Hypolipidemic activities of hydroalcoholic extract of avocado fruit on high cholesterol fed diet in rats and its antioxidant effect *in vitro*.

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Abstract: The present study was conducted to investigate the antioxidant effect of hydroalcoholic extract of avocado as a natural antioxidant at different concentration (0.1% and 0.3%) during heating cotton seed oils to different periods of time (7, 14, 21 days). Its antioxidant activity was studied in vitro by determination of peroxide, TBA and DPPH. The results showed that after heating for 21 days, the peroxide value of cotton seed oil, samples treated with different concentration of avocado extract (0.1% and 0.3%) and BHT were 12.9±0.70, 7.20±0.30, 6.73±0.47 and 9.66±0.32meq/kg, respectively. Also, this work aimed to study the anti-hyperlipidemic activities of avocado extract on rats. Hyperlipidemia in the animal was induced by feeding high cholesterol diet for 8 weeks. The results revealed that the rats consumed avocado extract showed significant decreases in serum ALT, AST, Urea, Creatinine, Uric Acid and Bilirubin levels. Also, significant decreases in serum cholesterol, triglycerides (TG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) were observed while there was an increase in serum HDL-C.
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Key words: Avocado extract - antioxidant- DPPH- hypolipidemia- rats.

1- Introduction

A high level of serum cholesterol has been identified clearly as a risk factor for atherosclerosis and coronary heart disease **(Chen *et al.*, 2004).** High cholesterol diet is however regarded as an important factor in the development of cardiac diseases since it leads to development of hyperlipidemia, atherosclerosis, and ischemic heart disease. Moreover, hypercholesterolemia is shown to be one of the major risk factors of atherosclerosis by increasing plasma low-density lipoprotein (LDL) levels **(Levine *et al.*, 1995).** Consumption of fruits and vegetables is associated with a lowered risk of cardiovascular diseases and even cancer **(Block *et al.*, 1992)** Natural medicines have been used empirically to lower the cholesterol levels. Avocado (*Persea Americana* Mill; Family: Lauraceae) fruit is widely consumed throughout the world as food, as well as medicinal purposes. Avocado ranks as the 14th most commonly consumed raw fruit in the United States **(Duester, 2001).** The health benefits of avocado may be due to its contents of over 20 essential nutrients and various disease-curing potential phytochemicals. Avocado fruit and leaves have been used in Latin American folk medicine, including Mexico to treat a variety of diseases. Hot water infusion from its leaves has been used as a diuretic, to induce menstruation and to treat hypertension **(Adeboye *et al.*, 1999).** A previous study among Mexican population has shown that avocado consumption decreases serum total cholesterol, LDL-cholesterol and triglycerides, and increases HDL-cholesterol levels compared to control diet subjects **(LópezLedesma *et al.*, 1996).** Avocado oil-rich diet has been shown to modify the fatty acid content in cardiac and renal cell membranes. Some studies on lipophilic extarcts of avocado inhibited prostate cancer cell apoptosis **(Butt *et al.*, 2006)** and suppressed liver injury **(Kawagishi *et al.*, 2001).**

2- Material and Method

2.1- Material:

Avocado was obtained from local market. Cotton seed oil (obtained from Misr Oil and Soap Company, Mansoura, Egypt). Saturated fat (meat fat) and eggs were obtained from local market, El Dakhlia, Egypt. Twenty female rats weighing 163±4.2g were obtained from Agriculture Research Center, Giza, Egypt. The standard diet prepared according to **NRC (1995)**.

2.2- Method:

2.2.1- Extraction of the hydro-alcoholic extract:

The extraction procedure for the hydro- alcoholic extract was carried out according to **Charles *et al.*(1993)** which modified by **El-Badrawy (1996).** About 250 grams of fruit slices were blended and macerated in 500 ml of methanol over night at room temperature, then filtered and the methanolic crude extract was collected. Another portion of 500 ml of methanol were added to the plant residue and boiled for two hours under reflux condenser in a water bath and then filtered. The filtrate was collected to the

previous crude extract. In the same manner 500 ml portion of water were added to the residue plant and left at room temperature over night, then filtered. The filtrate was added to the previous crude extract. Another volume of water was added to the residue, boiled for two hours under reflux condenser and filtered. The hot water filtrate and the methanolic crude extract obtained previously were gathered to form the hydro-alcoholic crude extract. The solvents were evaporated under vacuum using rotary evaporator. The crude extract was obtained, kept in dark bottles and stored in a deep freezer until use.

