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An experimental study to investigate the impact of Aspirin and Vitamin C therapy on fructose induced hepatic and pancreatic damage

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ABSTRACT

It is assumed that excessive fructose consumption is associated with the risk of developing various diseases, especially metabolic disease. The aims of this study were two fold: 1) Does liver and pancreatic damage occur due to excessive fructose consumption 2) If damage occurs, can we reduce this damage by using (ASA) and Vit. C. The rats were divided randomly into five groups of eight as follows: Group1- control; Group2- corn syrup (Fructose: F; 30% F solution); Group3- F and ASA (F+10 mg/kg/day, ASA, oral); Group4- F and Vit. C (F+200 mg/kg/day, Vit. C, oral); Group5- F, ASA and Vit C (F+A+C -same dose administration, respectively). The rats were sacrificed 24 h after the last application at the end of the 6th week, and their blood serum, liver and pancreas tissues were taken and evaluated histologically and biochemically. It was found that serum cholesterol and AST levels were significantly lower in the F+C and F+A+C groups, and ALT and TG levels were significantly lower in the F+A+C group compared to the F group ($p<0.05$). ASA and Vit.C significantly decreased TNF-alpha, which is responsible for inflammatory cytokines, and amylase, which is an indicator of pancreatic damage, compared to group F ($p<0.05$). As a result of histopathological examinations, the protective effects of ASA and Vit. C against the damage and fattening caused by fructose in the liver and pancreas tissues were observed. The use of antioxidants seems to be an important and promising option in preventing the development of nonalcoholic fatty liver disease due to excessive fructose consumption and the accompanying diseases.

Key words: Aspirin, Vitamin C, Corn syrup, Fructose, Rat, Organ damage

1. INTRODUCTION

Corn syrup is preferred by manufacturers as an alternative liquid sweetener to sucrose and glucose syrups because it extends the shelf life of foods, is sweeter, prevents drying, crystallizes late, is suitable for fermentation, does not mask the original taste and is cheaper (1). High fructose corn syrup (HFCS) is obtained by chemical and enzymatic hydrolysis of corn starch containing amylose and amylopectin to corn syrup containing mostly glucose, followed by isomerization of glucose to fructose in corn syrup (2). Depending on the presence of glucose and fructose in free forms during HFCS production, different formulations (HFCS-42, HFCS-55 and HFCS-90) can be created by changing the fructose-glucose ratios (3,4).

Glucose and fructose are two sugars with the same chemical formula ($C_6H_{12}O_6$), and instead of the aldehyde group on the first carbon of glucose, there is a keto group on the second carbon of fructose (5). The sweetness ratio of fructose is approximately 2 times higher than glucose and 1.5 times higher than sucrose (1). Fructose is metabolized primarily in the liver and has the same energy load as glucose (6).

Fructose is metabolized differently from glucose. The fact that fructose is directly converted to fatty acids, unlike glucose, creates an important difference (7). Increased fatty acid synthesis can increase circulating fatty acids and storage fat, which may lead to lipotoxicity, which reduces the insulin sensitivity of cells due to the production of fatty acids in tissues other than adipose tissue (8). In addition, since fructose does not create a feeling of satiety like glucose, ready-made foods and beverages containing high fructose are consumed more (6).

The most widely used form of fructose is the HFCS form, which is used commercially as a sweetener (9). There are many studies showing that the consumption of sugar-sweetened foods such as HFCS above the daily requirement is associated with the development of metabolic syndrome, obesity, fatty liver, type 2 diabetes and hypertension. Previous studies suggest that potentially having unexpectedly more fructose in the diet could cause this (10-13). The World Health Organization (WHO) recommends sugar consumption less than 10% of daily calories and preferably less than 5% (14). The Food and Drug Administration (FDA) states that it supports the recommendation in the 2010 Dietary Guidelines for Americans to limit consumption of all added sugars, including HFCS and sucrose (15).

Studies have proven that various inflammatory mediators such as prostaglandin, tumor necrosis factor alpha (TNF-α) and reactive oxygen species (ROS) are responsible for organ damage (16). There are studies on the direct/indirect contribution of these mediators and oxidants to the damage caused by the long-term use of corn syrup (17-19).

The antioxidant agent Vitamin C commonly called as ascorbic acid, which is the subject of various studies, can also act as a prooxidant that initiates reactive oxygen radical reactions, especially in the presence of transition metals (20). Vit. C is also an effective antioxidant that can reduce or prevent H_2O_2-induced lipid peroxidation and the formation of 8-hydroxydeoxyguanosine in nucleic acids (21). Aspirin (acetylsalicylic acid; ASA) irreversibly inhibits cyclooxygenase (COX) through acetylation of a serine residue at the active site of COX and inhibits the formation of proinflammatory prostaglandins (22). Various mechanisms have also been suggested focusing on the potential role of ASA as an antioxidant (23). Theoretically, the anti-inflammatory effect is expected to restore the normal redox balance and abolish the oxidative stress associated with inflammation (24).

