European Review for Medical and Pharmacological Sciences 2023; 27: 2404-2418

Studying the actions of sage and thymoquinone combination on metabolic syndrome induced by high-fat diet in rats

S.M. ALANAZI, R.A. ALSAQER, F.I. ALSAEED, R.M. ALMAKHAYTAH, N.T. BUWASHL, M.E. MOHAMED, N.S. YOUNIS

Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa, Saudi Arabia

Abstract. – OBJECTIVE: High-fat diet is one of the most imperative risk factors for cardiovascular disorders. Thymoquinone (TQ) is one of the active pharmacological components of *Nigella sativa* (black cumin). *Salvia officinalis* L. (sage) has been demonstrated to have diverse pharmacological actions. The main objective of this study was to determine the effects of sage and TQ combination on hyperglycemia, oxidative stress, blood pressure, and lipid profile in rats fed with a high-fat diet (HFD).

MATERIALS AND METHODS: Wistar male rats were divided into five groups; normal diet (ND) and HFD, in which rats were fed with a normal diet or HFD for 10 weeks, respectively. In HFD+sage group, animals were administered sage essential oil (0.052 ml/kg) orally along with HFD. In HFD+TQ group, rats were administered TQ (50 mg/kg) orally with HFD. In HF+sage + TQ group, animals received sage + TQ along with HFD. Blood glucose (BGL) and Fast serum insulin (FSI) levels, oral glucose tolerance test, blood pressure, liver function tests, plasma, and hepatic oxidative stress markers, antioxidant enzymes, and glutathione content, and lipid profile were measured.

RESULTS: Sage and TQ combination decreased the final body weight, weight gain, BGL, FSI, and Homeostasis Model Assessment-Insulin Resistance (HOMA-IR). The combination also lowered systolic and diastolic arterial pressures and liver function enzymes. The combination deterred lipid peroxidation, advanced protein oxidation product, and nitric oxide amplification, as well as restoring the superoxide dismutase, catalase activities, and glutathione content in plasma and hepatic tissue. Sage and TQ combination reduced the plasma total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL) levels and amplified high-density lipoprotein (HDL).

CONCLUSIONS: The results of the current study verified that sage essential oil, together with TQ exhibited hypoglycemic, hypolipidemic, and antioxidant actions and thus could be a valuable addition to diabetes management.

Key Words:
Thymoquinone, Sage, High fat diet, Blood pressure.

Introduction

Metabolic syndrome (MeS) is characterized by hypertension, abdominal obesity, insulin resistance, and atherogenic dyslipidemia. MeS develops because of a continuing energy imbalance between energy intake and consumption which occur during a high-fat diet (HFD)[1]. MeS initiates atherogenic dyslipidemia, pro-inflammatory and pro-thrombotic state, high blood pressure, central obesity, and cardiovascular disease[2,3]. A high-fat diet is claimed to be the most imperative risk factor for cardiovascular disorders.

Thymoquinone (TQ) is one of the active pharmacological components of *Nigella sativa* (black cumin)[4]. TQ exhibited neuroprotective actions in acute and chronic forms of cerebral pathology[5,6]. TQ was established to have hypolipidemic[7], anti-cancer[8,9], anti-pyroptotic[10], and anti-inflammatory effects that protected against several diseases[11]. Furthermore, TQ showed numerous cardiovascular actions. For instance, TQ attenuated myocardial ischemia/reperfusion injury[12], ameliorated angiotensin II-induced hypertension[13], reduced cardiac damage caused by hypercholesterolemia in apolipoprotein E-deficient mice[14] and diminished diabetic nephropathy[15,16]. Furthermore, TQ protected against cardiac mitochondrial DNA loss, oxidative stress, inflammation, and apoptosis in isoproterenol-induced myocardial infarction[17], and against hyperlipidemia-induced cardiac damage in low-density lipoprotein receptor-deficient (LDL-R$^{-/-}$) mice[10]. TQ protected against doxorubicin-induced heart failure[18],

Corresponding Author: Nancy S. Younis, MD; e-mail: nyounis@kfu.edu.sa

cyclophosphamide-induced cardiotoxicity[19], and streptozotocin (STZ)-induced cardio myopathy[20]. Numerous preclinical and clinical studies[21] have demonstrated the anti-diabetic efficacy of TQ. For example, intragastric administration of TQ in STZ- nicotinamide-induced diabetic rats resulted in HbA1c reduction, insulin escalation, and hypoglycemic effects. Moreover, TQ combined with metformin demonstrated[22] a reduction in the levels of HbA1c and blood glucose compared to metformin alone.

Salvia officinalis L. (Sage) is a perennial round shrub in the family of *Labiatae*/*Lamiaceae*[23]. The major phytochemicals of *S. officinalis* are well-identified, including alkaloids, carbohydrates, fatty acids, glycosides, phenolic compounds, polyacetylenes, steroids, terpenes/terpenoids, and waxes[24,25]. In traditional medicine, the plant has been used[23] to manage diverse disorders such as seizures, ulcers, gout, rheumatism, inflammation, tremor, paralysis, and diarrhea. Sage was also verified to exhibit anti-cancer on different cancer cell lines[26], anti-oxidant[27,28], anti-inflammatory and anti-nociceptive[29], anti-septic[30], antifungal[31] cognitive and memory enhancing[28] and hypoglycaemic[28]. The metabolic impairments and tissue disorders in alloxan-induced diabetic rats were alleviated by *S. officinalis* L. essential oil[32]. Furthermore, the concomitant administration of sage essential oil with co-amoxiclav exerted a hepatoprotective effect *via* the antioxidant defense response[33]. The actions of sage on high-fat diet-fed animals were not documented before. Thus, we tried to explore this effect, especially in combination with TQ. The main objective of the current experiment was to determine the effects of sage and TQ combination on glycemic parameters, oxidative stress, blood pressure indices, and lipid profile, in Wistar rats fed with high-fat diet-induced MeS.

Materials and Methods

Ethical Approval Statement

Wistar male rats (180-220 g) were purchased from the Experimental Animal Research Centre, King Saud University, Riyadh, KSA. Rats were provided with the typical laboratory food and water *ad libitum* in properly ventilated cages system (12 h light/dark cycles, 20-23°C) for one week of acclimatization before the experiment. The Institutional Animal Care and Use Committee of King Faisal University permitted the experimental procedure (KFU-REC-2022-MAY-EA000633). All the experiments were executed according to the appropriate procedures and regulations of the Ethical Conduct for the Use of Animals in Research at King Faisal University.

