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Ameriolation of Aspartame Induced Hepatic Oxidative Damage by Co - Enzyme Q10 and Flaxseed Oil in Rats

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ABSTRACT

Aspartame, the most widely used nonnutritive sweetener allover the world. This study aimed to examine the hepatoprotective influence of Co-enzyme Q10 and flaxseed oil against aspartame hepatotoxicity in rats. 24 male albino rats (150-180g), 6 weeks of age were divided into 4 groups (n=6); control group (I) had free access to food and water, group II received aspartame (250 mg/l/day) dissolved in distilled water by gavage, group III received Co-enzyme Q10 (500 mg/kg/day) in distilled water with aspartame by gavage and group IV received 15 % flaxseed oil/day in diet with aspartame for 60 days. Aspartame caused a marked rise in serum liver transaminases, alkaline phosphatase, total protein and total bilirubin with reduction in serum albumin, also it caused decrease in liver reduced glutathione level, superoxide dismutase and catalase activities while it induced lipid peroxidation, which indicated by marked increase of malondyaldehyde and nitric oxide. These results reflect aspartame hepatic intoxication. Concurrent supplementation of either Co-enzyme Q10 or flaxseed oil with aspartame administration were successfully ameriolated liver damage caused by aspartame which detected by improvement in all affected parameters. These data strengthened with the histopathological and immunohistochemical examinations of the liver tissue which prove concurrent protection of hepatocytes as a result of co-treatment with Co-enzyme Q10 and flaxseed oil.

KEYWORDS Aspartame, Co-enzyme Q10, Flaxseed oil, Hepatotoxicity, α- SMA, HSCs.

1. INTRODUCTION

Aspartame (L-aspartyl-L-phenylalanine methyl ester, ASP) is considered the most popularly consumed non-saccharide sweetener worldwide; it is involved in thousands of products including pharmaceuticals, such as vitamins and sugar free cough drops [1]. Upon oral administration, ASP is promptly absorbed from the intestinal tract and metabolized to 50% phenylalanine, 40% Aspartate and 10% methanol. Methanol is considered responsible for the hepatic mitochondrial damage as it is oxidized to formaldehyde and later to formic acid provoking microsomal propagation and inducing reactive oxygen species generation (ROS). Formic acid causes cells to become too acidic and produces metabolic acidosis which damages healthy cells by causing enzymes to stop functioning [2-3].

ROS enhance the depletion of enzymatic and non- enzymatic antioxidants leave the hepatocytes unprotected, it can cause excessive damage to hepatocytes through lipid peroxidation and protein alkylation thereby causing damage to cellular membrane [4]. Oxidative stress can liberate mediators and assist in the activation of hepatic stellate cells (HSCs), which are the predominant fibrogenic cell type producing collagen type I in the liver [5-6]. Therefore, oxidative stress has been recognized as a fundamental factor in the pathological process of liver fibrosis. Mechanisms other than the production of ROS might be implicated in ASP induced cellular damage. In accordance with these suggestions, some authors reported that aspartame could be metabolized in cell mitochondria, inducing mitochondrial and nuclear DNA damage and affecting its ability to produce inadequate and incomplete energy metabolism, which gives rise to highly damaging free radicals [7].

Liver plays the role of detoxifier and excreter of destructive agents against body intoxication and as a result liver injury occurs and this could lead to hepatic damage and development of hepatic disorders [8], such disorders translated with increasing in liver enzymes, such as aspartate/alanine transaminase (AST/ALT), alkaline phosphatase (ALP), and total bilirubin level [9]. These enzymes find their way into the serum through leakage arising from altered membrane permeability as its measurements are considered valuable tools in clinical diagnosis. In order to assess the toxicity threshold of chemicals on the hepatic tissue, activities of these marker enzymes can be estimated in tissues and body fluids [10-11].

