Tropical Journal of Science and Technology, 4(2), 1 - 16, 2023
ISSN: 2714-383X (Print) 2714-3848 (Online) Available online at credencepressltd.com
DOI: http://doi.org/10.47524/tjst.v4i2.2

Effects of Avocado (*Persea Americana*) and Sesame (*Seasame Indicum*) Seeds in the Management of Dmba-Induced Neoplasm in Wistar Albino Rats

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Abstract
Cancer, a genetic disease, is one of the leading causes of death all over the world. Many plants have been reported as being useful in its management, as chemotherapy destroys the normal cells along with cancer cells. Alternative safer therapies such as herbal therapies are becoming increasingly more popular. This research determined the anti-neoplastic effect of avocado (*Persea americana*) and sesame (*Sesame indicum*) seeds on 7, 12-dimethylbenz[a]anthracene (DMBA)-induced neoplasm in albino rats. Standard methods were used to determine the phytochemical constituents, proximate and mineral composition of avocado and sesame seeds. Biochemical, haematological and histological studies were also carried out. Thirty albino rats divided into five groups of six each were used for the study. Result showed that tannins and flavonoids were present in both avocado and sesame seeds; whereas saponins were only detected in avocado. The protein content was higher in sesame (17.64%) than in avocado (1.47%). Mineral analysis showed the presence of potassium, sodium, calcium, iron, phosphorus, magnesium and zinc in both seeds. The total red blood cells of the animals ranged from 6.64 $x10^{12}$/L to 8.92 $x10^{12}$/L. The lymphocyte for control group, and groups 1, 2, 3 and 4 were 65%, 76%, 73%, 62% and 95% respectively. The amount of alkaline phosphatase (ALP) was highest in group 4 (1352 µ/L) and least in group 3 (230 µ/L). There was no necrosis in the liver, skin, voice cord and mammary gland, except the mucosal gland of the colon of the neoplastic rats that showed some levels of tissue damage. A proof that organ injuries might not have occurred were evident in Alanine Transaminase (ALT) and Aspartate Transaminase (AST) levels which were not significantly different between the treated and the control groups. It can be concluded from this study that avocado and sesame seeds exert significant anti-neoplastic properties in rats.

Keywords: Cancer cells, necrosis, neoplasm, *Persea americana* and tumours

1. Introduction
The body is made up of billions of tiny cells. Normally, cells grow and multiply in a tightly regulated fashion. New cells are only made when and where they are needed. When cancer occurs, the cells' growth cycle goes haywire making them multiply uncontrollably (Schwartsmann *et al*., 2012). Cells become cancerous because of damage to their DNA. This leads to formation of a lump/tumour that may be benign or malign (AAbiodun *et al.,* 2010).

A neoplasm is malignant thus cancerous. Cancer, also called malignancy, is an abnormal growth of cells (Abiodun *et al* 2010). It represents a group of several diseases characterized by uncontrolled cell division and growth resulting in tumours, if malignant, and may spread to other parts of the body. Neoplastic cells originate from differentiated and specialized cells through a process of regression and differentiation to a simpler, more primitive stage which divides continuously unlike the normal parent cells (Celik *et al*., 2009). The characteristic properties of neoplasia include sustained proliferation, evasion of growth suppressors, immortality, anaplasia, continued replication, angiogenesis, reprogramming energy metabolism, invasion, metastasis,

and escaping immune destruction (Abiodun *et al.,* 2010).

Cancer is the second leading cause of death and it is one of the most worldwide spread diseases (Kutluk and Kars, 1998). Out of about 10 million people diagnosed of cancer every year, about 6 million die of the disease (Pinar, 1998). Deaths from cancer worldwide are projected to continue rising with an estimated 12 million deaths by 2030 (Wang *et al*., 2007). The major predisposing causes of cancer are smoking, dietary imbalances, hormones and chronic infections leading to chronic inflammation (Celik *et al*., 2009). The most frequent types of cancer worldwide in order of the number of global deaths are: among men- lung, stomach, liver, colorectal, oesophagus and prostate; and among women- breast, lung, stomach colorectal and cervical (Abiodun *et al.,* 2010).

Treatment of cancer usually involves a combination of surgery, radiation therapy, and chemotherapy. Despite these therapeutic options, cancer remains associated with high mortality. Various cancer and cancer-related conditions have been treated for ages by local herbalists and many plants have been reported as useful in the management of such conditions. Since chemotherapy destroys the normal cells along with cancer cells during treatment, active components from plant extracts can serve as source of new drugs that would likely lead to better treatments for cancer. Plant extracts have been used to produce many anticancer drugs such as taxanes and vincristine and still serve as a veritable source of new products through the use of standard bioassay methods (Richardson, 2001).

