

Role of hydroxytyrosol in ameliorating effects of high fat diet on male rats CNS

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Abstract

Hydroxytyrosol (HT) , a virgin olive oil phenolic compound with many health benefits has been used as a strong antioxidant with scavenging activity to eliminate the free radicals which have been formed in the body exposed to oxidative stress , the present study aimed to investigate the role of hydroxytyrosol in ameliorating the harmful effect of high fat diet consumption in male rats CNS .Forty eight males Wistar rats about 3 months old with average weight 160 ± 15 g were divided randomly in to four equal groups and treated for 3 months as the follow : First group (C) control group were drenched with distilled water and were fed with normal pelleted diet , second group (T1) were treated with 100 mg/kg/BW of hydroxytyrosol , third group (T2) were given high saturated fat diet(60% fat) for the last 6 weeks of the current study the fourth group (T3) were treated by 100 mg/kg/BW of hydroxytyrosol for the first 6 weeks of the study then diet was changed to a high fat diet for the other 6 weeks of this study. Results of the current study revealed a significant difference ($P \leq 0.05$) represented by increase in serum TNF- α concentration in T2 group compared with other groups C,T1,T, also there was a significant decrease in TNF- α concentration in T3 group as compared with T2 group. On the other hand there was a significant difference ($p \leq 0.05$) represented by increase in serum MCP-1 concentration in T2 group as compared with other groups while there was a significant decrease in MCP-1 concentration in group T3 as compared with the previous group. The results of lipid profile(cholesterol, triglyceride, high density lipoprotein HDL-c, low density lipoprotein LDL-c and very low density lipoprotein VLDL-c) of T1 and T3 were showed an improvement in lipid profile values when compared with T2 group which were showed a highly significant increase ($P \leq 0.05$) in TG, LDL-c, VLDL-c and significant decrease in HDL-c. regarding results of gene expression of (NF-KB) gene were showed a significant increase ($P \leq 0.05$) in T2 group and significant decrease in T1 and T3 groups.

Keywords: hydroxytyrosol , high fat diet, NF-KB, CNS.

INTRODUCTION

One of the greatest factors contributing to the prevalence of obesity is choice of diet. A term to describe the unhealthy diet eaten by many people as well as other westernized populations is " the western diet "(1). Excessive fat intake contributes to the progression of metabolic diseases by cellular injury and inflammation, a process termed lipotoxicity was investigated by the role of lysosomal dysfunction and impaired autophagic flux in the pathogenesis of lipotoxicity.(2) obesity may cause oxidative stress , neuroinflammation , adipocyte derived adipokine changes, such as increased leptin and resistin, and reduced adiponectin that are associated with adverse effects on atherosclerosis (3). Antioxidants act by inhibiting or delaying the cellular damage through their free radical scavenging property and can safely interact with them and destroy the chain reaction before the cellular damage (4) . Olive oil constitutes the main Mediterranean diet, the importance of its poly phenols such as Hydroxytyrosol has been come from its scavenging the free radicals and draw attention due to their beneficial effect in most pharmacological fields it act as an antioxidant, anticancer, anti-inflammatory and neuroprotective substance (5). also it is able to reduce oxidative stress, inflammatory processes, improve the mitochondrial function in brain tissues and counteract lipid profile and insulin sensitivity (6). NF-KB, known as a hallmark of oxidative stress in brain is an inducible and ubiquitously expressed transcription factor responsible for regulating the expression of genes involved in cell survival, cell adhesion, inflammation, differentiation, and growth(7). The transcription factor NF-KB promotes immunity by controlling the expression of genes involved in neuroinflammation . Cytokines and pathogen-associated molecular patterns (PAMPs) stimulate cell surface receptors including toll-like receptors (TLRs) to initiate a signaling cascade resulting in the activation of NF-KB. NF-KB drives expression of target genes that mediate cell proliferation and release of antimicrobial molecules and cytokines to activate the immune response . Although NF-KB was first characterized in cells of the hematopoietic system, subsequent research has revealed that NF-KB activation can occur in most cell types. Indeed, a number of recent high-profile reports have demonstrated a key role for the NF-KB signaling pathway in the liver, adipose tissue, and central nervous system in the development of inflammation-associated

