Journal

J. Biol. Chem. Environ. Sci., 2009, Vol. 4(4): 125-137
www.acepsag.org

EFFECT OF SOME VEGETABLE OILS ON HYPERLIPIDEMIC RATS

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ABSTRACT

The effect of diets containing different oils; olive, sesame, palm, corn, apricot kernel and moringa oleifera on hyperlipidemic rats were studied. Physical and chemical constants ;refractive index, acid ,peroxide and iodine values ;and the fatty acid composition of the investigation oils were determined .The effect of diet containing different oils(mainly high –oleic acid content)being; corn oil (basal diet, control 1) , high fat diet (HFD, control 2), extra- virgin olive oil (G1),refined olive oil (G2), sesame seed oil (G3),Palm olein (G4), apricot kernel oil(G5), moringa oleifera oil(G6), on hyperlipidemic rats was studied .It is clear that G1 fed on extra –virgin olive oil has the higher decrease in cholesterol followed by G2, G5,G6, G3, and G4. It is clear that G1 fed on extra –virgin olive oil has higher decrease in triglyceride followed by G5, G6, G2, G3, and G4. Rats fed on different vegetable oils (control 2 and group 1 to 6) showed significant decrease in its HDL-cholesterol compared with control 1. Besides, there are no significant differences between the 6 groups under invistigation compared with control 2. It is clear that is there a significant difference in LDL-cholesterol content between G1, G3 and G4 except that between G2, G5 and G6 with G3 and G4 and G1 with G2. Groups of rats (G1 to G 6) which fed on different vegetable oils showed a significant decrease in ALT activity compared to that of HFD .It was observed the rats fed on apricot kernel oil had the highest decrease in ALT activity compared with control 2 and other oils. It is

clear that oils under investigation (G1 to G6) caused a significant decrease in AST activity compared with that of HFD group.

Keywords: Extra-virgin olive oil, refined olive oil, corn oil, sesame seed oil, apricot kernel oil, moringa oleifera oil, hypocholesterolemic, hypolipidemic.

INTRODUCTION

In early assessments of the effects of dietary fat on human cholesterolemia or experimental atherosclerosis, total fat was the dietary parameter regarded as being the determining factor. The positive and significant association between a diet rich in saturated fat and raised cholesterol and increased cardiovascular risk, has been well established (keys et al., 1986). Some studied have shown that plasma concentration of total cholesterol and low-density lipoprotein cholesterol are significantly higher after a palm oil-rich diet than those obtained with unsaturated edible oils such as high-oleic sunflower oil (Denke and Grundy , 1992 ;ketan et al.,1995 and Cater et al., 1997) ,soybean oil (Enas, 1996) and high-linoleic safflower oil (Mattson and Grundy ,1985). Others have presented favorable results showing that concentration of low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very LDL (vLDL) after palm oil-rich diets are comparable to those obtained after ingesting diets rich in sunflower, peanut, corn, olive and soybean oils in normal - and hypercholesterolemic subjects (Baudette et al., 1984 and choudhury et al., 1995) and significantly different from those on milk- and butter-rich diets. This investigation was under taken to study the effect of diets containing different oils; olive, palm, corn, sesame, apricot kernel and moringa oleifera on hyperlipidemic rats.

MATERIALS AND METHODS

Materials

The corn oil, refined olive oil, extra-virgin olive oil and palm olein were obtained from the local market. The moringa oleifera seeds were obtained from Horticultural Research Institute, Giza, Egypt. The apricot kernels were obtained from Food Technology Research

Institute, Giza, Egypt. The sesame seeds were obtained from Agricultural Research Center at El matana, Suhag, Egypt.

Solvents and chemicals were obtained as pure and analytical reagent. The solvents were purified and redistilled.

Methods

Different constants: Refractive index, acid value, iodine value, and peroxide value of the oils were determined according to A.O.A.C. (2000).