Several fruits and seeds abound in Nigeria and most West African countries where they are used partly as condiments or spices in human diets or as supplementary feeds to livestock such as rabbits, poultry, swine and cattle (Aremu *et al*., 2006). Lack of information on the specific nutrients and phytochemicals in a large number of the native fruits, seeds and vegetable with which Nigeria is richly endowed is partly responsible for their under-exploitation especially in areas beyond the traditional localities where they are found and consumed. The use of medicinal herbs in the management of some diseases has been of immense benefit to man because they are relatively safer, more affordable and sometimes offer better therapeutic value than synthetic drugs. Studies have shown that plants are rich in vitamins, flavonoids, saponins, carotenoids, many amino acids and organic acids (Schwartsmann *et al*., 2012).

Avocado (*Persea americana*) is classified as a member of the flowering plant family *Lauraceae* (Boning *et al*., 2006). The fruit has a green-skinned, fleshy body that may be pear or egg-shaped, or spherical. It is rich in several B vitamins, and vitamins K, C, and E, and potassium. It also contains phytosterols and carotenoids, such as lutein and zeaxanthin (Dreher *et al*., 2013). Beni or sesame seed (*Sesame indicum*) belongs to the family *Pedaliacea*. They are tiny spherical seeds that are edible. Whole sesame seeds are rich in several B vitamins and dietary minerals, especially iron, magnesium, calcium, phosphorus, and zinc (Odugbemi, 2006).

Due to the high frequency with which cancer affects the humans, the medical practitioners have made many efforts in the early diagnosis and more effective treatment of this disease. Several researches have attempted to develop experimental models of carcinogenesis, so that various stages of this diseases are better understood and provide advances in prevention and cancer control. Various *in vivo* and *in vitro* studies revealed that plants are enriched with active

anti-cancer substances (Mensah *et al*., 2008; Ogie-Odia and Oluowo, 2009; Park, 2012). The plants of interest are *Persea americana and Sesame indicum* because they contain vitamins and alkaloids that can be used in the management of cancer and are easily found in the environment (Schwartsmann *et al*., 2012). The main objective of this study was to investigate the anti-neoplastic effect of *Persea Americana* and *Sesame indicum* seeds on DMBA-induced neoplasm in albino rats.

2. Materials and Methods

2.1. Selection and Acclimatization of the Experimental Animal

A total of thirty albino rats weighing 120-140g were purchased from Nigerian Institute for Trypanosomiasis Research (NITR) Kaduna. The animals were acclimatized for three weeks in the Biological Sciences laboratory of Ribadu Cantonment, NDA. They were kept in steel metal cages under standard conditions (temperature, 26±2°c, 12 hours light and 12 hours dark cycle). All animals were fed with commercially formulated animal feed purchased from Vital Feed Nig. Ltd and were offered water *ad libitum.*

The animals were divided into five groups with six rats each. Their cages were cleaned of waste daily. All procedures involving the use of animals in this study complied with the guiding principles for research involving animals as recommended by the declaration of Helsinki and the Guiding principles in the care and use of animals (Uthman *et al*., 2013).

2.2. Sample Collection

Persea americana and Sesame indicum seeds were purchased from central market in Kaduna State. The seeds were identified and authenticated at the Department of Biological Sciences, NDA, after which voucher number were assigned to the seeds and deposited in the herbarium. The seeds of avocado were grated and air-dried for four weeks, and after which they were ground into pellets.

2.3.Phytochemical Screening

The method of Trease and Evans (1996) were adopted for phytochemical screening of *Persea americana and Sesame indicum* seeds. The seeds were tested for the presence of tannins, flavonoids, alkaloids, cardiac glycosides and saponins.

Test for Tannins

To a portion of the pellet, 3-5 drops of ferric chloride solution was added. A greenish-black precipitate indicated the presence of condensed tannins while hydrolysable tannins gave a blue or brownish-blue precipitate (Trease and Evans, 1996).

Test for Flavonoids

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute HCl, indicates the presence of flavonoids (Trease and Evans, 1996).

Test for Alkaloids

About 1 g of each powdered sample was separately boiled with water and acidified with 5 ml of 1 % HCl on a steam bath. The solution obtained was filtered and 2 ml of the filtrate was treated with few drops of the following reagents separately in different test tubes and observed (Trease and Evans, 1996). Filtrates were treated with Wagner‟s reagent (Iodine in potassium iodide). Formation of brown or reddish- brown precipitate was regarded as evidence for the presence of alkaloids in the extract (Trease and Evans, 1996).

