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Article

Study the consequences of nanoemulsion canola oil in overweight Iraqi volunteers on various biochemical indicators in Iraq.

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Abstract

Obesity is a significant problem in human health and increases the chances of various diseases such as type 2 diabetes, particularly heart disease, systemic hypertension, hyperlipidemia, cardiovascular and certain types of cancer, and arthritis. Obesity is a significant health problem in human beings. The overweight, overweight individuals of various ages (50-65y) and the BMI (kg/m2) were >25 visiting Al-Mustansiriyah University/Baghdad National Diabetes Centre. The research period was between October 2018 and June 2019. This was a randomized, controlled, uni-blind test with a parallel design (1 control and 2 therapy groups) comparing the effects on 75 overweight individuals and obese during a 12-week intervention period of standard Canola oil and nanoemulsion canola oil. In addition to the traditional Canola oil group (CCO, n=25) and the canola nanoemulsion groups (NCO, n=25) who used the customary dietary ingredients, the control group participants (CO, n=25). Confirmation of fatty acids showed that oleic acid affected the principal fatty acid by a mean percentage of 61.6±5.78, followed by linoleic and alpha-linoleic, respectively, representing 21.7±3.1 and 9.6±0.87. Nanoemulsion canola oil emulsified with a sonic effect by means of the tween 80 and a milipore 0,22 µm of tiny nonionic surfactant molecule. There has been no change in the body composition indices (body weight, fatty body, total slurry tissue and bone mineral density). Fasting glucose reduction is seen following nanoemulsion canola oil ingestion. Experimental findings showed no significant fluid profile alterations between and between treatments. However, in both canola oil groups, LDL-cholesterol reduced compared to sunflower oil while participant intake HDL-cholesterol rose. On the other hand, triglyceride in the canola oil group rose in the three groups with a similar total cholesterol concentration. The leptin level in the nanoemulsion canola oil group was significantly less than in the control group, but the leptin exclusion was insignificant in classical and canola oil groups. The leptin level was significantly reduced.

Keywords: Chronic conditions; nano-production; diet of feed; blood pressures.

Introduction

Canola oil is the world's third-largest vegetable oil, behind palm and soybean oil. The plant has bright yellow flowers that belong to the Brassicaceae family and Brassica napus spp and was previously known as rapeseed plant [1]. Canadian scientists improved the quality of plants through traditional plant breeding to reduce the harmful erucic acid and glucosinolates [2]. They registered the new name of canola to be commercially consumable cultivars.

Since 1985, the US Food and Drug Administration has specified canola oil as "Generally recognized as Safe" as a dietary oil [3]. Dietary containing fat serves as an energy source, is composed of essential fatty acids and carries fat-soluble vitamins. Also, fat contributes to the palatability of most food and the feeling of satiety. However, the amount and type of fat were implicated in developing cardiovascular disease, cancer, hypertension and obesity. As a result, Canada tends to shift significantly away from fats of animal origin to vegetable fats, especially canola oil, which accounts for 75% of all vegetable oils produced in Canada. Canola oil is composed of a shallow level of saturated fatty acids less than 7%, a high level of monounsaturated fatty acids and an intermediate level of polyunsaturated fatty acids, including 61% oleic acid, 21% linoleic acid and 11% alpha-linolenic acid [4, 5]. Also, omega6 and omega3 exist in the oil.

The benefit of reducing saturated fatty acid is accomplished by decreased cholesterol levels and low-density lipoprotein (LDL). The canola oil Diet contained ≤ 300 mg of higher plant sterols and about 700-1200ppm of tocopherol than the olive oil diet. It included a minimum of about 2 percent of the total fatty acids [6], all indicating they are cardio-protective substances.

Cholesterol intake decreased by ≈10%, while olive oil cholesterol and bile acid release increased by 8.5-50%. Serum cholesterol was reduced by more than 6.5 %, but cholesterol (lathosterol) and plant sterols were still used for the serum check [7]. Food industries introduced nanoemulsions comprising two immiscible liquids containing physical stabile droplets with very small sizes ranging from 200 nm [8, 9]. Nano-sized droplets can flow easily and are optically transparent with unique texture/ rheology properties [10, 11].

