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# Chemopreventive Potential Of Aqueous Extracts Of *Phyllanthus amarus* and *Euphorbia hirta* On Benzo (a) Pyrene Induced Lung Cancer In Albino Mice

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**Abstract:- Lung cancer, amongst other forms of cancer is heterogeneous diseases with diverse morphological appearances as well as chemotherapeutic responses due to associated significant limitations in safety and efficacy. The major risk factor for lung cancer is tobacco which accounts for 25–30% incidence and 71% of global lung cancer-related deaths. Tobacco contains Polycyclic Aromatic Hydrocarbons (PAHs) carcinogens such as benzo(a)pyrene (B(a)P) which can be activated by a P-450 enzymes and covalently bind to DNA at specific sites to form bulky adducts preceding mutation, carcinogenesis, apoptosis or nucleotide excision repair system error. Several plant materials have been considered as effective in cancer chemoprevention with negligible or no side effects. This current study was aimed at determining the Chemopreventive potentials of aqueous extracts of the whole plant of *Phyllanthus amarus* and *Euphorbia hirta* on B(a)P-induced lung cell proliferation in albino mice based on selected indices (phytochemical screening, heamatology and histopathology). *P. amarus* and *E. hirta*, Forty (40) Pathogen free Swiss albino mice weighing 16g-23g and B(a)P were used for the study. Decoction extraction method was employed in the preparation of aqueous extract of *P. amarus* and *E. hirta* whole plants. Quantitative phytochemical screening of aqueous whole plant extract was employed using standard procedure. The mice were blindly divided into eight (8) groups consisting of five mice (n=5) each per group. The first two groups are controlled groups (positive PC and negative NC) the PC received 20mg/kg B(a)P once weekly while other groups received 20mg/kg B(a)P once weekly and 50mg/kg, 100mg/kg and 200mg/kg extracts respectively once daily through oral gavaging. Haemo-analyzer was used to analyze blood sample collected by cardiac puncture into a pre-labeled EDTA sample bottles for WBC, LYM, NEUT and BAS while haematoxylin and eosin method were used for histological assay. The quantitative phytochemical analysis reveals the presence of some secondary metabolites, alkaloids, flavonoids, saponins, tannins, cardiac glycosides and total phenols. Total phenol was found to be present in the highest concentration (1044.17 ± 0.78, 2015.25 ± 0.01, 1859.12 ± 0.01; *P. amarus* > *E. hirta* > *P.amarus* and *E. hirta*) while Cardiac glycoside have the lowest concentration (0.19 ± 0.00, 0.17 ± 0.00, 0.31 ± 0.01; *P.amarus* and *E. hirta* > *P.amarus* > *E. hirta*). The haematological parameter reveals a slight increase in WBC and LYM in the treated groups which indicates the strengthening of the defense mechanism as well as immune response of the organism towards B(a)P induced cell proliferation. The histological sections of lung tissue revealed the presence of vessels with mild and focal lesions in the treated animal groups suggesting the extracts curative and suppressive effects to the proliferating cell-tissue and damages induced by B(a)P. This study has shown that the extracts of *P. amarus* and *E. hirta* could be used as a prophylactic against B(a)P-induced cell proliferation in the lung tissues of mice. It also identifies new areas of research for development of better therapeutic and chemopreventive agents against carcinogenesis and other infectious diseases. Lastly, this study serves as a resource base for more research on molecular indices, biochemical screening and isolation of active compounds to determine the therapeutic and chemoprevention efficiency of the plants in lung cancer treatment in human.**

***Keywords:-*** *Lung cancer, B(a)P, Phyllanthus amarus, Euphorbia hirta, chemoprevention, histopathology.*

## I. INTRODUCTION

Cancer is a disease of abnormal gene expression characterised by multistage mechanistic process of DNA insults and abnormal gene transcription or translation culminating in cell function defects and tumorigenesis. Carcinogenesis initiation involves an alteration in a cell DNA due to carcinogens or damage to a DNA repair mechanism. During promotion, the mutant cell reproduces abnormally by asexual reproduction to forms a population of highly proliferative tumor cells outnumbering their normal cell counterparts (Pezzuto et al., 2005; 2006) in other words, carcinogenesis involves uncontrolled cell growth resulting from the activation of oncogenes and/or the deactivation of tumor suppression genes, leading to dysregulation of cellular differentiation, excessive proliferation and

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resistance to apoptosis (Hanahan and Weinberg, 2011; Sibaji et al., 2013).

