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Effect of Avocado on Serum Lipids of Hyperlipidemic Rats

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ABSTRACT

The present research was conducted to study the effect of dietary supplementation with Avocado fruits under various concentrations to give more protection against hyperlipidemia disease. Twenty eight male albino rats were used in this experiment. These rats were put on ideal diet for two weeks before then divided into four groups, seven rats each, and fed on diets for 45 days. The first group was fed on basal diet as a (negative control group). The second group (positive control group) was fed on basal diet + 2% cholesterol to induce hyperlipidemia. The third and fourth groups fed on high fat diet containing fortified with Avocado at 15% & 25%. At the end of the experimental period rats were fasted over night and sacrificed; blood samples were collected from the aorta to determine total cholesterol and other lipids, also for liver and kidney functions. Besides, nutritional parameters were recorded including body weight gain, feed consumption and feed efficiency ratio. Also, heart was removed surgically for histopathological observation. From the obtained results we concluded that group of rats fed on high fat diet were considered as a major risk factor for hyperlipidemia disease. Our results could be summarized that diet fortified at 15% and 25% Avocado can reduced blood cholesterol and other lipids as well as reducing hazards on liver and kidney function compared with positive control group. It means that diet with 25% Avocado improved the body weight gain, feed intake, serum lipid levels and both liver and kidney function. Histopathological observation proved that the last group is considered as a negative control group. We can conclude that fortified diet with 25% Avocado realized the best effects on hyperlipidemia rats.

Key words: Hyperlipidemia, rats, Avocado, serum lipids, liver function, kidney function, histopathology.

Introduction

Hyperlipidemia is a powerful and extremely one of the major causes of the development of cardiovascular disorders (Jain *et al*., 2010). Over the last few years the changes in the type of diets have led to an increased frequency of lifestyle related disorders such as hyperlipidemia, diabetes mellitus and atherosclerosis, (Santoshkumar *et al*., 2013). High fat diet is the term used to denote raised serum levels of one or more of total cholesterol, low-density lipoprotein cholesterol and triglycerides (Sivaiah & Reddy, 2012 and Duraipandiyan *et al*. 2016).

Nowadays, the treatment of hyperlipidemia and cardiovascular diseases with plants has increased in recent years, consumer demand for healthy, safe, natural, and fresh foods that require only a minimum effort and time for their preparation has increased, published by Ramos *et al*., (2012). In fact, dietary guidelines around the world recommend increased consumption of fruit and vegetables because they have low-to-medium energy density and are important contributors of major shortfall nutrients, different kinds of fruit and vegetables are being reevaluated and classified as excellent sources of nutraceutics (Fulgoni *et al*., 2013).

Colorful fruits contain hundreds of photochemical that work together with nutrients to promote health and prevent disease. Avocado is listed among the most commonly fruits in the world, due to their physicochemical properties and indigestible characteristics. Consuming Avocado is an effective way of inhibiting blood clotting and can improve cardiovascular risk profiles in healthy adults published by Ferguson & Fillion, (2003) and Ghasemnezhad *et al*., (2013). In fact, Limit research has also been published regarding incorporation of Avocado with higher degree of health benefits.

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Therefore, it is important to studying the protective effect of Avocado fruits, and the mixture dietary supplementation on hyperlipidemia.

Materials and Methods

Materials:

Fresh Avocado (*CV*. Fuerte) was purchased from Field Crops Research Institute, Ministry of Agriculture Giza, Egypt. Casein was obtained from Miser Scientific Company, Dokki, Giza, Egypt. Cholesterol was purchased from El-Gomhoria, Pharmaceutical Company, Cairo, Egypt. Cellulose, vitamins and minerals were purchased from Morgan Company Ind., Cairo, Egypt. Corn oil and starch were obtained from the local market.

Twenty eight adult male albino rats (Sprague-Dawley) were obtained from the laboratory animal colony, Ministry of Health & Population. Weighting were approximately between (120±10g). Kits used to determine Cholesterol, Triglycerides, LDL-C, Uric acid, Urea nitrogen, Creatinine, and Transaminases produced by Egyptian American Company for laboratory service and supplied by Alkan Company.

