

Full Length Research Paper

The effect of aqueous seed extract of *persea americana* (avocado pear) on serum lipid and cholesterol levels in rabbits

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The research work focused on the effect of aqueous extract of the seed of *Persea americana* (Avocado) on the cholesterol, triglycerides, low density lipoproteins levels in the blood of rabbits. A total of thirty rabbits were used of ten in each of groups A, B and C. Group A served as control. The test groups (B and C) were fed *ad libitum* with starter finish before and after administration of the extracts (w/w) by intubation. Two concentrations of the extracts of 100mg/kg body weight and 200mg/kg bodyweight were administered to groups B and C animals respectively. After two months of feeding, the animals were sacrificed and their serum samples used for the various determinations.. Results showed that for cholesterol, the mean cholesterol levels for the control group A and test groups B and C were: 1.54 ± 0.07 , 1.30 ± 0.06 and 0.93 ± 0.03 mmol/L respectively. The triglyceride concentrations were as follows: 1.69 ± 0.03 , 0.96 ± 0.1 and 0.65 ± 0.02 mmol/L. For the low density lipoprotein (LDL), the values were: 0.84 ± 0.12 , 0.81 ± 0.02 and 0.72 ± 0.01 mmol/L. The total lipid concentrations were as follows: 4.01 ± 0.01 , 2.62 ± 0.00 and 2.40 ± 0.01 mmol/L. Phytochemical analysis revealed the presence of the following and values expressed in percentage (%): tannins 21.66 ± 0.0 , saponins 51.0 ± 0.0 , flavonoids 21.0 ± 0.0 , alkaloids 9.43 ± 0.2 and cyanogens glycosides 4.86 ± 0.10 mg . The result of this work shows that the extract of the seed of *Persea americana* reduced the cholesterol, triglyceride, the LDL cholesterol and the total lipid levels significantly in the sera of the test animal and the effect of the extract was concentration dependent. Moreover, this seed often thrown away as waste, may prove to be an effective remedy for the management of atherosclerosis, hypertension, hyperlipidemia, hypercholesterolemia and other abnormalities of lipid metabolism in humans. The extract of *Persea americana* could be of immense therapeutic relevance in the management of hypertension and other cardiovascular episodes.

Key words: Aqueous seed extract, *Persea americana* , hypercholesterolemia , hypertension.

INTRODUCTION

Cholesterol is a lipid waxy steroid found in the cell membranes of humans and transported in blood plasma of all animals [Emma 2009]. It is an essential component of mammalian cell membranes where it determines its fluidity. It is the principal steroid synthesized by animals but small quantities are stored by other eukaryotes like plants and fungi. It is almost

completely absent in prokaryotes, which include bacteria [Pearson et al 2003]. However, high levels in blood circulation depending on the type of lipoprotein transporting it are strongly associated in the progression to atherosclerosis. Cholesterol is an important precursor molecule for the synthesis of vitamin D and other steroid hormones including the adrenal hormones, cortisol and aldosterone , as well as the sex hormones such as testosterone, progesterone and estrogen. Some research findings indicate that cholesterol may act as an antioxidant [Smith LL 1991]. Major dietary sources of

