Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 5, 838-844

Effect of fennel (*Foeniculum vulgare*) on some pathophysiological alterations in induced hyperlipidemic male Albino Rats

Walaa S. El-Din Raslan[1], Yasmeen Magdy[2*], Mohamed. M.S. Gaballa[3], Maha Mamdouh[1], Shimaa A.E. Atwa[4], Mona M. Abdel Mohsen[5], Amgad kadah[1]

[1]*Department of Physiology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt.*
[2]*Department of Anatomy and Embrylogy, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt.*
[3]*Department of Pathology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt.*
[4]*Veterinary Teaching Hospital, Biochemical Department, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt.*
[5]*Chemistry of Medicinal Plants Department, National Research Centre, 33 El-Bohouth St., Dokki, Giza 12622, Egypt.*

ARTICLE INFO

Recieved: 24 November 2023

Accepted: 07 January 2024

***Correspondence:**

Corresponding author: Yasmeen Magdy
E-mail address: dr.vet_yma@yahoo.com

Keywords:

Fennel
Pathophysiology
Obesity
Dyslipidemia
Oxidative Stress

ABSTRACT

Obesity alters lipid profile, blood glucose, liver enzymes, oxidative stress, and hormonal balance. Fennel has shown anti-obesity effects in preliminary studies, but its role in ameliorative metabolic complications of obesity needs further research. This study assessed the provisional influences of fennel extract against high-fat diet-provoked metabolic deviations in rats. Forty male albino rats were allocated into standard diet control, obese control, low dose fennel (100 mg/kg) and high dose fennel (300 mg/kg) groups. Obesity was triggered by 4 weeks of high-fat nourishment. Fennel extract was applied orally for 6 weeks. Parameters considered were body weight, lipid profile, blood glucose, liver enzymes, antioxidant status, thyroid hormones, leptin, and hepatic insulin receptor gene expression. Fennel significantly diminished body weight, total cholesterol, triglycerides, LDL-cholesterol, liver enzymes, glucose, and leptin while increasing HDL-cholesterol, antioxidant enzymes, and thyroid stimulating hormone compared to obese controls. Histological examination has shown alleviation of fat accumulation and intracellular changes in the liver. Insulin receptor gene expression was also significantly increased by fennel extract. This study demonstrated that fennel extract reverses obesity-induced metabolic syndrome, oxidative stress, endocrine disruption, and histopathological change in the liver of rats. The hypolipidemic, hypoglycemic, antioxidant, and hepatoprotective properties of fennel may be beneficial in managing obesity-associated metabolic complications.

Introduction

Obesity has caught epidemic proportions globally, with over 650 million adults being obese, as per WHO estimates. WHO has pointed out that a large portion of the population of the world is overweight, with obesity putting to death more people than underweight (Andrade-Cetto *et al*., 2023). Obesity happens from a disproportion across energy intake and expenditure, leading to abnormal or exaggerated fat build-up in adipose, hepatic, muscular tissues, and other organs (Yous *et al*., 2021). It is combined with various metabolic complications: insulin resistance, dysglycemia, low and dysfunctional high-density lipoprotein, development of small dense and oxidized low-density lipoprotein, and exalted circulating levels of free fatty acids, aside from cardiovascular disorders (Conte, 2021).

Adipose tissue has an endocrine role by liberating various cytokines incriminated in appetite regulation, lipid metabolism, insulin sensitivity, and inflammation. These cytokines encompass tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), and adiponectin, besides leptin, which are involved in appetite regulation, lipid metabolism, insulin sensitivity and inflammation (Flöck *et al*., 2022; Samy *et al*., 2022). Obesity generates a chronic low-grade inflammatory state, which impacts metabolic syndrome characterized by visceral adiposity, dyslipidemia, hypertension and hyperglycemia (Alonso-Pérez *et al*., 2022; Lee, 2022).

The connection between obesity and thyroid function is likely both ways, with hypothyroidism leveraging weight and obesity leveraging thyroid function (Fontenelle *et al*., 2016). Obesity also causes hypothyroidism-like changes characterized by elevated T3 and T4 levels and lessened TSH due to hyperleptinemia and leptin-induced central thyroid hormone resistance (Sanyal and Raychaudhuri, 2016). Moreover, Leptin levels may carry a role in the hyperthyrotropinemia of obesity and heighten liability to thyroid autoimmunity and successive hypothyroidism (Zakharova *et al*., 2013).

Obesity and insulin resistance are frequent triggers of hepatic steatosis and are often associated with hyperlipidemia (Alshammari *et al*., 2023). Hyperlipidemia refers to elevated blood levels of lipids involving total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) cholesterol. Various factors contribute to the advance of hyperlipidemia, such as unhealthy lifestyles, heavy alcohol consumption, pathological disorders like diabetes and renal disease, and definite medications (Ezeh *et al*., 2021). Managing hyperlipidemia involves comprehension of lipids and their metabolism and approaches to their management, such as the interrelationship of LDL-C and non-HDL cholesterol with detrimental cardiovascular events (Nirosha *et al*., 2014).

Pharmacological therapeutics for obesity have drawbacks and are often accompanied by adverse effects (Aghabeiglooei *et al*., 2023). In recent years, herbal solutions and nutraceuticals have become safer and more effective options for managing weight (Balkrishna *et al*., 2023). One such herb is Fennel (*Foeniculum vulgare*), routinely used for various digestive issues. The seeds of fennel are rich in compounds like anethole, dianethole, photoanethole, and alpha-pinene, which have demonstrated antioxidant, anti-inflammatory, anti-diabetic, and hypolipidemic attributes (Vyshnevska *et al*., 2022). Animal studies point out that fennel can reduce weight gain, lower lipid levels, and augment insulin sensitivity in models of diet-induced obesity (Elghazaly *et al*., 2019). Nevertheless, further investigation is required to understand its potential to alleviate complications, among others, Non-Alcoholic Fatty Liver Disease (NAFLD), hormonal discrepancies, and related molecular alterations.

This research targeted to fully assess the effects of fennel extract complementarity on high-fat diet-provoked obesity in rats with a focus on lipid profile, glycemic control, hepatic structure and function, oxidant-an-

ISSN: 2090-6277/2090-6269/

tioxidant balance, thyroid and metabolic hormones, and expression of genes tangled in hepatic insulin and lipid metabolic process. Illuminating the impact of fennel on obesity-associated metabolic derangements may present a scientific rationale for its use in managing this global epidemic.

Materials and methods

Preparation of fennel extract

Plant material

The fennel seeds (*Foeniculum vulgare*) employed in this study were procured from a local market. These seeds were subjected to grinding and sieving practices to attain a fine powder. The powder was then extracted by applying boiling water for 60 minutes. The resulting extract was subjected to dryness at 60°C utilizing a rotational evaporator, a technique adapted from Boyadzhieva and Angelov (2014).

Quantitative analysis of fennel extract carbohydrates and sugars

Sugars extraction

A 1g sample was extracted using hot 80% ethanol (2x5 ml). The supernatant was gathered and vaporized on a water bath set at 80°C. The sugars were then dissolved in 10 ml of water, sticking to the methodology outlined in recent studies (Zali *et al*., 2023).

Carbohydrate extraction

A 25 mg sample was weighed and mixed together with 250 µL of 72% (w/w) sulfuric acid. The mixture was incubated at 30±3°C for 1 hour, with intermittent vortexing. Post-incubation, the sample was diluted with 7 mL of water and then autoclaved at 121°C for an hour. The sample was then neutralized to a pH between 6 and 8 using calcium carbonate, as per established protocols (Chen *et al*., 2021).