Test for Cardiac Glycosides

To a portion of the pellet of each sample, 1ml of 2% solution of 3, 5-Dinitrobenzoic acid was added into 95% alcohol. The solution was made alkaline with 5% sodium hydroxide, appearance of purple-blue colour indicated the presence of cardenolides (Trease and Evans, 1996).

Test for Saponins

About 10 ml of distilled water was added to a portion of the pellet and was then shaken vigorously for about 30 seconds. The tube was allowed to stand in a vertical position and observed for about 30 minutes. A honeycomb froth that persisted for 10 to 15 minutes indicated presence of saponins (Trease and Evans, 1996).

2.4. Proximate Analyses of Seeds

The proximate analyses of the samples for moisture, total ash, crude fat, and crude protein were carried out using the AOAC (2005) procedures.

Moisture Content

This involved drying to a constant weight at 100°C and calculating moisture as the loss in weight of the dried samples. The crucible was thoroughly washed and dried in an oven at 100°C for 30 minutes and allowed to cool inside desiccators. After cooling, they were weighed using weighing balance and their various weights recorded as W1. Then, 2.0 g of the finely ground samples were put into the crucibles and weighed to get W2. Thereafter, the sample plus crucible were placed inside the oven and dried at 100°C for 4 hours, cooled and weighed at the same temperature for 30 minutes until constant weights were obtained to get W3. Then, the moisture content of the sample was calculated from the equation:

$$\% \text{ moisture} = \frac{(W2 - W3) \times 100}{(W2 - W1)}$$

Where, W1 = Initial weight of empty crucible, W2 = Weight of crucible + sample before drying and W3 = Final weight of crucible + sample after drying.

Determination of Fat

Total fat in the sample was determined using Soxhlet extraction for 4 hours starting with methanol and ethanol. About 250 ml clean boiling flasks were dried in an oven at 105 – 110°C for about 30 minutes and cooled in a desiccator. Approximately, 2.0 g of samples were weighed accurately into labeled thimbles. The dried boiling flasks were weighed correspondingly and filled with about 300 ml of petroleum ether (boiling point 40 - 60°C). The extraction thimbles were plugged tightly with cotton wool. After that, the Soxhlet apparatus was assembled and allowed to reflux for 6 hours. The thimble was removed with care and petroleum ether collected from the top container and drained into another container for re-use. After that, the flask was dried at 105 – 110°C for 1 hour when it was almost free of petroleum ether. After drying, it was cooled in a desiccator and weighed. Then, % fat in the sample was computed using the formula below:

$$\% \text{ fat} = \frac{\text{Weight of fat x 100}}{\text{Weight of sample}}$$

Total Ash Determination

Total ash of the sample was determined by Furnace Incineration based on the vaporization of water and volatiles with burning organic substances in the presence of oxygen in the air to CO_2 at a temperature of 600°C (dry ashing). About 1.0 g of finely ground dried sample was weighed into a 277 tared porcelain crucible and incinerated at 600°C for 6 hours in an ashing muffle furnace (Model 1184A Fisher Scientific, Houston, TX) until ash was obtained. The ash was cooled in a desiccator and

reweighed. The % ash content in the sample was calculated as:

$$\% \text{ Ash} = \frac{\text{Weight of Ash x 100}}{\text{Weight of original sample}}$$

Determination of Protein

The crude protein content of the samples was determined using the Microkjeldahl technique of AOAC (2005), which involved protein digestion and distillation.

Determination of Crude Fibre

About 2.0 g of the sample was hydrolyzed in a beaker with petroleum ether after which it was boiled under reflex for 30 min with 200 ml of a solution containing 1.25% H_2SO_4 per 100 ml of solution. The solution was filtered through a filter paper onto a fluted funnel. After filtration, the samples were washed with boiled water until they were no longer acidic. Then, the residue was transferred onto a beaker and boiled for another 30 min with 200 ml of solution containing 1.25% NaOH per 100 ml. The boiled samples were washed with boiled distilled water. The residues were filtered through Gooch filter crucible, dried at 100°C for 2 hours in an oven, cooled and washed. The percentage crude fibre in the rice sample was calculated as per the formula:

$$\% \text{ Crude fibre} = \frac{\text{Weight after drying x 100}}{\text{Weight of original sample}}$$

Determination of Total Carbohydrate

The total percentage carbohydrate content in the sample was determined by the difference method as reported by Onyeike *et al.* (2002). This method involved adding the total values of crude protein, lipid, crude fibre, moisture and ash constituents of the sample and subtracting it from 100. The value obtained is the percentage carbohydrate constituent of the sample. Thus:

% carbohydrate = 100 – (% moisture + % crude fibre + % protein + % fat + % ash).

