ORIGINAL ARTICLE

Examining the Effect of Aqueous Extract of Iranian Edible Asparagus in Prevention of Alcoholic Liver Disease in Adult Male Rats

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Abstract

Introduction: Alcoholic liver disease (ALD) is an important cause of morbidity and mortality worldwide. An advanced liver disease that causes severe liver failure. Edible asparagus has antioxidant properties and liver protective effects. Given the increasing prevalence of alcoholic fatty liver (AFL) disease and the positive effect of several herbal medicines on this disease, the present study aimed to evaluate the effect of aqueous extract of edible asparagus in the prevention of AFL disease. **Methods:** In this experimental study, 40 adult male Wistar rats were studied. The five groups consisted of one control group, one sham, and three experimental groups. The first experimental group received 25% ethanol, the second experimental group received 500 mg/kg asparagus extract, and the third experimental group received 25% ethanol and 500 mg/kg asparagus extract (for 70 consecutive days). Serum concentrations of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), tumor necrosis factor-alpha (TNF-α), and glutathione peroxidase (GPX) were measured. The collected data were analyzed using ANOVA and Duncan's test at $P \leq 0.05$ significance level. **Findings:** No significant difference was found between control and sham groups in terms of all measured parameters. Mean serum concentrations of ALT, AST, ALP, and TNF-α were significantly higher in the first treatment group compared to control and sham group. However, mean serum concentration of GPX was significantly lower in the first treatment group compared to control and sham group. Mean serum concentrations of ALT, AST, ALP, and TNF-α were significantly lower in the second treatment group compared to control and sham group. However, mean serum concentration of GPX was significantly higher in the second treatment group compared to control and sham group. Mean serum concentrations of ALT, AST, ALP, and TNF-α were significantly higher in the third treatment group compared to control and sham group. However, mean serum concentration of GPX was significantly lower in the third treatment group compared to control and sham group. Mean serum concentrations of ALT, AST, ALP, and TNF-α were significantly lower in the third treatment group compared to the first experimental group. However, mean serum concentration of GPX was significantly higher in the third treatment group compared to the first experimental group. **Conclusion:** The results showed that aqueous extract of edible asparagus with antioxidant properties has liver protective effect and prevents AFL disease.

Key words: Alcoholic liver disease, edible asparagus, rat

INTRODUCTION

Alcoholic liver disease (ALD) is an important cause of morbidity and mortality worldwide. ALD has different clinical and laboratory symptoms. These symptoms include asymptomatic fatty liver, alcoholic hepatitis, and complete liver failure. Unfortunately, many patients are initially asymptomatic and can only be diagnosed in case of advanced disease that has progressed to complete liver failure.[1]

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Although pathogenesis of ALD is not clearly defined yet, pre-disposing factors include metabolism of alcohol to toxic substances in the body, which causes oxidative stress. Another predisposing factors are acetaldehyde production, abnormal methionine metabolism, malnutrition, endotoxin activation, and impaired regeneration of liver cells.[2]

Kupffer cells produce various cytokines in the liver as exposed to alcohol. This factor is involved in pathogenesis of ALD. Tumor necrosis factor-alpha (TNF-α) is the most important inflammatory cytokine produced in acute alcoholic liver injury (disease). Chronic alcohol consumption causes hepatocyte damage due to TNF-α production, which ultimately leads to apoptosis and phagocytosis of liver cells.[3]

Laboratory symptom of alcoholic liver is two-fold increase in aspartate transaminase (AST) levels specifically. Although alanine transaminase (ALT) level increases in ALD, ALT serum levels are usually lower than AST levels.[4]

Treatments for ALD depend on stage of the disease. Only supportive treatments are used to reduce complications at the stage of liver cirrhosis, which are usually associated with irreversible complications. The best treatment for ALD is stop drinking alcohol to prevent development of alcoholic hepatitis. Treatment with corticosteroids can reduce the inflammatory process by reducing the production of inflammatory cytokines. Other medications are currently used to treat ALD including pentoxifylline-infliximab-etanercept Vitamin E.[5]

Herbal medicines are prescribed by traditional medicine practitioners in Iran and other countries for the treatment of ALD. Edible asparagus (*Asparagus officinalis* L.) is an important garden vegetable planted in areas with temperate and subtropical climates. Asparagus is an herbaceous perennial dioecious species belonging to the order Asparagales, the family Asparagaceae, and the genus Asparagus. Asparagus species are grown around the world from Eastern Mediterranean to the Caucasus Mountains as the origin of this species.[6]

Asparagus contains asparagine, coniferin, inositol, tannin, gallic acid, asparagose, and succinic acid.[7] Other studies have shown that extract of this herb contains amino acids and minerals, which prevent drowsiness and malaise and protect liver cells against toxins. Another study showed that asparagus contains large amounts of Vitamin K and folate (Vitamin B9).[8]

Hewawasam *et al.* evaluated liver protective effect of asparagus in the rats treated with acetaminophen. Asparagus reduces acetaminophen-induced liver damage, which is attributed to antioxidant properties of this herb.[9]

Flavonoid accounts for 60–80% of total phenolic content of green or purple asparagus. This element is the main antioxidant of this herb. Larger amounts of amino acids and inorganic minerals are found in the leaves than buds.[10]

Hep G2 cells were cultivated in different culture media containing ethanol, hydrogen peroxide, and carbon tetrachloride in a study conducted in 2009. The results of the former showed that cellular damage is significantly reduced in case of treatment with asparagus extract in the medium.[11] In addition, two key enzymes are actively involved in metabolism of alcohol in the liver, namely, alcohol dehydrogenase and aldehyde dehydrogenase. Enzymatic activity increased by two-folds in treatment with asparagus extract.[11-13] The present study aimed to evaluate protective effect of aqueous extracts of asparagus on liver pathology under the influence of chronic alcohol consumption. Since asparagus has high antioxidant properties, this herb can be an effective alternative to chemical medications.

