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Protective Effect of Lycopene on Aspartame Induced Oxidative Stress and Histopathological Changes in Liver of Male Rats

Mohammad S. Al-Harbi*

Biology Department, Faculty of Science, Taif University, Taif 888, Saudi Arabia.
*dr_reham_z@yahoo.com

Abstract

Aspartame (ASP) is used with the people that suffering from obesity and diabetes mellitus. The study aimed to evaluate the biochemical responses as well as histopathological changes of ASP alone or with Lycopene (LY) in liver of rats. Rats were divided into six groups according to the treatment into control low dose of ASP (LD; 75 mg/Kg), high dose of ASP (HD; 150 mg/Kg), Lycopene (LY; 20 mg/Kg), ASP-LD plus LY and ASP-HD plus LY. All the Animals were treated orally for 30 successive days. ASP increased alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and γ-glutamyl transpeptidase (γ-GT) activities and changed the levels of lipid profile as well as creatinine and uric acid levels. It marked decreased antioxidant enzyme activity of SOD and increased the levels of lipid peroxidation (MDA), C-reactive protein and interleukins. LY prevented the ASP-induced liver injury as indicated by improving all the parameters previously illustrated. LY prevented the ASP-induced liver and kidney injury as indicated by improving all the parameters previously illustrated. Histopathological results confirm the biochemical finding and the ameliorating effect of LY on liver toxicities. In conclusion, co-treatment of LY possessed different protective mechanisms against ASP-induced liver toxicity. So, the intake of ASP should be restricted and taken with LY when it is used in food or beverages to decrease its toxicity.

Keywords: antioxidant, aspartame, hepatic, histological, lycopene, oxidative

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Introduction

Aspartame (L-α-aspartyl-L-phenylalanine, 1-methyl ester) is a widely used as artificial sweeteners among a variety of foods (soft drinks, gelatins, chewing gum, yogurt etc.), drugs and hygiene products. It is used by diabetic and human those need to lose weight [8].

There are many cellular enzymes were leakage into the serum due to change in the cell membrane permeability. Therefore, measuring serum activities of marker enzymes as alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and γ-glutamyl transpeptidase (γ-GT) are very important in clinical diagnosis to assess the toxicity of any chemical on tissue. Oxidative damage in the tissue takes place when there is an imbalance between prooxidants and antioxidants of the cell. The level of lipid peroxidation is taken as evidence for oxidative stress[15].

Lycopene (Ly), a phytochemical belonging to carotenoid family, is a red-colored pigment, acyclic and a polar carotenoid. It is abundantly found in red-colored vegetables and fruits, such as tomatoes, papaya fruit, pink grape-fruit, pink guava, carrots and watermelon, with the concentrations ranging from 9 to 42 mg/kg depending on the variety [10].

Ly displays a range of unique and distinct biological properties owing to its acyclic structure, hydrophobicity and large array of conjugated double bonds. Recently,

* *Author for correspondence*

diverse studies have been reported that lycopene exerts powerful antioxidant activity both in vitro and in vivo against the oxidation of proteins, lipids and DNA, and also has the potential of quenching singlet oxygen 100 times more efficiently than vitamin E and 125 times more than glutathione (GSH) [13].

Most metabolisms of xenobiotics occur in the liver that can cause hepatic damage. Moreover, Increasing the levels of these catabolites used as renal function impairment[8]. Based on this evidence, the present study was conducted to evaluate the possible ameliorative effects of Lycopene against hepatic toxicity induced by aspartame in male rats. This could be fulfilled through the biochemical histological, as well as disequilibrium between prooxidants and antioxidants analysis of liver tissues.

Materials and Methods

Chemicals

Pure aspartame (ASP) powder and Lycopene (LY) was purchased from Sigma-Aldrich chemical; St. Louis, USA, and all other chemical used were of analytical grade obtained from Sisco Research Laboratory, Mumbai, India.