Experimental Design

The experimental rats were divided into five groups, each comprising six rats; normal diet (ND) (Group 1), in which rats received a normal diet and water for 10 weeks. The second group is the HFD group, in which animals were fed with HFD for 10 weeks. The third group is HFD + sage in which animals were administered sage essential oil (W300100, Sigma Aldrich, St. Louis, MO, USA) dissolved in carboxymethyl cellulose (CMC) at a dose of 0.052 ml/kg[33] orally using gastric gavage along with HFD for 10 weeks. The fourth group is HFD + TQ in which rats were administered TQ (50 mg/kg)[22,34] orally with HFD for 10 weeks. The fifth group is HFD + sage + TQ in which animals received Sage + TQ along with HFD for 10 weeks. The body weights of all rats were measured before HFD feeding and at the end of the experiment (10 weeks). HFD was prepared in the laboratory with normal diet (ND), animal fat (ghee), sugar, and casein[35]. ND was crushed into powder and mixed with animal fat (20%), sugar (10%), and casein (20%). The formed paste was divided into small round pellets and left to solidify in a tray under shade and then stored to be used within 4 weeks.

Measurement of Glucose and Insulin Levels

At the end of the 10th week, the metabolic phenotype of the rats was estimated by assessing the body weight changes, insulin resistance, and fasting blood glucose (FBG) levels. Blood glucose level (BGL) was determined using a commercial kit from Sigma-Aldrich (St. Louis, MO, USA) according to the manufacturer's instructions. Fast serum insulin (FSI) levels were assayed using rat insulin ELISA kit from Thermo Fisher Scientific (Frederick, MD, USA).

The Oral Glucose Tolerance Test (OGTT)

OGTT and Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) were performed after overnight fasting. HOMA-IR was determined using the following formula: FSI (μIU/mL) X

FBG (mg/dL)/405[36]. OGTT was performed on all groups after completing the experimental feeding period as described previously[37] to assess the glycemic activity after the consumption of the HF diet. Simply glucose solution was administered (2 g/kg), and BGL was determined at different time intervals 0, 30, 60, and 120 min and monitored. The area under the glucose curve (AUC) was calculated using trapezoid method to estimate OGTT. The AUC was calculated using the following formula: AUC = [(value at 0 min + value at 30 min) × 0.25] + [(value at 30 min + value at 60 min) × 0.25] + [(value at 60 min + value at 120 min) × 0.5].

Measurement of Blood Pressure

Blood pressure was measured using a noninvasive tail-cuff system following the manufacturer's instruction. Briefly, rats were placed on a hot plate at 35°C for 10 min and then placed in a restrainer. A cuff with a pneumatic pulse sensor was attached to the rat's tail. Blood pressure values were recorded on a CODA high throughput Noninvasive Blood Pressure system (Kent Scientific, Torrington, CT, USA) on a heating pad and were averaged from 12 consecutive readings obtained from each rat.

Blood and Organs Collections

At the end of the experiment, all animals were sacrificed using pentobarbital anesthesia (90 mg/kg) in the peritoneal region[38]. Blood samples were collected from the abdominal aorta. All other internal organs, including the heart, adipose tissues, spleen, kidney, intestine, and liver, were immediately collected, weighed, and stored at -20°C for further biochemical studies. The obtained blood was centrifuged at 8,000 rpm for 15 min at 4°C to separate plasma which was stored at -20°C until analysis.

Histopathological Investigation

Hepatic tissues were harvested and fixed with neutral buffered formalin. The liver tissues were embedded in paraffin, sectioned at a 5 μm thickness, and stained with hematoxylin/eosin (H&E) to visualize the architecture of hepatic tissue. Ten stained sections were examined and photographed under a light microscope at 40X magnification.

Liver Function Tests

Plasma alanine aminotransferase (ALT, ab105134/K752-100), aspartate aminotransferase (AST, ab263883), and alkaline phosphatase (ALP, ab83369) using the diagnostic kits obtained from Abcam Inc. (Cambridge, UK) were evaluated using the protocols provided by the manufacturer.

Measurement of Plasma and Hepatic Oxidative Stress Markers

Hepatic samples were mixed with 10 mL of phosphate buffer (pH 7.4) and centrifuged at 8,000 rpm for 15 min at 4°C to obtain the supernatant on which protein and enzymatic analyses were executed. Hepatic lipid peroxidation was assessed quantifying thiobarbituric acid reactive substrates (TBARS) calorimetrically at 535 nm[39]. Nitric oxide (NO) levels were measured as nitrate concentrations at 540 nm and reported as nmol/g of tissue as mentioned before[40]. Advanced protein oxidation product (APOP)levels were determined following the procedures mentioned[41] earlier with slight modifications.

Measurement of Plasma and Hepatic Levels of Antioxidant Enzyme and Glutathione Content

Superoxide dismutase (SOD; MBS036924), catalase (MBS726781) and glutathione content (GSH, MBS265966) ELISA kits were obtained from MyBioSource (San Diego, CA, USA). All the procedures were performed in agreement with the manufacturer's directions.

Measurement of Lipid Profile

Serum cholesterol, triglyceride (TG) and high-density lipoprotein (HDL) cholesterol were estimated by colorimetric procedure using the commercially available kit from Biodiagnostics (Cairo, Egypt). Low density lipoprotein (LDL) was calculated using Friedewald formula: LDL (mg/dL) = total cholesterol (TC) (mg/dL) - [HDL (mg/dL) - TG (mg/dL)/5][42].

Statistical Analysis

Data are presented as mean ± SD. For multiple comparisons, one-way ANOVA followed by Tukey-Kramer as a post-hoc test was performed. The 0.05 level of probability was used as the significance level ($p < 0.05$). All statistical analyses were performed using Graph Pad software version 5 (San Diego, CA, USA).

Results

Effects of Sage and TQ Alone and Their Combination on Body Weight (BW), Liver wet weight in HFD fed Animals

Body weight (BW) gain is an important indicator in evaluating the outcome of a HFD on obesity development, prevention, and treatment. Before feeding different diets, there was no difference between the body weights of all groups. Primary and final animals' body weights in the different groups were shown in Table I. HFD-fed animals disclosed an amplified final body weight and subsequent BW gain was significant ($p<0.05$) when related to the normal standard diet (ND) fed animals. Sage and TQ supplementation with HFD significantly decreased the final body weight and subsequent BW gain ($p<0.05$) compared to the HFD-fed rats. Organ weights, especially the liver wet weight, are important parameters for attaining an overview of HFD-induced obesity and metabolic syndrome. Wet weights of the liver from rats in each group were presented in Table I. Liver wet weights were significantly amplified in the HFD group compared to the control rats ($p<0.05$). Sage and TQ combination resulted in a reduction in the liver wet weight compared to HFD-fed animals ($p<0.05$).