It has been proven through several studies that compounds with significant antioxidant properties are capable of rendering protection to liver tissues against adverse effects caused by ROS [12-13]. Supplementation with antioxidants in free radical intoxication could be considered as the alternative method for chelation therapy [14]. Co-enzyme Q10 (CO Q10) also known as Ubiquinone, is the only endogenously produced lipid-soluble substance with powerful antioxidant properties. It was earlier thought that its only main function was to play the role of electron carrier in the mitochondrial respiratory chain. Later, it was found that it is highly effective in preventing lipid, protein and DNA oxidation [15]. In recent years, CO Q10 has gained considerable attention as a dietary supplement capable of influencing cellular bioenergetics and counteracting some of the damage caused by ROS [16]. The protective role of CO Q10 in various models of oxidative stress is evident from previously published data. It was achieved that CO Q10 has therapeutic

effects; as anti-oxidant and anti-inflammatory effects, against hepatotoxicity as well as metabolic-stress-induced liver damage by inhibition of apoptosis in hepatocytes [17-18].

Flaxseed (FSO) contains 35% of its mass as oil, of which 55% is alpha-linolenic acid (ALA, n-3, omega-3 fatty acid). Dietary omega-3 polyunsaturated fatty acids (PUFAs) have been shown to reduce the severity of numerous ailments. Flaxseed oil, Linum unisatissimum (FSO), has a history of food use in Europe and Asia for its potential health benefits, which include anticancer, antiviral, bactericidal and anti-inflammatory effects, ion reduction, laxative uses and reduction of atherogenic risks [19]. FSO (Omega nutrition) suppresses oxygen radical production by white blood cells with lowering of serum lipids and decreasing oxidative stress [20].

Although considerable toxicological studies were carried out on ASP, exposing its ability to induce hepatic oxidative stress and structural deformities on the long run, chemoprevention by using nutrients is the subject of intense study. Among the many compounds examined, antioxidants are being investigated because of their ability to reduce disease formation by either induction or inhibition of key enzyme systems [21]. We, therefore, underlined the possible protective effect of CO Q10 and FSO against hepatic injury induced by ASP under laboratory conditions.

2. MATERIALS AND METHODS

2.1. Experimental Materials

Aspartame (Diet sweet) was purchased in the form of tablets (20 mg/tablet) from Al-Ameriya pharmaceutical industries, Alexandria, Egypt. The tablets were dissolved in distilled water and administered to the rats. Co-enzyme Q10 (Ubiquinone, 30 mg) was obtained from MEPACO Pharmaceutical Company, Egypt. Flaxseed oil was obtained from local market of Herbs and Medicinal plants, Cairo, Egypt. All the other chemicals and reagents used were of analytical grade were purchased from Biodiagnostic co. Egypt.

2.2. Experimental Animals

Male Wistar albino rats (n=24) with an average weight of (150-180g), 6 weeks old were purchased from the lab animal house in the faculty of veterinary medicine, Zagazig University, Egypt. Animals were kept in light and temperature-controlled (24°C ± 2) room with 12-h dark-light cycles; they were acclimatized for two weeks before the onset of the experiment to exclude any inter current infection. Rats were maintained on stock diet adlibitum and clean water was continuously available. All animal procedures are carried out according to the specifications stated for the proper care and use of laboratory animals [22].

2.3. Experimental Protocol

Rats were divided equally into four groups (each of 6), rats in group I were kept as negative control and had free access to food and water. Rats in group II were kept as positive control and received 250 mg/L ASP dissolved in distilled water daily for 60 days, given orally by gavage [23]. Rats in group III were received 500 mg CO Q10/kg/day dissolved in distilled water along with ASP daily by gavage for 60 days [24]. Rats in group IV were received 15% FSO in combination with ASP daily by gavage for 60 days [25].

2.4. Blood Sampling

At the end of the experiment, animals were maintained off food overnight then anesthetized using diethyl ether, blood samples were collected from medial canthus of the eye in sterilized plain tubes and left to clot at room temperature for 20 minutes. Sera were separated by centrifugation at 3000 rpm for 10 minutes and the clear serum was collected and kept frozen at -20° C for various biochemical assays.

2.5. Tissue Sampling

Liver tissues were immediately homogenized in ice-cold phosphate buffer saline (PBS) 5% (1 g tissue in 5 ml) using Omni international homogenizer (USA) at 22,000 rpm for 20 s each with 10 s intervals. The homogenates were centrifuged (Hettich, Germany) at 3000 rpm for 15 minutes at 30°C. The yielded supernatants were saved for enzyme assays. The UV/vis Spectrophotometer (JENWAY 6505, UK) was used for the measurements of enzyme activities and oxidative stress parameters at 25° C.