The most common sites for carcinoma include lung cancer, breast cancer, prostate cancer, cervical cancer, colorectal cancer, and stomach cancer (IARCGlobocan, 2008). IARC estimates provide a breakdown of the leading sites of cancer deaths. These include cancer of the lung (approximately 1.4 million deaths each year), stomach (740,000), liver (700,000), colorectal (610,000), breast (460,000), cervix (275,000) and prostate (260,000); showing that lung cancer is the most diagnosed cancer cases and the leading cause of cancer-related death worldwide in both sexes. Tobacco consumption a major risk factor for lung cancer accounts for 25–30% incidence (Bray et al., 2018) and 71% of global lung cancer deaths attributable to tobacco (WHO, 2009). Tobacco is a multi-organ carcinogen causing oral, liver, bladder, pancreatic cancers and leukaemia (Danaei et al., 2005) because it contains Polycyclic Aromatic Hydrocarbons (PAHs) carcinogens such as benzo(a)pyrene (B(a)P) and the tobacco-specific nitrosamine known as nicotine-derived nitrosoaminoketone (NNK) which can be activated by aniline hydroxylase, a P-450 enzymes and might bind covalently to DNA at specific sites to form bulky adducts preceding apoptosis or nucleotide excision repair system error (Gazdar, 2007). However, most common cancers does not develop overnight but takes gradual process often evolving over many months and/or years before DNA mutations accumulate and result into a detectable premalignant lesions presaging the development of full blown malignant cancer (Bennett *et al.,* 1999; Klein, 2008). Any chemical compounds capable of preventing, blocking or reversing these processes and/or inhibits cellular events associated with tumor initiation, promotion, and progression are potential candidates for cancer chemoprevention (Pezzuto, 1993; Kinghorn et al., 2004). Chemoprevention, the most direct ways to reduce morbidity and mortality involves the prevention of cancer by ingestion of chemical agents which can reduce the risk of carcinogenesis.

The Plants kingdom provides an enormous potential for newer plant-based chemical compounds that can be used in chemopreventive approach against cancer (Taneja and Qazi 2007). Plant based chemotherapeutic agent acts by killing cells that divide rapidly, which are characteristic of most cancer cells. Medicinal plants used in traditional medicine have over the years contributed many novel compounds for preventive and curative medicine to modern science (Desai et al., 2008; Umadevi et al., 2013), because most chemotherapeutic treatments have be reported for various kinds of toxicities and intrinsic problems (Desai et al., 2008). Therefore, there is demand for an alternative medicine for the treatment of cancer. Herbal medicines have received greater attention worldwide as alternative to clinical therapy in recent times leading to subsequent increase in their demand (Sushruta *et al*., 2006; Ogbonnia *et al*., 2010). Medicinal plant products usage in the management or arresting the carcinogenic process offers an alternative to the use of allopathic conventional medicine for treatment of the disease.

The medicinal healing power of plants had been recognized since creation and botanical herbal medicine is one of the oldest practiced professions by mankind (Hugo and Russell, 2003). Medicinal plants have been found useful as anti-sickling, anti-malarial, anti-microbial, anti-convultant, anti-helmintic, anti-hypertensive, and molluscidal agent (Prescott *et al*., 2002). The curative potentials of these plants are locked up and embedded in some chemical components that effects physiological response in man (Edeoga *et al*., 2005). Polyphenolic compounds have been attributed to the plant chemopreventive properties with higher anticancer treatment potentials. Many pharmaceutical industries depend on plant-based products for medicaments in the treatment of various cancer/ailments (Abraham, 1981) for instance; four classes of plant-derived anticancer agents are vinca alkaloids (vinblastine, vincristine and vindesine), epipodophyllotoxins (etoposide and teniposide), taxanes (paclitaxel and docetaxel) and camptothecin derivatives (camptotecin and irinotecan) (Taneja and Qazi 2007). Plants still have enormous potential to provide newer drugs and as such are a reservoir of natural chemicals that may provide chemoprotective potential against cancer. The World Health Organisation (WHO) (2002a,b; 2009) estimate that more than 80 percent of the world population relies on traditional herbal medicines as their first source of health care due to their pharmacological properties and about 85 percent of such traditional medicine used have compounds derived from medicinal plants extracts. Phytochemical molecules from natural products such as alkaloids, flavonoids, tannins and phenols capable of exerting a physiologic action on the human body forms the effective ethno pharmacological information in the discovery of new anti-infective agents from medicinal plants (Duraipandiyan, et al., 2006). However, such plants should be investigated to better understand their properties, safety, and efficiency.

The aim of this study is to determine the Chemopreventive potentials of aqueous extracts of the whole plant of *phyllanthus amarus* and *Euphorbia hirta* on Benzo(a)pyrene induced lung cell proliferation in albino mice based on selected indices such as phytochemical screening, heamatology and histopathology in order to determine the efficiency of these extracts in the management of lung cancer in human.

*Phyllanthus amarus* is a small, erect, annual herb belonging to the family Euphorbiaceae, having large number of phytochemicals that are attributed to its leaves, stem and roots. *P. amarus* is used in Thai folk medicine for the treatment of fever, jaundice, ascites, heamorrhoid and diabetes (Pongboonrod, 1976). Several reports also showed that *P. amarus* had anti-hepatitis B virus effect (Thyagarajan *et al*., 1988), hypoglycemic effect (Moshi *et al.*, 1997), antinociceptive effect (Santos *et al.,* 2000), the increase in life span of rats with hepatocellular carcinoma (Rajeshkumar and Kuttan, 2000), antitumour, antimutagenic and anticarcinogenic effect (Sripanidkulchai *et al.* and Rajeshkumar *et al*., 2002), anti-inflammatory effect (Kiemer *et al*., 2003; Kassuya *et al.,* 2006) and chemoprotective

