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Anti-Tumor Activities of *Allium Sativum* on Serum IL-33, TNF-α and Breast Tissues in Cancer-Induced Wister Albino Rats

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Abstract

Background: *Breast cancer is an uncontrolled growth of breast tissue, resulting from mutations in genes regulating the growth of cells. It's the most common neoplastic disease in female. Despite the cost in anti-cancer drugs productions, most drugs have not yielded the desired results.*

Aims/Objective: *This research is aimed to investigate the effects ofAllium Sativum treatment, on some serum cytokines, in cancer-induced female albino rats.*

Materials/Methods: *Fresh Allium sativum was subjected to plant identification/authentication, extraction sand cytotoxicity testing. Twelve rats(12) were used in the determination of lethal dose, using lorke's method. The total of 25 rats were divided into 5 groups of 5 rats each. The groups were normal and positive controls, Acetylsalisalic acids, Preventive, and Treated group. With the exception of Group 1, all the groups were inducted with 65 mg/kg-1b.w. of 7,12 Dimethyl benzene-(a) anthracene (DMBA)and observed for 14 days, before treatment with 500mg of ASA (III), and A. sativum(IV,V). Therats were sacrificed, 24hrs after the last treatment, the blood and mammary glands were collected for ElISA and histological analysis.*

Results: *The extracts down-regulated serum IL-33, and TNF-α expressions as well as restoring the normal architecture of the mammary gland. Therefore, A. sativum may serve as a better preventive and therapeutic options in the management of Breast cancer.*

Conclusion: *The present study indicated that the ethanolic extract A. sativum posses an anti-tumour and immunomodulatory effects, so it's recommended that clinical trials should be undertaken to see if the result could be replicated in human.*

Keywords: *Allium Sativum, IL-33, TNF-α, Cytokine,and Breast Cancer*

Introduction

Cancer is a group of diseases involving abnormal cell growth with the potential to invade to other parts of the body. Currently, one in six deaths are due to cancer worldwide and around 70% of deaths from cancer occur in low and middle income countries[1]. These makes cancer an important health problem which requires effective prevention and treatment measures[2].

Breast cancer is an uncontrolled cell proliferation which leads to tumor formation and the development of a multifactorial disease. In breast cancer, tumors may grow in different parts of breast, such as lobules, ducts, and connective tissue, however, the hallmark of cancer pathology is not the tumor itself, but the migration of transformed cells to different tissues[3].

Cancer ranked the 7th leading cause of death in Africa in 2004, with an expected annual incidence of 1.28 million cases and 970,000 deaths by 2030[4]. In breast cancer, a patient with a primary tumor has a 5-year survival rate of 99%, whereas when metastatic tumors are present, this rate decreases to up to 23% [5]. Pregnancy reduces breast cancer risk, through breast cell maturation and diminishing the estrogen exposure during gestation[6]. Breast cancer occur in women and rarely in men, symptoms include a lump in the breast, bloody discharge from the nipple and changes in the shape or texture of the nipple or breast[7]. The fundamental issue in breast cancer control is prevention, which depends on identification of the determinants of the disease, in terms of initiation and promotion. The possibility of using biomodulators of breast carcinogenesis, such as selective estrogen receptor modulators (SERMS), aromatase and cyclooxygenase inhibitors, and dietary factors is very promising[8].

Mammary glands of several rat strains, mainly Sprague-Dawley and Wistar-Furth, are susceptible to transformation induced by chemical carcinogens, and the two most widely used active chemical inductors of mammary carcinogenesis are 7,12dimethylbenz(a) anthracene (DMBA) and N-methylnitrosurea[9]. Over the past two decades, there has been a slight improvement in cancer statistics due to diagnostic and therapeutic progresses and a better understanding of tumor biology[10]. However, cancer remains associated with very high mortality rates, which indicate still existing difficulties of effective treatment. The role of herbal medicine in the treatment of many diseases was proven[11], Medicinal plants constitute an effective source of both traditional and modern medicine[12].

Allium sativum commonly known as Garlic, is a member of the family Amaryliaceae and a species in the onion genus. It is a bulbous plant with hermaphrodite flowers growing up to 1.2m in height from a single underground bulb,the plant divides forming in time a cluster of plants. It is grown in temperate and tropical regions of the world[13,14]. The importance of garlic (*Allium sativum*) was recognized as a cure of variety of diseases including acute and chronic infections, gastritis, dysentery, typhoid fever, cholera, tuberculosis, pneumonia, diabetes mellitus, heart diseases, hypertension and cancer[15]. Laboratory investigations have provided adequate evidence that garlic-containing substances inhibits a variety of chemically-induced tumors/cancer in animals[16]. The anticancer effects of garlic are linked to several garlic-derived bioactive compounds, including saponins, terpenoids, garlic oils and Sulphur compounds such as alliin. Animals and *in vitro* studies have demonstrated that garlic bioactive compounds may prevent cancer through mechanisms such as inducing cell cycle arrest, promoting apoptosis, and inhibiting angiogenesis in different cancer cells[17].

Cytokines are low molecular weight regulatory proteins or glycoprotein that modulates the intensity and duration of immune response by stimulating or inhibiting the activation, proliferation, and/or differentiation of target cells[18]. Cytokines may include chemokines, interferons, interleukines, lymphokines and tumournecrosis factor. Different cytokines are known to have diverse role in breast cancer initiation and progressionas well as regression, the irregular cytokines levels can shift the immune responses from being beneficial to being harmful[19].