2.2.2- Evaluation of antioxidant activity for cotton oil: To assess the antioxidant activities of avocado extract, crude cotton seed oil having initial peroxide value of 1.5 meq/g, was taken for present investigation. This oil is the most frequently used edible oil. Four Petrie dishes were used in this experiment to study the antioxidant activity of avocado extract, each one contains 100g of cotton seed oil. One dish containing cotton seed oil only was used as control, another one was treated by BHT (40mg) as reference whereas the other two dishes were treated by 0.1% and 0.3% of avocado extract. All the dishes were incubated at 60 C for 21 days. Peroxide, TBA values were determined in all incubated dishes at intervals of 7 days during the incubation period. The antioxidant activity of extracts was evaluated by comparing them with the control and the activity of the reference antioxidant (BHT).

2.3- Chemical analysis.

2.3.1- Peroxide values: were determined according to the **AOAC (2000).**

2.3.2- **Determination of thiobarbituric acid value (TBA):**Thiobarbituric acid value (TBA) was determined according to the method described by **Sidwell *et al.* (1954).**

2.3.3- Scavenging effect on DPPH : The DPPH assay constitutes a quick and low cost method, which has frequently been used for the evaluation of the antioxidative potential of various natural products **(Soler *et al.*, 2000).** Due to its odd electron, DPPH gives a strong absorption band at 516 nm (deep violet color). In the presence of a free radical scavenger, this electron becomes paired, resulting in the absorption loss and consecutive stoichionetric decolaoization with respect to the number of electron acquired. The absorbance change produced by this reaction is assessed to evaluate the antioxidant potential of the test sample. Scavenging effect on DPPH radical was determined by the method reported earlier by some authors **(Miller *et al.*, 1997, Sanchez *et al.*, 1998)** with minor modifications. The extract (2.0, 1.5, 1.0 and 0.5mg) in methanol (1 ml) was mixed with 4 ml of 0.004% methanolic solution of DPPH. The mixture was shaken vigorously and left to stand for 30 min in dark at 30 C, and the absorbance was then measured at 517 nm in a spectronic 20 D Milton Roy spectrophotometer.

The percent of DPPH decoloration of the samples was calculated according to the formula

Antiradical activity = absorbance of control-absorbance of sample / absorbance of control

2.4- Biological evaluation:

Avocado extract was dissolved in saline solution just before use and given daily by stomach tube in a dose of 130,150 mg/kg/rat. Fat meat had been submitted to heat to obtain strength of liquid to be able to mix with diet as 10% with removal of corn oil in standard diet. Eggs had been boiled and yolks were separated, rubbed and distributed in trays then dried at 44° C for 4 h in hot oven. After cooling, yolks were ground to obtain powder which added as 10% of diet.

2.4.1- Experimental design: All rats were housed in well aerated cages under hygienic conditions and fed on basal diet for one week for adaption period before using the experimental diets. Food and water were provided *ad-libitum*. In this experiment, five rat served as control (-ve) and 15 rats consumed hypercholesterolemic diet all over the period of the experiment which classified into control (+ve) and treated groups with avocado extract. The trial period was 8weeks. Daily food intake and weekly body weight were recorded. Food efficiency ratio (FER) was calculated at the end of experiment as following:

FER= Body weight gain (gm) / Food intake (gm)

2.4.2- Biochemical analysis: At the end of the experimental period, the rats were anaesthetized by diethyl ether and sacrificed. Blood samples were collected in clean test tubes and left for coagulation then centrifuged at 3000 rpm for 15 minutes to obtain serum. Total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDLc) in serum were determined according to the methods of **Richmond (1973), Fassati** and **Principe (1982)** and **Gordon (1977),** respectively. Low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLC) were calculated using the method of **Hatch and Lees (1968)** and **Friedewald *et al.* (1972),** respectively. Atherogenic indices were obtained by dividing TC / HDLc or dividing LDLc / HDLc according to **Castelli and levitar (1977).** Serum alanine aminotransferase (ALT) and aspartate aminotransferase enzymes (AST) were determined according to the method described by **Reitman and Frankel (1957).** Serum urea and creatinine were determined according to the method described by **Fawcett and Scott (1960)** and **Bartles *et al.* (1972),** respectively. Serum uric acid was estimated an enzymatic method according to **Trinder (1969).**

2.5- Statistical analysis: All obtained data were statistically analyzed by SPSS computer software. The

calculated results were accorded by analysis of variance ANOVA and follow up LSD (SPSS) Computer program variation.

