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Reduction of Biochemical Marker Changes in Obese Rats by Nutraceutical Formulas

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Abstract: The idea of nutraceutical can replaced chemical to enhanced heath status. Supplementation of animal diets produced good trend to experiment beneficial foods. This research investigates the regulated effect of propolis, *Nigella sativa* and chia seeds mixture on obese rats. The biological and biochemical profiles of normal and obesitic rats were determined. Basal diet was fed to main first group. But, main second group were fed on high fat diet for 8 weeks, then diet supplemented with mixed formula with 2, 4 and 8 %, respectively for successive 4 weeks. The results revealed to mixed formula degradations percent have significant spike which improve feed intake, body weight, feed efficiency ratio, serum lipids, liver enzymes, kidney functions, antioxidants (SOD, GPx), lipid peroxidation (MDA) and leptin hormone levels. Collectively, the results indicated a beneficial role of propolis, *Nigella sativa* and chia seeds formulas supplementation for ameliorate biological and serum biochemical status in rats diet-induced obesity.

Key words: Propolis · *Nigella sativa* seeds · Chia Seeds · SOD · MDA · Leptin Hormone

INTRODUCTION

Obesity is often defined simply as a status of abnormal or excessive fat accumulation in adipose tissue, which can be extent to impaired health in some cases. Globally, obesity has reached epidemic proportions with more than one billion adults overweight at least 300 million of them clinically obese. Obesity is a major contributor to the global burden of chronic disease and disability [1]. Obesity is a complex condition often coexisting in developing countries with under nutrition, with numerous social and psychological dimensions, affecting virtually of all ages and socioeconomic communities. Excessive consumption of more energy-dense, nutrient poor foods with high levels of saturated fats and sugar, combined with reduced activity, have led to obesity rates that have risen three fold or more status all over the world. The obesity epidemic is often faster in developing countries than in the developed world. The health consequences range from increased risk death premature and child obesity, to serious chronic diseases that reduce the overall quality of life [2]. Nutraceuticals is a broad umbrella term that is used to include any product from food sources with extra health benefits in addition to basic nutritional value of foods. They can be considered non-specific biological therapies used to promote health, control symptoms and prevent malignant processes. A nutraceutical may be a naturally nutrient-rich or medicinally active food. The term "nutraceutical" combines two words "nutrient" (food component) and "pharmaceutical" (a medical drug). The abbreviation was coined in 1989 by Stephen DeFelice [3]. Their role in human nutrition is one of the most important areas of investigation. They can be classified on categories: dietary supplements, functional food, medicinal food, pharmaceuticals. A dietary supplement contains nutrients derived from food products and is concentrated in liquid, capsule and powder or pill form, which regulated by the FDA as foods [4].

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Thousands of years ago, ancient civilizations were used propolis for its medicinal properties. Assyrians put it on wounds and tumors to fight infection and help the healing process. Egyptians used it to embalm mummies. Propolis is sticky greenish-brown product made by bees as a coating to build beehives. The majority compounds are polyphenols called flavonoids as antioxidants that fight disease and damage in the body. Propolis is thought to have antibacterial, antiviral, antifungal and anti-inflammatory properties. But scientific research on propolis is limited [5]. *Nigella sativa* (black cumin) have been used as a seasoning spice and food additive in the Middle East and Mediterranean areas. Many literatures showed the biological activities of *Nigella sativa* seeds ashypolipidemic, cytotoxic, hypotensive, hepatoprotective, extensive, citing bronchodilative, anti-inflammatory, antinociceptive, antibacterial and antidiabetic effects. The active ingredients of *N. sativa* are concentrated in fixed or essential oil of seeds, which are responsible for most health benefits [6]. Chia seeds (*Salvia hispanica* L.) are an excellent ingredient for dieters because it has bioactive effects, such as reducing blood cholesterol, blood glucose, modifying insulinaemic responses, improvement of the intestine function and antioxidant activity [7]. Leptin is a hormone that produced by the body's fat cells. It is referred to satiety hormone or the starvation hormone. Leptin's primary target is excreted from the brain, particularly an area called the hypothalamus [8]. Therefore, the aim of this study used formula contents of propolis, *Nigella sativa* and chia seeds as nutraceutical to modifying biological and biochemical activities of obese rats.