2.6. Biochemical Determinations

2.6.1. Assessment of Liver Function

Commercial colorimetric assay kit (Biodiagnostic co.) was used for the estimation of serum aspartate transaminase (AST) and alanine transaminase (ALT) [26]. Alkaline phosphatase (ALP) in the serum was assayed [27].

2.6.2. Assessment of Serum Total Protein, Albumin and Total Bilirubin

Estimation of serum total protein, albumin and total bilirubin was carried out according to Walter and Gerarde (1970); Lowry and Rosebrough (1951) and Szasz (1969) respectively [28-30].

2.6.3. Assessment of Hepatic Oxidative Stress Markers

Reduced glutathione (GSH) in liver tissue homogenates was determined based on the reduction of 5,5` dithiobis (2 - nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm. The level of GSH was explicit as mg of GSH/ g. tissue [31].

Catalase enzyme activity in tissue homogenates was measured, as catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase (HRP), remaining H_2O_2 reacts with 3,5- Dichloro -2-hydroxybenzene sulfonic acid (DHBS) and 4 aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample, absorbance measured at 510 nm. The activity of CAT is expressed as U/ g. tissue [32].

The activity of superoxide dismutase (SOD) was measured colorimetrically at 560 nm for 5 minutes at 25° C [33]. The activity was explicit as U/ g. tissue.

Lipid peroxidation (LPO) Levels was assayed colorimetrically as Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95° C for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534 nm and expressed as nmol/ g. tissue [34].

Nitric oxide (NO) level was measured colorimetrically; in acid medium and in the presence of nitrite the formed nitrous acid diazotise sulphanilamide and the product are coupled with N-(1–naphthyl) ethylenediamine. The resulting azo dye has a bright reddish – purple color which can be measured at 540 nm and expressed as µmol/ L [35].

2.7. Histopathological Examination

After thorough washing with ice-cold 5 % phosphate-buffered saline (PBS), Tissue specimens from the liver of rats in different groups were collected and immediately fixed in 10% neutral buffered formalin, cleared in xylol, embedded and thin paraffin sections (4-5µ) thick were prepared and stained with routine stain hematoxylin and eosin and special stain (Masson's trichrome) [36].

2.8. Immunohistochemical Study

The standard Immunohistochemical methods were adopted [37]. Universal systems were the biotin-streptavidin (BSA) system used to visualize the markers [38]. Diaminobenzidine (DAB) was used as a chromogen since it allows a permanent preparation. Hematoxylin counter stain was done.

2.9. Statistical analysis

The collected data were statistically analyzed using the one-way analysis of variance (ANOVA) (SPSS Software, version 22,

SPSS Inc., Chicago, USA). Quantitative data were calculated as mean ± standard error (SE). The significance of the data was estimated by the P value; and the differences were considered significant at P≤ 0.05.

3. RESULTS

3.1. Biochemical Changes

3.1.1. Effect of ASP, CO Q10 and FSO on Serum Biochemical Parameters in rats

The results of the current study revealed that oral ASP administration (250 mg/l/day for 60 days) caused significant increase in the level of serum AST, ALT and ALP (P≤0.05). ASP group also showed induction of total protein (TP), total bilirubin (TB) and reduction of serum albumin (Alb) in comparison with control group. Animals received CO Q10 (500 mg/kg/day for 60 days) day for 60 days) along with ASP and FSO (15% /diet/ day for 60 days) along with ASP showed reduction of levels of serum AST, ALT, ALP, TP and TB with increasing of serum Alb compared with ASP group. All tested serum biochemical parameters of CO Q10 were nearer to normal values than that of FSO but without statistical significance except for Alb which was nearer to normal value in CO Q10 than FSO group with statistical difference **(Table 1).**

