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The potential role of dill leaves extract in protection against high fat diet-induced nonalcoholic hepatosteatosis in the obese male rats

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Abstract: This study evaluated whether dill leaves extract (100 mg/kg BW) could protect against obesity and associated liver fatty disease, with a particular focussing on underlying mechanisms. Feeding male rats (170 ± 10 g) on high fat diet (HFD) for 3 months exhibited notable obesity indicated by increased body mass index, abdominal circumference, adiposity index and body weight gain. Results also showed significant elevation of serum glucose, lipids , insulin, leptin and insulin resistance with decreased insulin sensitivity. This goes with significant increases in relative liver weight, hepatic triglycerides, fatty acid synthase, NADPH, oxidative stress indices (CYP2E1**,** Acyl-CoA oxidase, H_2O_2, MDA), and serum liver enzymes (AST, ALT, ALP, GGT), accompanied with reduction in endogenous antioxidants (SOD, CAT, GSH), serum total proteins and albumin levels. Increased hepatic proinflammatory cytokines (NF-κβ, TNF-α, IL-1β, IL-6), Kupffer cell markers; CD68 and CD163, and fibrotic mediators (TGF-β1, FN, COL-I) were also noticed, indicating progression of hepatosteatosis. Oral administration of dill leaf extract along with HFD seemed to protect against body weight gain, hepatic fat deposition, oxidative injury, inflammation and fibrotic response. Thus, dill extract can be considered in the future therapy for obesity and associated NASH.

Keywords: high fat diet, obesity, fatty liver disease, insulin resistance, fibrosis, dill leaves extract

1 Introduction

1.1 General background

Fatty liver disease (alcoholic or non-alcoholic) refers to a variety of illnesses characterized by excessive fat accumulation in the hepatocytes **[1]**. Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases worldwide, affecting about 25% in the general population and can reach 90% in the obese individuals **[2]**. Obesity is well recognized to play a pivotal role in developing NAFLD and its pathogenic switch to a more serious form called nonalcoholic steatohepatitis (NASH) **[3]**. Contributing causes are primarily linked to dyslipidemia, insulin resistance, hepatic fat accumulation, redox imbalance, and inflammation which can lead to fibrosis with evolution to NASH **[4]**. Alarmingly, in patients with NASH, cirrhosis and hepatic carcinoma can also develop with increased hepatic related mortality **[5]**. Therefore, it is probable that targeting obesity and related outcomes will also slow the development of fatty liver disease **[6]**.

Now, herbal remedies have drawn a lot of attention as a promising therapeutic options for a number of liver illnesses **[7]**. *Anethum graveolens* commonly known as dill is an annual herb of the family Apiaceae, growing widely in the Mediterranean region **[8]**. It has been used since ancient times for medicinal and food purposes **[9]**. In traditional medicine, dill is applied for the management and prevention of digestive diseases **[10]** and breath problems **[11]**. It has also been used as a carminative, preservative **[12],** antiviral **[13]**, antifungal and antibacterial agent **[14]**. Dill is a rich source of

bioactive constituents, including polyphenols, flavonoids, saponins, alkaloids, and tannins **[15]**. In this concern, dill has known to provide a number of pharmacological benefits, including anti-inflammatory, antioxidant, antidiabetic **[10]** and anticancer properties **[9]**. Dill is also capable of stimulating milk flow in lactating women and curing urinary complaints **[16]**. Besides, dill could be used in decreasing blood lipids **[17]** and as a cardioprotective agent **[18]**.

Given this information and the lack of data regarding dill effect on obesity-associated liver disease, the current study was designed to evaluate the possible protective effect of dill leaves extract against induced liver changes in an experimental murine model of obesity.

2. MATERIALS AND METHODS

2.1. Chemicals

Cholesterol was purchased from Techno Pharmchen Company (152, Vardhman City Center, New Delhi, India) and Folin-Ciocalteu reagent was obtained from Fluka Biochemical Inc. (Bucharest, Romania), while gallic acid and aluminum chloride were provided by Biomedical Inc. (Orange City, FL, USA) and Andenex-Chemie (Hamburg, Germany) respectively. The other chemicals; catechin, vanillin, hydrochloric acid, ascorbic acid, and 2.2 diphenyl-1-picryl hydrazyl (DPPH$^{\bullet}$) were obtained from Sigma-Aldrich (St. Louis, USA), while ammonium hydroxide solution was obtained from Alpha Chemika (Panvel, Maharashta, India).