METHODS

Sample collection and extraction method

Roots of asparagus were washed to remove soil remains. Cleaned roots were dried in darkness in the laboratory environment. The samples were completely dried and pulverized using an electric mill. The resulting powder was mixed 5 times the size of the plant and thoroughly stirred using a rotodoxy device for 24 h at room temperature to obtain a uniform solution. Then, the solution was passed through a filter and dried for 48 h at room temperature to obtain a solid extract. Solid extract was refrigerated until use.[14]

Rat classification

The project was registered in Ethics Committee of Jahrom University of Medical University under 2991/c/d dated 03/04/2014. All ethical issues were observed in treating laboratory animals. Male Wistar rats with an average weight of 180–200 g were used in this study. The rats were kept at animal breeding room in Jahrom University of Medical Sciences for a week to adapt to the environment. Light-dark cycle consisted of 12 h of light and 12 h of darkness. Humidity varied from 50% to 55% in the room.

The rats were weighed and kept in special cages (4 rats per cage). They were given water bottles. The cages were cleaned and disinfected 3 times a week cages. Asparagus extract was fed to the rats at 10 am every day.

Studied groups

Control

The group received no treatment during the experiment (70 days). Water and food were available to them ($n = 8$).

Sham

This group received 1 cc/kg normal saline by gavage with respect to body weight for 70 days during the experiment. Their diet was based on Lieber-DeCarli formula ($n = 8$).

The first experimental group (AFL)

This group was given 25% ethanol (5 ml/kg body weight) by gavage for 70 days with respect to body weight. Their diet was based on Lieber-DeCarli formula ($n = 8$).

The second experimental group (Asparagus 500 mg/kg)

This group received asparagus extract by gavage for 70 days with respect to body weight (500 mg/kg). Their diet was based on Lieber-DeCarli formula ($n = 8$).

The third experimental group (AFL + Asparagus 500 mg/kg)

This group received asparagus extract (500 mg/kg) and 25% ethanol (5 ml/kg body weight) by gavage for 70 days with respect to body weight. Their diet was based on Lieber-DeCarli formula ($n = 8$).

At the end of the project (the 71st day), blood samples were directly taken from heart of the rats. Serum samples were collected by centrifugation (3000 rpm for 15 min) and kept at −20ºC to measure AST, ALT, ALP, TNF-α, and GPX levels using Pars lab kit (made by Iran) and Diametra lab kit (made by Italy).

Liver tissues of the rats were isolated and fixated in 10% formalin solution. Tissue processing procedures were performed using an automatic tissue processor. Tissue dehydration, impregnation, replacement, and embedding were done. A rotary microtome was used to prepare 5 µ sections. Prepared sections were stained with hematoxylin and eosin and examined by light microscopy.

Induction of alcoholic fatty liver (AFL)

The group receiving ethanol was given 25% ethanol for 70 days (made by Merck Co. in Germany). The rats received ethanol at a rate of 5 ml/kg of body weight by gavage in a daily manner (intragastric intubation).[15]

Statistical analysis

ANOVA was used for data analysis. Data distribution was normal based on Kolmogorov–Smirnov test. Therefore, parametric tests were used. Parametric tests were used by Duncan post hoc test. SPSS 18 was used for statistical analysis. Significance level was considered as $P < 0.05$. The collected data were shown as mean ± standard error of mean. Excel was used for plotting graphs.

FINDINGS

Results of changes in ALP, AST, ALT, and TNF-α serum levels in various groups

No significant difference was found between sham group and control group.

A significant increase was observed in measured parameters in the first experimental group (AFL) compared to control and sham group.

A significant decrease was observed in measured parameters in the second experimental group (500 mg/kg asparagus) compared to control, sham group, and the first experimental group.

A significant increase was observed in measured parameters in the third experimental group (AFL + Asparagus 500 mg/kg) compared to control, sham group, and the second experimental group. However, a significant decrease was observed in the third experimental group compared to the first experimental group [Tables 1, Charts 1-5 and Figures 1-3].

The results of changes in GPX serum concentration in different groups

No significant difference was observed in measured parameters between control and sham groups.

Table 1: Comparison of studied groups in terms of all parameters

Groups/-Parameters	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TNF-α (U/ml)	GPX (U/ml)
Control	139.12±5.94^{b}	63.62±3.70^{b}	372.87±4.65^{b}	238.50±4.18^{b}	132.29±125.89^{c}
Sham	138.62±5.73^{b}	67.01±2.76^{b}	375.87±5.45^{b}	239.77±4.59^{b}	134.25±129.54^{c}
AFL	363.01±24.20^{d}	113.50±1.99^{d}	614.87±7.18^{d}	345.56±8.06^{d}	96.37±89.02^{a}
Asparagus 500 mg/kg	114.88±5.03^{a}	49.02±1.93^{a}	304.25±6.97^{a}	176.82±8.47^{a}	188.21±172.71^{d}
AFL+Asparagus 500 mg/kg	202.01±19.37^{c}	78.01±1.53^{c}	413.62±7.91^{c}	260.17±2.63^{c}	109.16±98.94^{b}