effect (Kumar and Kuttan, 2005), anti-bacterial (Mazumder *et al.,* 2006, Melendez and Capriles, 2006, Oliveira *et al.,* 2007), anti-parasitic (Zirihi *et al.,* 2005, Hout *et al.,* 2006), anti-viral (Venkateswaran *et al.,* 1987, Yang *et al.,* 2005, Balasubramanian *et al.,* 2007), anti-oxidation (Bhattacharjee and Sil, 2007, Chatterjee and Sil, 2006, Kumaran and Karunakaran, 2007), hypoglycaemic properties (Lawson-Evi *et al.,* 1997), antispasmodic properties (Gbeassor *et al*., 1988).

*Euphobia hirta* is a slender- stemmed, annual hairy plant belonging to the family *Euphorbiaceae*, used in traditional medicines ingredient as arrow poisons. E. hirta possesses antibacterial, anthelmintic, antiasthmatic, sedative, antispasmodic, antifertility, antifungal, antimalarial, antioxidant, anti-inflammatory and anti-cancer properties (Basma *et al.,* 2011).

## II. MATERIALS AND METHODS

### Collection and identification of plant materials

Whole fresh Plants of *P. amarus*, and *E. hirta* were collected from the Biological Sciences Garden, Kogi State University, Anyigba, Nigeria between July and September, 2019. The plants were taken in separate polythene bags to the herbarium, Plant Science and Biotechnology Department, Kogi State University, Anyigba for identification by a taxonomist. The plants were cleaned of extraneous matter, and the necrotic parts were removed and washed with clean water then air-dried for two weeks. Equal weight of the air-dried plants parts were measured and coarsely milled separately into powdery form using a milling machine for easy extraction.

### Preparation of Aqueous Plant Extracts

The grounded plants were subjected to decoction extraction method as described by Yadav and Agarwala (2011) with little modifications. 200g each of ground *P. amarus* and *E. hirta* were soaked in 2 litres of deionized water and boiled for 10 minutes separately. The decoctions were initially filtered using a sieve mesh and the filtrate was further sifted using cotton wool and finally using Whatman® no.1 (11µm) filter paper. The filtrate of each preparation mixture was concentrated under standard temperature and pressure using a water bath evaporator at 100°C to obtain a semi-solid form of the crude extract which was then stored in an air tight container and refrigerated at 4°C until use.

### Phytochemical screening of the plants extract

The aqueous extracts of *P. amarus* and *E. hirta* were used for quantitative phytochemical screening for the presence of secondary metabolites as per the standard methods outlined by Horborne (1973); Sofowara (1993); Falodun *et al* (2005) and Edeoga *et al.,* (2005).

### Animal Collection and Housing

Forty (40) Pathogen free Swiss albino mice aged six-eight weeks, weighing 16g-23g were purchased from the Veterinary Medicine Department, University of Nigeria, Nsukka. The animals were transported to the animal house, Department of Animal and Environmental Biology, Kogi State University, Anyigba, and housed in a neat and well ventilated plastic cage. Their bedding which was of saw dust was regularly changed and they were supplied with commercially pellet Feed and drinking water *ad libitum*. The animals were exposed to 12h periodic lights.

### Tumor induction

Lungs tumors were induced by a freshly prepared single dose of 20 mg of B(a)P weekly diluted in distilled water and given by oral-gavage method as described by Alfredo et al., (2004) and Minari et al., (2016). All the albino mice received the chemical carcinogen once every week for the period of the experiment. Exposures to B(a)P has been reported to induce tumor or implicated for development of lung cancer in animal species such as mice (IARC, 2010).

### Experimental design

The study was designed in accordance with the procedures provided by Builders *et al.,* (2012), Rajina and Shini (2013) and Onoja et al., (2019). The animals were made to fast over-night prior to drug administration to allow fast cellular absorption. The B(a)P and tested plants constituent were administered to animals in a sequential routine using syringe and canula via oral route. The animals were blindly divided into eight (8) groups consisting of five mice (n=5) each per group. The first two groups are controlled groups while the others are received homogeneous suspensions of the B(a)P once weekly and extracts once daily through oral gavaging for three (3) weeks.

- ❖ **Negative Control (NC):** Control Animals treated with distilled water only.
- ❖ **Positive Control (PC):** Animals treated with 20mg/kg B(a)P once per week.
- ❖ **Group I** (*P. amarus* (PA) and *E. hirta* (EH) respectively): Animals treated with 20mg/kg B(a)P weekly and 50mg/kg Extracts daily.
- ❖ **Group II** (*P. amarus* (PA) and *E. hirta* (EH) respectively): Animals treated 20mg/kg B(a)P weekly and 100mg/kg Extracts daily.
- ❖ **Group III** (*P. amarus* (PA) and *E. hirta* (EH) respectively): Animals treated with 20mg/kg B(a)P weekly and 200mg/kg daily.