Animal model

Animal experiments were carried out after following the European community Directive (86/609/EEC) and national rules on animal care that were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals 8th edition. The experimental animals were healthy, inbreed adult male Wistar albino rats, weighing approximately 200-250 g (12 weeks of age). The animals were maintained under standard laboratory conditions and were allowed to have food and water ad libitum (standard rat feed pellets). All the rats were housed under the condition of controlled temperature 26±2 °C with 12 h light and 12 h dark exposure.

Experimental Design

The Wistar rats were divided into six groups (n=8). Group I was the control animals which were administrated distilled water as a vehicle. Group II was treated with low dose of ASP (ASP-LD) and group III was treated with high dose of ASP (ASP-HD) (75 and 150 mg/Kg, respectively) according to[16]. Group IV was treated with Lycopene (LY; 20 mg/Kg) as recommended by[18]. Groups V and VI were treated with ASP-LD + LY and ASP-HD + LY, respectively. All the Animals were treated orally for 30 successive days.

Sample collection

The blood samples were collected from fasted animals overnight by retro-orbital puncture using blood capillary under ether anesthesia. Samples were incubated at room temperature for 10 minutes and centrifuged at 3000 r.p.m for 20 min. Serum was collected and kept at –80°C until biochemical investigation.

Preparation of tissue homogenates for measurement of redox state

Liver tissues (0.23 g) were used for the analysis of oxidative stress and antioxidant parameters. Tissues were perfused with a 50 mM of sodium phosphate buffer (100 mM Na_2HPO_4/NaH_2PO_4, pH 7.4), 0.25 M sucrose and 0.1 mM ethylene diamine tetra-acetic acid (EDTA). Then, tissues were homogenized in 5 mL cold buffer/g tissue using a Potter–Elvehjem homogenizer. The homogenates were centrifuged at 10,000 xg for 20 min at 4°C for estimating enzymatic assays and was centrifuged at 2500 xg for lipid peroxidation level, and the resultant supernatant transferred into Eppendorf tubes that preserved in a deep freeze until being used.

Hepatic and renal functions determination

Serum lactic dehydrogenase (LDH) activity (EC. 1.1.1.27) was determined by the method of[19] . Aspartate and alanine aminotransferases (EC. 2.6.1.1, EC. 2.6.1.2) were assayed by the[29]. Alkaline phosphatase (ALP) activity (EC. 3.1.3.1) was estimated according to[33]. γ-GT activity (EC. 2.3.2.2) was determined by[25]. Albumin level was determined according to a method of [4]. The total protein content was estimated by the method of [5].

The serum total cholesterol (TC) and triglycerides (TG) were determined by the method of [7]. Methods of[31] was used to determine the high-density lipoprotein–cholesterol (HDL–c). Serum low-density lipoprotein–cholesterol (LDL–c) level was calculated by formula: LDL-c = ¼ Total cholesterol levels – (Triglyceride concentration/5) – HDL-c concentration while VLDL-c = triglyceride/5.

Levels of creatinine and uric acid were determined

according to the methods of [11]. The level of urea was estimated according to the method of [26]. Bilirubin level was determined by using Sigma – Aldrich kit (MAK 126).

Inflammation markers

The levels (IL-6 and TNF-α) were measured in liver homogenates by using a quantitative enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies specific for rat (IL-6 and TNF-α). They were purchased from Immuno-Biological Laboratories Co., Ltd (IBL) the USA. Serum C-reactive protein (CRP) was determined according to the method reported by [32] using SEA821- Enzyme-linked Immunosorbent Assay Kit.

Prooxidation and antioxidant

In the liver tissues, lipid peroxidation was determined as described by[24]. Superoxide dismutase (SOD) was measured according to[21]. Myeloperoxidase (MPO) activity was measured by the method of[30]. Xanthine oxidase (XO) activity was assayed according to the method described by [20].