3.1.2. Effect of ASP, CO Q10 and FSO on Hepatic Oxidative Stress Parameters in rats

In comparison with control group, ASP (250 mg/l/day) treated group for 60 days showed depletion of liver enzymatic super oxide dismutase (SOD) and catalase (CAT) activities and non enzymatic reduced glutathione (GSH) antioxidants with increasing of both lipid peroxidation (MDA) and nitric oxide (NO) levels. Animal received CO Q10 (500 mg/kg/day for 60 days) for 60 days along with ASP and FSO (15%/diet/day) for 60 days along with ASP showed increase in SOD, CAT and GSH with reduction of MDA and NO levels compared to ASP treated group. All tested hepatic oxidative stress parameters of CO Q10 were nearer to normal values than that of FSO with statistical significance except for MDA and NO **(Table 2).**

3.2. Histopathological Results

Liver of the control rats showed normal polyhedral hepatic cells which arranged in cords that radically arranged around the central veins. Most hepatocytes showed vacuolated cytoplasm in ASP treated group. The vacuoles were irregular with ill-defined cell boundaries indicating hydropic degeneration and some other vacuoles with distinct boundaries indicating fatty change. Dilated congested blood vessels and sinusoids were observed. Some hepatic lobules showed a disturbed parenchymal architecture. Hepatic areas showed mild infiltration of lymphocytes and mild to moderate proliferation of fibroblasts. In CO Q10 treated group, liver showed pronounced improvement of hepatocytes with no proliferation of fibroblasts. Only mild vacuolar degeneration of hepatocytes was observed. While treatment with FSO showed a mild fibroblast proliferation and mild to moderate vacuolar degeneration of hepatocytes **(Figure 1).**

Masson's Trichrome Stain for Fibrous Tissue:

The liver sections stained by Masson's Trichrome stain revealed absence of fibrosis in the hepatic areas in control and CO Q10 treated rats. Liver of ASP treated rats showed mild proliferation of fibroblasts in hepatic areas that stained blue. In FSO treated rats, the sections stained by Masson's Trichrome stain revealed minimal blue fibrous tissue in portal areas **(Figure 2).**

3.3. Immunohistochemical results:

Control and CO Q10 groups revealed normal α –smooth muscle actin (α-SMA) positive stain around the central vein of the liver sections. No intralobular immunoreactivity was observed. ASP group showed increased expression of α-SMA positively stained brown and appeared around the hepatic areas. FSO group showed mild increased positively stained brown expression of α-SMA that appeared as thin septa **(Figure 3).**

4. DISCUSSION

ASP has been recognized as a low caloric dietary sweetener used in day life. Although it has been approved as an additive in many dry food applications, there are controversies about its safety [39]. The hepatotoxic effect of ASP could be attributed to its ability to induce reactive oxygen species (ROS) formation with induction of oxidative stress [40-41]. This oxidative stress may lead to oxidation of polyunsaturated fatty acids present in biomembranes, thereby causing destruction of structural and functional organization of these membranes [42]. Recent studies pointed out ASP ability to induce hepatic oxidative stress and structural deformities on the long run [43]. Up to our knowledge, no study assessed the protective effect of both the naturally occurring CO Q10 and FSO antioxidants against hepatic injury induced by ASP.

Biological compounds with antioxidant properties can protect cells and tissues against deleterious effects of ROS and other free radicals like superoxide anion and hydrogen peroxide molecules [44]. Recently, the use of botanicals and naturally occurring antioxidants for prevention of toxic effects of various toxicants and environmental agents is gaining interest [45]. These products have many pharmacological effects such as being anti-inflammatory, anti-hepatotoxic and antioxidant [46]. The antioxidants inhibit ROS, which are capable of causing damage to DNA, associated with many health problems [47-48]. CO Q10 is a naturally occurring antioxidant which involves antioxidative (free radical scavenging) function by decreasing oxidative phosphorylation and preventing increased hydrogen peroxide production [49]. Moreover, FSO is a good source of dietary fiber and phytoestrogenic lignans that are believed to have antioxidant properties [50]. The oxygen radical scavenging properties of FSO lignans were shown in vitro by either direct hydroxyl radical scavenging activity or inhibiting lipid peroxidation [51].

