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Effects of Aqueous Seed Extract of *Persea americana* Mill. (Avocado) on Blood Pressure and Lipid Profile in Hypertensive Rats

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Abstract: The effects of aqueous seed extract of *Persea americana* Mill. (avocado), var. Fuerte, on blood pressures, plasma and tissue lipids of albino rats were investigated. Twenty-five rats were divided into 5 groups of 5 rats each. Group 1 (normal), group 2 (hypertensive), group 3 (hypertensive + 200 mg/kg b. wt of extract), group 4 (hypertensive + 500 mg/kg b. wt of extract) and group 5 (hypertensive + 700 mg/kg b. wt of extract). Except for group 1, which received 100% growers mash, all other groups received 92% growers mash and 8% NaCl as their daily meal for 4 weeks. The different dose of *P. americana* aqueous extract, significantly ($P < 0.05$) reduced blood pressures of the hypertensive rats. Reduction in total cholesterol, LDL and triacylglycerol levels were observed at the 500 mg/kg b. wt of seed extract in the plasma, kidney, liver and heart. These results suggest that the use of aqueous seed extract of this plant in the treatment of hypertension may produce a favourable lipid profile at the 500 mg/kg dose level.

Key words: *Persea americana* • Lipids • Cholesterol • Hypertensive • Blood pressure

INTRODUCTION

High blood pressure or hypertension is defined in an adult human as a blood pressure greater than or equal to 140 mmHg systolic pressure or greater than or equal to 90 mmHg diastolic pressure. It is a disease of disordered autoregulation of blood pressure [1]. Presently, drugs used in the management of hypertension in most developing countries are imported and expensive, prompting many people to seek alternative management which include the use of herbal preparations. Some plant phytochemicals such as cafestol, flavonoids and carotenoids have been reported to influence plasma cholesterol levels [2 - 4]. Some plant preparations have been reported to produce increase in blood cholesterol levels e. g crude ethanolic extract of *Aplotaxis lappa* significantly increased cholesterol levels in rabbits [5], raw and treated *Mucuna pruriens* (velvet bean) seed increased blood cholesterol levels in pigs [6].

Elevated cholesterol levels predispose to a condition known as hypercholesterolemia. Hypercholesterolemia is not a disease but a metabolic derangement that can be secondary to many diseases and can contribute to many

forms of disease, most notably, cardiovascular diseases [7]. *P. americana* of the family, lauraceae, is a native fruit of Mexico and Central America. The fruit is commonly referred to as avocado pear, alligator pear or butter fruit. The plant is a tall evergreen tree that can grow up to 65 feet in height. There are dozens of varieties of avocados which fall into three main categories, Mexican, Guatemalen and West Indian, which differ in their size, appearance, quality and susceptibility to cold. The most popular type of avocado in the United States is the Hass variety, which has brown - black skin, another common type of avocado is the Fuerte, which is larger than the Hass and has smooth dark green skin and a more defined pear shape [8].

Much works have been done on the use of plants in the treatment of hypertension [9, 10]. An ideal anti hypertensive agent should not elicit any toxic alteration of the blood following prolonged use [11]. Extracts of avocado pulp have been shown to reduce blood cholesterol levels [12] and the risk of prostate and breast cancer [13]. Leaf juices and concoctions have been employed as antibiotics, treatments for hypertension, diarrhoea, sore throat and to regulate menstruation [14, 15].

Aqueous seed extract of this plant is used by most alternative medicine practitioners in Nigeria to treat hypertension.

This study was carried out to justify the use of this seed extract in the treatment of hypertension and to determine the effect of such usage on cholesterol levels.

MATERIALS AND METHODS

Source of plant materials; Fresh fruits of *P. americana* were purchased from Oba market, Benin City, Edo state, Nigeria, West Africa.

Preparation of Plant Extract: The dried seeds were pulverized, 1Kg of the powdered seeds was macerated in 5:7:10 distilled water. The homogenate was filtered and the filtrate evaporated to dryness with a rotary evaporator at reduced pressure. The concentrate was stored at 4°C.