Statistical analysis

Data are expressed as a mean ± standard error (SE). All data were analyzed with the SPSS for windows statistical package (version 20.0, SPSS Institute Inc., Cary, North Carolina). Statistical significance between the different groups was evaluated by One Way-Analysis of Variance (ANOVA). When the groups showed significant difference then Tukey's multiple comparisons was followed and values of $P < 0.05$ were considered as significant.

Results

Biomarkers of hepato-renal assessment

Serum ALT activities of ASP-LD and ASP-HD groups were increased significantly when compared with control group (Table 1). Treatment of the rats with 20 mg/kg LY with ASP decreased the activity of ALT significantly as compared with ASP-LD and ASP-HD, respectively. The same observation has been noticed in the AST, ALP, LDH and γ-GT activities that elevated by increasing the dose of ASP and decreased by the treatment of the rats with LY combined with ASP (Table 1). The total protein and albumin levels decreased in ASP-treated rats depend on the dose but increased in the groups treated with the LY and ASP (Table 1).

The lipid profile (TG, TC, LDL-c, and VLDL-c) were increased (Table 2). Administration of LY to the ASP-treated groups restored all the parameters cited above.

Table 1. Hepatic and renal functions in male rats treated with aspartame or/and Lycopene

Parameters	Control	LY	ASP-LD	ASP-HD	ASP –LD +LY	ASP –HD+Y
ALT (U/L)	14.33±0.32	12.38±0.62	89.36±2.64[a]	169.88±10.39[a,b]	18.65±2.698[c]	23.88±2.39
AST (U/L)	16.36±1.36	14.95±1.36	165.36±4.69[a]	180.69±11.69[a]	20.39±3.25[c]	36.39±4.25
ALP (U/L)	63.59±3.68	60.36±4.23	135.98±5.68[a]	169.29±8.69[a,b]	70.36±4.36[c]	80.36±5.36[b]
LDH (U/L)	193.56±7.69	187.95±9.36	532.69±8.69[a]	785.69±9.65[a,b]	202.69±6.39[c]	220.98±5.36[b]
γ-GT U/L	5.36±1.02	5.20±1.06	8.69±2.36[a]	9.02±2.36[a]	5.98±2.36[c]	6.25±1.69[b]
Total proteins (g/dL)	8.43±1.36	8.68±1.69	5.69±1.63[a]	4.32±1.02[a]	7.39±1.69[c]	6.25±2.02[b]
Albumin (g/dL)	4.39±0.65	4.61±0.69	3.68±0.69[a]	2.98±0.98[a]	4.01±0.96[c]	3.02±0.54[b]
Urea (g/dL)	20.36±3.65	18.36±3.23	29.35±5.23[a]	38.39±6.25[a]	24.23±3.25[c]	28.36±3.25[b]
Uric acid (g/dL)	13.02±2.02	13.65±3.02	24.02±4.25[a]	28.36±5.02[a]	18.03±2.62[c]	20.36±3.02[b]
Creatinine (g/dL)	0.52±0.09	0.51±0.07	1.68±0.34[a]	2.35±0.45[a]	0.67±0.32[c]	0.87±0.16[b]
Total bilirubin (mg/dL)	1.25±0.32	1.20±0.22	2.15±0.32[a]	2.77±0.35[a]	1.56±0.32[c]	1.68±0.65[b]

Values are expressed as means ± SE; n = 8 for each treatment group.
LY; Lycopene, ASP- LD; low dose of aspartame, ASP-HD; high dose of aspartame, ALT; alanine transaminase, AST; aspartate transaminase, ALP; alkaline phosphatase, LDH; lactic dehydrogenase, and γ-GT; gamma glutamyl transferase. a significant difference as compared to control, b significant difference as compared to ASP-LD and c significant difference as compared to its relative group of ASP ($P \leq 0.05$).