One of the novel processing tools to modify the functional properties of lipids is the ultrasonic wave, which has advantages over other alternative nanotechnologies because it can be applied to concentrated and optically opaque emulsions without the need for extra-sample preparation. Iraq has the highest sunflower and Palm oil [intake 12, 13]. However, attempts were made to introduce canola oil as a food ingredient in the Iraqi population to reduce fat-linked problems such as obesity, bone disorder and cardiovascular disease. So, this research aimed to evaluate the efficiency of canola oil and nano-canola in reducing obesity, cholesterol, low-density cholesterol and hormones like insulin and leptin.

Material and Methods

Preparation of Canola oil from Brassica napus seeds

Canola oil was extracted from Brassica napus seeds cultivated in semi-arid soil in the Agricultural Research Department-AL-Zafarania/ Baghdad-Iraq in 2017-2018, applying traditional fertilizer of N/P/K at recommended formula [14]. Seeds were cleaned to remove unwanted material and undeveloped seeds, crushed using a mortar and heated to 90C for 30 min using a laboratory electric oven. Canola oil was extracted using hexane as a solvent, which was then removed in a rotary evaporated at 30C [15]. The essential fatty acids constituents were analyzed by gas chromatography-mass spectrometer GC-MS following [16].

Nanoemulsion preparation of Canola oil extract:

Oil in water nanoemulsion was formulated using canola oil in water at a concentration of 8% and nonionic surfactant tween 80 to final formula 8:18:74 of oil: tween 80: water, the mixture was stirred at magnetic stirrer at 400 rpm. Input power was gained by a 13 mm sonication probe (manufactured by Ultrasonic Japan). Soni- probe was dipped in the mixture, equipped to give 20 kHz with utmost power with output 750 W. Oil-water emulsion was passed through Millipore filter 0.22 µm to achieve cold sterilization and obtain nanoemulsion following (JNN).

Experimental design

The current study is a dose-response randomized, controlled, single-blind trial with a parallel design. Overweight and obese 75 participants of different ages (50- 65 years) and had a BMI (in kg/m2) >25 visited The National Diabetes Center, Al-Mustansiriyah University / Baghdad. The period of study was from October 2018 to June 2019. The participants were divided into three groups. Each group had 25 Participants and were examined for 12 weeks. The control group participants consumed sunflower oil in their food diet (CO, n= 25), and the second group was designed to consume conventional Canola oil (CCO, n= 25). In contrast, the third group was dieted with the nanoemulsion of canola oil (NCO, n= 25). Participant interventions and codes blinded researchers who assessed study outcomes. Participants of groups 2 and 3 needed to be instructed on how and when to consume the canola and nanoemulsion canola oil. They were advised to retain their normal lifestyle and diet throughout the study. Those in the control group were advised to continue their regular routine and avoid consuming any canola oil during the study period. Participants were given a calendar to self-report their oil consumption for compliance analysis.

Measurement of Body Mass Index

Body weight was measured without shoes and light clothing and recorded in Kg. Body height was measured without a shoe and recorded in the meter. Anthropometric measures such as body weight and height were recorded using a stadiometer (Wedderburn), and the waist-to-hip ratio was assessed using a measuring tape according to WHO criteria [17]. Depending on these two anthropometric parameters, a mathematical formula was designed by Quetelet in the 1830s.

$$\text{BMI} = \frac{weight\ (kg)}{height\ (m^2)} \quad (2)$$

Body composition measurements, FM, LM, and fat percentage were analyzed using the Hologic QDR Discovery A Bone densitometry (dual-energy X-ray absorptiometry [DXA]). Bone mineral density (BMD) was measured at the FN, LS (L1–L4), trochanter, Ward's triangle, and total hip. The DXA machine was calibrated every morning for all the measurements and at the end of each day. The in vivo reproducibility of the coefficient of variation ranged between 0.34% and 0.70% for all measured sites. The reported BMD values were calculated as means of four measured values from L1 to L4. Apex System Software version 4.5.3 was used for analyzing the DXA scans. According to the WHO, osteoporosis was defined as a T-score ≤2.5 and osteopenia as a T-score between −1.0 and −2.5.