2.2. Plant collection and extract preparation

Fresh leaves of dill were obtained from local market in Mansoura city, during August 2020 and the botanical identification and authentication were confirmed in the Botany Department, Mansoura University, Egypt. Leaves were washed with clean water, dried at room temperature for 10 days and crushed with an electric grinder. The dry powder leaves (100g) was soaked with 80% ethanol for 3 days, then filtered and concentrated by rotary evaporator under reduced pressure at 40°C. The yield of dry extract (8% "w/w") was kept at 4°C for the further experimental work **[19]**.

2.3.Determination of phytochemical constituents of dill extract

The main phytochemical constituents of dill ethanolic extract were identified using standard analytical methods (Table 1). The test was conducted on the plant extract to determine and quantify its phenolic content applying Folin-Ciocalte reagent method **[20]**. Total phenolic contents were determined on the basis of the standard curve of gallic acid (GA) and expressed as mg GA/g dry extract. The content of flavonoids was assessed using the standard curve of catechin (CA) as described by **[21]**. The plant flavonoids were calculated as mg CA/g dry extract. Total tannin content was determined following the procedure of vanillin-hydrochloride assay **[22]**. The attained value was calculated as milligram tannic acid (TA) and expressed as mg TA /g dry extract. Total saponin contents were estimated using sodium chloride solution for precipitation **[23]**. The yield of total saponins was determined as mg saponins/g dry extract. Likewise, total alkaloids were assessed using a solution of sodium hydroxide for precipitation and the residue of alkaloids was expressed as mg alkaloids/g dry extract **[24]**.

2.3. Determination of antioxidant scavenging activity of dill extract

The antioxidant scavenging activity of dill extract was investigated based on the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical by the plant samples and the decolorization of the violet DPPH$^{\bullet}$ color **[25]**. Samples at the different concentrations were mixed with DPPH$^{\bullet}$ solution and then kept in dark room temperature for 30 minutes. The absorbance (A) of each sample was measured using a spectrophotometer at 517 nm. As a measure of scavenging activity for the free radical DPPH$^{\bullet}$, percentage of inhibition was estimated by applying the equation:

% DPPH$^{\bullet}$ remaining = $[DPPH^{\bullet}]_T / [DPPH^{\bullet}]_{T=0} \times 100$, where T is the final concentration and T=0 is the initial concentration of DPPH$^{\bullet}$. Results are expressed as IC_{50} values, which are the concentrations of antioxidants required to scavenge 50% of DPPH$^{\bullet}$.

2.4. Animals

This study was carried out using adult male Wistar rats weighing 170 ± 10 g obtained from

the animal unit of VACSERA, Egypt. Rats were kept in stainless steel cages with wooden chips bedding under a controlled conditions (23 ± 2ºC, relative humidity of 55 ± 5%, and 12 h light/dark cycle), with *ad libitum* rodent diet and drinking water. The experimental study was carried out in accordance with Mansoura University's local experimental animal ethics committee (Sci-Z-2020-14).

2.5. Experimental design and dietary protocol

Rats were randomly divided into five groups (n = 6) after acclimatization for one week and treated as following: rats in the first group, served as a control, which were given a regular commercial diet. Rats in the second group were given distilled water (1 ml/kg BW) by gavage and served as a vehicle. While, rats of the third group were given dill extract (100 mg/Kg BW) orally, dissolved in 1 ml distilled water **[19]**. In the fourth group, rats were fed HFD composed of 68% commercial diet mixed with, 30% animal abdominal fat and 2% cholesterol **[26]**, whereas, rats in the fifth group were given HFD plus dill as described in the previous groups. Rats of different groups received their respective treatment daily for 3 months. Rat's BW (g) was registered at the beginning and at the end of the experimental period to obtain body weight gain. Then rats were anesthetized by light ether for measurements of anthropometric parameters.

2.6. Anthropometric measurements

2.7.1. Abdominal circumference

Rats were positioned ventrally, and the abdominal circumference (AC) was measured (cm) on the biggest region of the rat abdomen using a plastic tape **[27]**.

2.7.2. Body mass index

The body length (nose-anus length) was measured and the body mass index (BMI) was determined as the following: BMI = body weight (g)/the square of the body length (cm^2) **[28]**.

2.7. Collection of blood and tissue samples

At the end of experiment, the rats were slaughtered with a scalpel, then their heads were cut off and the blood samples were drawn into sterile centrifuge tubes, allowed to clot, and centrifuged for 15 minutes at 3000 rpm. The clear non-hemolyzed sera were quickly removed for different biochemical analysis. Rats were then dissected and the total body fat and the liver from each rat were excised, rinsed with ice-cold normal saline and weighed. Each liver tissue was immediately homogenized for various biochemical assays.

2.8. Adiposity index

The adiposity index was calculated by dividing the weight of body fats (retroperitoneal, visceral, epididymal and subcutaneous fat) by body weight ×100 **[29]**.