Table 2. Serum hepatic antioxidant enzymes and thiol level in male rats treated with aspartame or / and Lycopene

Parameters	Control	Ly	ASP-LD	ASP-HD	ASP –LD +LY	ASP –HD+LY
Hepatic myeloperoxidase (MPO) (nmol/min/mL)	18.06±1.36	18.36±2.30	27.06±2.36[a]	30.66±3.62[a,b]	24.85±2.36[c]	23.08±3.02[b]
Hepatic xanthine oxidase (XO) (U/g)	16.03±2.36	16.20±1.36	30.69±3.03[a]	36.06±4.36[a,b]	22.36±2.03[c]	23.89±3.08[b]
Hepatic thiol (µmol/g)	7.39±2.03	7.33±1.36	4.03±1.03[a]	4.69±1.96[a,b]	6.58±1.69[c]	6.20±1.87[b]

Values are expressed as means ±SE; n = 8 for each treatment group.
LY; Lycopene, ASP- LD; low dose of aspartame, ASP-HD; high dose of aspartame, HDL-c; high density lipoprotein of cholesterol, LDLc; low density lipoprotein of cholesterol, and VLDL-c; volatile low density lipoprotein of cholesterol. a significant difference as compared to control, b significant difference as compared to ASP-LD and c significant difference as compared to its relative group of ASP ($P \leq 0.05$).

Urea, uric acid, creatinine, and bilirubin were measured to investigate the renal functions (Table 1). The significantly increased levels of all parameters in ASP administration groups at all tested doses were observed. The increase in urea level was noticed by increasing the doses as compared to control for ASP-LD and ASP-HD, respectively. The uric acid levels were increased significantly in ASP-LD and ASP-HD as compared to control, respectively. Treatment the rats with ASP-LD and ASP-HD elevated the creatinine levels as compared to control, respectively. The total bilirubin was increased in dose-dependent manner as the effect of ASP. Treatment the rats with LY and ASP improved the level renal functions parameters significantly as compared with each its relative ASP-group.

Hepatic antioxidant

Changes in serum hepatic antioxidant enzymes and thiol level in male rats treated with aspartame or/and Lycopene were presented in Table 2. Serum MPO and XO activities were increased as the effect of ASP while the thiol level decreased. They were improved when rats treated with LY and ASP.

Treatment with ASP alone caused an increase in C-reactive protein, these elevations were dose-dependent (Fig. 1). The presence of LY in combination with SA modulated the elevation in C-reactive protein and maintained the levels closer to the normal values.

Data presented in Fig. 2 showed that treatment with SA caused a significant increase in the level of TNF-α. It was increased significantly as compared to control for 75 and 150 mg/kg b.w of ASP, respectively. However, the concurrent administration of LY to the rats treated with ASP significantly decreased the levels of TNF-α as compared with its relative ASP treatment group only. In dose-dependent manner, IL-6 levels increased in ASP-LD and ASP-HD as compared to control, respectively (Fig. 2). However, LY returned the IL-6 levels to almost the normal level when treated before the administration of ASP.

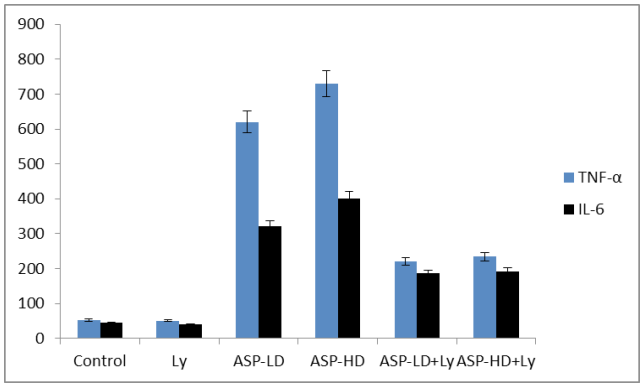


Figure 1. Changes of C-reactive protein in male rats treated with aspartame or / and Lycopene.
Values are expressed as means ±SE; n = 8 for each treatment group. LY; Lycopene, ASP- LD; low dose of aspartame, and ASP-HD; high dose of aspartame. [a] significant difference as compared to control, [b] significant difference as compared to aspartame-low dose group (ASPLD) and [c] significant difference as compared to its relative group of ASP.