Separation of fatty acids and unsaponifiable matters:

Lipid was saponified with ethanolic KOH (20% w/v) for 24 h at room temperature. The unsaponifiable matters were extracted three times with diethyl ether. The aqueous layer was acidified by HCI (20% w/v), and the liberated fatty acids were extracted with diethyl ether. The combined extracts of unsaponifiable matters and fatty acids were washed several times with distilled water, until the washing was neutral to phenolphthalein indicator, and then dried with anhydrous sodium. Sulfate (A.O.A.C., 2000).

Methylation of fatty acids:

Fatty acids of standards and samples were converted to methyl ester using ethereal solution of diazomethane. Fatty acids were dissolved in 0.5 ml anhydrous diethyl ether and methylated by drop wise addition of diazomethane solution until the yellow color persisted (Vogel, 1975). The mixture was then left at room temperature for 15 min and the solvent was evaporated on a water bath maintained at 60 ^{o}C. Finally the methyl esters of fatty acids were dissolved in pure chloroform and aliquots of this solution were subjected to GLC analysis.

GLC of fatty acid methyl esters:

The methyl esters of fatty acids, and standard compounds were analyzed by using a GCV pye Unicom gas chromatograph, equipped with dual flame ionization detector and dual channel recorder.The fractionation of fatty acid methyl esters was conducted by using a coiled glass column (1.5m x 4 mm) packed with Diatomite C.(100-120 mesh) and coated with 10% polyethylene glycol acclimate (PEGA). The column oven temperature was programmed at 8^{o}C/min from 70 ^{o}C to 190^{o}Cfor 45 min with nitrogen at 30 ml/min (A.O.A.C., 2000).

Biologicalexperiments:

Total of forty (40) of adult's male Albino rats (weights 100-120 g) raised in the animal house in Research Institute of Ophthalmology, Giza, Egypt. The animals were housed in stainless steel Cages with wire mesh bottoms in a room maintained at 25-30^{o}C). Animals were kept under normal healthy conditions for one week and fed on uniformly basal diet which containing salt mixture 4% and vitamin mixture 1%. The animals were allowed free excess of tap water and were fed on uniformly. After feeding on basal diet for one week, rats were divided into two major groups. The first main group (5 rats) was fed on the basal diet for another 7 weeks and was considered as normal control 1 group. The second main groups (35 rats) were fed hypercholesterolemic diet for three weeks (10% sheep tail fat and 1% cholesterol + 0.25 % bile salts) then divided into seven (7) sub groups. The first subgroup (5 rats) was continued to be fed hypercholesterolemic diet until the end of the experiment and considered as hypercholesterolemic control 2. The second subgroup (5 rats) was fed on basal diet containing 10% (10 g) extra virgin olive oil orally given daily. The third subgroup (5 rats) was fed on basal diet containing (10 g) Refined olive oil orally given daily. The fourth subgroup (5 rats) was fed on basal diet containing (10 g) sesame seed oil orally given daily. The fifth subgroup (5 rats) was fed on basal diet containing (10 g) palm olein oil orally given daily. The six subgroup (5 rats) was fed on basal diet containing (10 g) Apricot kernel oil orally given daily. The seven subgroup (5 rats) was fed on basal diet containing (10 g) Moringa oleifera kernel oil.

Statistical analysis:

Statistical analysis was carried out according to Fisher (1970). LSD (Least squares difference) test was used to compare the significant differences between means of treatment (Waller and Duncan, 1969).

RESULTS AND DISCUSSION

Physical and chemical constants of oils:

The physical; refractive index and chemical; aicd, peroxides, Iodine values constants and the stability (rancimate) of the investigated oils were determined and their results are shown in Table (1).

Table (1) Physical and chemical constants of the investegated vegetable oils :

Constants / Samples	Refractive index at 25°C	Acid value mg KOH/g	Peroxide value M. eqv/Kg	Iodine value $gI_2/100g$	Stability (hours)
Corn oil	1.4699	0.07	2.7	120.63	14.15
Extra virgin olive oil	1.4650	0.54	7.49	79.50	32.1
Refined olive oil	1.4653	0.19	12.86	79.50	11.55
Sesame seed oil	1.4689	0.38	4.44	107.30	5.11
Palm olein	1.4620	0.03	0.00	58.10	37.6
Apricot kernel oil	1.4670	1.86	0.00	106.99	21.75
Moringa olifera oil	1.4624	0.23	1.35	67.53	85.2

Fatty acid composition of the investigation oils:

The relative percentages of the fatty acids were determined by G.C for extra virgin olive oil, refined olive oil, corn, sesame seed, palm olein, apricot kernel and moringa olifera oils. The results are illustrated in Table (2).