HPLC analysis

High-Performance Liquid Chromatography (HPLC) was implemented via an Agilent Technologies (1100 series) liquid chromatograph, provided with an autosampler plus a refractive index detector (RID). The analytical column used was a Shim-pack SCR-101N. Ultrapure water served as the mobile phase, with a 0.7 ml/min flow rate. The 20 minutes run time set with isocratic elution. Sugar concentrations were determined by comparing the integrated areas with standard curves of sucrose, glucose, fructose, and arabinose, as described (Lai *et al*., 2022).

Total phenolic content

The overall phenolic was settled in line with the Folin-Ciocalteu practice, as described by Žilić *et al*. (2012). A 500 µl extract aliquot was blended with 250 µl of Folin-Ciocalteu reagent and allowed to oxidize for 5 minutes. The admixture was then neutralized with 1.25 ml of 20% aqueous Na_2CO_3 solution. Subsequent to 40 minutes, the absorbance was determined at 725 nm counter to a solvent blank. The phenolic content was stated as mg of gallic acid corresponding (GAE) per gram of sample.

Total flavonoid content

The overall flavonoid was calculated following the methodology described by Muflihah *et al*. (2021). A 250 µl aliquot of 5% $NaNO_2$ was blended with 500 µl of the extract. Later, after 6 minutes, 2.5 ml of a 10% $AlCl_3$ solution was included. The mixture was then centrifuged for 10 minutes, and the absorbance of the supernatant was quantified at 510 nm against a solvent blank. The flavonoid content was stated as mg of quercetin equivalent (QE) per gram of sample.

Radical DPPH scavenging activity

The radical scavenging activity was settled via 1,1-Diphenyl-2-picryl-hydrazyl (DPPH•) with a final concentration of 50 µM, as described by Hwang and Thi (2014). The absorbance at 517 nm was calculated counter to a blank of pure methanol at 60 minutes. The percentage impediment of DPPH free radical was measured using the formula:

Inhibition (%) = 100 × (Acontrol−Asample)/Acontrol

Experimental animals

The animal study was performed on healthy adult male albino rats (Rattus albinus) weighing 160-180 g. The rats were secured from the animal breeding facility at the Faculty of Agriculture, Benha University, Egypt.

Prior approval was obtained from the Ethics Committee, Faculty of Veterinary Medicine, Benha University, with approval number (BUFVTM 14-04-23).

The rats were kept in clean polypropylene pens in a well-ventilated room maintained at 25.0±2.0°C temperature, 55.0±5.0% relative humidity, and a 12-hour light/dark cycle. Standard rodent meal and clean water were supplied ad libitum. The animals were acclimatized in the laboratory environment for 2 weeks ahead of the start of the experiment.

Experimental diets

The experimental diets for rats were fabricated according to two primary formulations: a standard meal and a high-fat meal. These formulations were based on the research conducted by Das and Choudhuri (2020). The ingredients were sourced from El-Gomhoria Company in Cairo, Egypt. The standard diet for the control group contained fat (5%), carbohydrates (65%), proteins (20.3%), fiber (5%), salt admixture (3.7%), and vitamin admixture (1%). In contrast, the high-fat diet was constituted of fat (46%), carbohydrates (24%), proteins (20.3%), fiber (5%), salt admixture (3.7%), and vitamin admixture (1%).

The high-fat meal offered 46% energy from fat compared to 5% in the standard diet. Both diets were compiled weekly, kept at 4°C and provided fresh daily ad libitum.

Experimental design

After acclimation, the rats were arbitrarily partitioned into four test categories (n=10 per grouping) as follows:

Group I: Normal control - Standard rodent food.

Group II: Obese control - High-fat meal for 4 weeks .

Group III: Fennel low dose - High-fat meal for 4 weeks followed by oral fennel extract 100 mg/kg body weight daily for next 6 weeks.

Group IV: Fennel high dose - High-fat diet for 4 weeks followed by oral fennel extract 300 mg/kg body weight daily for next 6 weeks.

The fennel extracts were administered to respective groups by oral gavage once daily. An equal quantity of distilled water was applied to normal and obese control groups.

Experimental investigation

Blood collection and serum separation

Blood samples were procured from rats subsequent to an overnight fasting period of 12-14 hours. The samples were drawn from the retro-orbital venous plexus and separated into two aliquots. The first aliquot was collected into tubes containing sodium fluoride for the quantification of blood glucose levels. The second aliquot was placed into sterile, dry

centrifuge tubes and centrifugated at 300 rpm for 15 minutes at ambient temperature to isolate serum for later biochemical analyses.

Biochemical Analysis

Serum lipid profile

Serum total cholesterol levels were established employing the protocol recognized by Allain *et al*. (1974). Serum triglyceride concentrations were quantified in accordance with the method illustrated by Fossati and Prencipe (1982). High-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels were assessed based on the guidelines set forth by Lopes-Virella *et al*. (1977).

Liver function and glucose analysis

Serum levels of ALT and AST aminotransferases were determined, as illustrated by Huang *et al*. (2006). Fasting blood glucose readings were esteemed via the glucose oxidase-peroxidase (GOD-POD) endpoint technique, following the protocol by Trinder (Trinder, 1969).

Evaluation of oxidative stress markers

Oxidative stress markers were calculated in liver tissue homogenates matching the manufacturer's instructions using Bio-diagnostic assay kits (Giza, Egypt). Malondialdehyde (MDA) levels were calculated using the thiobarbituric acid reactive substances (TBARS), as per Ohkawa *et al*. (1979). Activities of catalase (CAT), besides superoxide dismutase (SOD), were determined via the methods described by Aebi (1984) anf Nishikimi *et al*. (1972), respectively.

Hormonal assays

Triiodothyronine (T3), Thyroxine (T4), Thyroid-stimulating hormone (TSH), and leptin in serum were quantified utilizing rat-specific ELISA kits, as described by Barker (1948); Considine *et al*. (1996); Fisher (1996) and Gharib *et al*. (1971).

Isolation (RNA), synthesis (cDNA), and Real-Time (PCR) analysis

Liver tissues were collected from the rats and subjected to RNeasy Mini Kit (Thermo Qiagen, #74104) for total RNA isolation as per the producer's recommendation. The isolated RNA concentration and purity were subsequently checked using a Quawell nanodrop Q5000. About 5 mg of this RNA was then reverse transcribed utilizing Quantiscript reverse transcriptase to synthesize cDNA. This cDNA served as the template for real-time PCR, which was conducted on a Step One Plus system (Applied Biosystems, USA). Primers specific for the insulin receptor gene (INSR) and β-actin were employed.

The INSR primers had the sequences: Forward - TTTGTCATGGATGGAGGCTA, and Reverse - CCTCATCTTGGGGTTGAACT.

The β-actin primers had the sequences: Forward - ATGGTGGGTATGGGTCAG, and Reverse - CAATGCCGTGTTCAATGG.

β-actin was utilized as the standard gene for normalization purposes. The relative expression levels of the INSR gene were quantified thanks to the $2^{-\Delta\Delta Ct}$ system, as outlined by Livak and Schmittgen (2001).