It is thought that it will be possible to reduce the harmful effects that may occur due to excessive consumption of corn syrup, which is frequently used in the food industry, with prostaglandin inhibitor ASA (25,26) and/or antioxidants (27,28), which are substances that inhibit inflammatory mediators.

2. MATERIALS AND METHODS

2.1. Chemicals

ASA was purchased as Aspirin (500 mg tablets) from Bayer (Istanbul, Türkiye). Vit. C was purchased as Redoxon (1000 mg tablets) from Bayer (Istanbul, Türkiye). Fructose, which is a commercial form of corn syrup (approximately 24% fructose and 28% dextrose) was obtained from Toposmanoglu (Isparta, Türkiye).

2.2. Animals

40 healthy approximately 15-week-old 250–350 g male Sprague-Dawley rats obtained from Suleyman Demirel University Experimental Animals and Medical Research Application and Research Center were used. The experimental protocol was approved by Suleyman Demirel University Faculty of Medicine, Laboratory Animals Ethics Committee (Protocol # 2012/01). The rats were maintained with a 12 h light:12 h dark cycle at 21 °C with free access to food and water *ad libitum*. The rats were kept in Euro type 2 cages with only a rat in each cage for 6 weeks. All rats in the experiment were fed a standard commercial chow diet.

2.3. Experimental design

Animals were divided randomly into five groups of

Table 1. Experimental design

Groups	Rats	Admistration	Dose	Time
1: Control (C)	8	-	-	6 weeks
2: Fructose (F)	8	Fructose	30% F solution	6 weeks
3: (F+A)	8	Fructose + Aspirin	30% F solution + 10 mg/kg/day-oral	6 weeks
4: (F+C)	8	Fructose + Vitamin C	30% F solution + 200 mg/kg/day-oral	6 weeks
5: (F+A+C)	8	Fructose + (Aspirin+Vitamin C)	30% F solution + 10 mg ASA + 200 mg vit. C/kg/day-oral	6 weeks

eight as follows: group 1-control (C- limitless feed and water); group 2-corn syrup (Fructose: F, 30% F solution); group 3-corn syrup and Aspirin (ASA) (30% F + 10 mg/kg/day, oral); group 4-corn syrup and Vitamin C (Vit C) (30% F + 200 mg/kg/day, oral); group 5-corn syrup, ASA and Vit C (30% F + 10 mg/kg/day, oral + 200 mg/kg/day, oral). 30% F solution was administered to each of the rats except the control group for 6 weeks in drinking water (Table 1). In order to evaluate the consumption of corn syrup we added to the drinking water, the drinking water was changed twice a week and the amount consumed by the rats was monitored. The treatment course lasted 6 weeks for all groups. All rats were sacrificed by intraperitoneal ketamine 10% (Alpha, Alfas IBV) and 2% xylazine (Alfaz's, Alfas IBV) anesthesia at a day after last drug administration. At the end of the experiment, blood, liver and pancreas were taken and evaluated biochemically and histologically. The obtained serum and tissue samples were stored at -80 °C until the date of analysis.

2.4. Biochemistry

Biochemical analyzes were carried out at Suleyman Demirel University Faculty of Medicine, Department of Medical Biochemistry Laboratories. Blood samples were collected from the abdominal aorta, centrifuged (Heraeus Biofuge Stratos; Kendo Laboratory Products, Osterode- Germany) at 3.000 × g for 10 min and serum was collected. Liver function tests, lipid profile levels, fasting blood glucose and insulin levels, TNF-α, amylase and lipase levels were evaluated in the serum samples. The pancreas and liver tissues, which were placed in phosphate buffer, were weighed separately and diluted 10-fold with 50mM phosphate buffer (pH 7.4). Homogenization was completed by sonicating with Janke&Kunkel Ultraturrax T-25 (Germany) brand tissue shredder and then UW-2070 Bandeun Electronic (Germany) brand sonicator. Tissue samples were centrifuged by cooled centrifugation at 5.000 rpm for 15 min and the supernatant was taken. Total oxidants and antioxidants status were evaluated in the tissues supernantants.

