Caesalpinia pulcherrima lowered serum carcinoembryonic antigen and antigen 125 in 7,12-Dimethylbenz[*a*]anthracene-induced Mammary Carcinogenesis in Female Albino Rats

Abraham Ehinomhen Ubhenin [a], Adedotun A. Adefolalu [a], Kelly Oriakhi [b], Fatima Adis Adamude [a], Emeka John Dingwoke [c], Joshua Onyeka Ikebuiro [a], Benjamin Comfort Chiwendu [a], Mabruqah Liman Muhammad [a], Kingsley Omage [d],*

[a] *Department of Biochemistry, Faculty of Medical Sciences, Federal University Lafia, Nasarawa State, Nigeria*
[b] *Department of Medical Biochemistry, Faculty of Medical Sciences, University of Benin, Benin City, Nigeria*
[c] *Department of Tropical Diseases, UNESCO-International Center for Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria*
[d] *Division of Nephrology and Hypertension, Department of Medicine, Oregon Health and Science University, Portland, United States*

A R T I C L E   I N F O

A B S T R A C T

Aim: This study is aimed at evaluating the anticancer effect of the aqueous extract of *Caesalpinia pulcherrima* (L.) Sw in 7,12-Dimethlbenz[*a*]anthracene (DMBA) – induced mammary cancer.
Methods: Tumors were induced via a single intraperitoneal injection of DMBA (dissolved in olive oil) at a dose of 80 mg/kg body weight to the test rats and allowed to develop for about four months. They were treated with cyclophosphamide and an aqueous extract of *Caesalpinia pulcherrima* at doses of 10 and 250 mg/kg body weight, respectively, for 28 days. Serum levels of cancer antigen 125 (CA125), carcinoembryonic antigen (CEA) activity, cyclooxygenase-2 (COX-2), and cytochrome p450 oxidase (cytp450) activity, as well as other diagnostic enzymes, were estimated.
Results: The result revealed that DMBA is associated with a significant ($p < 0.05$) increase in the serum levels of CA125, CEA, COX-2, cytp450, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) of the rats, thus suggesting tumor-promoting and hepatotoxic effects of DMBA. There was also a significant ($p < 0.05$) reduction of serum levels of these cancer and liver biomarker enzymes in the groups treated with cyclophosphamide and *Caesalpinia pulcherrima* compared to the untreated group, thus suggesting anticancer activity of *Caesalpinia pulcherrima*. The anticancer effect of *Caesalpinia pulcherrima* was further confirmed by the disappearance of infiltrative fibrous cells and the absence of inflammatory cells from the photomicrographs of the rats treated with *Caesalpinia pulcherrima*.
Conclusion: Our findings show that *Caesalpinia pulcherrima* possesses anticancer activity, and could protect against mammary cancer.

* Corresponding author. Division of Nephrology and Hypertension, Department of Medicine, Oregon Health and Science University, Portland, United States.
E-mail addresses: ehibram@yahoo.co.uk (A.E. Ubhenin), aadotman@gmail.com (A.A. Adefolalu), kelly.oriakhi@uniben.edu (K. Oriakhi), fatteeyjibamin@gmail.com (F.A. Adamude), dinhimself@yahoo.com (E.J. Dingwoke), ikebuirojoshua@gmail.com (J.O. Ikebuiro), chinwebenjamin5@gmail.com (B.C. Chiwendu), mabarukaliman@gmail.com (M.L. Muhammad), omagekingsley@yahoo.com (K. Omage).

1. Introduction

Cancer is a group of related diseases caused by mutation of the genes controlling cell divisions and it is characterized by excessive proliferation of cells and evasion of apoptosis [1,2]. Myriad studies have linked smoking, alcohol intake, obesity, exposure to certain chemicals, and radiation to carcinogenesis. Other predisposing factors may include viral infections and genetic abnormalities [1,2]. Death due to breast cancer among women have become issues of global concern [3,4]. Globally, breast cancer is the second most common type and accounts for 11.6 % of cancer incidence [4]. It is the fifth leading cause of cancer deaths globally and the leading cause of cancer – related deaths in women, with a death incidence of 6.6 % [4]. The prognosis of breast cancer in Nigeria represents about 12 % and accounts for 25 % of the total cancer incidence [5]. Today, breast cancer is the leading cause of cancer deaths in Nigerian women, overtaking cervical cancer [6,7].

