Original Article

Effect of *Helichrysum plicatum Subs. plicatum* on Fructose-induced Nonalcoholıc Fatty Liver

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ABSTRACT

Background: The Helichrysum genus of the Asteraceae family comprises 500 species. Fifteen *Helichrysum* species are found in Turkey. This genus is used in traditional medicine for several beneficial effects.

Objectives: This study explored *Helichrysum plicatum subsp. plicatum* (HPsP) effects on fructose-induced nonalcoholic fatty liver disease (NAFLD).

Methods: Thirty-five adult male Wistar rats weighing 200–250 g were divided into 5 groups: Control, HPsP, fructose (F), HPsP+fructose, and double-dose HPsP+fructose. Fructose (40%) was then added to the water. HPsP extract was administered at doses of 4 and 8 mg/kg. All treatments were performed for 8 weeks.

Results: The F, F+HPsP, and double-dose groups exhibited higher palmitic acid (16:0) levels than the control group ($P<0.001$). F+HPsP treatment enhanced palmitoleic acid levels ($P<0.001$). The F and F+HPsP groups showed reduced levels of linolenic (18:3 n-3), eicosadienoic (20:2, n-6), and arachidonic (20:4 n-6) acids ($P<0.001$, $P<0.01$, and $P<0.05$, respectively). The F, F+HPsP, and double-dose groups showed lower docosahexaenoic acid (22:6, n-3) levels ($P<0.05$). Retinol and in HPsP treated group and ($P<0.05$) K1 vitamin levels increased in all groups compared to control group ($P<0.001$). The F, F+HPsP, and HPsP groups had lower vitamin K-2 and D-3 levels ($P<0.001$ and $P<0.01$, respectively). The F, HPsP, F+HPsP, and double-dose groups had higher cholesterol levels ($P<0.05$, $P<0.01$, $P<0.001$, respectively).

Conclusion: HPsP extract altered fructose-induced fatty liver composition and levels of reduced glutathione and vitamins A, D, E, and K. Unexpectedly, high liver cholesterol levels require treatment.

Keywords: Fructose, *Helichrysum plicatum*, Lipophilic vitamins, NAFLD, Omega-3

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Introduction

Nonalcoholic fatty liver disease (NAFLD) has been observed in alcohol-abstaining individuals. It is also associated with hypertriglyceridemia and insulin resistance. NAFLD comprises several stages of liver disease, from progression toward steatosis and steatohepatitis (NASH). They can eventually progress to liver cirrhosis. NAFLD is associated with hypertriglyceridemia, hypertension, dyslipidemia, insulin resistance, inflammation, lipid peroxidation, oxidative stress, and increased free-radical activity (Li et al., 2016) studies have highlighted the importance of calorie reduction and macronutrient composition (e.g, carbohydrate and fat) (Bernal et al., 2013).

With decreasing physical activity and increasing caloric intake from fast foods in industrialized countries, people's dietary behaviors have changed, and the prevalence of metabolic diseases has increased. Fructose is an essential component of plant carbohydrates and a critical component of the human diet (Köseler et al., 2018; Abbasi et al., 2021). While average human fructose consumption was 16-20 g/d in the past, industrialization and new diet styles have increased fructose consumption to approximately 85-100 g/d (Drewnowski & Bellisle, 2007; Bergheim et al., 2008).

High fructose consumption causes insulin resistance and increases triglyceride levels (Bergheim et al., 2008). Fructose is taken up by fructokinase in the liver and is converted to fructose-1 phosphate, which is then converted to triose phosphate by aldolase. Thus, fructose passes three steps before glycolysis, according to glucosides. Interstingly, 60% conversion of fructose to fatty acids—more than that of glucose—increases lipid levels more than glucose. Increased levels of liver fats due to excess carbohydrate consumption have raised the prevalence of many liver-damaging diseases, including steatosis, steatohepatitis, advanced fibrosis, and sometimes cirrhosis (Bergheim et al., 2008; Ketaby & Mohammad-Sadegh, 2023). Many ethnobotanical remedies have been studied for their potential to alleviate NAFLD symptoms. However, none have demonstrated the desired results in rigorously controlled studies (Ebrahimi-Mameghani et al., 2014; Kołota & Głąbska, 2021).

Approximately 500 species of *Helichrysum* belonging to the Asteraceae family have been annotated worldwide. Twenty *Helichrysum* species have been identified in Turkey; 15 are endemic. They are named by different local names like "altın otu," "ölmez çiçek," "sarı çiçek," by different regions' peoples in Turkey (Davis et al., 1988). *Helichrysum plicatum subsp. plicatum* (HPsP) is 4–42 cm tall and contains the highest levels of flavonoids among *Helichrysum* species. People in Turkey use it to treat wounds and burns, reduce kidney stones, enhance urinary function, and ameliorate ear pain (Ozturk et al., 2014; Aydin, 2020; Kültür et al., 2021).

Previous studies have demonstrated antimicrobial, antioxidant, anti-inflammatory, antidiabetic, and antimitotic properties of HPsP, probably due to its rich flavonoid content (Albayrak et al., 2010; Apaydin et al., 2017; Kramberger et al., 2021; Akinfenwa et al., 2021). The antilipidemic effects of HPsPs (grown in Anatolia) have not yet been studied on NAFLD. This study aimed to investigate the effects of HPsP on fatty acid composition and other biochemical parameters of NAFLD.

Materials and Methods

Animals and treatments

Theity-five 8-month-old male Wistar rats were obtained from the Fırat University Experimental Animal Research Center. The animals were housed in air-conditioned rooms on a 12:12 h dark: Light cycle with ad libitum access to rat chow and water. The animals were randomly divided into 5 groups, with 7 in each group. The control group (I) was fed a standard diet. Besides the standard diet, other groups were further treated as described. Group (II) received nutrients with extra HPsP (4 mg/kg by oral gavage). Group (III) was fed fructose (F) (40% w/v in drinking water). Group (IV) received nutrients with F and HPsP (4 mg/kg by gavage), and group (V) received nutrients with F and double dose HPsP (8 mg/kg by gavage). After 8 weeks of treatment, the rats were anesthetized with ether. Harvested tissues were stored at -85°C until further analysis. Vitamin levels were determined using high-performance liquid chromatography (HPLC), and fatty acid levels were determined using gas chromatography (GC).