Fasting Glucose estimation, Lipids profile and Hormones

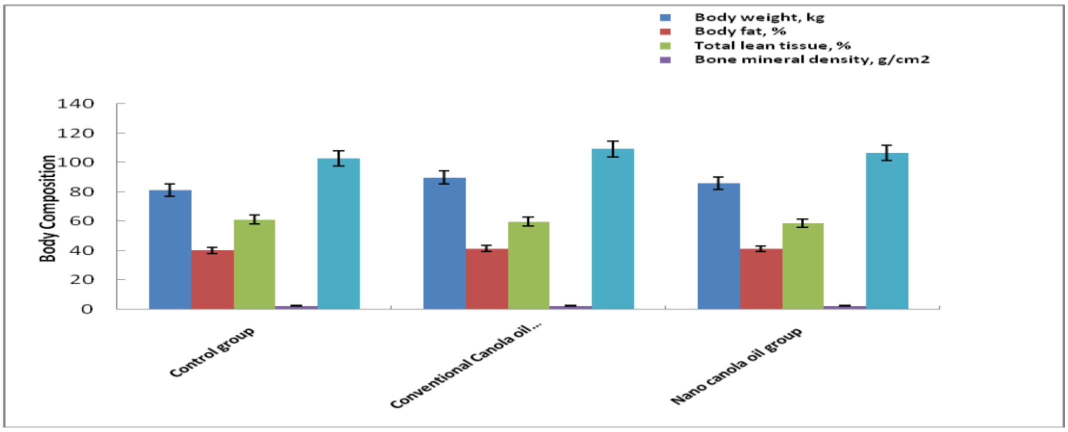
Blood samples were collected from fasting participants in a 10mL serum separation tube. After 30 min of incubation at room temperature, blood was centrifuged at 1300 × g for 10 min, and the separated serum was stored at −80°C until analysis. Fasting Glucose concentrations, Lipid profile (total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TGs)) and the waist-hip ratio (WHI) as the cardiovascular risk factors were measured by using an enzymatic assay in a chemistry analyzer (Indiko; Thermo Scientific) as per the manufacturer's protocol. Hormones were analyzed from fasting serum: assay of insulin, leptin, soluble leptin receptor and Adiponectin. The statistical analysis was done by using the Student's unpaired test.

Results

Fatty acid analysis of canola oil is presented in (Table 1); the findings of the work have shown the occurrence of different fatty acids in canola oil under conventional cultivation seeds in Baghdad. Confirmation of fatty acids indicated that oleic acid compromised the primary fatty acid, with a mean percentage reaching 61.6±5.78 followed by Linoleic and alpha-linolenic with means 21.7±3.1 and 9.6±0.87, respectively. The high oleic acid content in canola oil has better oxidative stability in deep frying applications and extended shelf life criteria, making it suitable for food consumption and application. Also, less percentage was estimated for each of the myristic (C14:0) 0.1±0.012, Palmitic (16:0) 3.6±0.024 stearic 1.5±0.11, Arachidic (20:0) 0.6±0.038, Behenic (22:0) 0.3±0.0028, Lignoceric (24:0) 0.2±0.012, Palmitoleic (16:1) 0.2±0.010, Gadoleic (20:1) 1.4±0.13, Erucic (22:1) 0.2±0.014, low total Saturated fatty acid 6.3±5.4, Total MUFA was 62.4±7.1 and Total PUFA 31.3±4.1.

Fatty acid	Conventional Canola oil; Mean± Standard Deviation
Myristic (14:0)	0.1±0.012
Palmitic (16:0)	3.6±0.024
Stearic	1.5±0.11
Arachidic (20:0)	0.6±0.038
Behenic (22:0)	0.3±0.0028
Lignoceric (24:0)	0.2±0.012
Palmitoleic (16:1)	0.2±0.010
Oleic (18:1)	61.6±5.78
Gadoleic (20:1)	1.4±0.13
Erucic (22:1)	0.2±0.014
Linoleic (18:2n-6)	21.7±3.1
Alpha Linolenic (18:3n3)	9.6±0.87
Total Saturated	6.3±5.4
Total MUFA	62.4±7.1
Total PUFA	31.3±4.1

