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Effect of *Spondias mombin* leaves' extracts on chemically induced tumor

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ABSTRACT

Background & Aim: A tumor is merely a mass of tissue that does not perform any physiological function; it can be either benign or malignant. Cancer is being regarded as a malignant ailment emerging from unrestricted cell division occurring in the body to make mass of tissues. *Spondias mombin* L. (Family- Anacardiaceae) grows in coastal areas and in the rain forest into a big tree of up to 15 – 22 m. This study investigated the efficacy of *S. mombin* aqueous and n-hexane extracts in chemically induced tumor-bearing animals.

Experimental: Twenty-eight (28) healthy rats were used and grouped into seven (7). Six groups were induced with trichloroacetic acid and ethidium bromide leaving out normal control group for 28 days prior to treatments. LBH589 (standard anticancer drug, 500 mg/kg) served as positive control. At the termination of the experiments, the animals were sacrificed; solid tumor volume was determined, blood and tissue samples were collected for hematology and histopathology respectively.

Results: The results showed very significant reduction in the solid tumour volumes of *S. mombin* leaves extracts treated groups compared to positive and untreated control groups. The result of hematological parameters showed that the hemoglobin content, red blood cell and white blood cell counts of all treated groups were near to normal control group. Normal histological features were observed in control group animals, the negative groups showed the manifestations of cancer, all the treatment groups showed significant levels of repair almost similar to normal features in control group particularly in intestines and lungs of the experimental animals.

Recommended applications/industries: The present data provided substantial evidence that *S. mombin* leaves suppressed tumor growth in experimental animals and suggested that the extracts are potential antitumorigenic agent inhibiting chemically induced lung and intestinal tumors.

1. Introduction

The use of plant and plant-based products has been a valuable and safe source of medicines for treating different kinds of diseases. This brought about the term herbal medicine, which denotes the use of herbs for their therapeutic or medicinal value (Acharya *et al.*, 2008). *Spondias mombin* (Family – Anacardiaceae and commonly known as Hog plum in English language) is a fruit-bearing tree that is indigeneous to the lowland moist forest of the Amazon (Adedokun *et al.*, 2010; Bicas *et al.*, 2011). The leaves are green, thin, 15 - 30 cm long, the majority of which are shed preceding the production of various little, white fragrant flowers. The

plant is found all over tropical America, Brazil, Nigeria, West Indies and other tropical rainforest of the earth (Duvall, 2006; Adedokun *et al.*, 2010; Bicas *et al.*, 2011). All parts of the plant have been reported to be medicinally useful (Uchendu and Isek, 2008) especially for the treatment of many inflammatory conditions (Nworu *et al.*, 2007). A tumor is merely a mass of tissue that does not perform any physiological function; it can be either benign or malignant. Cancer is being regarded as a malignant ailment emerging from unrestricted cell division occurring in the body to make mass of tissues (Musa *et al.*, 2007). These cells have the ability to penetrate neighboring or adjoining organs or extend to inaccessible parts of the body where they can continue multiplying wildly in this manner, causing critical diseased state and death. In any case, not every tumor or inflammation is cancerous; benign tumors are not assigned as being cancerous since they do not extend to other parts of the body. Cancer is positioned by Word Health Organization as the second principal reason for death universally (8.8 million deaths in 2015). In Africa, cancer was ranked as the 7th leading cause of death in 2004 (Nworu *et al.*, 2007). It is further projected that, by 2030, yearly occurrence and number of deaths will rise to 1.28 million cases and 970,000 respectively (Nworu *et al.*, 2007). Many remedy options for most cancers exist, with the major ones which includes; surgical treatment, chemotherapy, radiation remedy, hormonal therapy, targeted therapy, palliative care and immunotherapy. The choice of treatments used depends on the form, site, and level of the cancer as well as the person's fitness and desires. Approximately five decades of systemic drug discovery and development have resulted in the establishment of a large collection of useful chemotherapeutic agents. However, chemotherapeutic treatments are not devoid of their intrinsic problems. Most of the synthetic chemical agents currently being used in cancer therapy today are toxic and therefore potentially cause damage to normal cells (Hussein *et al.*, 2013). Many plants are been investigated in order to obtain new, effective and safe antioxidant and anticancer drugs, as well as to study their mode of action on cancer cell inhibition (Hussein *et al.*, 2013). Trichloroacetic acid is a colorless to white crystalline solid with a sharp, pungent odour. TCA is formed from organic material during water chlorination and has been detected in groundwater, surface water distribution systems, and swimming pool water. Ethidium bromide (EtBr) is an intercalating agent commonly used in biochemistry and molecular biology laboratories for a long period of time. It is well-known to be used for the nucleic acids and proteins observation. This study investigated the effect of *Spondias mombin* aqueous, diethyl ether, ethanol and n-hexane extracts in trichloroacetic acids and ethidium bromide induced tumor-bearing animals in order to ascertain the use of the plant ethnobotanically and to provide cheap and effective herbal remedy.

2. Materials and Methods

2.1. Collection of plant sample

Spondias mombin leaves were obtained from the Faculty of Pharmacy's premises, University of Benin, Benin City, Edo State and authenticated at the Department of Plant Biology and Biotechnology with voucher number UBH-S345. The plant was air dried at room temperature and pulverized into powdery form which was kept in air-tight containers ready for the analysis.