MATERIALS AND METHODS

Materials: Basal diet (g/1000g of diet) was composed of 12 g of casein; corn oil (10% fat); cellulose (4% fiber); minerals mixture (4% minerals); vitamins mixture (1% vitamins) and corn starch and water supply was given *ad-libitum* daily. Mineral mixture was prepared according to Hegsted *et al.* [9]. Composition of vitamin mixture presented as (g/kg) according to Campbell [10]. Composition of high energy diet were composed of the same component of basal diet (g/1000g of diet) with replace corn oil as 8% and add 44% sweetened condensed milk [11] and tap water supply was given *ad-libitum* daily. Propolis purchased as drops (Bioflavonoids 30ml/bottle) from Eco Bee farms product number YSO-91201. All *Nigella sativa* and chia seeds were purchased from local seeds market from Cairo city. *Nigella sativa* and chia seeds were grinded in to fine powder [12]. Male albino rats (*Sprague Dawley Strain)* (weight 160±10g, aged 10 weeks) were brought from the laboratory animal house of Faculty of Science, Cairo University.

Experimental Protocol: Thirty male albino rats acclimatized for one week to laboratory condition, 22 - 25°C and humidity 50 - 55 % with a 12 h light/ dark cycle. Rats kept in metal cage as single rat and classified into two main groups. Fist main group: kept as normal rats (6 rats per group) fed on basal diet. Second main group: rats received high energy diet (24 rats) for successive eight weeks to induce obesity. After this time, rats were divided to subgroups 6 rats per each subgroup. Subgroup (1): fed on basal diet only as obestic rats. Subgroup (2): rats fed on basal diet supplemented with mixed formula (propolis, *Nigella sativa* and chia seeds as 1:1/2:1/2) 20 g/kg diet. Subgroup (3): rats fed on basal diet supplemented with mixed formula 40 g/kg diet. Subgroup (4): rats fed on basal diet supplemented with mixed formula 80 g/kg diet for successive four weeks. Ethics committee's protocols for research experimental animals' care by Faculty of Science Cairo university was applied [13].

Biological Evaluation: The quantities of diet which were consumed (feed intake) and wasted were assessed every day. Body weight was recorded twice/week. On the last day of the experimental protocol, rats were fasted overnight and allowed free access to water. Feed intake, body weight gain (BWG %) and feed efficiency ratio (FER) were calculated [14]. Body weight gain and feed efficiency ratio were calculated using the following equations:

Feed intake = Initial Weight of diet (g) - Weight of diet lost (g).

Weight Gain (WG) (g) = Final Weight (g) – Initial Weight (g).

Feed efficiency ratio =Gain in body weight (g)/Feed intake (g).

At the end of the experimental period heart, kidneys and liver were removed carefully from each rat after an abdominal laparotomy, washed with saline solution, dried with filter paper and weighted [15]. Relative organ weight calculated by the following formula:

Relative organ weight (ROW) % = Organ Weight / Final Body Weight× 100.

Blood Samples and Biochemical Analysis: After the experimental period, all rats were fasted 12 hours then, anaesthetized by diethyl ether. Blood was collected by orbital sinus/plexus bleeding. Blood serum were separated from collected samples then centrifuged at 3000 revolutions/minute for 10 minutes. Serum was carefully separated by using a Pasteur pipette into dry clean Wasserman tubes. Serum was used freshly for determination of biochemical analysis. Kits used to determine biochemical analysis produced by Egyptian American Company for laboratory service from Alkan Company. Leptin hormone was measured using a commercial kit (Linco, St Charles, MO, USA). Serum total cholesterol (TC) and triglycerides (TG) were measured by using spectrophotometric method [16-19]. HDL-cholesterol was measured using a spectrophotometric method [20] and LDL-cholesterol were calculated by Friedwald formula, VLDL = TG/5, LDL = TC – (VLDL+HDL).