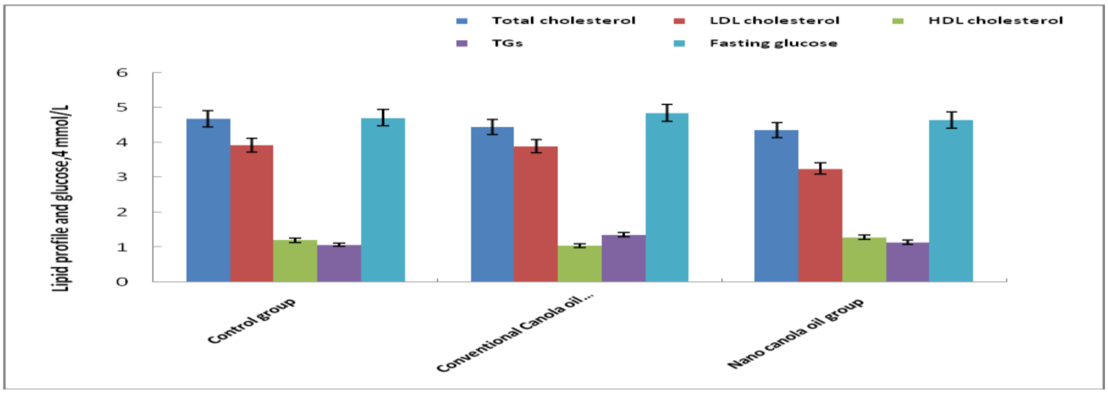
Table 1. The Mean of Fatty acid composition of conventional cultivar of Canola oil cultivated at semi-arid soil in Baghdad.



Parameters	Control group	Conventional Canola oil group	Nano canola oil group
Body weight, kg	81.09b	92.64a	85.72ab
Body fat, %	41.47a	41.97a	42.18a
Total lean tissue, %	61.51a	59.76a	60.68a
Bone mineral density, g/cm2	2.41a	2.72a	2.63a
Waist circumference, cm	103.12a	108.84a	106.63a

Figure 1. The differences in anthropometric parameters of obese participants after twelve weeks of oil consumption with significant levels based on the fishers test (P<0.05) Control group sunflower oil.

Canola oil extracted in the present study changed to nanoemulsion oil by sonication with the aid of small molecule nonionic surfactant the tween 80. This is vital in minimizing oil droplet diameter due to their rapid absorption onto the surface. Also, the Sonicator probe generated disrupted physical forces that reduced the droplet diameter from coarse emulsion to nanoemulsion, which passed through a Millipore filter 0.22 µm, indicating a low diameter drop ranging to 200 nm. Body composition indicators (body weight, body fat, total lean tissue, and bone mineral density) did not change among the three treatments. Figure 1 refers to the high food quality of canola oil and nanoemulsion canola oil as a substitute for traditional Iraqi sunflower oil in daily diet consumption.

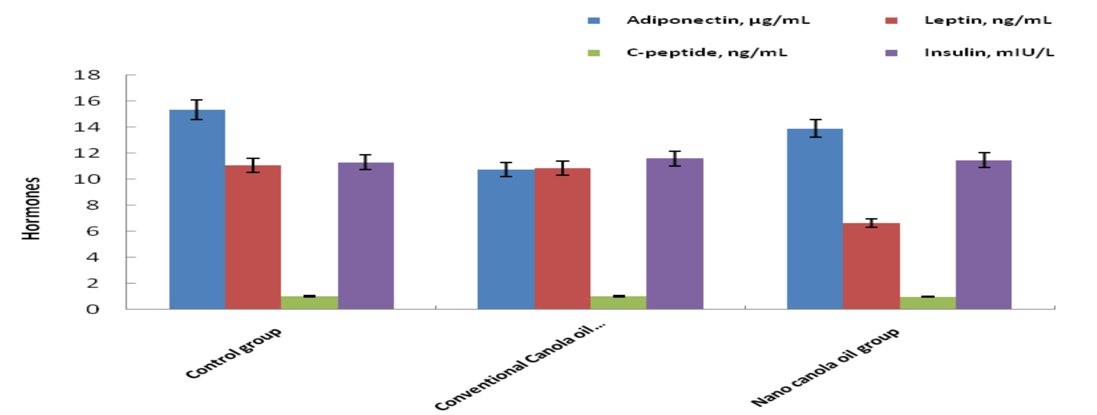


Parameters	Control group	Canola oil	Nanoemulsion canola oil
Total cholesterol	4.76a	4.55a	4.46a
LDL cholesterol	4.24a	3.60a	3.50a
HDL cholesterol	1.24a	3.94a	1.25a
TGs	1.06a	1.76a	1.55a
Fasting glucose	5.17a	4.86a	4.65a

Figure 2. Fasting glucose and Lipid profile after twelve weeks of oil consumption with significant levels based on fishers test (P<0.05) Control group sunflower oil.