2.9. Relative liver weight

The relative liver weight was obtained by dividing the absolute liver weight by the final body weight ×100 **[30]**.

2.10. Biochemical measurements

Serum glucose level, total lipids (TLs), triglycerides (TGs), total cholesterol (TC), HDL-C, total protein and albumin contents were estimated using kits from Bio-diagnostic Co. (Dokki, Giza, Egypt). Serum insulin and leptin were estimated by ELISA technique using kits from ALPCO (Salem, NH, USA) and DRG instruments GmbH Co. (Germany) respectively. Hepatic TGs, H_2O_2, malon-dialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were determined using commercial kits from Bio-diagnostic Co. (Dokki, Giza, Egypt). Fatty acid synthase (FAS) and cytochrome P450 2E1 (CYP2E1) were determined in the liver tissue using ELISA kits purchased from Cusabio (Baltimore, MD, USA). Nicotinamide adenine dinucleotide phosphate **(**NADPH), and acyl-CoA oxidase (ACOX) were determined using ELISA kits purchased from MyBioSource (San Diego, California, USA). Interleukin-1β (IL-1β), and interleukin-6 (IL-6) were estimated in hepatic tissue by ELISA kits from BioVision (Minneapolis, MN, USA). Tumor necrosis factor-α (TNF-α) was measured using ELIZA kit obtained from ALPCO (Salem, NH, USA). Nuclear factor-kappa β (NF-κβ), Kupffer cell markers (CD68, CD163), collagen type-Ⅰ (COL-Ⅰ), fibronectin (FN) and transforming growth factor-β1 (TGF-β1) were mineddeter in liver using ELISA kits obtained from Cusabio

(Baltimore, MD, USA). Low-density lipoprotein cholesterol (LDL-C) was calculated by the equation : LDL-C =TC – (HDL-C) – (TG/5) **[31]**. Very low-density lipoprotein cholesterol (vLDL-C) was calculated as: vLDL-C = TGs/5 **[32]**. Insulin resistance **[**Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)]was estimated from the following equation: I_O (µIU/ml) × G_O (mg/dl)/405, while insulin sensitivity [Quantitative Insulin Sensitivity Check Index (QUICKI)] was obtained by applying the following equation: 1/[(log I_O (µIU/ml)+ log G_O (mg/dl)] **[33],** where I_O is the fasting serum insulin and G_O represents the fasting glucose level.

2.10. Statistical analysis

Data were analysed by utilizing the Graph Pad Prism 5.0 software prepared by (Graph Pad Software Inc., San Diego, CA, USA). Results were presented as mean ± standard error (SE) (n = 6). The statistical comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test. P values less than 0.05 ($P < 0.05$) were considered statistically significant.

3. RESULTS

3.1. Assessmentofphytochemicalconstituents

As shown in Table 1, phytochemical screening of dill extract showed a high concentration of total phenolics, total flavonoids, and tannins, varying from 74.21 mg GA/g dry extract and 32.46 mg CA/g dry extract to 16.95 mg TA/g dry extract, respectively. Plant analysis also revealed notable presence of saponins (42.44 mg saponins/g dry extract), and alkaloids (17.17 mg alkaloids /g dry extract).

3.2. DPPH radical scavenging assay

The findings indicated that dill extract exhibited a potent radical scavenging activity (IC_{50} = 0.11 mg/ml). It is noteworthy that the antioxidant impact is inversely correlated with the elimination of $DPPH^{\bullet}$ and disappearance of violet color in the test samples, indicating that the higher the antioxidant activity, the lower the IC_{50} value (Table 1).

3.3. Obesity-related traits

Rats fed on HFD were more apparent to display obesity-related traits than control rats, as shown by a significant raise in body weight gain, BMI, AC and adiposity index. Dill extract administration, on the other hand, significantly reduced body weight and other obesity-related indices in animals fed an HFD when compared to obese rats. However, when control rats were given dill, no significant changes were seen (Table 2).

3.4. Serum biomarkers

Feeding rats on HFD resulted in a significant increase in serum levels of glucose, insulin, leptin, and insulin resistance, with decrease in insulin sensitivity compared to the control group. On contrary, the oral administration of dill to rats receiving HFD considerably reduced all of these alterations, however no apparent effect was shown when dill was given to untreated, normal rats (Table 3).

When comparing HFD-fed rats to the control group, it was observed that serum lipids (TLs, TC, TGs, LDL-C, and vLDL-C) were significantly elevated, whereas HDL-C, total proteins, and albumin were decreased. The administration of dill extract to the HFD group resulted in a significant reduction in values of all measured parameters except for HDL-C, total proteins, and albumin which demonstrated a significant raise compared to the non-treated group. The normal rats who received dill showed no obvious changes (Table 4).

