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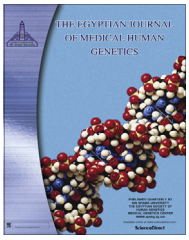

ORIGINAL ARTICLE

Antiproliferative potential of aqueous leaf extract of *Mucuna pruriens* on DMBA-induced breast cancer in female albino rats

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KEYWORDS

Breast cancer;
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Hyperplasia

Abstract *Background:* Breast cancer is the most common cancer and a major cause of death in women, makes up about one-tenth of all new cancer diagnoses worldwide. Breast cancer and other cancer forms are heterogeneous diseases with varied morphological appearances, molecular features, behavior, and response to therapies (using surgery, chemotherapy, radiation therapy and targeted therapy). Despite the achievement of objective responses, the available treatments are associated with significant limitations in safety and efficacy. Several plant products (barks, leaves, flowers, roots, fruits, seeds) have been characterized as effective in cancer chemoprevention with minimal or no side effects.

Aims: This study is aimed at evaluating the antiproliferative potential of aqueous leaf extract of *Mucuna pruriens* on 7,12-dimethylbenzeneanthracene (DMBA)-induced-breast cancer in female albino rats.

Materials and methods: *M. pruriens*, thirty (30) female albino rats and 7,12-dimethylbenzeneanthracene were used for this study. Hot extraction protocol was employed in the preparation of aqueous extract of *M. pruriens* leaves. Qualitative and quantitative phytochemical screening of aqueous leaf extract of *M. pruriens* and weight determination was employed using standard protocol. Comet assay protocol was employed to determine level of deoxyribonucleic acid (DNA) fragmentation while hematoxylin and eosin were used for histological assay.

Results: Phytochemical screening revealed the presence of alkaloids, reducing sugar, anthraquinones, flavonoids, saponins, tannins, cardiac glycosides, phenols and steroids. Flavonoid was quantitatively determined to be present in the highest amount. Histological assay revealed the presence

Abbreviations: DMBA, 7,12-dimethylbenzeneanthracene; *M. pruriens*, *Mucuna pruriens*; SCGE, single cell gel electrophoresis; DNA, deoxyribonucleic acid; dG, deoxyguanosine.

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of atrophy of seromucous glands with surrounding stromal fibrosis and hyperplasia of serous and mucinous. There was a significant weight difference between the control and treatment groups. Determination of length and weight of isolated tumor from rat induced with DMBA only was 2.4 cm and 2.8 g respectively. DNA smears obtained from single cell gel electrophoresis (SCGE) suggested possible DMBA-induced damage which was significantly prevented owing to the effect of the leaf extract of *M. pruriens*.

Conclusion: This study has shown that the leaf extract of *M. pruriens* could be used as a prophylactic measure against DMBA-induced cell proliferation in the breast tissues of female albino rats.

1. Introduction

Cancer is a cellular disease and mainly caused by the misbalance of the normal cellular growth maturation and multiplication [1]. Several chemicals and environmental toxins are responsible for changes in normal cellular DNA. Substances that cause DNA mutations are known as mutagens, and mutagens that cause cancers are known as carcinogens [2]. The most common types of cancer in males are lung cancer, prostate cancer, colorectal cancer, and stomach cancer, and in females, the most common types are breast cancer, colorectal cancer, lung cancer, and cervical cancer [3]. Breast cancer remains a major cause of death in the United States as well as the rest of the world [4]. Breast cancer is the most common malignancy affecting women in many parts of the world with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers). Breast cancer is the most frequent cause of cancer death in women in less developed regions (324,000 deaths, 14.3% of total), it is now the second cause of cancer deaths in more developed regions (198,000 deaths, 15.4%) after lung cancer [5]. In Nigeria, breast cancer is the most common cancer seen among women [6]. Breast cancer has recently overtaken cervical cancer as the most common female malignancy in Western and Eastern Nigeria. It is characterized by high mortality, younger age distribution, more advanced stage distribution and increased frequency of high-grade tumors, as found in African–American women [6–8]. Carcinogenesis involves uncontrolled cell growth, which follows the activation of oncogenes and/or the deactivation of tumor suppression genes. Development and progression of breast tumors involve a complex series of events, including dysregulation of cellular differentiation, excessive proliferation and resistance to apoptosis [9,10]. In those who have been diagnosed with cancer, a number of treatments may be used, including surgery, radiation therapy, chemotherapy, and targeted therapy [11]. Breast cancer is usually treated with surgery, which may be followed by chemotherapy or radiation therapy, or both [12,13]. Despite the achievement of objective responses, the available treatments are associated with significant limitations in safety and efficacy [14,15]. In view of the limited treatment options for patients with advanced breast cancer, preventive and novel therapeutic approaches play an important role in combating this disease [4,16]. The advent of modern drug-targeted therapies has undeniably improved cancer patients' cares. However, advanced metastasized cancer remains untreatable. Hence, continued searching for a safer and more effective chemoprevention and treatment is clearly needed for the improvement of the efficiency and to lower the treatment cost for cancer care [17]. In recent years the incidence of breast cancer has increased almost everywhere. Alternatives to therapy need to be developed for breast cancer control. Toward this end, chemoprevention constitutes a valuable approach. Cancer chemoprevention by phytochemicals may be one of the most feasible approaches for cancer control. It is mandatory to expand our efforts in identifying synthetic or naturally occurring agents that can inhibit the preneoplastic events preceding the occurrence of clinically detectable cancers [17–19]. In 1976, Sporn defined chemoprevention as "the use of pharmacologic or natural agents that inhibit the development of invasive breast cancer either by blocking the DNA damage that initiates carcinogenesis, or by arresting or reversing the progression of premalignant cells in which such damage has already occurred". The success of several recent clinical trials in preventive settings in selected high-risk populations suggests that chemoprevention is a rational and appealing strategy [20,21].

