (Fig. 11.12). Activated Shc binds the adaptor protein Grb2 in an IRS-1-independent manner, leading to the activation of the MAPK pathway. Because several investigators have suggested that ethanol affects the MAPK signaling cascade, the possible activation of ERK1/2, JNK, and p38 were investigated. Ethanol treatment induced JNK and ERK phosphorylation, and Hf-PS-1 pretreatment decreased JNK activation (Fig. 11.13), but p38 was not detected.

Moreover, GSH levels were observed to determine if Hf-PS-1 influenced the effect of ethanol metabolism on cellular redox status. Since a Western blot analysis demonstrated the activation of JNK by ethanol, the relationship between ethanol toxicity and JNK activation using the JNK inhibitor SP600125 was examined and found that ethanol treatment decreased GSH levels to 72.72% (±14.84%) of the control level, but co-treatment with ethanol and either Hf-PS-1 or SP600125 increased GSH levels to 118.18% (±10.49%) or 95.45% (±9.09%), respectively, of the control level (Fig. 11.14). These results suggest that Hf-PS-1 protects against ethanol-induced damage by inhibiting JNK phosphorylation and that JNK plays an important role in ethanol-induced toxicity.

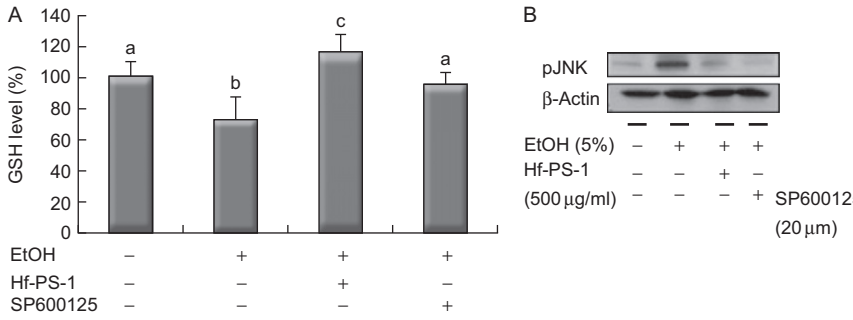

FIGURE 11.13 Effect of Hf-PS-1 on ethanol-induced oxidative stress. (A) GSH levels were measured as described in the materials and methods. Data were represented % of control (100%). Values are the mean ± SD. Different alphabets are significant values among the group by Duncan's multiple range test. (B) JNK phosphorylation by Hf-PS-1 or SP600125 pretreatment. Whole-cell extracts were prepared and analyzed by Western blotting using anti-phospho-JNK and anti-β-actin antibodies. This gel photograph is representative of three experiments.

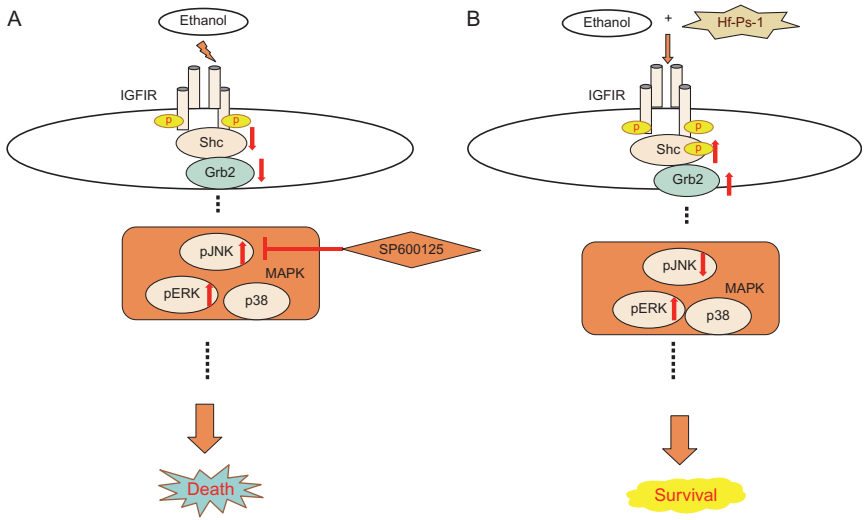

FIGURE 11.14 The proposed model of Hf-PS-1's protective mechanism on ethanol-induced damage in IEC-6 cells.