The means are presented in the form of means±SEM. $P<0.05$ is considered statistically significant. The means in each column with at least one letter in common are not significantly different based on Duncan's test. AFL: Alcoholic fatty liver, SEM: Standard error of the mean, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, TNF-α: Tumor necrosis factor-alpha, GPX: Glutathione peroxidase

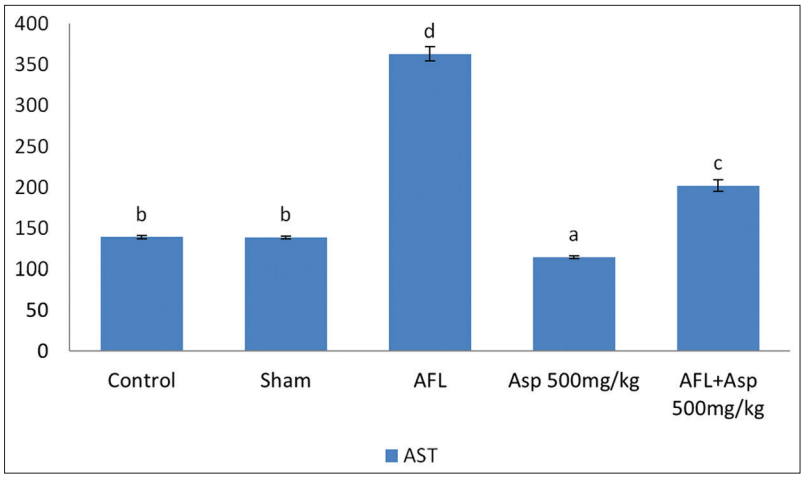

Chart 1: Aspartate transaminase mean serum concentration in the studied groups. The means in each column with at least one letter in common are not significantly different based on Duncan's test. AFL: Alcoholic fatty liver

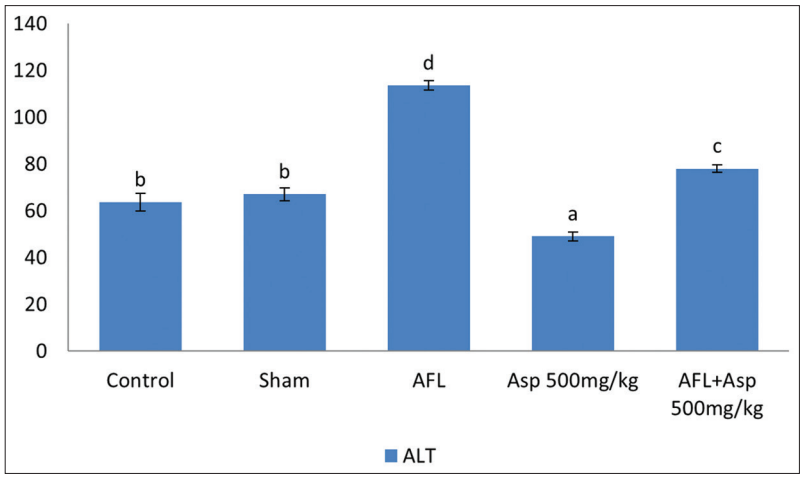

Chart 2: Alanine transaminase mean serum concentration in the studied groups. The means in each column with at least one letter in common are not significantly different based on Duncan's test. AFL: Alcoholic fatty liver

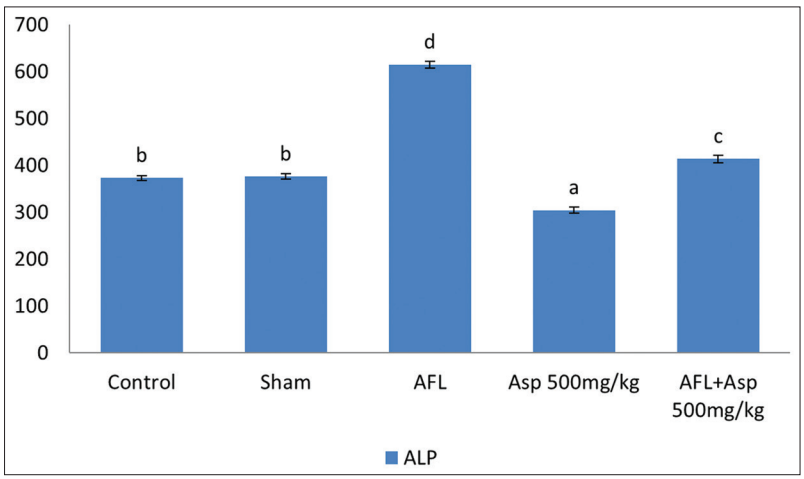

Chart 3: Alkaline phosphatase mean serum concentration in the studied groups. The means in each column with at least one letter in common are not significantly different based on Duncan's test. AFL: Alcoholic fatty liver

A significant decrease was observed in measured parameters in the first experimental group (AFL) compared to control and sham group.

A significant increase was observed in measured parameters in the second experimental group (Asparagus 500 mg/kg) compared to control and sham group.

