# Effects of *Asparagus officinalis* Extracts on Liver Cell Toxicity and Ethanol Metabolism

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**ABSTRACT:** ***Asparagus officinalis*** **is a vegetable that is widely consumed worldwide and has also long been used as a herbal medicine for the treatment of several diseases. Although *A. officinalis* is generally regarded as a supplement for the alleviation of alcohol hangover, little is known about its effects on cell metabolism. Therefore, this study was conducted to analyze the constituents of the young shoots and the leaves of asparagus and to compare their biochemical properties. The amino acid and inorganic mineral contents were found to be much higher in the leaves than the shoots. In addition, treatment of HepG2 human hepatoma cells with the leaf extract suppressed more than 70% of the intensity of hydrogen peroxide (1 mM)-stimulated DCF fluorescence, a marker of reactive oxygen species (ROS). Cellular toxicities induced by treatment with hydrogen peroxide, ethanol, or tetrachloride carbon ($CCl_4$) were also significantly alleviated in response to treatment with the extracts of *A. officinalis* leaves and shoots. Additionally, the activities of 2 key enzymes that metabolize ethanol, alcohol dehydrogenase and aldehyde dehydrogenase, were upregulated by more than 2-fold in response to treatment with the leaf- and shoot extracts. Taken together, these results provide biochemical evidence of the method by which *A. officinalis* exerts its biological functions, including the alleviation of alcohol hangover and the protection of liver cells against toxic insults. Moreover, the results of this study indicate that portions of asparagus that are typically discarded, such as the leaves, have therapeutic use.**

**Keywords:** ***Asparagus officinalis*****, ethanol metabolism, liver, toxicity**

## Introduction

*Asparagus officinalis* is a vegetable that is widely consumed worldwide and has also long been used as a herbal medicine due to its anticancer effects (Huang and Kong 2006). Several studies have demonstrated that *A. officinalis* possesses pharmacological properties including antifungal (Shimoyamada and others 1990), antimutagenic (Tang and Gao 2001), anti-inflammatory (Jang and others 2004), and diuretic activities (Schilcher and Rau 1988). Its roots and shoots consist of flavonoids, oligosaccharides, amino acid derivatives, and steroidal saponins (Huang and Kong 2006). Although the roots and shoots of *A. officinalis* are consumed as popular vegetables and homeopathic supplements, its other parts, such as its leaves, are generally not used.

Ethanol-induced fatty liver may occur in response to ethanol metabolism (Grunnet and Kondrup 1986). In addition, chronic alcohol use causes oxidative stress (DiLuzio and Hartman 1969) by inducing the production of cytochrome P-450E1 (CYP2E1) (Wu and Cederbaum 1996). Two major ethanol-metabolizing enzymes are alcohol dehydrogenase (ADH) and CYP2E1. Liver ADH is the principal enzyme responsible for ethanol oxidation. ADH metabolizes ethanol to acetaldehyde and then a 2nd enzyme, acetaldehyde dehydrogenase (ALDH), metabolizes the acetaldehyde to acetate. At higher concentrations, acetaldehyde causes toxic effects such as a rapid pulse, sweating, nausea, and vomiting (Eriksson 2001). Thus, the rapid- and efficient removal of excess ethanol and its metabolite, acetaldehyde, is important to prevent such toxicities in liver cells, which are primarily responsible for detoxification (Eriksson 2001).

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Therefore, the present study was conducted to analyze the composition of different parts (young shoot and leaves) of *A. officinalis* and to evaluate the effects of extracts of these plants on physiological functions in HepG2 human hepatoma cells. Additionally, it was determined whether the extracts might affect activities of ADH and ALDH from the biochemical assay using S9 rat liver postmitochondrial homogenate as the enzymatic source because HepG2 cells are deficient of those enzymes.

## Materials and Methods

### Materials

*A. officinalis* was obtained from several different farms in the Jeju region of Korea and the young shoots and leaves were removed and air-dried at room temperature. The dried samples (100 g) were then extracted with 1 L of boiling distilled water for 2 h at 100 °C and then the extracts were passed through a Whatman GF/C filter. The hot water extracts were then freeze-dried and stored at –20 °C until use. Two commercial hangover drinks (HOD1, Condition™, CJ, Korea; HOD2, Dawn808™, Glami, Korea) were also freeze-dried and used as positive controls to compare to the effects of the extracts of *A. officinalis*.

