Effect of avocado on lipid Profile of hypercholesterolemic rats

(تأثير الأفوكادو على صورة ليبيدات الدم للفئران المصابة بإرتفاع الكوليسترول)

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ABSTRACT

Avocados is one of function food . The present study aimed to investigate the effect of Avocado fruit on Serum lipid profile, liver function as well as histopathological changes of liver. Thirty male albino rats were used in this study. Rats were divided into five main groups (n=6 rats of each), group (1) was fed on basal diet and used as a control negative. Group (2) fed on the hypercholesterolemic diet and used as a control positive. Groups (3), (4) and (5) fed on the hypercholesterolemic diet formulated with 2.5, 5 and 10% dried avocado, respectively. At the end of experiment, blood samples were taken for biochemical analysis, while livers were removed for histopathological examination.

Results revealed that positive group had significant decreases in body weight gain (BWG) , fed efficiency ratio (FER) and serum HDL-c and significant increased in serum levels of total lipid (TL), total cholesterol (TC), triglycerides(TG), LDL-C,VLDL-c, AST and ALT compared to negative group. Hypercholesterolemic groups fed on different levels of avocado fruit had significant increased in food intake (except those treated with 2.5%), BWG, FER, and serum HDL-c while had significant decreased in serum levels of TL, TC, TG, LDL-c, VLDL-c, AST and ALT compared to positive group.

The present study concluded that Avocado fruit had beneficial effects of lipid profile and lipoprotein levels as well as it improved liver and kidney functions in hypercholesterolemic rats.

INTRODUCTION

Avocado (Persea americana) of the family Lauraceae is a tree native to the mainland of the western hemisphere from Mexico south to the Indian regions (wiley *et al*.,2010). Avocados are also called alligator pears because of their shape and the color of their skin. Avocado trees are large evergreens. An acre of avocado trees can produce a large amount of fruit, more than any other fruit crop in the world (Miller, 2011). Avocados have been cultivated for thousands of years (Steven *et al*.,2009). The avocado contains protein more than any other fruit. It is high in anganese, phosphorous, iron and potassium, but low in sodium, and also contains vitamin E, vitamin C, β-carotene, thiamin, riboflavin, nicotinic acid and folate (Rainey *et al*., 1994).The high concentration of monounsaturated fatty acids is responsible for beneficial effects on blood lipids and lowering cholesterol level (Coiquhoun *et al.,*1992).The edible pulp of avocado contains up to 33% oil rich in monounsaturated fatty acids (Ortiz *et al*., 2004) which are believed to modify the fatty acid contents in cardiac and renal membranes and enhance the absorption of alpha/beta-carotene and

lutein (Salazar *et al*., 2005). In addition to avocados are high in beta-sitosterol, a plant sterol that blocks the absorption of cholesterol from food, and the anti-cancer compound glutathione, a powerful antioxidant (Digest, 2010).

MATERIALS AND METHODS

1. Materials:

- **Rats:**

Male albino rats of Sprague Dawley strain weighing (170±5g) were purchased from Laboratory Animal Colony, Ministry of Health and Population, Helwan, Cairo, Egypt.

- **Chemicals:**

Casein, vitamin mixtures, mineral mixtures, cellulose, choline bitartrate , diethyl ether and Cholesterol were obtained as a pure yellow crystalline powder purchased from El-Gomhorya Company, for Drugs and Chemical Industries, Cairo, Egypt.

- **Kits:**

Kits for biochemical analysis of serum total lipid, total cholesterol, triglycerides, HDL-C, AST, ALT, uric acid, urea nitrogen and creatinine were obtained from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

- **Avocado fruit:**

Avocado was purchased from the local market, Cairo, Egypt.

2. Methods:

- **Preparation of dried Avocado:**

Avocado fruit were cleaned, outer skin was removed and sliced into ¼ inch, then dried in under vacuum in oven at 50 C° for 3hr. Then a grinder mill and sieves were used to obtain a powder particle size of less than 0.2mm (Reuven *et al.,* 1991).