All Concentrations Characterized by Units of mg/dL [21]. Atherogenic Index Was Calculated as Follows: Cardiac Risk Ratio (CRR) = TC/HDL-C; LDL-C to HDL-C ratio = LDL/HDL; Atherogenic Coefficient (AC) = (TC– HDLC)/HDL-C; Atherogenic Index (AI) = log (TG/HDL-C) [22]. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities were determined according to methods of Reitman and Frankel [23] and alkaline phosphatase enzymes (ALP) by King [24]. Serum urea was performed measured according to Patton and Crouch [25], serum creatinine was determined according to the method described by Kroll *et al.* [26]. The previous tests were measured by using auto analyzer (UV-1800VIS Spectrophotometer, Shanghai, China (Mainland). Enzymatic antioxidant activity as superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured by high performance liquid chromatography HPLC according to Leroy *et al.* [27], the mobile phase was 90% (0.5 mmoL/L) n-decyl sulfate sodium salt, disodium EDTA (0.1 mmol/L), phosphate buffer (10 mmol/L) at pH 2.5, and acetonitrile 10%. Flow rates were 1.2 and 0.3 mL/min of mobile phase and post column derivatizing reagent and separations were at 40°C. Excitation and emission wavelengths were 340 and 440 nm. Lipid peroxidation products were assayed by measuring malondialdehyde (MDA) according to Ohkawa *et al.* [28].The quantitative determination of leptin in human serum by an enzyme immunoassay method [29].

Statistical Analysis: Results are expressed as mean values with their standard deviation (S.D.) of each group. Data were evaluated for statistically signi?cant differences between groups were obtained by Duncan's multiple range tests. Differences were considered significant at $P < 0.05$by using (SPSS version 20.0; SPSS, Inc.) [30].

RESULTS

Biological Evaluation: As shown in Table 1, high energy diet caused significant decreases ($p = 0.05$) of FI in obesitic rats group as compared to normal rats group (21.58±.097and 21.90±.167 g/day). On the other hand, BWG% and FER ratio showed significant increases ($p \leq 0.05$) in obestic rats (9.66±1.72% and 0.64±0.99). Supplementation rats diet with mixed formula (propolis, *Nigella sativa* and chia seeds) appeared gradual improvement effects on FI, BWG % and (FER) of obese rats. From these results mixed formula especially high percent recoded the best values of biological evaluation compared to obesitic group.

In regarded to relative organs weight %, results recorded that high energy diet caused significant increases ($p \leq 0.05$) in heart and liver weights % (0.53±0.06 and 3.83 ±0.32%) as compared to normal rats (0.42 ±0.23 and 3.0±0.24%). In contrast, kidneys weight % recorded non-significant increases ($p = 0.05$) of obesitic group as compared to normal rats (1.54±0.10 and 1.22 ±0.23%). Supplemented diet with mix formula revealed to significant decreases of heart in high formula percent (0.44±0.07%) and significant decreases in all formulas percent of liver weights %.But, kidneys weight% induced non-significant decreases as compared to obesitic group (Table 2)

Biochemical Analysis

Lipid Profile: High energy diet showed significant increases ($p = 0.05$) of serum total cholesterol (TC), triglyceride (TG), LDL-c and VLDL-c (268±5.5, 210±4.3, 197±3.5 and 42±1.3 mg/dL, respectively) as compared to normal rats group (128±3.1, 100±2.4, 63 ±2.1 and 20±1.4 mg/dL, respectively). In contrast, the level of HDL-c illustrated significant decreases in obesitic rats group as compared to normal rats group (29±2.5 and 45±2.1 mg/dL).Supplemented diets with mixed formulas percent recorded significant decreased in TC, TG, LDL-c and VLDL-c meanwhile, results showed significant increase in HDL-c as compared to obesitic rats group. The best results were found in mixed formula 8%, which reduced the adverse effects of high energy diet nearly too normal rats of lipid profile (Table 3).