Correlation relationship between Biochemical Parameters

The correlation relationship of chemical analysis for the three treatments presented in Table (2) indicates a moderate percentage of total saturated fat, which could be sufficient for daily energy intake to avoid an imbalance in total energy. Reduction of any macronutrient in the daily consumption diet, such as saturated fatty acids, accompanied by consumption of an excess amount of carbohydrate to retain energy balance. However, a high-sugar diet may increase the risk of obesity and the second type of diabetes. Statistical analysis revealed that sunflower and traditional canola oil had the same relatedness in response to the parameters under study. Anthropometric parameters (BW, BF, TL, BN and WF), total cholesterol and HDL-cholesterol, Adiponectin and Insulin all had high relatedness.



Parameter	Control group	Canola oil group	nanoemulsion Canola oil group
Adiponectin, μg/mL	15.21a	10.52a	13.30a
Leptin, ng/mL	11.96a	10.89ab	6.48b
C-peptide, ng/mL	1.02a	1.35a	1.02a
Insulin, mIU/L	12.20a	11.50a	11.52a

Figure 3. Hormones profile after twelve weeks of oils consumption with significant levels based on fishers test ($P<0.05$) Control group sunflower oil.

While LDH-cholesterol had moderate low relatedness on bone parameters, TC, HDL-cholesterol, Adiponectin, and Insulin. This led to elevated fasting glucose, C- peptide and leptin and, as expected, linked to severe diseases such as diabetes and cardiovascular disease. This assumption was more apparent with sunflower oil. On the other hand, the behavior of HDL-cholesterol indicated its positive action on bone parameters, total cholesterol, Adiponectin and insulin; this parameter was suitable to conserve bone health, a diabetic and cardioprotective agent. Triglyceride played a vital role. They had no relatedness on all bone parameters, total cholesterol, HDL-cholesterol, Adiponectin, or insulin. Body weight showed the highest negative impact on C-peptide and that expected for obese persons. Many differences were observed in the analysis of the data of nanoemulsion canola oil; total leam (TL) and total cholesterol were negatively affected by anthropometric parameters BW, BF, BN, WF and with lipid profile (LDL-cholesterol, HDL- -cholesterol, TGs and FG) and with C- peptide as well as hormones leptin and insulin. Total leam would give moderate negative relatedness with body weight and body fat.

In contrast, body weight showed the highest relatedness on C-peptide, which gave the advantage to change traditional canola oil to nanoemulsion oil. This advantage might lead to decreased body weight with nanoemulsion canola and prevent many osteoporosis diseases linked to C- peptide. Total cholesterol was not linked with LDL-cholesterol, HDL-cholesterol, triglycerides and fasting glucose, leptin, C-peptide and Insulin.

Control group	**BW kg**	**BF, %**	**TLT, %**	**BMD, g/cm2**	**WC cm**	**TC**	**LDL C**	**HDL C**	**TGs**	**FG**	**A µg/mL**	**L, g/mL**	**C, ng/mL**	**I mIU/L**
Body weight, kg	1.000													
Body fat, %	0.894	1.000												
Total lean tissue, %	0.994	0.937	1.000											
Bone mineral density, g/cm2	1.000	0.904	0.996	1.000										
Waist circumference, cm	0.998	0.917	0.999	1.000	1.000									
Total cholesterol	0.981	0.964	0.996	0.985	0.990	1.000								
LDL cholesterol	-0.767	-0.397	-0.693	-0.751	-0.731	-0.627	1.000							
HDL cholesterol	0.984	0.960	0.997	0.988	0.992	1.000	-0.638	1.000						
TGs	-0.997	-0.927	-1.000	-0.998	-1.000	-0.993	0.713	-0.995	1.000					
Fasting glucose	-0.632	-0.217	-0.544	-0.613	-0.588	-0.469	0.982	-0.481	0.568	1.000				
Adiponectin, µg/mL	1.000	0.887	0.992	0.999	0.998	0.978	-0.776	0.981	-0.996	-0.643	1.000			
Leptin, ng/mL	-0.972	-0.764	-0.941	-0.966	-0.958	-0.908	0.896	-0.914	0.950	0.796	-0.975	1.000		
C-peptide, ng/mL	-1.000	-0.891	-0.994	-1.000	-0.998	-0.980	0.770	-0.983	0.996	0.636	-1.000	0.973	1.000	
Insulin, mIU/L	0.941	0.993	0.972	0.949	0.958	0.989	-0.505	0.987	-0.965	-0.333	0.936	-0.836	-0.940	1
Conventional Canola oil group														
Body weight, kg	1.000													
Body fat, %	-0.603	1.000												

