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Hepatoprotective activities of thyme (*Thymus vulgaris* L.) in rats suffering from obesity

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Abstract

Thyme (*Thymus vulgaris* L.) is medicinal plant in the lamiaceae family which is cultivated worldwide for cosmetic, culinary and medical uses. The current study aimed to investigate the impact of thyme on the nutritional parameters of obese rats. Phenolic and flavonoid components were detected in dry thyme powder by HPLC-UV and thyme essential oil was analyzed by GC-MS. In the biological experiment, the study included 30 male rats of weight approximately 200 grams which were divided into 5 groups; each group comprising 6 rats. Group (G1) rats were fed on basal diet, serving as a negative control group (N-control), while group (G2) rats were fed on a high-fat diet (fat percentage 15%) (P-control).The same diet used in (G2) was given to group (G3) but along with orlistat supplementation (2mg/Kg BW rat/day) using a stomach tube. Similarly, the rats of groups (G4 and G5) received HFD and supplemented with thyme powder (3g/100g diet) and thyme essential oil (0.6g/100g fat), respectively. The obtained results indicated that, dry thyme powder composition included high concentration of phenolic compounds such as ellagic acid and salicylic acid, as well as high concentration of flavonoids was hesperidin. The major constituent of thyme essential oil was thymol. After 8 weeks, the rats fed on HFD and supplemented with different treatments (G3, G4, and G5) showed a significant decrement in glucose levels. In addition, the treatments also showed significantly enhanced lipid profiles, liver functions, and total antioxidant capacity. However, the activities of catalase and glutathione reduced were significantly elevated in the blood of thyme powder and essential oil-treated groups. Moreover, malondialdehyde was significantly decreased in the treated groups. The induction of redox enzymes serves as a dependable marker for assessing the antiperoxidative effect of herbs; hence, this study revealed the role of thyme powder and essential oil treatment in the reduction of histopathological and liver abnormalities associated with obesity.

Keywords: Thyme; essential oil; HPLC; GC-MS; obesity

1. Introduction

Obesity is a complicated multifactorial condition resulting from the excessive accumulation of adipose tissue which could lead to health deterioration. Also, it is an important risk factor for chronic and impairing diseases in the developing and developed countries and in all age and socio-economic groups [1]. Obesity has significantly negative effects on people's lives on the social, financial, and psychological levels which might result in depression [2]. Moreover, the pathology in obesity caused by the continuous accumulation of fat decreases the individual's life expectancy [3]. Similar to metabolic and inflammatory diseases, obesity is characterized by its strong correlation with oxidative stress [4]. The inadequate neutralization of large amounts of free radicals via antioxidants in the body leads to cumulative damage [5].

Nowadays, one of the approaches of controlling obesity is using some conventional drugs; however, the utilization of these drugs is limited by their low availability and negative side effects. Therefore, safe, effective, economical, and easily-available alternatives are needed for treating and controlling obesity. Plant-derived medicines are regarded as the first-line defense for preserving health [6]. Other than the well-known vitamin C, vitamin E, and carotenoid

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antioxidants, many herbs contain phenolic antioxidants which are made of phenolic acid, flavonoids [7]. These phenolic compounds can quench lipid peroxidation, prevent oxidative damage which possibly affects the DNA, and destroy reactive oxygen species (e.g. superoxides, hydrogen peroxides, and hydroxyl radicals) [8]. Using synergistic polyherbal preparations effectively will strengthen the anti-obesity effect by acting on multiple targets. In addition to their effect against obesity, these plants have numerous health benefits [9].

Thyme is a perennial medicinal plant in the Lamiaceae family which is cultivated worldwide for cosmetic, culinary, and medical uses. This herb has distinctive antispasmodic, antiseptic, expectorant, antioxidant activities, and antimicrobial [10]. In addition, *thymus vulgaris* contains polyphenols, flavonoids, tannins, saponins, and triterpenes. Flavonoids include luteolin, apigenin, naringenin, eriodictyol, cirsilineol, salvigenin, cirsimaritin, thymoine, and thymusine. Triterpenes include ursolic and oleanolic acids [11].

In folk medicine, *thymus vulgaris* has been used as anti-asthmatic and antibacterial agent, bronchodilator, expectorant, antiseptic, antispasmodic, antitussive, carminative, secretomotor, anthelmintic, astringent, antifungal, antiviral, antiprotozoal, and antioxidant in addition to being used for treating dyspepsia, chronic gastritis, diarrhea and enuresis in children [12]. In addition, the extracts of this herb are reported to be immunomodulators and anti-inflammatory agents [13] as well as antioxidant and free radical scavenging, antiplatelets, anti-thombins, anti-hyperlipidemics, vasorelaxants, antihypertensives and anti-diabetic agents [14]. On the other hand, Vetvicka and Vetvickova [15] revealed that thymus-derived essential oils have weak immunomodulatory and anti-inflammatory activities in addition to their limited actions in proliferation of the human breast cancer cell line ZR-75-1 and liver protection.

The current study aimed to investigate the nutritional and protective effects of thyme (*Thymus vulgaris* L.) on obesity in rats after 60 days.

2. Materials and methods

2.1. Chemicals and plant material

Thyme (*Thymus vulgaris* L.) herb was purchased from the Center of Medicinal and Aromatic Plants Research, Faculty of Pharmacy, Cairo University. Beef tallow was purchased from the local market. Orlistat was purchased from a pharmacy. All colorimetric kits for biochemical parameters were purchased from Biodiagnostic Co, Cairo, Egypt. All remaining kits and chemicals were of analytical grade.

2.2. Extraction of essential oil

The essential thyme oil was extracted by water distillation in a Clevenger-type apparatus for 4 hours. The obtained volatile oil was dried over anhydrous sodium sulphate and then was kept in completely filled a glass bottle at -20°C until use [16].

2.3. Analysis of dry thyme powder using HPLC-UV

We used a high-performance liquid chromatography (HPLC) system equipped with a variable wavelength detector (Agilent Technologies, 1200 series, Berlin, Germany). In addition, it was equipped with an autosampler, quaternary pump degasser, and column compartment with the temperature set at 35 °C. A C18 reverse phase (BDS 5 µm, Labio, Czech Republic) packed in stainless-steel column (4 x 250 mm, I.D.) was used for the analyses.