3- Results and Discussion

3.1- Inhibitory effect of avocado extract on peroxide formation in cotton seed oil:

Peroxide value (PV) is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. As it is known, peroxide value is one of the most tests used for the measurement of primary oxidation in oils and fats. In this work, oxidation degree on cotton seed oil samples were determined by measuring peroxide value in the heated oil samples containing avocado extract and BHT every week during the heating period (21days). The results revealed that peroxide value increased linearly with heating time. Cotton seed oil samples without the antioxidant (control) reached maximum peroxide value of 12.9±0.70 meq/kg after 21 days heating. Different results in peroxide value were observed among the control and cotton seed oil samples containing avocado extract and synthetic antioxidant (BHT). After heating for 21days, the peroxide value of cotton seed oil samples treated with different concentration of avocado extract (0.1% and 0.3%) and BHT were7.20±0.30, 6.73±0.47 and 9.66±0.32.

The fruit of *avocado(Persea Americana)* is eaten in many parts of the world and has been shown to possess medicinal properties. The carotenoid content of *Persea americana* has been reported to play significant role in reducing cancer risk **(Lu *et al.*, 2005).** The aqueous leaf extract has also been demonstrated to possess analgesic and anti-inflammatory activities **(Adeyemi *et al.*, 2002).** Other medicinal properties of *Persea americana* are wound healing **(Nayak *et al.*, 2008)** and hepatoprotection **(Kawagishi *et al.*, 2001).**

Table (1): Inhibitory effect of avocado extract on peroxide value

	Zero Time	7 days	14 days	21 days
Oil control	1.58±0.46	5.66±0.70	8.8±0.26	12.9±0.70
Oil + BHT		4.06±0.30*	7.29±0.45**	9.66±0.32**
0.1% Avocado extract		3.76±0.58*	6.10±0.75**	7.20±0.30***
0.3% Avocado extract		3.61±0.38**	5.76±0.41***	6.73±0.47***

Each value is the Mean ± SD Significant with control group* $P > 0.05$ ** $P > 0.01$ *** $P > 0.001$.

3.2- Inhibitory effect of avocado extract on malonaldehyde formation in cotton seed oil measured by using TBA method:

Malonaldehyde formation was determined every 7 days, the effects of avocado extract under study on malonaldehyde formation for cotton seed oil in terms of incubation times versus TBA value at 60C was shown in Table (2). After 7 days the reductions in TBA values caused by all the treated groups were significant as compared to control oil, the highest reduction (0.35) was caused by 0.3%avocado extract followed by 0.1%avocado extract (0.39) while BHT was (0.42).The avocado pear is high in oleic and linoleic acids which play significant roles in human nutrition according to **Golkalp *et al.* (1983**).

Table (2): Inhibitory effect of avocado extract on malonaldehyde formation in cotton seed oil as measured by means of TBA value.

	Zero Time	7 days	14 days	21 days
Oil control	0.13±0.01	0.50±0.03	0.61±0.08	0.66±0.12
Oil + BHT		0.42±0.01**	0.56±0.02	0.61±0.01
0.1% Avocado extract		0.39±0.01**	0.54±0.02	0.57±0.01
0.3% Avocado extract		0.35±0.04**	0.47±0.02*	0.57±0.02

Each value is the Mean ± SD Significant with control group* $P > 0.05$ ** $P > 0.01$ *** $P > 0.001$.

3.3- Scavenging effect of avocado extract on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (%):

The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) is a stable radical with a maximum absorbance at 517 nm that can readily undergo reduction by an antioxidant. Because of the ease and convenience of this reaction, it now has widespread use in the free radical scavenging activity assessment **(Brand Williams, 1995).** The results revealed that the scavenging effect of avocado extract with a concentration of 2mg was 96.06% while it was 90.43, 87.46 and 59.73% at concentrations of 1.5, 1.0 and 0.5mg, respectively. **Wang *et al.* (2010)** described that, phenol compounds from avocado extracts playing a major role in their antioxidant effects against free radicals. **Jaime *et al.* (2011)** stated that avocado (*Persea Americana*) is considered a healthy fruit for its monounsaturated oil similar to olive and content of tocopherols, phytosterols and polyphenols. The fruits were analyzed at the level of pulp, skin and seed.