2.5. Oxidative stress biomarkers

Microprotein levels in the supernatants of the homogenized samples were measured spectrophotometrically using the Beckman Coulter AU 5800 (United States) autoanalyzer. TAS and TOS parameters were studied by spectrophotometric method in tissue supernatants using Rel Assay Diagnostic Assay kits and Beckman Coulter AU 5800 (United States) autoanalyzer. The results were calculated by dividing by the microprotein level. Establishing of OSI, which is an determinative parameter of oxidative stress level, the ratio of TOS to TAS was calculated using the following formula:

$$\text{OSI (AU)} = [(\text{TOS,}\mu\text{mol H2O2 Eqv./ L})) / (\text{TAS,}\mu\text{mol Trolox equivalent/L}) \text{ X } 100]$$

2.6. Histopathology

Histological samples from liver and pancreas tissues were stained with Hematoxylin and Eosin (H&E). It was examined histopathologically according to the previous study of Abdel Wahhab et al., (29). The slides were examined using a light microscope (Olympus BX50) and photographed. Classification of degeneration compared to normal tissue was evaluated according to the method of Refaiy et al., (30).

Histological (structural) evaluation of experimental parameters was scored.

- (negative score): No structural changes,
+ (1 positive score): Mild,
++ (2 positive scores): Moderate,
+++ (3 positive scores): It represents a serious structural change.

2.7. Statistical Analysis

Statistical analyzes of the experiment were made using the SPSS 15.0 program. All results are expressed as mean ± standard error. In histological analysis, Kruskal-Wallis test was used for semi-qualitative evaluation and non-parametric Mann-Whitney U test was used for pairwise comparisons. The valuation of *p* below 0.05 were considered significant.

3. RESULTS

3.1. Biochemistry

3.1.1. Liver Function Tests, Lipid Profile, Fasting Blood Glucose and Insulin Levels

It was observed that the administration of (F) (103.40±8.17 mg/dL) significantly increased the cholesterol level compared to the control (75.29±6.60 mg/dL) group ($p<0.05$). Cholesterol levels in the (F+C) (90.88±5.49 mg/dL) and (F+A+C) (87.31±8.37 mg/dL) groups were found to be significantly lower when compared with the (F) group ($p<0.05$) (Table 2). Compared to the control (137.13±15.35 U/L) group, the AST level was significantly increased in the fructose-administered group (20.5.38±37.61 U/L) ($p<0.05$). When the (F+C) (106.25±14.05 U/L) and (F+A+C) (104.63±12.60 U/L) groups were compared with the (F) group, AST levels were found to be significantly lower. ($p<0.05$). It was observed that the ALT level (F+A+C) (42.50±8.47 U/L) group decreased significantly compared to the (F) group ($p<0.05$) (Table 2). It was found that there was no significant change in ALP and GGT levels between the groups ($p>0.05$). It was observed that the triglyceride level increased significantly in the (F) (42.81±7.45 mg/dL) group compared to the control (29.19±15.12 mg/dL) group ($p<0.05$). When we compared the triglyceride levels in the (F+A)

Table 2. Liver biochemical parameters of the groups

	Group 1	Group 2	Group 3	Group 4	Group 5
Glucose (mg/dL)	93.88±21.25	181.57±22.41[a]	157.25±30.41	164.86±31.74	179.43±25.57
Insulin (mg/dL)	0.12±0.06	0.28±0.13[a]	0.45±0.30	0.42±0.14	0.53±0.12
HOMA indeks	0.57±0.29	2.55±0.89[a]	2.66±1.12	3.80±2.50[b]	4.62±2.47[b]
Cholesterol (mg/dL)	75.29±6.60	103.40±8.17[a]	100.10±4.08	90.88±5.49[b]	87.31±8.37[b]
AST (U/L)	137.13±15.35	205.38±37.6[a]	125.63±16.43	106.25±14.05[b]	104.63±12.60[b]
Triglyceride (mg/dl)	29.19±15.12	42.81±7.45[a]	32.34±9.09[b]	36.65±11.14	32.03±8.99[b]
ALT (U/L)	52.25±10.46	53.50±12.88[b]	45.00±13.67	44.88±10.34	42.50±8.47[b]
ALP (U/L)	141.63±18.49	164.71±64.64	152.12±35.85	164.14±17.53	167.43±65.59
GGT (U/L)	1.13±0.35	1.50±0.76	1.63±0.74	1.50±0.53	1.50±0.53

Group 1: (C), **Group 2:** (F), **Group 3:** (F+A), **Group 4:** (F+C), **Group 5:** (F+A+C) a: $p<0.05$ vs Control, b: $p<0.05$ vs Fructose