### Composition analysis

The amino acids, inorganic minerals, and nutrients in *A. officinalis* were analyzed using a custom analytical service provided by Scientec Lab Center Co. (Daeduk, Korea).

### Cell culture

HepG2 cells used in this study were obtained from the Korean Cell Line Bank (Seoul, Korea) and grown in DMEM containing 100 units/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum (FBS). The cells were maintained in a humidified incubator under 5% $CO_2$ at 37 °C. For the experiments, the HepG2




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cells were plated in 35-mm tissue culture dishes and incubated under the aforementioned conditions for 48 h and then the cells were serum-starved for 24 h and then treated with different reagents for purposes. The cells were then quickly frozen in liquid nitrogen and stored at 70 °C until further analysis.

### Measurement of reactive oxygen species (ROS)

2′, 7′-dichlorofluorescin diacetate ($H_2$DCFDA), a cell-permeable fluorogenic probe that is useful for the detection of ROS, was used to measure the degree of ROS accumulation within cells. Briefly, the cultured cells were washed once with D-PBS and then they were incubated in D-PBS containing 10 mM $H_2$DCFDA for 10 min at 37 °C. The fluorescence intensity was then measured using a Spectrafluor multiwell fluorescence reader (Tecan, Seestrasse, Mannedorf, Austria) at excitation and emission wavelengths of 485 and 535 nm, respectively, under constant conditions to allow quantitative comparisons of the relative fluorescence intensity from cells of different groups.

### Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) assay

The ADH and ALDH activities were measured using the method described by Blandino and others (1997) and Bostian and Betts (1978), respectively, with minor modifications. Briefly, S9 rat liver postmitochondrial (cytoplasmic) homogenate (Moltox, Boone, N.C., U.S.A.) was used as the sources of ADH and ALDH fraction for enzymatic assay. S9 rat liver homogenate was dissolved in 0.1% bovine serum albumin and aseptically filtered. The reaction mixture used for the ADH assay consisted of 0.25 M Tris-HCl (pH 8.8), 0.2 mM $NAD^+$, 1.7 M ethanol, 5% (v/v) ADH fraction, and test sample solution (3 mg/mL). The mixture was incubated at 30 °C for 5 min and then the absorbance at 340 nm was measured to verify the NADH production rate. The reaction mixture for the ALDH assay consisted of 0.1 M Tris-HCl (pH 8), 0.7 mM $NAD^+$, 0.03 M acetaldehyde, 0.1 M KCl, 0.01 M 2-mercaptoethanol, 3% (v/v) ALDH fraction, and test sample solution (3 mg/mL). After incubation for 5 min at 30 °C, the production rate of NADH from $NAD^+$ was determined at 340 nm.

### Analysis of cytotoxicity

MTT analysis was conducted as previously described (Mossman 1983) using cells that were seeded in 24-well plates. At the end of incubation, the medium was removed from the wells and 200 $\mu$L of MTT reagent (Sigma, St. Louis, Mo., U.S.A.) with a concentration of 1 mg/mL in RPMI-1640 medium without phenol red was added to each well. After incubation with samples for 1 h at 37 °C, the cells were lysed by the addition of 1 volume of 2-propanol and shaken for 20 min. The absorbance of converted dye was then measured at 570 nm. In addition, the cells were stained with a DNA-specific fluorescent dye (H33342) and then observed under a fluorescent microscope equipped with a CoolSNAP-Pro color digital camera (Media Cybernetics, Silver Spring, Md., U.S.A.) to examine the degree of nuclear condensation. The amount of lactate dehydrogenase (LDH) that leaked from the damaged cells into the culture medium was measured using reagents from Takara (Otsu, Shiga, Japan).

### Statistical analysis

All results shown represent the mean ± SE. Differences among groups were determined by the Student's *t*-test. A $P$ value $< 0.05$ was considered as significant.