Lipid extraction

Lipids were extracted from liver tissue using the approach established by Hara and Radin (1978), in which aliquots containing lipids were esterified in 2% methanolic sulfuric acid (Christie, 1989). GC was used to assess fatty acid composition.

Determination of fatty acid composition

To identify and quantify fatty acid methyl esters, 1 mL of n-hexane was added to each sample. Samples were

then vortexed and placed in vials. A Shimadzu GC-2010 plus chromatograph FID, an AOC-20i auto-injector, and an autosampler were used for GC analysis. Fatty acids were separated using Permabond (Machhery–Nagel, Germany) silica-coated capillary columns 60 m×0.25 mm I.D., 0.20 µm (Supelco, Sigma, USA). After the column temperature reached 120˚C, it was adjusted to 220˚C at a rate of 5˚C/min, then held constant at 220˚C for 15 minutes. Temperature settings for the injector and FID were 240˚C and 280˚C, respectively. Nitrogen was used as the gas phase, with a column flow rate of 1.2 mL/min, 43 cm/s. Comparisons were made possible by identifying methyl esters and analyzing standard external mixtures under the same conditions.

Determination of lipophilic vitamins

Tissue samples were homogenized by adding 5 mL of isopropyl alcohol/hexane (2:3: v/v) per gram. Samples were then vortexed and centrifuged for 10 minutes at 6000 rpm at 4˚C. Supernatants were collected and evaporated with nitrogen before adding 1 mL of an equal volume of acetonitrile/methanol mixture to each container. A SUPELCOSIL™ LC-18-DB HPLC column (250×4.6 mm, 5 µm)_was employed to examine lipophilic vitamins. A mixture of 75% acetonitrile and 25% methanol was used as the mobile phase. The mobile-phase flow rate was maintained at 1 mL/min. The column's temperature was maintained at 30˚C. Detection wavelengths were 320 nm for retinol (vitamin A) and 215 nm for vitamins K1, tocopherol (Toc), D2, and D3. Analysis results were presented as g/g (Katsanidis & Addis, 1999).

Cholesterol analysis

Isopropanol/ethanol/acetonitrile (30:70, v/v) was used to extract liver samples. The extracts were vortexed and centrifuged at 6000 g and 4˚C for ten minutes. Aliquoted supernatants were deposited in vials for HPLC analysis (Shimadzu). The mobile phase was a mixture of isopropyl alcohol and acetonitrile (30:70 v/v). The flow rate was set at 1 mL/min (Bragagnolo & Rodriguez, 2003). HPLC analyses were conducted using a SUPELCOSILTM LC-18 250×4.6 mm, 5 µm column. Detection was made by UV absorption at 202 nm, and column oven temperature was set at 30˚C (Katsanidis & Addis, 1999) tocotrienols, and cholesterol in muscle tissue were developed. Method A involves basic saponification of the sample, but causes losses of the γ- and δ-homologs of vitamin E. Method B does not involve saponification, thereby protecting the more sensitive homologs. Both permit rapid analysis of multiple samples and neither requires specialized equipment. These methods may provide techniques useful in simultaneous assessment of oxidative stress status (OSS. Class VP software was used to quantify external standardization.

HPLC and malondialdehyde analysis

Tissues were homogenized in a homogenizer by adding 1 mL of pure water and 1 mL of 10% perchloric acid to 0.5 g of liver tissue. Homogenized samples were placed in the centrifuge tubes (ISOLAB Garmany) and centrifuged at 10000 rpm for 5 min. Supernatants were removed and placed in vials for HPLC analysis. The malondialdehyde (MDA) mobile phase solution consisted of 0.05 g of dihydrogen phosphate, 110 mL of methanol, and 500 mL of purified water. Phosphoric acid was added to the MDA mobile phase solution to adjust pH to 3.50. Amounts determined in these analyses were reported in nmol/g.

The Karatepe method was modified for malondialdehyde analysis. A C18-ODS-3.5 µm, 4.6×250 mm column, and UV-Vis SPD-10 AVP detector for Shimadzu HPLC were used to measure MDA (Sedlak & Lindsay, 1968).

Glutathione analysis

Glutathione (GSH) content was determined using a UV spectrophotometer with a modified Sedlak and Lindsay method against pure water at a wavelength of 412 nm (Sedlak & Lindsay, 1968).

Statistical analyses

SPSS software, version 16 was used for statistical analysis. Experimental results are reported as Mean±SD and compared with control group results using analysis of variance (ANOVA) and the LSD test.

Results

Liver fatty acid compositions

All experimental groups were compared with the control group (Table 1). Oleic acid (18:1, n–9c) and eicosatrienoic acid (20:3, n-3) levels increased in the F, F+HPsP, and double-dose groups ($P<0.0001$, $P<0.01$, $P<0.0001$, $P<0.05$, respectively). Behenic acid (C 22:0) levels increased in the F and F+HPsP groups ($P<0.0001$ and $P<0.01$, respectively). Alpha-linolenic acid (18:3, n-3) and arachidonic acid (20:4, n-6) levels decreased in the F and F+HPsP groups ($P<0.0001$ and $P<0.01$, respectively). Alpha-linolenic acid (18:3, n-3) levels also decreased in the double-dose group ($P<0.0001$). Gamma linoleic acid (18:3, n-6) and docosahexaenoic

Table 1. Liver fatty acid compositions (%)