The use of plants whether herbs, shrubs or trees on parts or whole in the treatment and management of diseases and disorders date back to prehistoric days [22]. A medicinal plant as defined by the World Health Organization (WHO), is a plant which one or more parts of it contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [23]. The plant kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice in the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs, antihepatotoxic compounds [24]. Levy [25]; Van den Bogaard and Stobberingh [26] and Smolinski et al. [27] asserted that medicinal plants are increasingly gaining acceptance even among the literates in urban settlements, probably due to the increasing inefficacy of many modern drugs used for the control of many infections such as typhoid fever, gonorrhea, and tuberculosis as well as and the increasing cost of prescription drugs, for the maintenance of personal health. Arunkumar and Muthuselvam [28] in their study corroborate the reports of the World Health Organization (WHO) that medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicines, which have compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency.

Mucuna pruriens belongs to the species Fabaceae, commonly known as cowage plant. It is a popular Indian medicinal plant, which has long been used in the Ayurvedic system of medicine. All parts of *M. pruriens* possess valuable medicinal properties. Roots, leaves and seeds of the plant are commonly used in the treatment of impotence, diabetes mellitus and cancer [29]. *M. pruriens* is used extensively for its medicinal value for the treatment of diabetes mellitus in Nigeria [30].

The overall aim of this research was to determine the antiproliferative properties of aqueous leaf extract of *M. pruriens* and its effect based on selected biochemical and molecular indices such as phytochemical screening, histopathology and DNA fragmentation in order to determine the efficiency of this extract in the treatment of breast cancer in human.

2. Materials and methods

2.1. Plant material

A sample of fresh leaves of *M. pruriens* were collected within the Botanical Garden, University of Lagos, Lagos in the month of August, 2014. The plant was identified and authenticated by a taxonomist (Herbarium unit of Botany Department), UNILAG, Lagos where a voucher specimen number (LUH 6235) was deposited.

2.2. Chemicals used

7,12-Dimethylbenz(a)anthracene (DMBA) was purchased from (Sigma Aldrich, Germany), Low Melting Point Agarose (LMPA), Ficol and histopaque 1077. All other chemicals and drugs used were obtained commercially and of analytical grade.

3. Methodology

3.1. Preparation of plant extract and extraction

The fresh leaves were dried under shade and then ground into fine powder using laboratory mortar and pestle. The aqueous extracts of the plant were obtained using the Hot Extraction Method as described by Yadav and Agarwala [24].

3.2. Tumor induction

Mammary gland tumors were induced by a freshly prepared single dose of 20 mg of DMBA diluted in soy oil (5 mL) given intragastrically by gavage method as described by Alfredo et al. [31]. All the 30 female albino rats, with an average weight of 182.9 g (161–213 g), received the chemical carcinogen at the age of 57 days. The susceptibility of the mammary gland to DMBA carcinogenesis is strongly age-dependent, being maximal when the drug is administered to rats between the ages of 45 and 60 days, which is the age of the beginning of sexual maturity [32]. The animals were bred in our laboratory under ideal conditions of temperature, humidity, and light, and they were fed with appropriate ration in pellets and filtered water.

3.3. Experimental design

Animals (30 female Sprague–Dawley albino rats) with average weight of 45–66 g were classified into 6 groups, of 5 animals each. The groups are as follows:

Group NC (Negative control) – DMBA induced rats only.
Group PC (Positive control) – Control experiment.
Group WA – DMBA induced rats treated with *M. pruriens* extract (50 mg/g b wt).
Group WB – DMBA induced rats treated with *M. pruriens* extract (100 mg/g b wt).
Group WC – DMBA induced rats treated with *M. pruriens* extract (200 mg/g b wt).
Group WD – DMBA induced rats treated with *M. pruriens* extract (300 mg/g b wt).

3.4. Plant extract administration and treatment

Rats were palpated weekly to check for tumor appearance (tumor not really detected in the DMBA induced rats only but recorded a total of 3 mortalities in the group). After induction with DMBA, group WA, WB, WC and WD were administered and treated with daily weekly doses of (50, 100, 200 and 300) mg/kg body-weight of the plant extract of *M. pruriens* leaves by the gavage method for a period of 10 weeks respectively with respect to the LD_{50} result of Bhaskar and Vidhya [33].

3.5. Experimental site

The work was carried out in the animal house (Botanical Garden), University of Lagos, Lagos in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiment with consent from the University of Lagos Ethics Committee guidelines for experiment with whole animals.

3.6. Phytochemical screening of the plant extract

Preliminary phytochemical screening (Qualitative and Quantitative analysis) of the aqueous leaf extract of *M. pruriens* was carried out by methods of analysis described by Sofowara [34] and Horborne [35].

3.7. Histological determination

For microscopic evaluation, mammary gland tissues were fixed in a fixative (10% formal saline) and embedded in paraffin, sectioned at 4–5 μm and subsequently stained with hematoxylin/eosin. Sections were studied under the light microscope at 40 and 100 magnifications. Slides of all the treated groups were studied and photographed.

3.8. Comet assay

The single cell gel electrophoresis protocol used for this study was a modified procedure of Ostling and Johanson [36]; Singh et al. [37]; Collins [38]. Briefly, Preparation of base slides was

done using 1% (500 mg per 50 ml PBS) and 0.5% LMPA (250 mg per 50 ml PBS) and 1.0% NMA (500 mg per 50 ml in Milli Q water). To the coated slide, add 75 μL of LMPA (0.5%; 37 °C) mixed with ~10,000 isolated lymphocytes in ~5–10 μL. Electrophoresis of Microgel Slides was done under pH > 13 alkaline conditions, 24 V (~0.74 V/cm), 300 mA for 30 min. For visualization of DNA damage, observations are made of Silver-stained DNA using a 40× objective on a light microscope.