Table (2) Fatty acids profile in different investegated oils :

Fatty Acids / Samples	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	Saturated FA	Un saturated FA
Corn oil	—	—	12.3	—	1.7	35.0	49.4	1.5	—	—	14	85.9
Extra virgin olive oil	—	—	13.3	1.07	1.8	73.2	11.0	0.8	—	—	15.1	86.07
Refined olive oil	—	—	12.1	0.8	2.4	73.9	10.05	0.7	—	—	14.5	85.45
Palm olein oil	0.33	0.9	37.7	—	3.02	45.8	12.08	—	—	—	41.95	57.88
Apricot kernel oil	—	—	4.8	0.7	0.8	66.3	27.1	0.1	—	—	5.6	94.2
Sesame seed oil	—	—	9.2	—	4.8	49.1	36.8	—	—	—	14	85.9
Moringa olifera oil	—	—	10.9	2.01	2.64	73.8	3.31	—	1.85	2.59	15.39	81.71

Biological evaluation of some vegetable oils:

The effect of feeding hypercholesterolemic albino rats on some vegetable oils (mainly high- oleic acid content) being; corn oil (based diet), extra virgin olive oil, refined olive oil, corn oil, sesame seed oil, apricot kernel oil, and moringa oleifera oil were studied. The rat's serum was analyzed for total cholesterol (mg/dl), triglyceride (mg/dl), HDL- cholesterol (mg/dl), LDL- cholesterol (mg/dl), beside ALT and AST activities (U/L). The results are tabulated in Tables (3-8).

Effect of vegetable oils on serum total cholesterol level of experimental rats:

It is clear that (Table 3) feeding of rats on high fat diet (HFD) for 8 weeks showed a gradual increase in the blood serum total cholesterol of these rats (control 2). Meanwhile, feeding of rats on the vegetable oils under investigation G_1, G_2, G_3, G_4, G_5, and G_6 showed a significant decrease in total cholesterol level in the serum of hypercholesterolemic rats.

Table (3) Effect of the investegated vegetable oils on serum total cholesterol * level of experimental rats:

Groups	Zero Time Mg/dl	After 4 weeks mg/dl	After 8 weeks mg/dl
Control 1	80.00±1.73 [a]	85.00±2.08 [b]	87.67±6.22 [f]
Control 2	81.67±0.88 [a]	224.00±1.52 [a]	293.00±4.04 [a]
G_1	81.00±1.52 [a]	221.00±5.56 [a]	125.67±3.48 [e]
G_2	83.33±2.40 [a]	219.33±3.52 [a]	130.00±2.30 [de]
G_3	82.67±1.20 [a]	221.67±4.09 [a]	161.67±1.33 [c]
G_4	80.00±1.15 [a]	218.33±4.63 [a]	180.00±7.00 [b]
G_5	81.00±2.00 [a]	217.00±2.60 [a]	139.00±4.04 [d]
G_6	83.00±1.15 [a]	217.00±2.64 [a]	153.33±3.84 [c]
L.S.D at 0.05%	4.7	10.7	12.6

*Control 1, corn oil; control 2, high fat diet (1% cholesterol+ 10% fat); G_1, extra virgin olive oil; G_2, refined olive oil; G_3, sesame seed oil; G_4, palm olein oil; G_5, apricot kernel oil; G_6, moringa olifera oil.

In addition, it is seen from Table (3) that there are significant differences between groups fed on vegetable oils G_1, G_2, G_3, G_4, G_5, and G_6 in reducing total cholesterol level compared with control (2). It

is clear that G_1 fed on extra virgin olive oil has higher decrease in cholesterol followed by G_2, G_5, G_6, G_3, and G_4. There are a significant difference between all groups except that between G_3 and G_6 and G_1 with G_2 and G_5. These results are in agreement with Baba et al. (2000) on olive oil, El-tahir et al. (2005) on sesame seed oil, Visvadiya (2008) on crud palm, Kritchevsky et al. (2001) on corn oil.