The results indicated that the tocopherol content was higher in Esther oil (10 mg/g lipid) in Hass (6.9 mg/g lipid) and *Negra La Cruz* (5.7 mg/g lipid). In *Esther* pulp, *it was* found more DPPH antiradical capacity, than the other varieties. On the other hand, the antiradical power of seed and skin of *Hass* was higher. They concluded that the different varieties of avocado and industrial wastes (seed and skin) may be used as good sources of antioxidant ingredients.

Table (3): Scavenging effect (%) of avocado extract on (DPPH) radical.

	2mg	1.5mg	1.0mg	0.5mg
Avocado extract	96.06±0.94	90.43±1.69	87.46±1.2	59.73±3.07

Each value is the Mean ± SD Significant with control group* $P > 0.05$ ** $P > 0.01$ *** $P > 0.001$.

3.4- Biological results.

3.4.1- Effect of avocado extract on body weight, food intake and food efficiency ratio of hyperlipidemic rats.

Data in Table (4) shows that the body weight of all the rats groups increased at the end the experiment. The highest weight gain was noticed in the positive control (37.4g), which revealed higher values than those of normal control group (29.4g). On the other hand, the results show that food efficiency ratio of the positive control (Hyperlipidemic rats) was 2.2 while it was 1.7 in negative control group. The food efficiency ratio of the groups received the avocado extract with concentration of 130 and 150mg were 1.6 and 1.9, respectively.

Table (4): Effect of avocado extract on body weight, weight gain, food intakeand food efficiency ratio of hyperlipidemic rats.

Groups	Initial weight(g)	Final weight(g)	weight gain(g)	weight gain %	Daily food intake(g)	food efficiency ratio
Control (negative)	163.20±4.2	192.60±5.17	29.40±6.94	18.04±4.55	16.54±0.60	1.74±0.45
Control (Positive)	162.2±2.16	199.6±13.9	37.4±14.01	23.04±8.6	16.46±0.6	2.2±0.88
Avocado extract (130mg/kg)	162.4±4.4	191.2±8.7	28.8±11.8	17.8±7.5	16.8±0.44	1.6±0.71
Avocado extract (150mg/kg)	158.2±5.4	191.4±8.5	33.2±10.4	21.06±6.9	16.44±0.5	1.96±0.61

Each value is the mean± SD of 5 rats. Significant with control group* $P > 0.05$ ** $P > 0.01$ *** $P > 0.001$

3.4.2- Effect of avocado extract on lipids profile of experimental rats.

The results recorded in Table (5) showed significant decrease in TC, TG, LDLc and VLDLc and significant increase in HDLc in groups received avocado extracts with concentrations of 130 and 150mg/kg compared to positive control group. These results agreed with that of **Wang *et al.* (2010)** who reported that avocados are rich in monounsaturated fatty acids, fiber, Vitamins B and E and phytosterols which have been shown to exert potent anti-oxidant activities. Avocado consumption also provides more β-sitosterol compared to other edible fruits **(Weihrauch and Gardner, 1978)**. β-sitisterol is also known as anticholesterolemic agent **(Moghadasian and Frohlich, 1999)**. The underlying mechanism of the serum cholesterol-lowering effect of phytosterols involves inhibition of intestinal cholesterol absorption and decrease in hepatic cholesterol synthesis **(Ikeda and Sugano, 1983).** Avocado is also reported to contain several carotenoids and is one of the richest sources of leutin among commonly eaten fruits. Avocado also contain -tocopherols and other bioactive phytochemical substances which are known to possess potent antioxidant properties and beneficial in chronic diseases including prostate cancer and heart diseases. **(Lu *et al.*, 2005).**

Table (5): Effect of avocado extract on serum lipid profile of experimental rats.