Energy Value

The calorific energy value was obtained according to the methods of Akinyeye *et al*. (2011). This was done by multiplying the value of carbohydrate, protein and crude fat by the Atwater factors of 17, 17 and 37 respectively.

Mineral Analysis

The mineral contents of the samples were determined using the method described by Akinyeye *et al*. (2011). Calcium, Magnesium, Sodium and Potassium were determined by Atomic Absorption Spectrometry. About 1.0 g of sample was first digested with 20 ml of acid mixture (650 ml Conc. HNO_3, 80 ml Perchloric acid, 20 ml H_2SO_4) by weighing the sample into a digestion flask followed by addition of the 20 ml acid mixture. The digestion flask containing the sample and the digestion acid mixture was heated until a clear digest was obtained. The digest was later diluted with distilled water to 500 ml mark. Aliquots of the clear digest were used for atomic absorption spectrophotometry with filters that matched the different elements. The concentration of Calcium, Magnesium, Sodium and Potassium were determined with their calibration curves prepared with their standard solutions. The percentage values were later calculated by multiplying the concentrations by 100.

Phosphorus, iron and zinc were determined by Molybdate Method (Akinyeye *et al*., 2011). About 0.5 ml of the mineral digest and 9.5 ml of 10 % trichloroacetic acid were put into a test tube. This was followed by agitation for 5 mins. and filtration through a filter paper. About 5 ml of the filtrate was then measured into a cuvette. Also, 5 ml of trichloroacetic acid and 5 ml of the working standard were measured into two cuvettes which served as

blank and standard, respectively. About 0.5 ml of molybdate reagent was then added to each test tube and mixed. Similarly, 0.2 ml of sulphuric acid reagent was added and the contents were stoppered, mixed and allowed to stand for 10 mins. The absorbance of the test sample and standard were read in a Spectrophotometer at 660 nm with the blank set at zero.

Neoplasm Inducement

A modified method of Okeniyi and Lawal (2007) was adopted for the neoplasm inducement. Neoplasm was induced with 7.5mg/ml of DMBA (7, 12 dimethyl benz-anthracene), purchased from Sigma Chemical Co, St Louis MO USA. It was dissolved in 5.0ml of olive oil as diluent according to instruction's manual. Three weeks after the animals have been on formulation ration, the first dose of the inducement was administered orally. The second dose of the treatment was administered after three weeks. The animals were palpated weekly for visible tumour detection.

2.5. Experimental Design

Randomized experimental design was employed for the experiment. Thirty (30) albino rats weighing between 120 g to 140 g were randomly distributed into five different experimental groups with six (6) per cage.

Control group: The animals were provided with standard pelletized animal feed from Vital Feed Nigeria Limited and water freely throughout the experimental period.

Group 1: The animals were fed with normal animal feed, and offered water *ad lib*. They were administered with 7.5ml/mg of DMBA in 5ml of olive oil. For all groups where the inducement was used, it was administered via oral gavage.

Group 2: The animals were fed with pelletized avocado seed, animal feed, and water was provided *ad lib*. The animals received 7.5mg/ml DMBA inside 5ml of olive oil once at the beginning of the experiment, and another 7.5mg/ml of DMBA inside 5ml of olive oil at three-weeks interval.

Group 3: The animals were fed with pelletized sesame seed and animal feed and water freely. The animals also received 7.5mg/ml of DMBA inside 5ml of olive oil once at the beginning of the experiment and another 7.5mg/ml of DMBA inside 5ml of olive oil at three-weeks interval.

Group 4: The animals were fed with equally mixed ration of pelletized avocado and sesame seeds and animal feed and water freely. The animals received 7.5mg/ml DMBA inside 5ml of olive oil once at the first day of experiment and another 7.5mg/ml of DMBA inside 5ml of olive oil at three-week interval via oral gavage.

The animals were palpated weekly to check for any visible tumour development or neoplasm development. The treatment lasted for six weeks when inducement was withdrawn from the animals, but the formulated feeding regime was maintained. Twenty-four hours (24 hours) after the last day of the sixth week, the animals were sacrificed. Blood was collected for liver function test haematological analysis. The liver, mammary gland, colon and vocal part tissue were harvested for histopathology.

2.6. Biochemical, Haematological, and Histological Studies

Blood samples were taken from the various groups for biochemical and haematological studies. The Packed Cell Volume (PCV) and

liver function test were carried out according to Okeniyi and Lawal (2007).

The harvested organs were removed by cervical dislocation, washed in saline, fixed in bouins fluid, processed histologically, and stained with heamatoxylin and eosin in order to demonstrate the structure of the tissue and examined under a light microscope. Photographs using digital camera were taken as described by Okeniyi and Lawal (2007). The photographs of the histological structures of the organs of the rats from the treatment groups were compared with that from Control.