Effects of Sage and TQ Alone and Their Combination on Fasting Insulin, Oral Glucose Tolerance Test (OGTT) and HOMAR-IR

As demonstrated in Figure 1, feeding the rats with HFD for 10 weeks resulted in a significant increase in levels of FSI (μIU/mL) as compared to ND-fed rats (8.52 + 0.24 *vs.* 3.015 + 0.15), respectively (Figure 1a); whereas sage or TQ and their combination significantly reduced the FSI compared to HFD-fed animals. The oral glucose tolerance test (OGTT) was executed to evaluate the ability of the HFD-fed animals to metabolize glucose. In ND-fed animals, the BGL was normalized within 60-120 min, whereas HFD-fed animals exhibited high blood glucose levels. On the other hand, sage or TQ significantly reduced the BGL compared to HFD-fed animals, whereas their combination causes a further reduction in BGL more than each alone. In addition, we evaluated HOMA-IR which is a valid measure to determine insulin-resistance[43]. HOMA-IR in HFD-fed rats was significantly higher as compared to ND-fed rats (5.63 + 0.12 *vs.* 1.96 + 0.3), respectively (Figure 1c), while the administration of sage or TQ and their combination significantly lowered HOMA-IR.

Effects of Sage and TQ Alone and Their Combination on Blood Pressure

There was no difference in the systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) between the groups before feeding the animals with different diets. Animals exhibited a significant intensification in SAP from 119.5 ± 2.55 in ND to 178.50 ± 3.41 mm Hg in HFD, whereas in DAP from 84.50 ± 3.3 in ND to 128.83 ± 3.21 mm Hg in HFD-fed animals ($p < 0.05$) (Figure 2 a-b). On the other hand, TQ, sage, and their combination lowered SAP and DAP significantly compared to HFD alone. However, there was no difference between TQ and sage when compared to each other.

Effects of Sage and TQ Alone and Their Combination on Histological Examination and Liver Function Tests

Histological examination of the ND group showed homogeneous unilocular adipocytes, whereas HFD hepatic examination revealed many hypertrophic adipocytes. On the other hand, TQ, sage and their combination minimized these hypertrophic adipocytes as illustrated in Figure 3a. HFD-fed animals showed fat deposition in the liver and developed hepatic damage. Liver damage was evaluated by quantifying the hepatic function enzyme activities as shown in Figure 3. Serum ALT, AST, and ALP activities were significantly

Table I. REffects of sage and thymoquinone (TQ) and their combination administration for 10 weeks on initial and final body weight and liver wet weight in HFD-induced metabolic changes.

	ND	HFD	HFD + Saga	HFD + TQ	HFD + Saga + TQ
Initial body weight (gm)	193.23 ± 5.14	185.52 ± 4.360	184.38 ± 5.43	189.30 ± 3.78	198.30 ± 4.11
Final body weight (gm)	246.40 ± 2.96	326.07 ± 3.78$^{¥}$	287.34 ± 5.32^{A}	296.53 ± 6.8^{A,Φ}	270.45 ± 7.34^{A,Φ,λ}
Liver wet weight (gm/100 g of BW)	2.5 ± 0.05	3.8 ± 0.062$^{¥}$	3.4 ± 0.045^{A}	3.2 ± 0.075^{A,Φ}	2.9 ± 0.068^{A,Φ}λ

All values are stated as mean± SD (n=6). $^{¥}$designates statistically significant compared to the ND group and ^{A}designates statistically significant compared to the HFD group, ^{Φ}designates statistically significant compared to HFD + sage ($p < 0.05$) and ^{λ}designates statistically significant compared to HFD + TQ using one-way ANOVA followed by Tukey's post-hoc test.

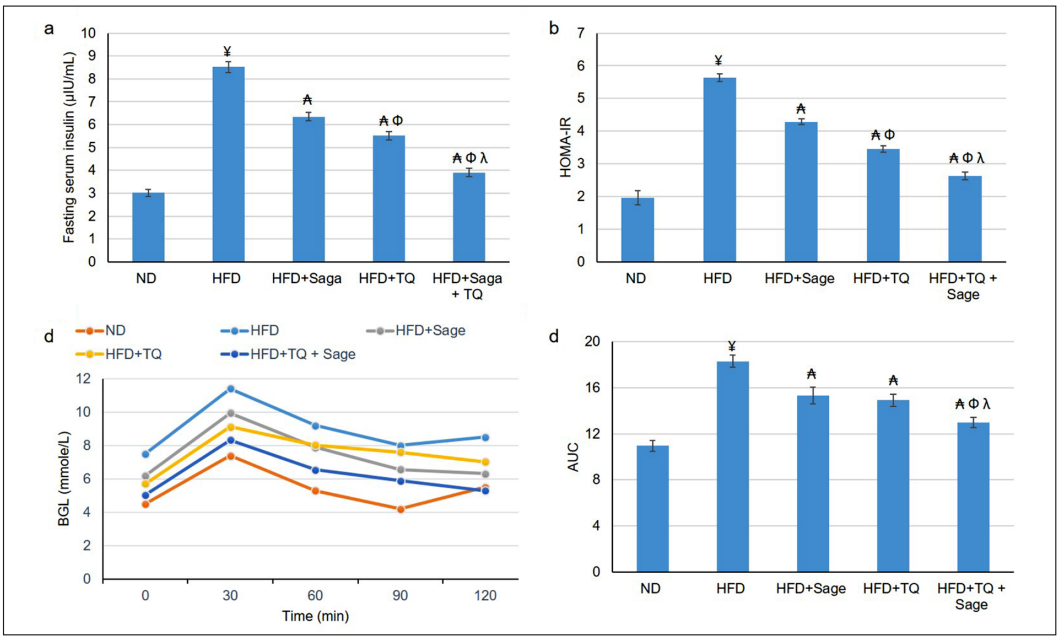

Figure 1. Effects of sage and thymoquinone (TQ) and their combination administration for 10 weeks on (**a**) fasting serum insulin, (**b**) HOMR-IR, (**c**) oral glucose tolerance test, and (**d**) AUC in HFD-induced metabolic changes. All values are stated as mean± SD (n=6). ¥ Designates statistically significant compared to the ND group, and Ѧ designates statistically significant compared to the HFD group, Φ designates statistically significant compared to HFD + sage ($p < 0.05$) and λ designates statistically significant compared to HFD + TQ using one-way ANOVA followed by Tukey's post-hoc test.