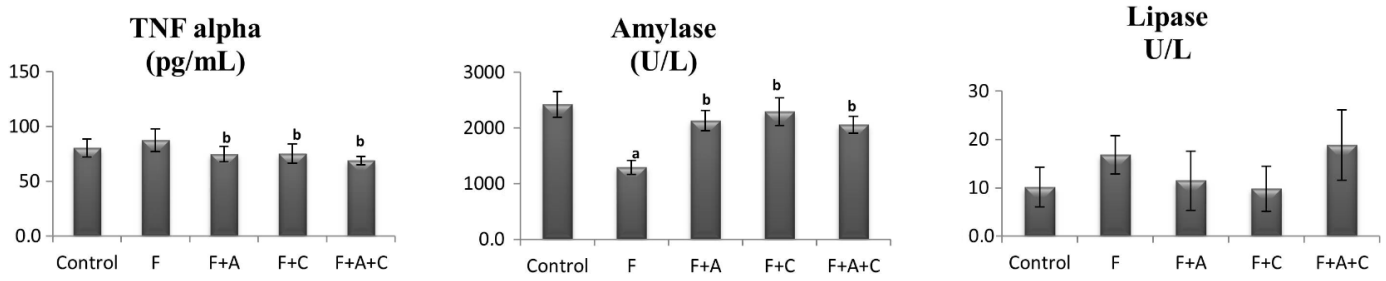
(32.34±9.09 mg/dL) and (F+A+C) (32.03±8.99 mg/dL) groups with the (F) group, it was observed that the triglyceride levels decreased significantly (p<0.05) (Table 2). It was observed that the fasting glucose level (F) (181.57±22.41 mg/dL) increased significantly in the control (93.88±21.25 mg/dL) group, but there was no significant change in the treatment groups (p<0, 05). The insulin level (F) (0.28±0.13 mg/dl) group increased significantly compared to the control (0.12±0.06 mg/dl) group (p<0.05), but there was no significant change in the treatment groups (p<0.05). Insulin resistance was calculated by using the HOMA model [HOMA-IR = fasting insulin (mU/mL)*fasting glucose (mmol/L)/22.5]. It was observed that Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) level (F) (2.55±0.89) group increased significantly compared to the control (0.57±0.29) group (p<0.05).

3.1.2. Serum TNF-alpha, Amylase and Lipase Levels

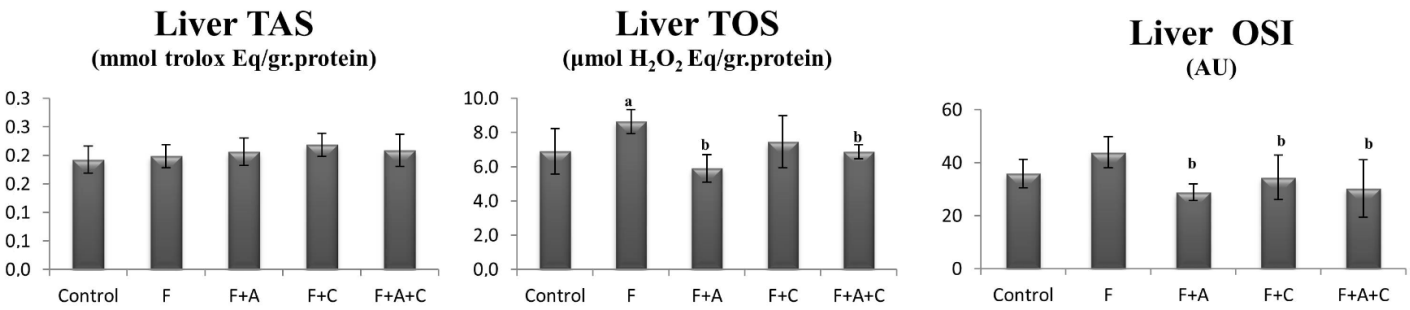
TNF-α levels of treatment (F+A) (74.73±7.19 pg/mL), (F+C) (75.23±7.19 pg/mL) and (F+A+C) (69.96±3.75 pg/mL) groups were found to be significantly lower when compared with the (F) group (p<0.05), while this decrease was found to be higher in the (F+A+C) group compared to the control group (p<0.05). Serum amylase levels (F) were found to be significantly higher in the (2423.25±230.72 U/L) group compared to the control (1294.00±126.14 U/L) group (p<0.05). Treated (F+A) (2133.50±180.47 U/L), (F+C) (2293.90±248.63 U/L) and (F+A+C) (2060.40±) 152.96 U/L) groups compared to the (F) group showed a significant decrease in amylase levels (p<0.05). It was observed that there was no significant change in serum lipase levels between the groups (Graphic 1).