Methods:

Fresh Avocado fruits were washed by using tap water to remove pathogenic microorganisms and dust, and then peeled off and the creamy pulps were homogenized using electric blender. These minced fruits were mixed with basal diet daily.

Chemical analysis of Avocado:

Moisture, fiber, ash, protein and fat content were determined according to the method outlined in AOAC. (2000). Total carbohydrates were determined by difference. Total fatty acids (saturated and unsaturated FA) were determined by the method described in AOAC, (2002).

Vitamins including (C, E, A and β -carotene) were assayed as recommended by J. Chrom. (2001 & 2005). Types and concentrations of flavonoids compounds were estimated as recommended by J. og. Agric. & Food Chem. (2000). Antioxidant activity was determined according to the methods described by Politeo *et al.,* (2006).

Experimental design:

Rats were adapted for two weeks prior to commencement of the experiment. Water was introduced ad-libitum. Rats were divided into four main groups and fed on diets for 45 days as follows: *Group 1*: Negative control group (7 rats) fed on basal diet. *Group2:* Positive control group (7 rats) contained the same composition, in addition 2% cholesterol to induce hyperlipidemia according to Hassarajani *et al*., (2007). *Group 3 and group 4:* (14 rats) obtained the same composition as positive diet, in addition fortified with 15% & 25% Avocado. During the experiment period, the quantities of diet, which were consumed and / or wasted, were recorded every day. In addition, rat's weight was recorded weekly, to determine Feed Intake, Body Weight Gain % and Feed Efficiency Ratio according to Chapman *et al*., (1959).

At the end of the experiment period, the rats were fasted overnight then the rats were anaesthetized and sacrificed, then the blood samples were collected from the heart. The blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum to determine total cholesterol (Allain *et al*., 1974), triglycerides (Fassati & Prencipe 1982), HDL-C (Lopes, 1977), LDL-C and VLDL-C were calculated by using the method of Friedewald *et al*., (1972). Uric acid, urea nitrogen and creatinine were determined according to the methods described by Fossati *et al*., (1980), Patton & Crouch (1977) and Bartels *et al*., (1972) respectively. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Retiman & Frankel (1957).

Histopathological Examination:

Tissues from heart of the sacrificed rats were examined as described by Bancroft *et al*., (1996).

Statistical analysis:

Results are expressed as mean ± SD. Data were statistically analyzed for variance using one-way analysis of variance "ANOVA" according to (Armitage & Berry, 1987). Computer software system SPSS (version 15) was used for these calculations.

Results and Discussion

Chemical composition of raw materials:

Avocado was investigated on fresh weight basis. The following parameters in Table (1) were determined for moisture, protein, ash, carbohydrate, fiber, fat and vitamins, the ratios were 63 .71; 02.12; 01.03; 09.87; 07.25 and 16.02 (g/100g edible portion) respectively. The same table pointed out that the contents of vitamin in Avocado including (C, total carotenoids, A, E and total antioxidant), the ratios were 13.17 (mg); 3.92 (mg); 65.10 (µg); 1.17 (mg) and 1.01 (µg/ml) respectively. The results in this study are related to that obtained by Fulgoni *et al*., (2013) revealed that the Avocado is one kind of fruit which is gaining a lot of importance because of its content of different components that are considered as nutraceutics. Avocados fruit meet the definition of a functional food as outlined by the American Dietetic Association in that they provide health benefits beyond basic nutrition. According to Venter (2013) showed that the natural antioxidants such as vitamins E, C and β-carotene prevent lipid peroxidation in the body this was supported by the fact that the low serum levels of antioxidant vitamins are associated with high risk of cardiovascular. In fact from our results showed that Avocado has major source of various types of nutrients with promising evidence of health benefits.