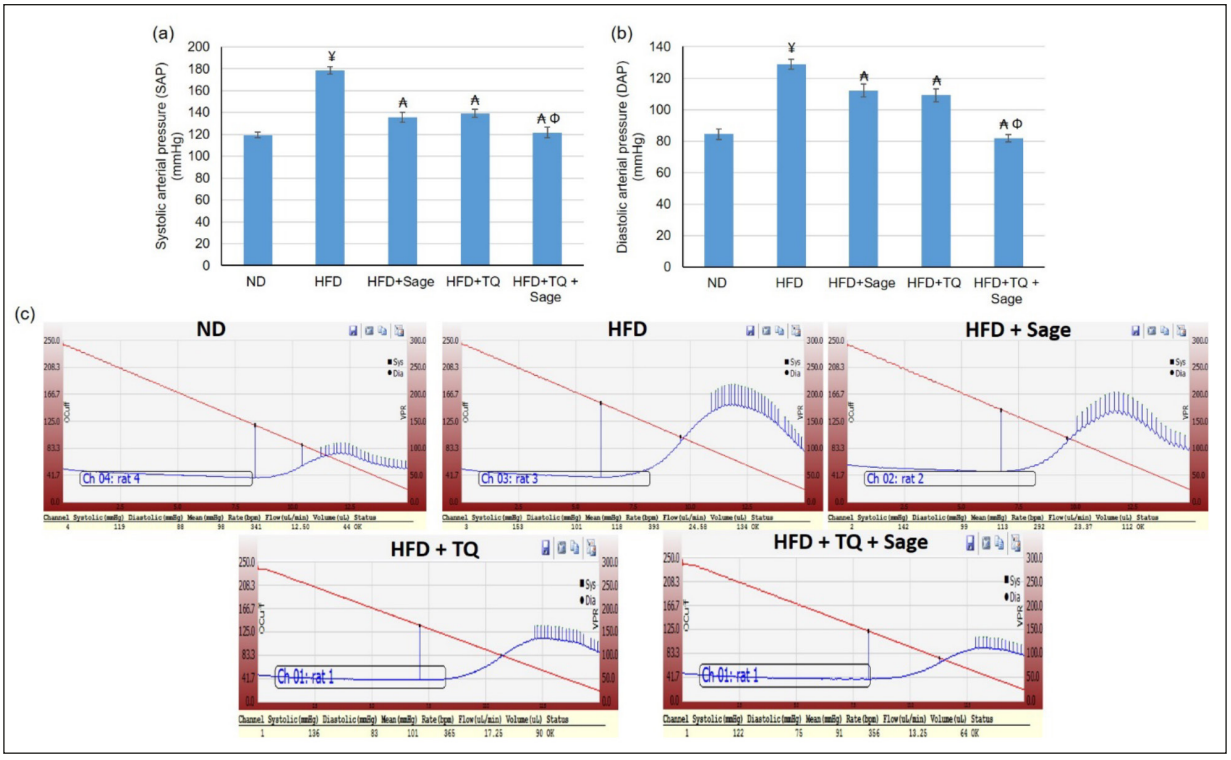

Figure 2. Effects of sage and thymoquinone (TQ) and their combination administration for 10 weeks on (**a**) SAP and (**b**) DAP in HFD-induced metabolic changes. **c**, Computerized tracings obtained during BP recordings from different groups. All values are stated as mean± SD (n=6). ¥ Designates statistically significant compared to the ND group and Ѧ designates statistically significant compared to the HFD group, Φ designates statistically significant compared to HFD + sage ($p < 0.05$) and λ designates statistically significant compared to HFD + TQ using one-way ANOVA followed by Tukey's post-hoc test.

