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Phytochemical, Toxicological, Biochemical and Haematological Studies on Avocado (*Persea americana*) in Experimental Animals

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ABSTRACT

The avocado tree belongs to the family lauraceae and is classified as *Persea americana*. The analysis of the fruits extract revealed the presence of considerable amounts of vitamins A, B_2, C, K, folic acid, lutein, zeaxanthin, coenzyme Q_{10} and beta-carotene. When administered to wistar rats for acute toxicity studies, the animals did not exhibit any sign of toxicity even when large doses were given. The maximum tolerable dose (MTD) was therefore determined to be ≥ 10g/kg body weight. The extract was found to significantly decrease ($p < 0.05$) the activity of liver and heart enzymes in the treated animals when compared with the control. The extract decreased total cholesterol (TC) by 37.97%, triglycerides by 37.87%, very low density lipoproteins(VLDL) by 47,41%, low density lipoproteins(LDL) by 59.57%, and at the same time increased high density lipoprotein (HDL) by 3.64%. The extract also decreased the prothrombin time (PT) and the partial prothrombo plastin time with kaolin (PTTK). These results are discussed with regard to the preventive and possible curative values of this extract as a potential inhibitor of cardiovascular diseases and in the regulation of blood clotting time due to its significant vitamin K content.

Keywords: *Persea americana*, maximum tolerable dose, phytochemicals, prothrombin time.

Introduction

Studies have long associated the consumption of fruits and vegetables with a reduced risk of various human diseases. The protective effect is attributed to the high levels of phytonutrients or phytochemicals plant compounds thought to have health protecting qualities that are often found in dark coloured fruits and vegetables.

The avocado tree belongs to the family lauraceae native to central Mexico, classified as Persea Americana (Chen *et al.*, 2008). Also called alligator pears, avocado pears are exotic plants that are quick to grow, and make attractive, evergreen houseplants with masses of dark green leaves. The fruits are a greenish, thick-skinned drupe that may be pear-shaped, egg-shaped, or spherical, the flesh of which has the consistency of firm butter and a faint nut-like flavor when ripe.

It is a firm conviction that this decade should see mainstream connection between food and health. Thus food that either heal or prevent sickness should be the focus of eating. Epidemiological data reflect that Africans would have to triple their intake of fruits and vegetables to reach the amounts consumed in parts of the Mediterranean during the 1960's in order to observe a low incidence of chronic diseases.

This work is therefore aimed at quantifying the chemical constituents of the crude extract of avocado fruits, carrying out acute toxicological studies and studying the effect of the extract on enzymes indicators of heart and liver necrosis and some haematological parameters in rats.

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Materials and Methods

Avocado pear *(Persea americana)* fruits were identified and purchased from the local market in Bamenda-Cameroon in the month of May.

Sample preparation and extraction

The fruits were peeled, the seeds removed, and the flesh was chopped finely, weighed, and air spread at the room temperature (28 – 30°C). The dried pears were blended, sieved (with a sieve of 1 mm mesh) for the flour and the latter weighed. The flour so obtained (40 g) was mixed with 400 cm^3 of ethanol (95%) and kept for 24 hours after which the mixture was filtered. The ethanol was evaporated out of the filtrate using a rotary evaporator.

Phytochemical analysis

Vitamin A, B_2, C, K, folic acid, lutein, zeaxanthin, coenzyme Q10 and β-carotene were quantified by dissolving 1 g of the flour in 10 cm^3 of appropriate solvents (methanol for vit. A, B2 and Folic acid; ethanol for vit. C, K, lutein, zeaxanthin, and CoQ10; then chloroform for β-carotene), then heating for 30 min at 40°C and filtered. The absorbance read at specific wavelength using the UV-visible principle of Keith and Kenneth, 1994.

Acute toxicological studies

In a preliminary study, a group of three Wistar rats was given free access to control powdered diet containing crude protein (14.5%), crude fiber (7.2%), fat (7.0%), calcium (9.8%), phosphorus (0.4%) and metabolizable energy (2,500Kcal/kg) and standard drinking water. Each rat in this group was additionally given 5 g/kg body weight daily of the prepared extract for three days and observed for evidence (s) of acute oral toxicity.

An additional oral acute toxicity study was designed as a "limit test", where the rats were administered 10 g/kg body weight two times a day in 24 hrs. All the animals were continuously observed for 6 h after each treatment.