3.5. Hepatic biomarkers

3.5.1. Adiposity biomarkers

Significant increases in the levels of hepatic TGs, FAS, NADPH, absolute and relative liver weights were demonstrated in HFD fed rats. However, a reverse pattern of changes was exhibited following administration of dill extract, where marked reduction in all mentioned parameters were noticed compared to the HFD-untreated animals. Indeed, after giving dill extract to the healthy untreated rats, no discernible changes were found (Table 5).

3.5.2. Oxidative stress and antioxidant biomarkers

Results revealed significantly higher levels of CYP2E1, ACOX, H_2O_2 and MDA in the liver of HFD-fed rats compared to control animals, along with a significant decrease in the antioxidant defense system, including SOD, CAT and GSH. However, when dill extract was

given to the HFD group, it exhibits significant improvement in all tested parameters. However, after giving dill to the healthy, untreated rats, no significant changes were noticed (Table 6).

3.5.3. Liver function enzymes

Serum levels of the liver enzymes; ALT, AST, ALP, and GGT were higher in the HFD-fed rats than in the control group. Indeed, these alterations seemed to be reduced when HFD-fed rats were administrated dill extract. Normal rats received dill showed no significant changes when compared to control rats (Table 7).

3.5.4. Hepatic inflammatory and fibrotic markers

Hepatic inflammatory cytokines (NF-κβ, TNF-α, IL-1β, IL-6) and Kupffer cell markers (CD68, CD163) along with fibrotic mediators; TGF-β1, FN, COL-Ⅰ (Table 8), were significantly increased in the liver of HFD fed rats. Administration of dill extract to HFD fed rats showed marked reduction in all these markers, however, normal rats when received dill extract only did not show any changes.

Table 1. Phytochemical analysis and scavenging activity of ethanolic dill extract

Phytochemical constituents	Content
Phenolics (mg GA/g dry extract)	74.21
Flavonoids (mg CA/g dry extract)	32.47
Tannins (mg TA/g dry extract)	16.95
Saponins (mg/g dry extract)	42.45
Alkaloids (mg/g dry extract)	17.18
$DPPH^{\bullet}$ (IC_{50} mg/ml)	0.11

The data represents total phenolic content expressed in mg equivalent gallic acid/g dry extract; total flavonoids content expressed in mg equivalent catechin/g dry extract, total tannins content expressed in mg equivalent tannic acid/ gram dry extract; total saponins content expressed in mg saponins/g dry extract; total alkaloids content expressed in mg alkaloids/g dry extract and scavenging activity of dill extract against $DPPH^{\bullet}$ radical expressed in IC_{50} value.

Table 2. Body weight gain, body mass index, adiposity index, and abdominal circumference in control and treated groups

	Control	Vehicle	Dill	HFD	HFD + Dill
Body weight gain (g)	106.50 ± 4.54	106.31 ± 3.72	101.60 ± 3.88	209.30^{a} ± 3.89	144.11^{ab} ± 3.47
BMI (g/cm^2)	0.54 ± 0.01	0.570 ± 0.01	0.56 ± 0.013	0.75^{a} ± 0.013	0.66^{ab} ± 0.00
AC (cm)	14.88 ± 0.15	14.78 ± 0.27	14.87 ± 0.24	17.98^{a} ± 0.31	15.75^{b} ± 0.34
Adiposity index (%)	1.91 ± 0.16	1.83 ± 0.11	1.82 ± 0.08	6.56^{a} ± 0.11	3.05^{ab} ± 0.18

Data are means (n = 6) ± SE. HFD = high fat diet. a: indicates significance when compared with control, while b: is significance when compared with HFD.

Table 3. Serum glucose, insulin, leptin, insulin resistance, and insulin sensitivity in control and treated groups**.**

	Control	Vehicle	Dill	HFD	HFD + Dill
Glucose (mg/dl)	90.42 ± 2.92	90.08 ± 3.98	89.50 ± 5.82	193.6^{a} ± 10.25	101.8^{b} ± 3.86
Insulin (µIU/ml)	58.72 ± 2.33	57.55 ± 2.30	57.53 ± 1.60	80.02^{a} ± 5.05	59.92^{b} ± 2.81
Leptin (ng/ml)	23.44 ± 0.51	22.62 ± 0.56	22.06 ± 0.70	38.18^{a} ± 0.63	28.12^{ab} ± 0.84
HOMA-IR (µIU × mg/dl)	13.15 ± 0.86	12.77 ± 0.66	12.72 ± 0.93	35.36^{a} ± 3.78	15.97^{b} ± 0.84
QUICKI (µIU×mg/dl)	0.26 ± 0.001	0.27 ± 0.002	0.27 ± 0.002	0.24^{a} ± 0.003	0.262^{b} ± 0.002

Data are means (n = 6) ± SE. HFD = high fat diet. a: indicates significance when compared with control, while b: is significance when compared with HFD.