metabolic diseases (8). HFD increased adiposity (body weight, fat mass, fat percent and adipocyte size), metabolic dysfunction (impaired glucose metabolism and insulin resistance) macrophage number, and gene expression of , T-cell markers, inflammatory mediators, and mRNA expression of MCP-1, tumor necrosis factor α (TNF- α) and interleukin-6 . However, contrary to the hypothesis, MCP-1 deficiency exacerbated many of these responses resulting in a further increase in adiposity (body weight, fat mass, fat percent and adipocyte size), metabolic dysregulation, macrophage markers , inflammatory cell infiltration.(9) . Rats fed a high-fat diet exhibited significant elevation of plasma triglyceride and total and low-density lipoprotein (LDL) cholesterol concentrations. These changes represented disorders of biosynthesis and metabolism of the primary bile acids, steroids, and fatty acids and mitochondrial fatty acid elongation pathways in diet-induced hyperlipidemia.(10). treatment with HT significantly suppressed NF-KB expression in THP-1 cells. These results support the implication of the NF-KB pathway in the neuroprotective effect of HT in the early brain injury .In addition to a reduction in neural apoptosis, caspase-3 activity was also ameliorated following HT administration . administration of Hydroxytyrosol restrained NF-KB protein expression following brain injury (11) . HT is the most powerful natural antioxidant protect cells from oxidative stress associated with metabolic fluxes . studies have demonstrated that HT inhibits the production of Tumor Necrosis Factor- α (TNF-a), a key inflammatory cytokine and inhibit MCP-1 production also (12) . Olive Mill Water extract significantly lowered the serum levels of TC and LDL-C while increasing the serum levels of high-density lipoprotein cholesterol (HDL-C), These results suggested that the hypocholesterolemic effect of hydroxytyrosol , might be due to its ability to lower serum TC and LDL-C levels as well as slowing the lipid peroxidation process and enhancing antioxidant activity (13).

The present study was carried out to investigate the role of Hydroxytyrosol extracted from olive oil As a potent antioxidant in ameliorating effect of high fat consumption in male Wistar rats through evaluation of the protective effect of hydroxytyrosol on brain tissues by assessing the expression level of mRNA of NF-kappa B gene , Serological assessment of the inflammatory markers TNF-alpha and MCP-1 by Elisa technique, Estimating

the role of hydroxytyrosol in combating high fat diet by the following biochemical tests ; total cholesterol ,Triglycerides TG, Low density lipoprotein LDL-c, High density lipoproteinHDL-c ,Very low density lipoprotein VLDL-c.

MATERIALS AND METHODS

Experimental animals In the current study, 48 male Wistar rats were used, ages ranged between 60-80 days old, and their weights ranged between 140-180g, and captured in plastic cages with dimensions 40-60cm and kept under controlled conditions about 12 hours light and 12 hours dark with 25°C. divided randomly in to four groups, each group included 12 animals and fed on basal diet and provided by drinking water , the animals were divided according to the following groups: Control Group: Fed on basal diet for 3 months .Treated Group T1: Fed on basal diet and were orally administered with Hydroxytyrosol 100 mg / Kg/BW once daily during the first 6 weeks of the study (14). Treated Group T2 : Fed on basal diet during the first 6 weeks of the study then the diet was changed to A high fat diet (HFD) 60 % fat (15) .for the last 6 weeks of this study. Treated Group T3: Fed on basal diet and were administered with 100 mg / Kg/ BW once daily of hydroxytyrosol during the first 6 weeks of the study then the diet was changed to high fat diet (HFD) 60 % fat for the last 6 weeks of the study.

Preparation of hydroxytyrosol and HFD: Hydroxytyrosol was obtained from(Transhuman Technologies/ UK) 100 Mg/ KG/BW dissolved in distal water at room temperature in dark bottle the solution was prepared every day for drenching. Diets were prepared weekly and were kept in sealed bags and drained of air, kept away from light , maintained at -20°C until administered, and were provided in pelleted form, HFD formulation and composition as showed in the table (1) (60% fat , 20% carbohydrate, 20% protein.

Table (1) High fat diet formula (saturated HFD 60% fat) .