Oxidative stress induced in rats in this study by oral administration of ASP (250 mg/l/day) was manifested by elevation in serum and tissue biochemical markers of hepatic function. The estimation of these parameters in the serum is useful quantitative markers of the extent and type of hepatic cellular damage [52-53]. These findings came in line with others who reported an elevation in serum marker enzymes AST and ALT levels in case of methanol intoxication due to ROS formation which in turn affect the secretory function of the liver and the structural integrity of the hepatocytes with mitochondrial deterioration. They also reported that methanol intoxication caused elevated serum ALP which indicates biliary tract damage and inflammation. These parameters indicated that ASP induced peroxidative damage to hepatocytes membrane [54-56].

As most blood proteins are synthesized in the liver, decreased levels can be found in liver injury because of decreased liver protein synthesis. The liver produces all of the proteins except that synthesized by the immune system (called gamma globulin or immunoglobulin); it does this by reassembling amino acids into protein. The main protein produced by the liver is albumin [57]. Normal albumin in the bloodstream is important for many physiologic functions; one of these functions involves the normal maintenance of fluid pressure in the arteries and veins. When the protein level falls below a certain point, the fluid in these vessels can leak out and pool in the abdominal or thoracic cavities cause ascites. In the present study, the increased bilirubin production may be a result of decreased uptake, conjugation, or increased bilirubin production; this reflects liver cell damage or bile duct damage within the liver itself [52-53,55] . On the other hand, our study reveals decrease in serum albumin level and this came in agreement with other study which mentioned that albumin represents a major synthetic protein in the liver and is a marker for the ability of the liver to synthesize proteins in which low level indicates that the synthetic function of the liver has been markedly diminished [58]. These changes may be attributed to the pathogenesis of ASP liver toxicity in which oxidative stress and free radicals production by methanol metabolite of ASP take place.

The cellular antioxidant status determines the susceptibility

to oxidative damage and is usually altered in response to oxidative stress. Hepatotoxicity could also be explained by the impaired antioxidant enzyme activities in the liver. Enzymatic and non enzymatic antioxidant defense systems are present in the cell to prevent the integrity of biological membranes from oxidative processes caused by free radicals [2,41]. Recent study mentioned that long term intake of ASP leads to deterioration in liver antioxidant status mainly through glutathione dependent pathway, which is also parallel to the present findings [59]. In our study, the oral administration of ASP (250 mg/l/day) led to a significant reduction of hepatic GSH level after 60 days of treatment far above the normal values. These findings came in agreement with Skrzydlewska (2003) who demonstrated that the depletion of liver GSH may be attributed to its conjugation with the electrophilic metabolites. Based on literature data, cellular GSH content and activities of related enzymes were decreased in liver during methanol intoxication because methanol metabolism depends upon GSH content [40, 60]. Depletion of cellular GSH increases cell vulnerability to oxidative stress [61] and when antioxidant defenses are weakened, body cells and tissues become more prone to develop dysfunction and/or disease [47].

CAT considered as most important H_2O_2 removing enzyme and also a key component of anti oxidative defense system, so with inadequate CAT activity to degrade H_2O_2, more H_2O_2 could be converted to toxic hydroxyl radicals that may contribute to oxidative stress due to methanol metabolite of ASP. SOD plays an important role in the biological defense mechanism and elimination of ROS through dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 and O_2 which are deleterious to polyunsaturated fatty acids and proteins, the decreased SOD activity is insufficient to scavenge the superoxide anion, produced during the normal metabolic process [2]. In the present study, the decrease in the activities of the hepatic antioxidant enzymes, CAT and SOD induced by ASP administration was previously reported by other studies which recorded that methanol administration caused a decrease in the enzymatic antioxidant (SOD and CAT) due to the formation of formaldehyde from the methanol [7, 62]. Other study elucidated that there was a dose-response relationship between formaldehyde concentration and SOD activity [63].

MDA is one of the main lipid peroxidation product, its elevated levels could reflect the degrees of lipid peroxidation which substantiates the generation of free radicals so contributes to the pathophysiology of liver damage [64]**.** A significant increase in the hepatic MDA, measured as an index of liver lipoperoxidation was observed in ASP treated. These findings came in agreement with other previous studies, which suggested that methanol as a byproduct of ASP metabolism could initiate the lipid peroxidation products which leads to degeneration of the biological membranes and hence cell death [54, 65].

