METABOLIC AND ANTIOXIDANT EFFECTS OF *NIGELLA SATIVA* OIL ON PREVENTION OF OBESITY DEVELOPMENT IN RATS FED HIGH-FAT DIET.

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The results of this study demonstrated that *Nigella sativa* seeds oil is beneficial for attenuating complications of obesity and possibly preventing it because of its essential polyunsaturated fatty acids; linoleic and linolenic acids and other nutrients such as liposoluble vitamins like tocopherols and also minerals, essential amino acids, some polyphenols, terpenoids and quinones, particularly thymoquinone that have shown potential medicinal properties in traditional medicine.

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Introduction

Obesity is rapidly increasing worldwide. It has become a leading health problem in the 21st century loading both in developed and developing countries.[1, 2] In 2009, the World Health Organization estimates that nearly 700 million the number of obese people by 2015, with an increase of 75% in 10 years.[3,4] Obesity and overweight can lead to a high risk of health complications such as cardiovascular disease, insulin resistance and type II diabetes mellitus, hyperlipemia, hypertension and cancers .[5]

Once of their many factors that involved obesity, consumption of energy-dense foods; rich in fat and/or carbohydrates, is considered a major contributing cause to this disease. Furthermore, an increased body weight is observed in the imbalance between the energy intake and the expenditure.[1,6,7] A high fat diet intake can contribute to the development of obesity and dyslipidemia in humans and rodents by altering cholesterol and triglyceride levels in plasma and tissues.[8] Several studies have reported that diet composition in type and amount of fat favors reduced hyperlipidemia[9] and mainly diet rich in saturated fatty acids favor an acute increase in insulin resistance and adiposity.[10] Some studies also show how the effect of n-3 polyunsaturated fatty acids (n-3 PUFAs), conjugated fatty acids, phytosterols, medium-chain triglycerides and phospho lipids as important modulators, can affect obesity.[11-13]

Numerous studies have found elevated biomarkers of oxidative stress in overweight and obesity. Oxidative stress can lead to damage of biomolecules such as lipids, proteins, and DNA.[14] Oxidative stress may be the mechanism underlying the development of inflammation and diseases associated with obesity such as insulin resistance, diabetes, hypertension, and metabolic syndrome. Different factors can contribute to oxidative stress in obesity, e.g., hyperglycemia, increased muscle activity because of the need to carry excessive weight, increased tissue lipid levels, inadequate antioxidant defenses, chronic inflammation and endothelial reactive oxygen species (ROS) production.[15]

There are several treatments developed to remedy this deadly disease and its complications, including hypo caloric diets, exercise, medication and also surgery.[7] Among these treatments, dieting, exercise and behavior modification are essential to the management of obesity; even though often, long-term results are disappointing. Various anti-obesity drugs, including rimonabant, Sibutramine, fenfluramine, phentermine, phendimetrazine, and diethylpropion have been withdrawn from the market owing to an unacceptable side effects that include headaches, vomiting, and heart attacks and psychiatric problems such as anxiety and depression. The history of anti-obesity drugs development is far from glorious and only a handful of agents are currently licensed for clinical use. Orlistat is the only weight loss agent approved for long-term clinical use in Europe.[16-18]

Many researchers in the field of food science have focused on the search for functional food ingredients and/or herbal extracts that can prevent the accumulation of body fat so achieving body weight loss,[19] improving the oxidative metabolism by alleviating glycemia and lipidemia and decreasing obesity-related oxidative stress by increasing tissue antioxidant levels.[15]

Vegetable oils are a good example that depicts the actions of dietary fat on obesity and their alterations. Among the various oil seeds, black cumin (*Nigella sativa* L.), an annual *Ranunculaceae* herbaceous plant, is of particular interest because it may be used for medicinal purposes as a natural remedy for a number of illnesses. The effects of black cumin seeds have been evaluated in clinical and animal studies. These seeds have been used as diuretic, antiulcerogenic, liver tonic, immunomodulative, anti-inflammatory, antitumor and antidiabetic.[20-22] Additionally, these seeds

have been subjected to a range of pharmacological investigations in recent years.[23,24] Besides the diversity of fatty acids that contained in this seed oil, thymoquinone (TQ) is known as a pharmacologically active compound that is shown to have a protective effect mainly against hepato-toxicity induced by either chemicals or diseases.[25]

The investigations of protective effect of *N. sativa* fixed oil seeds on lipid metabolism and their corrective effect of the alterations that follow obesity in animal models are limited.[26] Consequently, in order to explore the effect of vegetable oils for preventing and eventually treating obesity, the aim of this study was carried out to examine the possible beneficial effect of *N. sativa* seeds fixed oil on body weight, lipid metabolism, pancreatic and hepatic activity and investigate their effect on the antioxidant status by quantifying the plasmatic concentrations of antioxidant vitamins (A,C and E), malondialdehyde (MDA) levels, protein carbonyls as a markers of an oxidative stress in addition of catalase (CAT) and superoxide dismutase (SOD) activities, and this was carried out in growing obese male rats fed with high fat diet (HFD) and in non-obese rats were measured.

Materials and methods

Vegetable oils

Nigella sativa L. (*Ranunculaceae*) seeds oil was extracted using the following method: Seeds obtained from Tlemcen-Algeria were grounded using a crusher (Retsch RM 100). The lipid fraction was extracted using petroleum ether (40–60 °C) in a Soxhlet apparatus for 2 hours (Natural Products Laboratory, Tlemcen, Algeria). The solvent was then evaporated and the lipid fraction residues weighed. The yield oil content on seeds was found to be 35 ± 1.8 %.