7,12-dimethylbenz(*a*)anthracene (DMBA) is a polycyclic aromatic hydrocarbon (PAH) that is generally used to induce experimental tumors [8]. The suitability of DMBA as a chemical agent for the induction of mammary carcinogenesis comes from the fact that it produces pathological features that mimic the features accompanying cancerous growth in humans. Previous Studies have demonstrated that exposure to DMBA upregulates the transcription of numerous genes implicated in cellular metabolisms. One of these metabolisms is inflammatory, xenobiotic, cell cycle, and apoptosis pathways [9,10]. The initial events following the induction of the tumor by injection of DMBA involve its binding to the aryl hydrocarbon receptor (AhR) which may induce transcription of many genes after its combination with the cofactor known as aryl hydrocarbon receptor nuclear translocator (ARNT). AhR-ARNT complex is a standard transcription regulator for many genes. Notably, the gene coding for phase one metabolizing enzymes (e.g., cytochrome P450 oxidase) [10]. Biotransformation of DMBA by Cytochrome P450 resulted in the formation of a carcinogenic intermediate known as DMBA-3,4-dihydrodiol-1,2-epoxide. This intermediate is believed to be the mechanism of DMBA – induced carcinogenesis [9]. Recent studies have linked the mutations of the tumor protein p53 (p53), cyclin–dependent kinase interacting protein 1 (P21), and cyclin –dependent kinase inhibitor 1B (P27) to DMBA exposure [10,11]. The up-regulation of some genes, especially those coding for B-cell lymphoma-2 (Bcl2), cyclins and cyclin-dependent kinases (CDKS), nuclear transcription factor-kappa B (NF-kB), Cox-2 and Receptor tyrosine protein kinase (erbB2) have been reported to be associated to DMBA toxicity [10,11]. The consequence of the above cellular activation and mutation is the excessive proliferation of cells and evasion of apoptosis [12]. Therefore, inducing opposite pathways or inhibiting AHR activation appears to be the possible strategy for the anticancer activities of some medicinal plants.

The alarming prevalence of breast cancer in Nigeria has placed an imperative demand on scientists to provide better means of breast cancer management [13]. Advances have been made in cancer management; however, much work needs to be done [14]. In spite of considerable clinical efforts, cancer is still spreading rapidly, with increase in mortality. Moreover, during the last decade, the study revealed that synthetic chemotherapeutic agents currently used as therapies for cancer have not succeeded in fulfilling their expectations despite the considerable cost of their development [15]. The tremendous side effects of the current cancer therapies have necessitated the shift of research interest from synthetic chemotherapeutic agents to natural remedies from medicinal plants to provide a more effective treatment with less or no side effects and reduced cost of production [15]. Convincing evidence emanating from previous studies has proven that many phytochemicals from medicinal plants exert their effect on multiple molecular targets [16–18]. Modulating these molecular targets has been effective in inducing cellular responses that may give rise to the induction of apoptosis of mutant cells and blocking the mitotic division of tumor cells. Therefore, a continual search for more effective treatment with less or no side effects and reduced cost of production is sacrosanct. Medicinal plants are considered one of the most important sources of medicines [19–23]. Among the 250,000 higher plant species reported, more than 80,000 are used directly or indirectly for medicinal purposes [19,24–26]. Over the past few decades, herbal medicines have gained substantial global recognition because of their contribution to health and international trade [27]. Over three-quarters of the world's population, today depends on the plant and their extracts for health care [27]. Herbal forms of medication remain widely practiced for reasons including population increase, inadequate supply of drugs, high cost of treatments, and deleterious side effects of most synthetic medications [27,28]. In more recent history, the use of plants for medicinal purposes involves isolating and purifying their active compounds [29,30].

Caesalpinia pulcherrima (L.) Sw is a perennial shrub cultivated for ornamental and medicinal purposes. It belongs to the family of Fabaceae and is a native of the tropics and cold tropics part of the world. It is found as a shrub or small tree growing up to 3 m tall. This plant might grow more significantly in climates with few to no frosts [31,32]. Several secondary metabolites such as saponins, steroids, tannins, glycosides, phenolic, terpenoids, and alkaloids have been isolated from the plant [33]. Most notable among these phyto-chemicals are cassane-type diterpenoids [31]. Diterpenoids are vast chemicals recognized for their medicinal and industrial values [33]. Flavonoids possessing important biological activities have also been isolated from *Caesalpinia pulcherrima*. Five new flavonoids that had been isolated from the aerial part of the plant are 5,7-dimethoxy-flavanone, 5,7-dimethoxy-3′, and 4′-methylenediox--flavanone. isobonducellin, 2′-hydroyl-2,3,4′,6′-tetramethoylchalcone, and bonducellin [34]. Ethno-botanical evaluation and numerous research have confirmed the pharmacological potentials, as well as the antimicrobial activity of *Caesalpinia pulcherrima* [32–34]. This research aims to evaluate the anticancer activity of the aqueous root bark extracts of *Caesalpinia pulcherrima* in DMBA-induced mammary cancer in rats.

2. Materials and methods

2.1. Collection and identification of plant material

Fresh roots of *Caesalpinia pulcherrima* were collected in April 2021 from BukanKwator, in the Lafia local government of Nasarawa state, Nigeria. It was identified and authenticated in the department of plant science and biotechnology at the Federal University of

Lafia, where a voucher specimen number (FUL/0096) was deposited.