2.2. Plant extraction

The *Spondias mombin* leaves were cut into pieces, air-dried at ambient temperature and blended into powdery form. The powdered *Spondias mombin* was made into two extracts with n-hexane and deionized water. The suspension was agitated at an interval of 12 hours. After 72 hours, the n-hexane extract was then filtered and the filtrate was exposed to dryness at room temperature for seven days while aqueous extract was freeze dried. Dilutions of each dried crude extract were prepared to give final test concentrations of 10 and 30 mg/kg used for antitumor analysis.

2.3. Experimental animals

Healthy female albino rats (12 weeks-of-age; weighing 150 – 190 g) with an average weight of 170 g used for this study were obtained from the Department of Zoology and Environmental Biology, College of Medicine, Ibadan, Nigeria. The animals were maintained in the animal house of the Center for Bioinformatics and Drug Development, Adekunle Ajasin University, Akungba-Akoko where the study was carried out. They were subsequently allowed to acclimatize for two weeks during which they were fed with animal pellets and water *ad libitum* before the commencement of the experiment.

2.4. Induction of carcinogen

Trichloroacetic acid (TCA; 500 g, Abdel-Hamid *et al.*, 2011) was dissolved in distilled water and the volume was adjusted to 100 ml and 100 ml of Ethidium bromide was introduced into the solution. Each animal received 1 ml of the solution daily for 28 days with the use of oral cannula. Animals were confirmed cancerous by sacrificing one animal from each group in order to check for the presence of tumors prior to treatments. Figure 1 shows the formation of tumor (hyperplastic nodules) in the spleen, lung and intestine before the commencement of treatments.

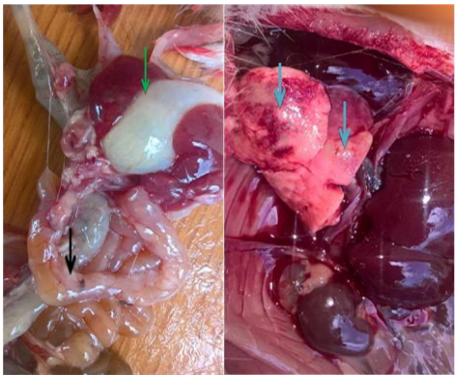

Figure 1. Formation of tumor (hyperplastic nodules) in the Spleen (green arrow), Lung (blue arrows) and Intestine (black arrow) before the commencement of treatments.

2.5. Experimental design

Twenty-eight animals were divided into seven groups of 4 rats each. Six groups (leaving out group one as healthy control group) were chemically induced with carcinogens for 28 days. Group 1 served as normal (basal) control and was administered water only. Group 2 served as the negative (diseased) control and was also administered water alone. Group 3 served as positive control and a standard anticancer drug (LBH589 at 500 mg/kg body weight) was administered. Groups 4 and 5 were given aqueous extract of *Spondias mombin* leaves dissolved in 1 ml of water at doses of 10 and 30 mg/kg body weight respectively while groups 6 and 7 received 10 and 30 mg/kg of n-hexane extract of *Spondias mombin* leaves respectively.

2.6. Sacrificing and collection of blood and tissues samples

At the end of the experiment (56 days), the animals fasted overnight and then anaesthetized using diethyl ether before sacrificing them. Blood was collected by cardiac puncture with a needle and syringe in bottles containing anticoagulant (EDTA), mixed properly and labeled appropriately for heamatology analysis. The liver, lungs, spleen, bile ducts, bone marrow and intestines were excised using sterilized dissecting kits and surgical blades. The radii of the tumor seen on the tissues excised were measured using Vernier Caliper.

The volume of the tumor was calculated using the formula:

$$V=4/3\ \Pi\ r1^2\ r^2.$$

Where ‘r1’ and ‘r’ represent the major and minor diameter respectively.

Photographs of the tissues were taking and weighed. Results were expressed as Mean ± SE. Statistical analysis was carried out by one-way ANOVA. Data were further subjected to Duncan test and differences between treated groups and controls were taken as significant at $P \leq 0.05$. The excised tissues were rinsed in physiological saline solution and preserved in sample bottles containing formalin for the histopathology analysis.

2.7. Histopathological test

Samples preserved were subjected to routine histology using Haematoxylin and Eosin staining method. The tissues were dehydrated in an ethyl alcohol series of ascending concentrations; they were cleared in xylene and embedded in paraffin. Further, the sagittal sections (5 µ thickness) were cut using a rotary microtome and mounted on glass slides. Sections were deparaffinized in xylene, hydratated in ethanol and stained with haematoxylin and alcoholic eosin for general histological evaluation. Photomicrographs of stained sections were made using photoelectron-microscope (XSZ-107BN, China). Photomicrographs of control groups were compared with those of exposed groups under the guidance of a pathologist.

2.8. Hematological analysis

The blood in the EDTA bottles was used for hematological indices. All assays were carried out within 24 hours of sample collection. The effects of the extracts on packed cell volume (PCV), hemoglobin (Hb) count, red blood cell (RBC) count, Mean Corpuscular Concentration Volume (MCV), Mean

Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), white blood cell (WBC), eosinophils, neutrophils, lymphocytes, monocytes, basophils and platelets counts were analyzed using an automated hematological analyzer (Dacie and Lewis, 2002). The PCV was estimated using microhaematocrit methods as described by Alexander and Grittiths (1993). All hematological parameters were analyzed at the Haematology Unit, Adekunle Ajasin University Health Centre, Akungba Akoko using the automated method with the automatic analyzer (Haematology auto analyzer Sysmex KX-21N).