A scheme summarizing the findings in a study is presented in Fig. 11.14. Ethanol induces the phosphorylation of IGF-IR but decreases the phosphorylation of Shc and the association between Shc and Grb2. Furthermore, although ethanol induces phosphorylation of JNK and ERK, Hf-PS-1 pretreatment decreases the phosphorylation of JNK but not of

ERK. Since the JNK inhibitor SP600125 inhibits ethanol-induced oxidative stress, the JNK activation is a critical factor in ethanol cytotoxicity. Hf-PS-1 pretreatment suppresses ethanol-induced cell death by increasing the phosphorylation of Shc and its association with Grb2 and by decreasing JNK phosphorylation. Therefore, the effect of Hf-PS-1 against ethanol-induced damage is a result of JNK downregulation. Hence, we suggest that Hf-PS-1 might provide a new, natural treatment option for ethanol-induced gastric damage in humans.

IV. CONCLUSION

Misuse of ethanol is associated with detrimental effects on several body organs and is one of several predisposing factors for gastrointestinal disorders. The pathophysiology of the gastric ulcer centers on an imbalance between aggressive and protective factors in the stomach; when aggressive factors (such as secretion of acid, pepsin, and emotional stress) have stronger effects than those of protective factors (such as cellular regeneration, prostaglandins, and EGF), gastric ulcer may occur. Many pharmaceutical products have been developed to treat gastrointestinal diseases such as ulcer hemorrhage and perforation (Higham *et al*., 2002). An increase in these disorders has been attributed to the increased use of NSAIDs, which have significant side effects despite the large amount of money and effort poured into their development by pharmaceutical companies. Hence, there is a need for new therapeutic compounds without side effects.

Polysaccharide extracted from *H. fusiformis* (Hf-PS-1) exhibited protective effects against ethanol-induced peptic injury and related mechanisms in rats. Experimental animals were divided into three groups: control, ethanol-only, and ethanol + Hf-PS-1. The ethanol-only group exhibited decreased levels of total GSH and increased levels of JNK phosphorylation relative to the control group, whereas levels were significantly increased and decreased, respectively, in the ethanol + Hf-PS-1 group. The ethanol-only group also exhibited increased levels of ERK 1/2 phosphorylation relative to the control group; these levels were not significantly different in the ethanol + Hf-PS-1 group. Hf-PS-1 appeared to reduce ethanol-induced gastric injury.

The signaling pathways related to the ethanol-protective effect of Hf-PS-1 in IEC-6 cells. Ethanol induced the death of IEC-6 cells in a dose-dependent manner, and pretreatment with Hf-PS-1 abrogated the ethanol toxicity. When examined whether the effect of Hf-PS-1 on ethanol cytotoxicity was associated with IGF-IR signaling pathways, involving MAPK, it was found that ethanol treatment decreased the phosphorylation of Shc and the binding of Grb2 to Shc, and Hf-PS-1 pretreatment

increased them. Ethanol treatment also induced the phosphorylation of JNK and ERK, whereas Hf-PS-1 pretreatment decreased JNK activation but not ERK activation. Using a JNK inhibitor (SP600125), GSH levels were evaluated to determine whether Hf-PS-1 pretreatment might protect against ethanol-induced gastric intestinal damage by downregulating JNK. Co-treatment with SP600125 and ethanol decreased GSH levels, indicating that JNK phosphorylation is a critical factor during ethanol-induced injury and that the effect of Hf-PS-1 occurs via JNK downregulation. Therefore, Hf-PS-1 may be useful as a biofunctional food source to protect against ethanol-induced gastrointestinal injury.

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