3.9. Statistical analysis

Statistical analysis of data was assessed by a One-way Analysis of Variance (ANOVA) using the GraphPad Prism software package 5.0 for windows from Graphpad Software Inc. Newman–Keuls Multiple Comparison Test was used to determine the group which showed the most significant reduction in weight as shown in the appendix.

Results were expressed as the mean ± SEM for the 5 rats in each group. Values of $p < 0.05$ were considered statistically significant.

4. Results

Results obtained for the qualitative phytochemical screening of the aqueous leaf extract of *M. pruriens* are shown in Table 1. The phytochemical screening revealed that alkaloids, reducing sugar, anthraquinones, flavonoids, saponins, tannins, cardiac glycosides, phenols and steroids were present while only terpenoid was absent. The quantitative phytochemical analysis of selected phytochemicals present in the aqueous extract of *M. pruriens* is shown in Table 2. Flavonoid was found to be present in the highest amount (21.14%) while alkaloid was found to be in the lowest amount (0.35%).

Plate 1 is an image showing 7,12-dimethylbenz(a)anthracene (DMBA) induced-breast cancer in an experimental rat. DMBA induced-breast cancer was seen at the neck region of one of the rats that received the DMBA only. This occurred 10 weeks after the administration of the carcinogen.

Table 3 shows the effect of different concentrations of aqueous extract of *M. pruriens* on absolute increase and variation in body weight of female albino rats administered with 7,12-dimethylbenz(a)anthracene. There was a significant reduction ($p < 0.05$) in the weight of rats that received 300 mg/g b wt dose of the extract when compared with those that took neither the extract nor DMBA (positive control) within the first 3 weeks. Animals that received DMBA only (negative control) showed a significant increase in weight ($p > 0.05$) within the 10th week. However, there was decrease in weight, sickling signs and weakness observed the first 3 weeks after DMBA administration.

The effects of aqueous leaf extract of *M. pruriens* on the survival rate of rat induced with breast cancer using DMBA is also shown in Fig. 1. Death was recorded in the NC (Negative control) group after 2 weeks of DMBA administration and no death occurrence as recorded in other groups throughout this experiment. The highest number of deaths was recorded in NC in the sixth week.

Table 1 Result showing qualitative analysis of aqueous extract of *Mucuna pruriens* leaves.

S. No.	Phytochemical components	Aqueous extract of *Mucuna pruriens* leaves
1	Alkaloids	+
2	Reducing sugar	+
3	Anthraquinones	+
4	Terpenoids	–
5	Flavonoids	+
6	Saponins	+
7	Tannins	+
8	Cardiac glycosides	+
9	Phenol	+
10	Steroids	+

Key: (+) Presence of phytochemical(s).
(–) Absence of phytochemical(s).

Table 2 Result showing quantitative analysis of aqueous extract of *Mucuna pruriens* leaves.

S. No.	Phytochemical components	% composition
1	Tannin	0.72 ± 0.008
2	Alkaloid	0.35 ± 0.006
3	Flavonoid	21.14 ± 0.013
4	Phenol	2.21 ± 0.006
5	Cardiac glycosides	13.32 ± 0.017
6	Saponin	5.06 ± 0.009

Mean values (%) of phytochemical screening of *Mucuna pruriens* leaves showing mean ± SEM.

Plate 1 DMBA induced-breast cancer at the neck region of rats treated with DMBA only. Arrows indicate the location of DMBA induced papilloma.

Table 3 Effects of an aqueous extract of *Mucuna pruriens* leaves on the weight trend of experimental animals over a period of 10 weeks.

Treatments	Total no. of rats	No. of mortality	No. of tumor	Absolute increase in body weight (g)									
				Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
NC	5	2	1	71.10 ± 3.44[b]	97.34 ± 8.63	115.35 ± 10.10	118.38 ± 12.82	136.28 ± 14.30	137.35 ± 15.40	144.63 ± 16.21	137.80 ± 15.20	167.73 ± 8.31	197.82 ± 16.91[b]
PC	5	0	0	76.72 ± 2.33	91.30 ± 4.70	110.74 ± 4.11	118.48 ± 5.46	136.54 ± 7.92	144.60 ± 9.60	145.30 ± 10.51	141.84 ± 11.02	153.28 ± 12.30	164.56 ± 14.58
WA	5	0	0	80.00 ± 3.22	110.76 ± 1.76	123.06 ± 3.58[c]	134.32 ± 2.39	138.18 ± 2.75	143.08 ± 3.91	142.82 ± 3.11	141.62 ± 3.18	150.50 ± 26.74	155.46 ± 5.01
WB	5	0	0	84.40 ± 1.07	119.42 ± 1.65	129.80 ± 3.68[a]	137.24 ± 4.09	147.24 ± 5.20	152.40 ± 5.83	151.70 ± 5.32	146.88 ± 6.45	150.42 ± 14.70	159.78 ± 6.80
WC	5	0	0	96.00 ± 0.45	126.92 ± 2.33[a]	139.30 ± 1.83[a]	134.50 ± 1.47	143.28 ± 1.90	148.94 ± 1.51	147.64 ± 0.76	143.82 ± 0.72	154.30 ± 0.87	154.88 ± 0.72
WD	5	0	0	102.00 ± 1.03	133.22 ± 1.70[a]	138.76 ± 2.59[a]	141.52 ± 4.36	144.68 ± 3.90	153.70 ± 6.19	152.94 ± 6.26	147.26 ± 5.63	155.20 ± 5.70	156.32 ± 5.59

Values are mean ± SEM ($n = 5$).
Values carrying superscript (a) showed significant reduction ($p < 0.001$).
Values carrying superscript (b) showed significant reduction ($p < 0.01$).
Values carrying superscript (c) showed significant reduction ($p < 0.05$).
NC (Negative control) – DMBA induced rats only.
PC (Positive control) – control experiment.
WA – DMBA induced rats treated with *M. pruriens* extract (50 mg/g b wt).
WB – DMBA induced rats treated with *M. pruriens* extract (100 mg/g b wt).
WC – DMBA induced rats treated with *M. pruriens* extract (200 mg/g b wt).
WD – DMBA induced rats treated with *M. pruriens* extract (300 mg/g b wt).