3.2. Oxidative stress biomarkers

When the TAS levels in the liver tissue were compared, no significant difference was found between the groups. It was observed that TOS levels in liver tissue (F) (8.6±0.71 µmol H_2O_2 Eq/gr.protein) group increased significantly compared to the control (6.9±1.33 µmol H_2O_2 Eq/gr.protein) group (p< 0.05). In the treated (F+A) (5.9±0.80 µmol H_2O_2 Eq/gr.protein) and (F+A+C) (6.9±0.41 µmol H_2O_2 Eq/gr.protein) groups (F) group, a significant decrease was observed in liver TOS levels (p<0.05). Treated (F+A) (28.79±3.12 AU), (F+C) (34.43±8.43 AU) and (F+A+C) (30.25±10.86 AU)) groups compared to



Graphic 1. TNF alpha, Amylase and Lipase results of the groups



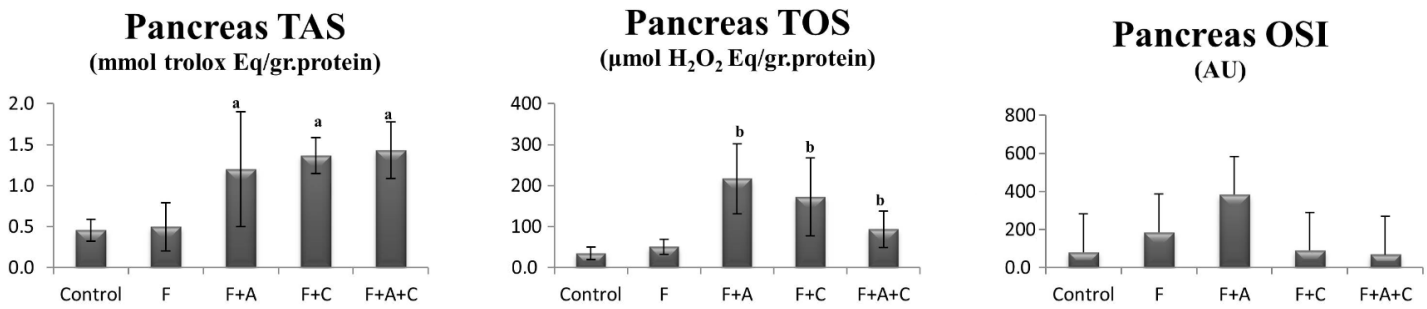
Graphic 2. Hepatic TAS, TOS and OSI values of the groups

the (F) group showed a significant decrease in liver OSI levels ($p<0.05$) (Graphic 2). Treated (F+A) (1.2±0.70 mmol trolox Eq/gr.protein), (F+C) (1.4±0.22 mmol trolox Eq/gr.protein) and (F+A +C) (1.4±0.34 mmol trolox Eq/gr.protein) groups, when compared with the (F) group, pancreatic TAS levels were found to be significantly increased ($p<0.05$). Treated (F+A) (216.9±85.37 µmol H_2O_2 Eq/gr.protein), (F+C) (172.7±95.12 µmol H_2O_2 Eq/gr.protein) and (F+A) +C) (93.6±44.72 µmol H_2O_2 Eq/gr.protein) groups, when compared with the (F) group, pancreatic TOS levels were found to be significantly increased ($p<0.05$). When the OSI calculations in the pancreatic tissue were examined, no significant difference was found between the groups ($p>0.05$) (Graphic 3).

3.3. Histopathology

While normal histological structures were observed in the liver tissue sections of the control group; statistically ($p<0.05$) significant macro and microvesicular adiposity, hemorrhage, mononuclear cell infiltrations, cells with pycnotic nuclei, granular degenerations in hepatocytes, bile duct proliferation and sinusoidal dilatation and degenerations were observed in the liver tissues of the fructose fed experimental group compared to the control group. A statistically significant improvement was detected in the group given (F+A) compared to the group (F) ($p<0.05$). In the group given (F+C), similar findings were obtained in the group given (F+A), but it was observed that the curative effect on fatty liver was not as effective as Aspirin. In the group given (F+A+C), it was determined that fatty liver decreased statistically ($p<0.05$) more (Figure 1).

As a result of the histological images of the pancreatic tissues of the control group, no pathological structure was found. Statistically significant ($p<0.05$) changes such as increase in intralobular duct diameter, increase in fat tissue in