- **Identification of phenolic compounds in Avocado:**

Separation and determination of avocado phenolic compounds were performed as described by (Goupy *et al.,* 1999) using HPLC (model 1100), a column Hyprsil DS (250x 4.6 mm). HPLC was equipped with auto-samples and a column compartment set at 35 ºC. Wavelength used for identified of phenolic compounds was 280nm.

- **Identification of flavonoids in Avocado:**

Determination of Avocado flavonoids were performed as described by (Mattila *et al.*, 2000) using HPLC 1100 adjusted at 330nm.

- **Preparation of basal diet:**

The basal diet (AIN-93M) was prepared according to (Reeves *et al.*, 1993).

Diet was formulated to meet recommended nutrients levels for rats as shown in table (1).

Table (1):
Composition of basal diet(AIN-93M) .

Ingredients	Amount
Casein	20%
Soybean oil	5%
Choline chloride	0.2%
vitamin mixture	1%
mineral mixture	4%
Fibers	5%
L-Cystine	0.18%
Sucrose	10%
corn starch	54.6%

Preparation of hypercholesterolemic diet:

Hyperlipidemic diet was prepared by formulated basal diet with cholesterol (1%) and bile salts (0.25% to induced hyperlipidemia in rats as described by (Cara *et al.*, 1992).

Experimental Designee:

Thirty male albino rats were used to achieve this study. Animals were maintained under standard conditions of humidity, temperature, and light, fed on basal diet and water *ad libitum* for one week before starting the experimental for acclimatization. After acclimatization period (one week), rats (n=30) were divided into five main groups (n=6 rats of each) as followed:

Group (1): Served as a control negative (normal rats) and fed only on the basal diet.

Group (2): Kept as a control positive (hyperlipidemic rats), and fed only on the hypercholesterolemic diet.

Group (3): Fed on the hypercholesterolemic diet formulated with 2.5% dried avocado fruit.

Group (4): Fed on the hypercholesterolemic diet formulated with 5% dried avocado fruit .

Group (5): Fed on the hypercholesterolemic diet formulated with 10 % dried avocado fruit.

Determination of food intake, body weigh gain and feed efficiency ratio in rats:

Food intake (FI) was calculated every other day. The effect of Avocado fruit were assessed by the determination of its effect on body weight gain and feed efficiency ratio (FER) at the end of the experimental period according to the method described by (Chapman *et al.,* 1959) using the following formulas:

Body weight gain = final body weight – initial body weight.

$$\text{Feed efficiency ratio (FER)} = \frac{\text{Body weight gain (g)}}{\text{Food consumed (g)}}$$

After the experimental period (6 weeks), rats were fasted for 12 hours then sacrificed. Blood samples were collected from the portal vein into dry clean centrifuge tubes and left to clot for 15 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 min. Then serum was frozen at –10ºC until further determination of tested parameter.

The organs such as liver, kidneys, spleen, and heart were removed, washed with saline, dried, and then weighted. Relative organs weight to body weight was calculated using the following equation:

$$\text{Relative organs weight} = \frac{\text{Organs weight}}{\text{Body weight}} \times 100$$

Liver and kidney of all rats were kept in formalin solution (10%) for histopathologically examine.

Chemical analysis of serum:-

1. Determination of Lipid Fractions :

1. Determination of Total Cholesterol:

Serum total cholesterol was determined according to the enzymatic method described by (Cohn *et al.*, 1988).

2. Determination of serum total lipids:

Serum total lipids were determined calorimetrically using sulfatphosphovalinic mixture as mentioned by (Schmit, 1964).

3. Determination of high density lipoprotein cholesterol:

Serum high density lipoprotein cholesterol (HDL-c) was calorimetrically determined according to the method described by (Young, 1995).

4. Determination of serum triglycerides:

Serum triglycerides were determined according to the method described by (Glick *et al*., 1986).

5. Determination of very low density lipoprotein cholesterol :

The concentration of serum very low-density lipoprotein cholesterol (VLDL-c) was calculated according to (Young, 1995)by using the following equation: VLDL-c = triglycerides/5.