## Results and Discussion

### Composition analysis

Based on the results of previous studies conducted to analyze the chemical composition of *A. officinalis*, flavonoids (Kartnig and others 1985), oligosaccharides (Fukushi and others 2000), amino acid derivatives (Kasai and Sakamura 1981), and steroidal saponins (Huang and Kong 2006) were isolated from the roots and shoots of *A. officinalis*. Table 1 shows the amino acid compositions of the different parts (young shoots and leaves) of *A. officinalis*. The levels of aspartic acid and asparagine in these parts were remarkably higher than any other amino acid species, whereas proline, tyrosine, methionine, and tryptophan were not detected. Although the young shoots of *A. officinalis* are preferred for dietary purposes, the levels of most amino acids were found to be several folds higher in the leaves than the young shoots. In addition, the contents of several inorganic minerals, protein, and carbohydrates were also higher in the leaves than the young shoots (Table 2). Taken together, these findings stress the importance of the practical use of the leaf as a nutritional source rather than the use of shoots alone for dietary purposes.

### ROS scavenging activity

Hydrogen peroxide ($H_2O_2$) was added to the culture medium and then it passively diffused into HepG2 cells and led to the

**Table 1—Amino acid contents in *A. officinalis*.**

| | Contents (mg/kg) | |
|---|---|---|
| Amino acids | Young shoot | Leaf |
| Aspartic acid | 921.17 | 4369.47 |
| Asparagine | 653.68 | 6967.50 |
| Threonine | 61.21 | 611.20 |
| Serine | 112.67 | 845.45 |
| Glutamic acid | 501.35 | 2933.77 |
| Proline | ND | ND |
| Glycine | 87.41 | 744.80 |
| Alanine | 143.53 | 919.58 |
| Valine | 113.80 | 835.70 |
| Isoleucine | 65.82 | 574.49 |
| Leucine | 106.94 | 995.94 |
| Tyrosine | ND | 416.76 |
| Phenylalanine | 95.54 | 517.61 |
| Histidine | 190.16 | 590.94 |
| Lysine | 40.85 | 1009.01 |
| Arginine | 62.11 | 933.21 |
| Cysteine | 108.19 | 347.14 |
| Methionine | ND | 145.71 |
| Tryptophan | ND | 227.21 |

The amino acid contents of freshly harvested young shoots and leaves of *A. officinalis* were determined by the Scientec Lab Center Co. (Daeduk, Korea).
ND = not detected.

**Table 2—Contents of inorganic minerals and organic nutrients.**

| | Contents (mg/kg) | |
|---|---|---|
| Constituents | Young shoot | Leaf |
| Calcium | 45.2 | 139 |
| Phosphorus | 96.1 | 501.6 |
| Potassium | 251.3 | 2574.8 |
| Iron | 2.7 | 8.5 |
| Sodium | 120.7 | 281.6 |
| Protein | 3000 | 20200 |
| Carbohydrate | 2600 | 31600 |
| Vit.B2 | 0.16 | 0.45 |

Freshly harvested young shoots and leaves of *Asparagus officinalis* were analyzed by the Scientec Lab Center Co. (Daeduk, Korea).

hydrolysis of $H_2$DCFDA (Figure 1). The DCF fluorescence observed after the hydrolysis of $H_2$DCFDA within the cells was used to determine the levels of ROS within the cell.

Pretreatment of the cells with the extracts of the leaves (Asp-L) and young shoots (Asp-S) markedly suppressed the intensity of DCF fluorescence that was induced by the addition of 1 mM $H_2O_2$, showing the maximal suppression (80%) by treatment with Asp-L. ROS scavenging activities were also observed in positive controls (HOD1 and HOD2) in which commercialized hangover drinks were used to evaluate the effects of *A. officinalis* on ethanol metabolism in the next experiments. *A. recemosus*, other members of the genus *Asparagus* increased ROS-scavenging activities (less than 30% increases of activities of liver catalase and superoxide dismutase) (Visavadiya and Narasimhacharya 2007) and *A. acutifolius* also contains plenty of water-soluble antioxidants (Salvatore and others 2005). Although we were unable to compare the antioxidant capacities of the genus *Asparagus* with other species of the genus *Asparagus* on the equal basis, the antioxidant activity of *A. officinalis* observed in this study was found to be potent enough. A recent study showed that asparagus-P, which is a product composed of equal proportions of the roots of *A. officinalis* and parsley leaves, inactivated ROS (Dartsch 2008). However, the antioxidant efficacy observed in this study was primarily attributed to the parsley leaves, while the asparagus roots appeared to only enhance the effects of the parsley leaves. The fruit of *A. officinalis* also contains carotenoid pigments (Deli and others 2000), which possess the antioxidant properties (Szajdek and Borowska 2008). Thus, the antioxidant properties of *A. officinalis* appear to be characterized in some specific parts of the plant. The results of the present study revealed that the extracts of Asp-S and Asp-L exert potent ROS scavenging activities, with those exerted by Asp-L being slightly higher. Although the levels of amino acids, minerals, and other constituents are high in the extracts of different parts of *A. officinalis*, further study is required to determine which constituents are responsible for its potent antioxidant properties.