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cholesterol include: cheese, egg yolks, beef, pork, poultry and shrimp. Human breast milk also contains significant quantities of cholesterol. Cholesterol is not present in plant-based food sources; peanuts contain healthy cholesterol-like compounds called phytosterols, which are suggested to lower serum cholesterol levels [Gotto AM Jr. 2003]. Triacylglycerols stored in adipose tissues are a major form of energy store in animals and a form of fat in the blood stream of humans. People with higher triglycerides often have higher cholesterol levels. Several studies have shown that people with more than triglyceride levels (greater than or equal to 200mg/dl), have increased risk of heart disease [Gotto AM Jr. 2003]. In recent years, evidence has emerged showing that lipid signaling is a vital part of cell signaling [Wang X. 2004]. Lipid signaling may occur via the activation of G-protein coupled to nuclear receptors [Eyster, KM 2007]. One of these signaling molecules includes sphingosine-1-phosphate, a sphingolipid derived from ceramide, a potent messenger molecule involved in regulating calcium mobilization, cell growth and apoptosis [Hinkovska-Galcheva et al 2008, Saddough SA 2008]. Most vegetable oils are rich in linoleic acid, e.g. sunflower, corn oil. Alpha (α) – linoleic acid is found in the green leaves of plants and in selected seeds, nuts and legumes (flaxseed, walnut and soy). Fish oils are particularly rich in the longer chain Omega-3-fatty acid – docosahexanoic acid (DHA). A large number of studies have shown positive health benefits associated with the consumption of Omega-3-fatty acids especially on infant development, cancer, cardiovascular diseases and various illnesses such as depression, attention deficit, hyperactivity disorder and dementia [Riediger et al 2007]. Total fat plays a large role in blood cholesterol than the intake itself. Trans fat can be found in commercial food supplies, fast food, snack food, fried foods and baked foods, which is a risk factor for cardiovascular disease [Galli and Rise 2009, Micha and Mozaffarian 2008]. A charged diet may help reduce blood cholesterol levels in addition to other life style modifications. Avoiding animal products may decrease cholesterol levels in the body. Sugars and grains require insulin secretion which is a potent stimulus to the liver to produce triglycerides. High triglycerides in blood are often seen in overweight people. The right approach is to radically reduce the consumption of simple sugars and grains. It is really inexpensive to substitute sugars and grains with green-leafy vegetables which have low glycemic index.

High density lipoprotein (HDL) packets the cholesterol formed and helps to transport it from the blood to other body tissues, especially the liver. Low density lipoprotein (LDL), does not aid transportation of cholesterol out of body tissues but deposits cholesterol in the lumen of arterial walls. Low density lipoprotein (LDL) molecules contain much more cholesterol than HDL. LDL is called “bad” cholesterol and high levels of LDL in the body can clog the arteries and increase the risk of heart attack and

stroke. LDL causes a build up of cholesterol plaques in the walls of arteries of the heart and brain [Robert et al 2006]. Triglycerides are a form of fat in the blood stream and people with high triglyceride levels often have high level of total cholesterol. Many people with heart diseases have high triglyceride levels. Several clinical studies have shown that people with more than normal triglyceride levels, greater than (200mg/dl) have an increased risk of heart disease and people with diabetes mellitus and obesity also have high triglyceride levels [Gotto AM Jr. (2003)].

Persea americana, is a tree native to the Carribeans. The name “avocado” also refers to the fruit of the Kupa shell that contains a pill (hard seed casing), which may be egg shaped or spherical. High *Persea americana* (avocado) extract intake has been shown to have effect on serum cholesterol levels. Specifically, after a seven day diet rich in *Persea Americana* leaf extract, hypercholesterolemic patients showed a 107% decrease in total serum cholesterol levels. These subjects also showed a 22% decrease in both LDL, triglyceride levels and 11% in HDL levels [Micha and Mozaffarian 2008, Robert et al 2006]. The Canadian Hass Avocados (*Persea americana*) were found to contain the highest amount of Lutein among commonly eaten fruits as well as some measurable amounts of related carotenoids, such as Zeaxanthin; and α and β -carotenes. Lutein accounts for 70% of the measured carotenoids. The pear contains significant quantities of vitamin E [Lu et al 2005]. An active extract of avocado containing these carotenoids and tocopherols was shown to inhibit the growth of both androgen dependent (LNCap) and androgen independent (PC-3) prostate cancer cell lines *in vitro* [Lu et al 2005]. Researchers from South Africa proposed the use of *Persea americana* (Avocado) leaf extract for the management of childhood convulsions. The data from their study in rats suggested that avocado aqueous leaf extract produced its anticonvulsant effect by enhancing GABAergic neurotransmission and its action in the brain. Avocados are loaded with nutrients such as dietary fiber, vitamin E, potassium, magnesium and folate. They are also cholesterol and sodium free. It is an excellent source of monounsaturated fatty acids, vitamins B and K [Navel et al 2002]. The main objective of this work includes: (a) to demonstrate the presence of some phytochemicals present in the extracts and (b) to assess the effects of the extracts *in vivo* in lowering cholesterol and triglyceride levels in rabbits.