6. Determination of low density lipoprotein cholesterol:

The concentration of serum low-density lipoprotein cholesterol (LDL-c) was calculated according to (Young,1995) by using the following equation: LDL-c (mg/ dl) = T. C – (HDL-c + VLDL-c).

2. Determination of Liver function:

1. Determination of Serum aspartate amino transferase:

Serum aspartate amino transferase (AST) was measured using Spectrophotometer at 505nm according to the method described by (Young, 2001).

2. Determination of serum alanine aminotransferase:

Serum alanine Amino transferase (ALT) was measured colorimetric according to the method described by (Young,2001) using Spectrophotometer adjusted at 505nm.

Histopathological examination:-

The liver of the scarified rats were taken and immersed in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness, and stained with Heamtoxylin and Eosin stain for examination of the liver as described by (Carleton, 1979).

Statistical analysis:

The obtained results were expressed as Mean ± SD. Data were evaluated statistically with computerized SPSS package program (SPSS 9.00 software for Windows) using one-way analysis of variance (ANOVA). Significant difference between means was estimated at $p<0.05$ according to (Sandcor and Cochran 1986).

RESULTS AND DISCUSSION

Date in Table (2) indicated that, The positive group had significant decreases in food intake, body weight gain (BWG) and fed efficiency ratio (FER) compared to negative group. Hypercholesterolemic rats treated with different levels (2.5 , 5 and 10%) of avocado fruit had significant increased in food intake, BWG and FER as compared to positive control group. These results agreed with Pieterse *et al*., (2005) who demonstrated that avocado within an energy-restricted diet does not compromise weight loss. The increased in body weight of rats may be explained base 100g serving of avocado provides 120 calories and 10g fat (Dennis, 2004) . In another case, these results disagreement with (Naveh *et al*., 2002) who reported that consuming avocado pulp in diets with or without cholesterol reduced food intake and body weight gain. The results also, demonstrated that as the dietary levels of avocado pulp increased food intakes decreased further more in rats fed both fibers body weight gains decreased as the amount of the fiber increased taste. In addition, avocados are, when compared to other fruit, high in fat (17.33 . 23.5 g/100g) USDA,(2002) ; Kruger *et al*.,(1998) and are seen by many consumers to be fattening, and therefore excluded in energy restricted diets.

Table(2):

Effect of feeding different levels Avocado fruit on body weight gain, food intake and feed efficiency ratio in hypercholesterolemic rats :

Groups	Parameters as Mean ± S.D		
	FI (gm / day)	BWG (gm)	FER
(G1) Negative Group	ab 22.33 ± 1.03	a 27.33 ± 1.51	a 0.04 ± 0.004
(G2) Positive Group	b 20.67 ± 1.21	d 14.83 ± 2.23	c 0.03 ± 0.004

(G3) 2.5% Avocado	ab 22.17 ± 2.99	c 22.50 ± 1.87	b 0.04 ± 0.005
(G4) 5%Avocado	a 23.00 ± 0.89	bc 23.83 ± 1.72	b 0.04 ± 0.02
(G5) 10% Avocado	a 23.67 ± 1.03	ab 25.50 ± 2.07	b 0.04 ± 0.004
L.S.D.	1.975	2.44	0.143

- Non- significant differences between the values had the same letter in each column.
- Significant differences at p< 0.05.
- L.S.D. means Least Significant Difference.

Date in Table (3) indicated that, The present results revealed that, Hypercholesterolemic rats treated with different levels of avocado induced non significant change in liver and heart relative weight compared to the positive . In addition, kidney relative weight , date showed that supplemented diet with a low level of avocado fruit induced non significant changes compared to

the positive group. Moreover, supplemented diets with two levels (5 and 10 %) of avocado caused a significant decrease in kidney relative weight compared to the positive group . Hypercholesterolemic rats treated with lowest and the highest levels of avocado fruit caused non significant change in spleen relative weight compared to the positive group. but the medium level of avocado caused a significant decrease in spleen relative weight compared to the positive group.