2.9. Statistical analysis

Results were expressed as Mean ± SD. Data were analyzed with SPSS 12.0 software and Duncan's New Multiple Range Test (DNMRT) was performed to compare the means of the different groups. Significance was statistically acceptable at $P \leq 0.05$.

3. Results and discussion

A tumor is merely a mass of tissue that does not perform any physiological function; it can be either benign or malignant. Possible tumor indications include: a fresh protuberance, abnormal blood loss, persistent cough, unexplained reduction in weight, and, among others, expulsion of feces irregularity. Possible signs can appear as cancer and they can also arise owing to other problems. Some of the frequent cancer risk conditions that influence people are tobacco use, obesity, poor diet, lack of bodily activity and alcohol intake. Other causes include certain diseases, introduction to ultraviolent emission, sedentary lifestyle, occupational factors, microbes and ecological pollutants (Calle *et al.*, 2003). Cancer is not limited to people: creatures and other living beings have the tendency to get cancer.

The effects of carcinogen on the weight of experimental animals before and during treatments are shown in Tables 1 and 2.

Table 1. Effect of carcinogen on the weight of experimental animals before treatment.

Groups	Initial weight (g)	Final weight (g)	Weight difference (g)
Normal control	170.78 ± 13.46^{ab}	175.00 ± 13.70^{abc}	4.22 ± 0.64^{c}
Negative control	129.42 ± 16.95^{cde}	144.21 ± 21.95^{bcde}	14.79 ± 5.84^{bc}
Positive control	142.03 ± 21.85^{bcd}	153.53 ± 16.51^{abcd}	11.5 ± 6.22^{abcd}
10 mg/kg ethanol extract	130.44 ± 20.90^{cde}	142.35 ± 28.48^{bcde}	11.91 ± 7.57^{bc}
30 mg/kg ethanol extract	132.89 ± 10.53^{cde}	157.92 ± 24.64^{abcd}	25.04 ± 14.11^{ab}
10 mg/kg N-hexane extract	151.32 ± 4.22^{abc}	185.70 ± 0.14^{ab}	34.39 ± 4.08^{a}
30 mg/kg N-hexane extract	181.70 ± 4.03^{a}	191.88 ± 4.87^{a}	10.18 ± 0.84^{bc}

Note: Normal control received no toxicant. Data is represented as Mean ± SD. Mean values with the same superscript alphabets in the columns are not significantly different from each other at P>0.05 using Duncan's New Multiple Range Test (DNMRT).

Table 2. Effect of extracts on the weight of tumor bearing rats during treatments.

Group	Initial weight (g)	Final weight (g)	Weight difference (g)
Normal control	175.00 ± 13.70^{abc}	203.07 ± 9.48^{abc}	28.07 ± 5.46
Negative control	142.35 ± 28.48^{bcde}	176.69 ± 66.80^{abcd}	34.34 ± 38.32
Positive control	153.53 ± 16.51^{abcd}	145.26 ± 15.94^{abcd}	-8.27 ± 1.58
10 mg/kg ethanol extract	157.92 ± 24.64^{abcd}	182.30 ± 21.20^{abc}	24.38 ± 3.44
30 mg/kg ethanol extract	144.21 ± 21.95^{bcde}	160.19 ± 18.36^{bcd}	15.98 ± 4.75
10 mg/kg N-hexane extract	185.70 ± 0.14^{ab}	221.35 ± 24.16^{a}	35.15 ± 23.31
30 mg/kg N-hexane extract	191.88 ± 4.87^{a}	206.20 ± 5.57^{ab}	14.33 ± 0.70

Note: Normal control received no toxicant. Data is represented as Mean ± SD. Mean values with the same superscript alphabets in the rows are not significantly different from each other at ($P > 0.05$) using Duncan's New Multiple Range Test (DNMRT).

The result showed increase in weight of the animals before treatments which could be as a result of chronic inflammation and development of tumor (Casado *et al.*, 2011). The result of Table 2 showed increase in the weights of all the treated animals (except for positive control group) but as the concentration of the extracts increased, there were reductions in their weights. The reliable criterion for judging the value of any anticancer drug is through the reduction of solid tumor volume (Oberling and GueRin, 1954; Marklund *et al.*, 1982). Plant derived extracts containing antioxidant principles such as flavonoids, phenolic compounds and tannins showed cytotoxicity towards tumor cells (Marklund *et al.*, 1982) and antitumor activity in experimental animals (Li and Oberley, 1997). Tables 3 and 4 show efficacies of *Spondias mombin* leaves

extracts on solid tumor volume of intestines and lungs of the tumor bearing rats respectively. There was significant reduction in the tumor volume of ethanol and n-hexane treated groups when compared with the control groups. The result of intestines showed that n-hexane at 30 mg/kg had the lowest tumor density while tumors were absent in the lungs of animals that received ethanol (30 mg/kg) and n-hexane (10 mg/kg) extracts.

Table 3. Effect of *Spondias mombin* leaves extracts on solid tumor volume of intestine on tumor bearing rats.