After preparation, seed oil extract was refrigerated and covred with aluminium foil, to protect from light to assure its stability.

Olive and Sunflower oils are commercial local products (Olive oil is obtained by cold extraction and sunflower oil purchased from Cevital®). All chemicals used were commercially provided with a high purity level.

Thymoquinone quantification on *N. sativa* seeds oil

TQ quantification was carried out by High Performance Liquid Chromatography (HPLC) as described by Al-saleh *et al.,*[27] with some modifications, for this; oil sample (a triplicate) was dissolved in methanol (for HPLC analysis from Sigma-Aldrich) vortexed for 1 min and centrifuged 10 min at 3000 rpm. 20 µL of supernatant was injected on reverse-phase Waters column (150×4.6mm×3.5µm particle size), using an isocratic mobile phase of water: methanol: 2-propanol (50:45:5% v/v) at a flow rate of 1 mL min^{-1}. Analyses were made at room temperature.

UV monitoring was carried out at 275 nm. Calibration curve was constructed by TQ standard at the same conditions.

Evaluation of biological activity

Animals

The experimental protocol was approved by the Animal Care and Use Committee of Tlemcen University. Fifty male Wistar rats obtained from the Pasteur Institute (Algiers, Algeria) weighing 86.7 ± 1.3 g were considered at the beginning of the experiment. One-month-old male Wistar rats were housed in clear plastic cages with natural beddings and subjected to 12 hours light-dark cycles. Food and water were available *ad libitum*. Food was replaced daily and the uneaten portions weighed. The temperature was maintained at 24 °C and humidity kept constant at 60 %.

Experimental diets

The rats were divided randomly into six diet (D1-D6) groups. Each group was fed one of six diets (**Table 1**) for eight weeks:

D 1: Control diet, rats received 4 % Sunflower oil (n=10);

D 2: Obese group received high fat diet (HFD) with 32 % Sunflower oil (n=10);

D 3: received 4 % black cumin seeds oil (n=7);

D 4: were fed 28 % Sunflower oil and 4 % black cumin seeds oil (n=8);

D 5: were fed 4 % Olive oil (n=7);

D 6: were fed 28 % of Sunflower oil and 4 % Olive oil (n=8).

Oral glucose tolerance test (OGTT)

After the two weeks period of experimentation diets and following overnight fasting, the rats were being subjected to an oral glucose tolerance test (OGTT) by intragastric feeding with a glucose solution (2 g/kg body weight). OGTT was carried out during two (02) hours. Blood samples were collected from the tail vein at 0, 30, 60, 90 and 120 min after glucose administration. Because it's stressful, only two blood samples were done from the retro-orbital sinus, and this was to determine initial (0 min) and final (120 min) insulin blood concentration.

Blood glucose was determined using a glucometer (Accu-Check Active, *Roche,* Germany). Plasma insulin concentration was measured by radio-immunoassay using Rat Ultrasensitive Insulin ELISA (ALPCO Diagnostics, NH). The homeostasis model assessment score of insulin resistance (HOMA-IR) was calculated from the fasting glucose and insulin concentration using the standard formula as described by Matthews *et al.*[28] Insulin sensitivity was determined using the quantitative oral glucose and insulin sensitivity index OGIS and calculated from a spreadsheet that can be downloaded using this link: http://webmet.pd.cnr.it/ogis/download.php, which was based on glucose oral dose and insulin levels .[29,30]

Table 1. Ingredient composition of experimental diet groups (D1-D6).

Constituent, wt. %	D1	D2	D3	D4	D5	D6
Starch	60.3	32.3	60.3	32.3	60.3	32.3
Oil	4.0	32.0	4.0	32.0	4.0	32.0
Dietary fatty acids[a]						
ΣSFA[b]	0.89+/-0.01	7.10+/-0.02[a]	0.76+/-0.01	6.98+/-0.05[b]	0.73+/-0.01	6.95+/-0.02
ΣMUFA[c]	2.40+/-0.05	19.21+/-0.08[a]	0.83+/-0.01	17.64+/-0.10[b]	2.59+/-0.08	19.40+/-0.12
ΣPUFA[d]	0.63+/-0.01	5.05+/-0.02[a]	2.22+/-0.09	6.64+/-0.07[b]	0.71+/-0.01	5.13+/-0.02
P/S[e]	0.71+/-0.01	0.71+/-0.01[a]	2.92+/-0.09	0.95+/-0.01[b]	0.85+/-0.02	0.73+/-0.01
Energy values (kcal)	371.9	517.5	371.9	517.5	371.9	517.5

In addition, the common ingredients in all diet groups were: 16 % casein, 0.3 % methionine, 5 % saccharose, 5 % cellulose, 7.37 % mineral mix* and 2 % vitamin mix*. *Mineral mix composition (g/100 g of dry diet): Ca^{2+}, 4; K^{+}, 2.4; Na+, 1.6; Mg^{2+}, 0.4 Fe^{2+}, 0.12; trace elements: manganese, 0.032; copper, 0.05; zinc, 0.018. Vitamin mix composition (mg Kg^{-1} of dry diet): retinol, 1.8; cholicalciferol, 0.019; thiamine, 6; riboflavin, 4.5; pantothenic acid, 21; inositol, 5; ascorbic acid, 240; L-tocopherol, 51; nicotinic acid, 30; folic acid, 1.5; biotin, 0.09. Refer to text for diet groups. *a:* calculated from CG data, *b:* total saturated fatty acids, *c:* total monounsaturated fatty acids, *d:* total polyunsaturated fatty acids, *e:* polyunsaturated and Saturated fatty acids ratio. a: $p<0.05$ obese group versus controls. b: $p<0.05$ nigelle oil treated group versus obese and olive oil's treated group. c: $p<0.05$ olive oil's treated group versus obeses.