2.3.1. Identification of phenolic and flavonoid compounds

The preparation method of samples explained by Jakopic et al. [17] was used to identify phenolic acids and flavonoids. 10 ml of methanol was added to 100 mg of each sample in an ultrasonic bath for 45 min. Then, centrifugation of each sample was done for 7 min at 4200 rpm. The supernatant was filtered through polyamide filter Chromafil AO-45/25 and transferred into vial prior analyses.

The HPLC method was applied as follows: a linear gradient at a flow rate of 1.0 ml/min with mobile phase of water/acetic acid (98:2 v/v; solvent

A) and methanol/acetonitrile (50:50, v/v; solvent B), starting with 5% B and increasing it to the levels of 30% at 25 min, 40% at 35 min, 52% at 40 min, 70% at 50 min, and 100% at 55 min. Estimation of phenolic acids and flavonoids was done by plotting the chromatogram at both 280 and 330 nm, respectively. Identification and quantification of components were done by comparing peak areas with external standards [18].

2.4. Analysis of the essential oil using gas chromatography-mass spectroscopy (GC–MS)

The GC–MS analysis of essential oil was performed using Agilent Technologies equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Center, Cairo, Egypt. Samples were diluted with hexane (1:19, v/v). The GC was fitted with HP-5MS column (30 m × 0.25 mm internal diameter and 25 µm film thickness). Analyses were performed using helium as the carrier gas at a flow rate of 1.0 ml/ min, a split ratio of 1:30, injection volume of 1 µL, and the following temperature program: 40°C for 1 min; rising at 4 °C/min to 150 °C and held for 6 min; rising at 4 °C/min to 210 °C and held for 1 min. The injector and detector were held at 280 °C and 220 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV using a spectral range of m/z 40–550 and a 3 min-solvent delay. Different constituents of essential thyme oil were identified by comparing the spectrum fragmentation pattern with the patterns stored in Wiley and NIST Mass Spectral Library data [19].

2.5. Biological experiment

2.5.1. Animals

Thirty male albino rats weighing an average of 200 g + 0.5 g. were procured from the animal house of the Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. These rats were kept in normal healthy laboratory conditions where temperature was adjusted at 25 ± 2 ºC for 12 hour light – dark. Also, they were adapted on free access of water and fed on a basal diet for 1 week before starting the experiment. Basal diet was composed of the following (g/kg): Casein (21.7%), sunflower oil (15%), corn starch (58.1%), cholic acid (0.2%), salt mixture (4%), and vitamin mixture (1%) according to Zahra and Pooya [20], Hegested et al. [21], and Camplell [22]. Animal experiments were conducted according to the guidelines of the National Institute of Health Guide for laboratory animal care and use (NIH Publications No. 8023, revised 1978).

2.5.2. Experimental design

Rats were divided equally into 5 groups; each group comprising 6 rats. Each group was housed in a wire cage in an equal room temperature of 25 °C ± 2 and normal healthy conditions. Food consumption was tracked and weight gain was determined weekly. The first group of rats (G1) was fed on basal diet, serving as a negative control group (N-control). The other four groups were given 15% of beef tallow instead of fresh sunflower oil proportion in the basal diet where G2 served as the positive control group (p-control). Groups 3, 4 and 5 were supplemented with the following treatments: Orlistat (2mg/Kg BW rat/day by stomach tube) (drug group, G3) [23], thyme powder (G4) (3g/100g diet), and thyme essential oil (G5) (0.6g/100g fat), respectively.

2.5.3. Growth of rats

The gained weight was calculated as follows:
Body weight gain = the final body weight – the initial body weight.
Food Efficiency Ratio (FER) = Body weight gain / Food intake.

2.5.4. Biochemical assay

At the end of experiment's period (60 days), blood samples were taken from the eye plexuses of rats then put into a dry clean centrifuge glass tube without any coagulation for serum preparation. The samples were left for 15 minutes at the room normal temperature, then centrifugation of tubes were done for another 15 minutes at 300 rpm. The clean supernatant serum was kept frozen in a temperature of-20ºC till analysis.

Serum levels of glucose, total cholesterol (T-ch), high-density lipoprotein (HDL-ch), low-density

lipoprotein (LDL-ch), very low-density lipoprotein (VLDL-ch), triglycerides (TG), and atherogenic index (AI) were determined by the methods explained by Trinder [24], Waston [25], Assmann [26], Wieland and Seidel [27], Wallach [28], Fossati and Prencipe [29], and Kikuchi et al. [30], respectively.

Liver function: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in serum were estimated by the methods described by Bergmeyer and Harder [31]. The activity of alkaline phosphatase (ALP) was determined by the method described by Varley et al. [32].

Antioxidant parameters: Serum lipid peroxidation level (Malondialdehyde, MDA) was determined by the colorimetric method described by Meltzer et al. [33].Thiobarbituric acid reacts with MDA in acidic medium at temperature of 95ºC for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product is measured at 534 nm.

Glutathione reduced (GSH) measured in erythrocyte by the method described by Ellman [34]. The method based on the reduction of 5,5dithiobis (2-nitrobenzoic acid) with GSH to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm.

Catalase (CAT) activity was estimated calorimetrically in plasma by the method described by Aebi [35]. CAT reacts with a known quantity of H2O2, the reaction is stopped after exactly one minute with Catalase inhibitor. In the presence of Horseradish peroxidase, remaining H2O2 reacted with 3,5-Dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample.

Serum total antioxidant capacity (TA) was measured calorimetrically according to the method of Koracevic et al. [36]. The determination of the antioxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provided (H2O2). The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H2O2 was determined calorimetrically by an enzymatic reaction which involved the conversion of 3,5, dichloro-2-hydroxy benzensulphonate to a colored product.

2.5.5. Histopathological examination

After the experiment period, the sample tissues from livers of all groups were collected and stored in neutral buffered formalin of 10%. Then, they were dehydrated in alcohol, cleared in xylol, and embedded in paraffin. 4µm of thick hematoxylene and eosin stained sections were prepared [37].

2.6. Statistical analysis

Data were expressed as a mean ± standard deviation (n=6). P -value <0.05 was considered statistically significant. The statistical analysis of the present study results were performed using the standard analysis of variance (ANOVA) followed by least significant difference test using MSTATC software [38].

3. Results & discussion

3.1. Chemical composition

3.1.1. Chemical characteristics of thyme

The main purpose of the present study is to highlight the natural antioxidants originating from thyme and their effects on obesity. Identification of phenols and flavonoids was performed using the HPLC instrument.