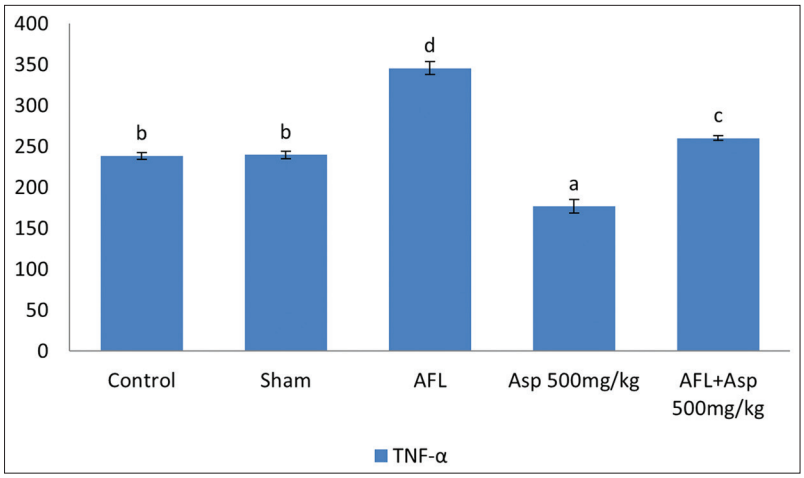

Chart 4: Tumor necrosis factor-alpha mean serum concentration in the studied groups. The means in each column with at least one letter in common are not significantly different based on Duncan's test. AFL: Alcoholic fatty liver

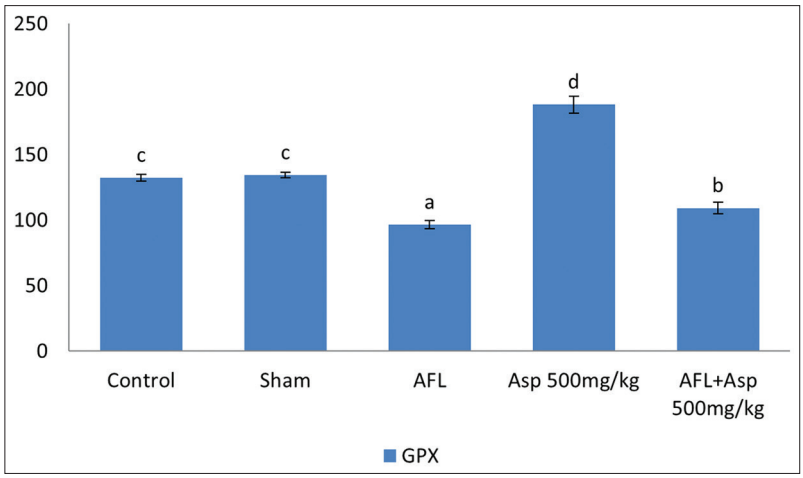

Chart 5: Glutathione peroxidase mean serum concentration in the studied groups. The means in each column with at least one letter in common are not significantly different based on Duncan's test. AFL: Alcoholic fatty liver

A significant decrease was observed in the third experimental group (AFL + Asparagus 500 mg/kg) compared to the second experimental group, control, and sham group. However, a significant increase was observed in the third experimental group compared to the first experimental group [Table 1 and Figures 1-4].

Pathological results

Liver tissues were normal and healthy in microscopic observations of control and sham group (normal structure of venule, central venous, sinusoid, and Kupffer cells; normal distribution of glycogen; absence of lymphocytic infiltration; and absence of congestion in vessels) [Figures 1-3,5].

Hepatocyte necrosis, infiltration of inflammatory cells around the portal space, central vein of the lobule and sinusoid spaces, cellular vacuolization, and transparency of cellular cytoplasm were detected in AFL disease group [Figures 4 and 6].

Liver cells were healthy and normal in the group receiving 500 mg of aqueous extract of edible asparagus [Figures 7 and 8].

Detrimental effects of non-AFL largely decreased in the group of AFL treated with 500 mg of aqueous extract of edible asparagus [Figures 9 and 10].

DISCUSSION

The results showed that TNF-α, AST, ALT, and ALP levels significantly decreased and GPX level significantly increased in asparagus group compared to control and sham group. These results indicate the effects of asparagus root extract on levels of these enzymes.

The liver is the main organ at the risk of damage due to alcohol consumption because portal blood directly flows into

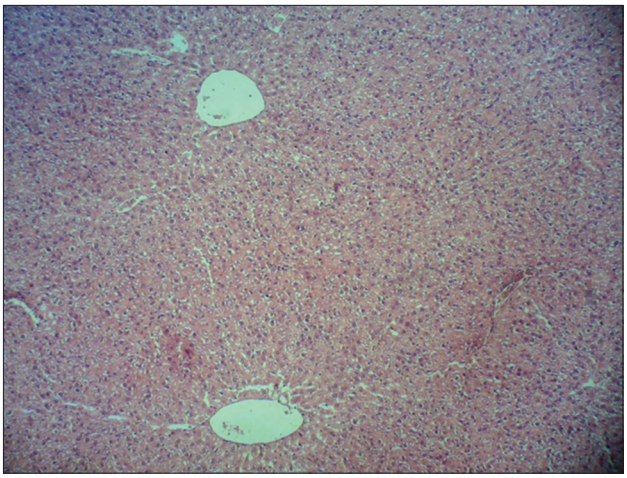

Figure 1: Control. With a natural structure and central venous congestion cannot be seen (hematoxylin and eosin, 100×)

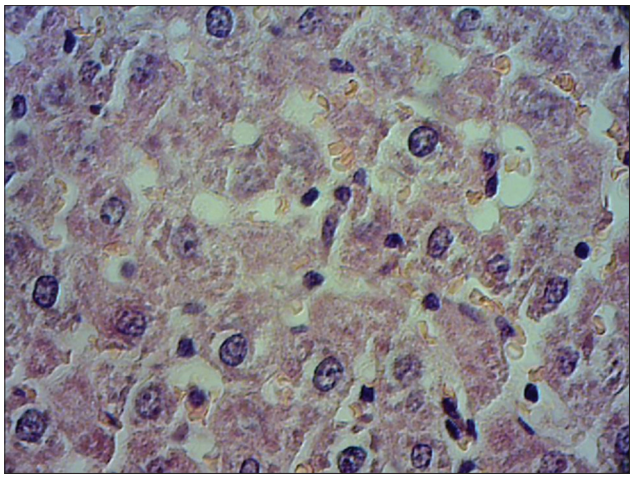

Figure 2: Control. With a natural structure and sinusoid infiltration and congestion cannot be seen (hematoxylin and eosin, 400×)