2.2. Processing and extraction of crude powdered samples

The fresh roots of *Caesalpinia pulcherrima* (Fig. 1) were washed, sliced into small pieces, air-dried, and ground to powder using a mortar and pestle. The ground plant material was then subjected to cold maceration by soaking 150 g of the powdered sample in four 4 L of distilled water in a flat bottom flask for three days (72 h) at room temperature, with occasional agitation of the flask. The aqueous extract was then filtered using a Buchner funnel and Whatman No.1 filter paper. The dry extract was obtained by evaporating the solvent under reduced pressure and temperature (40 °C) using the rotary evaporator. The extracts were stored in an air – tight container and kept in the refrigerator at 4 °C until use.

2.3. Chemicals used

DMBA was purchased from Sigma Aldrich (Germany), Di-ethanolamine buffer, Tris buffer, Paranitrophenol phosphate, and all other chemicals and kits used were obtained commercially and are of analytical grades.

2.4. Experimental site and animals

The work was carried out in the animal house (Botanical Garden) of the Federal University of Lafia, Nigeria, with adherence to the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments, and also with approval from the Federal University of Lafia, and Ahmadu Bello University Ethics Committee guidelines for experiment with whole animals (Ethical Code: ABUCAUC/2018/028).

Wistar albino rats aged 8–10 weeks old and weighing 70–100 g were obtained from the animal house at the National Veterinary Research Institute (NVRI) Vom, Jos, Plateau State of Nigeria. The rats were housed in well-aerated cages under hygienic conditions. Food and water were provided ad libitum. The animals were also subjected to a controlled environmental temperature of (28 ± 2 °C), relative humidity of (50 ± 5 %) and 12 h of light or dark. The handling procedures were conducted according to the Federal University Lafia ethical committee on experimental animals. The animals were allowed two weeks under these conditions.

2.5. Acute toxicity study

Acute oral toxicity (AOT) of aqueous extract of *Caesalpinia pulcherrima* was evaluated using albino rats according to the method of Lorke [35]. Before the experiment, the animals were fasted overnight and divided into five groups consisting of seven animals. Each animal in a group was given *Caesalpinia pulcherrima* orally at doses of 1, 2, 4, 6, and 8 g/kg body for groups 1, 2, 3, 4, 5, respectively. The animals were observed for two days for mortality (acute) and another 14 days for sub-chronic toxicity. The median lethal dose (LD_{50}) was calculated from the formula below.

$$LD_{50} = \sqrt{XY}$$

X stands for the highest dose without death, whereas Y stands for the lowest with death. The LD_{50} value was estimated above 8000 mg; therefore, 250 mg/kg body weight was chosen as effective dose for the experiment.
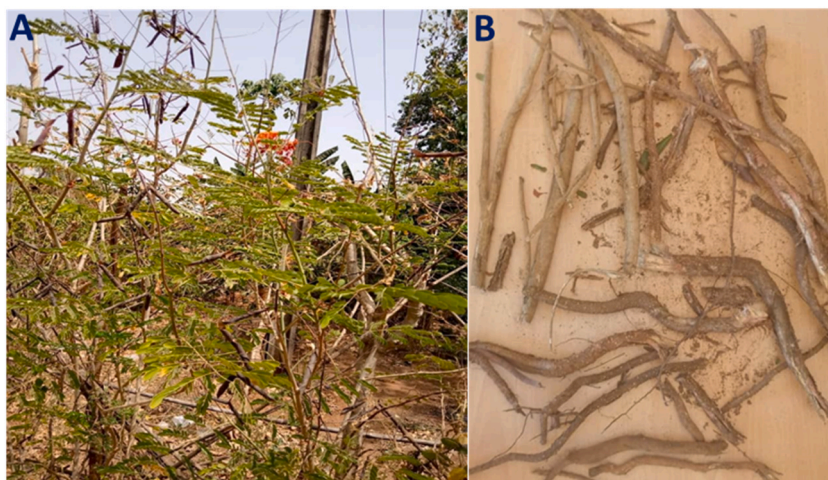

Fig. 1. *Caesalpinia pulcherrima* (A) – whole plant; (B) – dried roots. The pictures were taken during the flowering stage of the plant (towards the end of the raining season), at a local garden within the premises of the Federal University of Lafia, Nasarawa State, Nigeria.

2.6. Drug preparation/tumor induction

Induction of rat mammary gland tumor was done by intraperitoneal injection of a single dose of DMBA prepared in olive oil at a dose of 80 mg/kg body weight. Rats were palpated weekly to check for tumor appearance. The plant extract was administered orally at 250 mg/kg body weight.

2.7. Experimental design

The animals (20 female albino rats) were divided into four (4) groups of five animals each. The groups are as follows;
Group A: (standard control) received 0.5 ml of distilled water orally for five months.
Group B: received a single intraperitoneal injection of DMBA at a dose of 80 mg/kg body weight and 0.5 ml of distilled water for 28 days after four months of injection of DMBA.
Group C: received a single intraperitoneal injection of DMBA at a dose of 80 mg/kg body weight and treated with cyclophosphamide (a standard drug used for treating breast cancer) at a dose of 10 mg/kg body weight for 28 days after four months of induction of cancer.
Group D: received a single intraperitoneal injection of DMBA at a dose of 80 mg/kg body weight and treated with aqueous extract of *Caesalpinia pulcherrima* at 250 mg/kg body weight/day for 28 days after four months of induction of cancer.