Table 1: Nutraceutical effect of formula percent's on biological estimations of obesitic rats

Groups	FI (g/day)	BWG %	FER
Normal rats	21.90±.167[a]	7.11±0.61[b]	0.59±0.002[ab]
Obesitic rats	21.58±.097[c]	9.66±1.72[a]	0.64±0.99[a]
Mixed formula 2%	21.75±.089[b]	7.29±0.81[b]	0.62±0.05[ab]
Mixed formula 4%	21.64±.129[c]	6.84±1.94[b]	0.59±0.16[ab]
Mixed formula 8%	21.71±.041[b]	4.91±1.36[c]	0.39±0.08[c]

Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

Table 2: Nutraceutical effect of formula percent's on relative organs weight % of obesitic rats

Groups	Heart	Kidney	Liver
Normal rats	0.42 ±0.23[b]	1.22 ±0.23[a]	3.0±0.24[b]
Obesitic rats	0.53±0.06[a]	1.54±0.10[ab]	3.83 ±0.32[a]
Mixed formula 2%	0.50±0.10[a]	1.39±0.06[b]	3.42±0.34[c]
Mixed formula 4%	0.47±0.04[ab]	1.26±0.04[b]	3.21±0.26[c]
Mixed formula 8%	0.44±0.07[b]	1.13±0.07[b]	2.84±0.23[d]

Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

About atherogenic calculations cardiac risk ratio (CRR), LDL-C to HDL-C ratio, atherogenic coefficient (AC) and atherogenic index (AI) data showed significant increases ($p \leq 0.05$) of obesitic rats group (9.24±0.06 6.79±0.04 8.24±1.06 and 0.86±0.03 mg/dL, respectively) as compared to normal rats group (2.84±0.09, 1.40±0.03, 1.84±0.04 and 0.34±0.04 mg/dL, respectively). All supplemented diets with mixed formula recorded significant decreases of atherogenic calculation results as compared to obesitic rats group. High percent of mixed formula found as good result group, which closed to normal rats group data (Table 4).

Liver Enzymes: As shown in Table 5, serum liver enzymes (aminotransferases ALT, AST and alkaline phosphatase ALP) illustrated significant increases ($p \leq 0.05$) in obesitic rats group (48.3±2.2, 170.8±2.6 and 525.65±2.58 U/L) as compared to normal rats. The process of supplementation diet with mixed formula groups revealed to significant decreases of liver enzymes, especially in high percent of mixed formula group (23.2±2.5, 123.5±2.1 and 370.36±3.24 U/L, respectively) which closed to normal rats' group results.

Kidney Functions: Kidney functions as serum urea nitrogen and creatinine results revealed to significant increases ($p \leq 0.05$) of obesitic rats (39±3.5 and 0.99±0.13 mg/dL) as compared to normal rats group (28 ±1.8 and 0.75±0.15 mg/dL). All treated groups with mixed formula recorded significant decreases of serum urea and creatinine especially in mixed formula 8% group as compared to obesitic rats group (29.5±1.4 and 0.78±0.21 mg/dL), which closed to normal data results (Table 6).

Antioxidants, Lipid Peroxidation and Leptin Hormone: Antioxidant enzymes were expressed as serum SOD and GPx of rat groups. Data in Table 7 demonstrated significant decreases of SOD and GPx of obesitic rats group (1.60±0.47 U/ml and 0.34±0.04 mmol/L) as compared to normal rats (4.95±0.73 U/ml and 1.42±0.32 mmol/L). Mixed formula percent groups showed significant increases ($p \leq 0.05$) as compared to obesitic rats group. Lipid peroxidation as serum MDA showed significant increases in obesitic group as compared to normal rats

Table 3: Nutraceutical effect of formula percent's on serum lipid profile (mg/dL) obesitic rats