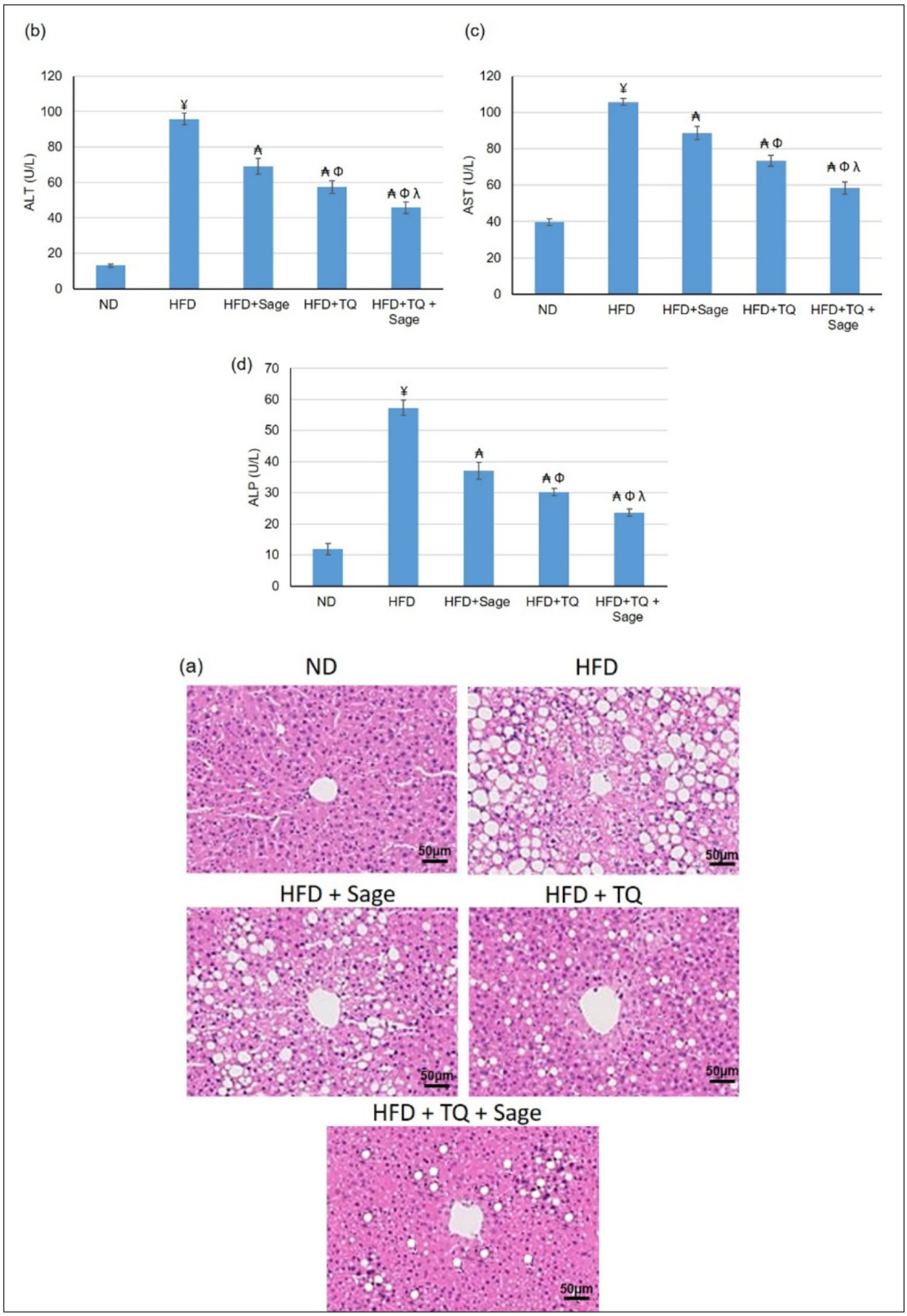

Figure 3. Effects of sage and thymoquinone (TQ) and their combination administration for 10 weeks on (**a**) histological examination (400 ×, scale bar is 50 µm) and on the hepatic function biomarkers including (**b**) ALT, (**c**) AST, and (**d**) ALP in HFD-induced metabolic changes. All values are stated as mean± SD (n=6). ¥ Designates statistically significant compared to the ND group, and Ⱥ designates statistically significant compared to the HFD group, Φ designates statistically significant compared to HFD + sage ($p < 0.05$), and λ designates statistically significant compared to HFD + TQ using one-way ANOVA followed by Tukey's post-hoc test.

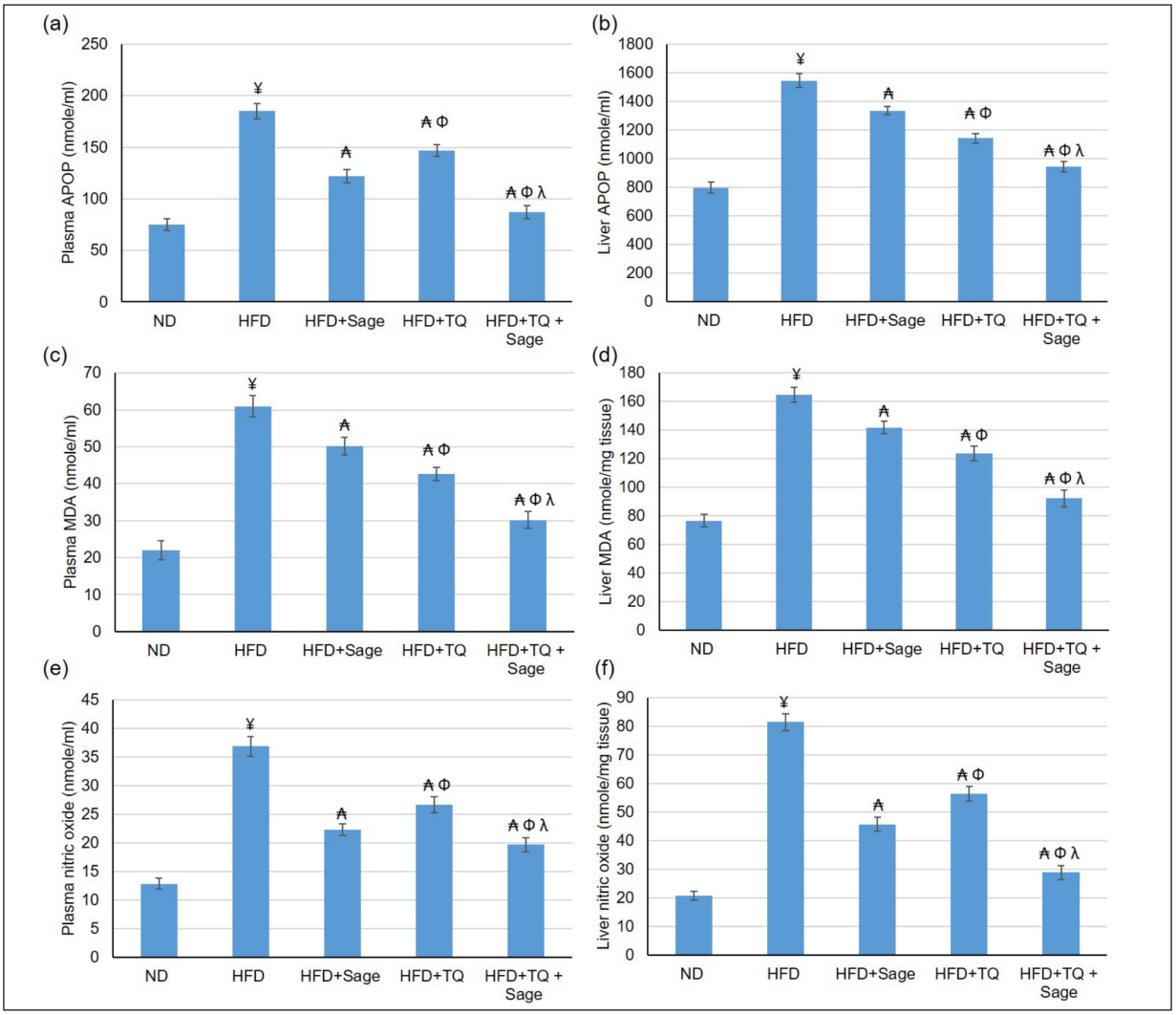

Figure 4. Effects of sage and thymoquinone (TQ) and their combination administration for 10 weeks on the oxidative stress markers including (**a-b**) advanced protein oxidation product (APOP), (**c-d**) malondialdehyde (MDA), and (**e-f**) nitric oxide (NO) in plasma and liver tissue respectively of HFD-induced metabolic changes. All values are stated as mean± SD (n=6). ¥ Designates statistically significant compared to the ND group, and Ⱥ designates statistically significant compared to the HFD group, Φ designates statistically significant compared to HFD + sage ($p < 0.05$) and λ designates statistically significant compared to HFD + TQ using one-way ANOVA followed by Tukey's post-hoc test.

amplified in the HFD-fed animals indicating hepatic damage when related to the ND-fed animals. Oral administration of sage or TQ and their combination for ten weeks reduced the serum ALT, AST, and ALP activities significantly ($p<0.05$) compared to animals that consumed the HF diet alone.