Treatment of Animals: Twenty - five albino rats were divided into 5 groups of 5 rats each. The animals were acclimatized for one week after which animals in group 1 (normal control) were given growers mash only and animals in other groups were given 92% growers mash and 8% NaCl to induce hypertension [24]. While groups 1 and 2 (hypertensive control) were given equivalent volume of water, animals in group 3 (hypertensive + 200 mg/kg) were given 200 mg/kg b. wt aqueous avocado seed extract, group 4 (hypertensive + 500 mg/kg), 500 mg/kg b. wt of extract while group 5 (hypertensive + 700 mg/kg) were given 700 mg/kg b. wt seed extract for a period of 4 weeks. The body weights of the animals were measured and recorded daily.

After 4 weeks the blood pressures of rats were measured using a 2 channel recorder (Gemini 7070). The rats were anaesthetized by intraperitoneal injection of urethane. The trachea and carotid artery (either left or right) were exposed and cannulated, the arterial cannula was then connected to a Cardio Tachograph which is connected to a 2 channel recorder. The respective systolic and diastolic pressures were then read using a sphygmomanometer [16]. The rats were dissected and blood, heart, liver and kidney samples were collected for further analysis.

Biochemical Analysis: Triacylglycerol, total cholesterol and HDL assay kits (Quimica Clinical Applicada Laboratories, Spain) were used. Tissue lipids were extracted using chloroform methanol mixture [17], total cholesterol was estimated using the Chod pap method [18]. Total cholesterol was determined after enzymatic

hydrolysis and oxidation by cholesterol esterase and cholesterol oxidase respectively. Phenol and 4 - amino antipyrine combined with hydrogen peroxide in the presence of peroxidase to produce the indicator, a red quinoneimine.

HDL was determined by the dextran sulphate mg(II) method [19]. LDL, VLDL and chylomicron fractions were precipitated quantitatively by the addition of dextran sulphate in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction which remained in the supernatant was determined. LDL was calculated using Friedewald equation [20]. Triacylglycerol was determined after enzymatic hydrolysis with lipase, glycerol kinase and glycerol phosphate oxidase [21].

Statistical Analysis: All data were expressed as mean \pm standard error of mean. Analysis of variance (ANOVA) and least significant difference tests were used to check the significant differences between various parameters at $p < 0.05$. Students' T test was used to compare the values further, within a parameter.

RESULTS

There was no significant difference in mean weight gain of all the tested rats compared with the normal control (Table 1). Mean systolic and diastolic blood pressures of tested rats were significantly ($P < 0.05$) higher than those of rats in the normal control group (Table 2), however there were 12.06, 24.7 and 45.2% reduction in systolic blood pressure of rats administered 200 mg/kg, 500 mg/kg and 700 mg/kg b. wt aqueous seed extract, respectively compared with the hypertensive control.

Table 1: Mean weight (g) gain of rats administered aqueous extract of *P. americana* seeds

Group	Weight Gain
Normal Control	19.3 \pm 3.1
Hypertensive Control	15.0 \pm 2.8
Hypertensive + 200mg/Kg	19.0 \pm 4.5
Hypertensive + 500mg/Kg	17.5 \pm 3.2
Hypertensive + 700mg/Kg	19.9 \pm 2.3

Results are expressed as mean \pm S.E.M. (n = 5)

Table 2: Blood pressure (mmHg) of rats administered aqueous seed extract of *P. americana*

Group	Systolic	Diastolic
Normal control	81.0 \pm 3.05	52.3 \pm 4.3
Hypertensive control	166.0 \pm 1.0*	128.6 \pm 6.1*
Hypertensive + 200 mg/kg	145.0 \pm 27.5*	76.6 \pm 11.6*
Hypertensive \pm 500 mg/kg	125.0 \pm 21.2*	66.6 \pm 2.3*
Hypertensive + 700 mg/kg	91.4 \pm 5.5*	56.4 \pm 4.0