Table 2. Effect of the different diets on plasma fatty acids composition

Plasma fatty acids, wt. %	D1 (*n*=10)	D2 (*n*=10)	D3 (*n*=7)	D4 (*n*=8)	D5 (*n*=7)	D6 (*n*=8)
C14:0	nd[α]	nd	0.11+/-0.03	0.17+/-0.03[b]	nd	nd
C16:0	26.27+/-0.01	24.22+/-0.03	25.66+/-0.04	14.34+/-0.04[b]	21.29+/-0.13	15.62+/-0.03
C18:0	19.22+/-0.01	16.68+/-0.01	10.44+/-0.03	16.52+/-0.06	9.70+/-0.09	14.16+/-0.01
C18:1	29.44+/-0.04	35.55+/-0.11[a]	35.10+/-0.18	32.07+/-0.04[b]	24.08+/-0.21	25.32+/-0.01
C18:2	11.27+/-0.05	21.7+/-0.03[a]	20.85+/-0.05	29.61+/-0.1[b]	27.37+/-0.06	35.16+/-0.01
C18:3	0.67+/-0.02	Nd	0.09+/-0.01		nd	nd
C20:0	nd	Nd	0.05+/-0.00	0.88+/-0.01[b]	nd	nd
C20:1	0.58+/-0.02	Nd	nd	nd	nd	nd
C22:0	nd	Nd	nd	nd	1.4+/-0.05	nd
C20:4	6.36+/-0.03	10.59+/-0.02[a]	7.08+/-0.03	12.09+/-0.01[b]	10.2+/-0.08	8.80+/-0.02
ΣSFA[β]	45.49+/-0.01	40.9+/-0.02[a]	36.26+/-0.02	31.91+/-0.12[b]	32.39+/-0.013	29.78+/-0.12
ΣMUFA[γ]	30.02+/-0.03	35.55+/-0.05[a]	35.1+/-0.04	32.07+/-0.08[b]	24.08+/-0.35	25.32+/-0.12
ΣPUFA[δ]	18.63+/-0.03	23.29+/-0.09[a]	28.02+/-0.04	41.7+/-0.10[b]	37.57+/-0.08	43.96+/-0.22

α not detected, *β* Total saturated fatty acids, *γ* Total monounsaturated fatty acids, *δ* Total polyunsaturated fatty acids. a: $p<0.05$ obese group versus controls. b: $p<0.05$ nigelle oil treated group versus obese and olive oil's treated group. c: $p<0.05$ olive oil's treated group versus obeses.

Body weight, liver, adipose tissues weight and blood parameters

Food intake and body weight were measured on a daily basis. At the end of the eighth (8^{th}) week, the control and treated groups were sacrificed, after anesthetization with intra-peritoneal injection of chloral hydrate 10 % (3 mL Kg^{-1}). These animals were treated, manipulated and killed according to the regulations of the *Animal Care Laboratory of Tlemcen University-Algeria*.

Blood was immediately collected from the abdominal aorta in heparined tubes and the plasma obtained after centrifugation was used for the determination of biochemical markers including alanine aminotransferase EC 2.6.1.2 (ALT), aspartate aminotransferase EC 2.6.1.1 (AST), lactate deshydrogenase EC 1.1.1.27 (LDH), alkaline phosphatase EC 3.1.3.1 (ALP) using enzymatic kits (Spinreact, Girona, Spain).

Albumin, globulin, total protein, blood urea, creatinine, blood glucose (BG), triglycerides (TG) and total cholesterol (TC) using enzymatic kits (Biomérieux, Lyon, France).

HDL-cholesterol (HDL-C) was measured using also enzymatic method after precipitating HDL by sodium phosphotungstate-magnesium.[31] VLDL-cholesterol (VLDL-C) was calculated by dividing triglyceride concentration by five.[32] LDL-cholesterol (LDL-C) concentration was calculated using the Friedewald–Levy–Fredrickson formula.[33]

Liver and visceral adipose tissues (abdominal's, kidney's and testis fats) were excised and weighted.

Measurement of total plasma fatty acids

The fatty acid profile of plasma was determined by gas chromatography (CG). Fatty acids were trans-esterified into methyl esters (FAMEs) following the Bligh and Dyer procedure using 14% boron trifluoride in methanol with some modifications.[34] FAMEs were identified by CG Varian CP-3380 using a capillary column (Alltech EC-Wax) (30m×0.53mm×1.2 µm film thickness) and equipped with a flame ionization detector (FID). Helium was used as the carrier gas. The oven temperature was kept constant at

250°C and the injected volume was 1µL. The temperature was kept at 180°C for two minutes and then increased to 220°C with 6°C/min heating rate. After this period, the temperature was kept at 220°C for 10 min. FAMEs were identified using FAMEs authentic standard (Grace AOCS Mix 3A) injected in the same conditions as the plasma FAMEs (a triplicate).

Oil FAMEs composition was also analyzed as described above. The results are shown in Table 2.