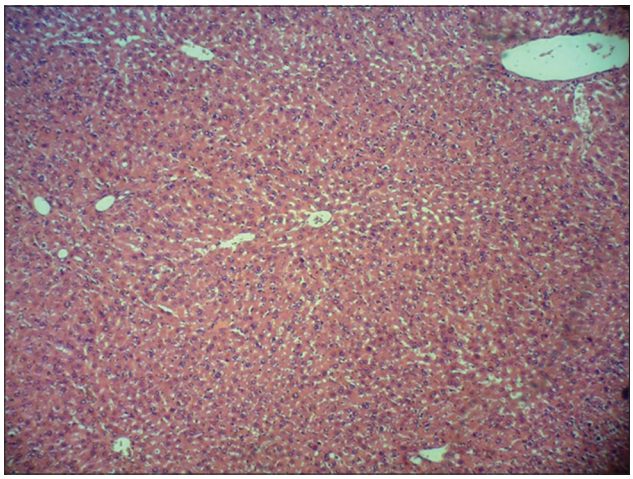

Figure 3: Sham. With a natural structure (hematoxylin and eosin, 100×)

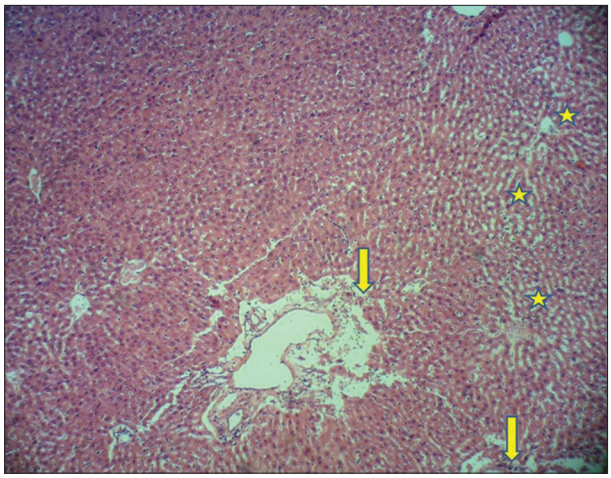

Figure 4: AFL. Infiltration of inflammatory cell around the portal apace (wide arrow), Infiltration of inflammatory cell in sinusoid spaces (Asterisk) (hematoxylin and eosin, 100×)

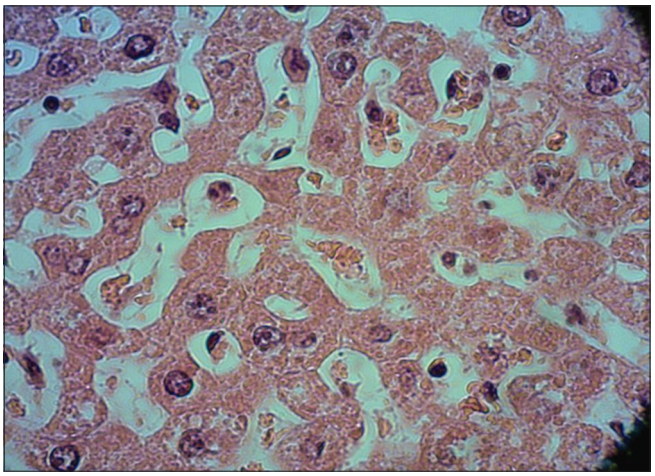

Figure 5: Sham. With a natural structure and leukocyte infiltration and congestion cannot be seen (hematoxylin and eosin, 400× magnification)

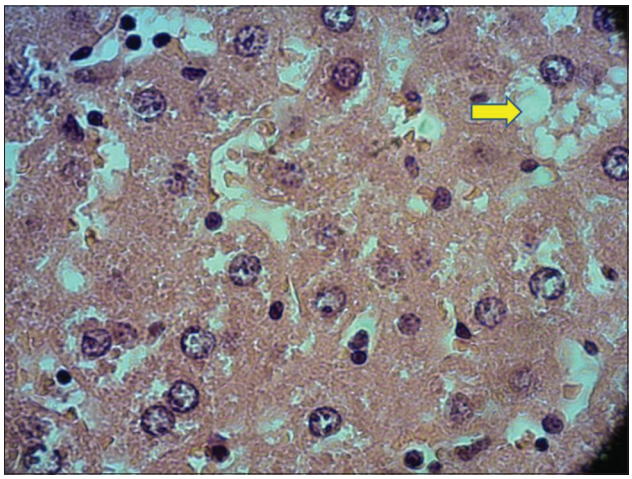

Figure 6: AFL. Cellular vacuolization (hematoxylin and eosin, 400×)

the liver from the gastrointestinal tract. Oral intake of ethanol can cause liver damage through different mechanisms such as microsomal damage[15] and release of such metabolites as malondialdehyde and acetaldehyde that synergistically bind