Graphic 3. Pancreatic TAS, TOS and OSI values of the groups

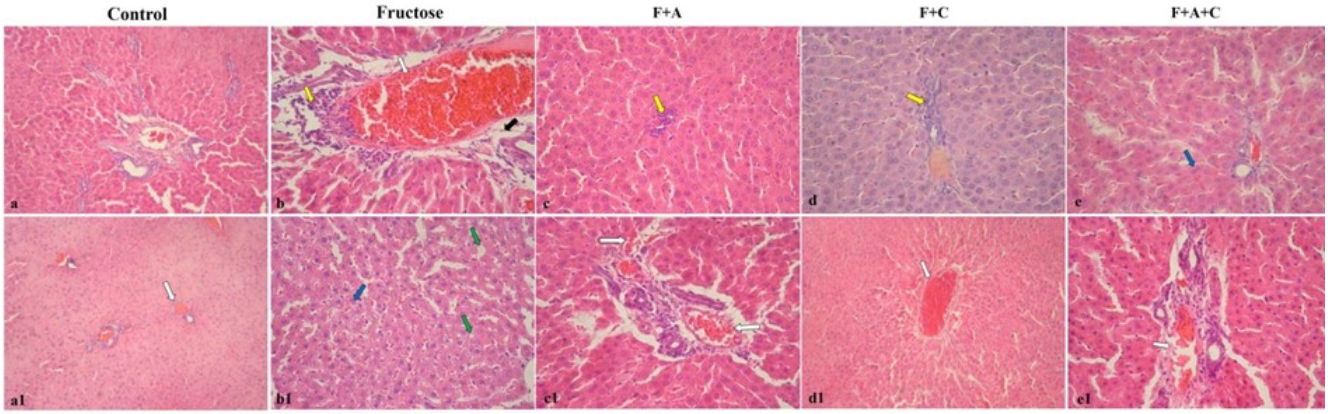


Figure 1. Control group liver tissue section, (a): portal area; (a1): central vein (white arrow). **(F) group liver tissue section,** (b): leukocyte migration (yellow arrow), hemorrhage and congestion (white arrow), connective tissue degeneration (black arrow); (b1): microvesicular lubrication (green arrow), pycnotic nuclei (blue arrow). **(F+A) group liver tissue section;** (c): leukocyte migration (yellow arrow); (c1): vascular congestion and hemorrhage (white arrow). (**F+C) group liver tissue section;** (d): leukocyte migration (yellow arrow); (d1): vascular congestion and hemorrhage (white arrow). **(F+A+C) group liver tissue section;** (e): pycnotic nuclei, (blue arrow); (e1): vascular hemorrhage (white arrow) (H-E X 20,40)

the exocrine region, and congestion and proliferation in the vessels in the connective tissue were detected in the pancreatic tissue of the fructose-treated rats compared to the control group. In the (F+A) group, a statistically significant ($p<0.05$) decrease was observed in the increase in the diameter of the intralobular canal and the increase in fat tissue in the exocrine region, while congestion and proliferation in the vessels in the connective tissue were higher than in the control and (F) groups. In the group given (F+C) compared to the group (F), the increase in fat tissue in the intralobular canal diameter and in the exocrine region, and a decrease in vascular congestion and proliferation in the connective tissue were observed. In the (F+A+C) group, compared to the other groups (except for the control), a decrease in the increase in the diameter of the intralobular canal, a decrease in the increase in fat tissue especially in the exocrine region, and a decrease in the congestion and proliferation of the vessels in the connective tissue ($p<0.05$) were determined as a result of statistical and microscopic examinations (Figure 2).

4. DISCUSSION

It is seen that fructose originating from corn, which is increasingly used in the food industry, has become an important threat to human health as a result of studies (31). In studies with a diet containing high fructose; it has been reported that there is a significant relationship between various pathological changes, oxidative stress, glucose intolerance, insulin resistance, type 2 diabetes, obesity, hypertension, cardiovascular and metabolic diseases (32-34).

The present study was carried out to evaluate whether the use of ASA and Vit. C can prevent or reduce possible organ damage due to high fructose consumption by examining different biochemical and histopathological parameters of liver and pancreatic tissues of rats. Aspirin and Vitamin C treatment in rats with nonalcoholic fatty liver disease induced by fructose consumption; histopathology of the liver and pancreas resulted in a remarkable improvement in liver function tests, lipid profile and serum proinflammatory cytokine levels.