Plate 2 showed the actual length of isolated tumor of DMBA-induced rats only, it measured as 2.4 cm. Plate 2 showed the weight of isolated tumor of DMBA-induced rats only which was given as 2.8 g.

Histological section of the breast tissue of experimental rats from the positive control (PC) is shown in Plate 3. This revealed the presence of normocellular serous glands at (HE-100×) magnification. Plate 4 shows the histological section of the breast tissue of DMBA induced-breast cancer in rats from negative control (NC). This revealed the presence of atrophy of seromucous glands with surrounding stromal fibrosis (Fibroadenoma). The histological section of the breast tissue of DMBA induced-breast cancer in rats treated with 50 mg/g b wt of aqueous leaf extract of *M. pruriens* is shown in Plate 5. This reveals the presence of hyperplasia of serous and mucinous glands while the presence of DMBA-induced atrophy of glands with periductal stromal fibrosis and fatty tissue were identified in Plate 6.

The histological section of the breast tissue of DMBA induced-breast cancer in rats administered with 200 mg/g b wt of aqueous leaf extract of *M. pruriens* were shown in Plate 7. The histological section reveals the presence of atrophy of seromucous glands with surrounding stromal fibrosis. Plate 8 shows histological section of the breast tissue of DMBA induced-breast cancer in rats treated with 300 mg/g b wt of aqueous leaf extract of *M. pruriens*. The histological section reveals the presence of atrophy of glands, within surrounding fatty tissue at a magnification of 100×.

Plate 9 shows the DNA fragmentation images for the control groups (NC and PC) and the treatment groups (WA, WB, WC and WD) obtained by single cell gel electrophoresis and Table 4 shows qualitative and quantitative analytical results of DNA fragmentation images obtained by single cell gel electrophoresis. The % DNA in tail, tail moment and tail length in the control and treatment groups were shown to be NC (50.13%, 9.35, 41), PC (0.25%, 0, 0), WA (31.26%, 4.99, 27), WB (18.59%, 2.14, 21), WC (12.48%, 1.15, 10) and WD (3.37%, 0.06, 2) respectively.

5. Discussion

Breast cancer is the commonest female cancer and most common cancer in both sexes [39]. Breast cancer is a kind of cancer that develops from breast cells. It is the most common type of cancer among women in Nigeria [40]. A major obstacle for cancer therapies is lack of targeted delivery of therapeutics. Therefore, there is a dire need to better understand the mechanisms by which drug resistance develops and to design new combined treatments that benefit patients [41]. To compensate, existing drug treatments rely on high dosages and frequent administrations. This results in adverse effects in the patient due to the drugs failure to discriminate between normal body cells and tumor cells [42]. Cancer chemoprevention involves the chronic administration of a synthetic, natural or biological agent to reduce or delay the occurrence of malignancy. The potential value of this approach has been demonstrated with trials in breast, prostate and colon cancer [16]. However, it is likely that many agents, particularly those that are dietary derived and multi-targeted, will have effects throughout the carcinogenic process [43]. Compounds that inhibit cancer initiation are traditionally termed 'blocking agents'. They may act

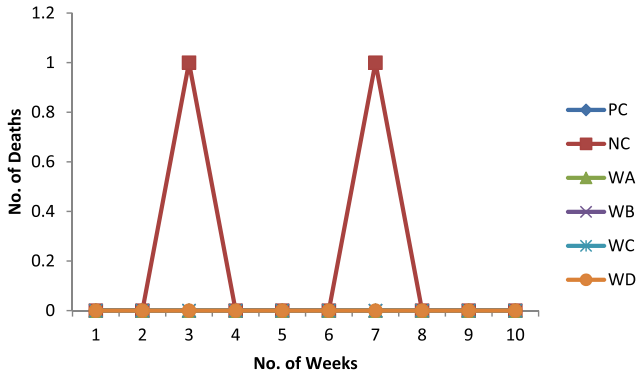


Figure 1 Effects of different concentrations of aqueous extract of *Mucuna pruriens* leaf on the survival rate of DMBA induced-breast cancer in rats.

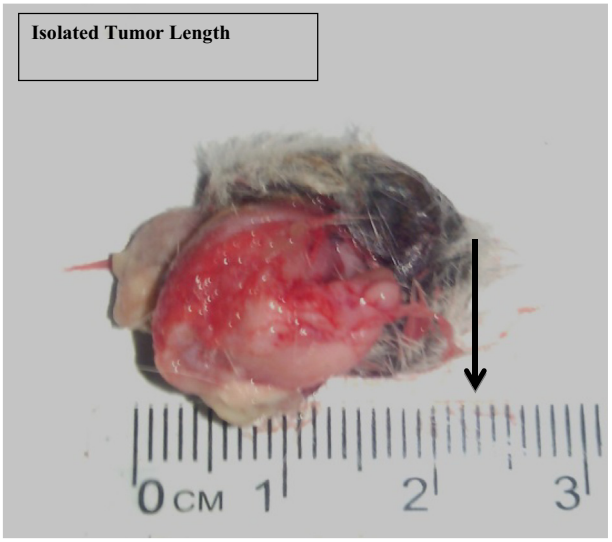


Plate 2 Length of isolated tumor of DMBA-induced rats only. (From the neck region.)