Groups	T.C.(mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Control (negative)	61.8±7.12	87.0±1.87	33.2±2.94	17.6±6.6	17.4±3.7
Control (positive)	118.2±17.65	145.0±56.4	29.2±2.5	67.2±18.5	29.0±11.2
Avocado extract (130mg/kg)	78.8±6.6**	83.8±25.5*	35.8±2.16**	19.4±7.3**	16.7±5.1*
Avocado extract (150mg/kg)	84.4±13.1***	80.4±13.7*	37.8±2.2**	17.0±5.0***	16.08±2.7*

TC:Total cholesterol - TG:Triglyceride - HDL:High density lipoprotein cholesterol -
LDL:Low density lipoprotein cholesterol - VLDLc:Very low density lipoprotein cholesterol
Each value is the mean± SD of 5 rats. Significant with control group* $P > 0.05$ ** $P > 0.01$ *** $P > 0.001$

3.4.3- Atherogenic indices of experimental rat groups at the end of the study

Belonging to the ratio of cholesterol/HDL-cholesterol, it was found that the treated groups (which received 150mg|kg and 130mg|kg avocado extract) revealed significant decreases in cholesterol/HDL-c ratio and LDL-c/HDL-c ratio as compared to hyperlipidemic control (positive control). It is now established that the high-cholesterol diet (HCD) and the resultant hyperlipidemia causes an increase in TC, LDL-cholesterol levels to enhance the risk for the development of atherosclerosis **(Genest *et al.*, 1992).** Additionally, serum TG is also considered a risk factor for atherosclerosis **(Jones and Chambliss, 2000).** High levels of LDL-cholesterol (bad cholesterol) are also a high risk factor of coronary heart diseases **(Smith *et al.*, 2004).** The association between hyperlipidemia and atherosclerosis has been demonstrated in many studies and trials **(Gordon *et al.*, 2007).** Hyperlipidemia may be manifested by elevation of total cholesterol, low density lipoprotein and triglycerides concentrations and a reduction in high density lipoprotein concentration. It has also been shown that reducing plasma level of LDL cholesterol sharply reduced the risk of coronary heart disease **(Superko and Krauss, 1994).**

Table (6): Atherogenic indices of experimental rats groups at the end of the study

Groups	Cholesterol/HDLc	LDL/HDLc
Control (-)	1.86 ± 0.19	0.53 ± 0.22
Control (+)	4.05 ± 0.68	2.30 ± 0.59
Avocado extract (130mg/kg)	2.20 ± 0.27**	0.54 ± 0.25**
Avocado extract (150mg/kg)	2.23 ± 0.32**	0.45 ± 0.14***

Each value is the mean± SD of 5 rats. Significant with control group* $P > 0.05$ ** $P > 0.01$ *** $P > 0.001$

Furthermore, high plasma levels of HDL cholesterol are associated with lower risk of coronary heart disease and it is widely believed that HDL protects against atherosclerosis by facilitating reverse cholesterol transport (Van *et al.*, 2009).

3.4.4- Effect of avocado extract on Serum ALT, AST, Urea, Creatinine, Uric Acid and Bilirubin of different experimental rats:

The effect of avocado oil on serum ALT, AST, Urea, Creatinine, Uric Acid and Bilirubin on the rats fed on high cholesterolemic diet are shown in Table (7). It was noticed that oral administration of high cholesterolemic diet elevated the ALT, AST, Urea, Creatinine, Uric Acid and Bilirubin levels in serum of positive group although the levels were within the normal levels. Treatment of rats with the two concentrations of avocado extracts (130 and 150mg/kg) significantly reduced the serum levels of ALT, AST, Urea, Creatinine, Uric Acid and Bilirubin. These results agreed with that of Butt *et al.* (2006) who reported that avocado oil-rich diet has been shown to modify the fatty acid content in cardiac and renal cell membranes. Some studies on lipophilic extarcts of avocado inhibited prostate cancer cell apoptosis and suppressed liver injury (Kawagishi *et al.*, **2001).**

Table (7): Serum ALT, AST, urea, creatinine, uric acid and bilirubin of different experimental rats at the end of the study

Groups	ALT (IU)	AST (IU)	Urea (mg/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)	Bilirubin (mg/dl)
Control -	34.6 ±5.59	20.0±5.56	25.4±2.9	0.51±0.10	2.8±0.41	0.48±0.05
Control +	47.0±9.0	26.0±3.3	33.60±4.5	1.66±0.34	4.02±0.46	0.82±0.22
Avocado extract (130mg/kg)	30.4±6.5*	18.8±4.81*	26.2±2.4**	0.58±0.31***	2.92±0.49**	0.47±0.04**
Avocado extract (150mg/kg)	30.60±4.8**	19.8±4.9*	25.6±3.2**	0.54±0.13***	2.44±0.56**	0.44±0.03**

ALT:alanine aminotransferase enzymes AST:aspartate aminotransferase enzymes

Each value is the mean± SD of 5 rats. Significant with control group* $P > 0.05$ ** $P > 0.01$ *** $P > 0.001$.