Group	Weight (g)	Tumor radius	Tumor volume	Tumor density
Negative control	5.86 ± 1.76^{a}	0.32± 0.08	0.15 ± 0.11	46.00 ± 14.14^{a}
Positive control	5.80 ± 0.27^{ab}	0.25 ± 0.14	0.10 ± 0.12	44.00 ± 5.00^{a}
Ethanol (10 mg/kg)	4.45 ± 1.59^{b}	0.18 ± 0.04	0.02 ± 0.01	19.50 ± 7.78^{b}
Ethanol (30 mg/kg)	6.13 ± 2.02^{ab}	0.18 ± 0.04	0.02 ± 0.01	34.50 ± 19.09^{ab}
N- hexane (10 mg/kg)	5.07 ± 0.52^{b}	0.15 ±0.07	0.02 ± 0.02	20.00 ± 2.82^{b}
N- hexane (30 mg/kg)	4.25 ± 1.22^{b}	0.23 ± 0.04	0.05 ± 0.03	17.00 ± 1.41^{b}

Note: Data is represented as Mean ± SD. Mean values with the same superscript alphabets in the rows are not significantly different from each other at (P>0.05) using Duncan's New Multiple Range Test (DNMRT).

Table 4. Effect of *Spondias mombin* leaves extracts on solid tumor volume on lungs of tumor bearing rats.

Group	Weight (g)	Tumor radius	Tumor volume	Tumor density
Negative control	1.47 ± 0.22	0.32 ± 0.08	0.15 ± 0.11	17.67 ± 4.51^{a}
Positive control	1.50 ± 0.12	0.15 ± 0.07	0.02 ± 0.02	10.50 ± 2.12^{ab}
Ethanol 10 mg/kg	1.08 ± 0.65	0.13 ± 0.04	0.06 ± 0.06	9.00 ± 2.83^{ab}
Ethanol 30 mg/kg	1.04 ± 0.14	0.00	0.00	0.00
N- hexane 10 mg/kg	1.06 ± 0.24	0.00	0.00	0.00
N- hexane 30 mg/kg	1.60 ± 0.15	0.15 ± 0.21	0.06 ± 0.08	4.00 ± 5.65^{b}

Note: Tumors were no longer present in rats of Groups with 0.00. Data is represented as Mean ± SD. Mean values with the same superscript alphabets in the rows are not significantly different from each other at (P>0.05) using Duncan's New Multiple Range Test (DNMRT).

The significant reduction and/or disappearance of the tumors (Tables 3 and 4) which indicated the potential of *S. mombin* leaves' extracts as antitumor agent could be attributed to the antioxidant activity of the plant either through induction of apoptosis or inhibition of angiogenesis (Ruby *et al.*, 1995; Ming *et al.*, 2002).

Blood parameters are key factors in diagnosing the actual physiological status of organisms. An organism must keep its blood composition and constituent relatively constant under natural conditions to function properly (Rodrigues and McNeill, 1992). Blood cancers can affect blood cell counts in a number of ways, either lowering or increasing measurements. When receiving treatment such as chemotherapy, drug therapy or radiation, blood counts will be affected because oral ingestion of medicinal compounds or drugs can alter the normal range of haematological parameters.

The effect of *Spondias mombin* leaves extracts on the haematological parameters of tumor bearing rats is presented in Table 5. There were reductions (P<0.05) in Packed Cell Volume (PCV), White Blood Cell count (WBC), Red Blood Cell count (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Haemoglobin (Hb) and Neutrophils of all the treatment groups compared to the normal control group. The result showed that treatment with *S. mombin* leaves extracts brought back the hemoglobin content, RBC and WBC counts near to normal. Blood counts usually return to normal after treatment is completed. The significant decrease in the Red Blood Cell (RBC), White Blood Cell (WBC), Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and haemoglobin levels are indicative of anemia, leukemia, malnutrition (Iron, folate, copper, vitamin B6 and vitamin B12 deficiencies), hemorrhage (bleeding), cancer, bone marrow failure and multiple myeloma.

Table 5. Hematological parameters of tumor bearing rats treated with *Spondias mombin* leaves extracts.

Parameters	Normal Control	Negative control	Positive control	Ethanol		N-hexane	
				10mg/kg	30mg/kg	10mg/kg	30mg/kg
PCV (%)	48.33±1.20^{b}	40.33±2.96^{a}	44.33±2.73^{ab}	43.50±0.50^{ab}	42.00±1.00^{ab}	43.00±0.00^{a}	41.00±2.00^{a}
WBC ×10^9	8.93±0.74^{b}	4.07±1.07^{a}	5.93±0.97^{ab}	5.50±1.80^{a}	3.85±0.35^{a}	6.60±0.40^{ab}	4.50±1.50^{a}
RBC ×10^12	10.37±0.64^{d}	6.07±1.04^{ab}	8.67±0.13^{cd}	6.70±0.00^{abc}	7.00±1.40^{abc}	6.30±0.20^{a}	7.20±0.20^{ab}
MCV (fl)	77.67±2.73^{b}	57.33±5.81^{a}	50.67±2.96^{a}	65.00±1.00^{ab}	62.00±1.00^{ab}	61.00±5.00^{a}	62.50±1.50^{a}
MCH	26.33±0.88^{b}	19.00±0.58^{a}	16.33±1.20^{a}	22.00±0.00^{ab}	20.50±3.50^{ab}	22.00±0.00^{b}	21.75±1.25^{b}
MCHC	33.77±0.33^{a}	33.67±0.33^{a}	33.00±0.58^{a}	34.00±0.00^{a}	33.00±0.00^{a}	33.00±0.00^{a}	34.00±0.00^{a}
Hb (%)	15.80±0.40^{b}	15.00±0.57^{ab}	15.47±0.37^{ab}	14.75±0.15^{ab}	14.80±0.40^{ab}	14.20±0.10^{a}	15.30±0.10^{ab}
Neut. (%)	45.67±0.88^{a}	44.47±.88^{a}	44.33±0.33^{a}	45.50±0.50^{a}	43.50±1.50^{a}	45.50±1.50^{a}	45.50±0.50^{a}
Lymp. (%)	47.33±0.67^{ab}	47.33±0.33^{ab}	47.67±0.67^{ab}	48.00±0.00^{ab}	47.50±0.50^{ab}	45.00±1.00^{a}	46.50±0.50^{ab}
Mono. (%)	5.33±0.88^{a}	5.00±0.58^{a}	5.33±0.33^{a}	4.50±0.50^{a}	4.50±0.50^{a}	6.50±0.50^{a}	5.50±0.50^{a}
Eos. (%)	1.67±0.33^{ab}	1.67±0.33^{ab}	1.33±0.33^{ab}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.50±0.50^{a}
Baso. (%)	1.00±0.00^{a}	1.00±0.00^{a}	1.33±0.33^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}
Plat. ×10^9	2.67±0.27^{a}	3.07±0.13^{a}	3.47±0.71^{a}	3.40±0.20^{a}	2.80±0.40^{a}	1.15±0.25^{a}	1.40±0.20^{a}