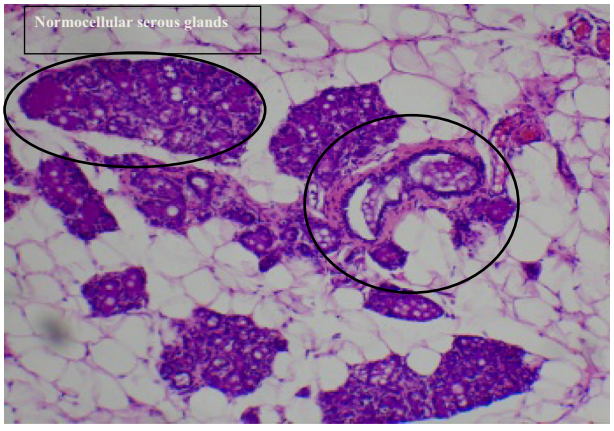


Plate 3 Histological section of the breast tissue of DMBA-induced breast cancer in rats from PC (HE-100×). (Normocellular serous glands.)

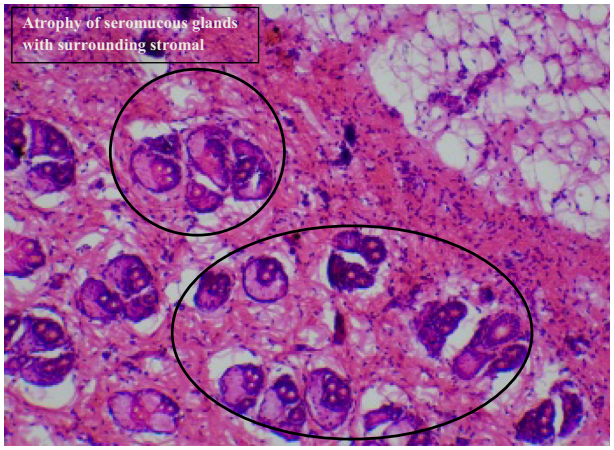


Plate 4 Histological section of the breast tissue of DMBA-induced breast cancer in rats from NC (HE-100×). (Atrophy of seromucous glands with surrounding stromal fibrosis.)

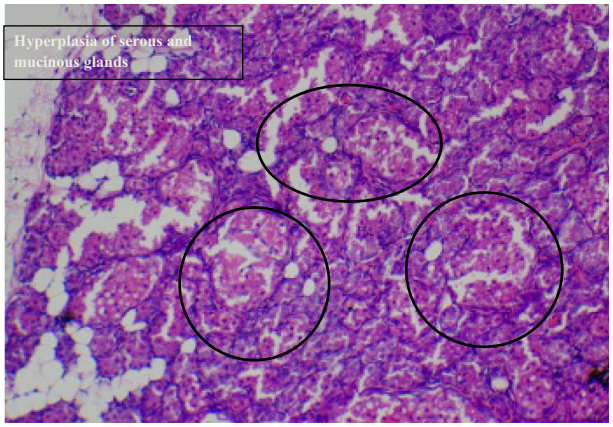


Plate 5 Histological section of the breast tissue of DMBA-induced breast cancer in rats from WA (HE-100×). (Hyperplasia of serous and mucinous glands.)

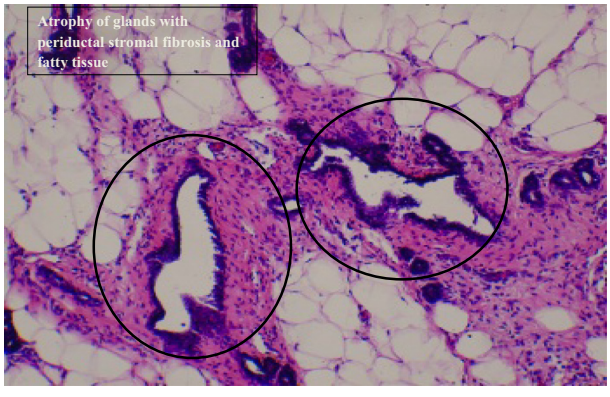


Plate 6 Histological section of the breast tissue of DMBA-induced breast cancer in rats from WB (HE-100×). (Atrophy of glands with periductal stromal fibrosis and fatty tissue.)

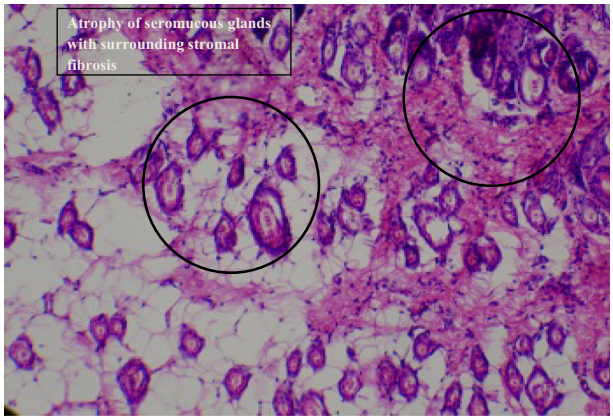


Plate 7 Histological section of the breast tissue of DMBA-induced breast cancer in rats from WC (HE-100×). (Atrophy of seromucous glands with surrounding stromal fibrosis.)

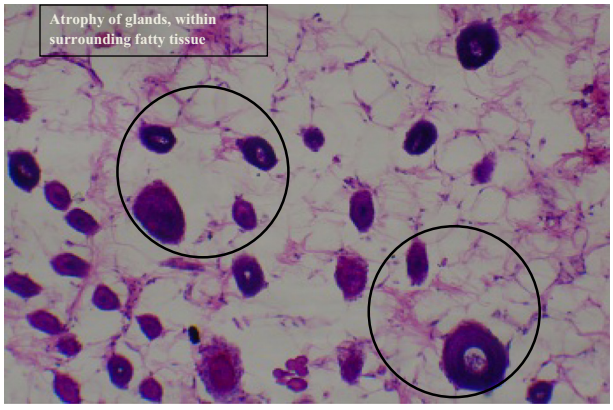


Plate 8 Histological section of the breast tissue of DMBA-induced breast cancer in rats from WD (HE-100×). (Atrophy of glands, within surrounding fatty tissue.)

by preventing the interaction between chemical carcinogens or endogenous free radicals and DNA, thereby reducing the level of damage and resulting mutations which contribute not only to cancer initiation but also progressive genomic instability and overall neoplastic transformation. Protection may be achieved as a consequence of decreased cellular uptake and metabolic activation of pro-carcinogens and/or enhanced detoxification of reactive electrophiles and free radical scavenging, as well as induction of repair pathways [43,44]. Owing to this development, 'Theanti-proliferative potential ofaqueous leaf extract of *Mucuna pruriens* on DMBA-induced breast cancer in female albino rats' was evaluated. Henry and Narendra [45] reported that numerous studies have shown that 7,12-dimethylbenz(a)anthracene (DMBA) can be used to induce experimental breast carcinomas in rats and that this process involves disruption of tissue redox balance; in turn, this suggests that biochemical and pathophysiological disturbances may result from oxidative damage.