Fatty Acids	Mean±SD				
	Control Group	Fructose Group (F)	F+HPsP	Double dose Group (F+HPsP)	HPsP Group
15:0	0.59±0.08	0.39±0.01^{d}	0.32±0.02^{d}	0.28±0.02^{d}	0.45±0.03^{b}
16:0	21.48±0.39	36.81±2.35^{d}	30.18±2.78^{d}	28.51±1.10^{d}	22.57±0.64
16:1 n-9	1.60±0.13	0.79±0.22^{d}	7.04±1.14^{d}	4.24±0.10^{c}	1.57±0.12
17:0	1.24±0.15	0.38±0.02^{d}	0.40±0.02^{d}	0.42±0.03^{d}	0.85±0.11^{a}
17:1	0.68±0.03	0.69±0.01	0.62±0.06	0.39±0.01^{d}	0.64±0.04
18:0	15.94±0.42	11.27±0.73^{b}	13.87±0.96	16.53±0.55	17.48±1.05
18:1, n–7c	8.72±0.27	18.34±1.31^{d}	14.74±0.89^{d}	13.51±0.78^{b}	8.28±0.48
18:2, n–6t	0.12±0.006	0.09±0.006	0.06±0.008	0.08±0.03	0.12±0.01
18:2, n–6c	18.78±0.33	8.68±0.69^{d}	9.85±1.15^{d}	9.71±0.36^{d}	19.13±0.72
18:3, n–6	0.21±0.01	0.25±0.05	0.12±0.07^{d}	0.13±0.01^{d}	0.19±0.01
20:0	0.10±0.06	0.14±0.09	0.04±0.03	0.05±0.02	0.33±0.16
18:3, n-3	0.39±0.01	0.13±0.06^{d}	0.12±0.09^{d}	0.10±0.05^{d}	0.43±0.02
20:2, n–6	0.46±0.01	0.05±0.02^{d}	0.12±0.01^{d}	0.09±0.06^{d}	0.44±0.04
20:3, n-3	0.53±0.04	1.01±0.06^{d}	1.12±0.05^{d}	0.85±0.08^{b}	0.70 ^{b} ±0.05
22:0	0.11±0.009	0.04±0.05^{d}	0.06±0.008^{b}	0.09±0.01	0.09±0.02
20:3, n-6	0.73±0.02	0.74±0.11	0.90±0.08^{b}	0.92±0.08^{b}	0.56±0.13c
20:4, n-6	19.25±0.58	12.86±0.78^{d}	14.49±0.68c	17.02±1.01^{b}	19.36±0.75
23:0	0.17±0.005	0.07±0.006^{d}	0.07±0.007^{d}	0.9±0.006^{d}	0.17±0.005
22:2, n-6	0.16±0.01	0.15±0.02	0.18±0.02	0.09±0.007	0.17±0.01
20:5, n-3	0.02±0.006	0.009±0.0^{d}	0.01±0.002^{c}	0.01±0.003^{c}	0.05±0.03 ^{d}
24:0	0.54±0.02	0.41±0.03	0.43±0.03	0.48±0.02	0.56±0.02
24:1, n-9	0.21±0.008	0.28±0.03	0.34±0.02^{b}	0.31±0.005^{a}	0.23±0.01
22:6, n-3	4.60±0.17	4.00±0.27^{b}	3.31±0.39^{b}	3.88±0.15^{b}	4.43±0.31
Monounsaturated fatty acids	11.68±0.73	16.62±2.85^{c}	20.98±3.29^{d}	18.63±0.75^{d}	11.34±0.50
Polyunsaturated fatty acids	45.20±0.68	24.73±3.16^{d}	30.31±3.12^{d}	32.85±1.34^{d}	44.24±1.76
Saturated fatty acids	56.88	49.05^{b}	45.37^{b}	47.26^{b}	42.5^{b}
∑ Total omega-3 (N3)	5.56±0.18	5.13±0.28	4.57±0.41	4.86±0.22	5.54±0.30
∑ Total omega-6 (N6)	39.11±0.52	22.65±1.22^{d}	25.45±2.74^{d}	27.88±1.28^{d}	39.61±0.84
∑N3/∑N6	0.14±0.003	0.22±0.02^{b}	0.18±0.008^{c}	0.17±0.009^{c}	0.14±0.01

Fatty Acids	Mean±SD				
	Control Group	Fructose Group (F)	F+HPsP	Double dose Group (F+HPsP)	HPsP Group
16:1,n-7/16:0	0.07±0.005	0.02±0.004[d]	0.22±0.02[d]	0.14±0.005[d]	0.06±0.004
18:1,n-9/18:0	0.57±0.05	1.64±0.14[d]	1.10±0.13[b]	0.82±0.07	0.48±0.04
18:3,n-6/18:2,n-6	0.01±0.0006	0.03±0.009[a]	0.01±0.002	0.01±0.002	0.009±0.0008
20:4, n-6/20:3, n-6	26.24±1.44	18.64±2.12[c]	15.97±1.16[d]	18.72±1.05[c]	43.80±8.05[d]

F: Fructose; HPsP: *Helichrysum plicatum subsp. Plicatum*.

[a, b]P>0.05, [c, d]P<0.01.

acid (22:6, n-3) levels decreased in the F+HPsP group (P<0.05, P<0.001, respectively). Eicosatrienoic acid (20:3, n-3) levels were significantly increased in both groups (P<0.0001 and P<0.01, respectively). Docosadienoic acid (22:2, n-6) levels were significantly decreased in the double-dose group (P<0.05) but did not show any significant change in the other groups. No significant changes were observed in levels of eicosatrienoic acid (20:3 n-6) and lignoceric acid (24:0) in any groups (P>0.05). Arachidonic acid (20:4 n6) levels decreased in the F and F+HPsP groups (P<0.001, P<0.01, respectively). Eicosapentaenoic acid (20:5, n-3) levels increased in the double-dose group and decreased in the other groups (P<0.0001, P<0.0001, P<0,001).