Note: Data is represented as Mean ± SEM. Mean values with the same superscript alphabets in the rows are not significantly different from each other at P>0.05 using Duncan's New Multiple Range Test (DNMRT).
PCV=Packed cell volume, WBC=White Blood Cell count, RBC=Red Blood Cells, Hb=Haemoglobin concentration, MCH=Mean Corpuscular Haemoglobin, MCV=Mean Corpuscular Volume, Neut=Nuetrophile, EOS=Eosinophil, Baso=Basophil, Mono=Monocytes, MCHC=Mean Corpuscular Haemoglobin Concentration, Plat=Platelets counts and Lymp= Lymphocytes.

The most common problems encountered in cancer chemotherapy are myelosuppression and anemia (Price, 1958; Marklund *et al.*, 1982). Anemia is found frequently in cancer patients (De Vita, 1993). The reduction in Red Blood Cell (RBC) or hemoglobin production (Tables 5) observed in all the tumor-bearing animals may be due to iron deficiency or hemolytic or other myelopathic conditions (Hoagland, 1982) caused by the action of the carcinogens. Treatment with *S. mombin* leaves extracts brought back the hemoglobin content, RBC and WBC counts near to normal. This indicates that the extracts have a protective effect on the hemopoietic system.

Figures 2- 4 reveal the results of histopathological effects of *Spondias mombin* leaves extracts on the livers, lungs, spleen and intestines of tumor bearing rats treated with standard drug (LBH589), *S. mombin* leaves ethanol and n-hexane extracts at 10 and 30 mg/kg b.w each.

Results of the histopathology revealed the presence of normal histological features in liver (Figure 2) which was divided into lobules, the center of which is the central vein (CV) from which anatomizing and branching cords of hepatic cells radiate; and at the periphery are the portal triads.

This section of the liver also showed hepatic sinusoids (S) surrounded by a discontinuous layer of flattened endothelial cells with a little amount of cytoplasm and flattened darkly stained nuclei (N). The lumen of these sinusoids was seen to be bulged into by the large and prominent-nucleid kuppfer cells (K). The hepatocytes (H) of the liver which have been reported to be polyhedral in shape (Rady and Amany, 2016) and hepatic cell plates (HCP) were clearly visible in the liver of the control animals. There were abnormal dilatation of central vein (DCV) and sinusoids (DS) in the group B section, and also the presence of marked distortion of sinusoidal patterns (S) with areas of complete necrosis (N), hyperactivation of kuppfer cells (AKC); prominent microvesicles with degenerating lipid cells (lipoid necrosis), which revealed incomplete resolution; all these were some of the manifestations of a cancerous liver. Others include distortion of hepatocytes, although not clearly visible in this group (B). In line with Kleiner (2015) study, these sinusoidal dilations may result from either impaired venous outflow or a blood-flow imbalance between the hepatic artery and portal vein of the portal triad which are manifestations observed in liver cirrhosis and carcinomas (Kakar *et al.*, 2004). It is also important to note that these sinusoidal dilations are usually accompanied by atrophy of the adjacent hepatocyte plates or by isolation of small groups. Necrosis which has to do with death of cells appeared minute but it is a symptom of hepatocellular carcinoma (HCC).