Determination of anti-oxidant status

Determination of plasma levels of vitamins A, C and E

Plasma α-tocopherol (vitamin E) and retinol (vitamin A) were determined by reverse phase HPLC and detected by an UV detector at 292 nm for vitamin E and 325 nm for vitamin A.[35] Vitamin C levels were determined in plasma using the method described by of Roe *and* Kuether.[36]

Measurement of malondialdehyde (MDA):

Analysis of lipid peroxidation was estimated by measuring thio-barbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde content, according to the method of Ohkawa *et al.,*.[37] Briefly, an aliquot of 200µl of plasma was mixed thoroughly with aqueous solution of thiobarbituric acid and heated at 95◦C for 30 min in a water bath. The suspension was then cooled to room temperature, centrifuged at 4000 rpm for 10 min, and the pink colored supernatant was taken for spectrophotometry measurement at 532 nm for MDA assay.

MDA concentration was calculated by the extinction coefficient of MDA–TBA complex $E^{M}_{532nm,1\mathrm{cm}} = 1.56\times10^{5}$ $M^{-1}cm^{-1}$.

Measurement of protein carbonyls

Plasma carbonyl proteins (marker of protein oxidation) were assayed by the 2, 4-dinitrophenylhydrazine (DNPH) reaction.[38] Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid and redissolved in DNPH and the absorbance was read at 370 nm.

Determination of antioxidant enzymes activities:

Analysis of catalase (CAT) (EC 1.11.1.6) activity was performed using the method of Aebi[39] where the enzyme-catalyzed decomposition of H_2O_2 is measured. The rate of H_2O_2 decomposition was measured at 240 nm. The activity was calculated utilizing the extinction coefficient of H_2O_2, $E^{M}_{240nm,1\mathrm{cm}} = 0.0394\ mM^{-1}cm^{-1}$. Superoxide dismutase (SOD) (EC 1.15.1.1) activity was determinate according to the method described by Sun *et al.*[40] The principal of the method is based on the inhibition of nitroblue tetrazoium (NBT) by the xanthine oxidase system as a superoxide generator. SOD activity (1U) was defined as of the enzyme amount causing 50% inhibition in the NBT reduction rate.

Statistical analysis

The results were expressed as the mean value ± standard deviation. The comparison between the mean values of each two groups was established by Student's test "t". Significance was set at $p < 0.05$. The significance of differences between all the parameters was assessed by the one way ANOVA analysis and Tukey's post hoc test was used for the comparisons; *p-values* < 0.05 were considered statistically significant. Multiple regression analysis was used to estimate the relationship between FBW (final body weight), glycemia, insulinemia, food intake, AGPI intake, HDL-C, TG, HOMA score and OGIS index; while all confidence limits represent 95% intervals. A Pearson correlation was also made to compare all the parameters.

Results

Body weight, lipid intake and energy supply

From the first week of the experimentation (Fig. 1), body weight of rats fed by an HFD (Diet 2) started to increase significantly compared to those fed an isocaloric diet (Diet 1, 3 and 5) which suggests in at first sight the installation of obesity. Also, the weight of rats fed an HFD with 4% Nigel oil or Olive oil (Diet 4 and 6 respectively) decreased significantly from the third week compared to group 2. On the other hand, liver and abdominal adipose tissue weights (Table 3) show a significant increase in obese *versus* control rats which are positively correlated to FBW. Only for liver weight, a significant decrease in Diet 4 and 6 (p<0.05) compared with obese group was noted. No differences were observed between the other adipose tissues (*see* **Table 1**).

The data showed that all rats received an HFD (Diets 2, 4 and 6) presented an increase in lipid intake compared to isocaloric fed rats (Diets 1, 3 and 5). Consequently, the total energy supply (TES) was significantly higher on the HFD fed rats.

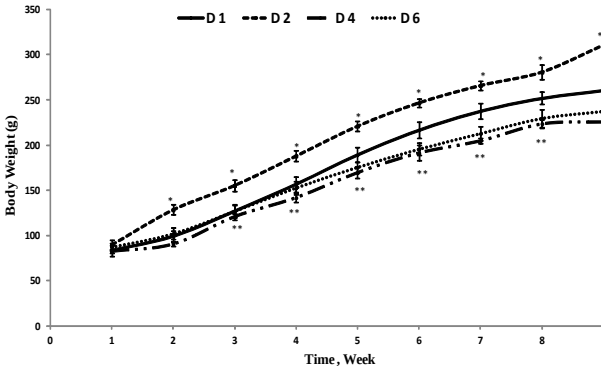

Fig 1. Changes in body weight per week of rats of different diet groups: control (D1), obese (D2), nigella (D4) and olive oil (D6) treated groups. (D3) and (D5) are not represented because of their superposition on the control curve (D1). Refer to Table 2 for diet groups. *($p < 0.05$): Significant difference between control and obese group. **($p < 0.01$): Significant difference between obese and treated groups.

Table 3. Effect of the different diets on initial and final body weight, food intake and organs weights.