HFD Formula		
Nutrient substance	weight (g)	energy kcal
Tallow (Saturated fat)	310	2480
Maltodextrin	160	340
Casein	265	1000
Sucrose	90	360
Cellulose	95	0
Sun Flower Oil	30	240
Table Salt	26	0
Vitamin Mix	15	60
Mineral Mix	4	0
Amino acid Mix (premix) Methionine , Lysine	5	0

Serum lipid profile estimation: All specimens were left at room temperature, the concentrations of serum Total Cholesterol, Triglycerides, HDL-c ,VLDL-c, LDL-c(mg / dL) in male rats were estimated using the clinical Chemistry automated analyzer Cobas c 311, an open reagent system for consolidating routine and special chemistry workloads, on board capacity of 45 tests and throughput of up to 480 test per hour.

Pro inflammatory Cytokines Estimation in Male rats serum by using ELISA techniques Serum samples were taken and ELISA assay to proinflammatory cytokines tumor necrosis factor TNF- α and monocyte chemoattractant protein (MCP-1) were achieved according to the method described by the manufactures company assay protocol (Elabsience.USA).

Estimation of Relative gene Expression for Nuclear Factor Kappa B (NF-KB)

Total RNA extraction :Total RNA were extracted from brain samples by using (Accuzol® reagent kit. Bioneer. Korea) and

done according to company instructions as follow; 300 μ l of blood sample was placed in 1.5ml eppendorf tube and 1 ml of Accuzol reagent was added and the tubes shaken vigorously for 1 minute. Then, 200 μ l chloroform added to each tube and shaken vigorously for 15 seconds. Then the mixture was incubated on ice for 5 minutes, and then centrifuged at 12000 rpm, 4°C, for 15 minutes. The supernatant transferred into a new eppendorf tube, and 500 μ l isopropanol was added. Then, mixture mixed by inverting the tube 4-5 times and incubated at 4°C for 10 minutes. Then, centrifuged at 12000 rpm, 4°C for 10 minutes. The supernatant was discarded, and 1ml 80% Ethanol was added and mixed by vortex again. Then, centrifuge at 12000 rpm, 4°C for 5 minutes. The supernatant was discarded and the RNA pellet was left to air to dry. Finally, 50 μ l DEPC water was added to each sample to dissolve the RNA pellet, and then the extracted RNA sample was kept at -20. The extracted total RNA was assessed and measurement by Nanodrop spectrophotometer (THERMO. USA)

DNase I Treatment: The extracted RNA were treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by using samples (DNase I enzyme kit) and done according to method described by Promega company, USA instructions as following table (2):

Table 2: DNase I Treatment master mix preparation

Mix	Volume
Total RNA 100ng/ul	10ul
DNase I enzyme	1ul
10X buffer	4ul
DEPC water	5ul
Total	20ul

After that, The mixture was incubated at 37°C for 30 minutes. Then, 1 μ l stop reaction was added and incubated at 65°C for 10 minutes for inactivation of DNase enzyme action

cDNA synthesis : DNase-I treatment total RNA samples were used in cDNA synthesis step by using AccuPower® RocketScript RT PreMix kit that provided from Bioneer company, Korea and done according to company instructions as following table:

Table 3: RT master mix for cDNA synthesis

RT master mix	Volume
Total RNA 100ng/ul	10ul
Random Hexamer primer (10 μ mol)	1ul
DEPC water	9ul
Total	20ul

This RT PreMix was placed in AccuPower RocketScript RT PreMix tubes that contains lyophilized Reverse transcription enzyme at form. Then dissolved completely by vortex and briefly spinning down The RNA converted into cDNA in thermocycler under the following thermocycler conditions

Table 4: Thermocycler conditions for cDNA synthesis

Step	Temperature	Time
cDNA synthesis (RT step)	42 °C	1 hour
Heat inactivation	95 °C	5 minutes

Quantitative Real-Time PCR (qPCR) :qPCR was performed for quantification of nuclear factor kappa B subunit 1 (Nfkb1) gene , relative gene expression analysis was carried out by using (2^{- $\Delta\Delta$ CT} Livak method) (17). The qPCR reaction was done on a Real-Time PCR system (BioRad. USA) by using SYBER Green dye qPCR master mix that used in detection and amplification of (Nfkb1) target gene and GAPDH housekeeping gene for normalization of gene expression(16) Primers were designed using the primer3 plus (Primers sequences are listed in Table 5)