Table (3):

Effect of feeding different levels of Avocado fruit on organs weight as a percent to body in hypercholesterolemic rats:

Groups	Parameters as Mean ± SD			
	Liver (%)	Kidney (%)	Heart (%)	Spleen (%)

(G1) Negative Group	a 2.65±0.08	a 0.63±0.06	a 0.24±0.02	c 0.16±0.01
(G2) Positive Group	a 3.23±0.76	ab 0.58±0.04	a 0.24 0.24±0.02	a Kkk 0.30±0.06
(G3) 2.5% Avocado	a 2.84±0.56	bc 0.55±0.08	a 0.23±0.03	a 0.28±0.02
(G4) 5%Avocado	a 2.80±0.56	d 0.47±0.05	a 0.26±0.03	b 0.23±0.04
(G5) 10% Avocado	a 2.74±0.76	cd 0.49±0.04	a 0.24±0.02	ab 0.27±0.04
L.S.D.	.722	.066	.038	.044

- Non- significant differences between the values had the same letter in each column.
- Significant differences at $p< 0.05$.
- L.S.D. means Least Significant Difference.

Date in Table (4 and 5) indicated that, The present results revealed that, Hypercholesterolemic rats treated with different levels of avocado fruit had significant decrease in serum levels of total lipid , total cholesterol, triglycerides, LDL-c and VLDL-c ,while had significant increase in serum level of HDL-c compared to rats in the positive group. These results agreed with (Carranza *et al*., 1995) who reported that Avocado is an excellent source of monounsaturated fatty acids in diets designed to treat hypercholesterolemia with some advantages over low-fat diets with a greater amount of carbohydrates, they found a significant decline in total cholesterol and LDL-c concentration with no change in HDL-c in healthy and hypercholesterolemic subjects, as well as a significant decline in TG levels in moderately hypercholesterolemic patients, either with hypertriglyceridemia (combined hyperlipidemia) or with normal serum TG levels (Kris-Etherton *et al*.,1999). Also, these results agreed with (Liz

scott, 2003) who reported that, the type of fat avocados contain, the monounsaturated variety, may actually lead to improved HDL cholesterol, lower triglycerides. These results agreed with (Charles,2008) who reported that, feeding Avocado fruit induced a significant decrease in serum cholesterol, triglycerides, total lipid , LDL-c and VLDL-c and an increase in HDL-c . These results agreed with (Carranza *et al*.,1997) who reported that, Avocado- enriched diet produced a significant reduction in low-density lipoprotein and total cholesterol in patients with high cholesterol levels. These results agreed with (Perez Mendez *et al*., 2007) who indicated that the inclusion of avocado in the diet decreased plasma triglycerides and increased HDL-cholesterol plasma levels. These results agreed with (Steven *et al*., 2009; Moghadasian *et al*., 1999 ; Lopez *et al*., 1996 and Keda *et al* ., 1983) reported that a 1 week diet based on avocado in hypercholesterolemia patients can dramatically decrease bad cholesterol (LDL) and triglyceride levels by 22%. Furthermore good cholesterol (HDL) increased by 11% . Bernard, (2004) one study from Australia demonstrated how eating half to one avocados a day for three weeks could lower your total cholesterol by more than 8 percent without lowering your HDL cholesterol. During the same study, a low- fat, high-carbohydrate diet also lowered the participants total cholesterol – but slashed the good cholesterol by almost 14 percent. Not only avocados decrease your LDL cholesterol without lowering HDL cholesterol, they also can reduce the amount of triglycerides, another type of fat, in your blood. A high triglyceride level can be a warning sign of heart disease.

(Tinker *et al*., 1994 and Bricklin , 1993) reported that, fibers presented in avocado are soluble which decrease plasma cholesterol.