Table 4. Serum lipid profile, total proteins, and albumin in control and treated groups.

	Control	Vehicle	Dill	HFD	HFD + Dill
TLs (mg/dl)	229.7 ± 10.93	227.3 ± 7.33	226 ± 7.22	515.3^{a} ± 33.43	349.2^{ab} ± 13.28
TC (mg/dl)	70.75 ± 6.51	69.32 ± 6.03	73.88 ± 4.01	250.00^{a}± 13.02	118.20^{ab} ± 6.17
TGs (mg/dl)	78.23 ± 5.99	80.17 ± 6.21	78.35 ± 6.09	251.2^{a} ± 13.85	117.7^{ab} ± 7.83
HDL-C (mg/dl)	53.17 ± 2.96	52.00 ± 3.26	50.83 ± 2.83	29.17^{a} ± 1.17	40.50^{ab} ± 1.18
LDL-C (mg/dl)	17.19 ± 1.55	18.37 ± 2.42	16.97 ± 2.13	192.8^{a} ± 3.59	54.55^{ab} ± 3.93
vLDL-C (mg/dl)	18.67 ± 1.90	18.31 ± 1.81	18.43 ± 1.67	45.90^{a} ± 3.01	23.70^{b} ± 2.68
Total proteins (g/dl)	7.22 ± 0.16	6.93 ± 0.17	6.80 ± 0.15	5.00^{a} ± 0.32	6.16^{ab} ± 0.32
Albumin (g/dl)	3.97 ± 0.14	3.90 ± 0.22	3.85 ± 0.08	2.55^{a} ± 0.13	3.23^{ab} ± 0.08

Data are means (n = 6) ± SE. HFD = high fat diet. a: indicates significance when compared with control, while b: is significance when compared with HFD.

Table 5. Hepatic triglycerides, fatty acid synthase, nicotinamide adenine dinucleotide phosphate, absolute and relative liver weights in control and treated groups**.**

	Control	Vehicle	Dill	HFD	HFD + Dill
TGs (mg/dl)	87.50 ± 7.43	91.53 ± 9.03	92.17 ± 8.36	229.5[a] ± 15.06	135.7[ab] ± 7.37
FAS (ng/mg)	1.49 ± 0.04	1.16 ± 0.02	1.21 ± 0.04	9.15[a] ± 0.75	4.62[ab] ± 0.23
NADPH (pg/mg)	204.7 ± 10.08	199.6 ± 8.50	202 ± 8.74	568[a] ± 18.24	274.7[ab] ± 7.22
Absolute liver weight(g)	6.08 ± 0.28	6.33 ± 0.28	6.30 ± 0.25	10.45[a] ± 0.32	8.22[ab] ± 0.47
Relative liver weight (%)	2.19 ± 0.07	2.14 ± 0.04	2.20 ± 0.06	2.90[a] ± 0.05	2.52[ab] ± 0.11

Data are means (n = 6) ± SE. HFD = high fat diet. a: indicates significance when compared with control, while b: is significance when compared with HFD.

Table 6. Hepatic oxidative stress and antioxidant biomarkers in control and treated groups

	Control	Vehicle	Dill	HFD	HFD + Dill
CYP2E1 (ng/mg)	1.32 ± 0.12	1.30 ± 0.11	1.22 ± 0.11	7.00[a] ± 0.39	3.21[ab]± 0.26
ACOX (ng/mg)	1.38 ± 0.07	1.11 ± 0.00	1.16 ± 0.04	5.67 [a] ± 0.35	3.84 [ab] ± 0.16
H_2O_2 (mM/g)	3.65 ± 0.02	3.28 ± 0.02	3.18 ± 0.07	13.56[a] ± 0.42	6.67[ab] ± 0.13
MDA (nmol/g)	831.1 ± 7.364	831 ± 3.606	816.1 ± 5.982	1143[a] ± 37.69	928.4[ab] ± 16.37
SOD (U/g)	178.6 ± 1.34	178.8 ± 1.14	186.3 ± 3.11	123.8[a] ± 2.46	153.4[ab] ± 3.34
CAT (U/g)	193.5 ± 1.08	195.1 ± 1.21	195.9 ± 2.12	114.5[a] ± 3.11	172.5[ab] ± 3.39
GSH (mg/g)	4.54 ± 0.01	4.56 ± 0.01	4.61 ± 0.02	1.49[a] ± 0.05	3.43[ab] ± 0.19

Data are means (n = 6) ± SE. HFD = high fat diet. a: indicates significance when compared with control, while b: is significance when compared with HFD.