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [46]. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [47]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [48]. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [49]. In this sense chemopreventive phytochemicals are applicable to cancer therapy, since molecular mechanisms may be common to both chemoprevention and cancer therapy [50,51].

Phytochemical analysis of the result presented in this study confirm the presence of constituents such as alkaloids, reducing sugar, anthraquinones, flavonoids, saponins, tannins, cardiac glycosides, phenols and steroids (Table 1); which are known to exhibit remarkable medicinal as well as physiological activities showing specific mode of action. This is in agreement with the study review of Sherma et al. [52] on the phyto constituents and therapeutic uses on *M. pruriens*. However, the presence of alkaloid and absence of terpenoid contradicts the result of Agbafor and Nwachukwu [53] who reported the absence of alkaloid and presence of terpenoid. This could be prior to the fact that the location of the plant might be a significant factor in determining the presence or absence of essential phytoconstituents; other contributing factors that might also play a major role are the biosynthetic mechanism, environmental factors and interaction with micro or macro elements. The quantitative phytochemical screening of selected phytochemicals present in the aqueous extract of *M. pruriens* is shown in (Table 2). Flavonoid was found to be present in the highest amount (21.14%) while alkaloid was found to be in the lowest amount (0.35%). The relatively high amount of flavonoid (an antioxidants/free radical scavenger) could possibly be responsible for the significant reduction ($p < 0.05$) in weight and inhibition of tumorigenesis in rats administered with 300 mg/g b wt (WD). Many forms of cancer are the result of reactions between free radicals and DNA, resulting in mutations that can lead to malignancy. Free radicals and their metabolites are increasingly recognized for their contribution to tissue injury leading to both initiation and promotion of multistage carcinogenesis [54,55]. According to Slaga [54], antioxidants/free radical scavengers function as inhibitors of both tumor initiation and promotion. Flavonoids are simple phenolic compounds which have been reported to possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic, as well as ability to modify the gene expression [56,57].

In general, the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents [58]. The presence of phenol (2.21%), relatively confers its potential to also influence cancer progression in the breast tissues. Polyphenols particularly are among the diverse phytochemicals that have the potential in the inhibition of carcinogenesis [59].

The visible evidence of 7,12-dimethylbenz(a)anthracene administration on DMBA-induced-tumorigenesis was seen at

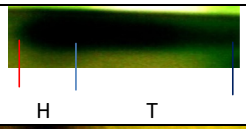
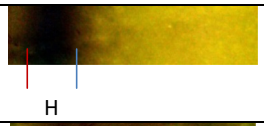
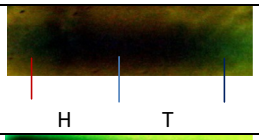
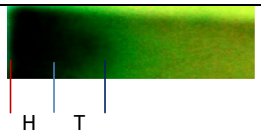
Image	Treatments	Comet images
1	**NC***(Negative control)* –DMBA Induced rats only	
2	**PC***(Positive control)*– Control experiment	
3	**WA**(DMBA Induced rats treated with *M. pruriens* extract *(50 mg/g b wt)*)	
4	**WB**(DMBA Induced rats treated with *M. pruriens* extract *(100 mg/g b wt)*)	
5	**WC**(DMBA Induced rats treated with *M. pruriens* extract *(200 mg/g b wt)*)	
6	**WD**(DMBA Induced rats treated with *M. pruriens* extract *(300 mg/g b wt)*)	

Plate 9 DNA fragmentation images obtained by single cell gel electrophoresis.

Table 4 Qualitative and quantitative analytical results of DNA fragmentation images obtained by single cell gel electrophoresis (SCGE).

Image	% DNA in tail	Tail moment	Tail length
NC	50.13	9.35	41
PC	0.25	0	0
WA	31.26	4.99	27
WB	18.59	2.14	21
WC	12.48	1.15	10
WD	3.37	0.06	2

% – (Percentage).
NC (Negative control) – DMBA induced rats only.
PC (Positive control) – Control experiment.
WA – DMBA induced rats treated with *M. pruriens* extract (50 mg/g b wt).
WB – DMBA induced rats treated with *M. pruriens* extract (100 mg/g b wt).
WC – DMBA induced rats treated with *M. pruriens* extract (200 mg/g b wt).
WD – DMBA induced rats treated with *M. pruriens* extract (300 mg/g b wt).

the neck region of one of the rats administered with DMBA only (Plate 1) about 10 weeks after DMBA induction. This occurred earlier than reported by Amin et al. [60]. This could be prior to the fact that the modified protocol of Alfredo et al. [61] used for this study was of a relatively high concentration and reason for development of tumor at the neck region could be due to the spread of the breast cancer cells to the lymph nodes in the chest, under the arm, above or below the collar bone which are connected to the lymphatic vessels from the breast. According to the American Cancer Society [62], most breast cancers begin in the cells that line the ducts (ductal cancers). Some begin in the cells that line the lobules (lobular cancers), while a small number start in other tissues. The lymph system is important to understand because it is one way breast cancers can spread. Breast cancer cells can enter lymphatic vessels and begin to grow in lymph nodes. Most lymphatic vessels in the breast connect to lymph nodes under the arm (axillary nodes). Some lymphatic vessels connect to lymph nodes inside the chest (internal mammary nodes) and those either above or below the collarbone (supraclavicular or infraclavicular nodes).