Liver cholesterol, lipophilic vitamins, MDA, and GSH levels

Liver tissue cholesterol levels increased remarkably in the F, HPsP, F+HPsP, and double-dose groups compared to the controls (P<0.05, P<0.01, P<0.001, and P<0.0001, respectively). Increased retinol levels were observed in the HPsP group (P<0.05). Vitamin K-1 levels increased in the F, HPsP, F+HPsP, and double-dose groups (P<0.05, P<0.0001, P<0.001, and P<0.0001, respectively), and vitamin K2 levels decreased in the HPsP, F, F+HPsP, and double-dose groups (P<0.05, P<0.0001). Vitamin D3 levels decreased in the F, F+HPsP, and double-dose groups (P<0.01, P<0.0001). Levels of α-Toc levels markedly reduced in the double dose group (P<0.05). MDA levels were considerably higher in the F and HPsP groups (P<0.0001) and did not change in the other groups. GSH levels decreased markedly in the F, HPsP, and F+HPsP groups (P<0.0001 and P<0.01, respectively) and increased appreciably in the double-dose group (P<0.0001) (Table 2).

Discussion

Helichrysum species produce flavonoids, acetophenones, phloroglucinols, pyrones, triterpenoids, and sesquiterpenes as secondary metabolites. Flavonoids are critically important phenolics that act as free radical scavengers in higher plants. Some plant species that are exceptionally rich in flavonoids are used to treat elevated free radical-mediated diseases, including NAFLD with excessive liver fat levels (Acet et al., 2020; Aguilera et al., 2016; Sun & Qu, 2019).

Lipid-reducing treatments are recommended for NAFLD patients, with 10% weight loss recommended in the first stage. Due to lifestyle changes, a complete treatment protocol has not yet been developed. Several scientific studies have been conducted on antioxidant and phytotherapeutic effects (Guvenc et al., 2017; Choi et al., 2019). Although the initial stages of this disease are not typically severe, death may occur in later stages due to fluid retention, increased inflammation, and cirrhosis. Many studies have investigated NAFLD caused by excessive fructose consumption (Choi et al., 2019).

In this study, with the application of HPsP, the 16:1 to 16:0 ratio, as a sign of Δ9 stearoyl-coenzyme A desaturase (SCD) synthesis, surprisingly decreased in the fructose group and increased in F+HPsP and double dose groups. The insulin resistance promoted by palmitic acid (16:0) is reversed by palmitoleic acid (16:1, n-9) (Puri et al., 2007). Stearic acid (18:0) is turned over to oleic acid (18:1 n-9) by Δ9 desaturase. Δ9 desaturase activity is raised in HPsP and double dose groups. The decrease in palmitoleic acid (16:1, n9) and the increase in its advanced product, vaccenic acid (18:1, n7), are signs of enhanced Δ9 SCD synthesis.

Table 2. Changes in vitamins A, D, E, and K, cholesterol levels, and other biochemical parameters in liver tissue

Parameters	Mean±SD				
	Control Group	Fructose Group (F)	F+HPsP	Fructose+HPsP (Double Dose)	HPsP Group
Retinol (µg/g)	1.16±0.26	1.40±0.33	1.4±0.23	0.96±0.26	2.50±0.36[b]
K1 (µg/g)	0.93±0.19	2.41±0.35[b]	3.74±0.82[b]	4.87±0.32[b]	6.38±0.65[c]
K2 (µg/g)	3.86±0.26	2.24±0.17[b]	2.17±0.16[b]	1.81±0.14[b]	3.13±0.21
D3 (µg/g)	10.79±0.35	8.66±0.57[b]	8.24±0.42[b]	7.42±0.28[c]	10.14±0.35
α-Toc (µg/g)	48.53±3.38	39.88±2.88	40.97±2.42	31.33±5.35[a]	46.12±2.46
Cholesterol (nmol/g)	4.24±0.8	11.22±2.82[b]	16.27±5.36[c]	24.18±6.02[d]	8.04±0.87[d]
MDA (nmol/g)	0.22±0.01	0.39±0.02[d]	0.22±0.01	0.22±0.007	0.37±0.03[d]
GSH (µmol/g)	0.28±0.01	0.21±0.01[d]	0.25±0.01[b]	0.36±0.01[d]	0.14±0.01[d]

Abbreviations: F: Fructose; HPsP: *Helichrysum plicatum subsp. Plicatum*; MDA: Malondialdehyde; GSH: Glutathione.

[a, b]P>0.05, [c, d]P<0.01.

Reduced stearic acid (18:0) and palmitoleic acid (16:1, n-9) levels in fructose groups may be in response to fatty acid accumulation as a sign of increased lipogenesis in F and F+HPsP groups compared with the control. A rise in the monounsaturated fatty acid/saturated fatty acid ratio is related to increased lipogenesis (Puri et al., 2007).

Linoleic (18:2, n-6c) and linolenic (18:3, n-3) acids are the first substrates of n6 and n3 polyunsaturated fatty acid (PUFA) metabolism, and are essential PUFAs. Linoleic acid levels (cis-18:2, n-6) decreased in the F, F+HPsP, and double-dose groups (P<0.0001). HPsP affected gamma-linolenic (18:3, n-6) and dihomo-γ-linolenic (20:3, n-6) acids, the advanced metabolites of linoleic acid (18:2, n-6) which requires Δ6 desaturase (Δ6DS) for synthesis. Δ6DS activity is affected by HPsP treatments to varying degrees. It causes an increase in the γ-linolenic acid to linoleic acid (18:3, n-6/18:2, n-6) ratio in fructose groups (P<0.05) (Guvenc et al., 2017; Ghazali et al., 2020).

Dihomo-γ-linolenic acid (20:3, n-6) levels also support these changes. Levels of their advanced product, the essential fatty acid arachidonic acid (20:4, n-6), were elevated in the F+HPsP and double dose groups. Phospholipase A2 releases arachidonic acid (20:4, n-6) from cell membranes, and phospholipase C releases arachidonic acid from phosphatidylinositol bisphosphate. The released arachidonic acid (20:4, n-6) is converted into pro-inflammatory prostaglandins, thromboxanes, and leukotrienes. The increased synthesis of leukotrienes and inflammatory prostaglandins can be explained by the elevated consumption of arachidonic acid (20:4, n-6) in the fructose groups. The increase in arachidonic acid (20:4, n-6) levels in the F+HPsP group was surprising (Guvenc et al., 2017; Ghazali et al., 2020).