3.1.1.1. Determination of phenolic and flavonoid components of dry thyme powder

Flavonoids are the largest group of plant phenols; they constitute over half of the 8000 naturally-occurring phenolic compounds [39]. Phenolic (21) and flavonoid (14) constituents of thyme are demonstrated in Table (1). Our results revealed that thyme composition comprises phenolic components including gallic acid, 4-amino benzoic acid, protocatchuic acid, chlorogenic acid, catechol, vanillic acid, p-coumaric acid, isoferulic acid, α-coumaric acid, 3, 4, 5 methoxycinammic, coumarin, and cinammic acid with concentration ranging from 10 to 100 ppm. This is consistent with the findings of Varga et al. [40], Köksal et al. [41], El-Newary et al. [42], and Rtibi et al. [43] who reported that typical thyme constituents included pyrogallol, caffic acid, ferulic acid, and benzoic acid. Furthermore, thyme

composition includes high concentration of some other compounds such as catechin, caffeine, p-OH benzoic, ellagic acid, and salicylic acid [42, 43, 44]. Meanwhile, hesperidin was high concentration in flavonoid. These data are consistent with the results of [40, 42]. In general, the growth of different thyme herbs in different regions with different conditions lead to the variation in their composition of phenolic and flavonoid compounds. Moreover, previous studies have revealed some phenolic and flavonoids compounds, which have anti-oxidant [10], anti-inflammatory [13] and anti-cancer activity [45]. The various active ingredients of thyme play an effective role in preventing numerous diseases where they possess anti-obesity, hepatoprotective, antibacterial, anti-inflammation, and neuroprotective effects as well as anti-diabetic, gastroprotective and anti-tumor activities [14].

Table (1): Phenolic and flavonoid constituents detected in *Thymus vulgaris* using HPLC.

Phenolic constituents	Concentration (ppm)	Flavonoid constituents	Concentration (ppm)
Gallic acid	14.29	Luteolin-7-glucoside	1653.96
Pyrogallol	165.81	Naringin	888.42
4-Amino benzoic acid	38.89	Rutin	204.38
Protocatchuic acid	12.53	Hesperidin	11199.35
Catechin	367.40	Rosmarinic acid	32.47
Chlorogenic acid	42.99	Apigenin-7-glucoside	81.50
Catechol	60.29	Qurectrin	175.05
Caffeine	305.33	Querctin	20.73
P-OH benzoic acid	371.86	Kaempferol 3,7-dirhamnoside	300.26
Caffic acid	171.58	Acacetin	87.76
Vanillic acid	81.21	Narengenin	17.50
P-Coumaric acid	37.49	Hesperitin	139.88
Ferulic acid	216.35	Kampferol	71.05
Isoferulic acid	99.25	Apigenin	26.07
Ellagic acid	617.54		
α – Coumaric acid	18.01		
Benzoic acid	245.31		
Salicylic acid	1126.52		
3,4,5 Methoxy cinammic	64.02		
Coumarin	36.02		
Cinammic acid	10.30		

All results expressed as (ppm = µg/g of dry weight).

3.1.1.2. Chemical composition of thyme essential oil

Fractionation and identification of the chemical constituents of thyme essential oil were done using gas-liquid chromatography/mass spectroscopy (GC-MS) technique (Table 2). A total of 44 components were isolated from thyme essential oil which could be categorized into ten chemical classes: monocyclic terpenes (11.81%), bicyclic terpenes (5.15%), aliphatic hydrocarbons (1.88%), aromatic hydrocarbons (20.54%), oxides (3.08%), alcohols (8.14%), esters (0.53%), sesquiterpenes (5.13%), phenol/phenol ethers (42.08%), and ketone (0.82%). These compounds represent a percentage of 99.16% of thyme essential oil's chemical composition, while the remaining unknown part represents 0.84%. The first chemical group detected in thyme essential oil composition was monocyclic terpenes; it included 4 compounds: α-terpinene (1.71%), d-limonene (0.66%), ɤ-terpinene (9.26%), and α-terpinolene (0.18%). This chemical group was reported as a constituent of thyme essential oil composition by Mahboubi et al. [46], and Schott et al. [47]. The second chemical group was bicyclic terpenes; it included 7 compounds: α-thujene (1.51%), α-pinene (1.25%), camphene (0.78%), sabinene (0.1%), β-pinene (0.46%), δ-3-Carene (0.1%), and cis-sabinene hydrate (0.95%). This chemical group was reported as a constituent of thyme essential oil composition by Ainane et al. [48], and Nemati et al. [49]. The third chemical group was aliphatic hydrocarbons; it included β-Myrcene (1.88%). This chemical group

was reported as a constituent of thyme essential oil composition by Nemati et al. [49]. The fourth chemical group was aromatic hydrocarbons; it included 2 compounds: M-cymene (20.34%) and naphthalene (0.2%). The fifth chemical group was oxides; it included 2 compounds: 1,8-cineole (2.12%) and caryophyllene oxide (0.96%). This chemical group was reported as a constituent of thyme essential oil composition by Vetvicka and Vetvickova [15], Schott et al. [47], and Ainane et al. [48]. Moreover, alcohols group was the sixth identified chemical group in thyme essential oil composition; it included 11 compounds:1-octen-3-ol (0.87%), linalool (3.17%), (E)-p-2-menthen-1-ol (0.1%), borneol (1.27%), terpinen-4-ol (1.44%), p-cymen-8-ol (0.09%), α-terpineol (0.28%), geraniol (0.17%), cubedol (0.14%), 8-epi-ɤ-eudesmol (0.33%), and tau-cadinol (0.28%). Several previous studies indicated that these alcohols are the common constituents of thyme oil [15, 46, 49]. The seventh detected chemical group was esters; it included 4 compounds: isobornylformate (0.05%), α-terpinyl acetate (0.14%), thymyl acetate (0.25%), and nerolidyl acetate (0.09%) [47]. The eighth chemical group was sesquiterpenes; it included 9 compounds: α-ylangene (0.11%), β-bourbonene (0.12%), caryophyllen (3.51%), β-copaene (0.06%), humulene (0.13%), ɤ-muurolene (0.34%), germacrene-d (0.43%), α-muurolene (0.06%), and δ-cadinene (0.37%). This chemical group was also reported as a constituent of thyme essential oil composition in previous studies, specifically the studies of Ainane et al. [48], and Nemati et al. [49]. The ninth chemical group was phenol/phenol ethers; it included 4 compounds: thymyl methyl ether (9.93%), carvacrol methyl ether (2.95%), thymol (27.41%), and phenol 2-methyl-5-(1 methylethyl) (1.79%). Thymol was reported in previous studies to be the major constituent of thyme essential oil [49]. Finally, the tenth identified chemical group in thyme essential oil's composition was ketones; it included one compound: camphor (0.82%) which was recorded in previous studies to be a constituent of thyme essential oil, specifically the studies of Vetvicka and Vetvickova [15], Schott et al. [47], and Ainane et al. [48]. Collectively, as per the literature, constituents of thyme essential oil vary according to different environmental conditions or growing regions.

