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Role of *Persea americana* and Vitamin E in Reducing the Tendency for Prostate Cancer Growth

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Abstract

Persea Americana Avocado is traditionally used for the treatment of various health problems. With its wide claim of medicinal benefits, it is imperative to determine the effects of the extract on some biochemical parameters of the male wistar rats and its tendency to reduce prostate cancer growth. It's also to determine the extract effect on the histology of the reproductive organs (testes) of the rats. Twenty-four (24) male wistar rats of an average weight of 170g were used for the experiment. The rats were divided into four groups (n=6): Group 1 (control), group II (150mg/kg of avocado extract), group III (300mg/kg of avocado extract, and group IV (200mg/kg of Vitamin E). The administration was for 56 days, and on the 57th day all the animals were sacrificed and their testes harvested. The histology and enzyme histochemistry of the testes conducted. There was a significant decrease in vitamin Glucose-6-phospodehydrogenase (G-6-PDH) and lactate dehydrogenase (LDH) in rats of groups II and III compared to the control group. Prostatic acid phosphotase (P-AcP) also significantly decreased in groups II and III when compared with the group IV. Avocado and Vitamin E increased body weight and reduce oxidative stress. It also decreased LDH and P-AcP levels in the body, and consequently reducing tendency for cancerous cells growth, and particularly prostate cancer. However, unlike Vitamin E which improved on spermatogenesis, Avocado exert negatively on testicular architecture, germ cells and spermatogenesis.

1 Introduction

Traditional Medicine has been brought into focus for meeting the goals of a wider coverage of primary health care delivery, not only in Africa but also, to various extents, in all countries of the world particularly in Nigeria. Due to the increased awareness of the significance of traditional medicine in human and animal healthcare, researches into the efficacy of some of the herbs used in the treatment of some illness would be worthwhile. It is equally very important to determine what side effects these traditional medicines have on the body in the course of their use as therapy in treating these diseases.

Avocado plants products are one of many that have been used for the treatment of various health problems. It is also one of the most important sources of drugs discovery and development used in traditional medicine to treat various diseases and conditions such as in vaso-relaxant activity, analgesic, anti-inflammatory activity, hypotensive activity, anticonvulsant effect, antiviral activity, wound healing activity, anti-ulcer effect, anti-hepatotoxic activity, antioxidant activity, hypoglycaemic activity, and increase of body weight.[1]

Biochemical parameters describe the chemical constituents of living organisms and their reactions underlying life processes. The set of methods, assays, and procedures that enable scientists to elucidate these chemical substances is referred to as biochemical analysis. A good knowledge of this analysis further gives an insight on how some plants/herbs could be used in treating some ailments.

2 Materials and Methods

2.1 Materials

Dissecting blade/fine scalpel, Dissecting board, Dissecting kit, Dissecting set, Enzyme assay kit, Feed, Fixative (normal saline),

Four rats cages, Freezer, Glass slides, Glass slip, Gloves, Hormonal assay kits, Laboratory coat, Light microscope, Lithium heparin bottle, Measuring cylinder, Methanol, Microtomes, Neuber haemocytometer chamber, Olive oil, Organ bottle, *Persea americana* (avocado), Plastic bottles, Rotary evaporator, Stains [hematoxylin and eosin (H&E), GFAP-stained], Syringe, Trinity biotech reagent, Twenty male wistar rats, Vitamin E, Waste basket, Water, Water bath, Weighing balance, Weighing scale, 10% normal saline, 100% ethanol, 5% sucrose solution, Alcohol, Automated tissue processor, Basket, Beaker, Cannula, Centrifuge, Chem-well chemistry auto analyser, Clean bottles, Cotton wool, Disinfectant.

Experimental Animals

Twenty-four (24) male wistar rats of average weight of 170g were used in the present study. The rats were procured from University of Jos Animal House and brought to Bingham University Animal house to acclimatize for two weeks and maintained over husk bedding in well ventilated (air spaced) cages. Throughout the experiment period, the rats were fed with a balanced pellet diet, water.