Also, the development of tumor at the neck region in the experimental rat (NC) supports the claims of Clark [63]; Russo et al. [64] and Balogh et al. [65] that several tissues are capable of activating DMBA. In a recent study conducted by Minari and Okeke [66], DMBA-induced-papilloma was seen toward the hind limbs. The increase in death rate was observed in the sixth week in the NC group of rats administered with DMBA only may also be due to the toxicity of the carcinogen.

The significant reduction ($p < 0.05$), in the weight of experimental rats that took 300 mg/g b wt (WD) of the extract when compared with the positive control is an indication of the possible toxicity of aqueous extract of *M. pruriens* leaf suggesting the possible interaction between phytochemical components due to increase in concentration (Table 3). This is in agreement with the work of Ngatchic et al. [67] on toxicity level of *M. pruriens*. Kumar and Saha [68] documented in their review that this plant has no such toxicological consensus but has several

limitations. It was further stated that it is toxic to humans and animals due to the presence of 3-(3,4-dihydroxyphenyl)-1-alanine (L-DOPA) and tryptamines.

The isolated tumor shown in Plate 2 is also a clear indication of the carcinogenic effect of induced DMBA (a polycyclic aromatic hydrocarbon) in the experimental rats which supports the studies of Ko et al. [69] that animal models have helped demonstrating different classes of chemicals to act as initiators and induce mammary cancer. Polycyclic aromatic hydrocarbons (PAHs) are toxic compounds commonly found in the environment. PAHs are genotoxic and capable of forming carcinogen–DNA adducts in human or animal tissues. Once these chemicals are consumed, our body will metabolize and transform these compounds into DNA attacking mutagens. Epidemiological studies have revealed that PAHs can form adducts in humans. Higher amounts of benzyl-[a]-pyrene like DNA adducts have been found in human breast tumors than in normal breast tissues. As one of the PAHs, DMBA has been shown to affect cellular signaling pathways to induce apoptosis. It is also known to induce cytotoxicity in various cell types, including mouse epidermis, hepatoma, and fibroblasts.

In human breast carcinomas, proliferation occurs almost exclusively in epithelial cells [63–65].

The histopathological classification of breast disease is subjective and, despite an attempt to provide clear guidelines, the inter-observer variability is known to be high [70]. The presence of DMBA-induced atrophy of seromucous glands with surrounding stromal fibrosis, hyperplasia of serous and mucinous glands, atrophy of glands with periductal stromal fibrosis and fatty tissue and atrophy of seromucous glands with surrounding stromal fibrosis in the histological sections of breast tissues of DMBA-induced breast cancer of rats that were administered with 7,12-dimethylbenz(a)anthracene in (Plates 5–8) respectively suggest that DMBA-induced rat mammary carcinomas have been shown to arise from the ductal elements of the mammary gland and significant variability in tissues induced with the carcinogen. This corresponds with the studies of Chow et al. [71]. The mammary tumors in rats arise in the epithelium of the terminal end buds, which are comparable structures to the terminal ductal lobular units in the human breast [32]. Nicolas et al. [72] in an experimental study confirmed that the most common histologies of the DMBA-induced mammary tumors were squamous or adenosquamous carcinomas, accounting for 85% of the mammary tumors, with other tumors being of the Wnt type, or scirrhous tubular, spindle cell, or papillary carcinomas.

The single cell gel electrophoresis results as shown in Plate 8 and Table 4 indicates largely the chemopreventive potential of the leaf extract of *M. pruriens* on possible DMBA-induced DNA damage. The % DNA in tail, tail length and moment of the WB (18.59%, 2.14, 21), WC (12.48%, 1.15, 10) and WD (3.37%, 0.06, 2) groups respectively were significantly reduced at their respective concentration of the leaf extract of *M. pruriens* when compared with the NC (50.13%, 9.35, 41) group; which might be due to the presence of antioxidants in the plant with the potential to scavenge free radicals and inhibit oxidative DNA damage by DMBA. Polycyclic aromatic hydrocarbons (PAH) such as 7,12-dimethylbenz(a) anthracene (DMBA) have been shown to form free radicals and these compounds play a critical role in carcinogenesis [73]. This role is accompanied by direct interaction with DNA and the generation of reactive oxygen species (ROS), such as peroxides, hydroxyl and superoxide anion radicals, which induce cellular oxidative damage through oxidation of biomolecules such as proteins and nucleo-bases, DNA strand breaks and lipid peroxidation [74]. Oxidative injury may produce unrepaired DNA damage and result in the accumulation of mutations. DNA mutation is a critical step in carcinogenesis, and elevated levels of oxidative DNA lesions such as 8-OH-dG have been noted in various tumors, strongly implicating such damage in the etiology of cancer [75]. Moreover, natural antioxidants contained in medicinal and aromatic plants, fruits and vegetables may be useful in preventing the deleterious consequences of oxidative damage caused by ROS and therefore they are considered as possible chemopreventive agents. They can possess a variety of biological activities, e.g., antimutagenic, anti-carcinogenic, anti-proliferative, scavenging of free radicals or activated mutagens/carcinogens, they can modulate DNA repair and other enzyme activities or even regulate gene expression [76,77].

6. Conclusion

The present study showed that the aqueous extract of *M. pruriens* leaves can be used as an anti-proliferative measure against DMBA induced breast cancer in female albino rats.

Histological assay revealed the presence of atrophy of seromucous glands with surrounding stromal fibrosis, and hyperplasia of serous and mucinous glands which suggest the presence of cells undergoing initial proliferation stage preceding carcinogenesis.