Table (2): Chemical components of *Thymus vulgaris* essential oil fractionated and identified by GC/Mass.

Chemical compounds	Retention time (min)	Concentration (%)
1- Monocyclic terpenes		
α-Terpinene	10.728	1.71
D-Limonene	11.195	0.66
ɤ-Terpinene	12.291	9.26
α-Terpinolene	13.299	0.18
Total		**11.81**
2- Bicyclic terpenes		
α-thujene	7.709	1.51
α-Pinene	7.902	1.25
Camphene	8.362	0.78
Sabinene	9.231	0.1
β-Pinene	9.3	0.46
δ-3-Carene	10.484	0.1
Cis-Sabinenehydrate	12.57	0.95
Total		**5.15**
3- Aliphatic hydrocarbons		
β-Myrcene	9.889	1.88
Total		**1.88**
4-Aromatic hydrocarbons		
M-Cymene	11.096	20.34
Naphthalene	27.095	0.2
Total		**20.54**
5- Oxides		
1,8-Cineole	11.271	2.12
Caryophyllene oxide	29.602	0.96
Total		**3.08**
6- Alcohols		
1-Octen-3-ol	9.551	0.87
Linalool	13.812	3.17
(E)-P-2-Menthen-1-ol	14.517	0.1
Borneol	16.242	1.27
Terpinen-4-ol	16.493	1.44
P-Cymen-8-ol	16.895	0.09
α-Terpineol	17.076	0.28
Geraniol	19.641	0.17
Cubedol	26.932	0.14
8-epi-ɤ-Eudesmol	30.896	0.33
tau-Cadinol	31.764	0.28
Total		**8.14**
7- Esters		
Isobornyl formate	18.236	0.05
α-Terpinyl acetate	22.386	0.14
Thymyl acetate	22.549	0.25
Nerolidyl acetate	28.932	0.09
Total		**0.53**
8- Sesquiterpene		
α-Ylangene	23.214	0.11
β-Bourbonene	23.488	0.12
Caryophyllene	24.601	3.51
β-Copaene	24.886	0.06
Humulene	25.644	0.13
ɤ-Muurolene	26.365	0.34
Germacrene-d	26.501	0.43
α-Muurolene	27.095	0.06
δ-Cadinene	27.783	0.37
Total		**5.13**
9- Phenols and phenol ethers		
Thymyl methyl ether	18.568	9.93
Carvacrol methyl ether	18.854	2.95
Thymol	20.771	27.41
Phenol, 2-Methyl-5-(1-Methylethyl)	20.97	1.79
Total		**42.08**
10- Ketones		
Camphor	15.263	0.82
Total		**0.82**
11-Unknown		
Unknown	10.291	0.23
Unknown	18.737	0.09
Unknown	20.44	0.52
Total		**0.84**

3.2. Biological activities

The present study was carried out to examine the protective and nutritional impacts of thyme powder and essential oil in experimental rats fed on a high-fat diet by supplementing male albino rats with thyme powder and essential oil with concentrations of (3g / 100g diet) and (0.6g / 100g fat), respectively for 2 months. Numerous parameters were investigated in this study including chemical components of thyme, food consumption, body weight gain, relative liver weight in addition to a number of biochemical tests and histopathological examination of the liver in normal and obese rats. Their effects and constituents are demonstrated in the tables of the study.

3.2.1. Effect of thyme powder and essential oil on food intake, body weight gain, and relative liver weight in obese rats

The impact of high-fat diet intake for 60 successive days with or without thyme powder and essential oil supplementation on body weight gain of rats and its results had been demonstrated in Table (3). The data in this table indicates that the initial body weights of rats were not significantly different among groups. At the end of experiment, regardless of the diet variation, decrements were observed in food intake, body weight gain, and relative organs weight in all the tested groups except for the positive control group where a significant decrement was observed in food intake/day (15.94%) as well as a significant increment in body weight gain (155.87 %) compared to negative control group fed on basal diet. Meanwhile, the groups supplemented with orlistat, thyme powder, and thyme essential oil demonstrated significantly increased food intake/day (8.34%, 12.29%, and 15.12%, respectively) and decreased body weight gain (30.33%, 36.76%, and 40.52%, respectively) compared to the positive control group. On the contrary, the relative liver weight was not significantly different among rats except that rats feed on high fat diet (G2 and G3) groups (Table 3). High-fat diet intake for 60 successive days significantly increased body weight gain and food intake. These findings are consistent with those of Bhandari et al. [50]. Thyme powder and essential oil supplementation with high-fat diet intake appeared to reduce the inhibitory impact of high fat on body weight gain and food intake. In this respect, Chooi et al. [51] defined the obesity as an enlargement in the mass of fatty tissue which leads to high risk of metabolic diseases such as cardiovascular diseases, non-insulin-dependent diabetes, and stroke as well as higher incidence of morbidity. The rat group treated with orlistat exhibited a significant decrement in body weight. This is attributed to the drug's activity where it blocks fat absorption by suppression of gastric and pancreatic lipase enzymes, leading to steatorrhea [23]. The rat groups supplemented with thyme powder and essential oil showed decrements in food intake, weight gain, and fat mass; these decrements may be attributed to the decreased serum leptin level which consequently decreases appetite [52]. Thyme's composition includes salicylic acid which caused a decline in food intake and elevation in thermogenesis where the thermogenic effect stimulated body weight loss [53]. Moreover, thymol, a major phenolic compound in thyme, decreases plasma lipids by altering hepatic triglyceride and fecal fat secretion as well as inhibiting the hepatic 3-hydroxy-3-methyle glutaryl coenzyme A (HMG-CoA) reductase activity which is a key regulatory enzyme in cholesterol synthesis. Also, this compound seems to have the ability to decrease the gene expression of proinflammatory cytokines, hence decreasing the formation of fatty streaks [54]. Pancreatic lipase has a significant role in lipid digestion [55]. Thyme powder and essential oil inhibit pancreatic lipase activity which helps in decreasing body weight and obesity [56]. The useful effect of antioxidants administration against high-fat toxicity in terms of body weight observed in the current study asserts the previous results reported by Yu et al. [54] who concluded that antioxidants supplementation may have a significant role against the toxic effects of excessive fats because of their prophylactic activity. In addition, Thamer et al. [57] found that thyme powder composition includes numerous compounds such as phenolic components and flavonoids which are responsible for its antioxidant effect. No difference in relative liver weight was observed in the group of rats supplemented with thyme powder and essential oil. However, the positive control and orlistat-treated groups demonstrated an increase in the relative liver