Effects of Sage and TQ Alone and Their Combination on Plasma and Liver Levels of Oxidative Stress Markers in HFD-Fed Animals

Oxidative stress was evaluated *via* quantifying advanced protein oxidation product (APOP), malondialdehyde (MDA), and nitric oxide (NO) concentrations in both the plasma and liver tissue. HFD-fed animals exhibited considerably enlarged MDA, signifying augmented lipid peroxidation ($p>0.05$) compared to the standard diet-fed animals, whereas sage and TQ and their combination deterred the lipid peroxidation amplification in HFD-fed rats (Figure 4). NO is a crucial element contributing to the progress of nitrosative stress in HFD-fed rats. Results of the present study demonstrated that NO concentrations in both plasma and liver were significantly ($p>0.05$) intensified in HFD-fed animals when correlated with the standard diet fed group. Sage and TQ and their combination significantly ($p<0.05$) diminished the NO levels in the plasma and liver in HFD fed group (Figure 4).

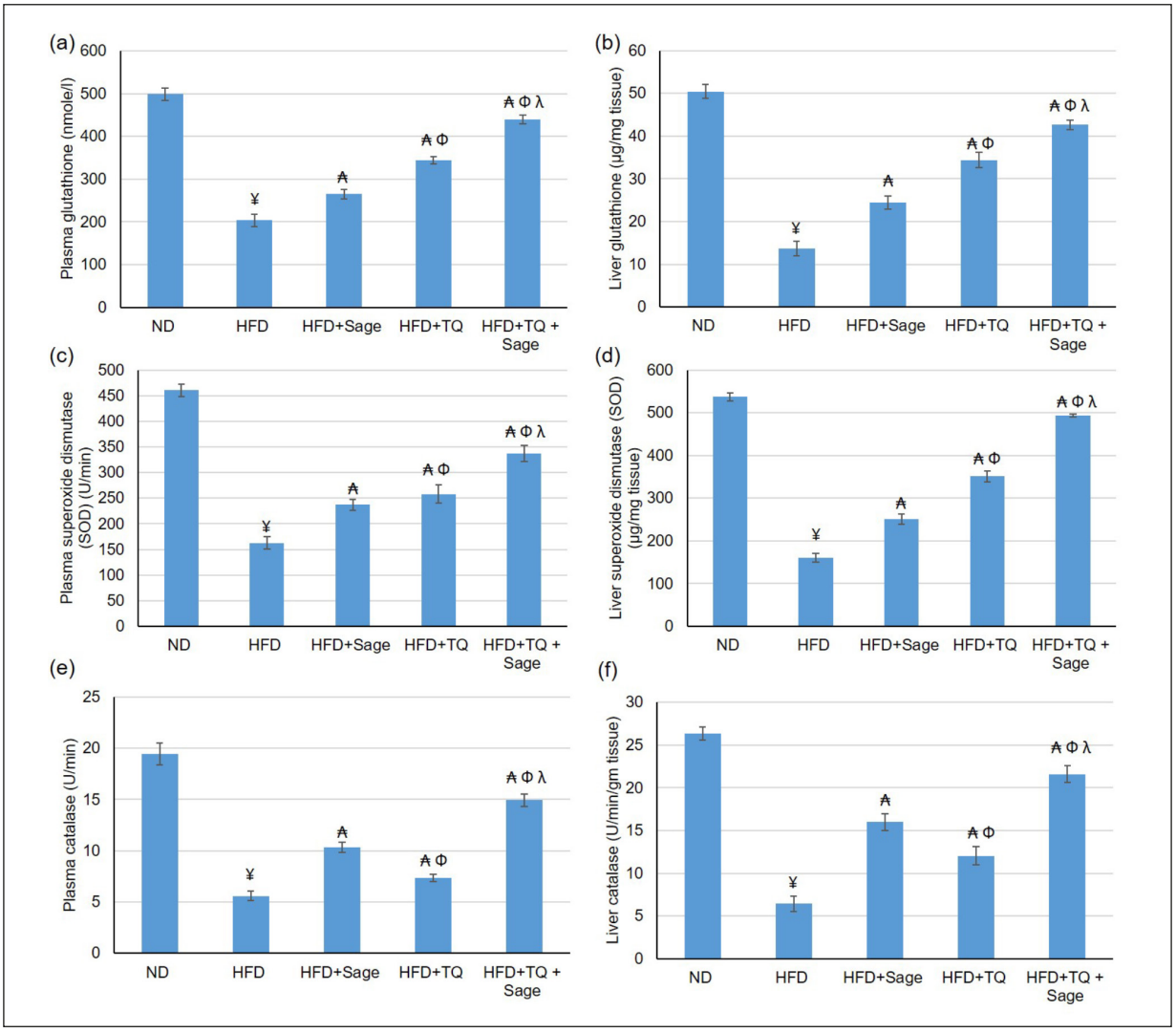

Figure 5. Effects of sage and thymoquinone (TQ) and their combination administration for 10 weeks on (**a-b**) glutathione content and antioxidant enzyme including (**c-d**) super oxide dismutase (SOD), and (**e-f**) catalase in plasma and liver tissue respectively of HFD-induced metabolic changes. All values are stated as mean ± SD (n=6). ¥ Designates statistically significant compared to the ND group, and Ⱥ designates statistically significant compared to the HFD group, Φ designates statistically significant compared to HFD + sage ($p < 0.05$), and λ designates statistically significant compared to HFD + TQ using one-way ANOVA followed by Tukey's post-hoc test.

Furthermore, APOP concentrations were also substantially amplified in HFD-fed animals when compared to standard diet-fed animals ($p>0.05$). Sage and TQ alone and their combination significantly reduced APOP levels ($p>0.05$) in the plasma and liver in HFD fed rats (Figure 4).

Effects of Sage and TQ Alone and Their Combination on Plasma and Liver Levels of Antioxidant Enzyme and Glutathione Content in HF Diet-Fed Rats

There are some naturally produced cellular antioxidants that are responsible for reducing oxidative stress, such as superoxide dismutase (SOD), catalase (CAT) activities, and glutathione (GSH) content. These cellular antioxidants were severely compromised in HFD-fed rats because of the amplified oxidative stress, as shown earlier. SOD, CAT activities, and GSH concentration were significantly lowered ($p<0.05$) in both the plasma and liver of HFD-fed rats compared to the standard-fed group. SOD, CAT activities, and GSH concentration in both the plasma and liver were significantly ($p<0.05$) restored by sage and TQ and compared to HFD-fed group (Figure 5).