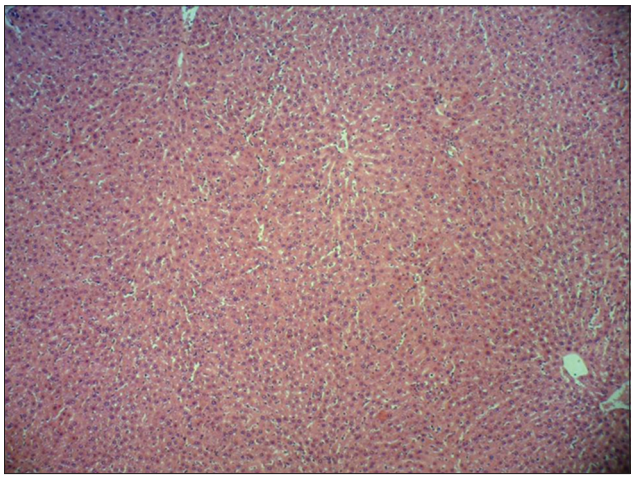

Figure 7: Asparagus 500 mg/kg. With a natural structure (hematoxylin and eosin, 100×)

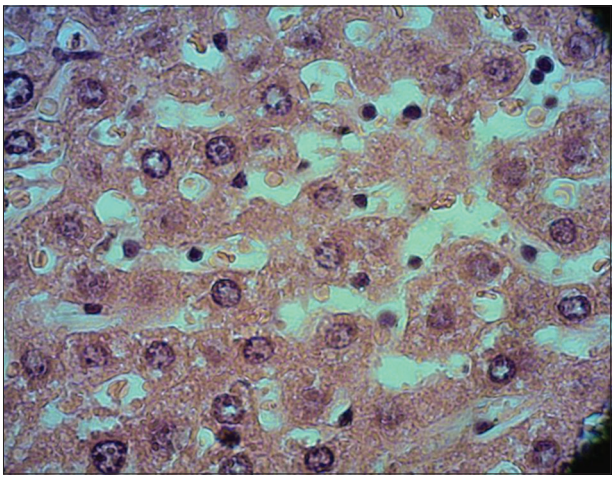

Figure 8: Asparagus 500 mg/kg. With a natural structure (hematoxylin and eosin, 400×)

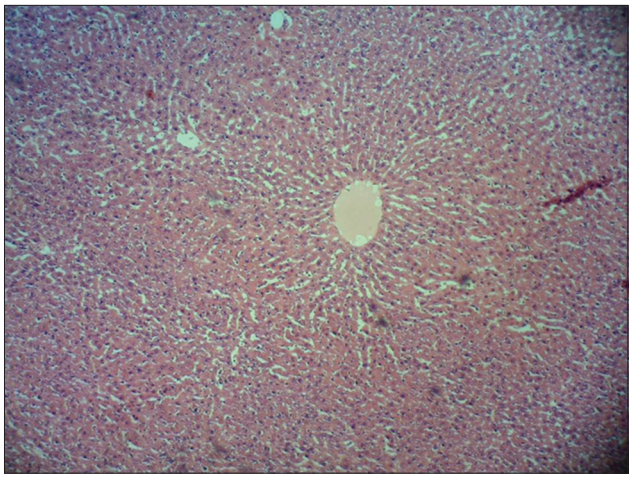

Figure 9: AFL + Asparagus 500 mg/kg. With a natural structure (hematoxylin and eosin, 100×)

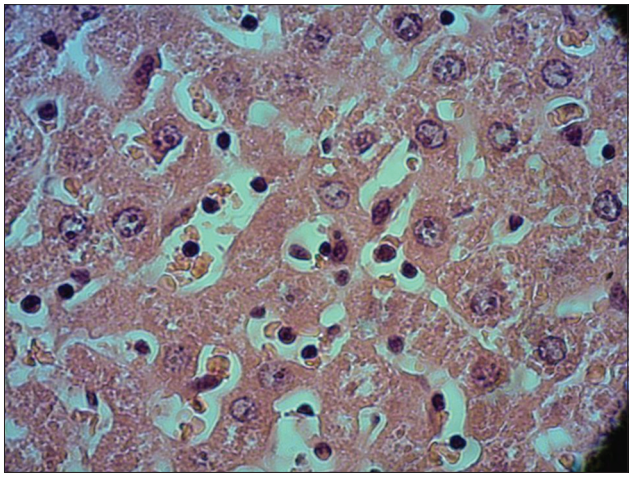

Figure 10: AFL + Asparagus 500 mg/kg. Decreased cellular vacuolization (hematoxylin and eosin, 400×)

to protein and form malondialdehyde-acetaldehyde hybrid complex.[14]

These proteins induce an inflammatory response in liver endothelial cells.[16] Release of free radicals from ethanol (1-hydroxyethyl) causes liver damage[17] and induces p450E1cytochrome.[18] As a result, Kupffer cells become sensitive to endotoxin (lipopolysaccharide). Consequently, these cells produce TNF-α, which increases serum levels of AST and ALT. This ultimately leads to progression of liver damage.[19]