Surprisingly, n-3 fatty acid changes were also detected. Linolenic acid (18:3, n-3), eicosapentaenoic acid (20:5, n-3), and docosahexaenoic acid (22:6, n-3) decreased in the fructose group. HPsP increased eicosapentaenoic (20:5n-3) acid levels but did not affect linolenic (18:3, n-3) and docosahexaenoic (22:6, n-3) acid levels. Docosahexaenoic acid (22:6, n-3) and eicosapentaenoic acid (20:5, n-3) have anti-inflammatory, antiproliferative, immunomodulatory, and metabolic effects (Guvenc et al., 2017; Ghazali et al., 2020).

The diet's increased omega-6 to omega-3 fatty acids ratio exacerbates NAFLD development (Allard et al., 2008). The n-6/n-3 ratio of F, F+HPsP, and double-dose groups also decreased. Allard et al. (2008) reported reduced levels of hepatic n-6 and n-3 PUFAs, as also noted by Allard et al. (2008). HPsPs increased cholesterol levels in a dose-dependent manner. However, the mechanism remains unclear.

The anti-inflammatory and antifibrotic properties of Vitamin D and its role in cell regulation and differentiation likely function via metabolic processes. Vitamin D deficiency is associated with insulin resistance and metabolic syndrome (Kitson & Roberts, 2012; Rhee et al., 2013; Kaywanloo et al., 2022). Vitamin D levels in the groups receiving F decreased, similar to previous studies

compared to those in the control group. This decrease in vitamin D levels may be due to a decrease in vitamin D-binding protein levels. HPsPs do not affect liver vitamin D levels (Rhee et al., 2013; Jablonski et al., 2013).

As an electron donor, GSH is the most important cellular antioxidant, which scavenges free radicals in its reduced form. Decreased GSH levels correlate strongly with increased free radical production, increasing oxidative stress. Decreased GSH levels, antioxidant activity, and accumulation of hepatic inflammation are essential sources of ROS overproduction in NAFLD (Liu et al., 2015). Increased oxidative stress due to low GSH levels may cause oxidative modification of lipoproteins. This modification can accumulate cholesterol and triglycerides, causing insulin resistance (Jain et al., 2014).

The antioxidant defense system is weakened by the overproduction of free radicals that deplete antioxidant levels via lipid peroxidation, making the liver susceptible to oxidative damage. MDA levels, which can indicate lipid peroxidation, were considerably higher in the F and HPsP groups than in the control groups, as expected from the results of other studies, and the application of HPsP improved MDA and GSH levels. In particular, the double dose significantly increased GSH levels. Toc levels indicate free radicals (Kitson & Roberts, 2012; Serviddio et al., 2013).

Vitamin E is an effective chain-breaking antioxidant that scavenges peroxyl, superoxide, and hydroxyl radicals. It also protects cells against lipid peroxidation. Vitamin E consists of tocopherols (α, β, γ, δ) and tocotrienols (α, β, γ, δ). α-Toc is the most abundant tocopherol and is important in inhibiting lipid oxidation. In cell membranes, low-density lipoprotein and polyunsaturated lipids are protected from oxidation by α-Toc (Li et al., 2016).

Decreased antioxidant defense levels have been generally observed in liver disease. Decreased α-Toc levels in liver diseases have been observed, consistent with our results, and HPsP has demonstrated no effect on α-Toc levels (Di Sario et al., 2007). Vitamin E supplementation slows the progression of NAFLD to NASH (Elizabeth et al., 2010). Fructose application decreased Toc (important in clearing free radicals during metabolism) levels in all groups. Surprisingly, HPsP did not show the expected effect.

Vitamin K is a fat-soluble vitamin formed from phylloquinone (K1) and menaquinone (K2) synthesized by intestinal bacteria. Menaquinones are of bacterial origin and are produced by microbiota (exp., the Firmicutes). The distal intestine synthesizes endogenous vitamin K2 (Knapen et al., 2018; Wagatsuma et al., 2019). Vitamin K2 is mainly found in fermented milk products and is produced by lactic acid bacteria in the gut. Phylloquinone (K1) is of plant origin and binds to the membranes of plant chloroplasts. In animals, vitamin K3 (menadione) is metabolized to vitamin K2. Vitamin K3 is a synthetic vitamin added to animal feed as a source of vitamin K (Wagatsuma et al., 2019). Leafy green vegetables, including soybeans, cottonseed, canola, and olives, are good sources of vitamin K and may be consumed by overweight and obese individuals (Booth & Suttie, 1998; Kimmons et al., 2006). Vitamin K positively influences glucose regulation. Decreased insulin resistance is associated with elevated plasma levels of vitamin K1 (Varsamis et al., 2021).

Vitamin K deficiency is associated with increased body fat, and triglyceride-rich lipoproteins elevate circulating vitamin K1 levels (Shea et al., 2010). Low vitamin K levels decrease urinary gamma-carboxyglutamic acid excretion (Ferland et al., 1993). Owing to its ability to increase insulin production and sensitivity, diets containing high amounts of vitamin K are associated with a decreased incidence of type 2 diabetes mellitus. Another study has suggested that vitamin K2 intake may benefit metabolism (Booth, 2012). NAFLD is often associated with metabolic syndrome. The leading phyla in the gastrointestinal microbiota are Firmicutes, Bacteroidetes, and Proteobacteria. The elevated triglyceride levels and central obesity significantly reduced diversity among these phyla. Steatosis and obesity have been linked with the Firmicutes/Bacteroidetes ratio. NAFLD increases Firmicutes that may cause hepatic steatosis by regulating fatty acid inflow and lipogenesis, potentially exacerbating hepatic steatosis (Raman et al., 2013; Chen et al., 2019).