Nitric oxide (NO) is a crucial molecule for biological systems. The damaging effects of NO on cellular proteins, DNA and lipids can lead to cell death, tissue injury and organ failure [66]. Our current study showed elevated liver NO levels in ASP treated rats which indicates membrane disruptions and free radicals production, this came in agreement with others who proved that free radicals are potent source of oxidative injury represented by NO induction [67, 68]. Our findings also came in agreement with other study which proposed that a high level of nitric oxide is associated with acute liver injury [69].

ASP induced hepatic structural alterations were evidenced by histopathological changes. Cytoplasmic vacuoles in hepatocytes might be responsible for the physical changes in the structure of plasma membranes of protein and lipids of different organelles. This might occur as a result of the release of the free radicals secondary to the production of methanol and aspartic acid after aspartame ingestion [70]. In the present work, damage of liver cells might be secondary to the activation of Kupffer cells that secrete tumor necrosis factor alpha, interleukins, reactive oxygen, nitrogen species, proteases, and prostaglandins. These mediators could act directly on hepatocytes to cause cell death. This was in accordance with others, where they proved that Kupffer cells might cause hepatocellular damage in acetaminophen hepatotoxicity [71]. In contrast, it was found that Kupffer cells played a protective role in hepatic injury. Chronic ASP intoxication is selectively enhances chemokine release by Kupffer cells and hepatic sinusoidal cells to induce the migration of inflammatory cells to liver which evidenced by significant neutrophil infiltration to the liver parenchyma of ASP administered group along with notable changes in nuclear condensations and microvacuole formation in the cytoplasm [72]. These findings came in agreement with present study.

These previously mentioned data were strengthened by increased expression of α-SMA which indicates liver fibrosis. In liver fibrosis, HSCs serve as the major source of extracellular matrices. During the activation process, HSCs undergo phenotypic changes in cellular morphology to a myofibroblast like cell type with expression of α-SMA [73].

In the present study, oral administration of CO Q10 at a dose of (500 mg/kg/day) for 60 days with ASP was able to ameliorate all tested parameters; serum biochemical parameters (AST, ALT, ALP, TP and TB) and hepatic oxidative stress parameters (GSH, CAT, SOD, MDA and NO) toward the normal values. The hepatoprotective action of CO Q10 may be attributed to the reduction of oxidative stress by the effectiveness in suppressing both the increased lipid peroxidation and destruction of the hepatocytes membrane in the regenerating liver cells of rats as reported previously [74]. The present study is in agreement with other studies which confirmed that CO Q10 significantly decreases the level of lipid peroxidation in vivo and in vitro [75-76]. Moreover, CO Q10 exhibits protection against liver damage by lowering thiobarbituric acid reactive substances (TBARS) and alanine release [77]. This effect of CO Q10 may be related to its antioxidant activity since it significantly decreased ROS generation. Furthermore, the present work demonstrated that CO Q10 also provided a significant protective effect against ASP hepatotoxicity in albino rats as indicated by complete restoration of hepatic tissue against ASP damage observed by histopathological and immunohistochemical examinations and these findings came in agreement with Choi et al. (2009) who reported that CO Q10 inhibit liver fibrogenesis by suppressing transforming growth factor-β1 expression in mice [78].

Present study revealed that FSO was able to ameliorate all tested parameters; serum biochemical parameters and hepatic oxidative stress parameters, toward the normal values. This could be attributed to its high content of the omega-3 fatty acid, alpha-linolenic acid (ALA) and phytoestrogenic lignans that carry antioxidative potential effects [20, 79]. Naqshbandi et al. (2012) reported that FSO ameliorated hepatoxictity and other deleterious effects due to its intrinsic biochemical antioxidant properties [80]. Rajesha et al. (2006) revealed the beneficial antioxidant components of FSO help to detoxify free radicals [81]. Furthermore, it was demonstrated that dietary FSO increases antioxidant defenses through both reduced ROS generation and increased ROS detoxification [82]. However, Lee and Prasad (2003) concluded that flaxseed oil (Omega Nutrition) suppresses oxygen radical production by white blood cells due to its powerful antioxidant activity [83]. It is also reported that FSO has high content of omega-3 polyunsaturated fatty acid (PUFA) which exerts lipid lowering effect and reduction of tissue lipid peroxide (MDA) [84]. Moreover, the present work demonstrated that FSO also provided a protective effect against ASP hepatotoxicity in albino rats but less than that observed with CO Q10 based on tested parameters (biochemical and oxidative stress), histopathological and immunohistochemical examinations.