Effect of vegetable oils on serum triglyceride of experimental rats:

From Table (4) it could be observed that feeding of rats on high fat diet (HFD) caused a pronounced increase in serum triglyceride than that fed on basal diet (corn oil) for 4 weeks, and 8 weeks. Also Table (4) indicates that rats fed on vegetable oils under investigation (G_1 to G_6) showed a significant decrease in the triglyceride in the serum of hypercholesterolemic rats (control 2) after feeding period (8weeks). It is clear that G_1 fed on extra virgin olive oil has the higher decrease in triglyceride followed by G_5, G_6, G_2, G_3, and G_4. There are significant differences between all groups except that between G_1 and G_5 with G_2 and G_6. These results are in agreement with Baba et al. (2000) on olive oil, Ladeia et al. (2008) on sesame seed oil, Wilson et al. (2005) on red palm oil.

Table (4) Effect of the investegated vegetable oils on serum triglyceride * of experimental rats:

Groups	Zero Time Mg/dl	After 4 weeks mg/dl	After 8 weeks mg/dl
Control 1	78.67±2.02 ^{ab}	77.67±2.02 ^{c}	79.33±2.02 ^{g}
Control 2	75.67±2.33 ^{ab}	265.00±2.30 ^{ab}	283.33±2.06 ^{a}
G_1	78.67±1.45 ^{ab}	262.67±6.48 ^{b}	168.67±2.33 ^{f}
G_2	75.33±2.18 ^{b}	270.00±1.20 ^{ab}	179.67±4.40 ^{de}
G_3	77.00±3.21 ^{ab}	271.00±0.57 ^{ab}	201.00±3.21 ^{c}
G_4	82.00±1.52 ^{a}	269.67±2.02 ^{a}	214.67±5.92 ^{b}
G_5	77.00±2.64 ^{ab}	268.67±0.88 ^{ab}	172.00±2.08 ^{ef}
G_6	76.33±1.45 ^{ab}	272.33±1.45 ^{a}	182.67±2.60 ^{d}
L.S.D at 0.05%	6.5	8.2	10.3

*Control 1, corn oil; control 2, high fat diet (1% cholesterol+ 10% fat); G_1, extra virgin olive oil; G_2, refined olive oil; G_3, sesame seed oil; G_4, palm olein oil; G_5, apricot kernel oil; G_6, moringa olifera oil.

Effect of vegetable oils on serum HDL- cholesterol * of experimental rats:

Feeding of rats on HFD showed a pronounced decrease in HDL-cholesterol in the serum of rats, while feeding rats on different vegetable oils under investigation (control$_2$ and G_1 to G_6) showed significant decrease in its HDL- cholesterol (Table 5) compared with control 1 . Besides it is clear that there is no significant difference between the 6 groups under investigation compared with control (2). These results are in agreement with Baba et al. (2000) on olive oil, EL-tahir et al. (2005) on sesame seed oil, Kritchevsky et al. (2001) on palm oil and corn oil.

Table (5) Effect of the investegated vegetable oils on serum HDL-cholesterol * of experimental rats:

Groups	Zero Time Mg/dl	After 4 weeks mg/dl	After 8 weeks mg/dl
Control 1	46.33±1.45 [a]	46.33±0.88 [a]	46.33±1.76 [a]
Control 2	48.00±0.57 [a]	36.33±2.02 [b]	25.00±1.73 [b]
G_1	46.33±0.88 [a]	32.33±1.20 [b]	24.67±0.88 [b]
G_2	44.67±2.40 [a]	33.00±1.15 [b]	26.33±1.20 [b]
G_3	44.67±2.02 [a]	35.67±2.02 [b]	24.67±2.60 [b]
G_4	44.33±1.20 [a]	34.00±1.15 [b]	27.33±0.88 [b]
G_5	47.00±1.15 [a]	35.33±1.20 [b]	26.33±1.20 [b]
G_6	43.33±2.40 [a]	34.33±0.88 [b]	25.33±1.76 [b]
L.S.D at 0.05%	4.9	4.15	4.8

*Control 1, corn oil; control 2, high fat diet (1% cholesterol+ 10% fat); G_1, extra virgin olive oil; G_2, refined olive oil; G_3, sesame seed oil; G_4, palm olein oil; G_5, apricot kernel oil; G_6, moringa oleifera oil.