Table 5: RT-qPCR primers with their sequence

Primer	Sequence (5'-3')		Product Size
Nfkb1	F	TGGTGGTTGGCTTTGCAAAC	76bp
	R	ATCCGTGCTTCCAGTGTTC	
GAPDH	F	AGTTCAACGGCACAGTCAAG	70 bp
	R	TGGAAGATGGTGATGGGTTTC	

qPCR master mix was prepared for Nfkb1 target gene and GAPDH housekeeping gene according to (AccuPower™ 2XGreen Star qPCR master mix kit. Bioneer .Korea) instructions as following table (6)

Table 6: qPCR master mi preparation

qPCR master mix	volume
cDNA template (100ng)	2.5µL
Forward primer(10pmol)	1 µL
Reverse primer (10pmol)	1 µL
qPCR Master Mix	10 µL
DEPC water	5.5 µL
Total	20 µL

After that, qPCR master mix reaction component that mentioned above placed in qPCR white tube strips and mixed by (Exispin vortex centrifuge, Bioneer. Korea) for 3 minutes, than the strips placed in MiniOpticon Real-Time PCR system BioRad USA as following thermocycler conditions table (7)

Table 7: qPCR thermocycler conditions

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	95 °C	5min	1
Denaturation	95 °C	20 sec	45
Annealing\Extention Detection(scan)	60 °C	30 sec	

Table (8) effect of hydroxytyrosol and HFD on serum TNF-α concentration in male rat (pg/ml)

Groups	TNF-α Concentration (pg/ml)
C	424.58 ± 109.87 ^A
T1	335.46 ± 74.42 ^A
T2	1767.32 ± 145.28 ^B
T3	783.20 ± 79.78 ^C
LSD 0.05	313.80

Table 9: effect of hydroxytyrosol on serum MCP-1 concentration in male rats

Groups	MCP-1 Concentration (ng/ml)
C	2.33 ± 0.53
T1	2.24 ± 0.45 ^a
T2	8.90 ± 0.55 ^c
T3	5.18 ± 0.43 ^b
LSD 0.05	1.45

Table 10: effect of hydroxytyrosol and HFD on serum lipids in male rats

Parameters	Cholesterol Mg/dl	TG Mg/dl	LDL Mg/dl	HDL Mg/dl	VLDL Mg/dl
C	64.75±2.33 a	77.53± 5.31 a	14.98±0.82 a	41.54±1.88 a	18.24±1.89 a
T1	60.91±2.14 ab	60.09±4.44 a	8.96±0.66 b	45.16±2.51 a	11.89±0.97 a
T2	68.65±2.68 ac	214.91±28.63 c	18.05±1.14 c	35.42±1.37 b	46.33±6.75 b
T3	65.96±3.47 a	85.90±4.43 a	9.51±0.7 db	39.18±0.97 ab	17.45±1.66 a
LSD 0.05	7.66	44.02	2.50	5.26	10.62

Numbers = mean ± S.E ,Different litters = significant differences (p≤0.05) ,C group = control group ,T1 group = Fed on basal diet and were orally administered with Hydroxytyrosol 100 mg / Kg/BW once daily during the first 45 days of the study. T2 group = : Fed on basal diet during the first 6 weeks of the study then the diet was changed to A high fat diet (HFD) 60 % fat for the last 6 weeks of the study .T3 group = Fed on basal diet and were administered with 100 mg / Kg/ BW once daily of hydroxytyrosol during the first 6 weeks of the study then the diet was changed to high fat diet (HFD) 60 % fat for the last 6 weeks of the study .

Table 11: effect of hydroxytyrosol and HFD on NF-KB gene expression

Groups	Fold change mRNA transcript level
T1	0.29± 0 .048 ^a
T2	10.77± 0.87 ^b
T3	2.56± 0.43 ^c
C	1.35± 0.19 ^{bc}
LSD 0.05	1.45

Statistical analysis of the results by using computer program (SPSS),Version 23one-way analysis of variance (ANOVA) were used, the difference was considered significant at P < 0.05 Descriptive statistics :mean± stander error, statistical analysis of data was performed on the basis of ANOVA (one way analysis of variance) with least significant difference LSD was detected to compare between groups.