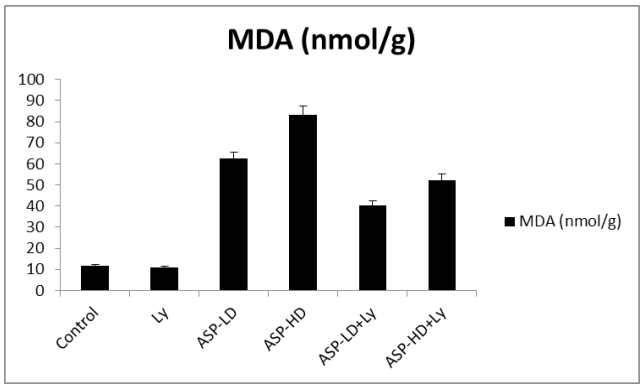


Figure 2. Changes of hepatic TNF-α and IL6 in male rats treated with aspartame or / and Lycopene.
Values are expressed as means±SE; n = 8 for each treatment group. LY; Lycopene, ASP- LD; low dose of aspartame, and ASP-HD; high dose of aspartame. [a] significant difference as compared to control, [b] significant difference as compared to aspartame-low dose group (ASPLD) and [c] significant difference as compared to its relative group of ASP.

The results revealed an increase of LPO in the liver of the ASP-treated group in dose-dependent manner as evidenced by the enhanced malondialdehyde levels in the liver homogenates of adult rats (Fig. 3). The Administration of LY combined with ASP alleviated LPO significantly and modulated the MDA levels in the liver.

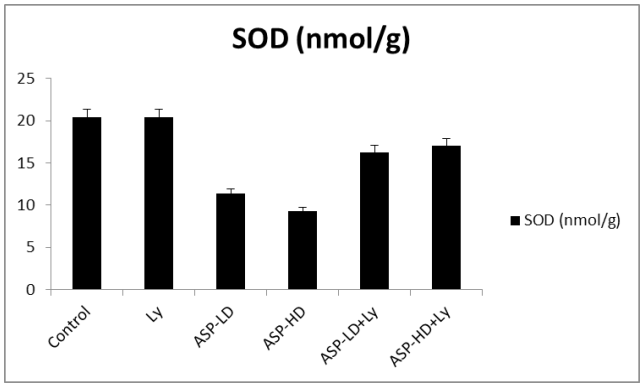


Figure 3. Changes of hepatic lipid peroxidation (MDA) levels in male rats treated with aspartame or / and Lycopene. Values are expressed as means ± SE; n = 8 for each treatment group. LY; Lycopene, ASP- LD; low dose of aspartame, and ASP-HD; high dose of aspartame. [a] significant difference as compared to control, [b] significant difference as compared to aspartame-low dose group (ASP-LD) and [c] significant difference as compared to its relative group of ASP.

In the liver homogenates of ASP-treated rats, SOD activity decreased significantly, when compared to controls (Figs. 4). Administration of LY with ASP ameliorated the antioxidant enzymes group when compared to ASP-group.

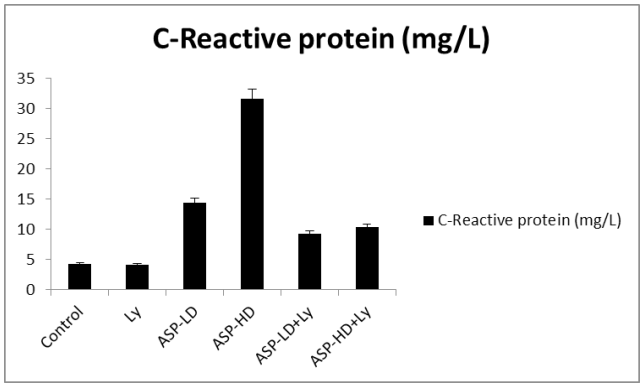


Figure 4. Changes of hepatic superoxide dismutase (SOD) levels in male rats treated with aspartame or / and Lycopene. Values are expressed as means ± SE; n = 8 for each treatment group. LY; Lycopene, ASP- LD; low dose of aspartame, and ASP-HD; high dose of aspartame. [a] significant difference as compared to control, [b] significant difference as compared to aspartame-low dose group (ASP-LD) and [c] significant difference as compared to its relative group of ASP.