2.7. Statistical Analyses

Statistical analysis was carried out using the statistical package for the social sciences (SPSS). T-test was used to determine the significance between the proximate composition of *P. americana* and *S. indicum* seeds. Significant difference between the mineral composition of *P. americana* and *S. indicum* seeds was determined. Analysis of Variance (ANOVA) was used to determine the significant difference between the: (i) full blood count (ii) liver function parameters of the experimental groups. The probability level of significance for all the analyses was 0.05.

3. Result and Discuss

3.1. Presentation of Results

3.1.1. Phytochemical Screening of P. americana and S. indicum Seeds

The result of phytochemical screening of *P. americana* and *S. indicum* as presented in Table 1, shows that tannins and flavonoids were present in both *P. americana* and *S. indicum* seeds; whereas saponins were only detected in *P. americana*. Alkaloids and Cardiac Glycosides were not detected in both seeds.

Table 1. Phytochemical screening of *P. americana* and *S. indicum* seeds

Phytochemicals	*P. Americana*	*S. indicum*
Tannins	-	+
Flavonoids	+	+
Alkaloids	-	-
Cardiac Glycosides	-	-
Saponins	+	-

+ Present; - Absent

3.1.2. Proximate Composition of P. americana and S. indicum Seeds

As shown in Table 2, the protein content was higher in *S. indicum* (17.64%) than that of *P. americana* (1.47%). The ash content of *P. americana* and *S. indicum* seeds were 2.63% and 6.03% respectively. *S. indicum* had a higher fat content when compared with *P. americana*, with values of 42.40% and 8.40% respectively. On the other hand, there was a noticeable higher amount of moisture content in *P. americana* (11.96%) than in *S. indicum* (5.05%). The crude fibre of *P. americana* was 4.11% and that of *S. indicum* was 3.47%. The total carbohydrate was 75.54% in *P. americana* and 28.88% in *S. indicum*. The energy value was higher in *S. indicum*than in *P. americana*, with values of 567.68 and 383.64 kcal/100g. T-test showed that the proximate composition of *P. americana* and *S. indicum* seeds are not significantly different, with a P-Value of 0.7877.

Table 2. Proximate composition of *P. americana* and *S. indicum* Seeds

Samples	Protein (%)	Ash (%)	Fat (%)	Moisture (%)	Crude Fibre (%)	Total Carbohydrate (%)	Energy (kcal/100g)
P. americana	1.47	2.63	8.40	11.96	4.11	75.54	383.64
S. indicum	17.64	6.03	42.40	5.05	3.47	28.88	567.68
T-Test = 0.2754							
P-Value = 0.7877							

3.1.3. Mineral Composition of P. americana and S. indicum Seeds

The result presented in Table 3 shows the mineral composition of *P. americana* and *S. indicum* seeds. Potassium was comparatively higher in *P. americana* (106.7 mg) than in *S. indicum* (22.11 mg/g) whereas sodium was lower in *P. americana* (3.83 mg/g) than in *S. indicum* (43.73 mg/g). Sodium content was 36.1 mg/g in *P. americana* and 21.12 mg/g in *S. indicum*. Also, calcium was higher in *P. americana*, with a value of 106.7 mg/g relative to that in *S. indicum*, with a value of 10.23 mg/g. Phosphorus content in *P. americana* was 157.0 mg/g while that in *S. indicum*was 28.06 mg/g. Among all the minerals, magnesium had the least values, occurring 1.03 mg/g in *P. americana* and 1.04 mg/g in *S. indicum*. The value of zinc in *P. americana* and *S. indicum*were 4.46 mg/g and 2.04 mg/g respectively. T-test revealed that the mineral composition of *P. americana* and *S. indicum* seeds are not significantly different, with a P-Value of 0.1534.

Table 3. Mineral Composition of *P. americana* and *S. indicum* seeds

Samples	**K**	**Na**	**Ca**	**Fe**	**P**	**Mg**	**Zn**
P. Americana	106.7	36.1	281.1	3.83	157.0	1.03	4.46
S. indicum	22.11	21.12	10.23	43.73	28.06	1.04	2.04
T-Test = 1.634							
P-Value = 0.1534							