In conclusion, the present study suggest that oral administration of either CO Q10 or FSO to rats with ASP oxidative stress improves hepatic function, reduces lipid peroxidation and nitric oxide level and enhances the activity of tissue antioxidant enzymes with restoration of hepatic architecture. This improvement might be secondary to the antioxidant properties of such

substances, which attacks ROS and neutralizes their harmful effects on the liver tissue. Also, supplementation of either CO Q10 or FSO in ASP treated rats showed protective effects by reducing the extent of hepatic damage and restoring architecture and near-normal levels of antioxidants. But CO Q10 had more protective effect than FSO. However, the persistence of some histological changes in animals treated with CO Q10 and FSO might be a result of the increased production of ROS by aspartame, which overwhelms the capacity of intrinsic defense mechanisms in the cells. In addition to this, the recommended doses of CO Q10 and FSO in our study might not be sufficient to protect the liver from the toxic effect of aspartame.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Tables

Table 1: Effect of Aspartame (ASP), Co-enzyme Q10 (CO Q10) and Flaxseed oil (FSO) on Serum Biochemical Parameters in Rats.

Parameters / Group	AST (u/ml)	ALT (u/ml)	ALP (u/ml)	TP (gm/dl)	Alb (gm/dl)	TB (mg/dl)
Control	57.63^{C} ± 0.52	64.48^{C} ± 0.62	258.3^{bc} ± 1.12	0.142^{C} ± 0.01	1.975^{a} ± 0.03	0.236^{b} ± 0.01
ASP	86.26^{a} ± 0.95	102^{a} ± 0.62	380.9^{a} ± 5.27	0.613^{a} ± 0.01	1.495^{b} ± 0.08	0.303^{a} ± 0.01
CO Q10	62.04^{bc} ± 2.07	80.16^{b} ± 2.43	252.0^{C} ± 7.42	0.408^{b} ± 0.05	1.605^{b} ± 0.10	0.258^{b} ± 0.01
FSO	63.89^{b} ± 2.09	83.00^{b} ± 1.02	271.4^{b} ± 1.61	0.35^{b} ± 0.04	1.70^{b} ± 0.04	0.255^{b} ± 0.01

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; TP: total protein; Alb: albumin; TB: total bilirubin. Values are expressed as Mean ± SE, values in each column with different superscripts (a, b, c) are significantly different at P≤ 0.05. n = 6 rats/group.

Table 2: Effect of Aspartame (ASP), Co-enzyme Q10 (CO Q10) and Flaxseed oil (FSO) on Hepatic Oxidative Stress Parameters in Rats.

Parameter / Group	GSH (mg/g. tissue)	CAT (U/g. tissue)	SOD (U/g. tissue)	MDA (nm/gm tissue)	NO (µmoles/l)
Control	94.36^{b} ± 1.2	9.243^{b} ± 0.21	2.795^{b} ± 0.06	57.07^{c} ± 1.28	27.95^{C} ± 0.61
ASP	64.87^{d} ± 0.48	7.141^{d} ± 0.117	2.026^{c} ± 0.01	75.85^{a} ± 0.73	64.22^{a} ± 0.93
CO Q10	98.05^{a} ± 0.62	9.815^{a} ± 0.11	3.055^{a} ± 0.08	65.08^{b} ± 1.97	52.42^{b} ± 2.51
FSO	79.84^{c} ± 1.98	7.91^{c} ± 0.16	2.685^{b} ± 0.04	60.57^{bc} ± 2.05	50.65^{b} ± 1.69

GSH: reduced glutathione; CAT: catalase; SOD: sodium oxide dismutase; MDA: malondyaldehyde; NO: nitric oxide. Values are expressed as Mean ± SE, values in each column with different superscripts (a, b, c) are significantly different at P≤ 0.05. n = 6 rats/group.