Effect of vegetable oils on serum LDL- cholesterol of experimental rats:

Feeding on hypercholesterolemic rats on the oils under investigation G_1 to G_6 caused a significant decrease in LDL-cholesterol (Table 6). From the some table there is a significant difference between G_1, G_3 and G_4 except that between G_2, G_5 and G_6 with G_3 and G_4 and G_1 with G_2. These results are in agreement with

EL-tahir et al. (2005) on sesame seed oil, Ladeia et al. (2008) on palm oil.

Effect of vegetable oils on serum ALT and AST activities of experimental rats:

Tables (7-8) show the serum ALT and AST activities in the serum of rats fed on basal diet B.D., HFD and other oils under investigation. Rats fed on HFD showed a gradual increase in ALT and AST activities. Groups of rats (G_1 to G_6) which fed on different vegetable oils showed a significant decrease in ALT activities compared to that of HFD (on level $p<0.05$), this mean that rat fed on apricot kernel oil had the highest decrease in ALT activity compared with control (2) and other oils. There are significant decreases between G_1, G_3, G_4 and G_5 and insignificant decrease between G_1, G_2 and G_6, and G_2, and G_3 this result are in agreement with El sayed (1995) and Mahmoud (1995).

Table (7) Effect of the investegated vegetable oils on serum ALT activities * of experimental rats:

Groups	Zero Time Mg/dl	After 4 weeks mg/dl	After 8 weeks mg/dl
Control 1	32.67±1.76 ^{ab}	32.00±1.15 ^{b}	35.67±1.45 ^{e}
Control 2	37.33±1.76 ^{a}	67.00±1.52 ^{a}	74.33±2.96 ^{a}
G_1	33.67±1.45 ^{ab}	67.67±3.17 ^{a}	45.67±2.96 ^{d}
G_2	34.67±1.45 ^{ab}	68.67±3.75 ^{a}	50.67±2.40 ^{cd}
G_3	36.67±2.40 ^{ab}	67.67±3.17 ^{a}	55.00±1.73 ^{c}
G_4	33.67±1.45	68.67±3.17 ^{a}	65.00±1.52 ^{b}
G_5	32.00±1.15 ^{ab}	64.67±2.40 ^{a}	36.33±1.45 ^{e}
G_6	35.00±1.73 ^{ab}	66.00±2.64 ^{a}	45.00±2.88 ^{d}
LSD at 0.05%	5.04	8.15	6.8

*Control 1, corn oil; control 2, high fat diet (1% choresterol+ 10% fat); G_1, extra virgin olive oil; G_2, refined olive oil; G_3, sesame seed oil; G_4, palm olein oil; G_5, apricot kernel oil; G_6, moringa olifera oil.

Table (8) .Indicates that oils under investigation (G_1 to G_6) showed significant decrease in AST in comparison that of HFD group. Also, significant difference between G_1 to G_6 was observed, while

there is no significant difference between G_1, and G_3 with between G_5 and G_6 and G_1, G_2, and G_3. These results are in agreement with El sayed (1995) and Mahmoud (1995).