It can be concluded that avocado fruit pulp has a definite antioxidant, anti-hyperlipidemic and urono-hepatic protective potential. So it is recommended to do further investigations to determine and isolate the main functional ingredients in the fruit to be used in food and pharmaceutical fields.

References

1. A. O. A. C., (2000): Association of Official Agricultural Chemists. Official Method of Analysis. 17th Ed. Vol. 11. Washington U.S. A.
2. Adeboye J.O; Fajanyomi M.O.; Makinde J.M. and Taiwo O.B. (1999). A preliminary study on the hypotensive activity of Persea Americana

leaf extracts in anastehesized normotensive rats. Fitoterapia, 70:15020.
3. Adeyemi, O.O., Okpo, S.O.and Ogunti, O.O. (2002). Analgesic and anti-inflammatory effects of the aqueous extract of leaves of Persea americana (Lauraceae). Fitoterapia, 73, 375- 380.
4. Bartles, H.; Bohmer, M. and Heirli, C. (1972): Colorimetric kinetic method of determination of creatinine, J.Clin.Chem. Acta, (37): 193.
5. Block G.; Patterson B. and Subar A. (1992). Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. Nutr. Cancer,18: 1-29.
6. Brand Williams, W.; Cuvelier, M.E. and Benset, R. (1995), Use of free radical methods to evaluate antioxidant activity. Lebensm Wiss Technol. 28, 25-30.
7. Butt A.J; Roberts C.G.; Seawright A.A.; Oelrichs P.B. and Macleod J.K. (2006). A novel plant toxin, persin, with in vivo activity in the mammary gland, induces Bim-dependent apoptosis in human breast cancer cells. Mol. Cancer Ther., 5: 2300- 9.
8. Castelli, T. and Levitar, Y. (1977): Atherogenic, indices, J.Curr Presc p39.
9. Charles, D.J.;Morales, R. and Simon,E. (1993): Essential oil content and chemical composition of hydroalcoholic extract of fennel, New crops,570-3
10. Chen C.; Chou F.P.and Ho YC (2004). Inhibitory effects of Hibiscus sabdariffaL. extract on low-density lipoprotein oxidation and antihyperlipidemia in fructose-fed and cholwesterol-fed rats. J. Sci. Food Agric., 84: 1989-96.
11. Duester K.C. (2001).Avocado fruit in a rich source of beta-sitosterol. J.Am. Diet Assoc., 101: 404- 5.
12. El-Badrawy, El S. El B.Y. (1996), Biochemical studies on some natural plant products. Ph.D. Thesis, Fac. of Agric., Mansoura Univ., Egypt.
13. Fassati, P. and Principe, L. (1982): Enzymatic colorimetric method for the determination of triglycerides, J.Clin. Chem., (28):2077.
14. Fawcett, J.K. and Scott, J.E. (1960): Determination of serum urea, J. Clin. Path., (13): 156-9.
15. Friedwald, W.T., Leve., R.I. and Frederickson, D.S. (1972): Estimation of the concentration of low-density lipoprotein separated by three different methods. Clin. Chem., 18: 499-502.
16. Genest JJ.; Martin-Munley SS.; McNamara JR. and Ordovas JM.(1992): Familial lipoprotein disorders in patients with premature coronary artery disease. Circulation, 85: 2025-33.
17. Golkap, HV.; Ockerman, HW.; Plimpton RF. and Harper WJ.(1983): Fatty acids of neutral and phospholipids: rancidity scores and TBA values as influenced by packaging and storage. J Food Sci.; 48: 829.
18. Gordon, T. M. (1977): HDL–cholesterol (determination after separation high-density lipoprotein lipid). Amer. J. Med. (62):707.
19. Gordon, T., Castelli, W.P., Hjortland, M.C., Kannel, W.B.and Dawber, T.R. (2007). High density lipoprotein as a protective factor against coronary heart disease: The Framingham study. Am. J Med., 62(5), 705-714.
20. Hatch, F. T. and Lees, R. S. (1968): Practical methods for plasma lipoprotein analysis. Adv. Lipid Res., (6):1–68.
21. Ikeda I. and Sugano M. (1983): Some aspects of mechanism of inhibition of cholesterol absorption by beta-sitosterol. Biochim. Biophys. Acta., 732: 651-8.
22. Jaime Ortiz;Nalda Romero;Rodolfo Gárate and Fernando Osorio.(2011) Department of Food Science and Chemical Technology,Faculty of Chemical and Pharmaceutical Sciences, University of Chile. V. Mackenna 20-
23. Jones JD. and Chambliss ML (2000): Hypertriglyceridemia and coronary heart disease. Arch. Fam. Med., 9(2): 189-190.
24. Kawagishi H.; Fukumoto Y.; Hatakeyama M. and Sugiyama K. (2001). Liver injury suppressing compounds from avocado (Perseaamericana). J. Agric. Food Chem., 49: 2215-21.
25. Levine G.N.; Keaney JF. and Vita JA. (1995). Cholesterol reduction in cardiovascular disease. Clinical benefits and possible mechanisms. N. Engl. J. Med., 332: 512-21.
26. LópezLedesma R.; FratiMunari A.C.; Hernández Domínguez BC. and Cervantes Montalvo S. (1996). Monounsaturated fatty acid (avocado) rich diet for mild hypercholesterolemia. Arch. Med. Res., 27: 519-23.
27. Lu QY.; Arteaga JR.; Zhang Q.; Huerta S. and Heber D. (2005): Inhibition of prostate cancer cell growth by an avocado extract, Role of lipid-soluble bioactive substances. J. Nutr.Biochem., 16: 23-30.
28. Miller, N.; Rice, D. and Evans, E. (1997), Factors influcencing the antioxidant activity determined by ABTS, radical cation assay. 26, 195-9.
29. Moghadasian MH. and Frohlich JJ. (1999): Effects of dietary phytosterols on cholesterol metabolism and atherosclerosis, Clinical and experimental evidence. Am. J. Med., 107: 588-94.