The extract was administered using the orogastric tube for 28 consecutive days. The next day, after an overnight fast, all the animals were euthanized under isoflurane anesthetization, followed by an incision on the abdomen, and blood samples were collected via cardiac puncture. Afterwards, the experimental rats were sacrificed. The blood samples were put in plain sample bottles, and sera were obtained from them by allowing them to stand for 2 h at room temperature, followed by centrifuging at 2000 rpm. The Serum was used for the estimation of various biochemical parameters. The mammary gland and liver were dissected and immediately fixed in formol saline for tissue histopathological analysis.

2.8. Histopathological analysis

The mammary gland and liver were examined in all the dissected rats and were preserved in 10 % buffered formalin, dehydrated in ethanol (70–100 %), cleared in xylene, and embedded in paraffin. All tissue sections were examined under a light microscope after staining with hematoxylin (H) and eosin (E) [36].

2.9. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) kits (Hangzhou, Zhejiang, China) were used for the serum estimation of cyclooxygenases activity (COX-2), Cancer Antigen 125 (CA125), carcinoembryonic antigen (CEA) and cytochrome p450 (cytp450) activity.

2.10. Assessment of liver function

Serum Aspartate and Alanine Transferases (AST and ALT): The activities of these enzymes were estimated by the method of Reitman and Frankel [37]. The value of AST and ALT were calculated from a series of standard curves.

Alkaline Phosphatase (ALP): Serum alkaline phosphatase activity was measured following the method of King and Armstrong using disodium phenyl phosphate as substrate. The color developed was read at 510 nm. Activities are expressed as U/L [38]. Albumin (ALB): Serum total bilirubin was estimated following the method of Doumas et al. [39].

Total Protein and Bilirubin: The total protein content in the Serum was determined by the Biuret method [40], whereas bilirubin was estimated using assay kits (Randox Laboratories LTD. United Kingdom BT294QY) by the method described by Jendrassik and Grof [41].

2.11. Statistical analysis

Data were expressed as (Mean $\pm$ SD) of six replicates and were subjected to one-way analysis of variance (ANOVA) using SPSS version 10.0 and the individual comparisons were obtained by the Duncan multiple range test (DMRT). $P < 0.05$ was considered to indicate a significant difference between groups.

3. Results

The oral toxicity test shows that the extracts are safe and not toxic, judging from their high LD_{50} (above 8000 mg/kg).

Fig. 2 shows that intraperitoneal administration of DMBA at a dose of 80 mg/kg body weight led to significant ($p < 0.05$) increases in the serum activities of CA125 and CEA in the induced group (Group B) as compared to group A (normal group). Treatment, however, with cyclophosphamide and aqueous extract of *Caesalpinia pulcherrima* at doses of 10, 250 mg/kg body weight, respectively, led to significant ($p < 0.05$) decreases in their serum level in the treated groups (group C and D) as compared with group B.

In Fig. 3, administration of DMBA at a dose of 80 mg/kg body weight led to significant ($p < 0.05$) increases in the serum activities of ALT, ALP, and LD; however, treatment with cyclophosphamide and extract of *Caesalpinia pulcherrima* at 10, and 250 mg/kg body

significantly ($p < 0.05$) decrease in their levels in the Serum. There were no significant ($p > 0.05$) changes in the serum levels of AST, TB, and TP upon administration of DMBA and after treatment as compared to Groups A and B, respectively (see Fig. 4).

4. Discussion

Breast cancer is the most common type of cancer and is the leading cause of cancer death among Nigerian women [6,15]. Phytochemicals are considered beneficial in the treatment of cancer. Elevation of antioxidant status, inhibition of matrix metalloproteinases, and proliferation have been recognized as cancer-antagonistic mechanisms of some plant-derived anticancer drugs. Other mechanisms regulate the immune and modify phosphokinase pathways [42–46]. Oral toxicity test shows that the extracts are safe and not toxic, judging from their high LD_{50} (above 8000 mg/kg).