	D1 (*n*=10)	**D2 (*n*=10)**	**D3 (*n*=7)**	**D4 (*n*=8)**	**D5 (*n*=7)**	**D6 (*n*=8)**
Initial BW, g	86.20+/-5.98	89.5+/-5.80	87.5+/-1.98	82.4+/-2.07	87.7+/-5.45	87.2+/-3.94
Final BW, g	202.0+/-7.05	310.5+/-4.13[a]	228.0+/-22.63	225.7+/-6.26[b]	237.0+/-2.54	237.2+/-9.64[c]
Food intake, g d^{-1} 100 g BW^{-1}	11.90+/-0.66	12.8+/-066	13.1+/-0.86	13.4+/-0.99	11.2+/-0.22	9.80+/-1.27[c]
Lipid intake, g d^{-1} 100 g BW^{-1}	0.48+/-0.03	5.10+/-0.27[a]	0.53+/-0.03	5.30+/-0.39	0.45+/-0.01	3.90+/-0.51[c]
TES, kcal d^{-1} 100g BW^{-1}	43.34+/-1.82	63.48+/-7.16[a]	47.52+/-1.66	57.78+/-10.32	40.56+/-1.67	49.85+/-10.40[c]
Liver weight, rel. %	3.37+/-0.12	3.82+/-0.20[a]	3.6+/-0.08	2.96+/-0.32[b]	3.31+/-0.10	3.26+/-0.11
Abdominal fat, rel. %	0.91+/-0.02	1.54+/-0.09[a]	1.20+/-0.06	1.35+/-0.02[b]	1.28+/-0.01	1.11+/-0.05[c]
Peri-testis fat, rel. %	1.46+/-0.10	1.48+/-0.02[a]	1.40+/-0.03	1.34+/-0.09[b]	1.48+/-0.15	1.46+/-0.13
Peri-renal fat, rel. %	0.24+/-0.10	0.31+/-0.02[a]	0.27+/-0.05	0.24+/-0.03[b]	0.22+/-0.07	0.20+/-0.02[c]

BW: Body weight, TES: Total energy supply; d: day; W: watt; rel: relative weights; a: $p<0.05$ obese group versus controls; b: $p<0.05$ nigelle oil treated group versus obese and olive oil's treated group; c: $p<0.05$ olive oil's treated group versus obeses rats.

Table 4. Effect of the different diets on the biochemical parameters.

	D1 (*n*=10)	**D2 (*n*=10)**	**D3 (*n*=7)**	**D4 (*n*=8)**	**D5 (*n*=7)**	**D6 (*n*=8)**
Glycemia, mmol L^{-1}	4.30+/-0.01	8.10+/-0.03[a]	4.70+/-0.07	4.20+/-0.04[b]	4.60+/-0.05	4.50+/-0.04[c]
Total cholesterol, g L^{-1}	0.45+/-0.02	0.73+/-0.25[a]	0.57+/-0.07	0.53+/-0.08[b]	0.49+/-0.02	0.53+/-0.03[c]
Triglycerids, g L^{-1}	0.40+/-0.03	0.47+/-0.01[a]	0.73+/-0.16	0.29+/-0.06[b]	0.33+/-0.06	0.37+/-0.01[c]
HDL-C, g L^{-1}	0.34+/-0.03	0.28+/-0.01[a]	0.34+/-0.02	0.34+/-0.05[b]	0.37+/-0.05	0.36+/-0.02[c]
LDL-C, g L^{-1}	0.03+/-0.01	0.36+/-0.07[a]	0.08+/-0.05	0.13+/-0.07[b]	0.05+/-.01	0.10+/-0.03[c]
VLDL-C, g L^{-1}	0.08+/-0.00	0.09+/-0.01	0.15+/-0.03	0.06+/-0.01	0.07+/-0.01	0.07+/-0.00
Total proteins, g dL^{-1}	5.66+/-1.15	5.62+/-1.73	5.60+/-0.02	6.70+/-0.87	5.25+/-3.20	5.26+/-0.58
Albumin, g dL^{-1}	2.65+/-0.21	2.70+/-0.08	2.80+/-0.16	3.60+/-0.12	2.95+/-1.08	2.75+/-0.05
Globulin, g dL^{-1}	2.98+/-0.23	2.88+/-0.09	2.74+/-0.06	3.06+/-0.19[b]	2.28+/-0.09	2.51+/-0.19
A/G ratio	0.88+/-0.12	1.43+/-0.13[a]	1.02+/-0.11	1.17+/-0.21[b]	1.29+/-0.12	1.09+/-0.15[c]
TGO, UI L^{-1}	112.7+/-1.87	94.1+/-5.50[a]	77.5+/-2.37	115.0+/-1.68[b]	106.2+/-7.10	87.5+/-3.15
TGP, UI L^{-1}	42.03+/-30.11	52.2+/-11.30[a]	38.8+/-6.55	45.5+/-6.54[b]	44.8+/-1.34	60.2+/-6.70
TGO/TGP ratio	2.34+/-0.20	3.20+/-0.7[a]	2.50+/-0.60	2.08+/-0.14[b]	2.30+/-0.22	2.30+/-0.19
LDH, UI L^{-1}	3421.4+/-132.34	3093.9+/-320.39[a]	3320.2+/-264.13	3181.5+/-710.43[b]	3171.32+/-530.04	3223.8+/-567.65
ALP, UI L^{-1}	412.5+/-23.33	305.2+/-11.67	396.2+/-0.18	448.8+/-14.0[b]	405.7+/-19.45	367.9+/-53.7[c]
Creatinine, mg L^{-1}	9.10+/-0.43	6.80+/-0.035[a]	6.70+/-0.18	7.05+/-1.06	7.10+/-0.24	7.40+/-0.10
Urea, g L^{-1}	0.66+/-0.02	0.50+/-0.01[a]	.38+/-0.01	0.40+/-0.01	0.34+/-0.01	0.41+/-0.01

a: $p<0.05$ obese group versus controls; b: $p<0.05$ nigelle oil treated group versus obese and olive oil's treated group; c: $p<0.05$ olive oil's treated group versus obeses rats. Data represents means+/-SD.

Table 5. Effect of *Nigella sativa* and olive oils oxidative stress biomarkers.