Discussion

The present study concerned on the biochemical and histopathological changes to prove that LY has an ameliorative effect on ASP induced hepatic toxicity. Increasing the activities of AST, ALT, ALP, LDH and γ-GT in serum indicated that damage has occurred in the membrane structure that could be due to the presence of reactive oxygen species (ROS) produced during the oxidative stress. These results are in agreement with[8] that found the ASP change the biochemical parameters of rats as well as[1] who noted that the activities of AST and ALP increased significantly after administration of ASP 100 mg/kg to rats for 2 weeks. These elevations could be due to oxidative stress induced by ASP and causing an imbalance in the membrane permeability. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membranes in the liver are indicators of tissue injury.

The decreasing levels of serum albumin and protein also suggest an adverse effect of ROS that produces during ASP metabolism on the formation ability of the liver.

Hypercholesterolemia has been considered a risk factor for hepatic injury as all the lipid profile parameters were changed. The liver is the main organ responsible for cholesterol metabolism. The present study showed that ASP induced changes in lipid metabolism, and could be involved in the development of hypercholesterolemia as reported by[27]. [17]found that hypercholesterolemic atherosclerosis was associated with increases in ROS, which plays an importance role in the development of atherosclerosis and cardiovascular diseases as the effect of ASP.

The significant increase of urea and creatinine could be due to the toxic effect of ASP on kidney and that could cause renal dysfunction. These effects reduced glomerular filtration rate and retention of urea and creatinine as reported by[2].

Many pathological changes extending to cellular damage occur if the balance between oxidants and anti-oxidants cannot be maintained in tissues. Oxidative stress damage with free radical generation and overwhelming LPO are indication of ASP toxicity. In the current study, MDA and C-reactive protein levels in ASP group were significantly increased and accompanied by an attendant decrease in the activities of antioxidant enzymes (SOD,

CAT, GPx) as well as GSH and total protein levels in the liver tissues when compared with control group.

The current results were similar previously reported by [23] who demonstrated that ASP may induce an oxidative stress in the liver of rats. Elevation of LPO levels in ASP-treated rats indicated the generation of free radicals and had been used as indirect biomarkers of oxidative stress. Antioxidant enzymes such as SOD that is decreased in ASP treated groups are considered to be primary defense enzymatic antioxidants that prevent the oxidative damage by ROS to macromolecules.

The antioxidant enzymes CAT and GPx protect SOD against inactivation by H_2O_2. Reciprocally, SOD protects CAT and GPx against superoxide anion through dismutation of endogenous cytotoxic superoxide anion to O_2 and H_2O_2 . However, overload of free radical could have been disturbing these regulations. Furthermore, the decrease in SOD and CAT activities may be due to the formation of formaldehyde from the methanol as the ASP metabolism. This is in accordance with [14]who indicated that formaldehyde exposure leads to a decrease in SOD and CAT activities in the liver tissue.

In the present work, the administration of ASP induced major alterations in the light structures of the liver. The number of vacuoles increased in hepatocytes that could probably represent a cellular defense mechanism against ASP, as previously described the toxic substances were accumulated in the vacuoles to prevent their interaction with cellular metabolism [9].

The damage of hepatocytes could be secondary to the activation of Kupffer cells that secrete TNF-α and IL-6 as well as increasing the levels of LPO and decreasing the antioxidant enzymes which cause cell death. This was in accordance with others, where they proved that Kupffer cells might cause hepatocellular damage in ASP hepatotoxicity [9].