Total lean tissue, %	-0.702	0.991	1.000											
Bone mineral density, g/cm2	-0.367	0.964	0.920	1.000										
Waist circumference, cm	-0.719	0.988	1.000	0.910	1.000									
Total cholesterol	-0.655	0.998	0.998	0.943	0.996	1.000								
LDL cholesterol	0.425	-0.978	-0.943	-0.998	-0.934	-0.962	1.000							
HDL cholesterol	0.970	-0.779	-0.854	-0.582	-0.867	-0.819	0.632	1.000						
TGs	0.314	0.568	0.455	0.768	0.434	0.511	-0.726	0.074	1.000					
Fasting glucose	-0.688	0.994	1.000	0.927	0.999	0.999	-0.949	-0.844	0.472	1.000				
Adiponectin, µg/mL	-0.770	0.973	0.995	0.876	0.997	0.987	-0.905	-0.902	0.364	0.993	1.000			
Leptin, ng/mL	-0.690	0.993	1.000	0.926	0.999	0.999	-0.948	-0.845	0.470	1.000	0.993	1.000		
C-peptide, ng/mL	-0.341	0.956	0.909	1.000	0.898	0.934	-0.996	-0.560	0.785	0.917	0.863	0.916	1.000	
Insulin, mIU/L	0.661	-0.997	-0.998	-0.940	-0.997	-1.000	0.960	0.824	-0.504	-0.999	-0.988	-0.999	-0.931	1
Nano canola oil group														
Body weight, kg	1.000													
Body fat, %	0.970	1.000												
Total lean tissue, %	-0.670	-0.470	1.000											
Bone mineral density, g/cm2	0.974	1.000	-0.484	1.000										
Waist circumference, cm	0.970	0.882	-0.831	0.889	1.000									
Total cholesterol	-0.989	-0.923	0.774	-0.929	-0.995	1.000								

LDL cholesterol	0.933	0.992	-0.358	0.990	0.817	-0.868	1.000							
HDL cholesterol	0.995	0.941	-0.741	0.946	0.989	-0.999	0.893	1.000						
TGs	0.893	0.976	-0.265	0.972	0.757	-0.816	0.995	0.844	1.000					
Fasting glucose	0.983	0.909	-0.796	0.915	0.998	-0.999	0.850	0.996	0.795	1.000				
Adiponectin, µg/mL	-0.980	-0.999	0.509	-1.000	-0.902	0.939	-0.986	-0.955	-0.965	-0.926	1.000			
Leptin, ng/mL	0.985	0.913	-0.789	0.919	0.997	-1.000	0.856	0.997	0.802	1.000	-0.931	1.000		
C-peptide, ng/mL	0.980	0.999	-0.510	1.000	0.902	-0.939	0.986	0.956	0.965	0.927	-1.000	0.931	1.000	
Insulin, mIU/L	0.999	0.981	-0.633	0.984	0.957	-0.980	0.949	0.989	0.914	0.973	-0.989	0.975	0.989	1

Table 2. Correlation relationship between biochemical parameters based on fishers test ($P<0.05$) Control group sunflower oil; conventional and Nano canola oil group.

Discussion

Estimation of the ratio of linoleic acid to linolenic acid (Omega6/ Omega3) reached 2.260, and it was more desirable almost 2:1, indicating that health assistance in canola oil; the lower ratio is supposed to be a vital factor in reducing the risk of much chronic disease via reducing LDL- cholesterol and overall cholesterol [18,19]. However, the extraction oil contained erucic acid at 0.2%, but it was less than many other reported data, which reached 9.4% for the Egyptian canola seed cultivar. The existence of erucic acid in the extracted canola oil may be due to environmental factor such as high temperature in Iraq during cultivation season; future attempts to reduce and eliminate erucic acid, which have negative cardiac health implication, would be necessary through plant breeding techniques to the successful selection of seed mutants expressing higher oil content and a lower percentage of erucic acid.