Groups	TC	TG	HDL-c	LDL-c	VLDL-c
Normal rats	128±3.1 [c]	100±2.4 [c]	45±2.1 [c]	63 ±2.1 [d]	20±1.4 [b]
Obesitic rats	268±5.5 [a]	210±4.3 [a]	29±2.5 [a]	197±3.5 [a]	42±1.3 [a]
Mixed formula 2%	166±2.3 [b]	168±3.5 [b]	35±2.3 [b]	97.4±2.3 [b]	33.6±1.3 [b]
Mixed formula 4%	150±2.4 [b]	128±3.1 [b]	39±2.4 [b]	85.4±2.4 [c]	25.6±1.1 [b]
Mixed formula 8%	136±3.2 [c]	114±3.4 [c]	44±3.2 [c]	69.2±2. 2 [cd]	22.8±1.2 [b]

Mean± SD values, means in the column with different letters are significantly different ($p = 0.05$).

Table 4: Nutraceutical effect of formula percent's on atherogenic calculations (mg/dL) of obesitic rats

Groups	CRR	LDL/HDL	AC	AI
Normal rats	2.84±0.09 [c]	1.40±0.03 [c]	1.84±0.04 [c]	0.34±0.04 [c]
Obesitic rats	9.24±0.06 [a]	6.79±0.04 [a]	8.24±1.06[a]	0.86±0.03 [a]
Mixed formula 2%	4.74±0.04 [b]	2.78±0.05 [b]	3.74±0.02 [b]	0.68±0.02 [b]
Mixed formula 4%	3.84±0.05 [b]	2.18±0.03 [b]	2.84±0.03 [b]	0.51±0.01 [b]
Mixed formula 8%	3.09±0.04[bc]	1.57±0.02 [c]	2.09±0.04 [c]	0.41±0.03 [c]

Mean± SD values, means in the column with different letters are significantly different ($p = 0.05$).

Table 5: Nutraceutical effect of formula percent's on ALT, AST and ALP enzymes of obesitic rats

Groups	ALT U/L	AST U/L	ALP U/L
Normal rats	20.8±1.4[d]	129. 9±2.3[c]	330.42±1.32 [cd]
Obesitic rats	48.3±2.2[a]	170.8±2.6[a]	525.65±2.58 [a]
Mixed formula 2%	38.2±2.6[b]	148.6±2.3[b]	463.74±2.87 [b]
Mixed formula 4%	29.6±2.4[c]	134.9±2.2[bc]	420.45±3.16 [b]
Mixed formula 8%	23.2±2.5[cd]	123.5±2.1[c]	370.36±3.24 [c]

Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

Table 6: Nutraceutical effect of formula percent's on urea and creatinine of obesitic rats

Groups	Urea mg/dL	Creatinine mg/dL
Normal rats	28 ±1.8[c]	0.75±0.15[bc]
Obesitic rats	39±3.5[a]	0.99±0.13[a]
Mixed formula 2%	34±1.3[b]	0.90±0.19[b]
Mixed formula 4%	31.9±2.3[b]	0.83±0.15[b]
Mixed formula 8%	29.5±1.4[bc]	0.78±0.21[b]

Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

Table 7: Nutraceutical effect of formula percent's on enzymatic antioxidants, lipid peroxidation and leptin hormone of obesitic rats

Groups	SOD U/mL	GPx mmol/L	MDA nmol/L	Leptin ng/mL
Normal rats	4.95±0.73[ab]	1.42±0.32[b]	2.40±0.3[c]	6.60±1.7[b]
Obesitic rats	1.60±0.47[d]	0.34±0.04[c]	5.75±0.2[a]	9.38±1.4[a]
Mixed formula 2%	2.50±0.43[c]	1.02±0.31[b]	5.01±0.1[b]	6.82±1.3[b]
Mixed formula 4%	3.75±0.45[b]	1.23±0.42[b]	4.29±0.3[b]	6.23±1. 5[bc]
Mixed formula 8%	5.34±0.53[a]	1.54±0.52 [a]	3.65±0.2[b]	5.62±1.3[c]

Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

(5.75±0.2 and 2.40±0.3 n mol/L). Formula supplemented diet groups recorded significant decreases values as compared to obesitic group, the best results found in mixed formula 8% group followed 4% and 2% groups. Leptin hormone recorded significant increases in obesitic group as compared to normal rats (9.38±1.4 and 6.60±1.7 ng/mL). All mixed formulas reduced serum leptin levels as compared to obesitic rats, especially in high formula percent group (5.62±1.3 ng/mL).