The result of the study shows that intraperitoneal administration of DMBA to rats at a dose of 80 mg/kg body weight led to a significant increase in the serum levels of CA125, CEA, LDH, and ALP as compared to group A. Treatment, however, with cyclophosphamide and aqueous extract of *Caesalpinia pulcherrima* at doses of 10 and 250 mg/kg body weight, respectively, led to a significant decrease in the serum levels of CA125, CEA, LDH, and ALP in the treated group as compared to with the induced group. CA125 and CEA are tumor markers (Fig. 5). Their levels are usually low in the blood of an average individual but elevated to about 80 % and 40 %, respectively, in metastatic breast cancer [47]. CEA is a glycoprotein with a molecular weight of about 200 kDa, a proven prognostic marker in differentiating between benign and invasive carcinomas [48]. Some researchers claim that CEA is the most sensitive indicator of metastatic liver disease [49,50]. CA125 is a transmembrane glycoprotein that effectively modulates cell adhesion, protein-protein interaction, and immunity. High expression of CA125 plays an essential role in malignant transformation and carcinogenesis [51]. It has been reported that CA 125 mediates heterotypic cell adhesion necessary for invasive metastasis [52]. The result suggests that *Caesalpinia pulcherrima* contains some phytochemicals capable of mitigating or inhibiting the synthesis of cell adhesive and metastatic apparatus. The marked increase in the serum level of CA125 shows that it might be the best indicator of a breast cancer diagnosis.

Lactate dehydrogenase is a cytoplasmic enzyme in almost all tissues but in high muscle, Liver, and kidney. Red blood cells also contain a moderate concentration of this enzyme, an essential enzyme of the anaerobic metabolic pathway. It catalyzes the reversible conversion of lactate to pyruvate with concomitant reduction of NAD^+ to NADH [53]. The elevated Serum level of this enzyme following the intraperitoneal injection of DMBA suggests a high dependence on the anaerobic pathway for ATP production by the cancer cells. The marked increase confirms the energy requirement of the cancer cell to proliferate [54,55]. Elevated serum activities of alkaline phosphatase and transferases have been reported in liver diseases such as acute hepatitis, cancers, and jaundice [56]. The Liver synthesizes protein and total bilirubin, and these molecules' serum levels may correlate with the Liver's metabolic status and the individual's nutritional status. They are used to ascertain the synthetic functions of the Liver [56].

An alkaline phosphatase is a group of isozymes located on the outer layer of the cell membrane; they catalyze the hydrolysis of organic phosphate esters present in the extracellular space [57]. Intraperitoneal administration of DMBA at a dose of 80 mg/kg body weight led to elevated serum activities of Alanine aminotransferase, Aspartate aminotransferase, and Alkaline phosphatase. In contrast, there was no significant change in the serum levels of protein and total bilirubin. The results suggest hepatocyte toxicity of DMBA.

DMBA – peroxidation of the plasma membrane and consequent outflow of these liver enzymes into the blood is well documented [58]. Administration of an aqueous extract of *Caesalpinia pulcherrima* for 28 days led to the reversal of these liver enzymes to normal
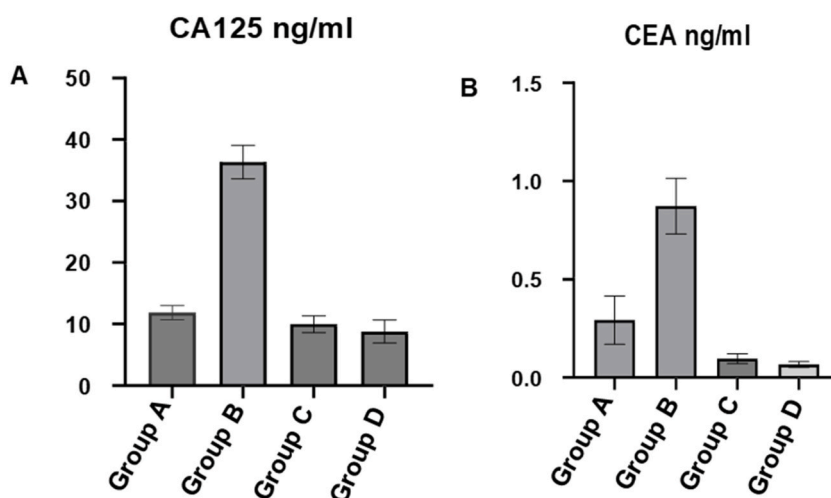