Table 7. Liver function enzymes in control and treated groups

	Control	Vehicle	Dill	HFD	HFD + Dill
ALT (U/L)	36.25 ± 2.02	37.42 ± 2.89	37.92 ± 3.12	72.50[a] ± 2.21	47.25[ab] ± 2.09
AST (U/L)	31.58 ± 1.15	33.83 ± 1.78	32.50 ± 1.88	120.80[a] ± 7.02	46.83[ab] ± 0.79
ALP (U/L)	207.9 ± 12.38	207.30 ± 12.43	206.10± 10.16	305.80[a] ± 3.61	253.20[ab] ± 2.50
GGT (U/L)	23.75 ± 2.83	24.00 ± 3.14	22.83 ± 2.99	63.67[a] ± 4.43	44.83[ab] ± 3.07

Data are means (n = 6) ± SE. HFD = high fat diet. a: indicates significance when compared with control, while b: is significance when compared with HFD.

Table 8. Hepatic inflammatory and fibrotic markers in control and treated groups**.**

	Control	Vehicle	Dill	HFD	HFD + Dill
NF-κβ (pg/mg)	18.35 ± 0.74	18.84 ± 0.59	17.7 ± 0.43	29.99[a] ± 0.98	23.29[ab] ± 0.60
TNF-α (pg/mg)	12.94 ± 0.55	12.29 ± 0.50	11.84 ± 0.41	37.59[a] ± 2.38	17.98[ab] ± 0.38
IL-1β (pg/mg)	71.45 ± 3.14	71.21 ± 2.68	72.75 ± 1.73	288.40[a] ± 4.04	153.70[ab] ± 3.26
IL-6 (pg/mg)	2.20 ± 0.03	1.97 ± 0.01	2.09 ± 0.07	7.33[a] ± 0.15	3.90[ab] ± 0.11
CD68 (ng/mg)	12.82 ± 0.44	12.54 ± 0.76	12.59 ± 0.35	33.51[a] ± 1.05	21.62[ab] ± 1.04
CD163 (ng/mg)	29.21 ± 2.094	29.05 ± 2.179	28.11 ± 2.18	106.5[a] ± 3.729	82.47[ab] ± 1.325
TGF-β1(pg/mg)	8.69 ± 0.38	8.25 ± 0.41	8.17 ± 0.15	31.71[a] ± 0.72	21.47[ab] ± 0.44
FN (ng/mg)	3.92 ± 0.14	3.748 ± 0.11	3.597 ± 0.13	19.69[a] ± 0.79	14.16[ab] ± 0.62
COL-I (pg/mg)	79.65 ± 1.68	78.22 ± 2.03	78.31 ± 0.91	298.3[a] ± 6.73	160.71[ab] ± 7.69

Data are means (n = 6) ± SE. HFD = high fat diet. a: indicates significance when compared with control, while b: is significance when compared with HFD.

4. Discussion

Immoderate exposure to dietary fats has been determined as a key attribute in increasing prevalence of obesity and related NAFLD. Although, considerable studies have been established in this regard, there is still critical need for more research **[34]**. In the present study, feeding rats a HFD for 3 months developed obesity indicated by significant elevation of BMI, waist circumference, adiposity index and weight gain, compared to normal rat group. Dietary fat contributed also to a number of metabolic changes including, hyperglycemia, hyperinsulinemia, insulin resistance, and impaired insulin sensitivity, accompanied by hyperlipidemia involving significant raise in serum TGs, TC, LDL-C and vLDL-C, with reduction of HDL-C levels, which together may derive development and progression of hepatic steatosis **[35]**.

On this basis, it can argue that massive expansion of adipose tissue with obesity is