Sub-acute toxicity studies

The experimental animals were divided into two groups of 8 each: both groups were fed with control powdered diet and allowed free access to standard drinking water. One group was given the prepared avocado daily at a concentration of 10 g/kg body weight for 8 weeks while no extract was administered to the other.

Four animals in each group were used for biochemical analysis and the remaining (four) in each group for haematological analysis.

Biochemical analyses methodology

Cholesterol: Free cholesterol is oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide combines with hydroxybenzoic acid (HBA) and 4-aminoantipyrine to form a chromophore (quinoneimine dye) which is then quantified at 500 – 550 nm (Akhtar *et al.*, 1997).

Triglycerides: Triglycerides are enzymatically hydrolyzed by lipase to free fatty acids and glycerol. The glycerol is phosphorylated by adenosine triphosphate (ATP) with glycerol kinase (GK) to produce glycerol-3-phosphate and adenosine diphosphate. Glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate (DAP) by glycerolphosphate oxidase (GPO) producing hydrogen peroxide (H_2O_2). The H_2O_2 reacts with 4-aminoantipyrine (4-AAP) and 3, 5-dichlorobenzene sulforate (DHBS) to produce a red coloured dye which absorbance is proportional to the concentration of triglycerides present in the sample (Akhtar *et al.*, 1997).

Alkaline phosphatase (ALP): The ALP-Tris/carbonate method of Moss and Henderson, 1994 uses p-nitrophenyl-phosphate as a substrate. At optimum pH of the reaction, P-nitrophenoxide(p-NPO) has intense yellow colour. The reaction is monitored by measuring the rate of increase in absorbance at 405 nm which is proportional to the activity of ALP in the serum.

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST): The ALT and AST reagents are based on the recommendations of the IFCC, 1986. The amino acid group is enzymatically

transferred by ALT and AST present in the sample from alanine to the carbon atom of 2-oxoglutarate yielding pyruvate and L-glutamate. The pyruvate is reduced to lactate by LDH present in the reagent with the simultaneous oxidation of NADH to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH.

Gamma-Glutamyltransferase (GGT) (Jo *et al.*, 2009) present in the sample catalyses the transfer of the glutamyl group from the substrate to glycylglycine forming glutamylglycylglycine and 5-amino-2-nitrobenzoate. The rate of formation of 5-amino-2-nitrobenzoate is proportional to the activity of GGT present in the sample and is measured kinetically at 405 nm.

Creatine Kinase (CK) (Klatt *et al.*, 1982): During the first stage, sample incubates with the thiol compound N-acetyl cysteine (NAC) which reactivates the CK molecule by rapidly reducing oxidized sulfydryl compounds at the active site. In the second stage, the substrate creatine phosphate initiates a series of catalyzed reactions. In the 1st of these reactions, CK catalyses the formation of ATP from creatine phosphate and ADP. ATP formed is used to form glucose 6-phosphate (G-6-P) in a reaction catalyzed by hexokinases. G-6-P produced is oxidized to 6-phosphogluconate (6PG) and NADP+is reduced to NADPH in a reaction catalyzed by glucose 6 phosphate dehydrogenase. AMP and p1p5di(adenosine-5-) pentophosphate (p1p5-diAP) are added to inhibit adenylate kinase (myokinase) activity.

Lactate Dehydrogenase (LDH-L) (Simaga *et al.*, 2005): LDH catalyses the oxidation of lactate reducing nicotiamide adenine dinucleotide (NAD+) to NADH. The activity of LDH is then determined by the rate of increase in absorbance at 340 nm as NADH is produced.

Haematological Analyses: The rabbit brain thromboplastin-calcium reagent was used for prothrombin time (PT) and the platelets/kaolin reagent for partial thromboplastin time with kaolin (PTTK) determination following the method of Dacie and Lewis, 1991.

Results and Discussion

No death occurred even in the highest doses administered, nor were there any signs of toxicity reported based on macroscopic alterations seen in the organs of the tested animals. The LD 50 value was determined to be higher than 5 g/kg body weight. The extract was classified as belonging to the practically non-toxic category. Clinical observations included the state of the skin, trembling, diarrhoea, fatigue (somnolence), and dysentery like stool after administration of 10 g/kg body weight twice daily. The results of the study indicate that the extract caused no toxic symptom or lethality during the observation period. Therefore, the maximal tolerable dose (MTD) to be administered was estimated to be $\geq$ 10 g/kg.