Histopathological examination

Liver tissue specimens were directly fixed in 10% neutral buffered formalin for optimal cellular preservation, followed by a graded ethanol series for dehydration. The tissues were then xylene-cleared and paraffin-embedded. Thin (5-7 µm) sections were ready employing a microtome and then stained using hematoxylin and eosin (H&E), a standard histological staining technique (Suvarna *et al*., 2019). These sections were subsequently examined for histopathological alterations using a computerized light microscope (Leica DM 3000 LED), allowing for high-resolution imaging of tissue structures.

Statistical analysis

Statistical evaluations were resulted utilizing the IBM SPSS (Version 16.0) program. A one-way analysis of variance (ANOVA) was considered to decide the variance within datasets at a significance level set at $\alpha = 0.05$. Post-hoc analysis was subsequently carried out using Fisher's Least Significant Difference (LSD) test. Further validation was executed through Duncan's Multiple Range Test, with a predefined significance level at $p < 0.05$. All quantitative data are stated as means ± standard errors (SE), and estimates were deemed statistically significant if $p \leq 0.05$.

Results

Quantification of bioactive components in fennel seed extract

The present study used standard spectrophotometric approaches to estimate the major bioactive phytochemicals in aqueous extract of fennel (*Foeniculum vulgare*) seeds.

Total phenolic content

The total phenolic defined by Folin-Ciocalteu assay was 16.14 mg gallic acid equivalents (GAE) per gram of fennel seed extract. This indicates an appreciable amount of phenolic compounds, which may offer the antioxidant potential of fennel seeds.

Total flavonoid content

The aluminum chloride colorimetric method estimated total flavonoids as 9.73 mg quercetin equivalents (QE) per gram of extract. Flavonoids are important natural antioxidants, and this substantial content denotes the radical scavenging ability of fennel extract.

Carbohydrates and sugars

Acid hydrolysis of the sample, subsequent by HPLC analysis, indicated total carbohydrates as 226.4 mg per gram extract. The significant component sugars were glucose (160.98 mg/g), fructose (50.71 mg/g) and arabinose (14.7 mg/g). Direct HPLC method without hydrolysis quantified total sugars as 41.96 mg/g extract comprising of sucrose (8.56 mg/g), glucose (5.88 mg/g) and fructose (27.52 mg/g).

Effect of fennel seed extract on body weight

There was a considerable increase ($p \leq 0.05$) in the body weight of obese control rats in contrast to normal control rats at all time intervals. Fennel supplementation at both low and high doses did not cause a considerable change in body weight, unlike obese controls at 1- and 2 weeks post-treatment. However, fennel treatment led to significantly lowered ($p \leq 0.05$) body weight relative to obese controls from 3 weeks onwards, although weights remained higher than normal controls throughout the study. The body weight-declining result was more clear with the high-dose fennel, as illustrated in Table 1.

Effect of fennel seed extract on lipid profile

The obese rats showed remarkably increased ($p \leq 0.05$) total cholesterol, triglycerides, LDL-cholesterol and lowered HDL-cholesterol, unlike normal controls at 2-, 4- and 6 weeks post-treatment. Fennel supplementation attenuated obesity-induced hypercholesterolemia and hypertriglyceridemia, with the high dose showing more significant hypolipid-

emic activity. Both fennel doses significantly decreased (p≤0.05) LDL and increased HDL versus obese controls at all time points, as illustrated in Table 2.

Effect of fennel seed extract on metabolic parameters

Obese rats exhibited significantly elevated (p≤0.05) blood glucose and serum transaminases (ALT, AST) along with diminished antioxidant enzymes (CAT, SOD) in comparison to normal controls at 2, 4 and 6 weeks. Serum MDA was also lowered by both fennel doses, indicative of reduced lipid peroxidation. Fennel supplementation at both high and low doses significantly mitigated (p≤0.05) obesity-induced hyperglycemia and hepatocellular damage suggested by restoration of ALT, AST and antioxidant activities towards normal levels, as illustrated in Table 3.

Effect of fennel seed extract on hepatic insulin receptor gene expression

Obese rats showed significantly lowered (p≤0.05) hepatic insulin receptor gene expression compared to normal controls at 2-, 4- and 6-weeks post-treatment. Fennel supplementation at concomitantly low and high doses substantially improved (p≤0.05) the insulin receptor gene expression compared to obese controls at all time points, suggesting enhancement of insulin sensitivity as illustrated in Table 4.

Effect of fennel seed extract on hormones

The obese rats showed remarkably increased (p≤0.05) serum T3 Fig. 3A, T4 Fig. 3B and leptin Fig. 3D but decreased TSH Fig. 3C relative to normal controls. Fennel supplementation considerably attenuated (p≤0.05) obesity-induced rise in T3, T4 and leptin at 4 and 6 weeks post-treatment. Fennel also normalized TSH levels versus obese controls, indicating a reversal of hormonal imbalance, as illustrated in Table 5.

Histopathological results

In the histopathological evaluation of hepatic tissue, distinct variations were observed across four experimental groups of rats, each exposed to differing dietary and treatment regimens. Micrographs corresponding to each condition are presented in Figure 1.

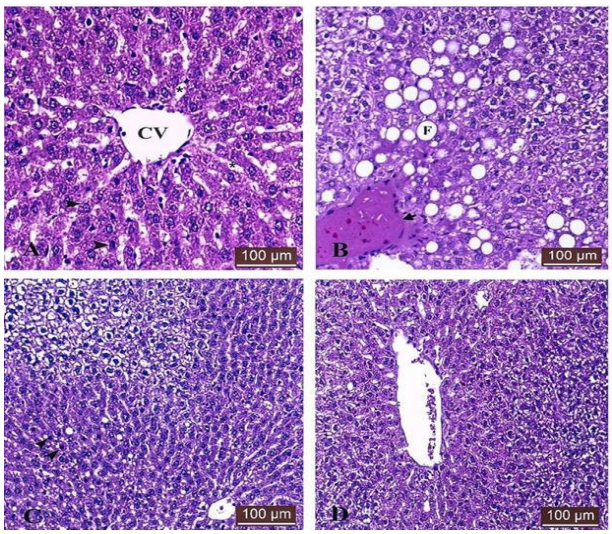

Figure 1. Histological sections of the liver in control and *Foeniculum vulgare*-treated groups of rats. (A) Photomicrograph of the liver section of rats of the control group showing a normal structure of hepatocytes (arrowhead), blood sinusoid (star) and central vein (CV). H&E stain, bar indicates magnification. (B) Photomicrograph of liver section of rats of an obese group showing swollen hepatocytes with vacuolated cytoplasm filled with fat droplet (F), congested central vein with RBCs (black arrow) and disappearance of blood sinusoids. H&E stain, bar indicates magnification. (C) Photomicrograph of liver section of rats of treated group (100 mg/ kg) showing mild recovery in the liver tissues. H&E stain, bar indicates magnification. (D) Photomicrograph of liver section of rats of treated group (300 mg/ kg) showing noticeable signs of recovery in the liver tissue of mice as some hepatocytes appeared normal. At the same time, some of them contained pyknotic nuclei (black arrowhead). H&E stain, bar indicates magnification.

Table 1. Effect of Fennel on Body Weight in High-Fat Diet-Induced Obese Rats.