Results are expressed in mean \pm S.E.M. (n=5). * Significant at $P < 0.05$ compared with normal control

Table 3: Plasma and tissue total cholesterol (mmol/L) levels of hypertensive rats administered aqueous seed extract of *P. americana*

Group	Plasma	Kidney	Heart	Liver
Normal control	30.6± 1.3	48.2± 2.6	46.7± 1.1	55.4±1.3
Hypertensive control	30.2±3.1	55.8±1.0*	61.7±1.4*	54.1±1.5
Hypertensive+200mg/Kg	29.3±2.1	58.9±0.4*	60.9±1.1*	55.8±0.8
Hypertensive+500mg/Kg	24.7±1.8*	27.7±0.5*	24.3±1.5*	41.8±3.0*
Hypertensive+700mg/Kg	40.1±0.5*	74.2±1.0*	77.1±11.5*	70.3±2.8*

Results are expressed in mean ± S.E.M. (n=5). * Significant at P < 0.05 compared with normal control

Table 4: Plasma and tissue HDL cholesterol (mmol/L) levels of hypertensive rats administered aqueous seed extract of *P. americana*

Group	Plasma	Kidney	Heart	Liver
Normal control	20.0±0.8	18.0±0.2	20.0±0.8	20.0±0.8
Hypertensive control	16.1±0.5	20.8±2.4	18.3±0.5	19.1±0.4
Hypertensive+200mg/Kg	21.9±1.2	15.7±2.5	24.0±1.8	17.6±2.4
Hypertensive+500mg/Kg	23.0±1.5	11.0±3.6*	14.7±3.2*	15.0±2.4*
Hypertensive+700mg/Kg	12.0±2.6*	18.5±3.3	17.7±1.4	12.3±3.5*

Results are expressed as mean ± S.E.M. (n=5). * Significant at P < 0.05 compared with normal control

Table 5: Plasma and tissue LDL cholesterol (mmol/L) levels of hypertensive rats administered aqueous seed extract of *P. americana*

Group	Plasma	Kidney	Heart	Liver
Normal control	10.6±3.2	30.2±5.1	26.7±1.4	35.5±2.3
Hypertensive control	14.1±1.3	35.0±2.2*	43.4±2.5*	35.0±2.2
Hypertensive+200mg/Kg	07.4±2.1	43.2±1.2*	36.2±4.5*	38.2±2.1
Hypertensive+500mg/Kg	01.7±0.8*	16.0±5.0*	09.5±3.6*	26.7±5.3*
Hypertensive+700mg/Kg	28.1±4.9*	65.9±2.3*	59.4±5.6*	58.9±5.1*

Results are expressed as mean ± S.E.M. (n=5). *Significant at P < 0.05 compared with normal control

Table 6: Plasma and tissue triacylglycerol (mmol/L) concentration of hypertensive rats administered aqueous seed extract of *P. americana*

Group	Plasma	Kidney	Heart	Liver
Normal control	10.5±1.7	19.2±3.1	02.3±5.5	37.3±4.3
Hypertensive control	26.7±2.3*	24.3±1.7	41.5±4.7*	48.4±3.4*
Hypertensive+200mg/Kg	26.9±3.7*	20.4±4.3	37.6±3.7*	45.3±5.5*
Hypertensive+500mg/Kg	03.3±3.1*	10.5±2.3*	15.9±3.4*	23.8±3.7*
Hypertensive +700mg/Kg	27.4±4.5*	54.0±11.4*	54.6±10.8*	62.6±49*

Results are expressed in mean ± S.E.M. (n=5). *Significant at P < 0.05 compared with normal control

Diastolic blood pressures were also significantly (P<0.05) reduced by 35.76, 51.32 and 56.14% in rats administered 200 mgkg⁻¹, 500 mgkg⁻¹ and 700 mgkg⁻¹ b. wt of seeds extract, respectively compared with the hypertensive control. cholesterol and LDL cholesterol in the kidney and heart were also significantly (P<0.05) increased at this dose compared with the normal control. HDL of the kidney and heart were not significantly altered, while triacylglycerol level of the heart was significantly (P<0.05) increased, kidney triacylglycerol was not significantly altered at this dose.