Permission was obtained from Bingham University Ethical Committee with number 156p

The rats were selectively grouped into four (groups one, two, three and four), each containing six animals.[2] Group one served as control, group two and group three were administered orally with 150mg/kg and 300mg/kg of *Persea americana* extract as low dose and high dose respectively[3]. Group four was administered orally with 200mg/kg of vitamin E [4].

2.2 Methods

2.2.1 Preparation of plant extract

Unripe fruits of *Persea americana* were collected from Enugu market in Enugu, Nigeria. A total number of 360 fruits were collected, with a total weight of 85kg. The fruit was sliced into two from top to bottom, and the seed removed and back peeled also, leaving the pulp of the fruit only for the extraction. The new weight of the avocado fruits after removing seed and back was 40kg. The fruit was then sliced into pieces and left to dry under shade foe three weeks[5] during which the following weights were taken after one, two and three weeks: 38kg, 25kg, and 10 kg respectively. When completely dried, the fruits were pounded into a powder with a final weight of the powder being 6.5kg[6,7].

3.5kg of the 6.5 kg of the powder form of the extract was then soaked in 5 litre of 70% methanol for 24 hours using a maceration process[8]. The extraction was done three times before evaporating the methanol content of the extract at 50^{o}C [9] for a day, after which a concentrated extract was obtained[9]. On the 5th day the extract weighing 58g was collected into a clean container and sealed tightly.

% Yield = wt of extract/wt of avocado powder

= 58/3500

= 0.017%

2.2.2 Vitamin E (standard drug)

The standard drug used was Vitamin E; each soft gel capsule is made up of 400IU and contains d-alpha Tocopheryl acetate. The excipients of vitamin E are soya bean oil, gelatin, and glycerin. The vitamin E was obtained from a Pharmacy shop at Mararaba town of Nassarawa state in Nigeria.

2.2.3 Administration of avocado extract

0.9g of the extract was dissolved in 10mls of distilled water to make a concentration of 0.09g/ml. And using a 2ml syringe with a cannula attached, the extract was then given to the groups of animals in the appropriate dosage of 150mg/kg[10] and 300mg/kg [11] as low and high dose respectively. The extract was administered for 56 days.

2.2.4 Administration of Vitamin E

10 tablet of vitamin E was dissolved in 4mls of olive oil and were given to rats in group 4 (200mg/kg). The vitamin E was also administered for 56 days [12].

2.2.5 Method of animal sacrifice

The final body weight of the wistar rats were obtained at the end of *Persea americana* extract and vitamin E administration, they were then sacrificed through cervical dislocation by applying a pressure on the neck separating the spinal cord from the skull causing loss of sensitivity to pain and dissection was easily carried out [13].

2.2.6 Biochemical analysis

2.2.6.1 Enzyme Assay

For the prostatic acid phosphatase (P-AcP) the method used for the assay was radioimmunoassay of which the test-tube were serially arranged and labeled 'BLANK' standard and samples respectively. 250 µl of the acid phosphate working reagent (Agappe) was added into the arrangement test-tubes. And then 2.0 µl of tartrate reagent (Agappe) was added respectively. It was then incubated for 5 minutes and read at 405-700 nm wavelengths spectrophotometrically using the CHEM-WELL chemistry auto analyzer[14].

For the glucose-6-phospodehydrogenase (G6PDH) the method used was an enzyme quantitative assay of which was tested on the homogenized left testis of the rats. 5.5ml of water was added to 10ml of G6PDH reagent (trinity biotech reagent). The content in the trinity biotech reagent was swirled to properly dissolve for 3 mins. 0.33 ml of the reagent prepared in previously was added to a test-tube. 20 µl of haemolysate was added and allowed to stand for 5 mins at 37 ˚C. 0.65 ml of G-6-PDH substrate solution was added and the content was well mixed by inverting the tubes several times. The tubes were placed in a water-bath at 37˚C for approximately 2 mins to attain thermal equilibrium. Initial

absorbance (A) of the test was read and recorded. Exactly 5 mins later, the final absorbance was read and recorded. The concentration of was then determined [15].