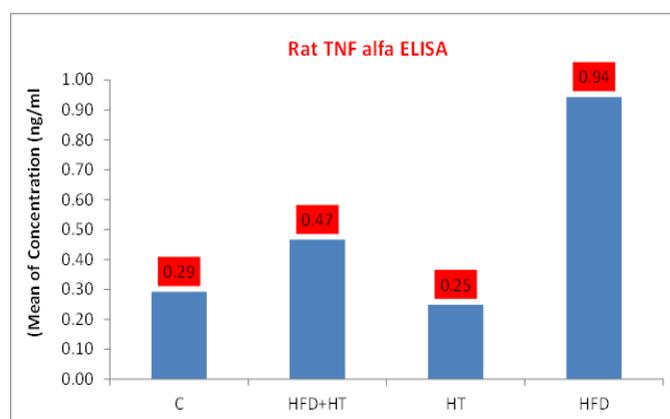


Figure 1: Diagram showing TNF-α concentration in rats serum (pg/ml)

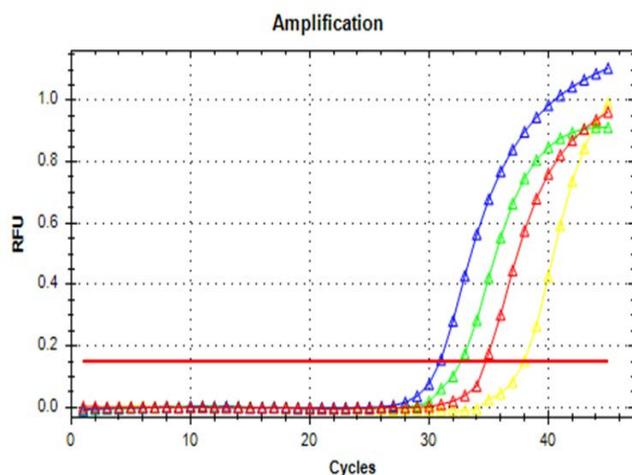


Figure 2 : Real time PCR amplification plot for nuclear factor – kappa B gene(NF-KB) in brain tissue of male rats that show difference in threshold cycle numbers (Ct value) between treatment and control groups.

Red plot : HT group Fed on basal diet and were orally administered with Hydroxytyrosol 100 mg / Kg/BW once daily during the first 45 days of study . Blue plot: HFD group Fed on basal diet during the first 6 weeks of the study then the diet was changed to A high fat diet (HFD) 60 % fat for last 6weeks of study . Green plot: HT+HFD group Fed on basal diet and were administered with 100 mg / Kg/ BW once daily of hydroxytyrosol during the first 6 weeks of the study then the diet was changed to high fat diet (HFD) 60 % fat for the last 6 weeks of study. Yellow plot: Control group

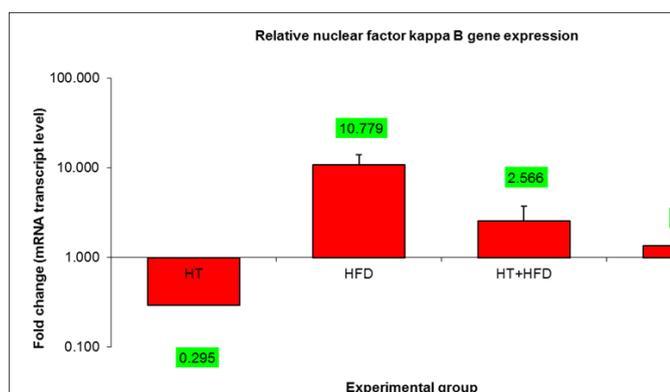


Figure 4: relative nuclear factor kappa B expression

RESULTS AND DISCUSSION

Effect of Hydroxytyrosol and HFD on serum Tumor Necrosis Factor-alpha (TNF- α) concentration in male rats .(pg/ml).