At dose level 200 mgkg⁻¹ b. wt of rats there were no significant differences in cholesterol, LDL, HDL and triacylglycerol levels in the plasma and tissues studied compared with the hypertensive control (Tables 3, 4 and 5), however, the results were more variable compared with the normal control, plasma and liver total cholesterol, LDL and HDL were not significantly altered, there were 61.9 and 17.6% increase in triacylglycerol respectively compared with the normal control. Total

The group of hypertensive rats administered 500 mgkg⁻¹ drug extract had 19.28, 42.53, 47.97 and 13.6% reduction in cholesterol levels in the plasma, kidney, heart and liver, respectively compared with the normal control and 19.7, 68.3, 76.8 and 22.7% reduction in cholesterol levels compared with the hypertensive control.

Significant reductions in LDL and triacylglycerol were observed also at the 500 mgkg⁻¹ dose level in both plasma and tissue studied compared with normal and hypertensive control. LDL levels were reduced by 89, 46.7, 64.4 and 31.2% in the plasma, kidney, heart and liver compared with normal control and by 87.2, 58.3, 78.1 and 23.7% in the plasma, kidney, heart and liver compared with hypertensive control, while triacylglycerol levels were reduced by 68.6, 45.3 and 36.19% in the plasma, kidney and liver, respectively compared with the normal control and by 87.6, 56.8, 61.7 and 50.8% in the plasma, kidney, heart and liver, respectively compared with the hypertensive control.

HDL cholesterol levels were not significantly altered in the plasma of rats administered 500 mgkg⁻¹ b. wt of seed extract but were significantly reduced in the kidney, heart and liver by 38.7, 26.6 and 25%, respectively compared with normal control. HDL levels of the plasma and heart differed insignificantly compared with the hypertensive control, while HDL level of the kidney and liver were significantly reduced by 47.8 and 21.47%, respectively compared with hypertensive control. However, there were 31, 35, 39.4 and 21.47% increases in cholesterol levels in the plasma, kidney, heart and liver, respectively of those rats administered 700mgkg⁻¹ b. wt of seed extract compared with normal control, 24.7, 24.8 20 and 23% increases in cholesterol levels were observed in the plasma, kidney, heart and liver, respectively of these rats compared with the hypertensive control. While LDL levels were increased by 62.3, 54.2, 55.1 and 39.7% in the plasma, kidney, heart and liver, respectively compared with the normal control, 49.8, 46.8, 26.9 and 40.6% increase, respectively, were observed compared with the hypertensive control.

Triacylglycerol levels were significantly ($P<0.05$) increased by 61.7, 64.4, 90 and 40.4% in the plasma, kidney, heart and liver, respectively, compared with the normal control in these group of rats administered 700 mgkg⁻¹ b. wt of seed extract, however plasma triacylglycerol levels were not significantly affected in these rats compared with the hypertensive control, 55, 24 and 22.7% increase were observed in the kidney, heart and liver, respectively compared with the hypertensive control.

HDL levels were significantly ($P<0.05$) reduced by 40 and 38.5% compared with the normal control, but significantly ($P<0.05$) increased by 25.5 and 35.6% in the plasma and liver, respectively compared with the hypertensive control. HDL cholesterol levels were not significantly altered in the kidney and heart compared with the normal and hypertensive control.

DISCUSSION

After four weeks of administering aqueous extract of *P. americana* seeds to hypertensive rats, the reduction in weights observed were not significant. Weight loss however had been associated with blood pressure lowering effect in overweight and hypertensive individuals [22]. Hypertension was successfully induced; this explains why the mean systolic and diastolic blood pressures of tested rats were significantly higher than that of the normal control.