### Blood Sample Preparation and Haematology parameter

At the expiration of the experiment, Blood sample was collected by cardiac puncture from the animals into a pre-labeled sample bottles containing ethylene diamine tetraacetic acid (EDTA) and was shaken gently to mix the sampled blood with EDTA to avoid clotting. The samples were analysed for the White Blood Cells, Lymphocyte, Neutrophil and Basophil using haemo-analyzer.

### Histological examination

At the end of experiment, the animals were sacrificed by cervical dislocation and the lungs removed, fixed in 10% formalin. The lungs tissues were processed using automated tissue processor (leica tp1020), dehydration and embedded in paraffin wax, serial sectioned at 4µm thickness using

rotatory microtome, deparaffinised and subsequently stained using haematoxylin and eosin (H & E) and examined microscopically under the light microscope at 40 and 400 magnifications. Slides of all the groups (treated and controlled) were studied and photographed (Kiernan, 1981; Avwioro, 2010; Onoja, et al., 2019).

**Data Analysis**

The result of the haematology study was subjected to the mean and standard deviation analysis analysed using the SPSS software program. ($P<0.05$) level of significance was considered using the Duncan Multiple Range Test.

## III. RESULTS

**Quantitative phytochemical screening**

Results obtained for the quantitative phytochemical analysis of selected phytochemicals present in the aqueous extract of *P. amarus* and *E. hirta* is shown in Table 1. Total phenol was found to be present in the highest concentration (1044.17 ± 0.78, 2015.25 ± 0.01, 1859.12 ± 0.01; *P. amarus* > *E. hirta* > *P.amarus* and *E. hirta*) while Cardiac glycoside was found to be in the lowest concentration (0.19 ± 0.00, 0.17 ± 0.00, 0.31 ± 0.01; *P.amarus* and *E. hirta* > *P.amarus* > *E. hirta*). There was a significant difference ($P < 0.05$) in the concentration of all selected phytochemicals.

**Table 1:** Result showing quantitative analysis of *P. amarus* and *E. hirta* whole plants aqueous extract.

| S/N | Phytochemical components | Concentration (mg/g) | | |
|---|---|---|---|---|
| | | ***P. amarus*** | ***E. hirta*** | ***P. amarus and E. hirta*** |
| 1 | Flavonoids | $144.11 \pm 0.11^{c}$ | $80.56 \pm 0.01^{a}$ | $95.10 \pm 0.01^{b}$ |
| 2 | Saponins | $8.36 \pm 0.02^{c}$ | $5.72 \pm 0.00^{b}$ | $3.58 \pm 0.00^{a}$ |
| 3 | Total Phenol | $1044.17 \pm 0.78^{a}$ | $2015.25 \pm 0.01^{c}$ | $1859.12 \pm 0.01^{b}$ |
| 4 | Tannins | $26.35 \pm 0.02^{b}$ | $24.11 \pm 0.01^{a}$ | $28.09 \pm 0.01^{c}$ |
| 5 | Cardiac glycoside | $0.19 \pm 0.00^{b}$ | $0.17 \pm 0.00^{a}$ | $0.31 \pm 0.01^{c}$ |
| 6 | Alkaloids | $4.22 \pm 0.02^{c}$ | $2.24 \pm 0.01^{a}$ | $2.86 \pm 0.01^{b}$ |

Mean ± S.E.M; values with different superscripts across a row are significantly different ($P < 0.05$)

**Haematological Analysis**

Table 2 shows the result of the four parameters components of the blood considered (white blood cell, lymphocyte, neutrophil and basophil). The result for groups treated with *P. amarus* revealed no significant difference ($P<0.05$) between the treatment groups and the control groups. However, the groups treated with *E. hirta* showed a significant difference between the treated groups when compared with the control groups for the white blood cell and lymphocyte but the neutrophils and basophil shows no significant difference at ($P<0.05$).

**Table 2:** Effect of B(a)P on the haematological parameter of Mice

| Treatment | | White Blood Cells | Lymphocyte | Neutrophil | Basophil |
|---|---|---|---|---|---|
| NC | | $6.36\times10^{9} \pm 6.51\times10^{8c}$ | $4.56\times10^{9} \pm 8.49\times10^{7b}$ | $41.00 \pm 1.41^{a}$ | $1.00 \pm 1.41^{a}$ |
| 50mg/kg | *P. amarus* | $6.12\times10^{9} \pm 3.57\times10^{8c}$ | $4.83\times10^{9} \pm 1.07\times10^{9b}$ | $48.25 \pm 0.96^{a}$ | $0.50 \pm 1.00^{a}$ |
| | *E. hirta* | $3.69\times10^{9} \pm 5.36\times10^{8b}$ | $6.34\times10^{9} \pm 5.39\times10^{8d}$ | $53.7 \pm 2.5^{a}$ | $2.0 \pm 0.0^{a}$ |
| 100mg/kg | *P. amarus* | $6.06\times10^{9} \pm 2.29\times10^{8c}$ | $4.76\times10^{9} \pm 4.27\times10^{8b}$ | $42.50 \pm 0.50^{a}$ | $0.50 \pm 1.00^{a}$ |
| | *E. hirta* | $3.57\times10^{9} \pm 5.37\times10^{8b}$ | $6.24\times10^{9} \pm 1.82\times10^{8d}$ | $45.2 \pm 0.5^{a}$ | $2.0 \pm 0.0^{a}$ |
| 200mg/kg | *P. amarus* | $6.09\times109 \pm 3.27\times10^{8c}$ | $4.53\times10^{9} \pm 8.10\times10^{8b}$ | $40.75 \pm 1.50^{a}$ | $0.50 \pm 1.00^{a}$ |
| | *E. hirta* | $3.41\times10^{9} \pm 4.05\times10^{8b}$ | $5.46\times10^{9} \pm 6.65\times10^{7c}$ | $40.2 \pm 0.5^{a}$ | $2.0 \pm 0.0^{a}$ |
| PC | | $6.23\times109 \pm 1.40\times10^{9c}$ | $4.60\times109 \pm 1.03\times10^{8b}$ | $40.75 \pm 2.99^{a}$ | $0.50 \pm 1.00^{a}$ |