weight. These findings are consistent with those of Bhandari et al. [50] where the relative organs weight of rats was reported to be significantly increased after high-fat diet intake. The dietary impacts of G2 and G3 on liver weight are in Table (3). When the liver weight was compared to final body weight, it was found that fat elevated the hepatosomatic index, resulting in liver damage. Uthandi and Ramasamy [58] reported that the potentially toxic components formed as a result of high-fat food intake in rats lead to higher hepatosomatic index.

Table (3): Effect of *Thymus vulgaris* powder and essential oil on initial and final weights, weight gain, food intake, FER, and relative liver weight in obese rats.

Treatment	Initial Weight (g)	Final Weight (g)	Weight gain (g)	(%)*	Food intake (g)	FER**	Liver (%)
G1: normal control group.	200.4^{a} ±0.55	254.4^{e} ±4.40	54.05^{e} ±3.85	26.97^{e} ±1.85	1176^{a} ±5.80	0.0459^{b} ±0.003	2.18^{d} ±0.08
G2: positive control group (HFD 15% fat).	200.4^{a} ±0.45	338.6^{a} ±3.60	138.3^{a} ±3.15	69.00^{a} ±1.41	988.5^{e} ±8.50	0.1398^{a} ±0.002	4.45^{a} ±0.15
G3: HFD + orlistat (2 mg/Kg body weight /day).	200.4^{a} ±0.45	296.8^{b} ±1.75	96.35^{b} ±1.30	48.07^{b} ±0.54	1071^{d} ±10.65	0.0899^{ab} ±0.001	3.70^{b} ±0.30
G4: HFD + thyme powder (3g/100g diet).	200.3^{a} ±0.40	286.8^{c} ±1.75	87.45^{c} ±1.35	43.16^{c} ±0.58	1110^{c} ±2.25	0.0778^{b} ±0.001	2.38^{c}±0.18
G5: HFD + thyme essential oil (0.6g/100g fat).	200.4^{a} ±0.85	282.6^{d} ±0.60	82.25^{d} ±0.25	41.05^{d} ±3.00	1138^{b} ±3.20	0.0722^{b} ±0.001	2.25^{cd}±0.10
LSD#	0.3420	2.905	3.072	1.466	6.642	0.05954	0.008

- Small letters (a, b, c, d, e) indicate the level of significance difference between means in the same column in which means within the same column followed by the same letter are not significantly different at $p < 0.05$.
- Means are followed by the corresponding standard deviation.

*(%): The percentage of overweight; **FER: Food Efficiency Ratio; #LSD: Least Significant Difference.

3.2.2. Effect of thyme powder and essential oil on serum glucose and lipid profile levels of obese rats

Serum glucose levels in all the included rats are shown in Table (4). The data revealed a significant elevation in blood glucose levels of positive control rats (96.88%) compared to negative control rats. The groups supplemented with orlistat, thyme powder, and thyme essential oil showed a significant decrease in the blood glucose levels (19.07%, 21.99% and 37.24 %, respectively) compared to the positive control group. Compared to negative control rats, the positive control rats demonstrated a significant elevation in serum total cholesterol (T-ch), low-density lipoprotein (LDL-ch), triglyceride (TG), very low-density lipoprotein (VLDL-ch), and atherogenic index (AI) (104.11%, 323.87%, 72.50%, 72.50%, and 382.53%, respectively), while they demonstrated a significant decrement (28.81%) in high-density lipoprotein (HDL-ch) level as displayed in Table (4). Supplementation of the tested drug, thyme powder, and thyme essential oil rendered the measured parameters to their normal ranges. Moreover, thyme powder and essential oil-treated rats showed low serum glucose concentrations compared to positive control rats which showed higher serum glucose concentrations. Thymol is a monoterpene phenolic compound which is considered as one of the main compounds in the chemical composition thyme essential oil. In this study, thymol improved

glycoproteins components, showed its improving insulin resistance where it promoted insulin to uptake glucose and reversed the altered glycoprotein levels in plasma as well as hepatic and renal tissues in HFD-induced diabetic mice [59]. T-ch and LDL-ch concentrations were significantly influenced by the high-fat diet in all groups where the positive control group had higher serum T-ch and LDL-ch concentrations than the negative control group and the rest of groups as well. On the other hand, thyme powder and essential oil-treated rat groups had lower serum T-ch and LDL-ch concentrations than the positive control group. These findings are consistent with the findings of Tuama [60]. HFD increase oxidative stress and the presence of oxidized LDL-ch and other lipoproteins. Oxidation converts LDL-ch to a form that is quickly taken up and degraded by macrophages and elevates the degradation of unoxidized LDL-ch. Antioxidants inhibit LDL-ch metabolism and decrease the toxicity of its oxidized form [61]. Oxidized lipoproteins may lead to development of atherosclerosis [62]. The increase in HDL-ch levels can be a result of the protective mechanism against oxidative stress caused by high fat diet. Also, it may be a mechanism to prevent oxidative alterations in other lipoproteins including LDL-ch [63]. Serum high density lipoprotein cholesterol (HDL-ch): Table (4) exhibits HDL-ch lower concentrations in the rat groups supplemented with thyme powder and essential thyme oil which contain natural antioxidants in comparison with normal control rats. Meanwhile, the positive control rats showed decreased serum HDL-ch concentrations compared to the rest of groups. Yu et al. [54] found that antioxidant rich foods decreased the response to oxidative damage in the pathogenesis of many diseases and also elevated HDL-ch levels. Serum triglyceride (TG) and very low-density lipoprotein cholesterol (VLDL–ch): TG and VLDL–ch concentrations were investigated in the study groups and results are demonstrated in Table (4). A significant elevation in TG and VLDL-ch concentrations was observed in positive control rats, while lower concentrations of both parameters were observed in the orlistat, thyme powder, and thyme essential oil-treated groups compared to the positive control group. Depressing impact of thyme on T-ch, LDL-ch, VLDL-ch, and TG might be attributed to the decrement in the activity of HMG-CoA reductase caused by active components of thyme (thymol and carvarcrol) or the formation of insoluble saponin-cholesterol complexes in gastrointestinal synthesis in liver because a significant correlation was revealed between plasma cholesterol and proportional abdominal fat weight. These findings agree with those of Saravanan and Pari [59]. Also, it was previously reported that high-fat diet accelerates the development of atherosclerosis [64].