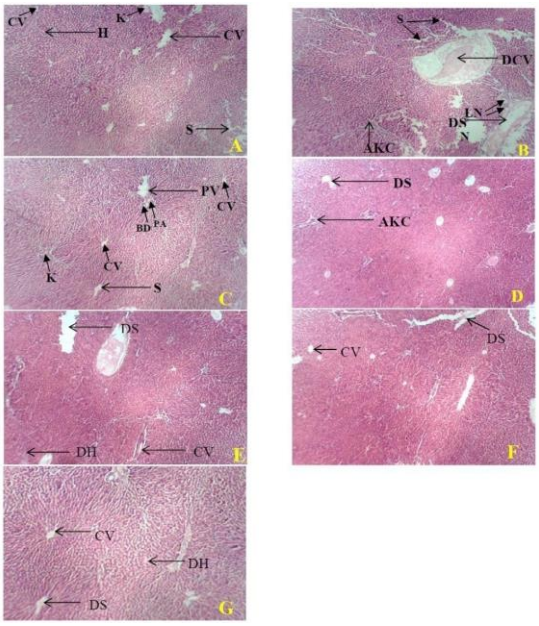

Figure 2: Photomicrographs of liver section of normal control (A), negative control (B), rat treated with LBH589 (positive control) (C), rat treated with 10 mg/kg b.w ethanol extract of *S. mombin* leaves (D), rats treated with 30 mg/kg b.w of ethanol extract (E), rats treated with 10 mg/kg b.w N-hexane extract (F) and rats treated with 30 mg/kg b.w N-hexane extract (G). (H=Hepatocyte, CV= Central Vein, K=Kuppfer cells, S= Sinusoid, DCV= Dilation of central vein, DS=Dilation of sinusoid, AKC=Activation of kupffer cell, DH= Degeneration of hepatocyte and CV=Cytoplasmic vacuolation, DCV= dilatation of central vein, LN= lipoid necrosis, N=Necrosis) (H & E 200X).

Spontaneous massive necrosis of HCC has been associated with narrowing in arties and portal veins as seen in Tominoa *et al*. (2014). The necrosis area consisted of sclerotic fibrous stroma and liquefaction also in line with this study, the formation of the fibrous capsule could also have led to reduced supply to the cancer nodule. Cytokines e.g., TNF-α, and other inflammatory cells produced in the tumor cells may also have resulted in thickening of the vessel wall intima which caused tumor hypoxia, which in turn caused the degeneration of HCC. Also, it was observed that the liver of animals treated with standard drug was not different from that of the normal animals. This positive control group showed the central vein and corresponding sinusoids as in a normal liver. Portal triad (normal portal artery, normal bile duct, mildly distorted portal veins) was also seen in this group (C). Other abnormality such as larger than normal kuppfer cells in the sinusoids was observed. These are suggestive of the repairing process manifested as a result of LBH589's potential to inhibit proliferation and metastasis of HCC via inhibition of gankyrin/stat3/akt pathway as reported by Song *et al*. (2013). The other groups D-G treated with *Spondias mombin* leaves extracts showed a prominent central vein with relatively large-sized nuclei with well-preserved cords of hepatocytes and the sinusoids were well demarcated, as in normal section. There were areas of fibrosis and localized mild necrosis, showing microvesicles and hepatocytes with hyperchromatic nuclei, which indicate inadequate hepatoprotection, infiltration of inflammatory cells, mostly neutrophils, along the portal tract and abundant mitotic bodies. These signify cellular regeneration and hepatoprotection provided by *S. mombin* extracts. Cytoplasmic vacuolation was also observed in these latter groups. There also seemed to be repair in progress in these groups, mainly as a result of its abundance in some of the various classes of secondary metabolites including phenolics, saponins, flavonoids with great antioxidant activities resulting in the scavenge of ROS.

The photomicrograph of lungs section (Figure 3) showed that each bronchus branches into narrower bronchi that eventually terminate in bronchioles. The wall of bronchioles consists of ciliated columnar epithelium (E) for larger bronchioles or cuboidal epithelium for smaller bronchioles and smooth muscle (SM). It is important to note that unlike the upper parts of the respiratory tract, it lacks hyaline cartilage. The main cell types of the bronchioles were similar to the ones in the bronchi; they were the bronchial cells (BCs) namely: basal cells, neuroendocrine cells, ciliated cells, serous cells, clara cells and goblet cells. Goblet and ciliated cells decreased in number as one approached the terminal bronchioles, whereas the number of clara cells increased proportionally. The clara cells have a secretory function and represent the main progenitor cell after bronchiolar injury. They are columnar to cuboidal in shape and project above the ciliated cells into the airway lumen. The pulmonary alveoli (lying in the alveoli sacs and composed of a single layer of squamous epithelium) were demarcated by septa, the inter alveoli septum (IAS) composed of a continuous layer of epithelial cells overlying a thin interstitium. Alveolar macrophages were also present on the epithelial surface. The interstitium contained capillaries

involved in gas exchange, as well as connective tissue and a variety of cells were involved in alveolar shape and defense. Group B section showed the various histological manifestations of cancer in the lungs; squamous cell carcinoma which ranges from well-differentiated squamous cell, neoplasm (N) with keratin (K) formation and intercellular bridges; adenocarcinoma varied with glandular differentiation or mucin (M) production by tumor cells, and the patterns were acinar, papillary, bronchiole alveolar and solid with mucin formation; it is also important to note that adenocarcinomas usually occurs in women and non-smokers and is characterized by a bronchiole alveolar growth pattern. Also, there was a proliferation of cells surrounding the bronchioles, leading to reduction of lumen (BL). There was also slight increase in the separating septa (IAS) between alveoli which could be attributed to epithelial cells proliferation lining the alveola wall; these abnormal and uneven-distributed proliferated cells could also have led to the change in shape of some alveolar sacs. The lungs of Group C animals administered with LBH589 were seen to be in a state of "repairing in progress", not showing great similarity with the negative control group, while tending towards the histomorphology of the normal control. Here, there was a widening of the bronchiole lumen as opposed to its reduction in the cancerous group; leading to the observation of less epithelial proliferation.