A minor increase in bone mineral density was observed under both canola-based oil treatments, with no significant difference between them internally. Our results were in accordance with other work [18], which referred to no significant differences in the anthropometric parameters and waist and hip circumference maintained during the test period. Similar results were noticed in fasting sugar with no significant differences between and among the three treatments (Fig. 2). However, the reduction in fasting glucose observed after consumption of nanoemulsion canola which refers to the importance of this treated canola oil in reducing serum sugar diets along with other dietary supplements such as Sylvestre gymnema, neem, Hordeum vulgare, Pterocarpus marsupium and Ocimum sanctum and Ballota [16, 20]. Recent experimental data revealed no significant lipid profile differences between treatments. However, LDL-cholesterol decreased in both canola oil groups compared to sunflower oil, while HDL-cholesterol increased with participants who consumed traditional canola oil. The three groups gave similar total cholesterol concentrations.

On the other hand, triglyceride increased in the canola oil group. Cholesterol is a risk factor that is elevated in serum and causes a block in blood vessels, leading to heart attack or stroke; two types of cholesterol exist in the serum. LDL-cholesterol is unhealthy for the heart because it leaves cholesterol on the blood vessels until blocked. The other type, HDL-cholesterol, can move some cholesterol out of blood vessels to avoid stroke, so a higher concentration of HDL-cholesterol benefits heart health. One of the most important factors is the high level of alpha-linolenic acid in extracted canola oil, which is known as one of the cardio-protective fatty acids besides 31.3%±4.1 of PUFA (Table) that is linked to reducing the harmful LDL-cholesterol.

Recent results are from other studies that referred to the importance of alpha-linolenic acid in reducing systolic blood pressure, diastolic blood pressure, LDL-cholesterol and total cholesterol [21, 22]. Also, [23] referred to the beneficial action of high content of PUFA in canola oil in reducing LDL- -cholesterol. Many other studies evaluated the positive impact of canola oil consumption on coronary heart diseases after reducing total cholesterol and cholesterol [24, 25]. Statistical analysis of the hormone profile found a significant decrease in leptin level observed in the serum of the nanoemulsion canola oil group compared to the control group. At the same time, there was no significant difference in leptin excretion between conventional and canola oil groups (Figure 3). This result might be an indicator of the effectiveness of oil in fluffiness, which might help in obesity in the long run; however, this conclusion needs more investigation in order to be verified. Results also illustrated a significant increase in Adiponectin between nano-oil and regular oil (similar levels were observed between nano-oil and control). This result might refer to the importance of nano-oil in diets, as Adiponectin is the protein hormone, and adipokine regulates glucose levels and fatty acid breakdown. In humans, it is encoded by the ADIPOQ gene, and it is produced primarily in adipose tissue, muscle, and even the brain [26, 27,29].

Conclusion

In recent years, consumption of canola oil has increased after it was approved by the United States Food and Drug Administration (FDA) [28]; also, its lower cost compared with olive oil and the perception that it shares its health benefits. Innovative foods produce nano nutrient-emitting in response to achieve, such as energy-related growth, improvement in cognitive functions, improvement in immunity and prolonging life. Dietary fat composition is suggested as a reason for many severe diseases such as cardiovascular disease, diabetes, obesity, and osteoporosis. As a result, evaluating the composition of fat in saturated and unsaturated fatty acids plays a vital role in decreasing mortality in different countries. Vegetable oils are sources of critical fatty acids. However, their composition depends on the source of oil and the technology process throughout their production. The most suitable technique was the mechanical ways with conditions that preserve the chemical composition of canola oil under study. The main conclusion was that canola oil could replace traditional sunflower consumed daily by the Iraqi population without any adverse effects. Converting canola oil to nanoemulsion was a promising step in reducing body weight, fasting glucose and leptin, as well as total cholesterol and harmful cholesterol. Studies focused on the relationship between total fats, SFAs and PUFA intakes and CHD have to be multifactorial because of many environmental factors and personal conditions that simultaneously impact the human metabolic pathways.

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