Table(4):
Effect of feeding different levels of Avocado fruit on total lipid, total cholesterol and triglycerides in hypercholesterolemic rats:

Groups	Parameters as Mean ± SD		
	Total lipid (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
(G1) Negative Group	e 321.50 ± 1.89	d 73.83 ± 2.21	d 41.67 ± 1.83
(G2) Positive	a	a 107.83 ± 3.74	a 66.50 ± 1.85

Group	383.71 ± 2.63		
(G3) 2.5% Avocado	b 356.00 ± 0.82	b 94.00 ± 3.46	b 55.50 ± 2.90
(G4) 5%Avocado	c 346.00 ± 2.16	c 86.50 ± 3.84	c 50.67 ± 1.70
(G5) 10% Avocado	d 335.00 ± 2.08	d 74.50 ± 3.15	d 43.17 ± 0.76
L.S.D	2.271	3.771	2.185

- Non- significant differences between the values had the same letter in each column.
- Significant differences at $p< 0.05$.
- L.S.D. means Least Significant Difference.

Table (5) :

Effect of feeding different levels of Avocado fruit on serum HDL, LDL and VLDL in hypercholesterolemic rats.

Groups	Parameters as Mean ± SD		
	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
(G1) Negative Group	a 35.17 ± 2.71	e 30.32 ± 4.20	d 8.33 ± 0.37
(G2) Positive Group	e 21.83 ± 1.22	a 72.70 ± 3.60	a 13.30 ± 0.37
(G3) 2.5% Avocado	d 24.00 ± 1.00	b 58.90 ± 4.04	b 11.13 ± 0.58
(G4) 5%Avocado	c 27.50 ± 1.26	c 48.87 ± 4.64	c 10.13 ± 0.34
(G5)	b	d	d

10% Avocado	31.00 ± 2.58	34.87 ± 3.02	8.63 ± 0.15
L.S.D.	2.151	4.456	.437

- Non- significant differences between the values had the same letter in each column.
- Significant differences at $p< 0.05$.
- L.S.D. means Least Significant Difference.

Date in Table (6) indicated that, Serum levels of AST and ALT increased significantly in the positive control group as compared to the control negative group. These results may be related to massive fatty change of hepatocytes which confirmed by histopathological examination. These results agreed with Sallie *et al*.,(1991) who reported that, the rise in levels of serum AST and ALT has been attributed to the damaged structural integrity of the liver, because these enzymes are cytoplasm in location and released into circulation after cellular damages. Karthikeyan *et al*., (2006) and Wittekind, (1995) also, demonstrated that Elevated levels of AST and ALT are often diagnostic of underlying cellular injuries. Recently, Jeong *et al*., (2010) who observed that rats fed on hypercholesterolemic diet had increase in liver enzyme ALT and AST concentrations. The elevation in serum AST and ALT may be results in response to oxidative process (Bansal *et al*., 2005). Medina and Moreno-Otero, (2005) also, demonstrated that oxidative stress is a common pathogenetic mechanism contributing to initiation and progression of hepatic damage in a variety of liver disorders. Cell damage occurs when there is an excess of reactive species derived from oxygen and nitrogen, or a defect of antioxidant molecules.

With regard to the effect of feeding different levels of avocado fruits, results indicated that diet supplemented with (2.5, 5 and 10%) of avocado fruit caused a significant decrease in serum levels of AST and ALT enzymes as compared to positive the control group. These mean that avocado fruits improved liver functions. The beneficial effect of avocado fruits may be attributed to its phenolic and flavonoids which have antioxidant properties. These results agreed with Ayano *et al*.,(2010) who indicated that, intravenous administration of syringic acid or vanillic acid significantly decreased the activities of AST and ALT. Therefore, syringic acid and vanillic acid might be promising oral agents for the prevention of liver disease. These results agreed with (Kawagishi *et al*., 2001) who reported that avocado showed extraordinarily potent liver injury suppressing activity. Kawagishi *et al*., (2001) reported that avocado compounds might help in the treatment of viral hepatitis (a virally caused inflammation of the liver). Recently, Liz scott, (2003) reported that, avocados contain potent

chemicals that may reduce liver damage and may be particularly promising for the treatment of viral hepatitis. These results confirmed with Art *et al*.,(2009) who reported that, the avocado contains several active components that reduce liver damage. Avocado contains many alkaloids, saponins, flavonoids, steroids. It has been reported that it slows liver damage in rats Kawagishi *et al*., (2001). Isolated compounds from avocado were showed to inhibiter the activities towards super oxide (O_2) and nitric oxide (NO) generation in cell culture system Kawagishi *et al*., (2001).