Kinetic method was used for the lactate dehydrogenase (LDH) assay using teco diagnostic reagent. The reagent composition was made up of lactate (4×40ml) for the reagent one while $NADH^+$ (4×40 ml) for reagent two. The tubes were arranged and the 25µl of the four calibrator labeled 25, 50, 75 and 100, were pipetted into the first four tubes respectively. Then 25 µl of samples were pipetted into the subsequent tubes of which 200µl of reagent one was pipetted into all the tubes respectively and then 40µl of reagent two was then pipetted into all the tubes respectively. It was the incubated for 12 minutes and read between 340 nm and 405 nm using chemwell auto analyser[16].

2.2.6.2 Tissue collection and preservation

The tissues were collected by harvesting the organ through the dissection of the rats, and then it has been preserved using formal saline solution for the testes used for histological analysis while 5% sucrose solution was used to preserve the testes used for enzyme analysis.

2.2.6.3 Histology of the testicular tissues

After samples of the testis were harvested from the Wistar rats, they were kept in a container with 10% normal saline to prevent autolysis, which will disrupt the histological structure of the testis. The testis tissues were then taken to the automatic tissue processor for proper processing of the tissues. The following steps were taken: fixation, dehydration, clearing and filtration[17].

Fixation: 1cm of fresh testicular tissue samples were preserved in normal saline[18]**.**

Dehydration: the blocks of tissue are transferred sequentially to 30%, 50%, 70%, 80%, 90%, 100% alcohols for about 12-24 hours each. The blocks are then placed in the second 100% ethanol solution to ensure that all water is removed.

Embedding: tissues are embedded in paraffin

Clearing: This was used to remove constituents of alcohol from the tissue and was achieved with the use of the agent xylene.

Infiltration: This is the immersion of the tissue in molten paraffin wax. Molten paraffin wax melted at 70^0c was used to provide support for tissue by filling the holes created by the clearing agents. It helps in proper embedding.

Embedding: After tissue processing, the tissue was then taken to the embedding machine with the use of the embedding mould. The embedding mould was filled with molten paraffin wax, the tissue was picked with forceps and oriented at the centre of the embedding mould and then the cassette was placed on it to allow solidification. The block of tissue was packed into the freezer for cooling and for easy removal of mould. The block is trimmed to remove excess wax. The block of tissue was placed in the microtome and trimmed to expose the organ surface after which the block was cooled on ice to allow easy sectioning. The microtome was set at 3-5 microns, and the testes were cut from the block of testis tissues. Sections were picked with forceps and placed on a slide. 20% of alcohol was used to float section in water bath to allow the section to spread well. The slide obtained was labeled with the aid of a pencil according to the number on the block. After labeling, the slide was placed on a hot plate for the section to stick properly to the slide.

Staining: The dried slide was arranged in a staining rack and then dewaxing was done in two changes of xylene for ten minutes of which it was then hydrated in descending grades of alcohol i.e. from absolute to 70%. Then it was Rinsed in water for a few minutes and it was Stained in haematoxylin afterward (this stains the nucleus blue), and then differentiating procedure was carried out in 1% alcohol. For ten minutes a blowing process was done slowly in a running tap. A counterstaining was then done in eosin for few minutes of which rinsing in water were then carried out after it; it was then dehydrated in ascending grades of alcohol i.e. in 70%, 80% and absolute alcohol (100%). After which it was dried in a hot plate. A clearing in xylene and the mounting was done using dibutyphostate xylene (DPX) mounting and coverslips. Finally, the slides were then allowed to dry before arrangement[17-19]

Photomicrography: Histological slides were then viewed using a light microscope with objective lens x10. Photomicrography was taken using an attached colour digital camera. Slides microscopes were viewed and Photomicrography taken at objective lens x40 and x100 using immersion oil - (cedar wood oil).

2.3 Phytochemical screening procedures

2.3.1 Test for Tannins

About 0.5g of the plants extract was stirred with 1ml of distilled water, filtered, and ferric chloride reagent added to the filtrate. A blue-black, green, or blue green precipitate was taken as evidence for the presence of tannins.