The extract of the studied fruit has a significant effect on the liver and heart enzymes in the blood of the treated animal compared to the untreated (Fig. 1). The aspartate aminotranferase (AST) and alanine aminotransferase (ALT) jointly known as transaminases were decreased respectively by 30% and 26.25%. This probably gives an insight for the possible therapeutic use of the extract in cases of liver disease since it was reported that most liver diseases are characterized by greater ALT elevations than AST elevations with normal values ranging from 0 to 40 IU/L for AST and to 45 IU/L for ALT in human (Melissa, 2004).

GGT and ALP known as Cholestatic enzymes were decreased ($p < 0.05$) by 54.07 % and 0.23% with the extract. The significant decrease of GGT by the sample unlike with ALP where there is only a mild decrease (Figure 1) shows the preventive and perhaps the curative values of this extract. This is because GGT is found predominantly in the Liver while ALP is mainly found in the bones and the liver but can also be found in many other organs such as intestines, kidneys and placenta. Therefore, elevated levels of ALP will indicate that something

is wrong with the liver only if the amount of GGT is raised as well. GGT can be elevated without ALP being elevated, as GGT is a sensitive marker of alcohol ingestion and certain hepatotoxic drugs (Melissa, 2004).

It is well known that diagnosis of cardiac enzymes is important. Serum CK activity is a more sensitive indicator in early stage of myocardial ischemia, while peak rises in LDH is roughly proportional to the extent of injury or integrity to the myocardial tissue (Chatterjea and Shinde, 2002). Also the integrity of the cardiac apparatus in drugs biotransformation and metabolism could be assessed by evaluating the levels of AST, CK and LDH in serum. The results of the effects of the used extract in this experiment at the maximum tolerable dose of 10 g/kg/day on these three enzymes (AST, CK, LDH), show that avocado significantly decreases ($p < 0.05$) them by 30.14%, 50.65%, 13.81% respectively (Figure 1).These results exhibit significant decrease of CK by all the extract in accordance with Chatterjea and Shinde (2002). Also, the significant decrease ($p < 0.05$) of LDH exalts the protective effect of the extracts on the integrity of the myocardial tissue.

The presence of a high amount of cholesterol in the diet has been demonstrated to elevate plasma cholesterol and may increase aortic atherosclerosis. Several studies have indicated that diet treatment importantly, regulates cholesterol thus, reducing subsequent CVD-associated mortality and morbidity (Kwiterovich, 1997). On the basis of this, great efforts have been made to reduce the risk of CVD through the regulation of cholesterol, thus the therapeutic benefits of plant foods have been the focus of many extensive dietary studies (Yokozawa *et al.*, 2006; Zhang *et al.,* 2007). The effect of the extract on lipid profile was assessed by measuring the levels of TC, Trig., HDL, VLDL and LDL. Avocado decreased TC by 24.91%, Trig by 37.97%, VLDL by 47.41%, LDL by 59.57% and HDL was increased by 14.54% (Figure 2). Since low level HDL cholesterol plays a direct role in atherogenic process, therapeutic intervention to raise HDL-cholesterol together with other risk factors is widely encouraged. This is probably the reason why the extract significantly reduced the levels of LDL in blood and at the same time increasing that of HDL (Figure 2).

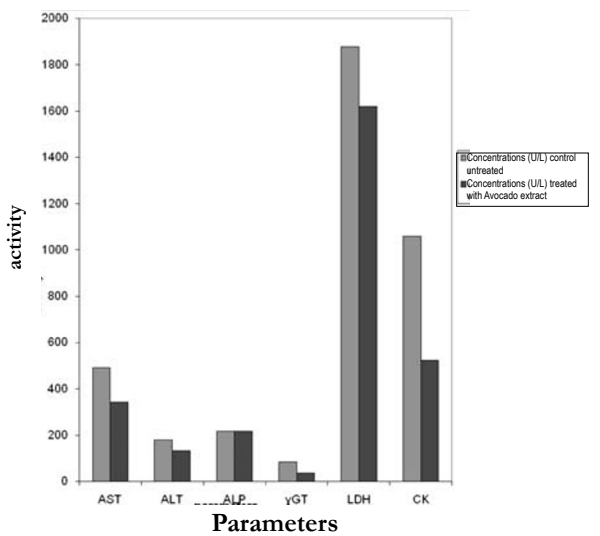

Fig. 1: Effect of ethanolic extract of Avocado *(Persea americana)* **on liver function tests (LFT) and heart function tests (HFT)**