MATERIALS AND METHODS

MATERIALS

The following reagents were used: alcoholic KOH, Hexane, Acetic acid, acetic anhydride, Sulfuric acid, Libermann-Buchard reagent for color development,

Table 1: Quantitative values of phytochemicals from (Avocado Pear) *Persea americana* seeds, expressed in percentage.

Sample glycosides	tannins	saponins	flavonoids	alkaloids	cyanogenic
<i>Persea americana</i> seed	21.66±0.0	51.00±0.0	21.0±0.0	9.43 ± 0.2	4. 86±0.1 mg

Values in the table are Means ±SD from triplicate (n=3) determinations.

Unicam Spectronic-20 DR, all supplied by BDH Biochemicals ,London.

DESIGN OF THE EXPERIMENT.

The research was carried out by having three groups of rabbits of ten (10) animals in each group These groups were designated A, B and C. The animals were first of all kept in separate cages for two weeks to be used to the environment .They were provided with light 12 hours/day and were fed *ad libidum*. The weights of the animals were from 3.35-5.1kg bw. Group A animals served as control. The test groups were groups B and C which were fed *ad libidum* with starter finish before and after administration of the extracts .Two concentrations of the extracts were used - 100mg/kg bw and 200 mg/kg bw. The feeding experiment lasted for 2 months. At the end of the feeding exercise, the rabbits were sacrificed and the serum samples collected and analyzed for triglycerides (TG), cholesterol and LDL-cholesterol (LDLC) respectively.

METHODOLOGY

(a) In the CDC reference method for triglycerides; the triglycerides were first extracted quantitatively with chloroform to remove water soluble interfering substances such as glucose and glycerol from the serum and the analysis carried out by the methods [Nador and Warnick 2006]

(b). Measurement of cholesterol.

The CDC reference method for cholesterol is based on a chemical reaction method devised by Abel and Colleagues [Deacon and Dawson 1979]. In the CDC version of this method, 0.5 ml aliquot of serum is treated with 5.0 ml of alcoholic KOH (0.36 mol/L) to hydrolyze the cholesterol esters. Total cholesterol is then extracted from the mixture with 10 ml of hexane for 15 minutes. An aliquot of the extract is dried in *vacuo* and the residue treated with 3.2ml of a mixture of acetic acid, acetic anhydride and sulfuric acid (Liebermann-Buchard reagent)

for color development. After 30 minutes, absorbance is read at 620 nm. Cholesterol concentration is expressed either in terms of moles (millimoles per liter (mmol/L) or mass (milligram per deciliter (mg/dl) [Deacon and Dawson 1979, Ellerbe et al 1990].

(c) Indirect Methods.

Indirect methods for measuring LDL- cholesterol are based on measuring a number of lipid related analytes followed by their use in calculating the cholesterol content of the serum. In the most widely used indirect method, cholesterol, triglyceride and HDL-cholesterol are measured and LDL-cholesterol is calculated from the primary measurements using the empirical equation of the Friedewald and colleagues [Friedwald 1972]. The Friedewald Equation is given and interpreted as follows

$$H = C - L - KT$$

Where, H = HDL cholesterol

L =LDL cholesterol

T = Triglyceride and

K = 0.20 if the quantities are measured in mg/dl and 0.45, if in mmol /L

C= Total Cholesterol

Phytochemical screening

Phytochemical screening and quantitation was carried out by the established methods of Harborne and co-workers [Durrington 2003] and the tests involved those of alkaloids, flavonoids, saponins, tannins and cyanogenic glycosides etc.

Statistical Analysis

Two statistical methods were applied to compare and validate the results. These included: (a) The determination of the Mean and standard deviation values (b) The use of students t -test to compare results.