Values are mean ± standard deviation of four replicates per group. Different alphabets superscripted in the same columns represent significant difference at P<0.05(DMRT=Duncan multiple range test).

**Histopathological Analysis of the Lungs**

Plate 1 shows the histology section of the negative control (NC) groups with normal lung architectures without structural changes. This indicates the state of health of the used animals as well as the conducts of the experimentation was under proper research conditions. Plate 2 shows the histological section of the positive control (PC) group lung tissue. This revealed the presence of vessels with mild to moderate congestion, the intra aveolar spaces show area of moderate hemmorhage, congestion and necrosis at (HE 400X) magnification. The animal groups induced with B(a)P and treated with *P. amarus* and *E. hirta* plant extract shows mild and focal lesions only at all dose levels used in the study (Plate 3, 4 and 5). This suggests that the extracts have some curative and suppressive effects to the proliferating cell-tissue and the damage induced by B(a)P.

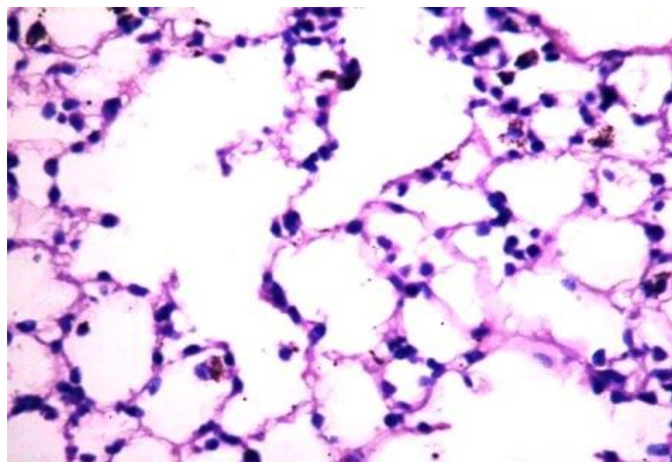

**PLATE 1:** NC 400X Photomicrograph of a lung section stained by Haematoxylin and Eosin showing normal bronchiole, intra aveolar spaces and alveolar ducts

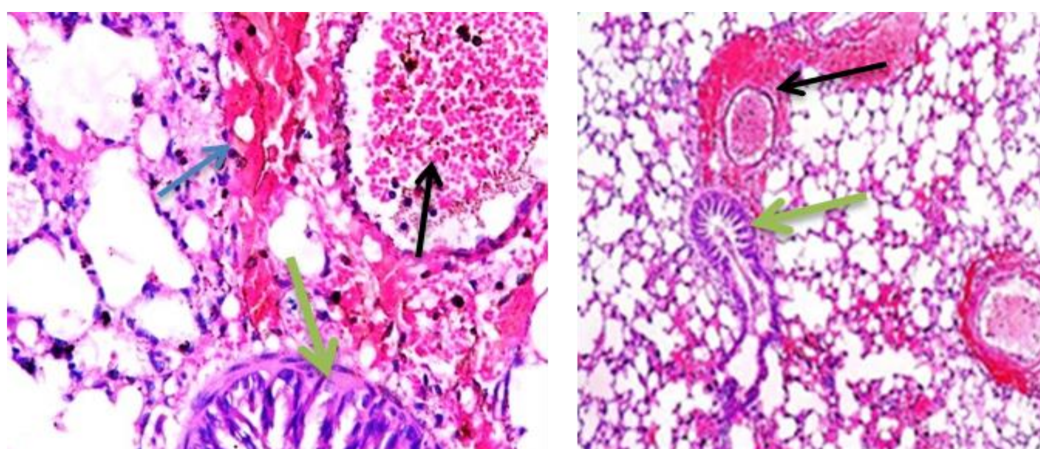

PLATE 2: PC 400X Photomicrograph of a lung section stained by Haematoxylin and Eosin showing normal bronchiole without infiltration of inflammatory cells (green arrow). there are vessels with mild to moderate congestion seen (black arrow). the intra aveolar spaces show area of moderate hemmorhage and congestion (blue arrow).