Table (6) :

Effect of feeding different levels of Avocado fruit on serum levels of AST and ALT in hypercholesterolemic rats.

Groups	Parameters as Mean ± SD	
	AST (U/L)	ALT (U/L)
(G1) Negative Group	d 35.17 ±1.49	c 11.67 ± 1.3
(G2) Positive Group	a 46.33 ± 2.33	a 26.67 ± 1.7
(G3) 2.5% Avocado	b 43.83 ± 2.50	b 16.67 ± 1.19
(G4) ٥%Avocado	c 40.33 ± 1.70	b 16.00 ± 1.53
(G5) 10% Avocado	c 39.83 ± 1.37	b 15.50 ± 1.12
L.S.D	2.183	1.58

- Non- significant differences between the values had the same letter in each column.

- Significant differences at p< 0.05.

- L.S.D. means Least Significant Difference.

الملخص العربى

الأفوكادو من الأغذية الوظيفية وتهدف هذه الدراسة الى التعرف على تأثير الأفوكادوعلى مستوى دهون الدم ، ووظائف الكبد (مستوى انزيمى ALT and AST) والتغيرات الهستوباثولوجية التى قد تطرأ على الكبد فى هذه الفئران .ولاتمام هذه الدراسة تم استخدام عدد ٣٠ فأر ذكر تم تقسيمهم

الى خمس مجموعات رئيسية (كل مجموعة ٦ فئران) . المجموعة الأولى : تم تغذيتها على الغذاء الأساسى وتستخدم كمجموعة ضابطة سالبة، والمجموعة الثانية : تم تغذيتها على حمية خاصة بإرتفاع الكوليسترول وتستخدم كمجموعة ضابطة موجبة، والمجموعات الثالثة والرابعة والخامسة : تم تغذيتهم على نظام غذائى خاص بإرتفاع الكوليسترول مع (٢,٥ ، ٥ ، ١٠ %) من الأفوكادو المجففة على التوالى خلال فترة التجربة. وفى نهاية التجربة تم ذبح جميع الفئران وتجميع الدم وفصل السيرم واستخدامه فى إجراء التحليلات البيوكيميائية. كما تم تجهيز الكبد لإجراء الفحص الهستوبثولوجى.

وقد أظهرت النتائج وجود نقص معنوى فى معدل وزن الجسم ومعدل التمثيل الغذائى ومستوى الليبوبروتينات عالية الكثافة خلال المجموعة الضابطة الموجبة كما سجلت النتائج ارتفاع معنوى فى مستوى الكوليسترول الكلى ، الجليسيريدات الثلاثية ، الليبوبروتينات منخفضة الكثافة والليبوبروتينات منخفضة الكثافة جدا ، وكذلك انزيمى AST and ALT مقارنة بالمجموعة الضابطة السالبة . والمجموعات المصابة بإرتفاع الكوليسترول والمتناولة نسب مختلفة من الأفوكادو سببت زيادة معنوية كبيرة فى تناول الطعام ومعدل وزن الجسم ومعدل التمثيل الغذائى وفى مستوى الليبوبرتينات عالية الكثافة، بينما أحدثت نقص معنوى فى مستوى الكوليسترول الكلى ، الجليسيريدات الثلاثية ، الليبوبروتينات منخفضة الكثافة والليبوبروتينات منخفضة الكثافة جدا وانزيمىAST and ALT مقارنة بالمجموعة الضابطة الموجبة .

وأكدت الدراسة أن الأفوكادو فاكهة كان لها آثار مفيدة على تحسن صورة دهون الدم (مستوى الكوليستيرول الكلى والجليسريدات الثلاثية ومستويات الليبوبروتينات) بالإضافة الى أنها أدت الى تحسن وظائف الكبد في الفئران المصابة بإرتفاع الكوليسترول.

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