RESULTS

Results are presented in tables 1-5. Table 1 shows the

Table 2. Cholesterol levels of rabbits in the experimental groups compared with the control: A(control),B(test)andC(test)

A std	C std	ABS Control (A)	ABS 100 mg/ kg bw	ABS 200 mg/kg bw	Conc. (A) control	Conc . (B) 100 mg	Conc . (C) 200 mg
0.212	5.18	0.063	0.055	0.039	1.53934	1.343868	0.952925
0.212	5.18	0.064	0.051	0.037	1.563774	1.246132	0.904057
0.212	5.18	0.059	0.055	0.039	1.441604	1.343863	0.952925
0.212	5.18	0.066	0.051	0.037	1.612642	1.246132	0.904057
				Mean (X)	1.54	1.30	0.93
				S.D	0.07	0.06	0.03
Values are cholesterol concentrations in serum in mmol /L					Control	100mg/kgbw	200mg/kgbw
					1.54±0.07	1.30±0.06	0.93±0.03

ABS = Absorbance kg/bw = kilogram per body weight.

There is significant difference between the cholesterol levels of the control group (A) and the test groups B and C at $p < 0.05$. The values in the table are the Mean \pm SD from quadruplicate determinations. Students t-test analysis shows that, there is significant difference between the cholesterol levels of the control and the test animals, $t = 4.57$ and $t_{\alpha} = 1.734$

Table 3. Triglyceride levels of rabbits in groups: A (control), B (test) and C (test)

A std	C std	ABS Control (A)	ABS 100 mg/kg bw (B)	ABS 200mg/kg bw (C)	CONC. (A) control	CONC. (B) 100mg	CONC. (C) 200 mg
0.234	2.26	0.179	0.1600	0.064	1.728803	0.965812	0.618120
0.234	2.26	0.171	0.0990	0.067	1.651538	0.956154	0.647094
0.234	2.26	0.179	0.1000	0.070	1.728803	0.965812	0.676068
0.234	2.26	0.171	0.0990	0.067	1.651538	0.956154	0.647094
				Mean (X)	1.69	0.96	0.65
				S.D	0.04	0.01	0.02
Values are triglyceride concentrations in serum in (mmol /L)					Control	100mg/kgbw	200mg/kgbw
					1.69±0.04	0.96±0.01	0.65±0.02

There is significant difference in the triglyceride (TG) concentrations of the Group (A) animals and the test Groups (B and C) at $p < 0.05$.

quantitative values of the phytochemicals identified in *Persea Americana* seed extract .Table 2 shows the cholesterol levels of rabbits in the three groups (A ,B and C) of animals used for the study. Table 3 shows the triglyceride levels (TG) of both the control and test groups. Table 4 depicts the LDL-cholesterol levels before and after administration of the plant extracts at two different concentrations of 100mg/kgbw and 200mg/kgbw respectively. Table 5 shows the VLDL cholesterol level , showing the effect of the seed extracts in lowering the levels of this form of cholesterol.

DISSUSSION

Cholesterol is a lipid, waxy steroid found in cell membranes and maintains the fluidity of the membrane. It can dissolve and flow in the water-based blood stream at exceedingly small concentrations. It is transported in the circulatory system with lipoproteins. Abnormal high dietary cholesterol levels lead to hypercholesterolemia, which is strongly associated with cardiovascular diseases because it promotes atherosclerosis [Durrington 2003]. In this study, we examined the effects of aqueous seed extract of *Persea americana* on serum cholesterol of rabbits compared with the values in normotensive (non-

Table 4. Low density lipoproteins (LDL) values of rabbits administered extracts of *Persea americana*