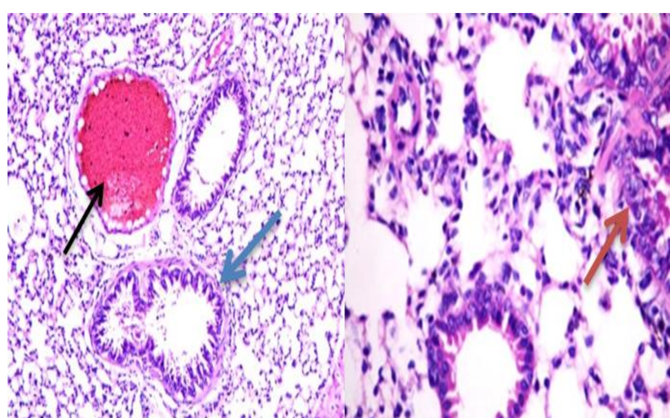

PLATE 3a: 50mg/kg *P. amarus* 400X Photomicrograph of a lung section stained by Haematoxylin and Eosin showing peribronchiolar lymphoid infiltration without infiltration of inflammatory cells (red arrow). there are vessels with moderate congestion seen (black arrow). the intra aveolar spaces is mildly infiltrated (blue arrow).

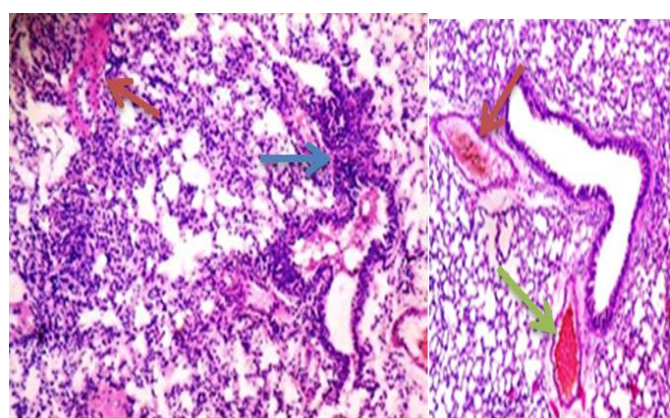

**PLATE 3b:** 50mg/kg *E. hirta* 400X Photomicrograph of a lung section stained by Haematoxylin and Eosin showing mild peri bronchiolar infiltration of inflammatory cells and epithelial hyperplasia intra aveolar spaces (blue arrow); alveolar ducts show area of edema and duct collapsed (red arrow); there are vessels with mild congestion seen (green arrow).

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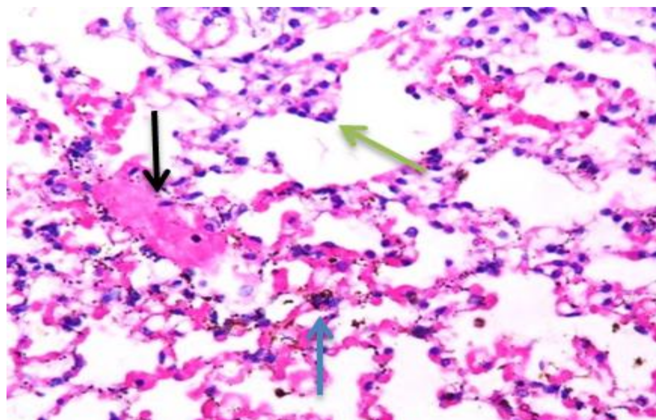

**PLATE 4a:** 100mg/kg P. amarus 400X Photomicrograph of a lung section stained by Haematoxylin and Eosin showing normal bronchiole without infiltration of inflammatory cells. There are vessels with moderate congestion (black arrow). the intra aveolar spaces is mildly infiltrated (green arrow),the alveolar ducts show mild necrosis (blue arrow)

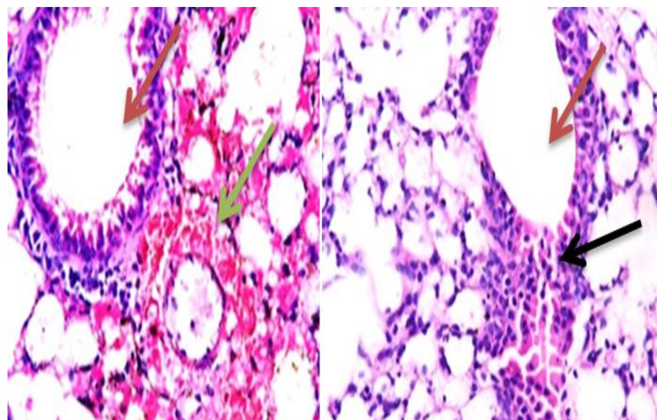

**PLATE 4b:** 100mg/kg E. hirta 400X Photomicrograph of a lung section stained by Haematoxylin and Eosin showing mild peri- bronchiolar infiltration of inflammatory cells (black arrow); there are vessels with mild congestion seen (green arrow); the intra aveolar spaces and alveolar ducts show moderate hemorrhage and scantly infiltrated (red arrow).