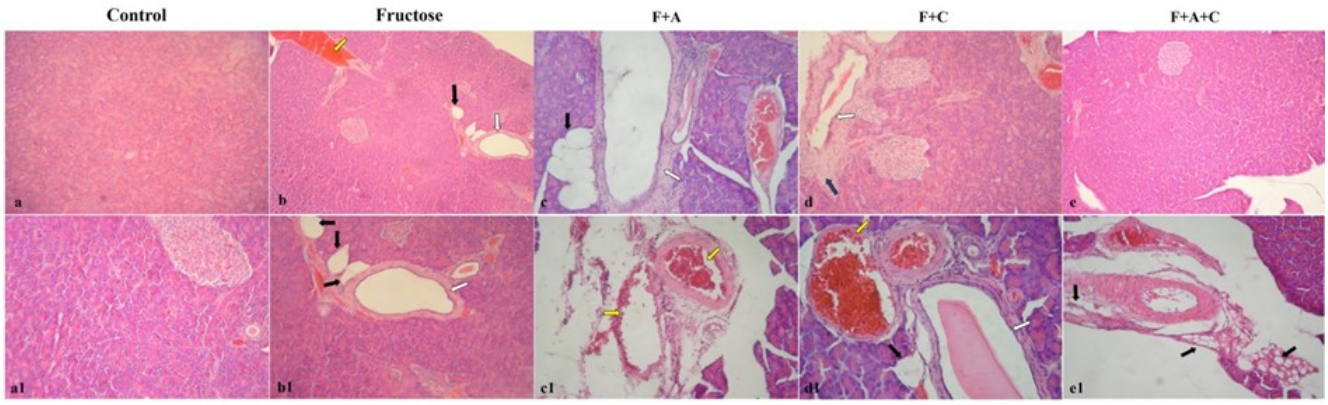


Figure 2. Control group pancreatic tissue section; (a): exocrine pancreatic region; (a1): exocrine and endocrine (islet of Langerhans) pancreatic region. **(F) group pancreatic tissue section;** (b): increase in intralobular canal diameter (white arrow), increase in fat tissue in the exocrine region (black arrow), congestion in vessels in the connective tissue (yellow arrow); (b1): increase in intralobular canal diameter (white arrow), increase in fat tissue in the exocrine region (black arrow). **(F+A) group pancreatic tissue section;** (c): increase in intralobular canal diameter (white arrow), increase in fat tissue in the exocrine region (black arrow); (c1): congestion and proliferation of vessels in connective tissue (yellow arrow). **(F+C) group pancreatic tissue section;** (d): fibrosis in the exocrine region (blue arrow), increase in intralobular duct diameter (white arrow); (d1): increase in intralobular canal diameter (white arrow), adipose tissue in the exocrine region (black arrow), congestion and hemorrhage (yellow arrow). **(F+A+C) group pancreatic tissue section;** (e): close to the control group; (e1): fibrosis and increased adipose tissue in connective tissue septa (black arrow) (H-E X 20,40)

Ascorbic acid is necessary for the catabolism of cholesterol in the liver. In its deficiency, the conversion of cholesterol to bile acids slows down and cholesterol accumulation occurs in the liver (35). It is known that Vit. C inhibits the oxidative processes of lipids and lipoproteins in the cell membrane with its antioxidant ability (36). The increase in cholesterol in the F group and significantly lower ($p<0.05$) in the F+C and F+A+C groups is due to the antioxidant property of Vit. C and its role in cholesterol metabolism (37). The increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels is the predominant laboratory finding in patients with fatty liver; usually, AST, ALT, or both are mild to moderately elevated. (38). Alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) levels may be elevated in nonalcoholic fatty liver disease (NAFLD) (39,40). There is some evidence that an increase in GGT is a sensitive marker of insulin resistance. Therefore, increased GGT is among the early biochemical findings for NAFLD (41). In present study, serum AST level was found in group F; significantly ($p<0.05$) higher than the control group; It was found to be significantly ($p<0.05$) lower in the F+C and F+A+C groups compared to the F group. According to this result, it can be said that Vit. C has a lowering effect on AST. In the AST F+A group; it was found to be significantly ($p<0.05$) higher than the F+C and F+A+C groups. This elevation may be due to salicylate hepatotoxicity that may occur due to the use of aspirin (42). Serum ALT level in group F; significantly higher than the control group; it was found to be significantly ($p<0.05$) lower in the F+A+C group compared to the F group. This decrease shows the positive effects of Vit. C on AST and ALT. While there was an increase in serum ALP and GGT levels compared to the control, no significant difference was found between all groups ($p>0.05$). Serum triglyceride levels in group F; significantly ($p<0.05$) higher than the control group; a significant ($p<0.05$) decrease was observed in the F+A and F+A+C groups compared to the F group. The earliest detected abnormality related to fructose beverage consumption was the elevation in triglyceride level. The liver is an organ that plays an important role in many physiological processes such as the regulation of systemic glucose and lipid metabolism. Since the liver is the place where fructose is metabolized to fatty acids, there is intra -organ adiposity (43,44).

Studies have shown an increase in insulin resistance in NAFLD compared to the normal population (41,45). In a study by Mager et al., it was found that the plasma AST, ALT, GGT, insulin, triglyceride and HOMA-IR levels increased significantly after 0, 3 and 6 months of observation by adding fructose to the diets of children aged 7-14 years diagnosed with NAFLD (46). In present study, serum fasting glucose levels were compared to the control group; It was found to be significantly higher ($p<0.05$) in the F, F+A, F+C and F+A+C groups. Serum insulin levels compared to the control group; it was found to be significantly higher ($p<0.05$) in the F, F+A, F+C and F+A+C groups. While HOMA-IR was found to be significantly ($p<0.05$) higher in the F group than in the control group, in the F+C and F+A+C groups; It was found significantly ($p<0.05$) higher than the F group. While adding fructose to the diet increases insulin resistance (47); it can be said that the administration of ASA and Vit. C did not have a positive effect on insulin resistance, and even increased insulin resistance.