Increased activity of liver enzymes (e.g., AST, ALP, and ALT) and TNF-α inflammatory factor and decreased serum levels of GPX indicate liver damage.[20] Since the above changes were previously reported in liver damage,[21] the present study aimed to examine serum levels of these enzymes. Changes in liver enzyme levels were detected in serum of rats with fatty liver disease, which indicate damage to liver Kupffer cells. These findings are consistent with the results of the study conducted by Chidambarama *et al*., in 2010.[20] Asparagus extract does not allow changes in serum levels of the above-mentioned enzymes as exposed to alcohol-containing diet. In other words, asparagus extract contains various antioxidants that do not allow changes in these enzymes. In this study, biochemical results were confirmed by histopathologic findings. Accordingly, severe hepatic steatosis was detected in the rats with alcohol-containing diet during 70 days. On the other hand, histopathologic assessments showed anti-hepatosteatosis effects of asparagus extract in alcohol-rich diet. Accordingly, asparagus extract significantly inhibits hepatocyte necrosis, infiltration of inflammatory cells around the portal space, central vein of the lobule and sinusoid space, cellular vacuolization, and transparency of cellular cytoplasm. Histopathological observations were consistent with biochemical findings. These findings were also consistent with the results of the study conducted by Wang *et al*., in 2009.[21] Evidence shows that pathological changes in liver tissue increase sensitivity of this tissue to other damaging factors such as oxidative stress, which leads to progression of steatosis to cirrhosis.[22] Given the relationship between alcohol-induced oxidative stress and liver tissue damage,[20]

the present study emphasized alcohol-containing diet causing liver damage. These results are consistent with the findings of the study conducted by Zou *et al*. in 2006.[23] The rats with alcohol-containing diet were treated with asparagus extract, which reduced the incidence of pathological changes. This intervention significantly decreased abnormal increase in serum levels of AST, ALT, ALP, and TNF-α and increased serum levels of GPX antioxidants. Histological changes in the liver of the rats with alcohol-containing diet were hepatocyte necrosis, infiltration of inflammatory cells around the portal space, central vein of the lobule and sinusoid space, cellular vacuolization, and transparency of cellular cytoplasm. Histological changes were consistent with the results of biochemical analyses of serum liver enzymes. These results showed that asparagus extract may prevent hepatosteatosis by reducing liver enzymes and inflammatory factors and increasing effective antioxidants in liver health. The liver is directly involved in the metabolism of alcohol. Hepatic steatosis indicates that liver fails to break down large amounts of alcohol in the diet.[24,25]

Asparagus roots are rich in such antioxidant as alcohol dehydrogenase and aldehyde dehydrogenase.[16] Various studies on alcohol dehydrogenase-containing and aldehyde dehydrogenase-containing media have shown that cellular damage has significantly reduced in treatment with asparagus extract. In addition, the activity of these two key enzymes (alcohol dehydrogenase and aldehyde dehydrogenase) has increased by two-folds in response to treatment with asparagus extract. These enzymes are involved in metabolism of alcohol in the liver.[11]

Rukkumani[25] studied the role of antioxidants in rat liver cells. The results of the former study showed that antioxidants are effective in reducing the release of free radicals from alcohol metabolites, reducing accumulation of glutathione, increasing glutathione peroxidase activity, increasing superoxide dismutase activity, and increasing catalase activity in liver Kupffer cells. These factors reduce damaging effects of alcohol on the liver. These results are consistent with the results of the present study.

Hussein[13] obtained similar results in another study. He showed that decreased levels of glutathione and superoxide dismutase reduce the effects of alcohol-containing diet in the liver of rats. These results are consistent with the results of the present study. Asparagus root contains amino acid compounds and its derivatives (e.g., L-carnitine).[9] This amino acid has anti-inflammatory effect on alcohol-induced fatty liver as manifested in decreasing fat oxidation, plasma levels of AST, ALT, and TNF-α, and production of TNF-α by liver Kupffer cells.[26,27] Hewawasam *et al*. (2008) evaluated protective effect of asparagus on the liver of the rats treated with acetaminophen. They showed that asparagus reduces damaging effect of acetaminophen on the liver. This was attributed to antioxidant properties of this plant. These results are consistent with the results of the present study.[9]

A similar study was conducted on the effect of asparagus extract on alcohol-induced fatty liver, which suggested a protective effect of asparagus on liver Kupffer cells. This was attributed to antioxidant properties of asparagus extract.[28]

The results of another study also indicated that asparagus extract may decrease the incidence of non-AFL by increasing serum lipid-derived antioxidants, which reduce inflammatory responses of liver Kupffer cells to fatty liver induction factors.[29]

In total, the results of this study showed that asparagus extract has protective effects on AFL disease, improves liver histological structure, reduces serum biomarkers of liver damage (liver disease), and increases effective antioxidants in liver damage caused by alcohol-containing diet. These effects were attributed to antioxidant activity of asparagus extract. It should be noted that this study was conducted on rats. Further studies should be performed to generalize these results to humans. The effect of various concentrations of asparagus extract on alcoholic liver fatty disease was not addressed in this study and should be investigated in future studies.

CONCLUSION

Given the increase in serum concentrations of liver enzymes caused by intake of asparagus extract in this study, asparagus may prevent alcohol-induced fatty liver disease.

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REFERENCES

1. Paula H, Asrani SK, Boetticher NC, Pedersen R, Shah VH, Kim WR, *et al.* Alcoholic liver disease-related mortality in the united states: 1980-2003. Am J Gastroenterol 2010;105:1782-7.
2. Haber PS, Warner R, Seth D, Gorrell MD, McCaughan GW. Pathogenesis and management of alcoholic hepatitis. J Gastroenterol Hepatol 2003;18:1332-44.
3. Kawaratani H, Tsujimoto T, Douhara A, Takaya H, Moriya K, Namisaki T, *et al.* The effect of inflammatory cytokines in alcoholic liver disease. Mediators Inflamm 2013;2013:495156.
4. Cohen JA, Kaplan MM. The SGOT/SGPT ratio–an indicator of alcoholic liver disease. Dig Dis Sci 1979;24:835-8.
5. Lucey MR, Mathurin P, Morgan TR. Alcoholic hepatitis. N Engl J Med 2009;360:2758-69.