Ly is a natural pigment, synthesized by plants and microorganisms. Red fruits and vegetables are the most common sources of Ly, which exhibits the highest antioxidant activity among all dietary carotenoids. Furthermore, the Mediterranean dietary pattern, which includes proportionally high consumption of vegetables and fruits with Ly, has shown notable benefits for non-alcoholic fatty liver patients[13].

Therefore, nowadays, the potential role of Ly in human health is beginning to be recognized, and the most important health benefits are hypothesized to occur through their ability to protect against oxidative damage. In vitro studies have demonstrated that Ly is an effective antioxidant and radical scavenger [13].

Ly is the most potent singlet oxygen quencher among natural carotenoids, due to its high number of conjugated dienes, and recent studies have shown that Ly is at least two times as active as β-carotene in protecting lymphocytes for $NO_2\cdot$ radical-induced membrane damage, which indicates that Ly is the most potent scavenger of ROS among other major dietary carotenoids [22].

In addition, Ly was shown to protect human LDL against photosensitized oxidative damage[28]. Thus, based on the benefits of Ly, the aim of the present study was to explore the effect of Ly in prevention of ASP-induced hepatic toxicity in a rat model. To the best of our knowledge, this is the first time research has attempted to explore the potent effects of Ly on ASP -induced hepatotoxic rats.

Treatment with Ly prevented the histopathological changes in liver of rats induced with ASP in either low or high doses. Thus, these results suggested that the inhibition of the elevation of liver function markers, obvious lipid-lowering and liver damage may related to the protective effect of Ly against ASP-induced hepatotoxicity.

Moreover, Ly enhanced the activities of SOD, increased GSH and diminished MDA against the ASP-induced hepatic toxicity in these animals, suggesting that the activity of antioxidants may play a role in the mechanism of its hepatoprotective effects.

TNF-α is a central proinflammatory cytokine, which is associated with a variety of physiological and pathological conditions, including cytotoxicity, growth stimulation, immune-modulation and pro-inflammatory activity. In addition, TNF-α is produced predominantly by the monocyte macrophage lineage in liver, and the main population of this lineage is Kupffer cells. Thus, increased TNF-α production by activated Kupffer cells may be responsible for ASP induced hepatic toxicity.

Furthermore, the most current studies have indicated that inhibition of TNF-α could decrease the content of hepatic fatty storage in the ASP-induced hepatic toxicity. In our study, the effects of TNF-α in damaged liver was evaluated. Compared to the normal group, rats treated with ASP showed up-regulated expression of TNF-α, while pretreatment with Ly led to down-regulated expression of TNF-α compared to the ASP treated group.

Elevation of serum ALP occurs in hepatobiliary diseases[6]because it is a membrane-bound glycoprotein enzyme with a high concentration in sinusoids and

endothelium. The results of the present study indicate that the ASP groups probably stabilize the hepatic plasma membrane as the effect of LY. Reduction of ALP levels with concurrent depletion of bilirubin levels suggests the stability of the biliary function as the effect of LY on ASP-treated group.

The reduction of total protein could be attributed to the damage of the endoplasmic reticulum which results in the loss of cytochrome P-450 enzymes leading to depletion in protein synthesis and accumulation of triglycerides leading to fatty liver [3]. LY with ASP enhanced the synthesis of TP which protect the hepatocytes and indicates the hepatoprotective activity of LY.

The results showed that using LY along with ASP administration show improving oxidative stress parameters in ASP groups. MDA was significantly decreased while, activities of CAT, GPx, and SOD, as well as total protein and GSH levels, were increased. These effects of LY may result directly from antioxidant effects against free oxygen radicals or from enhanced biosynthesis of enzymatic/non-enzymatic antioxidants.

Conclusion

In conclusion, the results showed that ASP causes Hepatic damage and consequently affects their functions as indicated by remarkable biochemical as well as histological changes. LY provided protection from these changes and decreased the injury induced by ASP. Therefore, the intake of ASP should be restricted and taken with LY when it is used in food or beverages.

Conflict of Interest: None Declared

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