Groups	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Normal Control	173.33±2.11^{b}	178.33±2.11^{b}	195.00±6.05^{b}	206.67±7.49^{b}	215.83±11.50^{b}	211.67±1.67^{c}
Obese Control	261.67±8.72^{a}	273.33±8.33^{a}	317.14±9.12^{a}	344.29±13.65^{a}	359.29±12.22^{a}	408.33±6.00^{a}
Fennel 100 mg/kg	253.33±1.05^{a}	272.50±2.14^{a}	330.71±10.32^{a}	357.86±12.43^{a}	345.71±12.31^{a}	293.33±6.67^{b}
Fennel 300 mg/kg	260.00±4.12^{a}	275.00±2.67^{a}	340.00±11.29^{a}	363.57±12.13^{a}	354.29±12.51^{a}	301.67±1.67^{b}

Values are displayed as Mean ± SEM (n=10); Means with different superscripts (a,b,c) differ significantly at p≤0.05 by one-way ANOVA and later by LSD test.

Table 2. Effect of Fennel on Lipid Profile in High-Fat Diet-Induced Obese Rats.

Parameters	Groups	2 weeks	4 weeks	6 weeks
Total cholesterol (mg/dl)	Normal Control	104.41±1.72^{c}	97.66±3.24^{b}	106.85±1.29^{bc}
	Obese Control	158.87±2.87^{a}	170.04±3.66^{a}	181.93±3.78^{a}
	Fennel 100 mg/kg	111.64±2.56^{b}	105.46±3.75^{b}	116.78±3.28^{b}
	Fennel 300 mg/kg	101.92±2.71^{c}	95.93±2.89^{b}	101.68±4.07^{c}
Triglycerides (mg/dl)	Normal Control	130.00±1.32^{c}	131.74±1.13^{b}	138.75±3.48^{b}
	Obese Control	179.37±2.53^{a}	186.96±1.78^{a}	209.01±3.58^{a}
	Fennel 100 mg/kg	148.74±3.01^{b}	125.24±2.62^{b}	128.32±3.29^{c}
	Fennel 300 mg/kg	136.09±3.01^{c}	103.58±2.57^{c}	122.01±1.65^{c}
HDL-C (mg/dl)	Normal Control	59.58±4.67^{a}	43.22±0.84^{a}	37.72±1.08^{a}
	Obese Control	40.24±1.20^{b}	37.15±2.12^{b}	29.87±1.94^{b}
	Fennel 100 mg/kg	42.48±1.35^{b}	38.37±2.07^{ab}	40.19±1.97^{a}
	Fennel 300 mg/kg	47.91±1.90^{b}	43.17±0.67^{a}	45.06±3.51^{a}
LDL-C (mg/dl)	Normal Control	14.83±3.39^{d}	28.10±2.87^{b}	41.38±2.01^{bc}
	Obese Control	82.76±2.16^{a}	95.50±5.73^{a}	110.25±5.54^{a}
	Fennel 100 mg/kg	39.41±3.34^{b}	42.04±5.42^{b}	50.92±4.03^{b}
	Fennel 300 mg/kg	26.79±3.49^{c}	32.04±4.03^{c}	32.22±7.20^{c}

Values are expressed as Mean ± SEM (n=10); Means with different superscripts (a,b,c) differ significantly at p≤0.05 by one-way ANOVA followed by LSD test.

Table 3. Effect of fennel on metabolic parameters in high-fat diet-induced obese rats

Parameters	Groups	2 weeks	4 weeks	6 weeks
Blood Glucose (mg/dl)	Normal Control	97.95±0.89^{b}	84.77±2.54^{b}	87.73±0.95^{b}
	Obese Control	105.55±1.82^{a}	96.14±1.10^{a}	97.48±0.39^{a}
	Fennel 100 mg/kg	101.27±0.93^{b}	84.04±3.36^{b}	85.46±0.69^{b}
	Fennel 300 mg/kg	98.27±0.80^{b}	81.60±2.86^{b}	77.88±0.92^{c}
ALT (U/L)	Normal Control	26.19±0.50^{b}	22.83±0.73^{b}	21.55±0.39^{b}
	Obese Control	30.34±0.99^{a}	26.24±0.77^{a}	23.23±0.35^{a}
	Fennel 100 mg/kg	27.89±1.18^{ab}	24.02±0.63^{b}	22.12±0.34^{b}
	Fennel 300 mg/kg	26.47±0.90^{b}	23.95±0.34^{b}	21.73±0.20^{b}
AST (U/L)	Normal Control	32.73±1.06^{bc}	30.62±0.27^{b}	28.65±0.53^{b}
	Obese Control	37.57±0.89^{a}	33.05±0.50^{a}	30.60±0.66^{a}
	Fennel 100 mg/kg	35.66±0.40^{ab}	30.03±1.07^{b}	28.39±0.54^{b}
	Fennel 300 mg/kg	32.57±1.08^{c}	29.59±0.75^{b}	28.04±0.12^{b}
CAT (U/ml)	Normal Control	37.26±1.42^{c}	44.47±0.30^{c}	45.23±0.28^{c}
	Obese Control	28.53±1.44^{d}	42.08±0.59^{d}	42.91±0.57^{d}
	Fennel 100 mg/kg	44.68±1.43^{b}	46.71±0.67^{b}	48.87±0.90^{b}
	Fennel 300 mg/kg	52.00±0.58^{a}	55.69±0.95^{a}	53.17±0.62^{a}
SOD (U/ml)	Normal Control	27.29±1.05^{b}	25.52±0.76^{b}	27.87±0.82^{b}
	Obese Control	25.48±1.09^{b}	23.00±0.43^{c}	23.74±0.44^{c}
	Fennel 100 mg/kg	27.64±0.83^{b}	25.30±0.75^{b}	26.92±0.57^{b}
	Fennel 300 mg/kg	38.17±0.38^{a}	29.60±0.75^{a}	31.09±0.88^{a}
MDA (nM/ml)	Normal Control	34.16±1.55^{c}	30.04±3.22^{b}	33.82±1.33^{b}
	Obese Control	63.96±3.54^{a}	63.62±4.20^{a}	62.52±1.91^{a}
	Fennel 100 mg/kg	45.25±1.89^{b}	42.62±3.79^{b}	37.81±1.05^{b}
	Fennel 300 mg/kg	39.99±1.53^{bc}	37.67±6.77^{b}	35.25±0.94^{b}

Values are expressed as Mean ± SEM (n=10); Means with different superscripts (a,b,c) differ significantly at p≤0.05 by one-way ANOVA followed by LSD test.

Table 4. Effect of fennel on hepatic insulin receptor gene expression in high-fat diet-induced obese rats.

Gene Expression (Fold Change) Groups	2 weeks	4 weeks	6 weeks
Normal Control	1.00±0.00^{a}	0.99±0.02^{a}	0.99±0.02^{a}
Obese Control	0.27±0.04^{c}	0.30±0.01^{c}	0.33±0.02^{c}
Fennel 100 mg/kg	0.48±0.03^{b}	0.54±0.02^{b}	0.59±0.03^{b}
Fennel 300 mg/kg	0.53±0.02^{b}	0.59±0.01^{b}	0.61±0.02^{b}

Values are expressed as Mean ± SEM (n=10); Means with different superscripts (a,b,c) differ significantly at p≤0.05 by one-way ANOVA followed by LSD test.

Table 5. Effect of fennel on metabolic hormones in high-fat diet induced obese rats.