TNF-α production is one of the earliest reactions in liver injury, triggering the production of other inflammatory cytokines, and an increase in mitochondrial permeability and reactive oxygen species (ROS) (48). As a result, apoptosis is accelerated and the susceptibility to damage increases in cells that do not undergo apoptosis (49). In present study, serum TNF-α levels were found to be higher in the F group when compared

to the control group (p>0.05), while TNF-α was decreased significantly in the F+A, F+C and F+A+C groups compared to the F group (p<0.05). It can be said that this decrease is due to the antioxidant properties of ASA and Vit. C. Demonstration of beneficial effects of antioxidants can be considered as indirect evidence for the role of oxidative stress in liver damage (50).

The main function of ascorbic acid as an antioxidant is to prevent the formation of lipid hydroperoxides (20). Vit. C is one of the most potent inhibitors of lipid peroxidation in human plasma and is an effective scavenger of various ROS such as superoxide hydroxyl radical (51). The study reveals that the administration of Vit. C together with ASA reduces the immunological, hematological and biochemical changes caused by ASA (52). In the study; considering the OSI calculations in liver tissue, in the F+A, F+C and F+A+C groups; it was found to be significantly (p<0.05) lower than the F group. This is an indicator of oxidative stress caused by fructose (53), and it can be said that ASA and Vit. C reduce oxidative damage in the liver.

Pancreatic isoamylase and lipase determinations together with amylase are widely used in pancreatic diseases (54). In studies, AST, ALT, Total Bilirubin and ALP were found to be statistically significantly higher in cases of biliary acute pancreatitis (55,56). In the study, serum amylase levels were found to be significantly higher (p<0.05) when we compared the F group with the control; In F+A, F+C and F+A+C groups; it was found to be significantly (p<0.05) lower than the F group. Serum lipase level of group F; It was found to increase, although not significantly, compared to the control group (p>0.05). In a study by Mollaoğlu et al., they found that the vitamin E+C combination against pancreatic damage decreased lipase activity and increased amylase activity; they argued that this increase in amylase may be due to Vit. C. When the OSI calculations in the pancreatic tissue were examined, no significant difference was found between the groups (57). The negative effects of high fructose nutrition on organs vary depending on the fructose concentration and the duration of feding (58).

Increased TNF-α expression triggers triglyceride production and leads to liver steatosis (59). It has been shown that fructose at a concentration of 30% and above can upregulate TNF-α expression, which is reflected in histopathological findings (60). When the histopathological results of liver were evaluated; statistically significant (p<0.05) macro and microvesicular adiposity, hemorrhage, mononuclear cell infiltrations, cells with pycnotic nuclei, granular degenerations in hepatocytes, bile duct proliferation and sinusoidal dilatation and degeneration were observed in group F compared to the control group. In the group in which ASA and Vit. C were given combined treatment, fatty liver was found to be statistically reduced (p<0.05). Histopathological results of applied F30 were similar to those of other studies (60,61). When the histopathological results of pancreas were evaluated; statistically significant (p<0.05) changes were detected in the F group compared to the control group, such as an increase in the diameter of the intralobular canal, an increase in fat tissue in the exocrine region and congestion and proliferation in the vessels in the connective tissue. Van Geenen et al. suggested that interlobular and total pancreatic steatosis is associated with NAFLD, and that total pancreatic steatosis is a statistically significant predictor for the presence of hepatic steatosis (62). Ou et al. showed that subjects had significantly more NAFLD and fatty pancreas with glycemic increase in a cross-sectional study (63). The data support the existence of nonalcoholic fatty liver disease as a strong predictor for the development of the metabolic syndrome, with potentially relevant clinical implications for the diagnosis, prevention and treatment of metabolic syndrome (64).

5. CONCLUSION

As a result; the obtained biochemical parameters and histopathological results supported each other and showed nonalcoholic fatty liver and nonalcoholic pancreatic adiposity due to corn syrup consumption. With ASA and Vit. C treatment in rats with nonalcoholic fatty liver due to fructose consumption; a remarkable improvement in liver function tests, lipid profile and serum levels of proinflammatory cytokines was observed in the histopathology of the liver and pancreas. Chronic consumption of fructose from corn syrup triggers many diseases along with fatty liver. The use of ASA and Vit. C together or trying different antioxidants seems to be an important and promising option in the prevention and treatment of nonalcoholic fatty liver disease and the diseases it will bring.

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