Fig. 2. Effect of aqueous extract of *Caesalpinia pulcherrima* on serum levels of CA125 (A) and CEA (B) in DMBA-induced mammary tumorigenesis in Female Albino Rats. Data are expressed as Mean ± standard error of mean (SEM) n = 7. a$p < 0.05$ as compared to the normal group (Group A), and b$p < 0.05$ as compared to the induced group (Group B).
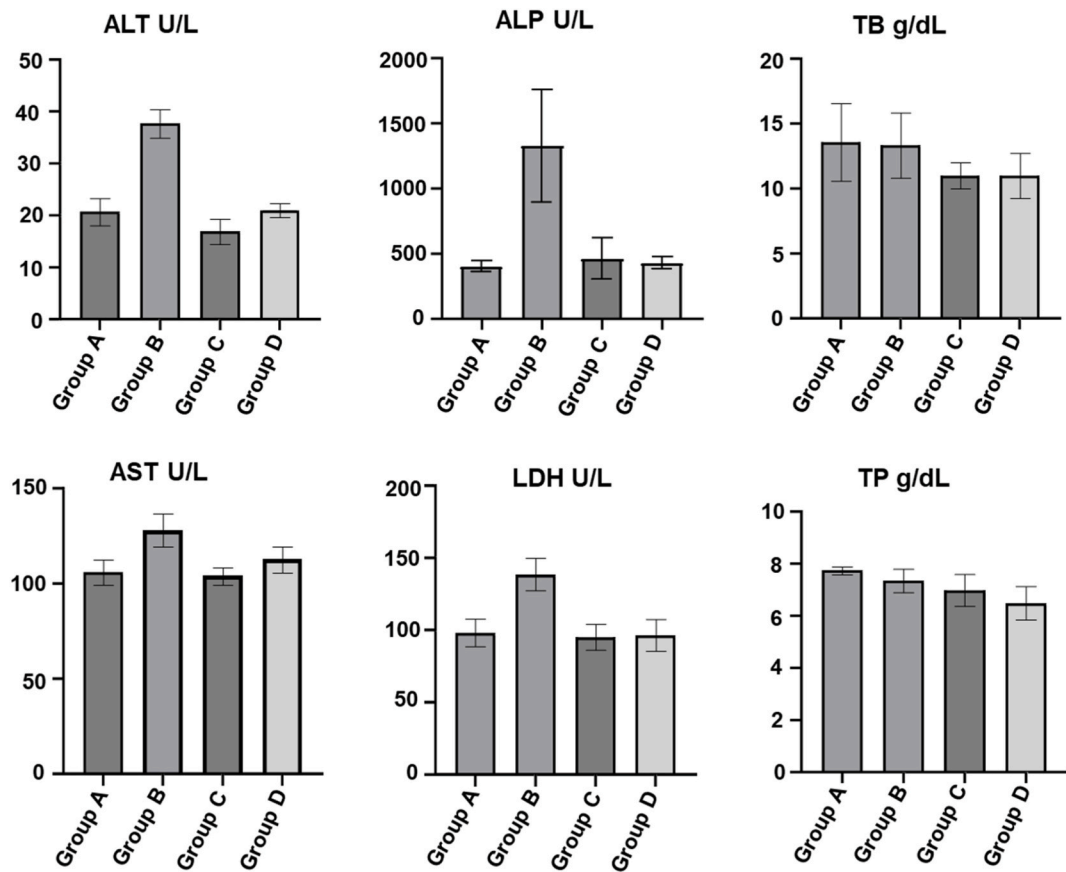
Fig. 3. Effect of aqueous extract of *Caesalpinia pulcherrima* on serum levels of ALT, AST, ALP, LDH, TB, and TP in DMBA-induced mammary tumorigenesis in Female Albino Rats. Data are expressed as Mean ± SEM n = 4 *ap < 0.05* as compared to the normal group (Group A) *bp < 0.05* as compared to the induced group (Group B).
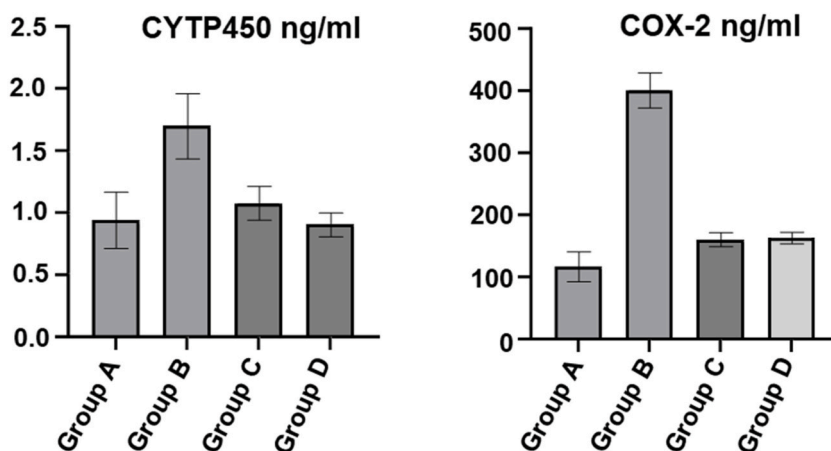

Fig. 4. Effect of aqueous extract of *Caesalpinia pulcherrima* on serum level of CYTOP450 and COX-2 in DMBA – induced mammary tumorigenesis in Female Albino Rats. Data are expressed as Mean ± SEM n = 4. *ap < 0.05* as compared to the normal group (Group A). *bp < 0.05* as compared to the induced group (Group B).