Parameters	Groups	2 weeks	4 weeks	6 weeks
Leptin (ng/ml)	Normal Control	22.57±1.54^{c}	22.98±2.20^{d}	25.70±1.54^{d}
	Obese Control	67.16±2.14^{a}	70.36±2.00^{a}	71.35±1.99^{a}
	Fennel 100 mg/kg	46.57±1.81^{b}	45.74±1.24^{b}	45.44±0.82^{b}
	Fennel 300 mg/kg	41.51±2.03^{b}	38.90±1.21^{c}	36.20±1.33^{c}
T3 (ng/ml)	Normal Control	0.70±0.06^{b}	0.72±0.09^{b}	0.64±0.09^{c}
	Obese Control	1.29±0.07^{a}	1.30±0.08^{a}	1.39±0.07^{a}
	Fennel 100 mg/kg	1.14±0.09^{a}	1.11±0.02^{a}	1.13±0.05^{b}
	Fennel 300 mg/kg	0.91±0.02^{b}	0.75±0.13^{b}	0.93±0.04^{b}
T4 (ng/ml)	Normal Control	4.14±0.21^{b}	4.04±0.10^{b}	3.93±0.20^{b}
	Obese Control	5.36±0.52^{a}	5.43±0.35^{a}	5.74±0.29^{a}
	Fennel 100 mg/kg	5.31±0.24^{a}	5.39±0.28^{a}	4.66±0.34^{b}
	Fennel 300 mg/kg	4.47±0.27^{ab}	4.45±0.29^{b}	4.59±0.43^{b}
TSH (µU/ml)	Normal Control	0.58±0.03^{a}	0.58±0.04^{a}	0.58±0.04^{a}
	Obese Control	0.30±0.04^{b}	0.22±0.02^{b}	0.21±0.01^{b}
	Fennel 100 mg/kg	0.50±0.06^{a}	0.48±0.05^{a}	0.46±0.04^{a}
	Fennel 300 mg/kg	0.52±0.04^{a}	0.57±0.07^{a}	0.56±0.04^{a}

Values are expressed as Mean ± SEM (n=10); Means with different superscripts (a,b,c) differ significantly at p≤0.05 by one-way ANOVA followed by LSD test.

Control Group: Rats in the control group received a standard diet demonstrated hepatic tissues with well-preserved structural integrity. The hepatocytes, blood sinusoids, and central vein appeared morphologically normal, as depicted in Figure 1(A).

Obese Group: The hepatic architecture of rats in the obese cohort presented with significant alterations. Specifically, hepatocytes were swollen and exhibited vacuolated cytoplasm laden with lipid droplets. Additionally, blood sinusoids were conspicuously absent, the central vein was congested, and hemolyzed red blood cells were observed within the vascular structures, as illustrated in Figure 1(B).

Low-Dose *Foeniculum vulgare* Treatment (100 mg/kg): Rats treated with a low dosage of *Foeniculum vulgare* manifested moderate hepatic tissue recuperation. The endothelial lining of the central vein appeared partially detached, and a heterogeneous population of hepatocytes was observed: some appeared morphologically normal, while others were vacuolated and displayed pyknotic nuclei, as shown in Figure 1(C).

High-Dose *Foeniculum vulgare* Treatment (300 mg/kg): In rats subjected to a high dosage of *Foeniculum vulgare*, palpable signs of hepatic recovery were evident. Hepatocytes proximal to the central vein were nearly normocellular, featuring eosinophilic cytoplasm. Furthermore, the blood sinusoids and central vein appeared unobstructed. Notably, bi-nucleated cells were also observed. However, peripherally, hepatocytes still exhibited vacuolization and pyknotic nuclei, as Figure 1(D) denoted.

Discussion

The current study provides important insights into fennel seed extract's anti-obesity and metabolic consequences in rats fed a high-fat diet. Obesity was effectively induced by 4 weeks of high-fat feeding, indicated by significantly increased body weights in the obese control group versus normal diet-fed rats, agreeing with preceding studies (Amin and Nagy, 2009; Shahat *et al*., 2012). Fennel supplementation for 6 weeks attenuated weight gain in obese rats, although their weights remained higher than normal controls. The body weight diminishing effect was more noticeable with the higher 300 mg/kg dose. Studies have ascertained that fennel seed extract can reduce body weight gain provoked by a high-fat diet in obese rats (You *et al*., 2014). Fennel could suppress appetite and weight gain by stimulating cholecystokinin release, as its seeds contain trypsin inhibitors (Schöne *et al*., 2006). Phytoestrogens like isoflavones in fennel also inhibit serotonin re-uptake to increase satiety signaling (Ofir *et al*., 2003). Enhanced fat metabolism by activating PPARγ receptors and raised thermogenesis by trans-anethole may also add to the anti-obesity effect of fennel (Lv *et al*., 2023).

The liver performs a key job in lipid and lipoprotein metabolism, including uptake, oxidation and secretion of fatty acids, cholesterol synthesis, and production of triglyceride-rich lipoproteins (Enjoji *et al*., 2016). Hepatic steatosis results when fatty acid input and de novo lipogenesis overwhelm the liver's ability to metabolize and export lipids. This is characterized by fat accumulation in hepatocytes along with mitochondrial dysfunction (Bhattacharjee *et al*., 2023). Additionally, fennel seed extract has been shown to exhibit hypolipidaemic activity, improving lipid profiles and hepatic enzyme activity in rats with high-fat diet-induced obesity (Nagamma *et al*., 2019). Fennel administration reversed high-fat diet-induced fat deposition in the liver, pointing to its potential to alleviate hepatosteatosis by modulating lipid homeostasis pathways.

Diet-induced obesity led to significant derangements in lipid profile, evidenced by exalted total cholesterol, triglycerides and LDL, and lowered HDL in the obese controls, as reported earlier (Galisteo *et al*., 2005; Shahat *et al*., 2012). Fennel supplementation attenuated dyslipidemia, improving the atherogenic risk profile. Increased faecal fat excretion by delayed gastrointestinal transit and reduced cholesterol absorption could mediate the lipid-lowering action (Lal *et al*., 2004). Anethole, the major component of fennel, suppressed HMG CoA reductase and enhanced LDL receptor expression in hepatocytes, thereby decreasing LDL and total cholesterol levels (Xie *et al*., 2009). The lipid peroxidation marker MDA was also significantly lowered by fennel, attributed to the antioxidant constituents like quercetin, kaempferol and anethole (Choi and Hwang, 2004). These findings suggest that fennel seed extract may have the potential to be a useful agent against obesity-related diseases (HANDA *et al*., 2005).

Obesity and insulin resistance are accompanied by non-alcoholic fatty liver disease (NAFLD), dominated by hepatomegaly and raised liver enzymes (EASL–EASD–EASO, 2018; Godoy-Matos *et al*., 2020). In this study, fennel supplementation reversed obesity-induced increments in serum AST, ALT and ALP, as well as restored the diminished antioxidant enzymes CAT and SOD in liver tissues. This highlights the hepatoprotective and antioxidant benefits of fennel in contravention of high-fat diet provoked liver dysfunction, in line with earlier reports (Al-Amoudi, 2017; Ozbek *et al*., 2003). Quercetin present in fennel scavenges free radicals, suppresses lipogenesis and improves mitochondrial function in the liver (Gayibov *et al*., 2021). Moreover, obesity is accompanied by long-lasting low-grade inflammation, which can lead to insulin resistance and metabolic disturbance (Monteiro and Azevedo, 2010). Adipose tissue macrophages are increased in obesity and release pro-inflammatory cytokines like TNF-α, IL-6 and IL-1β that inhibit insulin signaling (Grant and Dixit, 2015). Fennel has shown anti-inflammatory properties as its extracts suppressed LPS-induced TNF-α, IL-6 and NF-kB activation in macrophages (Vyshnevska *et al*., 2022). The anti-inflammatory phytochemicals like anethole can potentially counteract obesity-linked inflammation and improve insulin sensitivity.