2.3.2 Test for Saponnins

About 0.5g of the plant extract was shaken with water in a test tube. Frothing which persist on warming was taken as preliminary evidence of saponins.

2.3.3 Test for Flavonoids

2g of the powdered fruit was completely detained with acetone. The residue was extracted in warm water after evaporating the acetone on a water bath. The mixture was filtered while hot. The filtered was cooled and used for the following tests.

Lead acetate test for flavonoids: To 5ml of the detained water extracted was added lead acetate solution. A yellow colour precipitate indicates the presence of flavonoids.

2.3.4 Test for Alkaloids

About 0.5g of the powdered extract was stirred with 10ml of 1% aqueous hydrochloride acid on a steam bath; 1ml each of the filtrate was treated with a few drops of Mayer's reagent, dragendorff's reagent, and picric acid solution. Precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloid in the extract.

2.3.5 Test for Terpenes

5g of powdered pulp was extracted by maceration with 50ml of chloroform (95%) filtered and the filtrate was evaporated to dryness. The residue was dissolved in 10ml of anhydrous chloroform and the filtered filtrate was divided into two equal portions and the following test was carried out.

Using the Liebermann-burchard test, the first portion of the chloroform solution from above was mixed with 1ml of acetic anhydride, followed by addition of 1ml of concentrated sulphuric acid down the wall of the test-tube to form a lower layer. The formation of a reddish-violet colour in the chloroform layer indicates the presence of terpenes.

2.3.6 Test for Steroids

5g of powdered pulp was extracted by maceration with 50ml of chloroform (95%) filtered and the filtrate was evaporated to dryness. The residue was dissolved in 10ml of anhydrous chloroform and the filtered filtrate was divided into two equal portions and the following test was carried out.

Using salkowski's test, the second portion of the solution was mixed with 2ml of concentrated sulphuric acid carefully so that the acid forms a layer. A reddish brown colour at the interface indicates the presence of a steroidal ring.

2.3.7 Test for Glycosides

About 100mg of each extract was taken in a test tube and 2.5ml of dilute sulphuric acid was added and boiled in a water bath for 15 minutes. This was cooled and neutralized with 20% potassium hydroxide solution. 5ml of a mixture of Fehlings solution A and B was added and boiled for 3 minutes. A brick red precipitate indicates the hydrolysis of a reducing sugar, an indication of glycoside.

2.3.8 Test for Resins

5ml of petroleum ether extract was made using 0.1g of powdered pulp and filtered into a test-tube. An equal volume of copper acetate solution was added and shaken vigorously then allowed to separate. A green colour indicates the presence of resins.

0.5g of the powdered pulp was dissolved in acetic anhydride and 1 drop of concentrated sulphuric acid was added. A purple or colour indicates the presence of resins.

2.3.9 Test for Carbohydrates

3g of the powered pulp were boiled in 50ml of distilled water on a water bath for 3 minutes. The mixtures was filtered while hot and the resulting filtrate cooled and used for the following carbohydrate test

Using Molisch's general test; a few drops of molisch's reagent was added to 2ml of water extract obtained above then a small quantity of concentrated sulphuric acid was added and allowed to form a lower layer. A purple ring at the interface of the liquids indicates the presence of carbohydrates. The mixture was the shaken allowed to stand for 2 minutes and then diluted with 5ml of water. A purple precipitate also indicates the presence of carbohydrates[19,20].

2.4 Statistical analysis

All data are presented as mean ± standard deviation (SD). The significance of differences among different groups was assessed by paired student T test and one-way analysis of variance (ANOVA). The acceptance level of significance was $P \leq 0.05$. Data was evaluated by SPSS for windows (SPSS Inc., Chicago, Illinois, USA) version 12.0.1 with 95% confidence interval level.

3 Results

3.1 Phytochemical analysis

Results of the phytochemical analysis of *Persea americana* is given in table 1.

Table 1: Phytochemical analysis of *Persea americana*

Compounds	Results
Tannins	+
Saponin	+
Flavonoids	+
Alkaloid	–
Phenols	+
Terpenes	–
Steroids	–
Glycosides	–
Resins	+
Carbohydrates	+
Oil	+

Keys; positive (+) = present, negative (-) = absent

3.2 Initial and final body weight

Results of initial and final body weight of the plant are given in table 2.