In all groups receiving HPsP, K1 vitamin levels were elevated compared to the control group. Vitamin K2 levels decreased in the F and HSP groups. Currently, the effect of microbial diversity on intestinal bacteria has remained unclear. HPsP may also increase metabolic activity. The mechanism underlying the decrease in vitamin K2 levels in the F group is unknown but may involve the firmicute/bacteria ratio, which decreases with obesity or NAFLD.

The chemical composition and other properties of HPsP in Turkey have been investigated by several researchers (Demir et al., 2009; Taşkın et al., 2020). HPsH produces compounds such as helichrysums A and B, apigenin, naringenin, isoastragalin, isosalopurposide, isoquercitrin, dicaffeoylquinic acid, and luteolin. Apigenin protects

against nonalcoholic fatty liver disease by decreasing lipid accumulation and ameliorating insulin resistance (Jung et al., 2016). This study is the first to elucidate biochemical parameters for fructose-induced HPsP, including the liver's lipophilic vitamins and fatty acid composition. The study also demonstrated that HPsP application increases liver total cholesterol. Taken together, our results provide experimental confirmation of the health value of HPsP application to fructose-induced rats. The mechanisms by which HPsP application increases liver cholesterol are unclear and require further research.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Animal Ethics Committee of Fırat University (2014/122) and performed in accordance with the rules of the European Economic Community (EEC, 1986).

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Authors' contributions

Conceptualization, supervision, investigation and writing-original draft: Mehmet Guvenc, and Ibrahim Dogan; Writing–review & editing: Mehmet Guvenc; Data collection: Ibrahim Dogan.

Conflict of interest

The authors declared no conflicts of interest.

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References

Acet, T., Ozcan, K., & Zengin, G. (2020). An assessment of phenolic profiles, fatty acid compositions, and biological activities of two Helichrysum species: H. plicatum and H. chionophilum. *Journal of Food Biochemistry, 44* (2), e13128. [DOI:10.1111/jfbc.13128] [PMID]

Abbasi, M., Kaboutari Kataj, J., Cheraghi, J., & Zendehdel kheibari, M. (2021). Antihyperglycemic and antihyperlipidemic effects of hydroalcoholicextract of ferulago angulta in experimental hyperlipidemic rats. *Iranian Journal of Veterinary Medicine, 15*(2), 208-219. [DOI:10.22059/IJVM.2020.292328.1005042]

Aguilera, Y., Martin-Cabrejas, M. A., & González de Mejia, E. (2016). Phenolic compounds in fruits and beverages consumed as part of the mediterranean diet: Their role in prevention of chronic diseases. *Phytochemistry Reviews, 15,* 405-423. [Link]

Akinfenwa, A. O., Sagbo, I. J., Makhaba, M., Mabusela, W. T., & Hussein, A. A. (2022). Helichrysum genus and compound activities in the management of diabetes mellitus. *Plants, 11* (10), 1386. [DOI:10.3390/plants11101386] [PMID]

Albayrak, S., Aksoy, A., Sağdiç, O., & Budak, Ü. (2010). Phenolic compounds and antioxidant and antimicrobial properties of Helichrysum species collected from eastern Anatolia, Turkey. *Turkish Journal of Biology, 34* (4), 463-473. [Link]

Allard, J. P., Aghdassi, E., Mohammed, S., Raman, M., Avand, G., & Arendt, B. M., et al. (2008). Nutritional assessment and hepatic fatty acid composition in nonalcoholic fatty liver disease (NAFLD): A cross-sectional study. *Journal of Hepatology, 48*(2), 300–307. [DOI:10.1016/j.jhep.2007.09.009] [PMID]

Apaydin Yildirim, B., Kordali, S., Terim Kapakin, K. A., Yildirim, F., Aktas Senocak, E., & Altun, S. (2017). Effect of Helichrysum plicatum DC. subsp. plicatum ethanol extract on gentamicin-induced nephrotoxicity in rats. *Journal of Zhejiang University. Science. B, 18*(6), 501–511. [DOI:10.1631/jzus.B1500291] [PMID]

Aydin, T. (2020). Secondary metabolites of Helichrysum plicatum DC. subsp. plicatum flowers as strong carbonic anhydrase, cholinesterase and α-glycosidase inhibitors. *Zeitschrift fur Naturforschung. C, Journal of Biosciences, 75*(5-6), 153–159. [PMID]

Bergheim, I., Weber, S., Vos, M., Krämer, S., Volynets, V., Kaserouni, S., & McClain, C. J., et al. (2008). Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: Role of endotoxin. *Journal of Hepatology, 48*(6), 983-992. [DOI:10.1016/j.jhep.2008.01.035] [PMID]

Bernal, C., Martín-Pozuelo, G., Lozano, A. B., Sevilla, A., García-Alonso, J., & Canovas, M., et al. (2013). Lipid biomarkers and metabolic effects of lycopene from tomato juice on liver of rats with induced hepatic steatosis. *The Journal of Nutritional Biochemistry, 24*(11), 1870-1881. [DOI:10.1016/j.jnutbio.2013.05.003] [PMID]

Booth S. L. (2012). Vitamin K: Food composition and dietary intakes. *Food & Nutrition Research, 56,* 10.3402/fnr.v56i0.5505. [DOI:10.3402/fnr.v56i0.5505] [PMID]

Booth, S. L., & Suttie, J. W. (1998). Dietary intake and adequacy of vitamin K. *The Journal of Nutrition, 128*(5), 785–788. [DOI:10.1093/jn/128.5.785] [PMID]

Bragagnolo, N., & Rodriguez-Amaya, D. B. (2003). Comparison of the cholesterol content of Brazilian chicken and quail eggs. *Journal of Food Composition and Analysis, 16*(2), 147-153. [DOI:10.1016/S0889-1575(02)00129-1]