Table 1: Chemical composition of Avocado fruits (edible portion)

Chemical composition (g/100g)	Avocado	Vitamins (mg / 100g)	Avocado
Moisture	63.71±0.3 [a]	Vitamin C	13.17
Protein	02.12±0.2 [d]	Total carotnoids	3.92
Ash	01.03±0.1[d]	Vitamin A (µg)	65.10
Carbohydrate	09.87±0.2[c]	Vitamin E	1.17
Fiber	07.25±0.1[c]	Total antioxidant activity by DPPH% (µg/ml)	1.01
Fat	16.02±0.2[b]		

**Results are expressed as means ±SD.*
**Values in each raw which have different letters are significantly different ($p<0.05$).*

Types and concentrations of flavonoid compounds in Avocado give in Table (2). It obvious that Rutin, Hisperidin, Rosmarinic, Quercitin, Quercetin, Narinenin, kaempferol and Apignen were the abundant flavonoid compounds, which were at concentration of 275.12, 106.43, 265.31, 241.09, 189.09, 269.10, 258.14 and 043.25 (mg/100g), respectively. The results reported previously by Maria *et al*., (2012) that rutin and kaempferol were present in small amounts in Avocado fruit. On the contrary, Mahmoed & Rezq, (2013) obvious that querecitin and kaemferol were the abundant flavonoid compounds, which were at concentration of 9.447 and 8.993 (pmm), respectively. In fact, recent epidemiological studies have revealed that the intake of flavonoids is inversely associated with the risk of coronary heart disease Santoshkumar *et al*., (2013).

The amount of saturated and unsaturated fatty acid (monounsaturated and polyunsaturated) of Avocado may vary among individual fatty acid. The data presented in Table (3) showed the fatty acid composition of total lipids extracted from the Avocado by the Gas Chromatography (GC) were recognized that the most abundant saturated fatty acids was Palmitic acid, while Stearic acid present

at moderate ratio and trace amounts of the other saturated fatty acids like Arachidic acid; Myristic acid; Lauric acid and Caprylic acid (0.57, 0.16, 0.06 and 0.03%) respectively. Also, results given in Table (3) revealed that the oleic acid is the main monounsaturated fatty acid contained in the Avocado (64.76%), followed by the PalmitiOleic acid (3.90%), while the main polyunsaturated fatty acid contained in Avocado was linoleic acid (6.50%), and followed by the Linolenic acid (0.70%) and Arachidonic acid (0.50%). From our results, Avocado contain total saturated fatty acid at (23.64%) and total unsaturated fatty acid was (76.36%) respectively. These results are in agreement with those reported by other authors for Avocado (Moreno *et al*., 2007 and Dreher & Adrienne, 2013) they indicated that the Avocado contain a monounsaturated fatty acids with 71%, 13% polyunsaturated fatty acids, and 16% saturated fatty acids, which helps to promote healthy blood lipid profiles and enhance the bioavailability of fat soluble vitamins and phytochemicals which are consumed with Avocado. Also, as the Avocado fruit ripens, the saturated fat decreases and the monounsaturated oleic acid increase.

Table 2: Types and concentrations of Flavonoids compounds of Avocado fruits

Flavonoids (mg/100g)	Avocado
Rutin	275.12
Hisperidin	106.43
Rosmarinic	265.31
Quercitin	241.09
Quercetin	189.09
Narinenin	269.10
Kaempferol	258.14
Apignen	043.25

Table 3: Fatty acids in Avocado fruits (g / 100g edible portion)

Fatty acids (g/100 g FW)		Avocado
Saturated fatty acid	Caprylic acid	0.03
	Lauric acid	0.06
	Myristic acid	0.16
	Palmitic acid	21.36
	Stearic acid	1.46
	Arachidic acid	0.57
Mono-unsaturated fatty acid	Oleic acid	64.76
	PalmitiOleic acid	3.90
Poly-unsaturated fatty acid	Linoleic acid	6.50
	Linolenic acid	0.70
	Arachidonic acid	0.50
Total saturated fatty acid		23.64
Total unsaturated fatty acid		76.36

Biological evaluation:

Effect of feeding hyperlipidimic rats on diet fortified with Avocado fruits on body weight gain; feed intake and feed efficiency ratio:

The mean values of rats body weight gain (BWG); their feed intake (FI) (g/ day for each rat)) and feed efficiency ratio (FER) were summarized in Table (4). Data presented could be observed that there were significant increases in BWG; FI and FER for control positive group (1.43±0.08; 13.91±0.10 and 6.46±0.27) as compared to the healthy control group (negative), (1.08±0.14; 11.15±0.25 and 5.73±0.35), respectively. Groups fed on the hyperlipidimic diet containing Avocado fruits showed improving in body weight and feed intake compared to the positive control group, it seems that Avocado at 15% and 25% exert a protective effect against overweight. These results are in harmony, with those obtained by Naveh *et al*., (2013) and Fulgoni *et al*., (2013) indicated that Avocado consumption is associated with improved nutrient intakes and lower body weight and possibly reducing the risk of metabolic syndrome. Moreover, the feed efficiency ratio of treated

groups fed on hyperlipidimic diet with Avocado fruits at (15% or 25%) recorded significant decreased (P< 0.05), as compared to control positive group, this data agree with Makni *et al*., (2008) and Barakat, (2011).

Table 4: Effect of feeding hyperlipidimic rats on diet fortified with Avocado fruits on body weight gain; feed intake and feed efficiency ratio:

Groups / Parameters		(BWG %)	(FI/g/day)	(FER)
1	Control (-ve)	1.08 ± 0.14^{c}	11.15±0.25^{e}	5.73± 0.35^{c}
2	Control(+ ve)	1.43 ± 0.08^{a}	13.91±0.10^{d}	6.46± 0.27^{a}
3	Avocado15%	0.64 ± 0.02^{d}	16.38±0.23^{c}	2.26± 0.90^{b}
4	Avocado 25%	0.68 ± 0.01^{d}	18.65±0.21^{b}	1.85± 0.17^{a}

**Results are expressed as means ±SD.*
**Values in each column which have different letters are significantly different (p<0.05).*

Influence of Avocado fruits on lipid profile of hyperlipidimic rats:

It could be noticed that the positive control group fed on basal diet containing 2% cholesterol has shown a significant increase in the mean values of TC, TG, LDL-C and VLDL-C (191.40±12.15; 73.55±1.67; 162.30±9.05 and 14.71±1.31 mg/dl) respectively, compared with the control negative group fed on the basal diet (104.23±10.41; 43.05±1.08; 62.80±12.56 and 8.61±1.35 mg/dl) respectively. Concerning the mean value of serum HDL-C, the control positive group exhibited a markedly significant decrease (14.39±1.25), as compared to the negative control group (32.82±1.02). These findings are in agreement with Al-Dosari, (2011); Wang *et al*., (2012); Fulgoni *et al*., (2013) and Duraipandiyan *et al*., (2016) they revealed that feeding rats on the high cholesterol diet elevated serum triglycerides and total cholesterol levels are viewed as independent risk factors of coronary heart disease and diabetic diseases, Moreover, Dreher & Adrienne, (2013) showed that the Avocados have the highest fruit lipophilic antioxidant capacity, which may be one factor in helping to reduce serum lipid peroxidation and promoting vascular health.

Table 5: Effect of Avocado on lipid profile of male albino rats

Parameters / Groups	mg/dl				
	Cholesterol	Triglyceride	HDL-C	LDL-C	VLDL-C
Control negative group	104.23 ±10.41^{c}	43.05 ±1.08^{b}	32.82 ±1.02^{c}	62.80 ±12.56^{c}	8.61 ±1.35^{b}
Control positive group	191.40 ±12.15^{a}	73.55 ±1.67^{a}	14.39 ±1.25^{d}	162.30 ±9.05^{a}	14.71 ±1.31^{a}
Control positive group+15% Avocado	109.05± 11.01^{d}	56.00± 1.87^{b}	37.10± 1.05^{c}	60.75± 15.49^{e}	11.20± 1.104^{b}
Control positive group+25% Avocado	105.61± 12.05^{d}	44.75± 2.17^{b}	47.36± 2.20^{a}	49.30± 15.69^{ef}	8.95± 1.02^{b}

All results are expressed as means ±SD. Values in each column which have different letters are significantly different (p<0.05).