3.1.4. Haematological Studies

The total red blood cells ranged from 6.64 $x10^{12}$/L – 8.92 $x10^{12}$/L, with group 2 having the highest concentration. The control group and group 4 had small concentration of total platelet when compared with other groups. Group 2 had the highest concentration of total platelet (867 $x10^{9}$/L). Group 1 which was the positive control had the highest concentration of total white blood cell of 11.3 $x10^{9}$/L. There was a progressive increase in the PCV from negative control group to group 2; groups 3 and 4 had PCV of 39.3% and 47.7% respectively. The haemoglobin concentration of group 1 and 2 were the same and highest, with a value of 15.1 (g/dL). The mean corpuscular haemoglobin of the control group, groups 1, 2, 3, and 4 were 13.5pg, 17.9pg, 16.9pg, 18.5pg and 18.7pg respectively. The highest Mean Corpuscular Haemoglobin Concentration was highest in group 1 (318 g/dL) while the least was recorded in the control group (25 (g/dL). Also, the Mean Corpuscular Volume of the control group, group 1, group 2, group 3 and group 4 were 53.9fL, 56.2fL, 55.2fL, 59.2fL and 62.8fL respectively (Table 4). There is no significant difference in the Haematological Parameters (Full Blood Count) of the

Experimental Animals (Table 5). With the exception of lymphocyte, there was no observed value in the differential count of the experimental animals (Table 6). The lymphocyte of negative control group, group 1, group 2, group 3 and group 4 were 65%, 76%, 73%, 62% and 95% respectively.

Table 4. Haematological parameters (Full Blood Count) of the experimental animals

Experimental Animals	Total RBC ($x10^{12}$/L)	Total Platelet ($x10^9$/L)	Total WBC ($x10^9$/L)	PCV (%)	HB (g/dL)	MCH (pg)	MCHC (g/dL)	MCV (fL)
Control group	6.93	17	3.7	36	9.1	13.5	25	53.9
Group 1	8.45	580	11.3	47.5	15.1	17.9	318	56.2
Group 2	8.92	867	9.1	49.2	15.1	16.9	307	55.2
Group 3	6.64	670	10.3	39.3	12.3	18.5	313	59.2
Group 4	7.6	54	9.8	47.7	14.2	18.7	298	62.8
Mean	7.708	437.6	8.84	43.94	13.16	17.1	252.2	57.46

RBC- Red Blood Cell, WBC- White Blood Cell, PCV, Packed Cell Volume, HB- Haemoglobin, MCH- Mean Corpuscular Haemoglobin, MCHC- Mean Corpuscular Haemoglobin Concentration, MCV- Mean Corpuscular Volume.

Table 5. Analysis of variance of the haematological parameters (Full Blood Count) of the experimental animals

Source of variation	DF	SS	MS	F-value	P – value
Treatment	4	116337	29084	0.7286	0.579
Residue	35	1397169	39919		
Total	39	1513505			

Table 6. Haematological parameters (Differential Count) of the Experimental Animals

Experimental Animals	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
Control group	0.0	65	0.0	0.0	0.0
Group 1	0.0	76	0.0	0.0	0.0
Group 2	0.0	73	0.0	0.0	0.0
Group 3	0.0	62	0.0	0.0	0.0
Group 4	0.0	95	0.0	0.0	0.0

3.1.5. Biochemical Test

The liver function parameters of the experimental animals as presented in Table 7 shows that with the exception of group 4, there was a decrease in the total bilirubin of the experimental animals from control group to groups 1, 2 and 3. Group 4 had the highest conjugated bilirubin of 1.5 µmol/L whereas groups 2 and 3 had similar value of 1.3 µmol/L. The amount of alkaline phosphatase was highest in group 4 (1352 µ/L) and least in group 3 (230 µ/L). The aspartate transaminase in the experimental group ranged from 275 µ/L to 342 µ/L; which occurred highest in group 4. Group 4 had the highest concentration of alanine transaminase of 107 µ/L. The total protein of control group, groups 1, 2, 3, group 4

were 69 µ/g, 67 µ/g, 65 µ/g, 66 µ/g and 66 µ/g respectively. The albumin concentration was least in the control group (29 µ/g) but highest in groups 2 and 4 (33 µ/g). Analysis of variance showed no significant difference in the liver function parameters of the experimental group of animals (Table 8).

Table 7: Liver function parameters

	Total Bilirubin (µmol/L)	Conjugated Bilirubin (µmol/L)	Alkaline phosphatase (µ/L)	Aspartate Transaminase (µ/L)	Alanine Transaminase (µ/L)	Total protein(µ/g)	Albumin (µ/g)
Control group	31.5	0.6	846	287	93	69	29
Group 1	26.2	0.2	1063	335	83	67	32
Group 2	18.2	1.3	604	313	80	65	33
Group 3	14.9	1.3	230	275	66	66	30
Group 4	30.9	1.5	1352	342	107	66	33

Table 8: Analysis of variance of the liver function test

Source of variation	DF	SS	MS	F-value	P – value
Treatment	4	129199	32300	0.2995	0.8760
Residue	3	3235584	107853		
Total	34	3364783			

3.1.6. Histological Studies

There was no necrosis in liver, skin, voice cord, and mammary gland. However, the mucosal gland of the colon of the neoplastic rats showed some levels of tissue damage when viewed under the microscope (Fig. 1 and 2).