	D1 (*n*=10)	**D2 (*n*=10)**	**D3 (*n*=7)**	**D4 (*n*=8)**	**D5 (*n*=7)**	**D6 (*n*=8)**
Vitamin C, µg mL^{-1}	15.40+/-2.3	11.08 +/-1.9[a]	16.16 +/-1.2 b	16.27 +/-1.8[b]	15.80+/-1.06[c]	16.12 +/-1.07[c]
Vitamin E, µmol mL^{-1}	7.65+/-0.6	6.87 +/-0.7[a]	8.22 +/-0.65[b]	8.58 +/-0.8[b]	7.98 +/-0.54	8.08 +/-0.63[c]
Vitamin A, µmol mL^{-1}	16.09+/-3.12	10.03 +/-2.18[a]	14.08 +/-2.04[b]	14.55 +/-2.09[b]	14.15+/-2.02[c]	14.20 +/-1.78[c]
MDA, nmol mL^{-1}	2.14 +/-2.3	4.47+/-4.0[a]	2.13+/-5.0	2.40 +/-4.1 b	2.24+/-2.8[c]	2.50+/-2.4[c]
Protein carbonyls, mmol mL^{-1}	1.94 +/-0.3	2.63 +/-0.5[a]	1.24 +/-0.1[b]	1.42+/-0.1[b]	1.34 +/-0.2[c]	1.07 +/-0.6[c]
SOD, U mL^{-1}	10.02 +/-1.91	11.89+/-1.85[a]	09.74+/-2. 21[b]	10.11+/-0.96[b]	08.60+/-0.85[c]	09.25+/-0.99[c]
Cat activity, U mL^{-1}	18,43+/-2.6	21,93 +/- 4.2[a]	20,27+/-10.8[b]	19,83 +/-10.9[b]	20,93+/-13.9[c]	20,61+/-11.2[c]

Data represents means+/-SD. a: $p<0.05$ obese group versus controls; b: $p<0.05$ nigelle oil treated group versus obese and olive oil's treated group; c: $p<0.05$ olive oil's treated group versus obeses rats. Refer to Table 1 for diet groups.

Final body weight an Biochemical parameters

Concerning the investigated biochemical parameters presented in Table 4, the HFD group (D 2) points out a significant increase in alanin aminotransferase (ALT) and aspartate aminotransferase (AST) compared to the control group (D 1). In opposite, ALT and AST concentrations decreased ($p<0.05$) significantly when comparing the HFD group with obese treated groups (Diets 4 and 6). A statistical difference is also observed between the two treated groups D 4 and D 6 which present a difference in their fatty acid composition (*see* Table 1). Also ALP levels increased significantly after dietary treatment with NS and olive oil. In contrast, no statistical difference was noted in LDH levels.

Total proteins, albumin and globulin levels decreased significantly in the HFD group compared to the control group. In opposite, after 08 weeks of daily treatment with NS oil, these parameters raised up, although the A/G ratio decreased in the same treated group when compared with obese rats reflecting the production of albumin by the hepatic cells which are the site of albumin synthesis and that the liver gets back their functional status. Also, there was a significant decrease in urea and creatinine levels in the obese rats (D 2) group *vs.* the control group (D1) an there was no statistical difference observed between the other groups.

BG, insulin, TG and TC were significantly higher in the HFD group compared to the control group, but decreased significantly ($p < 0.05$) for rats that received NS and olive oils compared to the HFD group. The HDL-C concentrations tended to increase in the treated groups (D4 and D6) while LDL-C was decreased significantly.

Blood glucose, plasma insulin and insulin resistance

The results of OGTT are shown in Figure 2. HOMA and OGIS results are summarized in Figure 3. For the duration of 120 min of the test, the animals were manipulated carefully to avoid stress. During the OGTT (Figure 2), the blood glucose levels in the control (D1) and treated group rats (D4 and D6) remained higher and reached the fasting levels at 2 hours and no significant difference was noted between these two groups. In contrast, in a HFD group (D2) glucose levels were significantly higher than in Diet 4 ($p<0.01$) even at 2 hours of test. HOMA-IR score is correlated to insulin sensibility OGIS has found to give a very similar results compare to hyper-insulinemic-euglycemic clamp.

Based on a multiple regression model, we can predict at 80.1% of the final weight from the amount of lipid intake, the insulin and glucose levels taken during an oral glucose tolerance test.

Oxidative stress biomarkers

Results of vitamins (A, C and E), MDA and protein carbonyls contents, CAT and SOD activities are shown in Table 5.

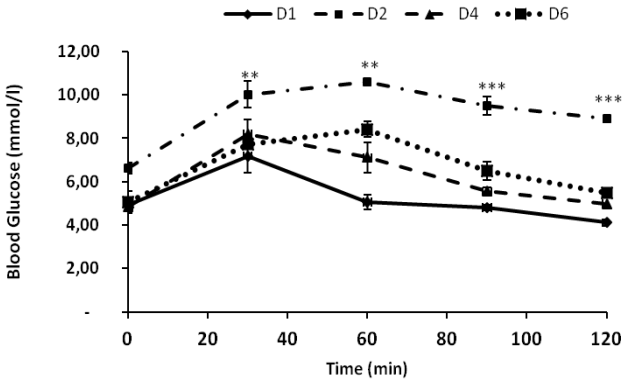

Fig 2. Evolution of blood glucose during *OGTT*. Results are expressed as means+/-SD. **($p<0.05$) ; ***($p<0.01$): significant difference between obese and control and treated obese groups.