Another interesting manifestation observed in this section is the presence of macrophages (M), a type of white blood cell, of the immune system, that engulfs and digests cellular debris, foreign substances, microbes, cancer cells, and anything else that does not have the type of proteins specific to healthy body cells on its surface (Ovchinnikov, 2008). These cells thus might also have played a role in phagocyting tumor cells induced by the cancerous agent. Some abnormalities were still seen, such as formation of keratin and inter alveolar septae; these were not as thin as those in the control group. The lungs of animals treated with *S. mombi*n extracts showed similarities with the "repair-in-progress" state observed in animals treated with standard drug which could be due to *S. mombin*'s anti-inflammatory and antioxidant activities. Some of these similarities include epithelial growth regression, increase in bronchiole and alveolar lumen, thinning of the inter- alveoli septae; but there were few abnormal manifestations present in each of these groups such as the perivascular inflammatory cells infiltration as observed in group E, severe epitheloid cells as present in group D, Necrosis as shown in group F. The persistent presence of these abnormalities could be due to the dose and duration of treatments, which if they had been higher and longer respectively, they would have been completely restored to normal lung histoarchitecture. The photomicrograph revealed that group G administered 30 mg/kg b.w. n-hexane extract of *S. mombin* showed less similarity to the control group, amongst other treatment groups.

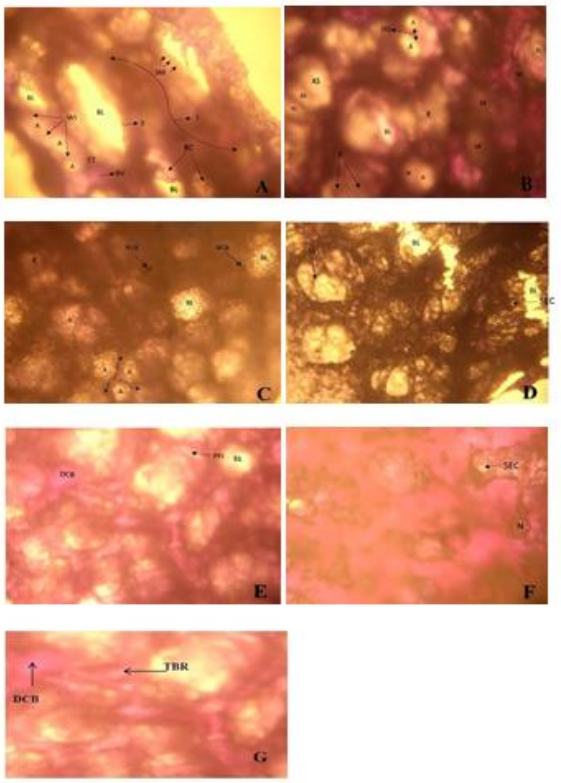

Figure 3. Photomicrographs of lung section of normal control (A), negative control (B), rat treated with LBH589 (positive control) (C), rat treated with 10 mg/kg b.w ethanol extract of *S. mombin* leaves (D), rats treated with 30 mg/kg b.w of ethanol extract (E), rats treated with 10 mg/kg b.w of N-hexane extract (F) and rats treated with 30 mg/kg b.w of N- hexane extract of *S. mombin* leaves (G). (A= Alveoli, AS=Alveolar sacs, IAS= Inter alveolar septae, BL= Bronchiole lumen, E= Epithelium, SM= Smooth muscle of the bronchioles, BV=Blood vessels, I=Interstitium, K=Keratin, M=Mucin Ma=Macrophages, TBR=Thickened bronchioles, PFI=Perivascular Inflammatory Cells Infiltration, DCB=Dilation and congestion of blood

vessel, BC= Bronchial Cells, SEC= Severe epithelioid cell and N=Necrosis) (H & E 200X).

Light microscopic examination of the normal spleen (Figure 4) revealed normal histology of red pulp (composed of connective tissue known also as the cords of billroth and many splenic sinuses that are engorged with blood, giving it a red color), white pulp (part of the immune system (lymphatic tissue) mainly made up of white blood cells), macrophages, the capillary channel, endothelial cells and splenic sinusoids while negative control group (B) showed hyperplasia of white and red pulp sinuses, in addition, there was proliferation of mononuclear cell around sinusoids; these were manifestations not seen in the normal control group. Hyperplasia, being the increase in the amount of organic tissue that results from cell proliferation; microscopically, the cells resembled normal cells but were increased in numbers. Also, congestion of red pulp was seen in this section, this was characterized by excessive distension of sinuses within the red pulp by erythrocytes.