Hyperleptinemia is implicated in obesity-associated development of leptin resistance and hyperthyroidism (Knight *et al*., 2010; Zhang and Scarpace, 2006). Leptin resistance refers to a state where the body becomes less responsive to the effects of leptin, a hormone that regulates body weight (Bossola and Tazza, 2011). Obese rats in this study exhibited elevated T3, T4 and leptin along with low TSH levels, indicating perturbed thyroid homeostasis. Fennel treatment corrected these hormonal imbalances, which could be mediated through the thyroid-inhibiting flavonoids like quercetin and kaempferol (Pistollato *et al*., 2019).

Obesity and fatty liver disease are coupled to defective hepatic insulin signaling (Bugianesi *et al*., 2005). This manifests as reduced insulin receptor expression and receptor tyrosine kinase activity, leading to insulin resistance (García-Ruiz *et al*., 2006). Obese rats in this study showed lowered insulin receptor gene expression in the liver versus controls, similar to Winer *et al*. (2011). Fennel supplementation significantly upregulated hepatic insulin receptor gene expression, denoting improved insulin sensitivity (Henagan *et al*., 2014).

Histological observations further validated the provisional influences of fennel toward high-fat diet-induced hepatic damage. Obese livers exhibited fat deposition, congestion, inflammation and necrosis. Fennel treatment dose-dependently ameliorated these manifestations, preserving near-normal liver architecture. Histological observations from the study (Al-Amoudi, 2017) showed that fennel positively affected the histopathological picture of the hepatorenal tissue. The phytochemicals like quercetin suppress inflammatory mediators and attenuate structural changes in hepatosteatosis (Panchal *et al*., 2012).

Conclusion

This study demonstrates fennel's promising anti-obesity and anti-diabetic outcomes in a rodent model of diet-induced metabolic dysfunction. Fennel supplementation attenuated weight gain, dyslipidemia, hormonal disturbances and oxidative stress associated with high-fat feeding. The hepatoprotective action was illustrated by the reversal of hepatomegaly and the restoration of liver enzymes and structure. Fennel also enhanced hepatic insulin receptor expression, denoting improved insulin sensitivity. These observed benefits can be attributed to fennel bioactives like anethole, quercetin, kaempferol, and phytoestrogens. The results provide a scientific rationale for using fennel as a hepatoprotective and weight management agent, especially against fatty liver disease and metabolic complications accompanying obesity. Further clinical evaluation in humans is recommended to substantiate its therapeutic utility.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Aebi, H., 1984. [13] Catalase in vitro, in: Methods in Enzymology, Oxygen Radicals in Biological Systems. Academic Press, pp. 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Aghabeiglooei, Z., Namazi, N., Karimi, M., Soleymani, S., Ayati, M.H., Rezaeizadeh, H., 2023. Effects of Komouni Formulation (Herbal Product of Persian Medicine) With a Low-Calorie Diet on Cardiometabolic Risk Factors in Overweight and Obese Women: A Triple-Blinded Randomized Clinical Trial. Iran. J. Pharm. Res. 22. https://doi.org/10.5812/ijpr-136114

Al-Amoudi, W.M., 2017. Protective effects of fennel oil extract against sodium valproate-induced hepatorenal damage in albino rats. Saudi J. Biol. Sci. 24, 915–924. https://doi.org/10.1016/j.sjbs.2016.10.021

Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W., Fu, P.C., 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20, 470–475.

Alonso-Pérez, A., Guillán-Fresco, M., López-Fagúndez, M., Pazos-Pérez, A., Crespo-Golmar, A., Piñeiro-Ramil, M., López, V., Jorge-Mora, A., Gómez, R., 2022. Effect and Regulation of Obesity-Associated Low-Grade Chronic Inflammation in Major Rheumatic Diseases. Rheumato 2, 114–125. https://doi.org/10.3390/rheumato2040016

Alshammari, G.M., Abdelhalim, M.A., Al-Ayed, M.S., Al-Harbi, L.N., Yahya, M.A., 2023. Concomitant Sub-Chronic Administration of Small-Size Gold Nanoparticles Aggravates Doxorubicin-Induced Liver Oxidative and Inflammatory Damage, Hyperlipidemia, and Hepatic Steatosis.