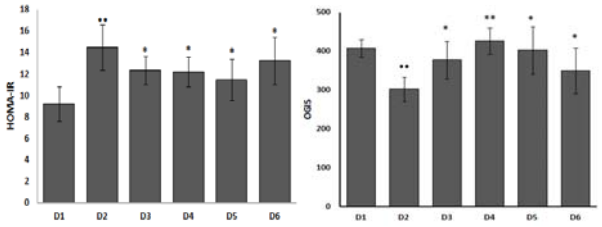

Fig 3. HOMA-IR score and OGIS values. • : Significant difference between between obese group and control ($p<0.05$); •• ($p<0.01$); ***:**Significant difference between obese and treated groups (*Nigella* or olive oil) ($p<0.05$); ****** ($p<0.01$).

We have noted a significant reduction on vitamins concentrations in obese rats compared to control group and treated groups with NS fixed oil and olive oil. A significant increase was observed in the levels of plasma MDA and protein oxidation in HFD group (D2) compared to control group (D1) and supplemented groups with nigelle and olive oils (D4 and D6). The SOD and CAT activities were significantly higher ($p<0.05$) in rats feeding with HFD. The treatment with NS oil induced a significant correction in SOD and CAT activities in both control (D3) and obese treated rats (D4).

Discussion

The aim of this study was to verify whether the supplementation with vegetable oils (*Nigella sativa* and olive oils) if it can prevent obesity. In fact, HFD induced obesity in growing rats during the 08 weeks of experimentation 32.02 % of weight gain was noted in the HFD group compared with the control group. It has been reported that HFD consumption may predispose individuals to obesity, and it can be explained that a high fat intake can contribute to excess of energy and consequently promote or support obesity.[41] It can also be explained that excessive weight gain may result from high energy intake resulting from the high caloric density of fat-enriched food or a failure in the ability to oxidize fat in obese rats.[42] These results can also be explained by the fact that high fat diets

are rich in SFAs which are considered as enhancing compounds in the development of adipose tissues.[43] On the other hand, it was shown the beneficial effects of PUFAs, due to the presence of double bonds ending up with low energy following their oxidation.[44] The oxidation rate of oleic acid is faster than that of linoleic acid and the increase in PUFAs induces a decrease in adipose tissue mass and prevents obesity development compared with MUFAs and SFAs .[45]

Moreover, the results obtained showed a significant body weight decrease which can also be explained by the high *P/S* ratio that is equal to 2.92, 0.85 and 0.71 in *N. sativa* oil, olive oil and sunflower oil, respectively. Actually, a high *P/S* ratio constitutes an important determinant for preventing HFD induced obesity. Liao and collaborators[45] reported similar results to ours in hamsters fed a low *P/S* ratio diet, which increased body weight gain and supported fat accumulation, whereas a high *P/S* ratio diet appeared to be beneficial in preventing white adipose tissue accumulation by increasing hepatic lipolytic enzyme activities involved in *β*-oxidation.

In fact, our results showed a significant decrease in the development of hyperglycemia and hyperlipidemia (total serum cholesterol and triglyceride levels) in groups treated with *N. sativa* and olive oils compared to the HFD group. The correction of lipid profile and glycemia comes to confirm that *N. sativa* fixed oil possesses favorable metabolic effects. Therefore in the study led by Houcher *et al.,*[46] the authors mentioned that an oil seed extract of *N. sativa* cause an important decrease in glycemia. This hypoglycemiant effect can be explained by either inhibition of intestinal absorption, the stimulation of insulin secretion or sensibility of tissues to insulin action which is confirmed by the HOMA-IR score and OGIS index, or by the inhibition of gluconeogenesis enzymes which must be verified. At the same time, the presence of thymoquinone on *N. sativa* as detected by the HPLC at the concentration of 6.01 ± 0.54 mg g^{-1} of oil can be another explanation to the corrective effect on the glycemia level; this is which Fararh *et al.,*[47] proved in their study. We find out that the proportion S/M/P of NS seed oil is about 1:1.1:2.6 close enough to the proportion cited in a recent study where the authors[48] mentioned that the proportion of different fatty acids in diets played an important role in metabolism and specifically, the proportion of S/M/P at 1:1:2 improved glucose and lipids metabolism, and increase insulin sensitivity.

Grundy[41] imputed the increased cholesterol and triglyceride levels to high intakes of SFAs when compared with UFAs, which can be explained by the fact that the effect of SFAs on the intestinal absorption of dietary cholesterol is greater than UFAs action.[49] Similar results were obtained by El-Dakhakhny *et al.*,[50] who found that *N. sativa* oil administrated to rats decreased significantly the serum levels of total cholesterol, TG, LDL-C and increased HDL-C. Also, Zaoui *et al*.,[51] indicated that oral treatment with *N. sativa* oil, reduced serum cholesterol and TG and glycemia levels compared with normal rats. Moreover, Meddah *et al.,*[52] showed that chronic *N. sativa* treatment improved glucose tolerance as efficiently as metformin and reduced body weight without any toxic effect.

Many studies correlate the level of serum triglycerides have been associated with insulin resistance.[29,53] In a recent study, Yang *et al.,*[54] showed that the proportion of different fatty acids in diets plays an important role in improving lipid and glucose metabolism and increasing insulin sensitivity. In fact, one of the destinies of PUFAs in cells is incorporated into membrane phospholipids. When in membranes, PUFAs contribute to their fluidity that is an important factor in the correct hormone-receptor binding. In fact, increasing in membrane fluidity might result in an enhanced number of insulin receptors that can explain by the opposite the insulin resistance, which might be associated with a rigid membrane, which limits the number of insulin receptors and decreased affinity of insulin to its receptors .[55]