Table 2: Initial and final body weight of control and experimental rats

Groups (mg/kg)	Initial weight (g)	Final weight (g)
Control	162.83±18.47	197.33±23.85
Low Dose (150)	190.67±4.18	211.00±8.00
High Dose (300)	201.33±4.32*	227.17±19.25*
Vitamin E (200)	225.67±7.00*	247.83±9.37*

Values are given as mean ± SEM. * Significant at p ≤ 0.05

3.3 Activities of testicular histochemistry of G-6-PDH, LDH and Serum

P-ACP in the control and experimental groups are shown in table 3.

Table 3: Activities of testicular G-6-PDH, LDH and serum PAP

Groups (mg/kg)	G6PD (u/g Hb)	LDH (U/L)	P-ACP (U/L)
Control	6.50±0.50	367.50±25.50	0.83±0.09
Low Dose (150)	3.00±0.00*	203.00±22.00*	0.70±0.06*
High Dose (300)	2.00±0.00*	137.00±11.00*	0.33±0.03*
Vitamin E (200)	4.50±0.50*	516.50±182.50*	0.20±0.06

Values are given as mean ± SEM. * Significant at p ≤ 0.05.

3.4 Histopathology

Testicular Photomicrograph of Control Group and different doses is shown in figure 1-4.

4 Discussions

The study showed a slight increase in the final body weight of the rats in the treated groups which was however not significant when compared with the initial body weight of the rats. The slight increase in the body weight of the rats could be due to the oleic acid present in the *Persea americana* (avocado) which controls appetite and weight loss[21].

The groups given low dose and high dose of Avocado extract showed a significant decrease in glucose-6-phosphate dehydrogenase (G-6-PDH) level when compared to the control group and vitamin E group at p ≤ 0.05. A slight decrease in glucose-6-phosphate dehydrogenase (G-6-PDH), which was however, not significant, was observed in the group given low dose of Avocado extract, when compared with the group given Vitamin E, at p ≥ 0.05. The decrease seen in the G-6-PDH suggests the absence of oxidative stress following the administration of the *Persea americana* extract and the vitamin E[21].

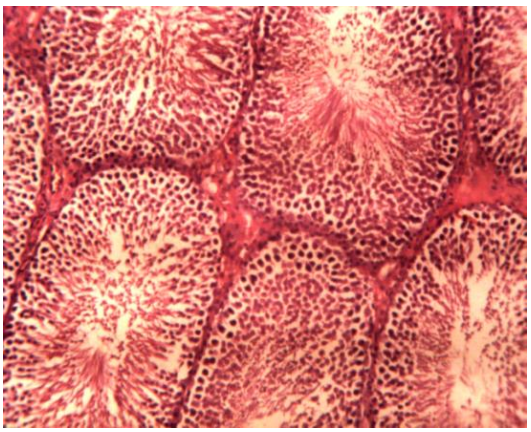

Figure 1: Light microscopic photomicrograph of left testis from adult rats in control groups showing normal spermatogenic epithelium composed of different spermatogenic cells, sertoli cells, leydig cells. Magnification: × 100

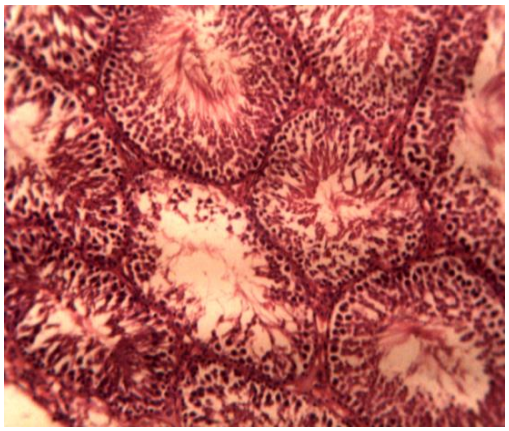

Figure 2: Light microscopic photomicrograph of left testis from adult rats in low dose (*Persea americana*) showing abnormal testicular architecture with focal areas of germ cells layer reduction and mild tubular thickness. Magnification: × 100