Chen, Y. H., Chiu, C. C., Hung, S. W., Huang, W. C., Lee, Y. P., & Liu, J. Y., et al. (2019). Gnotobiotic mice inoculated with Firmicutes, but not Bacteroidetes, deteriorate nonalcoholic fatty liver disease severity by modulating hepatic lipid metabolism. *Nutrition Research (New York, N.Y.), 69,* 20–29. [DOI:10.1016/j.nutres.2019.07.001] [PMID]

Choi, E., Jang, E., & Lee, J. H. (2019). Pharmacological activities of Alisma orientale against nonalcoholic fatty liver disease and metabolic syndrome: Literature review. *Evidence-Based Complementary and Alternative Medicine: eCAM, 2019,* 2943162. [DOI:10.1155/2019/2943162] [PMID]

Christie, W.W. (1989). *Gas chromatography and lipids: A practical guide.* Oily Publisher. [Link]

Davis, P., Miller, R., & Tan, K. (1988). *Flora of Turkey.* Edinburg: Edinburgh University Press. [Link]

Demir, A., Mercanoglu, B., Aslan, M., Yesilada, E., & Aykut, A. S. (2009). Antimicrobial effect of Helichrysum plicatum subsp. plicatum. *Pharmaceutical Biology, 47*(4), 289-97. [DOI:10.1080/13880200802590434]

Drewnowski, A., & Bellisle, F. (2007). Liquid calories, sugar, and body weight. T*he American Journal of Clinical Nutrition, 85*(3), 651-661. [DOI:10.1093/ajcn/85.3.651] [PMID]

Ebrahimi-Mameghani, M., Aliashrafi, S., Javadzadeh, Y., & AsghariJafarabadi, M. (2014). The effect of chlorella vulgaris supplementation on liver enzymes, serum glucose and lipid profile in patients with nonalcoholic fatty liver disease. *Health Promotion Perspectives, 4*(1), 107-115. [PMID]

Ferland, G., Sadowski, J. A., & O'Brien, M. E. (1993). Dietary induced subclinical vitamin K deficiency in normal human subjects. *The Journal of clinical investigation, 91*(4), 1761-1768. [DOI:10.1172/JCI116386] [PMID]

Ghazali, R., Mehta, K. J., Bligh, S. A., Tewfik, I., Clemens, D., & Patel, V. B. (2020). High omega arachidonic acid/docosahexaenoic acid ratio induces mitochondrial dysfunction and altered lipid metabolism in human hepatoma cells. *World Journal of Hepatology, 12*(3), 84–98. [DOI:10.4254/wjh.v12.i3.84] [PMID]

Guvenc, M., Guvenc, A. N., Yılmaz, Ö., & Tuzcu, M. (2017). Effect of resveratrol on fatty acid compositons and lipofilic vitamins of fructose induced nonalcoholic fatty liver. *Pakistan Journal of Zoology, 49* (6), 2103-21011. [DOI:10.17582/journal.pjz/2017.49.6.2103.2111]

Hara, A., & Radin, N. S. (1978). Lipid extraction of tissues with a low-toxicity solvent. *Analytical Biochemistry, 90*(1), 420–426. [PMID]

Izadi, A., Aliasghari, F., Gargari, B. P., & Ebrahimi, S. (2020). Strong association between serum Vitamin D and Vaspin Levels, AIP, VAI and liver enzymes in NAFLD patients. *International Journal for Vitamin and Nutrition Research, 90* (1-2), 59-66. [DOI:10.1024/0300-9831/a000443] [PMID]

Jablonski, K. L., Jovanovich, A., Holmen, J., Targher, G., McFann, K., & Kendrick, J., et al. (2013). Low 25-hydroxyvitamin D level is independently associated with nonalcoholic fatty liver disease. *Nutrition, Metabolism, and Cardiovascular Diseases: NMCD, 23*(8), 792–798. [DOI:10.1016/j.numecd.2012.12.006] [PMID]

Jain, S. K., Micinski, D., Huning, L., Kahlon, G., Bass, P. F., & Levine, S. N. (2014). Vitamin D and L-cysteine levels correlate positively with GSH and negatively with insulin resistance levels in the blood of type 2 diabetic patients. *European Journal of Clinical Nutrition, 68*(10), 1148-1153. [DOI:10.1038/ejcn.2014.114] [PMID]

Jung, U. J., Cho, Y. Y., & Choi, M. S. (2016). Apigenin ameliorates dyslipidemia, hepatic steatosis and insulin resistance by modulating metabolic and transcriptional profiles in the liver of high-fat diet-induced obese mice. *Nutrients, 8*(5), 305. [DOI:10.3390/nu8050305] [PMID]

Katsanidis, E., & Addis, P. B. (1999). Novel HPLC analysis of tocopherols, tocotrienols, and cholesterol in tissue. *Free Radical Biology and Medicine, 27*(11), 1137-1140. [DOI:10.1016/S0891-5849(99)00205-1]

Kaywanloo, M., Ahmadi Hamedani, M., Jebeli Javan, A., Emadi Chashmi, H., & Rakhshani Zabol, F. (2022). Effect of parenteral Vitamin D3 supplementation in several doses during a six-day period on total antioxidant capacity in healthy holstein bulls. *Iranian Journal of Veterinary Medicine, 16*(1), 79-86. [DOI:10.22059/IJVM.2021.314273.1005142]

Ketaby, M., & Mohammad-Sadegh, M. (2023). Pre- and postpartum serum concentration of adiponectin, leptin, and ghrelin and their ability to predict the reproductive performance and milk production indexes in holstein dairy cows. *Iranian Journal of Veterinary Medicine, 17*(3), 217-230. [DOI:10.32598/IJVM.17.3.1005266]

Kimmons, J. E., Blanck, H. M., Tohill, B. C., Zhang, J., & Khan, L. K. (2006). Associations Between Body Mass Index and the prevalence of low micronutrient levels among US Adults. *Medscape General Medicine,8*(4), 59. [PMID]