Table (4): Effect of *Thymus vulgaris* powder and essential oil on serum glucose, total cholesterol, HDL, LDL, VLDL, triglycerides, and atherogenic index in obese rats.

Treatment	Glucose (mg/dl)	Total Cholesterol (mg/dl)	HDL-ch. (mg/dl)	LDL-ch. (mg/dl)	VLDL-ch. (mg/dl)	Triglycerides (mg/dl)	Atherogenic index
G1: normal control group	84.45^{e} ±2.45	87.40^{e} ±1.40	44.60^{a} ±0.60	28.90^{e} ±0.90	13.75^{d} ±0.35	68.75^{d} ±1.75	0.956^{e} ±0.016
G2: positive control group (HFD 15% fat)	166.27^{a} ±1.27	178.40^{a} ±3.35	31.75^{e} ±1.75	122.50^{a} ±0.90	23.72^{a} ±0.72	118.60^{a} ±3.60	4.613^{a} ±0.204
G3: HFD + orlistat (2 mg/Kg body weight /day)	134.56^{b} ±3.56	143.60^{b} ±2.55	36.25^{d} ±0.25	87.73^{b} ±1.93	19.57^{b} ±0.37	97.85^{b} ±1.85	2.943^{b} ±0.045
G4: HFD + thyme powder (3g/100g diet)	129.70^{c} ±1.70	122.20^{c} ±3.15	41.50^{c} ±0.50	62.30^{c} ±2.20	18.16^{c} ±0.56	90.80^{bc} ±2.80	1.938^{c} ±0.043
G5: HFD + thyme essential oil (0.6g/100g fat)	104.35^{d} ±1.35	103.70^{d} ±1.65	42.75^{b} ±0.75	42.92^{d} ±0.60	17.98^{c} ±0.38	89.90^{c} ±1.90	1.407^{d} ±0.002
LSD[#]	0.915	1.644	1.087	1.376	0.3036	1.513	0.1975

- Small letters (a, b, c, d, e) indicate the level of significance difference between means in the same column in which means within the same column followed by the same letter are not significantly different at $p < 0.05$.
- Means are followed by the corresponding standard deviation.
[#]LSD: Least Significant Difference.

3.2.3. Effect of thyme powder and essential oil on the liver functions of obese rats

High-fat diet intake affected the liver functions of the rats negatively where a significant elevation in the actions of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) enzymes were observed in the positive control group (131.74%, 106.82%, and 129.76%, respectively) and orlistat-treated group (106.32%, 107.74%, and 88.05%, respectively) compared to normal controls. After 60 days, the groups treated with thyme powder and essential oil showed improved activities of ALT, AST, and ALP enzymes compared to the positive control group (Figure 1). Liver function can indicate the liver's state; therefore, liver function tests (LFTS) are used to identify the liver condition and if there is any impairment, assess the development of diseases, and monitor the impact of hepatotoxic drugs and necrosis on the animal liver. LFTS include serum aminotransferases, alkaline phosphatase, bilirubin, albumin, and prothrombin time [65]. ALT and AST are used to measure the concentrations of intracellular hepatic enzymes which leak into circulation. Normal levels of ALT and AST reflect the normal functioning of liver. The intracellular enzymes leakage from liver cytosol into blood stream elevates the serum ALP level, indicating hepatotoxicity in obese rats [66]. Moreover, the metabolic changes such as administration of toxin, liver cirrhosis, hepatitis, and cancer of the liver leads to the elevation of serum ALT, AST, and ALP levels [67]. Thus, these enzymes can be considered as markers for evaluating the degree of liver damage. In the present study, the reduction of ALP activity in thyme powder and thyme essential oil-treated groups, with respect to positive control group, indicates the existence of liver damage. Therefore, another significant effect which can be added to treatment with thyme is rendering ALP to its normal level. In this concern, there are some evidence that oxidative stress can mediate the impacts of oxidized fats [68]. Reactive oxygen species (ROS) formation might increase dietary oxidized fats and protein damage in the liver by promoting lipid peroxidation of the cell membrane and increasing ROS formation which could result in calcium homeostasis disturbances as well as elevated membrane fluidity and cell death.