levels. There were no significant changes in the serum levels of total protein and total bilirubin following the administration of DMBA, and even with treatment with *Caesalpinia pulcherrima* indicating that the synthetic function of the Liver was not affected following the administration of DMBA contrary to those reported by Refs. [59,60]. The hepatocyte curative effect of *Caesalpinia pulcherrima* might be
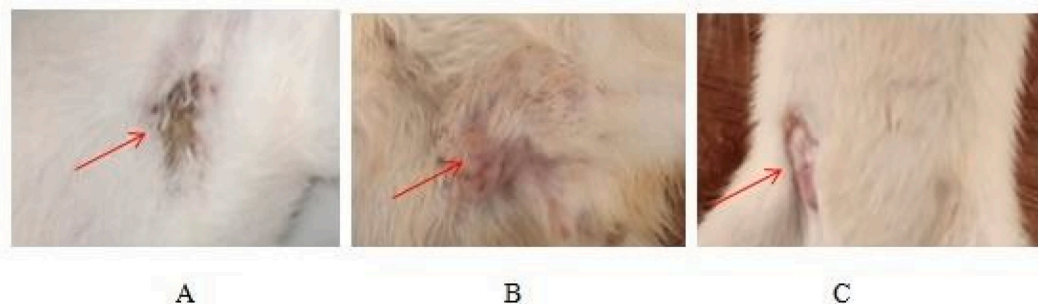
Fig. 5. A; An image of a rat induced with tumor via single intraperitoneal injection of DMBA. B; An image of a rat induced with tumor via single intraperitoneal injection of DMBA and treated with cyclophosphamide. C; An image of a rat induced with tumor via a single intraperitoneal injection of DMBA and treated with an aqueous extract of *Caesalpinia pulcherrima*.

attributed to its ability to either induce phase II metabolizing enzymes or inhibits the induction of phase I metabolizing enzymes, thereby limiting the formation of DMBA reactive intermediates.

In this study, intraperitoneal administration of DMBA to rats at a dose of 80 mg/kg was also accompanied by a significant increase in the cytop450 and Cox-2, but administration of *Caesalpinia pulcherrima* at the dose of 250 mg/kg body weight for 28 days resulted in the restoration of these enzymes to near normal serum levels. The elevated level of cytop450 upon administration of DMBA might be due to the upregulation of the cytop450 gene. Cytop450 is a phase I metabolizing enzyme that introduces a reactive group to xeno-biotics [61]. The conversion of arachidonic acid (AA) to prostaglandins (PGS) is achieved by the action of this COX-2. COX-2 controls inflammation and immune responses [62]. Upregulation of COX-2 has been reported to restrain apoptosis and induces metastasis in some tumor cells [62]. Numerous studies have shown that various resveratrol, fisetin, and β-carotene are potent inhibitors of COX-2 [63], and these phytochemicals have been used to target COX-2 in human ovarian cancer cells (OVCAR-3) and human colon cancer cells [63,64].

The tumor-promoting effect of DMBA was further confirmed by histological examination of a cross-section of the breast tissues. The micrograph of the DMBA-treated group shows circumscribed but focally infiltrative lesions composed of fibrous cells arranged in fascicles. Invasion of skeletal muscle fibers and surrounding breast tissue was seen. The inflammatory cells predominantly comprising lymphocytes signify inflammation due to invasion. In contrast, the breast tissue micrograph for normal rat and *Caesalpinia pulcherrima* treated breast tissues shows unremarkable tissue. There were visible tumors in the group induced with DMBA (Fig. 6). In contrast, the tumor that had developed in the other groups prior to treatments were externalized and reduced, confirming the anticancer activity of the *Caesalpinia pulcherrima*. The limitation of this study is that we did not identify the specific anticancer component of the plant extract. Further studies should be carried out to identify the specific anticancer components of *Caesalpinia pulcherrima* with a view to evaluating their structure-function relationships, as well as their mechanisms of action (Fig. 7).

5. Conclusion

Caesalpinia pulcherrima exhibits the capacity to lower the serum levels of cytP450, COX2, CEA, and CA125. Its anti-tumor or anti-cancer potential stems from its ability to cause the shrinkage of tumors in the breast tissues of the experimental rats.

Funding

This research was self-funded by the authors.

Availability of data and materials

Not Applicable.

Ethics statement

The ethical approval for this research was obtained from the Federal University of Lafia, and Ahmadu Bello University Ethics Committee on the guidelines for experiment with whole animals (Ethical Code: ABUCAUC/2018/028).

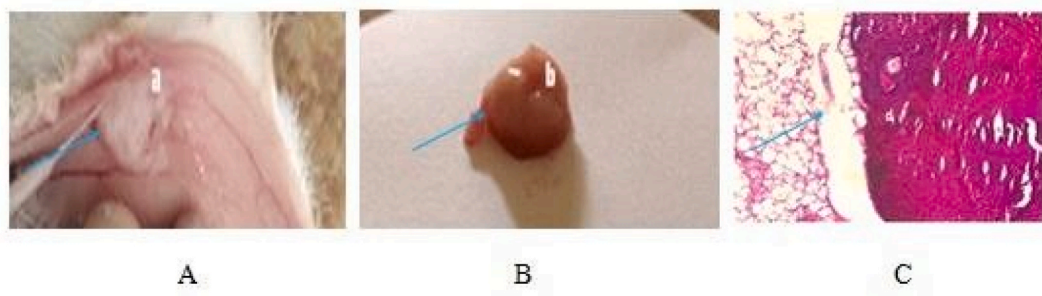## Consent to participate

Not Applicable.