Molecules 28, 796. https://doi.org/10.3390/molecules28020796
Amin, K.A., Nagy, M.A., 2009. Effect of Carnitine and herbal mixture extract on obesity induced by high fat diet in rats. Diabetol. Metab. Syndr. 1, 17. https://doi.org/10.1186/1758-5996-1-17
Andrade-Cetto, A., Islam, Md.S., Heinrich, M., De Feo, V., 2023. Editorial: Action mechanisms of traditional medicinal plants used to control type 2 diabetes or conditions of metabolic syndrome, volume II. Front. Pharmacol. 14. https://doi.org/10.3389/fphar.2023.1267677
Balkrishna, A., Rana, M., Mishra, S., Srivastava, D., Bhardwaj, R., Singh, S., Rajput, S.K., Arya, V., 2023. Incredible Combination of Lifestyle Modification and Herbal Remedies for Polycystic Ovarian Syndrome Management. Evid. Based Complement. Alternat. Med. 2023, 1–20. https://doi.org/10.1155/2023/3705508
Barker, S.B., 1948. Determination of protein-bound iodine. J. Biol. Chem. 173, 715–724.
Bhattacharjee, P., Carlson, C., Ryan, C., Fadlaoui, A., Sunny, N., 2023. Impact of sustained de novo lipogenesis on mitochondrial dysfunction and hepatocellular redox during fatty liver disease. Physiology 38, 5730204. https://doi.org/10.1152/physiol.2023.38.S1.5730204
Bossola, M., Tazza, L., 2011. Hyperleptinemia, Leptin Resistance, and Cognition in Hemodialysis Patients. Ren. Fail. 33, 1049–1050. https://doi.org/10.3109/0886022X.2011.618975
Boyadzhieva, S., Angelov, G., 2014. Optimization of water extraction of fennel seeds. Journal of Chemical Technology and Metallurgy 49, 447-450.
Bugianesi, E., McCullough, A.J., Marchesini, G., 2005. Insulin resistance: A metabolic pathway to chronic liver disease. Hepatology 42, 987–1000. https://doi.org/10.1002/hep.20920
Chen, Hanqin, Chen, Haiqin, Lu, H., Tang, X., Zhang, H., Chen, Y.Q., Chen, W., 2021. Carbohydrate analysis of Mortierella alpina by colorimetry and HPLC–ELSD to reveal accumulation differences of sugar and lipid. Biotechnol. Lett. 43, 1289–1301. https://doi.org/10.1007/s10529-021-03120-2
Choi, E.-M., Hwang, J.-K., 2004. Anti-inflammatory, analgesic and antioxidant activities of the fruit of *Foeniculum vulgare*. Fitoterapia 75, 557–565. https://doi.org/10.1016/j.fitote.2004.05.005
Considine, R.V., Sinha, M.K., Heiman, M.L., Kriauciunas, A., Stephens, T.W., Nyce, M.R., Ohannesian, J.P., Marco, C.C., McKee, L.J., Bauer, T.L., 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334, 292–295. https://doi.org/10.1056/NEJM199602013340503
Conte, C., 2021. Complications of Obesity, in: Luzi, L. (Ed.), Thyroid, Obesity and Metabolism: Exploring Links Between Thyroid Function, Obesity, Metabolism and Lifestyle. Springer International Publishing, Cham, pp. 95–116. https://doi.org/10.1007/978-3-030-80267-7_7
Das, S., Choudhuri, D., 2020. Calcium supplementation shows a hepatoprotective effect against high-fat diet by regulating oxidative-induced inflammatory response and lipogenesis activity in male rats. J. Tradit. Complement. Med. 10, 511–519. https://doi.org/10.1016/j.jtcme.2019.06.002
EASL–EASD–EASO, 2018. Non-alcoholic fatty liver disease (NAFLD). Qeios. https://doi.org/10.32388/138147
Elghazaly, N.A., H. Radwan, E., Zaatout, H.H., Elghazaly, M.M., Allam, N.E.D., 2019. Beneficial Effects of Fennel (*Foeniculum vulgare*) in Treating Obesity in Rats. J. Obes. Manag. 1, 16–33. https://doi.org/10.14302/issn.2574-450X.jom-18-2484
Enjoji, M., Kohjima, M., Nakamuta, M., 2016. Lipid Metabolism and the Liver. In: The Liver in Systemic Diseases. Ohira, H. (Ed.), Springer Japan, Tokyo, pp. 105–122. https://doi.org/10.1007/978-4-431-55790-6_6
Ezeh, K.J., Ezeudemba, O., Ezeh, K.J., Ezeudemba, O., 2021. Hyperlipidemia: A Review of the Novel Methods for the Management of Lipids. Cureus 13. https://doi.org/10.7759/cureus.16412
Fisher, D.A., 1996. Physiological variations in thyroid hormones: physiological and pathophysiological considerations. Clin. Chem. 42, 135–139.
Flöck, A., Ferrari, N., Joisten, C., Puth, M.T., Strizek, B., Gembruch, U., Merz, W.M., 2022. Cytokines and parturition: investigating adiponectin, TNF-α, and IL-6 in mother–newborn pairs. J. Matern. Fetal Neonatal Med. 35, 9249–9256. https://doi.org/10.1080/14767058.2021.2025356
Fontenelle, L.C., Feitosa, M.M., Severo, J.S., Freitas, T.E.C., Morais, J.B.S., Torres-Leal, F.L., Henriques, G.S., Marreiro, D.N., 2016. Thyroid Function in Human Obesity: Underlying Mechanisms. Horm. Metab. Res. 48, 787–794. https://doi.org/10.1055/s-0042-121421
Fossati, P., Prencipe, L., 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. 28, 2077–2080.
Galisteo, M., Sánchez, M., Vera, R., González, M., Anguera, A., Duarte, J., Zarzuelo, A., 2005. A Diet Supplemented with Husks of Plantago ovata Reduces the Development of Endothelial Dysfunction, Hypertension, and Obesity by Affecting Adiponectin and TNF-α in Obese Zucker Rats1. J. Nutr. 135, 2399–2404. https://doi.org/10.1093/jn/135.10.2399
García-Ruiz, I., Rodríguez-Juan, C., Díaz-Sanjuan, T., del Hoyo, P., Colina, F., Muñoz-Yagüe, T., Solís-Herruzo, J.A., 2006. Uric acid and anti-TNF antibody improve mitochondrial dysfunction in ob/ob mice. Hepatol. Baltim. Md 44, 581–591. https://doi.org/10.1002/hep.21313
Gayibov, U., Gayibova, S.N., Pozilov, M.K., Tuxtaeva, F.S., Yusupova, U.R., Djabbarova, G.M.-K., Mamatova, Z.A., Ergashev, N.A., Aripov, T.F., 2021. Influence of quercetin and dihydroquercetin on some functional parameters of rat liver mitochondria. J. Microbiol. Biotechnol. Food Sci. 11, e2924–e2924. https://doi.org/10.15414/jmbfs.2924
Gharib, H., Ryan, R.J., Mayberry, W.E., Hockert, T., 1971. Radioimmunoassay for triiodothyronine (T 3): I. Affinity and specificity of the antibody for T 3. J. Clin. Endocrinol. Metab. 33, 509–516. https://doi.org/2016092613333500708
Godoy-Matos, A.F., Silva Júnior, W.S., Valerio, C.M., 2020. NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. Diabetol. Metab. Syndr. 12, 60. https://doi.org/10.1186/s13098-020-00570-y
Grant, R.W., Dixit, V.D., 2015. Adipose tissue as an immunological organ. Obesity 23, 512–518. https://doi.org/10.1002/oby.21003
HANDA, T., YAMAGUCHI, K., SONO, Y., YAZAWA, K., 2005. Effects of Fenugreek Seed Extract in Obese Mice Fed a High-Fat Diet. Biosci. Biotechnol. Biochem. 69, 1186–1188. https://doi.org/10.1271/bbb.69.1186
Henagan, T.M., Cefalu, W.T., Ribnicky, D.M., Noland, R.C., Dunville, K., Campbell, W.W., Stewart, L.K., Forney, L.A., Gettys, T.W., Chang, J.S., Morrison, C.D., 2014. *In vivo* effects of dietary quercetin and quercetin-rich red onion extract on skeletal muscle mitochondria, metabolism, and insulin sensitivity. Genes Nutr. 10, 2. https://doi.org/10.1007/s12263-014-0451-1
Huang, X.-J., Choi, Y.-K., Im, H.-S., Yarimaga, O., Yoon, E., Kim, H.-S., 2006. Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques. Sensors 6, 756–782. https://doi.org/10.3390/s6070756
Hwang, E.-S., Thi, N.D., 2014. Effects of Extraction and Processing Methods on Antioxidant Compound Contents and Radical Scavenging Activities of Laver (Porphyra tenera). Prev. Nutr. Food Sci. 19, 40–48. https://doi.org/10.3746/pnf.2014.19.1.040