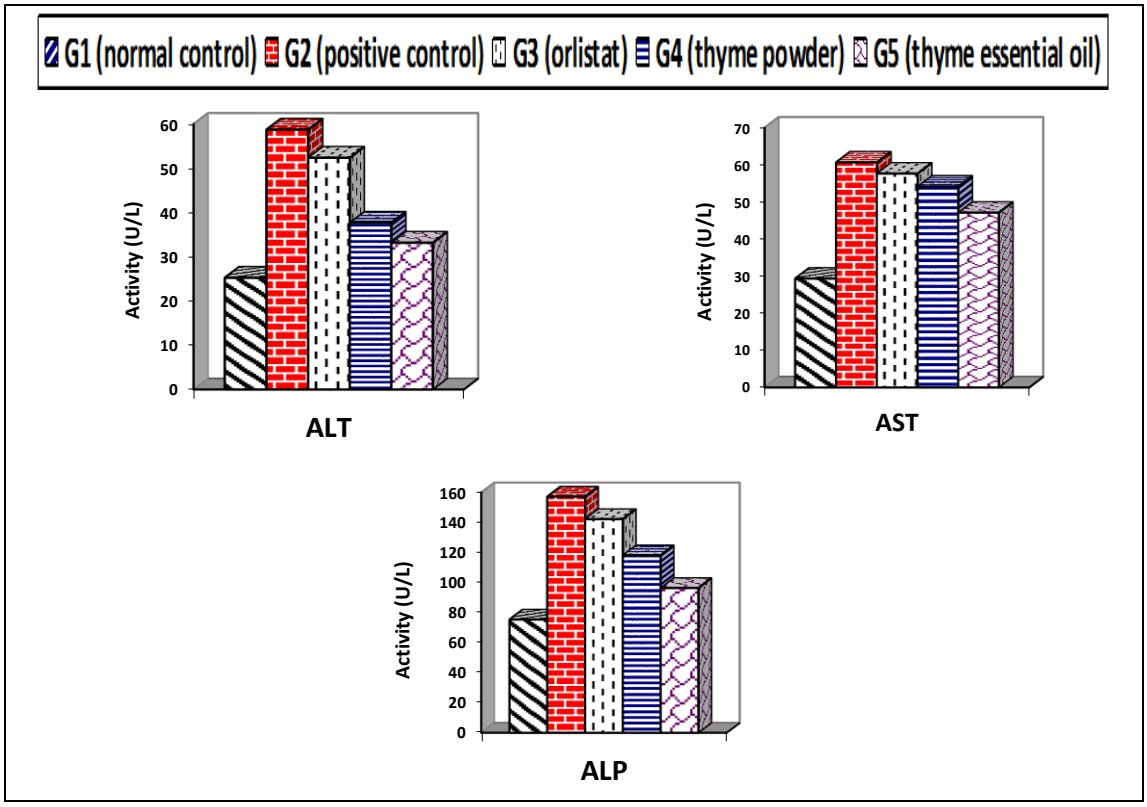

Figure (1): Effect of *Thymus vulgaris* powder and essential oil on liver functions of obese rats.
G1: normal control group; G2: positive control group (HFD 15% fat); G3: HFD + orlistat (2 mg/Kg body weight /day); G4: HFD + thyme powder (3g/100g diet); G5: HFD + thyme essential oil (0.6g/100g fat).

On the other hand, other previous studies reported that the composition of thyme powder and essential oil includes some elements which reduce the oxidation rate by eliminating free radicals or directing the breakdown of peroxides into stable substances which cannot promote further oxidation [42]. Histopathological examination of the liver in obese rats demonstrated kupffer cells activation as well as vacuolar degeneration of hepatocytes and fatty degeneration of centro-lobular hepatocytes [4]. Damaged membranes were recovered by the supplementation of herbs via promoting the status of antioxidants and decreasing lipid peroxidation [69].

3.2.4. Effect of thyme powder and essential oil on the level of malondialdehyde, enzymatic antioxidants, and non-enzymatic antioxidants activity in obese rats

Figure (2) exhibits levels of malondialdehyde (MDA) and total antioxidant (TA) in serum as well as enzymatic antioxidants, catalases (CAT), and non-enzymatic antioxidants such as glutathione reduced (GSH) in blood of experimental rat groups. Compared to normal control group, positive control group exhibited significant elevation in MDA level in serum (288.21%), while they exhibited significant decrement in TA level (59.23%). In addition, significant decrements were observed in the activities of blood enzymatic antioxidants CAT and non-enzymatic antioxidants GSH (52.91% and 64.08%, respectively) in the positive control group compared to the normal control. Thyme powder and essential oil administration in rats fed on HFD caused a significant decrement in MDA level and significant increments in total antioxidant level as well as the activities of enzymatic antioxidants CAT, and non-enzymatic antioxidants GSH in blood during the study period (60 days). Any abnormal condition causes the free radicals to be excessively produced, leading to oxidative stress an imbalance in the oxidant per antioxidant system. Generation of free radicals is an integral feature of normal cells in contrast to their excessive production and/or inadequate elimination which results in destructive and irreversible cell damage. In the normal state of the body, several enzymes such as CAT and non-enzymatic antioxidants such as GSH form a natural defense system which plays a significant role in detoxifying free radicals. Recently, the food rich in antioxidants or anti-oxidant food supplements are widely used due to the association of many diseases with oxidative stress [15, 54, 59, 69].

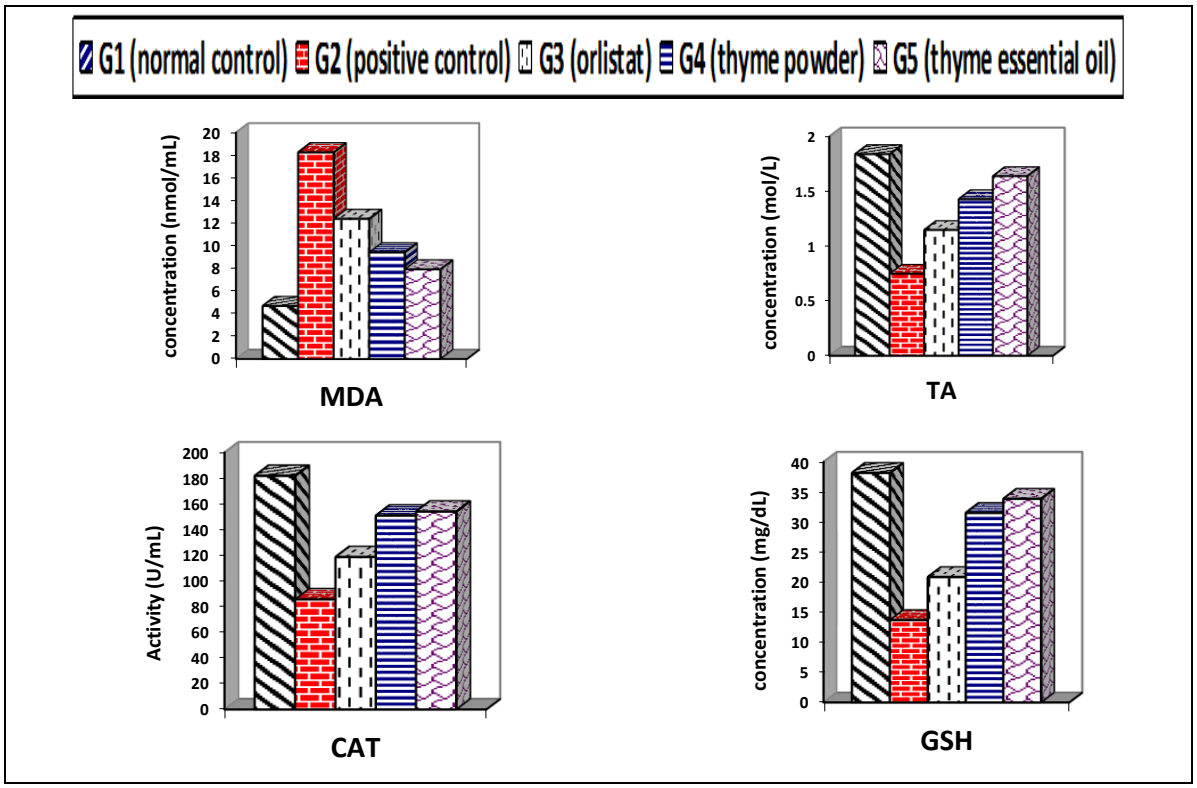

Figure (2): Effect of *Thymus vulgaris* powder and essential oil on malondialdehyde, total antioxidant, catalase, Glutathione reduced in obese rats.