Table (8) Effect of the investegated vegetable oils on serum AST activities * of experimental rats:

Groups	Zero Time Mg/dl	After 4 weeks mg/dl	After 8 weeks mg/dl
Control 1	42.00±1.15 [b]	47.00±1.15 [b]	47.67±1.45 [e]
Control 2	44.33±1.20 [ab]	109.67±4.09 [a]	123.00±1.73 [a]
G_1	45.67±2.40 [ab]	112.00±2.08 [a]	88.33±4.05 [cd]
G_2	42.67±1.45 [ab]	108.67±2.02 [a]	93.67±2.02 [c]
G_3	47.33±2.33 [ab]	111.00±1.52 [a]	88.67±1.76 [cd]
G_4	45.00±1.52 [ab]	115.00±3.21 [a]	102.00±1.15 [b]
G_5	46.67±2.90 [ab]	107.67±2.90 [a]	87.00±1.52 [d]
G_6	48.00±2.08 [a]	108.00±3.05 [a]	87.33±1.45 [d]
L.S.D at 0.05%	5.9	8.00	6.2

*Control 1, corn oil; control 2, high fat diet (1% choresterol+ 10% fat); G_1, extra virgin olive oil; G_2, refined olive oil; G_3, sesame seed oil; G_4, palm olein oil; G_5, apricot kernel oil; G_6, moringa olifera oil.

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تاثير بعض الزيوت النباتيه على الدهون فى الفئران

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**معهد بحوث تكنولوجيا الاغديه –الجيزه- مصر

تمت دراسه تاثير الاغذيه المحتويه على زيوت مختلفه مثل: زيت الزيتون- زيت السمسم- زيت النخيل – زيت الذره – زيت نوى المشمش- زيت بذور المورينجا على فئران التجارب المحتويه على ليبيدات مرتفعه فى السيرم كما درست الثوابت الفيزيائيه والكيماويه لتلك الزيوت مثل : معامل الانكسار – رقم الحموضه – رقم البيروكسيد – الرقم اليودى – وكذلك تركيب الاحماض الدهنيه ولقد قسمت فئران التجارب الى 8 مجموعات حيث تغذى المجموعه الاولى على العليقه الاساسيه

(كنترول 1) والمجموعه الثانيه على عليقه مرتفعه الدهن(كنترول 2) و المجموعه الثالثه (G1) تغذى على عليقه محتويه على زيت زيتون بكر و المجموعه الرابعه (G2) تغذت على زيت زيتون مكرر والمجموعه الخامسه(G3) تغذى على عليقه محتويه على زيت سمسم و العليقه السادسه (G4)تغذى على عليقه محتويه على زيت النخيل و المجموعه السابعه (G5) تغذى على عليقه محتويه على زيت نوى المشمش والعليقه الثامنه (G6) تغذى على عليقه محتويه على زيت بذور المورينجا . ولقد وجد ان التغذيه على زيت الزيتون البكر سببت اعلى نقص فى الكوليستيرول فى سيرم فئران التجارب ويليه المجاميع المغذاه على زيت الزيتون المكرر ثم زيت نوى المشمش ثم زيت بذور المورينجا ثم زيت السمسم.كما وجد ان التغذيه على زيت الزيتون البكر سبب اعلى نقص فى الجليسريدات الثلاثيه متبوعه بالفئران المغذاه على زيت نوى المشمش ثم زيت بذور المورينجا ثم زيت الزيتون المكرر .كما اتضح وجود فروق معنويه فى محتوى الكوليستيرول مرتفع الكثافه بين المجموعات تحت الاختبار مقارنه بمجموعه التى تغذت على العليقه الاساسيه.كما اتضح وجود فروق معنويه فى محتوى الكوليستيرول منخفض الكثافه بين المجموعات المغذاه على زيت زيتون بكر وزيت السمسم فى حين لا توجد فروق معنويه بين تلك المجموعات المغذاه على زيت الزيتون المكرروزيت نوى المشمش مقارنه بزيت بذور المورينجا و زيت السمسم وزيت النخيل وزيت الزيتون المكرر مقارنه بزيت الزيتون البكر .لوحظ انخفاض معنوى فى نشاط انزيم ALT فى المجموعات المغذاه على زيوت نباتيه مختلفه بالمقارنه بتلك المغذاه على عليقه مرتفعه الدهن ،كما لوحظ ان الفئران المغذاه على زيت نوى المشمش سببت اعلى نقص فى نشاط انزيم ALT.ايضا وجد ان الزيوت النباتيه تحت التجربه سببت نقص معنوى فى نشاط انزيم ASTمقارنه بالمجموعه المغذاه على عليقه مرتفعه الدهن .