In current study , results in table (8), figure(1), showed that there was a significant difference ($p \leq 0.05$) represented by increase in serum TNF- α concentration in T2 group (1767.32 ± 145.28) as compared with C , T1 and T3 groups . there was a slight decrease TNF- α concentration in T1 group (335.46 ± 74.42) compared with C group (424.58 ± 109.87) while there was a significant decrease in TNF- α concentration in T3 group (783.20 ± 79.78) which were received both HT and HFD as compared with T2 group which were received HFD only .Several studies confirm this fact that the cytokine tumor necrosis factor (TNF), a master regulator of the immune system, plays an important role in the propagation of inflammation due to the activation and recruitment of immune cells via its receptor TNF receptor 1 (TNFR1). Moreover, TNFR1 can directly induce oxidative stress by the activation of ROS and RNS producing enzymes .Nutrient stress is generally considered from the standpoint of how cells detect and respond to an insufficient supply of nutrients to meet their bioenergetic needs. However, cells also experience stress as a

result of nutrient excess, during which reactive oxygen species (ROS) production exceeds that required for normal physiological responses. This may occur as a result of oncogene activation or chronic exposure to growth factors combined with high levels of nutrient.(31). Both TNF-induced oxidative stress and inflammation interact and cooperate to promote neurodegeneration. However, TNF plays a dual role in neurodegenerative disease, since stimulation via its second receptor, TNFR2, is neuroprotective and promotes tissue regeneration (18), (9). Our results were very similar to a study reported by (19) . who measured the concentration of two pro-inflammatory cytokines (IL-1 β and TNF α) in the plasma of mice at week 16 of high fat mice feeding and found a significant increase of TNF α for mice fed a HFD compared with control mice. our results were in agreement with (20) . documented that the olive oil rich with antioxidant compounds (hydroxytyrosol and tyrosol) in dose of 0.75ml/kg/day in the cortex and striatum of rats attenuated TNF- α receptor -1expression significantly compared with the control (intact) group($P= 0.01$ and $P= 0.00$, respectively). The significant difference in lower doses of olive oil was not seen.

Effect of hydroxytyrosol and high fat on serum monocyte chemo attractant protein (MCP-1) concentration in male rats(ng/ml).

Results in table (9) figure (2) showed that there was a significant difference ($p \leq 0.05$) represented by increase in serum MCP-1 concentration in T2 group which were fed on 60% fat (8.90 ± 0.55) as compared with other groups while there was a significant decrease in MCP-1 concentration in group T3 which were received both HT and HFD 60% (5.18 ± 0.43) as compared with the previous group, on the other hand we found that there was no significant difference characterized by slight decrease between group T1 (2.24 ± 0.45) which were received only HT and control group (2.33 ± 0.53). gene expression and the protein secretion of MCP-1, was markedly enhanced in adipocyte and adipose tissue of obese animals, presumably contributing to the inflammatory milieu in obesity, monocyte chemoattractant protein-1 decreases insulin-stimulated glucose uptake in adipocytes, indicating the involvement of MCP-1 in insulin resistance (21). phenolic compounds of hydroxytyrosol also modulated TNF- α and MCP-1 plasma levels in high cholesterol fed male rats for 8 weeks compared to normal fed rats and they found that HT-Acetate and HT-Ether improved adipose tissue distribution and adipokine production, decreasing MCP-1 levels. results confirm the metabolic effects of HT, which are maintained and even improved by hydrophobic derivatives, particularly HT-Acetate (22).

Effect of hydroxytyrosol and high fat diet on serum lipids in male rats (mg/dl).

Table (10) showed a significant increase ($P < 0.05$) in cholesterol level in T2 group (HFD fed rats), (68.65 ± 2.68) as compared with T1 group (which were received HT) 60.91 ± 2.14 , while there was a slight increase in cholesterol value in T2 group as compared with other groups, C (64.75 ± 2.33) and T3 (65.96 ± 3.47) which were received HT and HFD) . In the same table with regarding triglycerides parameter we noticed a significant increase with TG level in T2 group (214.91 ± 28.63), as compared with other 3 groups Control group , T1 and T3 (77.53 ± 5.31), (60.09 ± 4.44), (85.90 ± 4.43) respectively , and showed a significant decrease of TG level in T3 group compared with T2 group. As for low density lipoprotein parameter (LDL), our results showed a significant increase in T2 (18.05 ± 1.14) compared with control and T1 groups (14.98 ± 0.82), (8.96 ± 0.66) respectively , on the other hand there was significant decrease between T2 and T3 group (9.51 ± 0.7). Regarding high density lipoprotein parameter (HDL) our results showed a significant decrease in T2 group (35.42 ± 1.37) compared with C and T1 groups , there was no significant difference between control group (41.54 ± 1.88) and T1