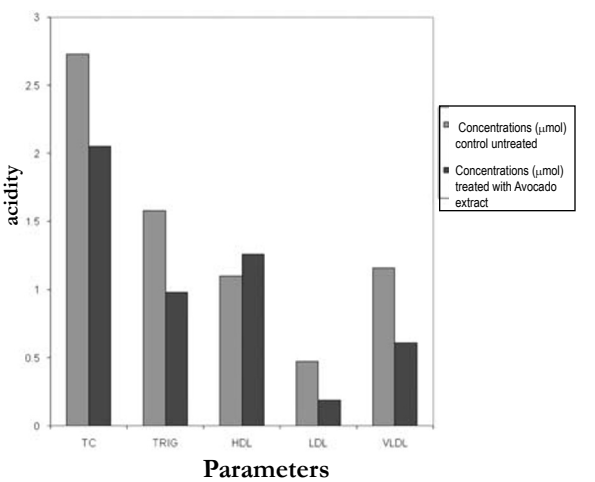

Fig. 2: Effect of ethanolic extract of Avocado *(Persea americana)* **on lipids profile**

Table 1: Concentrations of the identified parameters

Mean ± SD parameters	Avocado
Vitamin A(IU/100 g)	7.00 ± 0.071
Vitamin B_2 (mg/100 g)	47.00 ± 0.013
Vitamin C (mg/100 g)	10.519 ± 0.125
Vitamin K(mg/100 g)	0.171 ± 0.300
Folic acid(mg/100 g)	1.699 ± 0.071
Beta carotene (mg/100 g)	950 ± 0.017
Zeaxanthine (mg/100 g)	0.060 ± 1.001
Coenzyme Q10(mg/100 gl)	0.070 ± 0.076
Lutein (mg/100 g)	0.040 ± 0.012

Results are means ± standard deviation ($p < 0.05$).

Table 2: Effect of avocado extract on biochemical parameters

mean ± SD parameters (mean ± SD)	Avocado extract
AST (U/L)	342.67 ± 73.28
ALT (U/L)	132.50 ± 29.65
ALP (U/L)	218.00 ± 107.58
GGT (U/L)	38.50 ± 22.07
LDH (U/L)	1619 ± 118.79
CK(U/L)	523.25 ± 229.10
TC (µmol/L)	2.05 ± 0.10
TRIG (µmol/L)	0.98 ± 0.15
HDL (µmol/L)	1.26 ± 0.24
VLDL	0.61 ± 0.02
LDL (mol/L)	0.19 ± 0.19

Results are means ± standard deviation ($p < 0.05$)

The presence of considerable levels of coenzyme Q_{10} in the studied extract may justify the decrease in the level of total cholesterol in the animal's samples in accordance with Watt (2000), who suggested this vitamin – like antioxidant nutrient to safeguard sharp thinking by helping mitochondria in the complex process of transforming food into ATP. It also breaks down cholesterol thus, can be used in the treatment of congenital heart disease.

Table 3: Effect of avocado extract on PT and PTTK

	Pear group	Control group
PT(S) Mean ± SD	7.75 ± 0.35	22.75 ± 1.77
PTTK (S) Mean ± SD	18.5 ± 0.7	54 ± 2.83

Results are means ± standard deviation ($p < 0.05$).

Table 3 shows a significant decrease ($p < 0.05$) in prothrombin and partial thromboplastin time for the fruits extract studied. This may be due to the contributing factor of the significant amount of Vitamin K found in the samples. Vitamin K has been known for many years to be essential for the synthesis of prothrombin and several other clotting factors. The results of studies of the abnormal prothrombin synthesized in the absence of Vitamin K or in the presence of Vitamin K antagonists such as dicoumarol, revealed the mode of action of this vitamin (Jeremy *et al.*, 2002).

Conclusion

The crude extract of *Persea Americana* showed a significant decrease of AST, ALT, ALP, GT, LDH, and CK in the blood of the treated animals compared to the untreated (control). This reveals the means through which the incorporation of this fruit in significant amounts in a diet could protect the liver and the heart. In addition, the increase of HDL "the good cholesterol" to the detriment of TC, Trig., LDL makes this fruit a potential inhibitor of cardiovascular diseases and also a regulator of the blood clotting time due to its significant Vitamin K content.

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