6. Stajner N, Bohanee B, Javornik B. Genetic variability of economically important *Asparagus* species as revealed by genome size analysis and rDNA ITS polymorphisms. Plant Sci 2002;162:931-7.
7. Woo WS, Lee EB, Kang SS, Shin KH, Chi HJ. Antifertility principle of dictamnus albus root bark. Planta Med 1987;53:399-401.
8. Visavadiya N, Narasimhacharya A. *Asparagus* root vegulates cholesterol metabolism and improves antioxidant statusin hypercholesteremicrats, Evid Based Complement Altern Med 2009;6:219-26.
9. Hewawasam RP, Jayatilaka KA, Pathirana C. Effect of *Asparagus fa*lcatus on acetaminophen toxicity in mice: A comparison of antioxidative effect with N-acetyl cysteine. J Diet Suppl 2008;5:1-9.
10. Moreno R, Espejo J, Cabarera A, Millan T, Gil J. Ploidic and molecular analysis of morado de huetor asparagus (*Asparagus officinicalis* L.) population; a Spanish tetraploid landrace. Gen Resogur Crop Evol 2006;53:729-36.
11. Kim BY, Cui ZG, Lee SR, Kim SJ, Kang HK, Lee YK, *et al*. Effects of *Asparagus officinalis* extracts on liver cell toxicity and ethanol metabolism. J Food Sci 2009;74:H204-8.
12. Zhu X, Zhang W, Zhao J, Wang J, Qu W. Hypolipidaemic and hepatoprotective effects of ethanolic and aqueous extracts from *Asparagus officinalis* L. By-products in mice fed a high-fat diet. J Sci Food Agric 2010;90:1129-35.
13. Hussein JS, Oraby FS, El-shafey N. Antihepatotoxic effect of garlic and onion oils on ethanol-induced liver injury in rats. J Appl Sci Res 2007;3:1527-33.
14. Tuma DJ, Thiele GM, Xu D, Klassen LW, Sorrell MF. Acetaldehyde and malondialdehyde react together to generate distinct protein adducts in the liver during long-term ethanol administration. Hepatology 1996;23:872-80.
15. Lieber CS. Biochemical factors in alcoholic liver disease. Semin Liver Dis 1993;13:136-53.
16. Thiele GM, Duryee MJ, Willis MS, Sorrell MF, Freeman TL, Tuma DJ, *et al.* Malondialdehyde-acetaldehyde (MAA) modified proteins induce pro-inflammatory and pro-fibrotic responses by liver endothelial cells. Comp Hepatol 2004;3 Suppl 1:S25.
17. Reinke LA, McCay PB. Pin trapping of alcohol-initiated radicals in rat liver: Influence of dietary fat. J Nutr 1997;127 5 Suppl:8995-9025.
18. Gouillon Z, Lucas D, Li J, Hagbjork AL, French BA, Fu P, *et al.* Inhibition of ethanol-induced liver disease in the intragastric feeding rat model by chlormethiazole. Proc Soc Exp Biol Med 2000;224:302-8.
19. Enomoto N, Takei Y, Hirose M, Konno A, Shibuya T, Matsuyama S, *et al.* Prevention of ethanol-induced liver injury in rats by an agonist of peroxisome proliferator-activated receptor-gamma, pioglitazone. J Pharmacol Exp Ther 2003;306:846-54.
20. Chidambaram J, Carani Venkatraman A. Cissus quadrangularis stem alleviates insulin resistance, oxidative injury and fatty liver disease in rats fed high fat plus fructose diet. Food Chem Toxicol 2010;48:2021-9.
21. Wang JQ, Li J, Zou YH, Cheng WM, Lu C, Zhang L, *et al.* Preventive effects of total flavonoids of *Litsea coreana* leve on hepatic steatosis in rats fed with high fat diet. J Ethnopharmacol 2009;121:54-60.
22. Koteish A, Mae Diehl A. Animal models of steatohepatitis. Best Pract Res Clin Gastroenterol 2002;16:679-90.
23. Zou Y, Li J, Lu C, Wang J, Ge J, Huang Y, *et al.* High-fat emulsion-induced rat model of nonalcoholic steatohepatitis. Life Sci 2006;79:1100-7.
24. Burt AD, Mutton A, Day CP. Diagnosis and interpretation of steatosis and steatohepatitis. Semin Diagn Pathol 1998;15:246-58.
25. Rukkumani R, Aruna K, Varma PS, Rajasekaran KN, Menon VP. Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. J Pharm Pharm Sci 2004;7:274-83.
26. Bykov I, Järveläinen H, Lindros K. L-carnitine alleviates alcohol-induced liver damage in rats: Role of tumour necrosis factor-alpha. Alcohol Alcohol 2003;38:400-6.
27. Bertelli A, Cerrati A, Giovannini L, Mian M, Spaggiari P, Bertelli AA, *et al.* Protective action of L-carnitine and coenzyme Q10 against hepatic triglyceride infiltration induced by hyperbaric oxygen and ethanol. Drugs Exp Clin Res 1993;19:65-8.
28. Deli J, Matus Z, Tóth G. Carotenoid composition in the fruits of *Asparagus officinalis*. J Agric Food Chem 2000;48:2793-6.
29. Küçükgergin C, Aydin AF, Ozdemirler-Erata G, Mehmetçik G, Koçak-Toker N, Uysal M, *et al.* Effect of artichoke leaf extract on hepatic and cardiac oxidative stress in rats fed on high cholesterol diet. Biol Trace Elem Res 2010;135:264-74.

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