The knowledge obtained from this study serves as a resource base and can be scientifically exploited for future research in breast cancer chemoprevention. Finally, the present study identifies new areas of research for development of better therapeutic and chemopreventive agents for colon, lungs and prostate cancer, and other infectious diseases.

Conflict of interest

The authors declare that there is no conflict of interest with any organization or individual on the work.

Appendix

Week 1

ANOVA Table	SS	df	MS	
Treatment (between columns)	10,930	5	2187	
Residual (within columns)	23,000	54	425.9	
Total	33,930	59		
Newman–Keuls Multiple Comparison Test	Mean Diff.	*q*	Significant? $P < 0.05$?	Summary
NC vs PC	−26.38	4.042	Yes	**
PC vs WD	−14.22	2.180	No	ns
PC vs WC	−10.62	—	No	ns
PC vs WB	−9.592	—	No	ns
PC vs WA	−3.644	—	No	ns

Week 2

ANOVA Table	SS	df	MS	
Treatment (between columns)	3486	5	697.1	
Residual (within columns)	600.8	24	25.03	
Total	4086	29		
Newman–Keuls Multiple Comparison Test	Mean Diff.	q	Significant? $P < 0.05$?	Summary
NC vs PC	−5.620	2.512	No	ns
PC vs WD	−25.28	11.30	Yes	***
PC vs WC	−19.28	8.617	Yes	***
PC vs WB	−7.680	3.432	No	ns
PC vs WA	−3.280	—	No	ns

Week 3

ANOVA Table	SS	df	MS	
Treatment (between columns)	6824	5	1365	
Residual (within columns)	2214	24	92.25	
Total	9038	29		
Newman–Keuls Multiple Comparison Test	Mean Diff.	q	Significant? $P < 0.05$?	Summary
PC vs WD	−41.92	9.759	Yes	***
PC vs WC	−35.62	8.292	Yes	***
PC vs WB	−28.12	6.546	Yes	***
PC vs WA	−19.46	4.530	Yes	*
PC vs NC	−6.040	1.406	No	ns

Week 4

ANOVA Table	SS	df	MS	
Treatment (between columns)	8258	5	1652	
Residual (within columns)	10,870	24	452.9	
Total	19,130	29		
Newman–Keuls Multiple Comparison Test	Mean Diff.	q	Significant? $P < 0.05$?	Summary
NC vs PC	−18.46	—	No	ns
PC vs WC	−28.56	3.001	No	ns
PC vs WD	−28.02	—	No	ns
PC vs WB	−19.06	—	No	ns
PC vs WA	−12.32	—	No	ns

Week 5

ANOVA Table	SS	df	MS	
Treatment (between columns)	6664	5	1333	
Residual (within columns)	18,950	24	789.7	
Total	25,620	29		
Newman–Keuls Multiple Comparison Test	Mean Diff.	q	Significant? $P < 0.05$?	Summary
NC vs PC	−34.72	—	No	ns
NC vs WA	−33.20	—	No	ns
WA vs PC	−1.520	—	No	ns
PC vs WD	−9.100	—	No	ns
PC vs WB	−7.800	—	No	ns
PC vs WC	−4.340	—	No	ns

Week 6

ANOVA Table	SS	df	MS	
Treatment (between columns)	4730	5	946.0	
Residual (within columns)	20,500	24	854.3	
Total	25,230	29		
Newman–Keuls Multiple Comparison Test	Mean Diff.	q	Significant? $P < 0.05$?	Summary
NC vs PC	−29.60	—	No	ns
NC vs WA	−27.12	—	No	ns
WA vs PC	−2.480	—	No	ns
PC vs WD	−7.640	—	No	ns
PC vs WB	−6.400	—	No	ns
PC vs WC	−2.340	—	No	ns

Week 7

ANOVA Table	SS	df	MS	
Treatment (between columns)	4974	5	994.7	
Residual (within columns)	19,160	24	798.1	
Total	24,130	29		
Newman–Keuls Multiple Comparison Test	Mean Diff.	q	Significant? $P < 0.05$?	Summary
NC vs PC	−31.60	—	No	ns
NC vs WA	−31.38	—	No	ns
PC vs WD	−5.420	—	No	ns
PC vs WB	−5.040	—	No	ns
PC vs WC	−1.980	—	No	ns

Week 8

ANOVA Table	SS	df	MS	
Treatment (between columns)	11,410	5	2281	
Residual (within columns)	33,260	24	1386	
Total	44,670	29		
Newman–Keuls Multiple Comparison Test	Mean Diff.	*q*	Significant? $P < 0.05$?	Summary
NC vs PC	−52.64	—	No	ns
NC vs WA	−49.86	—	No	ns
WB vs PC	−2.860	—	No	ns
WA vs PC	−2.780	—	No	ns
PC vs WD	−1.920	—	No	ns
PC vs WC	−1.020	—	No	ns

Week 9

ANOVA Table	SS	df	MS	
Treatment (between columns)	6833	5	1367	
Residual (within columns)	45,920	24	1913	
Total	52,750	29		
Newman–Keuls Multiple Comparison Test	Mean Diff.	*q*	Significant? $P < 0.05$?	Summary
NC vs PC	−45.88	2.345	No	ns
WC vs PC	−9.680	—	No	ns
WA vs PC	−9.100	—	No	ns
WD vs PC	−8.240	—	No	ns
WD vs WB	−3.460	—	No	ns
WB vs PC	−4.780	—	No	ns

Week 10

ANOVA Table	SS	df	MS	
Treatment (between columns)	10,930	5	2187	
Residual (within columns)	23,000	54	425.9	
Total	33,930	59		
Newman–Keuls Multiple Comparison Test	Mean Diff.	*q*	Significant? $P < 0.05$?	Summary
NC vs PC	−26.38	4.042	Yes	**
PC vs WD	−14.22	2.180	No	ns
PC vs WC	−10.62	—	No	ns
PC vs WB	−9.592	—	No	ns
PC vs WA	−3.644	—	No	ns

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