Fig. 6. A; An image of a rat with a tumor developing in the epithelial cells of the mammary gland. B; image of tumor isolated from the rat injected with DMBA but left untreated; C; photomicrograph of the isolated tumor.
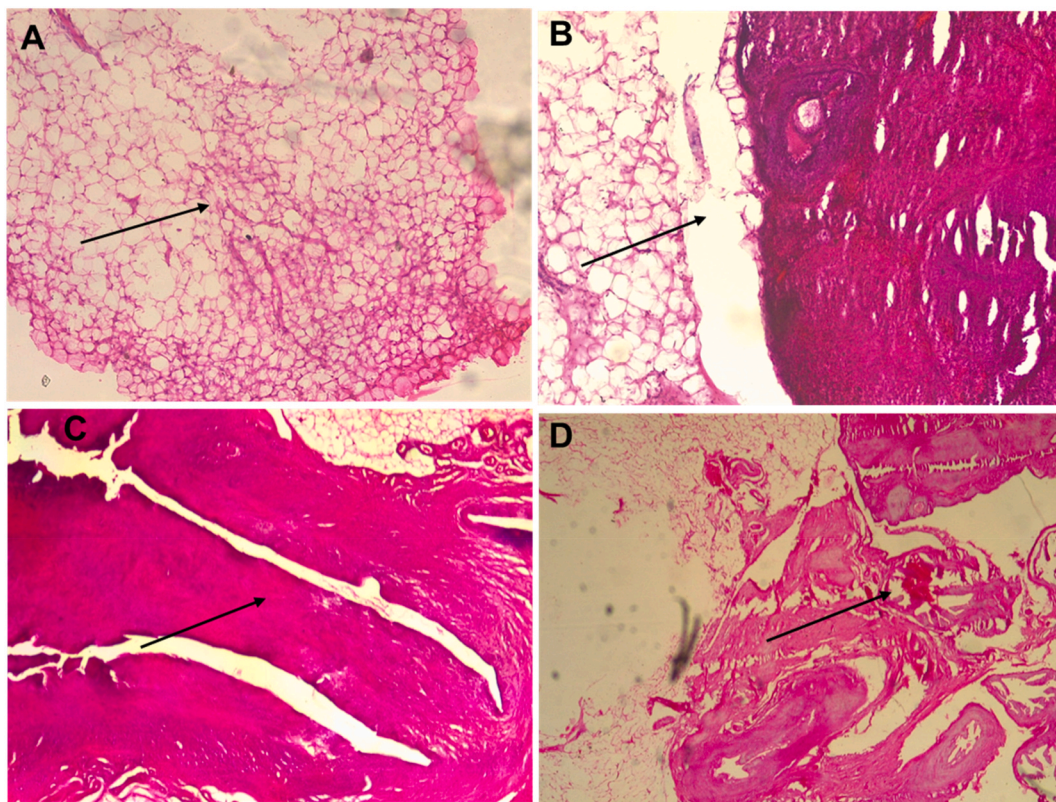

Fig. 7. Photomicrographs of the breast tissues. A: Photomicrograph of the breast tissue of a normal rat showing unremarkable breast and adipose tissue. B: Photomicrograph of the breast tissue of a rat injected with DMBA but left untreated showing papillary projection and distended breast tubule. C: Photomicrograph of the breast tissue of a rat injected with DMBA and treated with cyclophosphamide showing unremarkable breast and adipose tissues. D: Photomicrograph of the rat injected with DMBA and treated with aqueous extract of *Caesalpinia pulcherrima* showing unremarkable breast and adipose tissue.

Consent for publication

Not Applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Abbreviations

CEA   carcinoembryonic Antigen
CA125   antigen 125
DMBA   7,12-Dimethylbenz[a]anthracene
COX-2   cyclooxygenase-2
cytp450   cytochrome p450 oxidase
ELISA   Enzyme Linked immunosorbent assay
LDH   lactate dehydrogenase
ALP   alkaline phosphatase
AST   aspartate amino transferase
ALT   alanine amino transferase
TP   total protein
TB   total bilirubin
PAHs   polycyclic aromatic hydrocarbons
AhR   aryl hydrocarbon receptor
ARNT   aryl hydrocarbon receptor Nuclear translocator
P53   tumor protein p53
P21   cyclin – dependent kinase – interacting protein 1 P21
P27   cyclin –dependent kinase inhibitor 1B
Bcl2   B-cell lymphoma-2
CDKs   cyclins and cyclin-dependent kinases
NF-kB   nuclear factor – kappa B
erbB2   Receptor tyrosine protein kinase 2
AOT   Acute oral toxicity
SOD   sample optical density
STOD   Standard optical density
SEM   standard error of mean
ATP   adenosine triphosphate
NAD+   nicotinamide adenine dinucleotide oxidize form
NADH   nicotinamide adenine dinucleotide reduced form
OVCAR- 3   human ovarian cancer cells 3

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