G1: normal control group; G2: positive control group (HFD 15% fat); G3: HFD + orlistat (2 mg/Kg body weight /day); G4: HFD + thyme powder (3g/100g diet); G5: HFD + thyme essential oil (0.6g/100g fat).

3.2.5. Histopathological findings

The microscopic examination of liver in the present study rat groups are shown in figure 3. The untreated rat group (G1) had a normal histological structure of hepatic lobule slide (1). The liver of positive control group showed focal hepatocellular necrosis associated with inflammatory cells infiltration and fibroplasia in the portal triad in addition to newly formed bile ductuoles and cholangitis slide (2, 3 and 4). These findings are consistent with the findings of Song et al. [70]. However, orlistat-treated rats exhibited a slight cytoplasmic vacuolization of hepatocytes, slight activation of Kupffer cells, sinusoidal leukocytosis and fibroplasia in the portal triad, as well as appearance of newly formed bile ductuoles slide (5, 6 and 7). Thyme powder-treated rats (G4) exhibited a slight cytoplasmic vacuolization of hepatocytes (slide 8), while thyme essential oil-treated rats (G5) did not demonstrate any changes with apparent normal hepatocytes (slide 1). These findings are consistent with the findings of Newary et al. [42], and Elgaml and Hashish [69].

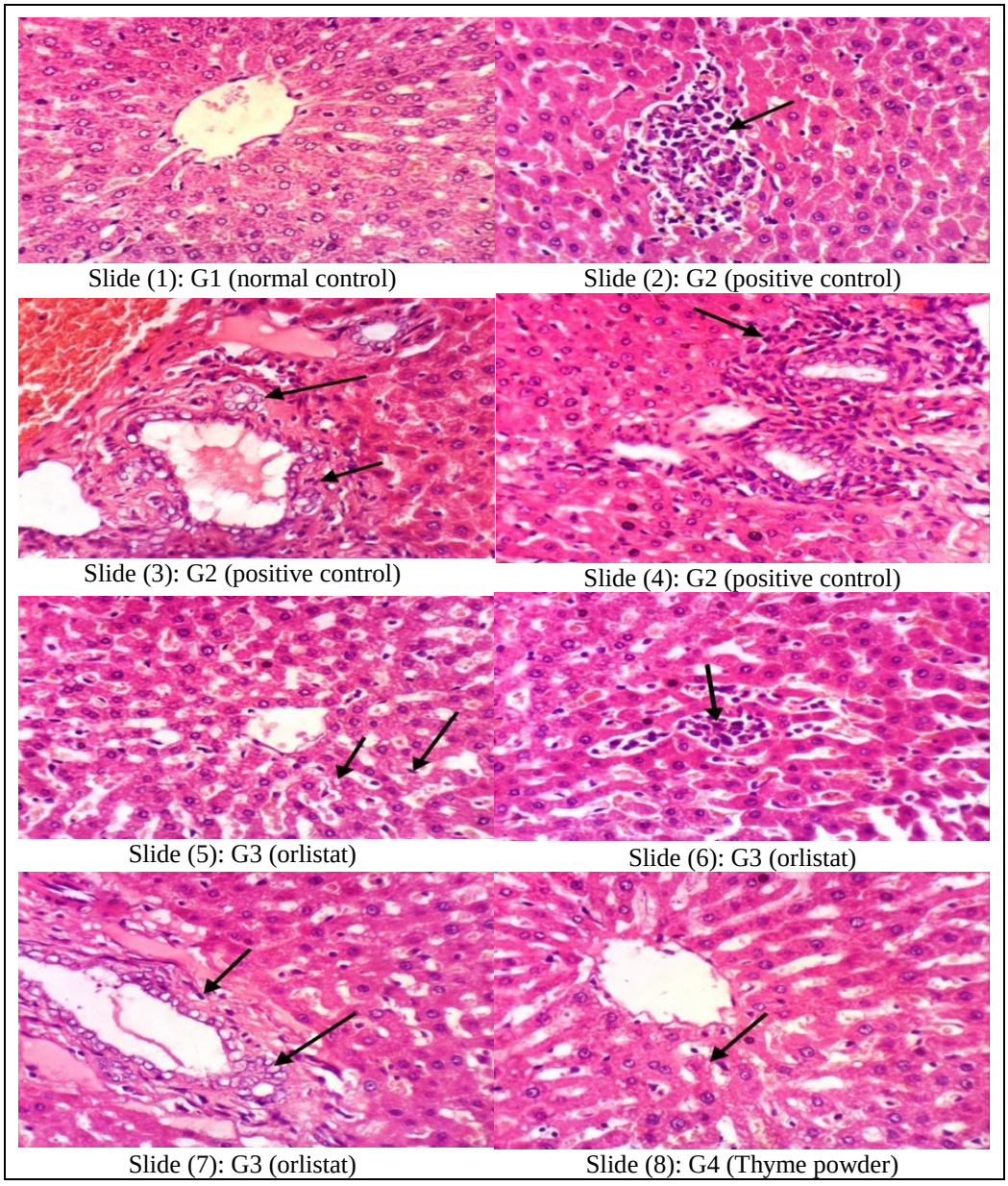

Figure (3): Histopathological changes in tissue sections liver.
Liver tissues were stained with H&E (X 400).

Slide (1) normal control group; slide (2, 3, 4) positive control group (HFD15% fat); slide (5, 6, 7) animals treated with HFD + orlistat (2 mg/Kg body weight /day); slide (8) animals treated with HFD + thyme powder (3g/100g diet).

4. Conclusion

This study concluded that lipid peroxidation is a successful indicator of the increment of free radicals caused by obesity. Consequently, administration of thyme powder and its essential oil significantly declined the levels of lipid peroxidation and thus prevent liver tissue damage.

5. Conflicts of interest

No potential conflict of interest was reported by the authors.

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