A std	C std	ABS Control (A)	ABS 100 mg/kg bw (B)	ABS 200mg/kg bw (C)	CONC. (A) control	CONC. (B) 100mg	CONC. (C) 200 mg
0.41	5.18	0.075	0.063	0.056	0.945225	0.794015	0.705791
0.41	5.18	0.058	0.066	0.058	0.730998	0.831825	0.730998
0.41	5.18	0.075	0.063	0.056	0.945255	0.794015	0.705791
0.41	5.18	0.058	0.066	0.058	0.730998	0.831825	0.730998
				Mean (X)	0.84	0.81	0.72
				S.D	0.12	0.02	0.01
Values are LDL-cholesterol- Concentrations in the serum in (mmol /L)					Control	100mg/kgbw	200 mg/kgbw
					0.84±0.12	0.81±0.02	0.72±0.01

The base line concentration of 0.84±0.12 is the LDL-concentration of the control. Compare with 0.81±0.02 and 0.72±0.01 at concentrations of 100 mg/ kg bw and 200 mg/ kg bw of the extracts respectively.

Table 5. Very low density lipoprotein levels (VLDL) of rabbits administered extracts of *Persea Americana*

A _{STD}	C _{STD}	ABS Control) A	ABS 100 mg/kgbw B	ABS 200 mg/kgbw C	CONC. (A) control	CONC. (B) 100 mg/kgbw	CONC. (C) 200 mg/kgbw
0.0468	0.452	0.0358	0.0320	0.0128	0.3457606	0.1931624	0.1236244
0.0468	0.452	0.0342	0.0198	0.0132	0.3303076	0.1912308	0.1294188
0.0468	0.452	0.0358	0.0200	0.0140	0.3457606	0.1931624	0.1352136
0.0468	0.452	0.0342	0.0198	0.0132	0.3303076	0.1931624	0.1294188
				Mean X=	0.34	0.19	0.13
				SD	0.00	0.00	0.04
Values are VLDL- cholesterol concentrations in nmol / L:					Control	100 mg/ kgbw	200 mg/kg bw
					0.34 ± 0.0	0.19 ± 0.0	0.13 ± 0.04

hypertensive) rabbits. Table 1 shows the phytochemical composition of *Persea americana* seeds. It can be seen that the seed is rich in saponins (51.0±0.0 %), tannins (21.66± 0.0%), flavonoids (21.00±0.0%) and alkaloids (9.43±0.2%). The medicinal effects of some phytochemicals like flavonoids, saponins and alkaloids have been documented. Flavonoids have been found to be an active principle in many herbal medicines [Bonilla and Gilbertsville 2009]. A lot of flavonoid containing herbs have been known for their anti-allergic, anti-inflammatory, anti-thrombotic and vasopressor properties. All these actions have been attributed to its action on arachidonic acid [Bonilla and Gilbertsville 2009]. Apart from flavonoids, saponins are another group of plant phytomedicines. They are steroidal compounds in plants and have been found to possess oral contraceptive and diuretic effects. Most drugs used in the management of hypertension possess the same mechanism of action such as ModureticTM and LasixTM. Flavonoids are

powerful antioxidants that may help to protect LDL cholesterol against oxidation and reduce platelet aggregation, making blood clots less likely [Hertog 1995, Fuhrman and Aviram 2001, Whitney et al 2002]. Table 2 shows the cholesterol levels of rabbits fed diets containing various concentrations of *Persea americana* seed extracts compared with the control animals in group (A). The mean cholesterol levels of the groups expressed in mmol/L were: Group A (control) 1.54±0.07, Group B (test group 1) 1.30±0.06 at 100 mg/kg bw; Group C (test group 2)-0.93±0.03 at 200 mg/kg bw. Table 3 shows the serum triglyceride concentration of rabbits treated with the same concentrations of *Persea americana* seed extracts. Results showed that the extracts decreased the serum levels of triglycerides in the two test groups in a concentration dependent manner. There is statistical difference between the TG levels of the test groups and the control at p≤ 0.05. Table 4 shows low density lipoprotein (LDL) levels in rabbits. The values were:

Group A (control)- 0.84 ± 0.12 , Group B (test group 1)- 0.81 ± 0.02 and Group C (test group 2)- 0.72 ± 0.00 . There is statistically, a significant difference between the test results and the control at $p < 0.05$. Administration of two different doses of 100 mg/kgbw and 200 mg/kgbw respectively to the test animals resulted in a significant reduction in serum TG levels. Suppression of TG levels implies that bile acid synthesis was reversed. By this TG reduction by the extract, VLDL cholesterol levels also decreased, indicating that increase in intracellular TG also leads to increased synthesis of VLDL levels [Asaolu et al 2010]. This is exactly consistent with our finding, as could be seen in table 5. Similar findings have been reported by other workers [Shirdel et al 2009]. VLDL is indirectly involved in LDL- cholesterol (LDLC) generation. The extract equally reduced the level of LDL, but not significantly enough as could be seen in table 3. There is an inverse relationship between plasma TG concentration and HDL cholesterol levels.

The triglyceride (TG) level is a predictor of HDL concentrations; hence HDL levels would increase when VLDL levels decrease; although not shown. Many workers have reported on the hypolipidemic effects of the leaf extracts of *Persea americana*. Findings from other research works have attested to the fact that extracts of the leaves of the plant possess the potential to reduce plasma lipid or triacylglyceride levels in rats fed hyperlipidemic diets. Leaf extracts of *Persea americana*, when administered intravenously, induced a marked fall in mean arterial blood pressure lasting 2-3 minutes [Shirdel et al 2009]. Other workers reported the pre-treatment of rats with aqueous seed extract of *P. americana*, over a period of ten (10) days which resulted in the reduction of mean arterial pressure in the rats [Ogochukwu et al 2009, Owolabi et al 2005, Ojewole and Amabeoku 2006, Ojewole et al 2007]. Phytochemical screening of the extracts revealed the presence of tannins. Tannins are polyphenolic compounds that possess vaso-relaxant effects. They are also reported to have physiological effects like anti-irritancy, antimicrobial and anti-diarrheal [Westendarp 2006, Stoclet et al 2004]. Mechanisms attributed to the vaso-relaxant actions of polyphenols include: the inhibition of protein kinase C, inhibition of cyclic nucleotide phosphodiesterases and decreased Ca^{2+} uptake [Herrera et al 1996]. Some polyphenols have been reported to possess contractile properties in vascular smooth muscles which may be responsible for the reduction of blood pressure in experimental rats [Senae et al 2003]. There are several medications that help to lower plasma lipids. Most medications can reduce LDL-cholesterol by 20-40% and may modestly increase HDL-cholesterol. However, these synthetic medications are not without adverse effects. In view of this, the quest for natural products with lipid lowering potential with or without side effects is warranted. The use of medicinal plants extracts more importantly, in the treatment of hypertension and other

related cardiovascular diseases is increasing because of their cheapness, availability, apparent lack of adverse effects and time-trusted efficacy [Fogari and Zoppi 2004]. The role of most plant sterols have been documented. One strategy adopted in the reduction of cholesterol levels involves the use of such plant sterols such as sitosterol, stigmasterol and campesterol, whose mechanism of action despite their structural similarity to cholesterol involves the inhibition of cholesterol absorption by the intestinal cells [Evans 2005]. Plant sterols administered in a soluble micellar form are more effective in blocking cholesterol adsorption than plant sterols administered in a solid/crystalline form. In this work, we have demonstrated the effectiveness of the seed extract in lowering the cholesterol level in rabbits at different concentrations. This is similar to the effect of some mixtures of plant sterols including soy bean sterols and β -sitosterol [Brai et al 2007, Ogochukwu et al 2009]-dihydrostigmasterol. Studies have shown that high *Persea americana* seed intake has an effect on blood serum cholesterol levels.

Results from this work have proved that crude aqueous seed extracts of *Persea americana*, can be used effectively to manage hypertension, cardiovascular diseases and other similar health conditions. Our choice for the use of aqueous crude extracts in the study was based on the reports of studies by many researchers and ethno-medicinal practitioners from Edo State and other places in Nigeria [Evans 2005] and United States of America on the potency of this fraction and moreover the patent of the extracts of the seeds in that country as conventional medication for hypercholesterolemia and treatment of hypertension.

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