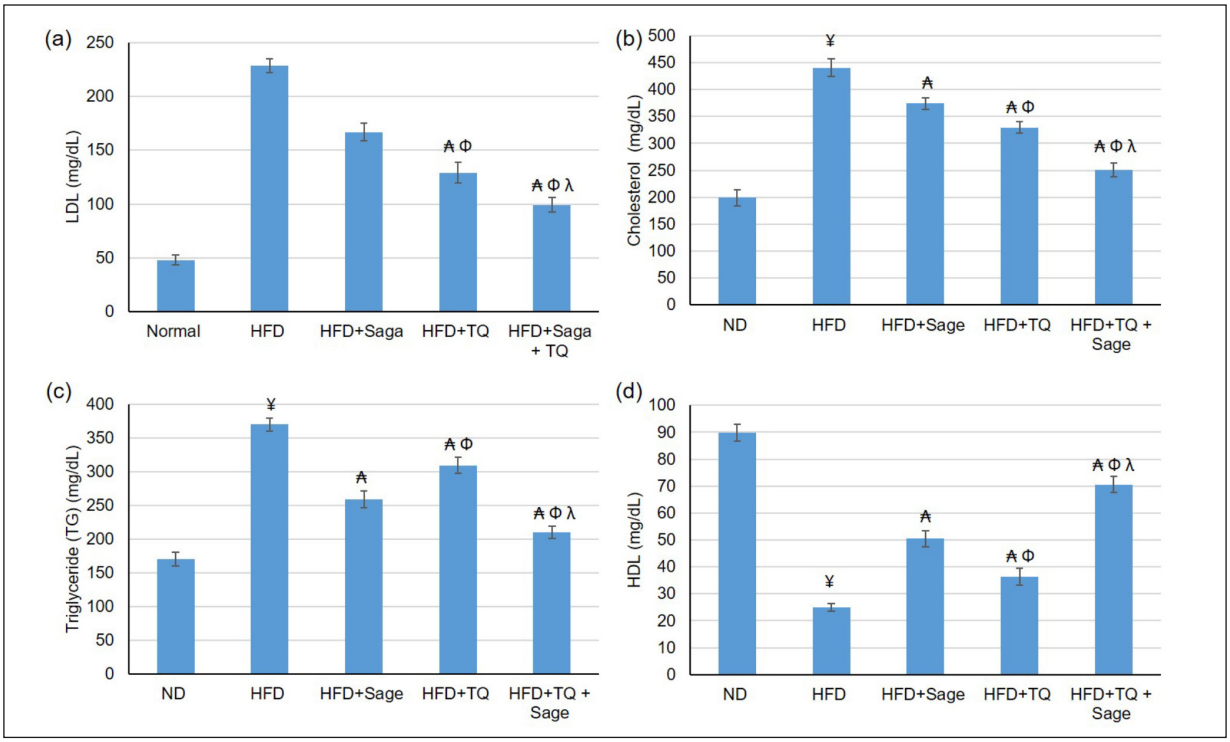

Figure 6. Effects of sage and thymoquinone (TQ) and their combination administration for 10 weeks on lipid profile including (**a**) LDL, (**b**) cholesterol, (**c**) triglyceride, and (**d**) HDL in HFD-induced metabolic changes. All values are stated as mean ± SD (n=6). ¥ Designates statistically significant compared to the ND group, and Ⱥ designates statistically significant compared to the HFD group, Φ designates statistically significant compared to HFD + sage ($p < 0.05$) and λ designates statistically significant compared to HFD + TQ using one-way ANOVA followed by Tukey's post-hoc test.

Effects of Sage and TQ Alone and Their Combination on the Lipid Profiles of HF Diet-Fed Rats

In the current investigation, we measured the level of total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL) cholesterol levels in the plasma of HFD-fed animals to assess the lipid-lowering effect of sage and TQ and their combination. Plasma TC, TG, and LDL levels were considerably augmented ($p<0.05$) in rats that consumed HFD. Sage and TQ and their combination significantly reduced the plasma TC, TG, and LDL levels in HFD-fed animals ($p<0.05$) (Figure 6). Furthermore, the high-density lipoprotein (HDL) cholesterol level was significantly diminished in HFD-fed animals ($p<0.05$), whereas sage and TQ alone and their combination significantly amplified the plasma level of HDL (Figure 6).

Discussion

Nowadays, there is a globally vast interest in the use of herbal remedies for the management of metabolic disorders. Thymoquinone (TQ) is the main active pharmacological component of *Nigella sativa* (black cumin) and has been demonstrated[44] to have a wide range of activities. Clary sage (*Salvia sclarea*) essential oil is widely used in the flavor and fragrance industry and in aromatherapy for its calming and immunomodulatory effect[44]. The consumption of a high-fat diet is the main cause of the development of obesity and obesity-associated complications.

In the current study, HFD-fed animals disclosed an amplified final BW, weight gain, and liver wet weight. In addition, feeding the rats with HFD for 10 weeks caused an escalation in FSI, BGL levels, and HOMA-IR, and developed glucose intolerance and were unable to properly utilize glucose. Numerous studies[7,45-47] showed that rats fed with HFD display amplified weight gain and liver wet weight, increased serum FSI, BGL concentrations, and HOMA-IR, whereas sage and TQ supplementation with HFD significantly decreased the final body weight, BW gain, and liver wet weight and reduced the FSI, BGL levels, and HOMA-IR compared to

HFD-fed animals. Sage and TQ combination improved the glucose utilization in this experiment, as evidenced by the outcomes from the OGTT. Alshahrani et al[34] showed that TQ attenuated hyperglycemia-induced insulin resistance in HFD and STZ-induced type 2 diabetes in rats. TQ ameliorated obesity-induced metabolic dysfunction and improved reproductive efficiency in Female C57BL/6 HFD mice[48]. Besides, TQ effectively adjusted glycemic control and reduces oxidative stress in STZ-induced diabetic rats with no damaging effects on renal function[49]. Furthermore, TQ combined with metformin revealed a reduction in the levels of HbA1c and blood glucose compared to metformin alone in diabetic patients[22]. Regarding sage essential oil, it was not tried in the management of HFD-induced metabolic changes but in other diabetes mellitus (DM) models and it showed promising results. For instance, Lima, et al[50] revealed that sage essential oil increased hepatocyte sensitivity to insulin and inhibited gluconeogenesis, thus suggesting that it may be useful as a food supplement in preventing T2DM. In addition, the metabolic impairments and tissue disorders in alloxan-induced diabetic rats were alleviated by sage essential oil[32].

