Effects of alpha-lipoic acid on high fructose-induced hepatic pathology

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Objectives:

Fructose is a sweet-tasting sugar that is found naturally in fruits and some vegetables. Its consumption has markedly increased [1]. Many prepared foods and drinks include high-fructose corn syrup (HFCS) in several countries [2]. Controversial reports have been published about fructose and HFCS with respect to their effects on metabolism and health based on the limited data from human or animal studies [1,3,4]. In general, these studies are in agreement with the high consumption of sugar-sweetened beverages and foods being associated with obesity and an elevated risk of type 2 diabetes [2-4]. There is a theoretical argument concerning the potential adverse metabolic effects of fructose consumption based on the well-established differences in hepatic metabolism between fructose and glucose [5]; there are significant differences between fructose and glucose metabolism in the liver. However, it is important to point out that the metabolic pathways of fructose and glucose in the liver are interactive [4]. Alpha-lipoic acid (ALA) is a strong antioxidant that reduces AGE level to improve insulin sensitivity in the skeletal muscle and liver. However, no studies have investigated the use of ALA as an antioxidant against HSCF-induced hepatic damage. Therefore, the aim of this study was to investigate the protective effects of ALA on hepatic damage induced by chronic HFCS consumption.

Methods:

This protocol was carried out according to the Animal Care and Use Committee guidelines of Suleyman Demirel University and approved by its Animal Ethics Committee (22/08/2013-03). For these experiments, 24 female Sprague Dawley rats weighing 250–300 g each were randomly allocated into three groups. HFCS was obtained from Toposmanoglu (Isparta, Turkey) and contained approximately 24% fructose and 28% dextrose in a syrup of 73% total solids. HFCS has been named according to the ratio of commercial fructose it contains (F30, F42, and F55 contain 30%, 40%, and 55% fructose, respectively). For this study, a prepared 30% solution of F30 was given in drinking water for 10 weeks. Thioctacid in 600 mg tablets (Meda Pharma, Turkey), which are a commercial form of ALA, were dissolved in distilled water and used for treatment. A single dose per day at 100 mg/kg was given orally for the last 6 weeks of the experiment. For the HFCS group, only the F30 solution was given in drinking water for 10 weeks. For the ALA group, the same dose of F30 was given and ALA was also administered during the last 6 weeks of the experiment (4 weeks after the first HCSF treatment). No drugs were administered to the control (CON) group. Each group comprised 8 rats, and at the end of 10 weeks, 24 h after the last ALA administration, they were euthanized.Liver samples were collected for biochemical analyses. For histopathological examination; liver samples were collected during necropsy and fixed 10% neutral formalin solution. After two days fixation samples were routinely processed and embedded in paraffin, 5µm sectioned by a Leica RM 2155 rotary microtome. Then sections were stained with hematoxylin- eosin (HE) and examined by light microscope. Selected liver sections were stained to demonstrate the presence of a caspase-3 using the streptavidin–biotin peroxidase technique.

Results:

The results of the biochemical analysis are shown in Table 1. Serum AST levels increased in the HFCS group compared with the CON group and decreased in the ALA-treated group. Parameters marking hepatic damage such as AST ($P < 0.05$) increased in the HFCS group in comparison with the CON group and decreased after ALA treatment ($P < 0.05$) in comparison with the HFCS group. MDA levels in the hepatic tissues increased in the HFCS group in comparison with the CON group ($P < 0.001$) and decreased in the ALA treatment group in comparison with the HFCS group ($P < 0.05$). CAT activities diminished in the HFCS-treated hepatic tissues ($P > 0.05$) and enhanced after ALA treatment ($P > 0.05$; Table 1). Body weights increased in the HFCS group and decreased in the ALA treatment group, but the difference was not significant. No abnormalities were observed in the liver of any of the groups at gross examination. Significant lipidosis was observed with numerous hepatocytes in the HFCS group ($P < 0.001$) when histopathologically examined. No lipid accumulation was observed in the CON group, whereas slight lipidosis was observed in the ALA treated group ($P < 0.001$). Immunohistochemistry revealed that apoptotic activity significantly increased in the HFCS group in comparison with the ALA-treated and the CON groups ($P < 0.001$; Fig. 1 and Table 3).

Table 1: Oxidative stress markers of hepatic tissues.

	MDA (µmol/mg protein)	CAT (ku/mg protein)
Control	0.024 ± 0.008	0.053 ± 0,007
HFCS	0.057 ± 0.014	0.048 ± 0,011
HFCS +ALA	0.038 ± 0.009	0.060 ± 0.007
P values	CON-HFCS (<0.05) CON-HFSC+ALA (NS) HFCS-HFSC+ALA (<0.05)	CON-HFCS (NS) CON-HFSC+ALA (NS) HFCS-HFSC+ALA (NS)

Table 2: Body weight and AST changes between the groups.