Our results indicate that the fortified diet with Avocado at 25% to the basal diets of hyperlipidimia resulted the decrease of lipid parameters has been shown to be a strong best treatment factor for high cholesterol diet. The reduction of TC, TG, LDL-C and VLDL-C were 44.82%; 39.16%; 69.62% and 39.16%, respectively, associated with a 69.62% increase of HDL-C in rats fed on a high-cholesterol diet when orally treated with diet fortified with Avocado 25% after 45 days might due to decrease of cholesterol absorption and biosynthesis and increase of fecal bile acid and cholesterol excretion. The data were in the line with those of Nwaoguikpe & Braide, (2011) and Wang *et al*., (2012) they found that Avocado intake has been shown to have effects on serum cholesterol levels. Specifically, after a seven day diet rich in Avocado, hypercholesterolemic patients showed a 107% decrease in total serum cholesterol levels. Furthermore, consumption of fruits and vegetables is associated with a lowered risk of cardiovascular diseases; Natural medicines have been used empirically to lower the cholesterol levels Al-Dosari, (2011) and Duraipandiyan *et al*., (2016).

Effect of Avocado fruit on liver functions of hyperlipidimic rats:

Results in Table (6) indicated that feeding on basal diet containing 2% cholesterol has shown a significant increase P< 0.05 in serum AST and ALT, as compared to healthy rats fed on basal diet (31.20±2.51 and 14.25±1.09 vs. 17.07±2.43 and 10.00±2.04 U/L), respectively. This may be referred into a direct excessive effect of feeding at high cholesterol diet on liver enzymes, at the same time; our results indicated that the high levels of AST and ALT in serum are indicators for liver dysfunction. These findings are in agreement with Al-Dosari, (2011) they revealed that the rats feeding on high cholesterol diet for 70 day showed significant increase in serum liver marker enzymes (GOT, GPT, GGT, ALP) and bilirubin levels. Our data indicated also that, feeding on high cholesterol diet with Avocado fruit which used in the above fortifications at 15% & 25% resulted in significant decrease p<0.05 in serum Aspartate aminotransferase and Alanine aminotransferase (AST and ALT) as compared to positive control group.

Table 6: Effect of Avocado on liver functions of male albino rats

Parameters / Groups	AST (u/l)	ALT (u/l)
Control (-ve)	17.07±2.43^{d}	10.00±2.04^{c}
Control (+ve)	31.20±2.51^{a}	14.25±1.09^{a}
Avocado 15%	24.10±1.52^{c}	9.09±1.04^{c}
Avocado 25%	21.03±1.40^{c}	8.23±1.34^{d}

**Results are expressed as means ±SD.*
** Values in each column which have different letters are significantly different (p<0.05).*

Effect of Avocado fruit on kidney functions of hyperlipidimic rats:

Kidneys remove metabolic wastes such as urea nitrogen, uric acid, creatinine and ions, so optimum chemical composition of body fluids is maintained. The concentrations of the metabolites increase in blood during renal diseases or renal damage may due to high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels (Barakat & Mahmoud, 2011). Results presented in Table (7) summarize the effect of tested different ratios of Avocado fruits on serum urea nitrogen; creatinine and uric acid in rats fed high cholesterol diets and compared with healthy group.

Table 7: Effect of Avocado on kidney function of male albino rats

Parameters / Group	Urea nitrogen	Creatinine	Uric acid
	mg/dl		
Control (-ve)	85.3±1.50^{c}	0.45±0.01^{c}	1.67±0.16^{d}
Control (+ve)	160.7±2.41^{a}	1.29±0.34^{a}	4.20±0.03^{a}
Avocado 15%	134.3±1.60^{b}	0.40±0.13^{c}	2.65±0.71^{c}
Avocado 25%	65.40±1.05^{d}	0.38 ± 0.12^{c}	1.90±0.23^{c}

**Results are expressed as means ±SD.*
**Values in each column which have different letters are significantly different (p<0.05).*

Serum urea nitrogen:

High cholesterol diet induced hyperlipidemic rats had the higher values of serum urea nitrogen reached to 160.7 ± 2.41 compared with negative control group 085.3±1.50 mg/ dl, may be related to with high cholesterol diet the kidneys are not functioning properly or if the body is using large amounts of cholesterol in the diet, the serum urea nitrogen level will rise. While, the level of urea nitrogen after 45 day decreased gradually according to the concentration, the ratios reached from 160.7 ± 2.41 to 134.40 ± 1.60 and 65.40± 1.05 mg/dl respectively, for Avocado fruits at 15% and 25%. It may also be an indication of dysfunction at the glomerular and tubular levels of the kidney, it is well known that, many biochemicals and histopathological findings confirmed renal damage by high fat diet, these findings are in agreement with Barakat & Mahmoud, (2011).

Serum creatinine:

Serum creatinine increased significantly ($p< 0.05$) in groups of rats fed on high cholesterol diet as compared to the negative control groups (1.29 ± 0.34 and 0.45 ± 0.01), confirmed by Tricia, (2007) who showed that high creatinine levels indicate a person is experiencing kidney failure, also may due to high activities of xanthine oxidase, lipid peroxidation, and increased cholesterol levels (Barakat & Mahmoud, 2011). From Table (7) the mean values and standard deviation of serum creatinine for different supplementation were 0.40 ± 0.13 and 0.38 ± 0.12 mg/dl respectively. These results indicated that there was non-significant difference between negative control group and other groups fed on diets contained 25% Avocado fruits.

Serum uric acid:

It could be observed that the control positive group fed on high cholesterol diet has shown a significant increase ($p< 0.05$) in serum uric acid compared with those of the control negative group fed on basal diet (4.20 ± 0.03 and 1.67 ± 0.16 mg/dl), respectively. From these results, it could observe that there was significant difference ($P< 0.05$) between positive control group and other groups. In fact, oral administration of 15% & 25% Avocado fruit reduced the uric acid level significantly; the concentration of uric acid was reduced by 36.90% and 54.76% respectively. Confirmed by Saleh *et al*., (2002) and Parrish, (2004) they published that the current trends demonstrate a significant increase in this segment of our healthcare population and gastroenterologists can anticipate increased interaction with patients who have hypercholesterolemia disease.

Histopathological results:

Heart:

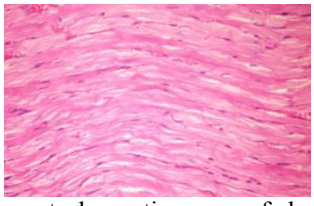

Fig. 1: Microscopically, heart of rat from control negative group fed on basal diet, showed no histopathological changes (H & E X 400).

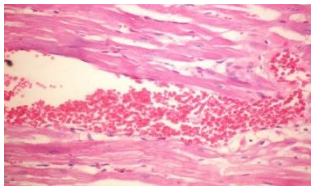

Fig. 2: Heart of rat from group fed on basal diet and 2% cholesterol revealed congestion of myocardial blood vessel (H & E X 400), and intermuscular oedema

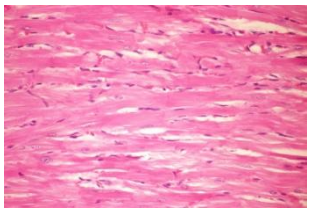

Fig. 3: Examined sections from group fed on positive group + 15% Avocado showed congestion of myocardial blood vessels, whereas, other sections from this group revealed no histopathological changes (H & E X 400).

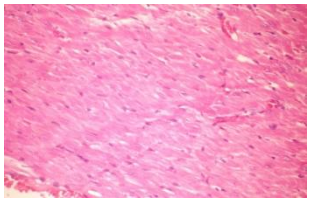

Fig. 4: Showed heart of rat from group fed on positive group + 25% Avocado revealed no histopathological changes (H & E X 400).

Recommendations

Avocado fruits might be recommended for production on a commercial scale in our meals, factories and medicines; such fruits have the capability to decline total cholesterol, triglycerides, LDL-C and increased HDL-C. At the same time, improve the functions of liver and kidney.

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