Kitson, M. T., & Roberts, S. K. (2012). D-livering the message: The importance of vitamin D status in chronic liver disease. *Journal of Hepatology, 57*(4), 897-909. [PMID]

Knapen, M. H. J., Jardon, K. M., & Vermeer, C. (2018). Vitamin K-induced effects on body fat and weight: Results from a 3-year vitamin K2 intervention study. *European Journal of Clinical Nutrition, 72*(1), 136-141. [DOI:10.1038/ejcn.2017.146] [PMID]

Kołota, A., & Głąbska, D. (2021). Dietary intake of milk thistle seeds as a source of silymarin and its influence on the lipid parameters in nonalcoholic fatty liver disease patients. *Applied Sciences, 11*(13), 5836. [DOI:10.3390/app11135836]

Köseler, E., Kızıltan, G., Türker, P. F., Saka, M., Ok, M. A., & Bacanlı, D., et al. (2018). The effects of glucose and fructose on body weight and some biochemical parameters in rats. *Progress in Nutrition, 20* (1), 46-51. [Link]

Kramberger, K., Jenko Pražnikar, Z., Baruca Arbeiter, A., Petelin, A., Bandelj, D., & Kenig, S. (2021). A comparative study of the antioxidative effects of helichrysum italicum and helichrysum arenarium infusions. *Antioxidants (Basel, Switzerland), 10*(3), 380. [DOI:10.3390/antiox10030380] [PMID]

Kültür, Ş., Gürdal, B., Sarı, A., & Melikoğlu, G. (2021). Traditional herbal remedies used in kidney diseases in Turkey: An overview. *Turkish Journal of Botany, 45*(4), 296-287. [DOI:10.3906/bot-2011-32]

Li, J., Cordero, P., Nguyen, V., & Oben, J. A. (2016). The role of vitamins in the pathogenesis of nonalcoholic fatty liver disease. *Integrative Medicine Insights, 11*, 19-25. [DOI:10.4137/IMI.S31451] [PMID]

Liu, W., Baker, S. S., Baker, R. D., & Zhu, L. (2015). Antioxidant mechanisms in nonalcoholic fatty liver disease. *Current Drug Targets, 16*(12), 1301-1314. [DOI:10.2174/1389450116666150427155342] [PMID]

Ozturk, B., Ozek, G., Ozek, T., & Baser, K. H. C. (2014). Chemical diversity in volatiles of helichrysum plicatum DC. Subspecies in Turkey. *Records of Natural Products, 8*(4), 373-384. [Link]

Puri, P., Baillie, R. A., Wiest, M. M., Mirshahi, F., Choudhury, J., & Cheung, O., et al. (2007). A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology, 46*(4), 1081-90. [DOI:10.1002/hep.21763] [PMID]

Raman, M., Ahmed, I., Gillevet, P. M., Probert, C. S., Ratcliffe, N. M., & Smith, S., et al. (2013). Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clinical Gastroenterology and Hepatology, 11*(7), 868–75.e753. [PMID]

Rhee, E. J., Kim, M. K., Park, S. E., Park, C. Y., Baek, K. H., & Lee, W. Y., et al. (2013). High serum vitamin D levels reduce the risk for nonalcoholic fatty liver disease in healthy men independent of metabolic syndrome. *Endocrine Journal, 60* (6), 743-752. [DOI:10.1507/endocrj.EJ12-0387] [PMID]

Di Sario, A., Candelaresi, C., Omenetti, A., & Benedetti, A. (2007). Vitamin E in chronic liver diseases and liver fibrosis. *Vitamins & Hormones, 76,* 551-573. [DOI:10.1016/S0083-6729(07)76021-1] [PMID]

Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry, 25*(1), 192–205. [PMID]

Serviddio, G., Bellanti, F., & Vendemiale, G. (2013). Free radical biology for medicine: Learning from nonalcoholic fatty liver disease. *Free Radical Biology and Medicine, 65,* 952-968. [PMID]

Shea, M. K., Booth, S. L., Gundberg, C. M., Peterson, J. W., Waddell, C., & Dawson-Hughes, B., et al. (2010). Adulthood obesity is positively associated with adipose tissue concentrations of vitamin k and inversely associated with circulating indicators of vitamin k status in men and women. *The Journal of Nutrition, 140*(5), 1029-1034. [DOI:10.3945/jn.109.118380] [PMID]

Sun, Y. S., & Qu, W. (2019). Dietary Apigenin promotes lipid catabolism, thermogenesis, and browning in adipose tissues of HFD-Fed mice. *Food and Chemical Toxicology, 133,* 110780. [DOI:10.1016/j.fct.2019.110780] [PMID]

Taşkın, T., Gezmiş, T., Çam, M. E., Taşkın, D., Çelik, B. Ö., & Şenkardeş, İ., et al. (2020). The in vitro and in vivo investigation of biological activities and phenolic analysis of Helichrysum plicatum subsp. plicatum. *Brazilian Journal of Pharmaceutical Sciences, 56.* [DOI:10.1590/s2175-97902019000418345]

Varsamis, N. A., Christou, G. A., & Kiortsis, D. N. (2021). A critical review of the effects of vitamin K on glucose and lipid homeostasis: Its potential role in the prevention and management of type 2 diabetes. *Hormones, 20*(3), 415-22. [DOI:10.1007/s42000-020-00268-w] [PMID]

Wagatsuma, K., Yamada, S., Ao, M., Matsuura, M., Tsuji, H., & Iida, T., et al. (2019). Diversity of gut microbiota affecting serum level of undercarboxylated osteocalcin in patients with crohn's disease. *Nutrients, 11*(7), 1541. [DOI:10.3390/nu11071541] [PMID]

Elizabeth, L. Y., Schwimmer, J. B., & Lavine, J. E. (2010). Nonalcoholic fatty liver disease: Epidemiology, pathophysiology, diagnosis and treatment. *Paediatrics and Child Health, 20* (1), 26-29. [DOI:10.1016/j.paed.2009.09.005]