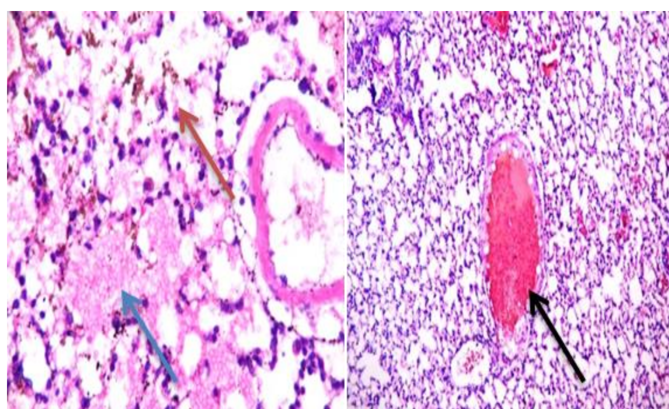

**PLATE 5a:** 200mg/kg P. amarus 400X Photomicrograph of a lung section stained by Haematoxylin and Eosin showing normal bronchiole without infiltration of inflammatory cells. there are vessels with moderate to severe congestion (black arrow). the intra aveolar spaces is mildly infiltrated (red arrow),some of the alveolar ducts are filled with fluid(blue arrow).

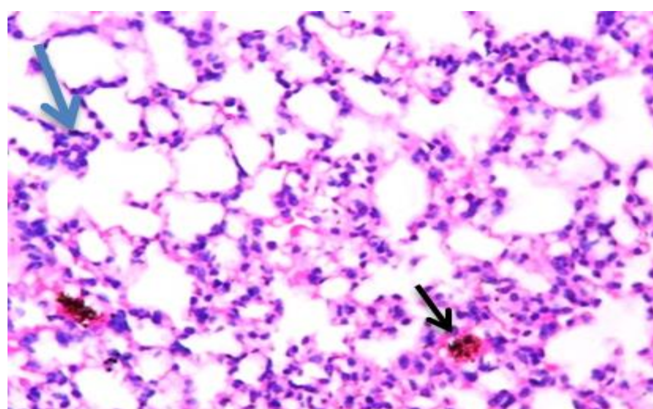

**PLATE 5b:** 200mg/kg E. hirta 400X Photomicrograph of a lung section stained by Haematoxylin and Eosin showing normal bronchiole without infiltration of inflammatory cells. There are vessels with mild congestion (black arrow). the intra aveolar spaces show moderate infiltration of inflammatory cells (blue arrow) and alveolar ducts are normal.

## IV. DISCUSSION

Lung cancer accounts for about 13% of total cancer cases diagnosed and the most common cause of death from cancer globally (Torre, et al., 2015). More than half of the cases are diagnosed at an advanced stage (Rami-Porta, et al., 2014). Fighting against a painful disease like cancer is very essential for public health.

Plants are the main sources for painkillers and the most important sources of active alternative clinical bio-substances with therapeutic potential to cure a range of human diseases (Gill *et al.*, 2010; 2011). The fast progress in the phytochemical study transforms plant products to popular anticancer sources. Plants produce a wide range of chemical compounds called secondary metabolite such as alkaloids, terpenoids, flavonoids, pigments, and tannins with biologic effects such as anti-inflammatory, anticancer, contraceptive, and different effects on hematopoietic cells, lipids and cardiovascular systems (Mansouri et al., 2015; Kooti, et al., 2014; 2016). Plants used as anticancer contains blocking agents capable of inhibiting initiation process by preventing carcinogens and DNA interaction, thereby reducing the level of damage and resultant mutations that contribute to cancer initiation as well as progressive genomic instability and neoplastic transformation (Yu and Kong, 2007).

This work investigate the potential chemoprevention activity of *Phyllanthus amarus* and *Euphorbia hirta* in lung cancer using animal model on Benzo(a)pyrene induced lung cell proliferation, phytochemical, haematology and histopathology analysis were used to reveal the curative ability of the plants extract. Studies has shown that B(a)P can be used to induce experimental lung carcinoma in mice (Shimada, 2006; IARC, 2010).