closely related to increased levels of circulating free fatty acids (FFAs) that directly affect insulin signaling, diminish glucose uptake by muscles**,** and induce gluconeogenesis, thereby generating a state of hyperglycemia **[36]**. The latter can promote compensatory hyperinsulinemia, down regulation of insulin receptors and insulin resistance which in turn stimulates lipolysis and increases circulating FFAs that are taken up by the liver. Increased FFAs uptake overwhelms the capacity for fatty acids oxidation, leading to intrahepatic fat accumulation **[37]**. In this study, consumption of HFD seemed to activate hepatic fat deposition in the form of TGs, accompanied by significant increase in the relative liver weight that may result from insufficiency of insulin action and disrupted lipid metabolism. Supplementation of dill extract to HFD fed rats showed favorable effects against obesity, insulin resistance and other metabolic abnormalities compared to untreated HFD group. Also, it was able to reduce increased liver weight and hepatic fat accumulation. This beneficial effects may be related to the fact that dill contains various bioactive constituents including, total phenolics, flavonoids, saponins and alkaloids. Flavonoids, in particular, have known for its ability to decrease body weight gain and liver weight in the HFD fed rats **[38]**. Flavonoids have also shown to maintain normal glucose homeostasis through improving insulin sensitivity and cellular glucose uptake, thereby reduce blood glucose levels **[39]**. Additionally, flavonoids may help in modulating altered lipid metabolism through enhancing catabolism of LDL-C and activating tissue lipases and/or inhibiting production of triglyceride precursors such as acetyl-CoA and glycerol phosphate production **[40], s**uggesting dill extract as an appropriate agent for management of hepatic steatosis.

Further interpretation may involve *de novo* lipogenesis (DNL), as a key pathway that promotes accumulation of hepatic TGs and development of fatty liver **[41]**. FAS is a major enzyme in *de novo* synthesis of long chain fatty acids from acetyl-CoA and malonyl-CoA, utilizing NADPH to provide reducing equivalents **[42]**. When nutrients are present in excess, FAS appears to catalyze production of TGs for storage in liver or secretion in the circulation as vLDL-C **[43]**. Numerous nutrients and hormones frequently control the transcriptional level of FAS. Among these, leptin is a specific adipocyte- derived hormone that regulates appetite and energy expenditure by the way of leptin receptors in the hypothalamus. In obesity, the anorexic effect of leptin is abolished due to disrupted leptin signaling pathway, resulting in hyperleptinemia and leptin resistance **[44]**. This cascade, consequently leads to high levels of FAS expression in the brain, thereby produces increased serum TGs and may also trigger hepatic fatty acids synthesis in response to dietary fats **[45]**. This can explain the present finding showing increased serum leptin with consequent elevation of hepatic FAS and NADPH that lastly lead to activation of DNL and progression of hepatic steatosis in HFD fed rats. Administration of dill extract was effective in normalizing levels of leptin, FAS and NADNP to reduce activated DNL in the liver of HFD fed rats. The exact mechanism may be ascribed to several dill active components, including saponins which are important for improving leptin signaling in the hypothalamus, thereby decreases the rate of leptin secretin and enhances its effect in the obese animals **[46]**. Other plant constituents particularly flavonoids, have identified as inhibitors of FAS activity **[47]**. Therefore, it can suggest that down regulation of these pathways by dill extract may be a viable option to combat liver steatosis associated with a fatty diet.

Now, it is widely accepted that hepatic fat accumulation is a key factor in creating unfavorable oxidative environment that derives NAFLD to a worse degree of liver disease **[48]**. Increased non-metabolized fatty acids in the hepatocytes and generation of ROS are ultimately linked to poor mitochondrial-oxidation. This effect is mediated in large part through increasing levels of CYP2E1 which is a member of cytochrome P450 oxidoreductase family serves for oxidizing fatty acids. According to **[49]** fatty acids increase the levels of CYP2E1 mRNA and protein in cell culture models and animals subjected to HFD. This is comparable with previous reports that CYP2E1 expression and activity have shown to be increased in the liver of obese humans and animals**,** leading to subsequent production of ROS such as H_2O_2 **[50]**. Add to this, ACOX is another enzyme belongs to the oxidoreductase family that plays a determining role in fatty

acids oxidation and increased ROS production **[51]**.

Under conditions of normal cellular homeostasis, production of ROS is kept in balance by sufficient amounts of scavenging antioxidants**,** however in states of excess ROS, this balance is shifted towards oxidative stress, resulting in cellular membrane damage and propagation of lipid peroxidation **[52]**. Free radicals mediated lipid peroxidation leads to accumulation of MDA, resulting in structural damage and loss of cell function **[53]**. In this line, the present findings showed increased hepatic activities of CYP2E1 and ACOX, concomitantly with elevation in levels of H_2O_2 and MDA, while the antioxidant defenses (SOD, CAT, GSH) were reduced. Further outcomes including raise in serum liver enzymes (AST, ALT, ALP, GGT) and a decline in albumin and total proteins were also observed, confirming propagation of liver oxidative damage. Surprisingly, oral administration of dill extract elicited decreased activity of hepatic CYP2E1**,** and ACOX, accompanied by reduction in both H_2O_2 and MDA, with elevation of the antioxidant defenses in the liver of HFD-fed rats. Thus, dill extract has suggested to possess strong antioxidant activity based on its efficacy in inhibiting the stable $DPPH^{\bullet}$ free radical with low IC_{50} value of 0.11 mg/ml. This effect may be a result of its high content of polyphenolic constituents, particularly flavonoids known for their potent antioxidant activity, by scavenging free radicals and/or chelating metal ions **[54]**. Flavonoids were found to be effective in increasing activities of ROS detoxifying enzymes and improving hepatic oxidative stress in rats fed on HFD **[55]**. Additionally, flavonoids can protect against NAFLD by controlling CYP2E1 activity and reducing mitochondrial dysfunction **[50]** Thus, dill extract may eventually aid in suppressing NAFLD, as presently reflected by improving alterations in serum hepatic enzymes, albumin and total protein.