DISCUSSION

In this study, a high-energy diet (HED) induced significant increases in FI, BWG% and FER. These results are agreement with, Levin and Dunn who confirmed that, when *Sprague-Dawley* rats exposed to a high-energy diet HED, they become obese [11]. Diet composition can interfere in development of obesity due to specific roles of some fatty acids, which can alter both fat oxidation and deposition rates, resulting in changes in body weight and composition. It is generally accepted that factors influence food intake and body fat accumulation can be conceptualized as stimulate appetite or eating per se and stimulate fullness or satiation [31]. Propolis has beneficial effects, which make it a potential preventive and therapeutic agent. Polyphenols and flavonoids are active components of propolis that have been identified [32]. *Nigella Sativa* decrement body weight by suppression of appetite, which associated to the neural circuits that regulate catecholaminergic, serotonergic and peptidergic system or *via* circulating leptin hormone signaling the brains satiety centre to produce hypophagic effects in animals [33]. Ingestion of chia seed may induce the sense of fullness, which may continue until the following meal. Fiber and an abundance of omega-3 fatty acids in chia seed may play a role in appetite suppression [34]. About blood analysis, Puska *et al.* found that, obesity lead to adverse metabolic effects on biochemical analysis markers and body weight [2].High energy or fat diets in general are associated with hyperphagia, but the type of dietary fat seems to be more important. Polyunsaturated fats, omega-3 and omega-6 seem to increment energy expenditure and decrement energy intake by specific mechanisms involving hormone-sensitive lipase. Also, obesity is also associated with disturbances of mitochondrial function [35]. Similar results were also obtained by Rokling-Andersen *et al.* [36] who demonstrated that a diet rich in omega-3 such as seeds reduced cholesterol triglycerides and phospholipids. While, elevated HDL-c levels have been conducted with a reduction in cardiovascular risk. The monounsaturated and polyunsaturated fat diets have a better result of lipid profile. These findings explain the