Salt loading had been shown to cause hypertension [23 - 25]. However, administration of the aqueous seed extract reduced the blood pressures of hypertensive rats with the highest percentage reduction occurring at the highest dose level. This demonstrates the blood pressure lowering effect of aqueous avocado seed extract. Blood pressure lowering effect of avocado pulp has been reported by some workers [26, 27].

The blood pressure lowering effect of avocado has been attributed to their high content of potassium [27]. Potassium dilates blood vessels and helps regulate blood pressure and sodium concentration. Adequate intake of potassium reduces the risk of cardiovascular disease incidence [28].

At 500 mg/kg b. wt of extract, cholesterol, triacylglycerol and LDL cholesterol levels were significantly reduced in the plasma and tissues studied compared with the normal and hypertensive control. Reduction in cholesterol, LDL cholesterol and triacylglycerol levels on ingestion of avocado pulp have been reported by some workers [12, 29]. The cholesterol and LDL reducing potential of avocado pulp has been attributed to high content of monounsaturated fatty acids, beta sitosterol, Carotenoids (e.g. Zeaxanthin, alpha carotene, beta carotene and tocopherols (Vit. E) present in the pulp of this fruit [30].

The original studies of Keys *et al.* [31] suggested that monounsaturated fats have neutral influence on blood cholesterol concentrations leading to neither a rise nor a fall when administered to volunteers, High levels of monounsaturated and polyunsaturated fatty acids could be responsible for the cholesterol lowering effect of avocado seeds [32 - 34].

Cholesterol lowering effect of avocado seeds may also be attributed to the presence of beta sitosterol and tocopherols in the seeds. Beta sitosterol is a natural plant sterol which maintains healthy cholesterol levels, it does this by interfering with cholesterol absorption.

Tocopherols are natural antioxidants, they protect tissues from lipid peroxidation by mopping up free radicals, thus they prevent the oxidation of LDL receptors hence facilitating the uptake of cholesterol into tissues [35].

In addition, avocados have been found to contain three times the amount of glutathione present in any other fruit [28].

Increase in cholesterol and triacylglycerol levels were not significant compared with the hypertensive control at the 200 mg/kg dose level, therefore increase observed compared with the normal control can be attributed to the hypertensive state of the rats. Hypertension frequently coincides with one of the following risk factors; high LDL or triacylglycerols, low HDL and dysglycemic obesity [29]. Heart study indicates that 80 % of hypertensives have one of these conditions [29].

Rats administered 700 mg/kg b. wt of seed extract showed 20 - 24 % increases in cholesterol levels compared with the hypertensive control. This effect may have been produced by alterations in the metabolism of some hormones or could be as a result of undesirable increase in antioxidant levels. The aqueous avocado seed extract may produce hypothyroidism, which is probably responsible for the increase in cholesterol levels [5]. Hypothyroidism may be produced by drug by disturbing the central regulation of the thyroid gland or by disturbing the hormonal control of this gland.

Reports from several studies have shown that antioxidants lose their beneficial effects when present in excess [36, 37]. For example, natural antioxidants like lycopene and carotenoids, which are prostate cancer preventive agents [13] have been found to lose their protective effect at high concentrations, high serum beta carotene levels has been associated with increased risk of prostate cancer [38 - 40].

High antioxidant level may produce free radicals that could oxidize LDL receptors and reduce the uptake of cholesterol into cells; this could lead to increase in the level of plasma total cholesterol and LDL cholesterol.

In Conclusion, although aqueous avocado seed extract reduced blood pressures of rats at all dose levels, the use of avocado seed in the treatment of hypertension should be dose dependent. High concentrations of this herbal drug could produce increase in cholesterol levels which causes atherosclerosis. Further work would be done on the proximate analysis and phytochemical screening of the avocado seed, to determine the chemical components responsible for its pharmacological and biochemical actions.

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