30. Nayak, B.S., Raju, S.S.and Chalapathi, A.V. (2008). Wound healing activity of Persea americana (avocado) fruit: a preclinical study on rats. J Wound Care, 17(3), 123-126.
31. NRC(1995): National Research council: nutrient requirements of laboratory animals, fourth revised edition, 29-30 national academy press.washington, DC.
32. Reitman, S. and Frankel, S. (1957): Determination of glutamate pyruvat transaminase and glutamate oxaloacetate transaminase. Amer. J. Clin. Path., 28: 56-63.
33. Richmond, N. (1973): Colorimetric method of determination of total cholesterol and high density lipoprotein cholesterol (HDLc). Clin. Chem, 19, 1350-1356.
34. Sanchez, M. and Calixto, F. (1998), A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Agric. 76, 270-6.
35. Sidwell, C.G.; Salwin, H.; Benca, M. and Mitchell, J.R., J. H., (1954): The use of thiobarbituric acid as a measure of fat oxidation. J. Am. Oil Chem. Soc.; 31 (12), 603 – 606.
36. Smith C.; Yndestad A.; Halvorsen B. and Ueland T. (2004): Potential anti-inflammatory role of activin A in acute coronary syndromes. J. Am. Coll. Cardiol., 21: 44: 369-75.
37. Soler- Rivas, C.; Espin, I. C. and Wichers, I. (2000), An easy and fast to compare total free radical scavenger capacity of food studys. Phytochem Anal 11; 330-8.
38. Superko, H.R.and Krauss, R.M. (1994). Coronary artery disease regression. Convincing evidence for the benefit of aggressive lipoprotein management. Circulation, 90, 1056-69.
39. Trinder, P. (1969) Ann Clin Biochem 6-24.
40. Van, E.C., Lievens, J., Jacobs, F., Feng, Y., Snoeys, J.and Geest, B.A. (2009). Apolipoprotein A-I and lecithin cholesterol acyltransferase transfer induce cholesterol unloading. Gene therapy, 16, 757-765.
41. Wang W, Bostic TR and Gu L (2010): Antioxident capacities, procyanidins and pigments in avocados of different strains and cultivar. Food Chem., 122: 1193-1198.
42. Weihrauch JL and Gardner JM (1978): Sterol content of foods of plant origin. J. Am. Diet. Assoc., 73: 39-47.

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