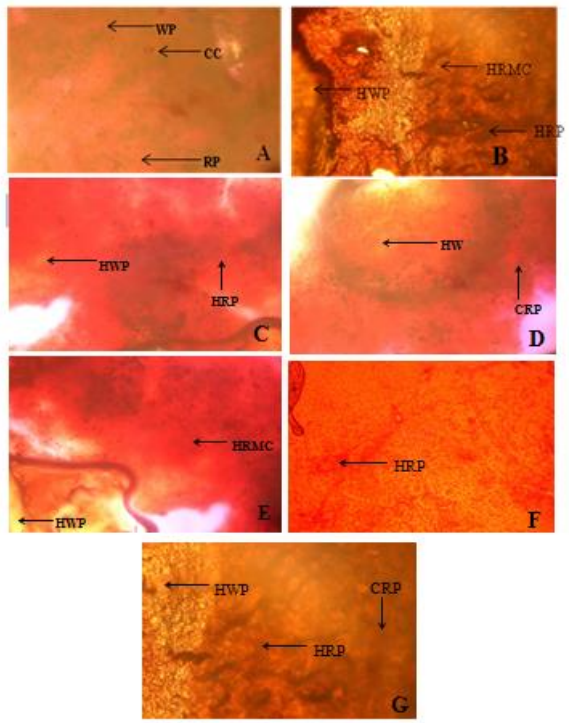

Figure 4. Photomicrographs of spleens section of normal control (A), negative control (B), rat treated with LBH589 (positive control) (C), rat treated with 10 mg/kg b.w ethanol extract of *S. mombin* leaves (D), rats treated with 30 mg/kg b.w of ethanol extract (E), rats treated with 10 mg/kg b.w of N-hexane extract (F) and rats treated with 30 mg/kg b.w of N- hexane extract of *S. mombin* leaves (G). (WP=White pulp, RP=Red pulp, CC=Central capillary channel, HWP=Hyperplasia of white pulp, HRP=Hyperplasia of red pulp, HRMC= Hyperplasia of red pulp with proliferation of Mononuclear cell around Sinusoids and CRP=Congestion of red pulp) (H & E 200X).

According to U.S. Department of Health and Human Services, some of the reported causes for this include mononuclear cell leukemia and may occur with hemangiosarcomas (which arises from the lining of blood vessels). The positive control group (C) showed reduced hyperplasia and all other test groups also seemed to manifest these hyperplastic changes, and some spleen congestions similar to the normal control animals. These are most likely due to the fact that *Spondias mombin* leaves extracts were not as effective in mitigating the cancerous effects induced in the experimental animals.

Normal histological section of intestines (Figure 5) were observed in group A animals, where epithelium of the villi was made up of tall columnar absorptive cells called enterocytes, and goblet cells, which secrete mucin, for lubrication of the intestinal contents, and protection of the epithelium; the 'brush border' on the apical surface of the enterocytes was also noticed.

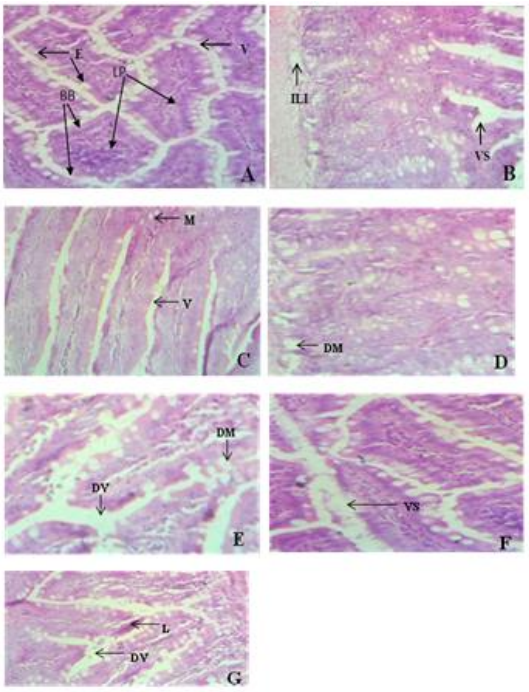

Figure 5. Photomicrographs of intestinal section of normal control (A), negative control (B), rat treated with LBH589 (positive control) (C), rat treated with 10 mg/kg b.w ethanol extract of *S. mombin* leaves (D), rats treated with 30 mg/kg b.w of ethanol extract (E), rats

treated with 10 mg/kg b.w of N-hexane extract (F) and rats treated with 30 mg/kg b.w of N- hexane extract of *S. mombin* leaves (G). (V=Villi, BB=Brush border, E=Enterocytes LP= lamina propria, ILI=Inflammatory Lymphocytic Infiltrates, VS=Villi Sloughing, M=Mucosa, DM=Dilation of mucosa, DV=Dilation of villi, and L=Lesion) (H & E 200X).

The lamina propria which underlies the epithelium had a rich vascular and lymphatic network that absorbed the digestive products. The intestinal crypt in group B animals manifested one particular abnormality, inflammatory lymphocytic infiltrates, which characterizes by lymphocyte or plasma cell infiltration into the intestinal lamina propria, this was as a result of abnormal immune response to environmental stimuli, caused by the cancerous agent. There was also manifestation of villi sloughing, hence, ineffective absorption as the intestinal villi increased the overall surface area of the intestinal lumen to permit maximal interaction of ingested nutrients with the epithelial cells for efficient absorption. Newly formed cells moved in a polarized fashion as a column of cells from the lower part of the intestinal crypt upward to an adjacent villus where migration occurred along the villus until the cells were shed at the villus tip. Group C showed similar histological manifestations with the normal control animals. Little or no studies are on the overall effects of LBH589 on the intestinal crypts, but results from the present study suggest its non-hazardous effects on the ileum; as the villi, mucosa and other histological appearances show no abnormality. The intestines of extracts treated animals seemed to show dilation in the mucosa and villi, although not as largely manifested in the negative group, suggesting the hypothetic effect of *S. mombin* leaves in curing cancer (Chukwuemeka *et al.*, 2011).

4. Conclusion

The present data of *Spondias mombin* leaves on ethanol and n-hexane crude extracts revealed antitumor property of the plant in dose-dependent degrees. The n-hexane extract of the leaves had significant antitumor activity compared to ethanol extract. The results suggested that the extracts are potential antitumorigenic agents inhibiting chemically induced lung and intestinal tumors.

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