Knight, Z.A., Hannan, K.S., Greenberg, M.L., Friedman, J.M., 2010. Hyperleptinemia Is Required for the Development of Leptin Resistance. PLOS ONE 5, e11376. https://doi.org/10.1371/journal.pone.0011376
Lai, Y.-C., Tsai, Y.-C., Shin, Y.-N., Chou, Y.-C., Shen, Y.-R., Lin, N.-C., Chang, S.-H., Kao, Y.-M., Tseng, S.-H., Wang, D.-Y., 2022. Inorganic arsenic speciation analysis in food using HPLC/ICP-MS: Method development and validation. J. Food Drug Anal. 30, 644–653. https://doi.org/10.38212/2224-6614.3432
Lal, A.A.S., Kumar, T., Murthy, P.B., Pillai, K.S., 2004. Hypolipidemic effect of Coriandrum sativum L. in triton-induced hyperlipidemic rats. Indian J. Exp. Biol. 42, 909–912.
Lee, J., 2022. Obesity, Metabolic Syndrome and Inflammation, in: Camara, N.O.S., Alves-Filho, J.C., Moraes-Vieira, P.M.M. de, Andrade-Oliveira, V. (Eds.), Essential Aspects of Immunometabolism in Health and Disease. Springer International Publishing, Cham, pp. 133–149. https://doi.org/10.1007/978-3-030-86684-6_7
Livak, K.J., Schmittgen, T.D., 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262
Lopes-Virella, M.F., Stone, P., Ellis, S., Colwell, J.A., 1977. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin. Chem. 23, 882–884.
Lv, S., Hu, T., Zhang, R., Zhou, Y., Yu, W., Wang, Z., Shi, C., Lian, J., Huang, S., Pei, G., Luan, B., 2023. Rhamnose Displays an Anti-Obesity Effect Through Stimulation of Adipose Dopamine Receptors and Thermogenesis. Diabetes 72, 326–335. https://doi.org/10.2337/db22-0552
Monteiro, R., Azevedo, I., 2010. Chronic Inflammation in Obesity and the Metabolic Syndrome. Mediators Inflamm. 2010, e289645. https://doi.org/20160818101504
Muflihah, Y.M., Gollavelli, G., Ling, Y.-C., 2021. Correlation Study of Antioxidant Activity with Phenolic and Flavonoid Compounds in 12 Indonesian Indigenous Herbs. Antioxidants 10, 1530. https://doi.org/10.3390/antiox10101530
Nagamma, T., Konuri, A., Nayak, C.D., Kamath, S.U., Udupa, P.E.G., Nayak, Y., 2019. Dose-dependent effects of fenugreek seed extract on the biochemical and haematological parameters in high-fat diet-fed rats. J. Taibah Univ. Med. Sci. 14, 383–389. https://doi.org/10.1016/j.jtumed.2019.05.003
Nirosha, K., Divya, M., Vamsi, S., Sadiq, M., 2014. A Review on Hyperlipidemia. International Journal of Novel Trends in Pharmaceutical Sciences 4, 81-92.
Nishikimi, M., Appaji Rao, N., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 46, 849–854. https://doi.org/10.1016/S0006-291X(72)80218-3
Ofir, R., Tamir, S., Khatib, S., Vaya, J., 2003. Inhibition of serotonin re-uptake by licorice constituents. J. Mol. Neurosci. 20, 135–140. https://doi.org/10.1385/JMN:20:2:135
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358. https://doi.org/10.1016/0003-2697(79)90738-3
Ozbek, H., Uğraş, S., Dülger, H., Bayram, I., Tuncer, I., Öztürk, G., Oztürk, A., 2003. Hepatoprotective effect of *Foeniculum vulgare* essential oil. Fitoterapia 74, 317–319. https://doi.org/10.1016/s0367-326x(03)00028-5
Panchal, S.K., Poudyal, H., Brown, L., 2012. Quercetin ameliorates cardiovascular, hepatic, and metabolic changes in diet-induced metabolic syndrome in rats. J. Nutr. 142, 1026–1032. https://doi.org/10.3945/jn.111.157263
Pistollato, F., Masias, M., Agudo, P., Giampieri, F., Battino, M., 2019. Effects of phytochemicals on thyroid function and their possible role in thyroid disease. Ann. N. Y. Acad. Sci. 1443, 3–19. https://doi.org/10.1111/nyas.13980
Samy, K., Porwal, Y.C., Yadav, A., 2022. Evaluation of Interleukin 6(Il-6), Tumor Necrosis Factor Alpha (Tnf-α) and Paraoxonase 1(Pon 1) in Obese and Non-Obese Metabolic Syndrome. J. Assoc. Physicians India 70, 11–12.
Sanyal, D., Raychaudhuri, M., 2016. Hypothyroidism and obesity: An intriguing link. Indian J. Endocrinol. Metab. 20, 554. https://doi.org/10.4103/2230-8210.183454
Schöne, F., Vetter, A., Hartung, H., Bergmann, H., Biertümpfel, A., Richter, G., Müller, S., Breitschuh, G., 2006. Effects of essential oils from fennel (Foeniculi aetheroleum) and caraway (Carvi aetheroleum) in pigs. J. Anim. Physiol. Anim. Nutr. 90, 500–510. https://doi.org/10.1111/j.1439-0396.2006.00632.x
Shahat, A.A., Ahmed, H.H., Hammouda, F.M., Ghaleb, H., 2012. Regulation of Obesity and Lipid Disorders by *Foeniculum vulgare* Extracts and Plantago ovata in High-fat Diet-induced Obese Rats. Am. J. Food Technol. 7, 622–632. https://doi.org/10.3923/ajft.2012.622.632
Suvarna, S.K., Layton, C., Bancroft, J.D., 2019. Bancroft's theory and practice of histological techniques, Eighth edition. ed. Elsevier, Amsterdam.
Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 6, 24–27. https://doi.org/10.1177/000456326900600108
Vyshnevska, L., Severina, H.I., Prokopenko, Y., Shmalko, A., 2022. Molecular docking investigation of anti-inflammatory herbal compounds as potential LOX-5 and COX-2 inhibitors. Pharmacia 69, 733–744. https://doi.org/10.3897/pharmacia.69.e89400
Winer, D.A., Winer, S., Shen, L., Wadia, P.P., Yantha, J., Paltser, G., Tsui, H., Wu, P., Davidson, M.G., Alonso, M.N., Leong, H.X., Glassford, A., Caimol, M., Kenkel, J.A., Tedder, T.F., McLaughlin, T., Miklos, D.B., Dosch, H.-M., Engleman, E.G., 2011. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat. Med. 17, 610–617. https://doi.org/10.1038/nm.2353
Xie, Z., Liang, G., Zhang, L., Wang, Q., Qu, Y., Gao, Y., Lin, L., Ye, S., Zhang, J., Wang, H., Zhao, G., Zhang, Q., 2009. Molecular mechanisms underlying the cholesterol-lowering effect of Ginkgo biloba extract in hepatocytes: a comparative study with lovastatin. Acta Pharmacol. Sin. 30, 1262–1275. https://doi.org/10.1038/aps.2009.126
You, J.S., Lee, Y.J., Kim, K.S., Kim, S.H., Chang, K.J., 2014. Anti-obesity and hypolipidaemic effects of Nelumbo nucifera seed ethanol extract in human pre-adipocytes and rats fed a high-fat diet. J. Sci. Food Agric. 94, 568–575. https://doi.org/10.1002/jsfa.6297
Yous, F., Zaabar, S., Aoun, O., Adjeroud-Abdellatif, N., Madani, K., 2021. Comparative mapping approach of energy intake and expenditure of obesity prevalence among school adolescents. Clin. Nutr. ESPEN 43, 397–407. https://doi.org/10.1016/j.clnesp.2021.03.017
Zakharova, S.M., Savelieva, L.V., Fadeeva, M.I., 2013. Ожирение и гипотиреоз. Ожирение И Метаболизм 10, 54–58. https://doi.org/10.14341/2071-8713-4826
Zali, M.A., Fonkoua, M., Daboy, C.D., Tazon, W.A., Goda, D., Akamba, D.A., Nanhah, J.V., Ngondi, J.L., 2023. Comparative Study of the Effect of Two Extraction Solvents on Polyphenols Content and Antioxidant Activity of Alstonia Boonei Bark. Asian J. Chem. Sci. 13, 7–17. https://doi.org/10.9734/ajocs/2023/v13i4245
Zhang, Y., Scarpace, P.J., 2006. The role of leptin in leptin resistance and obesity. Physiol. Behav., Proceedings from the 2005 Meeting of the Society for the Study of Ingestive Behavior 88, 249–256. https://doi.org/10.1016/j.physbeh.2006.05.038
Žilić, S., Serpen, A., Akıllıoğlu, G., Janković, M., Gökmen, V., 2012. Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. J. Cereal Sci. 56, 652–658. https://doi.org/10.1016/j.jcs.2012.07.014