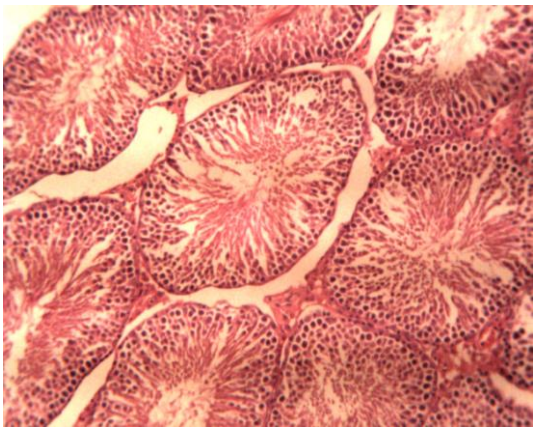

Figure 3: Light microscopic photomicrograph of left testis from adult rats in high dose (*Persea americana*) showing abnormal testicular architecture with focal areas of germ cells layer reduction and mild tubular thickness. Magnification: × 100

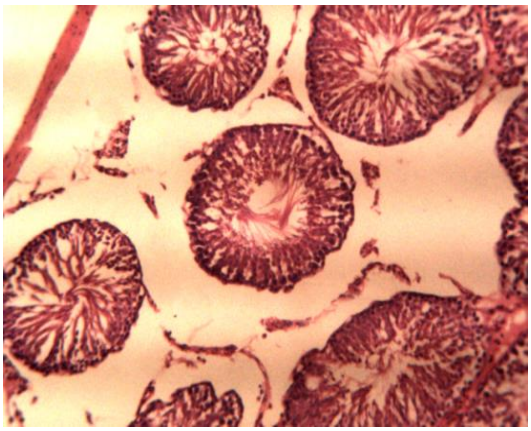

Figure 4: Light microscopic photomicrograph of left testis from adult rats in standard drug showing normal spermatogenic epithelium composed of different spermatogenic cells, sertoli cells, leydig cells. Magnification: × 100.

The groups given low dose and high dose of the extract showed a significant decrease in lactate dehydrogenase (LDH) when compared with the control group and vitamin E group at $p \leq 0.05$. The decreased level of the LDH was as a result of the absence of cell death and cancerous development as well as other diseases [22].

Result also showed a slight decrease in the prostatic acid phosphate (P-AcP) when the group given low dose and high dose of Avocado were compared with the control group. This however was not statistically significant at $p \geq 0.05$. But it showed a significant increase when compared to the vitamin E group at $p \leq 0.05$. The decrease in the level of P-AcP in the extracts and the vitamin E suggests reduced tendency for prostate cancer[23].

Photomicrograph of the testes showed that the control group had normal spermatogenesis with intact germ cell layers of the semineferous tubules, while both low dose and high dose groups of Avocado showed an abnormal testicular architecture with focal areas of germ cells layer reduction and mild tubular thickening, which could be a result of the phenol content of the extract [24] that may have exerted negatively on the germ cell and spermatogenesis in the high dose. In the vitamin E group, the photomicrograph showed a same structure with the control with normal spermatogenesis with intact germ cell layers of the seminiferous tubules, indicating normal spermatogenesis [25].

5 Conclusion

Avocado and Vitamin E increase body weight and reduce oxidative stress, they also decrease LDH and P-AcP levels in the body, and consequently reducing a tendency for cancerous cells growth, and particularly prostate cancer. However, unlike Vitamin E which improves on spermatogenesis, Avocado exerts negatively on testicular architecture, germ cells and spermatogenesis.

6 Conflict of interests

There is no conflict of interest among the authors regarding the publication of this article.

7 Author's contribution

This work was carried out in collaboration between all authors. Authors SOM and IOS were involved in concept and design of research. Author AOF and SAS were involved in data Analysis and interpretation. Author SAS handled the manuscript, involving critical writing and revising of the content. All authors read and approved the final version of this manuscript.

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