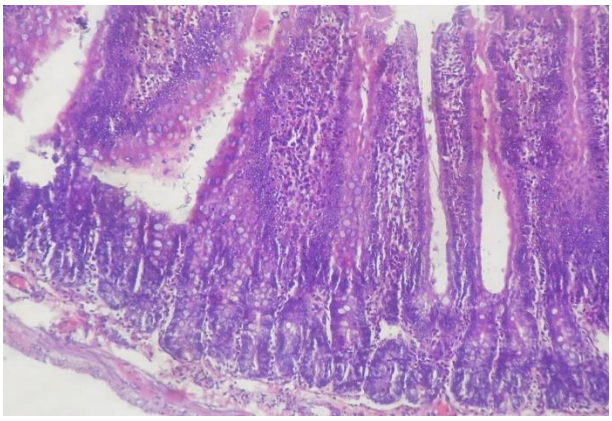

Fig. 1. Photomicrograph of colon showing necrosis of mucosal glands and villi

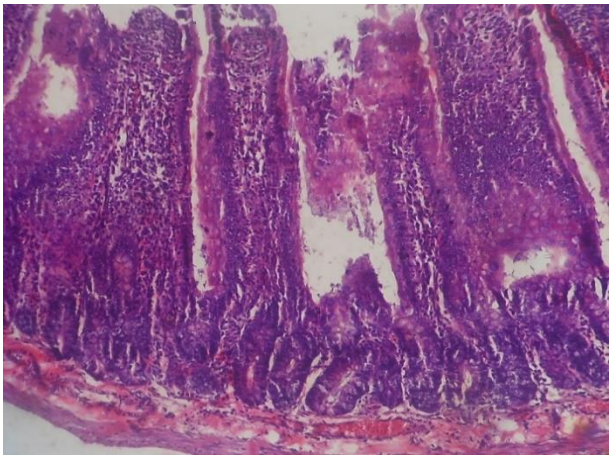

Fig. 2. Photomicrograph of colon showing necrosis of mucosal glands and villi

3.2. Discussion

The proximate composition revealed that avocado and sesame seeds are good source carbohydrates, proteins, lipids and fibres. The ash content when compared with the results obtained by Arukwe *et al.* (2012) were nearly same (2.40%). The proximate value of avocado seed seems to show semblance to those of *Jatropha* seeds in terms of the protein and lipid content as reported by Chikpah and Demuyakor, (2013). Except for the carbohydrate and moisture contents, every other nutritional parameter was lower for both the Hass avocado and Fuerte avocado as reported by Olaeta *et al.* (2007). The high values of total carbohydrate of avocado and sesame seeds portray them as alternative energy sources for metabolic processes. The low moisture contents showed that these seeds can be stored for a long period of time without spoilage, and may not be susceptible to microbial growth (Chikpah and Demuyakor, 2013). Low moisture in food samples implies they may not be inclined to decay, since foodstuffs with high dampness are more inclined to spoilage (Ortiz *et al*., 2004). The value of moisture content obtained in avocado seed was closely related to the moisture content of legumes (Abou-Garbia *et al*., 2000). Whereas that of sesame seed is related to 5.7% that was reported for cashew nut flour (Aremu *et al*., 2006) and 5.0-5.5% for pumpkins (Fagbemi and Oshodi, 1991).

The protein contents of avocado and sesame seeds obtained in this study is lower than that of protein-rich foods as soy beans, cowpeas, pigeon peas, melon, pumpkin and gourd seeds, all ranging between 23.1-33.0 % (Olaofe *et al* 1994). The crude protein content obtained in this study is also lower than the one obtained by Faqir *et al* (2012). They are less than the FAO recommendation value of 19.8% (Whitney and Rolfes 2005). Therefore, it can be established that it is unlikely that defatted seeds can supply the recommended daily intake of protein for children. The crude fibre contents obtained in this study are lower than the values obtained by Rehman *et al.* (2012). The ash content of avocado seed obtained in this study is within the acceptable ash content mean values of seed and nut of 1.0 to 5.0% recommended by Duke (1992). More so, the ash contents of sesame seeds are higher than that obtained by Ogungbenle (2011), Faqir *et al* (2012) and Gamal *et al.* (2012).