group (45.16 ± 2.51), and no significant difference between T2 and T3 group characterized by slight increase in T3 (39.18 ± 0.97). While VLDL parameter results were showed a significant increase in T2 group (46.33 ± 6.75) compared with other groups C, T1, T3 groups (18.24 ± 1.89), (11.89 ± 0.97), (17.45 ± 1.66) respectively, no significant differences between previous groups have been observed. Beef fat raises serum cholesterol concentrations. Because beef fat is 19% stearic acid, the cholesterol-raising potential of beef is not as great as predicted by its total saturated fatty acid content. However, beef tallow is hypercholesterolemic compared with fats containing less cholesterol-raising saturated fatty acid (23). male rats fed a diet containing 1% cholesterol or 15% lard, increased plasma cholesterol and triglycerides compared to controls (24). Moderate and High SFA presented reduction in insulin, leptin/adiponectin ratio, and increase in adiponectin and adiponectin/leptin ratio. Adiponectin/leptin ratio was predictor of total cholesterol and LDL-cholesterol reduced only in High SFA tertile, and was associated with SFA independent of visceral fat (26). Many trials have investigated the healthy benefits of hydroxytyrosol supplementation. These studies have demonstrated a decrease in the ratio of T-CHOL and increase in HDL-C, with a reduction of markers of oxidative stress such as IL-6 and CRP levels, and monocyte number with a high hydroxytyrosol levels it is considered indicative of one of the major anti-atherogenic polyphenol compounds in olive-oil (25). Another recent study were similar to our study showed that the treatment with supplementation of new nutraceutical NC which include hydroxytyrosol demonstrated a significant reduction of serum total cholesterol, LDL-cholesterol, triglycerides and a significantly increase of HDL-cholesterol levels in hypercholesterolemic patients (27).

Relative expression of Nuclear Factor – Kappa B (NF-KB) gene

Our results in table (11), figures (3, 4) showed that there was a significant difference ($P \leq 0.05$) represented by increase fold change of gene expression level in T2 group (10.77 ± 0.87) as compared with other groups, there was a significant decrease in fold change of gene expression level in T1 group ($P \leq 0.05$) (0.29 ± 0.048) as compared with T2 and T3 (2.56 ± 0.43), on the other hand there was no significant difference between T1 and control group (1.35 ± 0.19) also there was no significant difference between T3 and C group. Our results were showed a marked up regulation in NF-kappa B gene expression in T2 group which were exposed to a high fat diet for 6 weeks of the current study which indicate the close relationship between induced obesity and inflammatory signaling pathway regulated by NF-KB gene. at the same time our results were showed a marked decline in the same gene expression in T3 group which were given hydroxytyrosol and HFD, these findings prove the protective effect of HT against the oxidative stress induced by a HFD, also we found an obvious down regulation of NF-KB gene expression in T1 group, the one that was received only HT and these results confirm the fact that HT is a potent antioxidant which reduce the expression of NF-kb gene even within normal feeding cases.

The mediobasal hypothalamus regulates energy balance and prevents obesity by adjusting appetite and food intake in response to signals of metabolic status including insulin and leptin. To investigate how inflammatory gene expression contributes to central control of nutrient metabolism, the Cai group sought to define IKK β action in the hypothalamus. IKK β is constitutively expressed in the hypothalamus and directs NF-KB activation in the CNS of mice exposed to a high-fat diet. Forced expression of IKK β in the CNS interrupted leptin and insulin signaling, resulting in increased intake of high-fat food and weight gain, targeted disruption of IKK β in the hypothalamus lowered food intake and protected mice from obesity (28). IKK β activity in the

CNS was associated with augmented IL-6 production, and cytokine signaling, suggesting that NF-KB target genes mediate the metabolic changes observed in the CNS of IKK β . Several reports have linked over nutrition with stressed protein assembly pathways in the ER leading to inflammation and even the development of hepatic insulin resistance(29). The inhibitory effect of olive oil polyphenol compounds on NF-kB and TNFR1 protein level, this result highlights the inhibitory effect of olive oil on the destructive inflammatory process,(20). Regarding the effect of hydroxytyrosol in NF-KB attenuation research was reached a conclusion that phenolic compounds of olive oil prevent the blood-brain barrier integrity by suppressing free radicals, NF-KB activity and matrix metalloproteinase-9 expression (30).

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