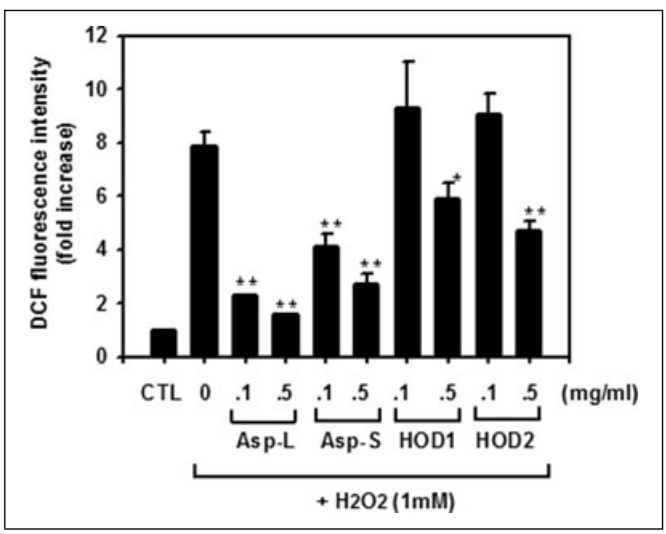


**Figure 1—Effects of the extracts of *A. officinalis* on intracellular ROS contents generated by treatment of HepG2 cells with hydrogen peroxide ($H_2O_2$). HepG2 cells were serum-starved overnight and then pretreated with leaf extract (Asp-L), shoot extract (Asp-S), or hangover drink 1 or 2 (HOD1 or HOD2, positive controls) for 30 min prior to the addition of $H_2O_2$ (1 mM) and $H_2$DCFDA. The DCF fluorescence intensity was measured after 30 min as described in the Materials and Methods section. *$P < 0.05$, **$P < 0.01$ compared to $H_2O_2$-alone.**

## Effects of *A. officinalis* on liver cell toxicity

The HepG2 cell line is a reliable *in vitro* model for the study of hepatotoxicity induced by different environmental, chemical, and pharmacological insults (Neuman and others 1993, 1995). $H_2O_2$ is a cytotoxic mediator in HepG2 cells (Srivastava and Chan 2007). The present study investigated the extracts of Asp-S and Asp-L to determine if they can protect HepG2 cells from $H_2O_2$-induced cytotoxicity. When cells were treated with $H_2O_2$ for 24 h, more than 70% were not viable; however, the presence of Asp-S or Asp-L in the culture medium attenuated the $H_2O_2$-induced cell death, with the maximal protection being observed in response to treatment with Asp-L (0.5 mg/mL) (Figure 2A). A number of apoptotic, condensed nuclei were also observed in cells that were treated with $H_2O_2$ for 24 h. However, treatment with Asp-S and Asp-L attenuated the apoptotic nuclear condensation that was induced by $H_2O_2$ (Figure 2B). These cytotoxic effects occurred in response to oxidative stress that was induced by the presence of excess ROS. Therefore, the protective effects of the extracts of Asp-S and Asp-L against $H_2O_2$-induced cytotoxicity likely occurred via potent ROS-scavenging properties.