The active principles of many plant-based drugs are secondary metabolites (Builders *et al*., 2012). Secondary metabolites are chemical compounds with imprecise function in countless areas such as human therapy, veterinary, agriculture, and scientific research (Vasu, et al., 2009). The phytochemical analysis presented in this study confirms the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, cardiac glycosides and total phenols (Table 1). Studies have shown that these constituents exhibit significant medicinal and physiological activities with specific mode of action. The identified phytochemicals were similar to those reported in other published reports (Sherma *et al.*, 2012; Agbafor and Nwachukwu, 2011) and may be responsible for the observed pharmacological activities. Phenolics compounds have been studied mainly for antioxidant properties in determining their role as protecting agents against free radical-mediated disease processes (Silva et al, 2007, Saxena et al., 2013), oxidative damage leading to degenerative diseases, such as cardiovascular diseases, inflammation and cancer. Indeed, tumour cells typically have higher levels of reactive oxygen species (ROS) than normal cells making them sensitive to oxidative stress (Mandal et al, 2010), as well as antimutagenic, anticarcinogenic, antiulcer, anti-inflammatory, cytotoxic and antitumor, antispasmodic and antidepressant activities and ability to modify the gene expression (Silva et al, 2007, Ghasemzadeh et al, 2010). Cardiac glycosides have been reported for their antitumor activity (Doskotct et al., 1972). Flavonoids have been reported to exert multiple biological property such as antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities but the best-described property is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species (Saxena et al., 2013). The tannin-containing plant extracts are used as natural healing agent, astringents, diuretics, against diarrhoea, stomach and duodenal tumours (De Bruyne et al, 1999), as well as antiinflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Dolara et al, 2005). Alkaloids have many pharmacological activities including antibacterial, antifungal, antihypertensive, antiarrhythmic, antimalarial, and anticancer actions (Wink et al., 1998). Some have stimulant property used as analgesic drug (Roa et al, 1978). The presence these secondary metabolites could possibly be responsible for the significant reduction and/or inhibition of tumorigenesis in mice administered with *P. amarus* and *E. hirta* plant extracts, as the extracts contains secondary metabolites that function as inhibitors of both tumor initiation and promotion. Cancer results from reactions between free radicals and DNA, leading to mutations that can cause malignancy. Free radicals and their metabolites are increasingly recognized for their contribution to tissue injury leading to both initiation and promotion of multistage carcinogenesis (Slaga, 1995, Kawanishi, et al., 2002).

The haematology parameter generally disclosed no noticeable changes in the target parameters because it remained within the normal limits of expected range for the rodents used in this study. However, the WBC and LYM showed a slight increase in the treated and PC group which indicates the strengthening of the organism defense mechanism against the tumor cells or immune response of the organism to the B(a)P induced cell proliferation. This suggests the immune potentiating effect of *P. amarus* and *E. hirta* against infectious diseases and foreign invaders (Onoja *et al*., 2019; Al-dulaimi *et al*., 2018). The decrease in the basophil and neutrophil is an indication of the curative and suppressive effect of the *P. amarus* and *E. hirta* extract.

The classification of lung histopathological lesion is subjective due to the high inter-observer unpredictability and uncleared guidelines (Sloane et al., 1994). The presence of B(a)P-induced severe haemmorhagic and congestion, lymphoid and inflammatory cells infiltration in the histological sections of lung tissues of mice that were administered with with different concentrations of B(a)P in (Plate 2-5) suggest that B(a)P-induced mice lung carcinomas have been shown to arise from the pulmonary neuroendocrine cells exposed to heavy carcinogen and/or significant changeability in tissues induced with the carcinogen (Gridelli, 2015). This work show no visible or macroscopic lung changes as evidence of B(a)P administration induced tumorigenesis in all groups treated. However, the histological result shows that B(a)P interfere with the cellular function thereby decreases cellular efficiency leading to cancer/ tumor initiation with ability to resist against care from blocking agents produced by the plants leading to genomic instability and regulatory signal disregard. That is, it ignores signals related to regulation of cell’s growth and obtains invasion characteristics and causing changes in surrounded tissues (Yu and Kong, 2007). Few cases of mild vessels congestion, infiltration, inflammatory cells and focal heammorhage suggests dose-dependent curative and anti-proliferative potential of *P. amarus* and *E. hirta* plant extracts. Anticancer activities of *P. amarus* and *E. hirta* plant maybe due to the presence of secondary metabolite and blocking agents capable of inducing cell cycle arrest, DNA repair interference and carcinogenic compounds metabolic activation inhibition (Rajeshkumar *et al.,* 2002).

## V. CONCLUSION

This research authenticate B(a)P-induce lung carcinogenic effects in mice through the oral route exposure. Lung cancer is a common malignancy the leading cause of cancer-related death worldwide in both sexes. Tobacco is a multi-organ carcinogen accounts for 25–30% lung cancer incidence and 71% of global lung cancer deaths because it contains Polycyclic Aromatic Hydrocarbons (PAHs) carcinogens such as benzo(a)pyrene (B(a)P) which can be activated and bind covalently to DNA at specific sites to form bulky adducts preceding apoptosis or nucleotide excision repair system error initiating mutations that causes lung cells proliferation. This study shows that the aqueous extracts of *P. amarus* and *E. hirta* can be used as inhibiting agents against cancer initiation as well as anti-proliferative measure against B(a)P induced lung cancinomas in mice. The haematological indices indicates immune response of the organism to the B(a)P induced cell proliferation as well as the curative and suppressive effect of the *P. amarus* and

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*E. hirta* extracts. The histopathological sections revealed reduction in tissue inflammation, this indicate curative and anti-proliferative potential of the *P. amarus* and *E. hirta* extracts against severe lesions such as congestion, heamorrhage, infiltrations amongst others in the PC. Furthermore, the chemoprevention activity of these plants cannot be concluded with this study only because other research such as cytotoxic and pharmacological testing is required to ascertain the effectiveness of the plants used.

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