In the existing study, HFD animals exhibited a significant intensification in SAP and DAP, while TQ, and sage combination lowered SAP and DAP significantly compared to HFD alone. Liu et al[51] demonstrated the protective effect of TQ through improving cardiovascular function and attenuating oxidative stress, inflammation and apoptosis by mediating the PI3K/Akt pathway in STZ-diabetic rats. Another study, performed by Enayatfard et al[52] proved that TQ ameliorated angiotensin II-induced hypertension in rats, probably due to reducing the cardiovascular effects of angiotensin II (Ang II). Furthermore, TQ attenuated hypertension and renal damage in nitric oxide deficient hypertensive rats[53]. A double-blind, randomized, controlled trial[54] carried out in 34 female patients with urinary incontinence investigated the effect of inhalation of *Salvia sclarea* (clary sage) essential oil vapors on autonomic nervous system activity. The study found that the sage group experienced a significant decrease in systolic blood pressure, diastolic blood pressure, and respiratory rate.

Hepatic oxidative damage leads to hepatocyte damage in HFD-fed rats, which causes these enzymes to be transported to the plasma[46,55,56]. Thus, AST, ALT, and ALP enzymes are contemplated as markers for liver dysfunction. As demonstrated in the current study, serum ALT, AST, and ALP activities were significantly amplified in the HFD-fed animals indicating hepatic damage, whereas oral administration of sage and TQ combination reduced the serum ALT, AST, and ALP activities compared to animals that consumed the HF diet alone. TQ lowered liver enzymes activities and exhibited hepatoprotective actions against numerous liver diseases, for instance high-fat, high-cholesterol diet-induced nonalcoholic fatty liver disease in rats[57], lipopolysaccharide (LPS)-induced liver fibrosis[58], acrylamide[59] and carbon tetrachloride[60]-induced liver damage. Besides, Belhadj et al[32] demonstrated that sage essential oil significantly lowered serum AST, ALT and LDH activities which may be due to the inhibitory effect of sage essential oil on α-amylase and lipase activities in both *in vitro* as well as *in vivo* studies. In addition, sage essential oil increased hepatocyte sensitivity to insulin and inhibited gluconeogenesis, suggesting it has a metformin-like effect on rat hepatocytes[50].

Oxidative stress triggered by the intake of a HFD was manifest in several investigational studies[61] as well as in patient subjects[62,63]. As revealed in the existing study, plasma and hepatic lipid peroxidation product, malondialdehyde, nitric oxide, and advanced protein oxidation product levels were amplified in HFD-fed animals. This escalation in oxidative stress markers was accompanied by a reduction in SOD and catalase activities and GSH content in plasma and hepatic tissue. These results are consistent with previous studies[64,65] showing that antioxidant defenses may be compromised in HFD-fed animals. Sage and TQ combination deterred the lipid peroxidation amplification and diminished NO and APOP levels, as well as restored SOD, CAT activities, and GSH content in plasma and hepatic tissue. Earlier studies[13,17,66] showed that TQ attenuated oxidative stress in numerous animal models signifying nephroprotective[13], cardioprotective[17], and neuroprotective[66] actions. Regarding hepatoprotective, administration of TQ reduced the oxidative stress damage in HFD-induced nonalcoholic steatohepatitis (NAFLD)[51], and in CCl_4[59], doxorubicin[67] and gentamicin[68] induced hepatotoxicity.

Evidence from several studies[27] suggests that *S. officinalis* has potent antioxidant activities. For instance, enriching the drinking water of rats with *S. officinalis* extract increases the resistance of rat hepatocytes against oxidative stress[27]. El-Hosseiny et al[33] verified oxidative stress mitigation by sage essential oil in co-amoxiclav in-

duced liver injury. In that study[33], Co-amoxiclav induced oxidative stress, including intensification in lipid peroxidation, as well as the depletion of both glutathione level and glutathione-dependent enzymes' activities, were all reversed by the sage essential oil.

In relation to lipid profile, the results of the current study showed that plasma TC, TG, and LDL levels were augmented, whereas HDL cholesterol level was diminished in rats who consumed HFD. On the other hand, the sage and TQ combination reduced the plasma levels of TC, TG, and LDL and amplified HDL levels. HFD-fed animals displayed amplified plasma TG and cholesterol levels, which ultimately trigger the development of lipotoxicity and lipid accumulation in the liver[37]. TQ reduced TC, TG, and LDL induced by HFD supplementation and thus can be considered as a promising agent in preventing the neuronal morphological changes in the cerebellum[69] and improving reproductive efficiency[48] by attenuating atherosclerosis development[70]. In a nonrandomized clinical trial[71], a diabetic patient administered *N. sativa* supplement for one year showed a decline in TC, LDL-C, TC/HDL-C, and LDL-C/HDL-C ratios, compared with the respective baseline data and the control group. The administration of sage infusion significantly decreased body weight, and serum triglycerides in HFD-fed rats[72]. The treatment with sage in STZ-diabetic rats induced amelioration in lipid profile parameters[73]. Ghowsi et al[74] investigated the possible lipid-lowering effects of *Salvia officinalis* (sage) tea in testosterone-induced polycystic ovary rats. The results showed that the serum LDL-C and total cholesterol levels were decreased in the polycystic ovary rats treated with sage tea. As for human trials, a pilot trial[75] (non-randomized crossover trial) with six healthy female volunteers (aged 40-50) was designed to evaluate the beneficial properties of sage tea consumption on blood glucose regulation, lipid profile, and transaminase activity in humans. The outcomes demonstrated that four weeks of sage tea treatment displayed an improvement in lipid profile as observed with lower plasma LDL cholesterol and total cholesterol levels as well as higher plasma HDL cholesterol levels with no hepatotoxic effects or other adverse effects were observed.

The attained results from this study evidenced that sage essential oil, together with TQ, exhibited hypoglycemic, hypolipidemic, and antioxidant actions thus could be a valuable addition for diabetes management.

Conclusions

This investigation provides evidence that sage and TQ combination may be an alternative approach to combat obesity-related complications. Sage essential oil together with TQ mitigated hyperglycemia, hyperlipidemia and oxidative stress associated with HFD in animals thus could be a valuable addition for diabetes management. Further research is warranted to conclude this favorable outcome on human subjects in a clinical trial.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

The authors are grateful to Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University.

Funding

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. 966]..

Ethics Approval

The Institutional Animal Care and Use Committee of King Faisal University allowed and permitted the experimental protocol (KFU-REC-2022- MAY -EA000633). All the animal handling and experiments, and tests were executed according to the appropriate guidelines and regulations of the Ethical Conduct for the Use of Animals in Research at King Faisal University. All experiment protocols were conducted in harmony with the relevant policies and regulations.

Informed Consent

Not applicable.

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