Ethanol is metabolized via 3 pathways in the human body that involve the following enzymes: alcohol dehydrogenase (ADH),

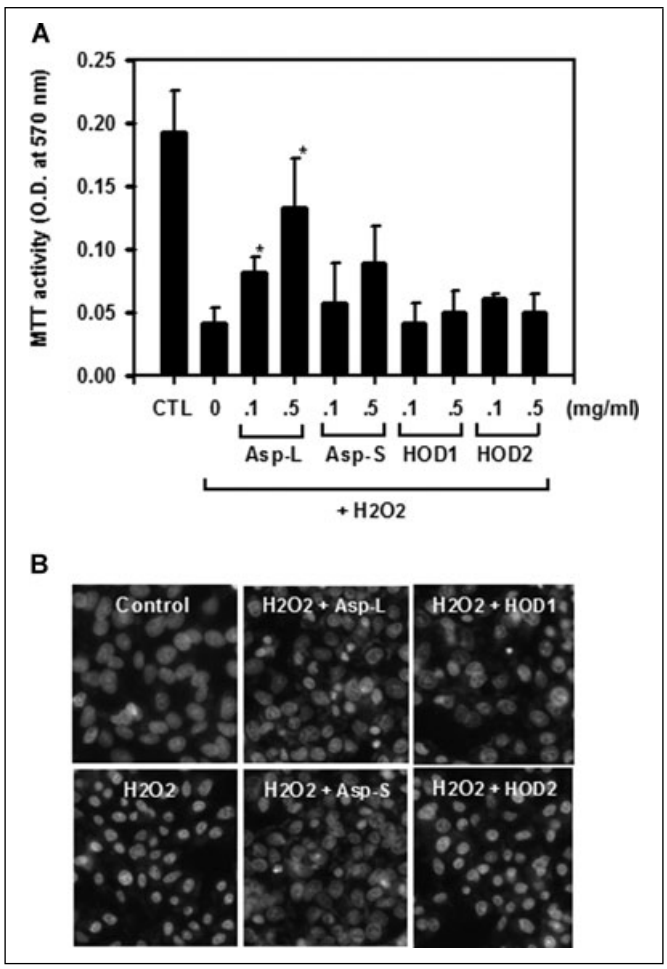


**Figure 2—Effects of the extracts of *A. officinalis* on the cytotoxicity induced by treatment of HepG2 cells with hydrogen peroxide ($H_2O_2$). HepG2 cells were serum-starved overnight and then pretreated with leaf extract (Asp-L), shoot extract (Asp-S), or hangover drink 1 or 2 (HOD1 or HOD2, positive controls) for 30 min prior to the addition of $H_2O_2$ (1 mM). An MTT assay (A) and H33342 staining (B) were then performed after 24 h of $H_2O_2$ treatment. *$P < 0.05$ compared to $H_2O_2$-alone.**

microsomal ethanol oxidation (MEOS), and catalase. During the ethanol metabolism, ROS can be generated. Although moderate amounts of ethanol are metabolized by hepatic ADH, excess ethanol is oxidized by the participation of the microsomal electron transport system via catalysis by cytochrome P4502E1 (CYP2E1), which results in the generation of ROS and can lead to various liver diseases (Subir and Vasudevana 2007). Therefore, the development of beneficial products to facilitate the metabolic hydrolysis of ethanol is crucial to protect the liver from the various harmful insults. Accordingly, we evaluated the extract of Asp-L to determine if it protected HepG2 cells from ethanol-induced cytotoxicity. The results of this study indicated that LDH leakage from cells and MTT activity were induced by treatment with excess (5%, v/v) ethanol, but that these effects were significantly ($P < 0.01$) reversed by pretreatment with 0.5 mg/mL Asp-L for 24 h (Figure 3A). In addition, Asp-L suppressed the degree of nuclear condensation that was induced in HepG2 cells by ethanol (Figure 3B). Cytotoxic responses in LDH leakage and MTT activity induced by tetrachloride carbon ($CCL_4$), a strong oxidative genotoxic reagent (Beddowes and others 2003), were also significantly ($P < 0.01$) suppressed by pretreatment with Asp-L (Figure 3C). However, ADH and CYP2E1 are less expressed in HepG2 cells (Dai and others 1993; Clemens and others 1995). Thus, ethanol-induced cytotoxicity in HepG2 cells might be independent of ADH or CYP2E1. Recent studies suggested that the diminished ADH activity facilitates nonoxidative ethanol metabolism and its products, fatty acid ethyl esters (FAEEs) cause apoptosis in HepG2 cells via intrinsic pathway (Kabakibi and others 1998; Wu and others 2006). Moreover, FAEEs also lead to ethanol-induced pancreatitis (Kaphalia and Ansari 2001). From this notion, it is suggested that the extracts of *A. officinalis* help to protect HepG2 cells against ethanol toxicity via other mechanisms than ADH and CYP2E1. Suppression of the nonoxidative ethanol metabolism and the subsequent synthesis of FAEEs, or the ROS-scavenging activity by extracts of *A. officinalis* per se might be an alternative mechanism.