On the other hand, the difference noted between the two fat supplemented diets (diets 4 and 6) can be explained by the difference in fatty acid composition of both oils. *N. sativa* oil is rich in ω6-PUFAs, which are present in low amount in olive oil. ω6-FAs have shown a cholesterol-lowering effect. They reduce insulin-resistance as well as the risk of type 2 diabetes. The main FA in olive oil is the monounsaturated oleic acid, which has a controversial action.[56] A recent study made on Spanish adult's shows that the risk of obesity increased with olive oil consumption when not controlled for total energy intake. The authors explain this result by the failure of energy compensation for olive oil consumption but at the isocaloric level, olive oil consumption did not affect obesity risk in plausible energy intake reporters.[57]

The effect of *N. sativa* on albumin synthesis was long recognized to be used as a liver disease cure. Several studies reported that treatment with *N. sativa* seeds increased the total proteins and especially the serum albumin levels in rats.[58,59] Also, in a recent study similar results were found by Tousson *et al.*,[26] where *N. sativa* supplemented diet increased plasma total protein, albumin, ALT and AST levels in rabbits and decreased total lipid serum concentrations. The change in albumin level reflects the change in the liver function and the presence of fatty acids may have an effect on the muscle protein synthesis through a prostaglandin-dependent mechanism. It is also possible as a hypothesis that the high albumin level in NS treated group may be due to an induction of their synthesis by the detected TQ and it is important to note a proof that TQ have a specific binding site on human albumin,[60] which remains to be confirmed in rodents. Moreover, Nagi *et al.,*[61] treated with an oral supplementation of TQ in acetaminophen-induced hepato-toxicity in mice and confirmed a corrected effect of ALT in a dose-dependent manner which is therefore protecting the liver.

In a recent study, the authors find for the first time that n-3 PUFA causes alterations in several novel functional proteins involved in regulating lipid, carbohydrate and protein metabolisms, and suggesting integrated regulation of metabolic pathways. These novel proteins are potential targets to develop therapeutic strategies against metabolic disorders such as obesity.[62]

Furthermore, in order to investigate the anti-oxidant effect of NS fixed oil, we analyzed plasmatic MDA and protein carbonyl contents, plasmatic vitamins levels and CAT and SOD activities in HFD rats and supplemented rats by this oil compared to olive oil supplementation. Lower vitamins

concentrations and a higher plasmatic MDA and protein carbonyls contents were found in obese rats compared with the control group, that reflect the amount of oxidative stress that rats have been exposed to during the time of experimentation. These results confirm the fact that obesity is associated with the oxidant stress increase. Possible mechanisms contributing to the obesity-associated oxidant stress include increased oxygen consumption *via* mitochondrial respiratory chain, increased fat deposition and cell injury causing increased rates of radicals and reactive oxygen species (ROS) formation such as H_2O_2.[63] Lee H.I. *et al.,*[64] reported that increased fat deposition results mainly from consumption of a hyperlipidemic diet, and is vulnerable to oxygen metabolism that can cause lipid peroxydation and consequently malondihaldehyde (MDA-TBARS) formation. The low plasma levels of vitamins could reflect their high utilization rate, suggesting that these vitamins may be used to reduce oxidative stress in obese rats. In addition and unexpectedly, an increase in SOD and CAT activities were observed in obese rats. These results suggest an up regulation of these antioxidants enzymes in our growing young obese rats. It's could be interpreted as a positive feedback mechanism reflecting a favorable response of the organism to oxidative stress if compared to old obese rats in other studies.[65,66]

Elevated levels of oxidant markers in obese rats could result from their insulin resistance state, hypercholesterolemia and abnormal metabolism. Actuality, recent studies, have proposed that ROS such as H_2O_2 are produced transiently in response to insulin stimulation and also act as a second messenger for insulin signaling in adipocytes. In fact, a brief increase of intracellular ROS is important for the insulin signaling pathway, while excessive and long-term exposure to ROS reduces insulin sensitivity and impairs glucose and lipid metabolism. These results suggest that increased ROS production caused by fat accumulation may prevent further lipid storage, but may simultaneously cause insulin resistance.[14]

In our study, a NS and olive oils supplementation in obese rats show a corrective effect in the antioxidant defense system. These results show the potential antitoxic effect of NS seeds oil mediated by their antioxidant properties. Many studies have reported that dietary supplementation by the small molecular-weight antioxidants and the free radical scavengers such as vitamins, minerals, polyphenols, quinones and PUFAs prevent or at least attenuate the damages due to ROS in the case of an oxidative stress.[19,67] In a recent study,[68] the authors found that the protective effect of NS against petrochemical-induced oxidative stress may be due to TQ which is the most potent in terms of antioxidant capacity. In addition, Mansour M.A. *et al.,*[69] suggested that TQ may act as an antioxidant agent and prevent the membrane lipid peroxidation and antioxidant enzymes in hepatocytes and that may well sustain our results.

Conclusion

The results of this study demonstrated that *Nigella sativa* seeds oil is beneficial for attenuating complications of obesity and possibly preventing it because of its essential polyunsaturated fatty acids; linoleic and linolenic acids and other nutrients such as liposoluble vitamins like tocopherols and also minerals, essential amino acids, some polyphenols, terpenoïds and quinones, particularly thymoquinone that have shown potential medicinal properties in traditional medicine.

Thus, *N. sativa* seeds oil supplement has shown a beneficial impact by ameliorating glucose tolerance, liver enzymes activities, pancreatic function in obese rats and metabolic rate as well. So, further investigations are encouraged in this way in order to unveil the molecular mechanisms of this oil on body cells and then show its importance for human use as a supplementary source of lipids and nutraceutical rich source to correct obesity and theirs consequences or to prevent their installation also in a favorable environment like a consumption of high caloric diet.

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