The relatively high amount of calcium, sodium and potassium in the samples indicate high nutritional value and fucntionality. Ozcan (2004) reported that

calcium is important in blood clotting, muscle contraction and in certain enzymes in metabolic processes. Sodium and potassium regulate water balance, heart rhythm, muscles contraction and nerve-signal conduction (Loscalzo, 2001). The high amount of iron and zinc found in sesame and avocado seeds indicate that the plant could be a good source of dietary iron to overcome nutritional deficiency of iron, if supplemented in the diet (Loscalzo, 2001).

The lack-of-effect on neutrophils, monocytes, eosinophils and basophils levels indicates that the seed extract may not have induced any inflammatory process since these cells are usually elevated in the course of inflammations (Formela *et al*., 1995). Additional proof that organ injuries might not have occurred was shown in alanine transaminase (ALT) and aspartate transaminase (AST) levels which were not significantly different between the treated and the control groups. Elevated levels of AST and ALT are often diagnostic of underlying cellular injuries (Wittekind, 1995; Karthikeyan *et al* 2006). There was a slight increase in the level of AST in groups 1, 2, and 4; but decrease in group 3 and negative control group. Increase in AST level agreed with the findings of Uthman *et al* (2013) who found elevation in transaminase enzymes (AST and ALT) in rat when treated with diazinon. Slight elevation in the serum level of this enzyme could be attributed to acute hepatocellular damage or extrahepatic dysfunction or persistent stress and be due to leakage of these enzymes from liver cytosol into the blood stream as a consequence of hepatotoxic effect of DMBA (Okeniyi, 2007). The level of ALP in group 2 and 3 are lower than that of the control groups. Fawzy *et al.* (2007) is of the opinion that decrease in ALP level could be as a result of the effect on absorptive and secretory surface of the cell membrane causing cellular leakage and thus reduced activity. Increased ALT level in group 4 could also be due to hepatoxicity causing permeability alterations (Okeniyi, 2007). The significate elevation in total proteins in group 1 and 4 when compared to the control group indicates a reduction in the synthetic function of the liver. Also, the slight decrease in the total protein level in group 1, 2 3 and 4 might be due to the catabolism of protein or malfunctioning of liver (Sarin and Gill, 1998). Swamy *et al*. (1992) have similarly reported that the decrease in total proteins indicates their metabolic utilization. Total bilirubin formed from the breakdown of red blood cells by hepatocytes, are used to determine the extent of hepatocellular damage (Sarin and Gill, 1998). The result of this study suggests that the DMBA caused some significant hepatocellular damage in groups 1 and 4 when compared with the negative control group, as there was a noticeable increase in their bilirubin level.

A good pathological and physiological indicator of animal health is the blood (Okeniyi 2007). In this study, an elevation in the level of RBC, PVC and haemoglobin levels in the course of administration of DMBA was not indicative of anaemia. This is not in agreement with the findings of Matsuoka *et al.* (2010) following intraperitoneal administration of DMBA. The MCH, MCV and HB values suggest that normocytic normochromic anaemia might have occurred. This could be attributed to the destruction of the red blood cells by DMBA beyond the production capacity of the bone marrow and the fall in the level of iron content (Matsuoka *et al* 2010).

An elevation in lymphocytes which was observed in this study is indicative of leukocytosis (Celik *et al*., 2009). This finding is in concordance with the observation of Jones (1996) who similarly

recorded an increase in the level of lymphocytes following the administration of DMBA. In this study, there was a noticeable increase in the level of blood platelets, which is in agreement with the findings of Celik *et al* (2009). Necrosis of the mucosal gland within the colon cells was evident. This necrosis was evidence of infiltration of the cells by toxic substances. The presence of necrosis may be related to the depletion of ATP, which finally leads to the death of the cells (Schwartsmann *et al.,* 2012). The absence of necrosis in the liver, mammary gland and skin showed that the avocado and sesame seeds had anti-neoplastic effects in the organs.

Conclusion

Results from this study indicate that the *P. americana* and *S. indicum* seeds are high in carbohydrate, fats, protein and fibre that can suggest its utilization in food or animal feed formulation. Also, this study shows the presence of flavonoids, tannins and saponins in *P. americana* and *S. indicum* seeds. These seeds contained some minerals like potassium, sodium, calcium, phosphorus, zinc, iron and magnesium, and that their mineral compositions are not significantly different. *P. americana* and *S. indicum* seeds exerted significant anti-neoplastic properties in rats. The findings of this study provide a pharmacological basis for the traditional use of avocado and sesame seeds in the management of cancer. However, further studies are required to identify the active ingredient responsible for the anti-neoplastic properties of the seed extract.

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