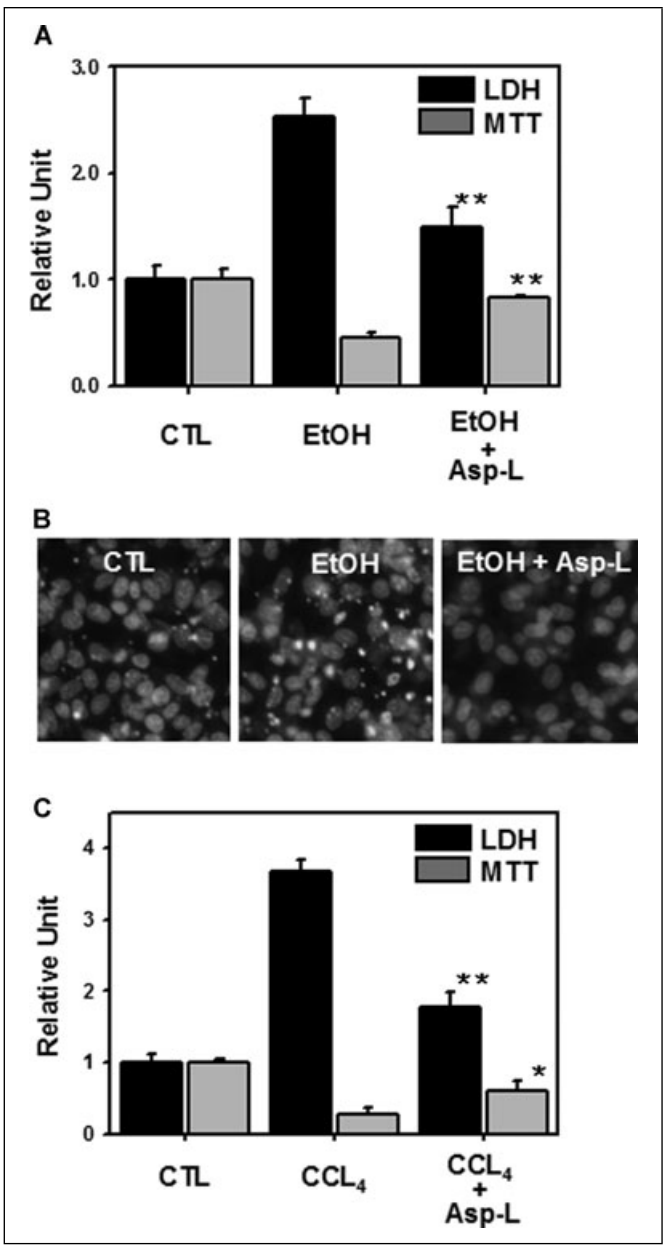


**Figure 3—Effects of extracts of *A. officinalis* on the cytotoxicity induced by the treatment of HepG2 cells with ethanol (EtOH, 5%, v/v) or carbon tetrachloride ($CCL_4$, 10 mM). HepG2 cells were serum-starved overnight and then pretreated with leaf extract (Asp-L, 0.5 mg/mL) prior to the addition of ethanol (EtOH, 5%, v/v) or carbon tetrachloride ($CCL_4$, 10 mM). MTT and LDH assays and H33342 staining were then performed after 24 h. $^*P < 0.05$, $^{**}P < 0.01$ compared to ethanol (A) or $CCL_4$-alone (C).**

Taken together, these results show that the extracts of *A. officinalis* have strong antioxidant- and cytoprotective activities that can overcome cellular toxicities induced by $H_2O_2$ and ethanol in the ADH- or CYP2E1-independent manners, respectively, in HepG2 cells.