mechanism of obesity to induced significant alteration in lipid profile (TC, TG, LDL-c and VLDL-c), its calculations (CRR, LDL/HDL, AC and AI ratios) and explain the importance of polyunsaturated fats of seeds in this study formula. The ethanolic extract of propolis enhanced reverse cholesterol transport and stimulated plasma HDL-c level and hepatic enzymes expression [37]. *Nigella sativa* seeds in diet had a desirable effect on the lipid profile by lowering TG, TC, LDL-c and improvement HDL-c as compared to controls [36]. *Nigella sativa* have reported that hypotriglyceridaemic effects are due to its cholerectic functions activity by reduced cholesterol synthesis in hepatocytes or by decremented fractional reabsorption from small intestine [38]. Reduction in LDL-c consistent could be due to increased production of LDL receptors [39]. Rats ingesting chia seeds showed a significant drop in TG and LDL-c concentrations and a spike in HDL and polyunsaturated fatty acids [40]. Turkish propolis, which is rich in flavonoids prevented alcohol-induced acute liver damage and lipid accumulation and induced beneficial changes of lipid profile [41]. *Nigella sativa* was described to enhance liver cell sensitivity [42]. Rats subjected to a diet heavy in fat and carbohydrates over an eight-week period supplemented diet with chia seeds improved insulin sensitivity and glucose tolerance and a reduction in visceral fat, fatty liver and heart and liver inflammation [43]. Also, chia seeds showed that it was able to prevent the development of hypertension, liver steatosis, hypertriglyceridemia and hypercholesterolemia. Normal triacylglycerol secretion and triacylglycerol clearance were accompanied by an improvement of de novo hepatic lipogenic and enzymatic activities, associated with an accretion of n-3 polyunsaturated fatty acids in liver homogenate [44]. Reactive oxygen species (ROS) are continuously produced through normal physiologic reactions and removed by antioxidant defense mechanism. In pathological conditions, ROS are produced in high levels and result in lipid peroxidation and oxidative damage [45]. Obesity is associated with endothelial dysfunction due to ROS-mediated inactivation of nitric oxide NO induced in obesity. Increased generation of reactive oxygen species produced NO inactivation [46, 47]. These reactions can illustrate our results of significant decreases of SOD, GPx and increases of MDA as results of HED of rats. Nutraceuticals means, Nutritive + Pharmaceutical: A food stuff (a fortified food or dietary supplement) that have health benefits. So, the idea of this study combined propolis with *Nigella sativa* and chia seeds. Mixed formula showed effective components of seeds and propolis in controlling of obesity induced by HED, our findings agreements with Rubin *et al.* [48] who using nutraceutical such as conjugated linoleic acid and fiber, which possess potential antiobese properties. The balance nutrient seems to involve a rigorous control to adjust intake towards oxidation. An increase in carbohydrate and protein consumption is accompanied to increased oxidation rates. On the other hand, the balance between fat consumption and oxidation rates is not tightly regulated [49] and depends on the type of fatty acids [50]. Schettler *et al.* [51] suggested that reduced antioxidant production was due to increased oxygen metabolities induced a decrease in the activity of antioxidant defense system. The propolis flavonoids are powerful antioxidants, which can be scavenging free radicals and hence protecting the cell membrane against lipid peroxidation [52]. Also, flavonoid can be scavenge free radicals, such as superoxide [53], protecting serum blood lipids from oxidation [54]. *Nigella sativa* supplement decremented the elevated MDA and also incremented the reduced SOD antioxidant enzyme activities [55]. Nutraceutical fraction of chia seed had higher percent's of antioxidant substances as omega-3 fatty acids in olein fraction were had the free radical scavenging activity greater than many potentially strong natural antioxidant sources. Reyes-Caudillo *et al.*[56] reported that chia seed with a wide range of antioxidant compound can be described as a great source of antioxidants. Tepe *et al.* [57] reported that phenolics of chia seed can play an important action to inhibit the lipid peroxidation phenomenon. The present study results are confirmed that propolis, *Nigella sativa* and chia seed powder may have antioxidant properties that will be favorable for therapeutic purposes. Leptin have a many roles in growth factor in cell types also a mediator of energy expenditure, interact with other hormonal mediators and regulators of energy status and metabolism [58]. These facts can detective the reason of significant spike in serum leptin in HED. Kohei *et al*. [59] found that propolis treatment indeed clearly increment of leptin mRNA production in visceral adipose tissues. Moreover, propolis extract directly elevated of leptin expression in differentiated adipocytes [60]. Parhizkar *et al.* [61] suggested that treatment with *Nigella sativa* extracts have a therapeutic and protective effect by modifying weight gain, improving lipid profile and blood glucose as well as hormonal level. Chai seeds with dietary supplement induced significant improvement in body mass, waist perimeter, body composition, blood sugar, insulin, lipid profile, leptin, adiponectin and C-reactive protein CRP [62]. The results

of the present study indicate that the preventive effects of propolis, *Nigella sativa* and chia seeds may be due to inhibition of lipid peroxidation as a result of its antioxidant nature.

CONCLUSIONS

High-energy diets could lead to changes in biological and biochemical analysis. These alterations seem to be very important in symptoms of obesity. Using nutraceutical formula is important to consider the positive effects of internal mechanisms in metabolism. Propolis, *Nigella sativa* and chia seeds consumption are primordial importance in the treatment of obesity. High concentration of nutraceutical formula represents the link between bioactive components leading to obesity reduction.

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