Rather than oxidative stress, enhanced inflammation may confer a high risk for pathogenic switch from NAFLD to NASH. Specifically, obesity- induced adipose tissue expansion with infiltration of macrophages provides high levels of monocyte chemoattractant protein -1 (MCP-1) capable of initiating hepatic inflammatory response **[56]**. More importantly, hepatic fat accumulation increases susceptibility of liver to more serious insults through activation of resident macrophages called Kupffer cells **[57]**. Signaling from Kupffer cells activates the transcription factor NF-κβ and expression of large quantities of cytokines (TNF-α, IL-1, IL-6, and IL-18) contributing to progression of NAFLD **[58]**. These events are further supported by the fact that activated Kupffer cells express increased surface receptors including CD68 and CD163 closely corroborated to hepatic inflammation in the obese populations **[59]**. At the histological level, a higher number of lobular and portal CD68+ macrophages were detected in liver biopsies of patients with NAFLD **[60]**. Similarly, there is a stepwise increase in CD163 expression with the prognosis of chronic liver inflammation in patients with NAFLD when compared to healthy controls **[61]**. In the current study, hepatic inflammatory cytokines (NF-κβ, TNF-α, IL-1β, IL-6) and Kupffer cell markers (CD68, CD163) were found to be significantly increased in the liver of HFD fed rats, highlighting enhanced inflammatory response. Indeed, this inflammatory state was improved when HFD-fed rats were administered with dill extract, which may help in suppressing NAFLD, as presently reflected by improved inflammatory cytokines and features of Kupffer cells activation. This effect can be explained by the fact that dill is known to contain beneficial compounds described for their anti-inflammatory activities, such as alkaloids **[62]**. It has been shown that administration of alkaloids to HFD-fed rodents infected with NAFLD, decreased the expression of IL-6, and TNF-α, which may occur through regulation of NF-κB pathway **[63]**. Other dill constituents, mainly saponins were identified as a potent anti-inflammatory substances. Similar evidence have been produced by using radix roots and flowers of ginseng which are rich in saponins and are able to inhibit the activity of several pro-inflammatory cytokines in various experimental models **[64]**. Based on this, it seemed logical to confirm the anti-inflammatory influence of dill extract which may aid in suppressing progression of NAFLD to more worse disease.

Other consequence of liver inflammation is fibrosis that constitutes a significant risk for

development of NASH **[65]**. Hepatic stellate cells (HSCs), a subtype of quiescent liver progenitor cells, are assumed to play a major role in controlling the fibrosis process. Long term liver injury and continuous supply of inflammatory cytokines can eventually cause activation of stellate cells **[66]**. Activated stellate cells enhances production of TGF-β1. that increases FN and COL-I deposition while also acting in a paracrine and autocrine manner to activate quiescent HSCs. In this way, the composition of extra cellular matrix changes quantitatively and qualitatively giving rise to an increase in scar tissue **[67]** that cannot self-repair or otherwise function. This cascade blocks or limits the blood flow within the liver, promoting further tissue scaring and fibrosis as a major hallmarks of NASH **[68]**. In support, the present study showed increased fibrotic markers; TGF-β1, FN and COL-I in the liver of HFD-fed rats, which was significantly reduced following administration of dill extract. This effect may be attributed to the plant alkaloids, which are very effective in preventing fibrosis and collagen synthesis, by suppressing collagen gene transcription. Also, flavonoids can alleviate hepatic fibrosis in rats, mostly due regulating TGF-β1 signaling pathway **[68]**. Thus, supporting dill extract as a potential hepatoprotective therapy with high ability to overcome hepatic fibrosis.

In conclusion, the present data concluded that dill extract could help to suppress the obesogenic effect of HFD and its hepatic outcomes through mediating altered metabolism, insulin resistance, inflammation and fibrotic response which are potential mediators for development of NASH. Therefore, regular intake of dill may be beneficial for the obese people with multiple hepatic complications.

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