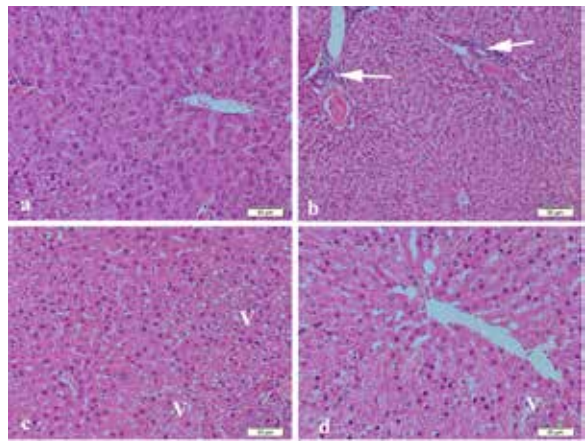

Figure 1. (a) Liver of control rats with intact normal hepatocytes (H&Ex40), (b) ASP group (250 mg/L/day) showed diffuse vacuolation and fibroblast proliferation with lymphocytic infiltration (arrows) (H&Ex40), (c) CO Q10 group (500 mg/kg/day) with normal hepatocytes and mild vacuoles (V) (H&Ex40), and (d) FSO group (15 %/diet/day) showed mild to moderate vacuolation (V) (H&Ex40).

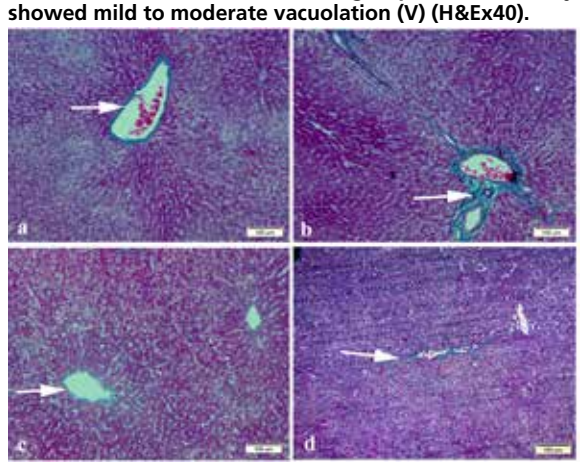

Figure 2. Masson's Trichrome of liver; (a) control normal group with intact normal hepatocytes, (b) ASP treated group (250 mg/L/day) with mild proliferation of fibroblasts in hepatic areas, (c) CO Q10 group (500 mg/kg/day) showed normal hepatocytes, and (d) FSO group (15%/diet/day) showed minimal proliferation of fibroblasts.

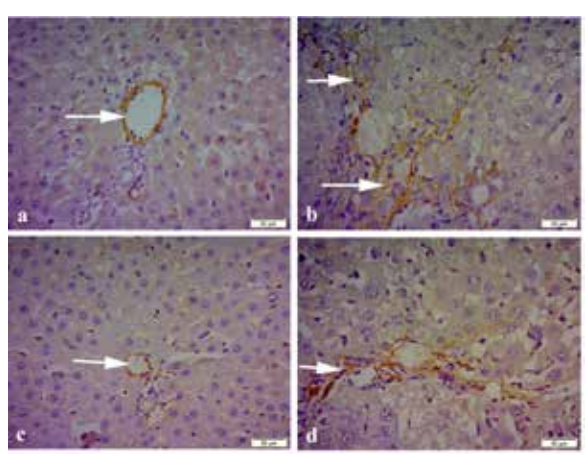

Figure 3. α-SMA Immunohistochemical reaction of liver;(a) control normal group, (b) ASP group (250 mg/L/day), (c) CO Q10 group (500 mg/kg/day), and (d) FSO group (15%/diet/day). Arrows refer to expression of α-SMA positively stained brown and appeared around the hepatic areas.

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