## Effects of *A. officinalis* on ADH and ALDH

The extracts of asparagus were evaluated to determine if they could affect ADH and ALDH in liver cells. From the enzymatic assays using S9 rat liver postmitochondrial homogenates as enzymatic sources, ADH and ALDH activities were higher in groups containing Asp-L and Asp-S (more than 2-folds over the control, respectively) than HOD1 and HOD2 on the basis of equal concentrations of the samples (Figure 4).

Because excess ethanol generates ROS in its metabolic pathways, ADH-dependent elimination of ethanol is the principle involved in the protection of cells from oxidative stress. These results suggest that the extracts of *A. officinalis* exert a wide spectrum of activities,

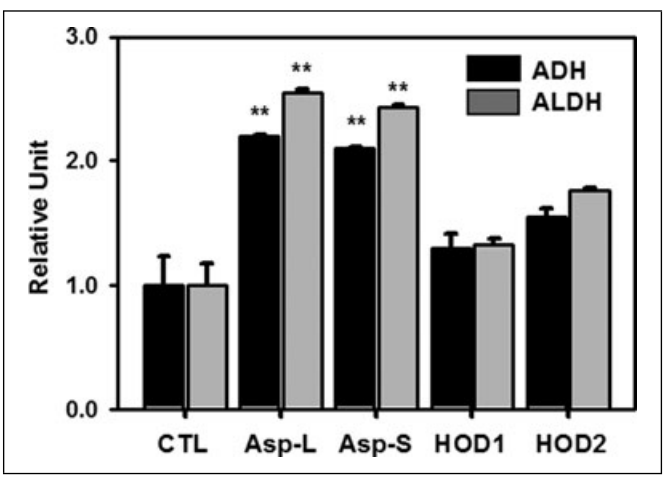


**Figure 4—Activities of ADH and ALDH. Enzyme activities were measured using the methods described in the Materials and Methods section. $^*P < 0.05$ compared to control (CTL).**

including strong antioxidant activity and the ability to act as a potent catalytic factor to stimulate the enzymatic activities required to metabolize ethanol.

## Conclusions

The results of this study demonstrate that the extract of the leaves of *A. officinalis* contained a higher level of amino acids and minerals than those of the young shoots. Furthermore, the extracts of asparagus were found to exert potent cytoprotective properties, including a strong, wide range of antioxidant activities in HepG2 cells. The extracts of asparagus also promoted activities of ADH and ALDH in the enzymatic assay using liver cell homogenate as an enzymatic source. Thus, the leaves of *A. officinalis*, which are normally discarded, have the potential for use in therapy designed to protect the liver from various harmful insults.

## References


Beddowes FJ, Faux SP, Chipman JK. 2003. Chloroform, carbon tetrachloride and glutathione depletion induce secondary genotoxicity in liver cells via oxidative stress. Toxicology 187:101–15.

Blandino A, Caro I, Cantero D. 1997. Comparative study of alcohol dehydrogenase activity in flor yeast extracts. Biotechnol Lett 19:651–4.

Bostian KA, Betts GF. 1978. Kinetics and reaction mechanism of potassium-activated aldehyde dehydrogenase from *Saccharomyces cerevisiae*. Biochem J 173:787–98.

Clemens DL, Halgard CM, Miles RR, Sorrell MF, Tuma DJ. 1995. Establishment of recombinant hepatic cell line stably expressing alcohol dehydrogense. Arch Biochem Biophys 321:311–8.

Dai Y, Rashba-Step J, Cedabaum AI. 1993. Stable expression of human cytochrome P4502E1 in HepG2 cells: characterization of catalytic activities and production of reactive oxygen intermediates. Biochemistry 32:6928–37.

Dartsch PC. 2008. The potential of Asparagus-P to inactivate reactive oxygen radicals. Phytother Res 22:217–22.

Deli J, Matus Z, Toth G. 2000. Carotenoid composition in the fruits of *Asparagus officinalis*. A Agric Food Chem 48:2793–6.

DiLuzio N, Hartman A. 1969. Role of lipid peroxidation in the pathogenesis of the ethanol-induced fatty liver. Fed Proc 26:1436–42.