		Control	HFCS	HFCS +ALA	P value
Body weight change (g)	Pre-experiment	261.00 ± 11.20	259.00 ± 12.00	262.10 ± 12.80	CON-HFCS (NS) CON-HFSC+ALA (NS) HFCS-HFSC+ALA (NS)
	Post-experiment	280.10 ± 10.90	293.50 ± 40.30	293.00 ± 43.50	CON-HFCS (<0.05) CON-HFSC+ALA (<0.05) HFCS-HFSC+ALA (NS)
	Change	19.10	34.50	30.90	CON-HFCS (<0.05) CON-HFSC+ALA (<0.05) HFCS-HFSC+ALA (NS)
AST (U/L)		95.81 ± 15.80	121.95 ± 27.29	99.99 ± 11.49	CON-HFCS (NS) CON-HFSC+ALA (NS) HFCS-HFSC+ALA (NS)

Table 3: Statistical analysis of histopathological and immunohistochemical scoring results.

	Control	HFCS	HFCS+ALA	P values
Histopathological scores	0.00±0.00	1.87±1.12	0.62±0.74	CON-HFCS (<0.001) CON-HFSC+ALA (NS) HFCS-HFSC+ALA (<0.05)
Caspase-3 immunohistochemisytry	0.42±0.53	1.87±0.99	0.50±0.75	CON-HFCS (<0.01) CON-HFSC+ALA (NS) HFCS-HFSC+ALA (<0.01)

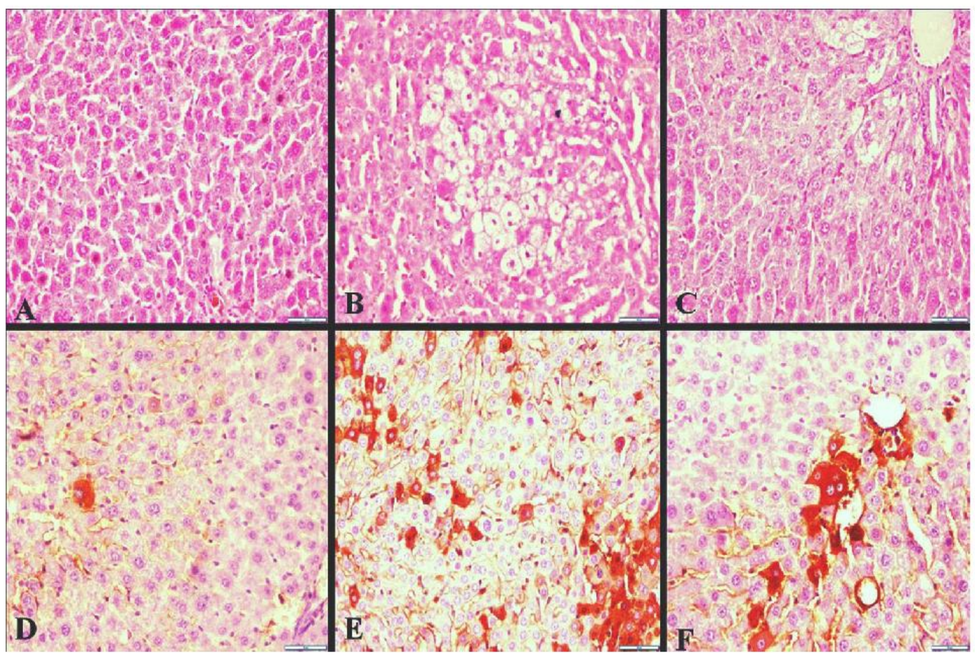

Figure 1: (A) Histopathological appearance of the liver in the control (CON) group, HE.; **(B)** Marked lipidosis in liver hepatocytes from the group treated with high-fructose corn syrup (HFCS), HE.; **(C)** In the alpha-lipoic acid (ALA) treated group, slight lipidosis in liver hepatocytes can be seen, HE.; **(D)** Caspase-3 activity of the CON group, as assessed by streptavidin biotin pull-down; **(E)** A marked increase in caspase-3 activity can be seen in the HFCS group, as assessed by streptavidin biotin pull-down; **(F)** A slight caspase-3 reaction can be seen in the ALA treated group as assessed by streptavidin biotin pull-down. Bars = 50 µm.

Conclusions:

In conclusion, chronic consumption of HFCS induced oxidative stress and apoptosis that caused hepatic damage. Research on hepatocytes and their role in nutrient metabolism has gained a renewed impetus in recent years.

References:

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