Eriksson CJ. 2001. The role of acetaldehyde in the actions of alcohol. Alcohol Clin Exp Res 25:15S–32S.

Fukushi E, Onodera S, Yamamori A, Shiomi N, Kawabata J. 2000. NMR analysis of tri- and tetrasaccharides from asparagus. Magn Reson Chem 38:1005–11.

Huang X, Kong L. 2006. Steroidal saponins from roots of *Asparagus officinalis*. Steroids 71:171–6.

Grunnet N, Kondrup J. 1986. The effect of ethanol on the beta-oxidation of fatty acids. Alcohol Clin Exp Res 10:64S–68S.

Jang DS, Cuendet M, Fong HHS, Pezzuto JM, Kinghorn AD. 2004. Constituents of *Asparagus officinalis* evaluated for inhibitory activity against cyclooxygenase-2. J Agric Food Chem 52:2218–22.

Kabakibi A, Christopher R, Morse R, Laposata M. 1998. Fatty acid ethyl esters and HepG2 cells: intracellular synthesis and release from the cells. J Lipid Res 39:1568–82.

Kaphalia BS, Ansari GA. 2001. Fatty acid ethyl esters and ethanol-induced pancreatitis. Cell Mol Biol 47:OL173–9.

Kartnig T, Gruber A, Stachel J. 1985. Flavonoid pattern from *Asparagus officinalis*. Planta Med 51:288.

Kasai T, Sakamura S. 1981. N-Carboxymethyl-l-serine a new acidic amino acid from Asparagus (*Asparagus officinalis*) shoots. Agric Biol Chem 45:1483–5.

Mossman T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63.

Neuman MG, Koren G, Tiribelli C. 1993. *In vitro* assessment of the ethanol-induced hepatotoxicity on HepG2 cell line. Biochem Biophys Res Commun 197:932–41.

Neuman MG, Cameron RG, Shear NH, Bellentani S, Tiribelli C. 1995. Effect of tauroursodeoxycholic and ursodeoxycholic acid on ethanol-induced cell injuries in the human Hep G2 cell line. Gastroenterology 109:555–63.

Salvatore S, Pellegrini N, Brenna OV, Del Rio D, Frasca G, Brighenti F, Tumino R. 2005. Antioxidant characterization of some Sicilian edible wild greens. Agric Food Chem 53:9465–71.

Schilcher H, Rau H. 1988. Nachweis der aquaretischen Wirkung von Birkenblatter- und Goldrutenauszugen im Tierversuch. Urologe [B] 28:274–80.

Shimoyamada M, Suzuki M, Sonta H, Maruyama M, Okubo K. 1990. Antifungal activity of the saponin fraction obtained from *Asparagus officinalis L.* and its active principle. Agric Biol Chem 54:2553–7.

Srivastava S, Chan C. 2007. Hydrogen peroxide and hydroxy radicals mediate palmitate-induced cytotoxicity to hepatoma cells: relation to mitochondrial permeability transition. Free Radic Res 41:38–49.

Subir KD, Vasudevana DM. 2007. Alcohol-induced oxidative stress. Life Sci 81:177–87.

Szajdek A, Borowska EJ. 2008. Bioactive compounds and health-promoting properties of berry fruits: a review. Plant Foods Hum Nute 63:147–56.

Tang XH, Gao J. 2001. Inhibitory effects of juice from *Asparagus officinalis L.* on cyclophosphamide (CTX)-induced mutagenic activities in mice. J Nanjing Univ (Nat Sci) 37:569–73.

Visavadiya NP, Narasimhacharya AV. 2009. Asparagus root regulates cholesterol metabolism and improves antioxidant status in hypercholesteremic rats. Evid Based Complement Alternat Med 248:59–65.

Wu D, Cederbaum AI. 1996. Ethanol cytotoxicity to a transfected HepG2 cell line expressing human cytochrome P450E1. Alcohol Clin Exp Res 23:67–76.

Wu H, Cai P, Clemense DL, Jerrells TR, Shakeel GA, Kaphalia BS. 2006. Metabolic basis of ethanol-induced cytotoxicity in recombinant HepG2 cells: role of nonoxidative metabolism. Toxicol Appl Pharmacol 216:238–47.