The aim of this study is to investigate the preventive and therapeutic activities of ethanolic extracts of *Allium sativum* on serum interleukin IL-33,TNF alpha and breast tissues in 7, 12 dimethyl benzene (a) anthracene (DMBA)-induced breast tumor in female albino rats.

Materials and Methods

Plant Collection and Identification

Fresh *Allium sativum* were purchased from Sokoto central market, Sokoto-Nigeria and was identified and authenticated at the herbarium Department of Pharmacognocy, Usmanu Danfodiyo University, where a voucher number was allocated (PCG/UDUS/AMAR/0001) and a voucher specimen was kept in the herbarium.

Preparation of Extract

All reagents use for the study were of an analytical grade. Ethanol used for extraction and phytochemical reagents were purchased from Science Technology CO., LTD. China.

Plant Extraction

The raw *Allium sativum* bulbs were peeled off to obtain garlic cloves, the cloves were crushed using kitchen manual blender. The extraction was obtained by soaking 876.133g in 1100.00ml of ethanol for 24-hrs at room temperature. The residue and the filtrate were obtained by filtering the soaked *Allium sativum* using separating funnel. The residue was dried on a cardboard paper and the filtrate was obtained as extract. Concentration of the extract was obtained by putting in to a thermostat oven at 50-60^{o}C for some days. Following evaporation, 7.31g volume of extract was obtained.

Experimental Animals

A total of 40 female Wistar Albino rats weighing approximately 100-140g aged 8 - 10 weeks old, were purchased from the Veterinary Department of Ahmadu Bello University (ABU), Zaria. They were housed in a well-ventilated cages under hygienic condition in animal house, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto (UDUS), in an environment of ambient temperature (25 ± 5^{0}C), humidity (55 ± 5%) and the lighting period of about 12 hours daily. They were fed with standard commercial pelletized grower's feed (Vital® feed, Jos, Nigeria) obtained from Grand Cereal Soil Mills Limited, Jos, Nigeria. They were allowed free access to clean drinking water ad libitum throughout the experimental period. They were kept in cages to acclimatize for a period of two weeks before commencing the experiment. Cleaning of the animal cages was carried out on daily and regular basis.

Ethical Statement

The ethical approval for the research was sort and obtained from the UDUS Research and ethical committee. This study was conducted in accordance with the Helsinki guide for the care and use of laboratory animals.

Experimental Design

The total of twenty five (25) rats were randomly divided into 5 groups of 5 rats each, the design is as follows:

Group I (n=5): Normal controls, rats were fed orally with Normal saline till the end of experiment.

Group II (n=5): Positive controls, rats were inducted with (65 mg/kg body weight) 7,12 - Dimethylbenzene (a) anthracene (DMBA) once and fed orally with normal saline till the end of experiment (28 days).

Group III (n=5): Acetylsalicylic (ASA) acid group, rats were inducted with single dose (65 mg/kg b.w.) of DMBA and observed for 14days, this was followed by an oral administration of acetylsalicylic (ASA) acid (600mg/kg/day) for 14 days.

Group IV (n=5): Preventive group, rats were treated with single oral dose (500mg/kg/day) ethanolic extract of *Allium sativum* for 14 days prior to DMBA induction (65 mg/kg b.w.) for another 14days.

Group V (n=5): Treated group, rats were inducted with single dose (65 mg/kg b.w.) DMBA and observe for 14 days this was followed by oral administration (500 mg/kg/day) ethanolic extract of *Allium sativum* for 14 days.

Determination of Lethal Dose (LD_{50})

Determination of lethal dose was carried out as describe by lorke[20]. Using Phase 1 and Phase 2. Twelve rats were used for the experiment.

Phase I: This phase requires nine animals, the rats were randomly divided into three (3) groups of three (3) rats each. Each group was administered with different doses (10, 100 and 1000 mg/kg B.W.) of test extracts (*A. sativum*) and placed under observation for 24 hours to monitor signs of behaviorial changes and mortality.

Phase II: This Phase requires three animals and the rats were randomly divided into three groups of one rat each. The rats were administered with higher doses (1600, 2900 and 5000 mg/kg B.W) of test extracts (*A. sativum*) using oral cannula. Observation of toxic symptoms and mortality were made and recorded within the first hour, four hours, twelve hours and

subsequently for 24 hours after the administration of the extract (*A. sativum*). Behavioral parameters and mortality were monitored.

Induction of Mammary Tumor

A single dose of dimethylbenzene (a) anthracene (DMBA) 65 mg/kg body weight was injected once into the rat mammary glands subcutaneously. The rats were observed for the development of breast tumor after 14days of induction.

Laboratory Analysis

Blood Sample Collection and Processing

The blood samples were collected 24 Hours after the last treatments, in a sterile plain and EDTA bottles. The samples collected in to a plain tube was allowed to clot at room temperature and later centrifuged at 3000g for 5 minutes. The clear unhaemolysed sera was transferred into labelled sterile serum bottles tightly caped and stored at -20◦C until use.

Tissue Processing

The animals were sacrificed immediately after blood collection by cervical dislocation. The breast tissues was excised, washed with normal saline and dried with tissue paper. The tissues were weighed using sensitive electronic compact balance and transferred to a well labelled wide mouth container of 10% formalin and stored appropriately until use. The carcass remnants of the animals were disposed of in a biohazard bag for incineration.

Analytical Methods

Estimation of Serum Cytokines Concentrations

Serum Cytokines Concentrations (IL-33, and TNF-α,) were measured using Sandwich-ELISA Technique (using test kit procured from Sunlong Biotech. CO., LTD China). The procedure was carried out with strict adherence to the manufacturer's instructional manual.

Histological Examination

Mammary gland

Tissue samples of mammary gland were manually processed at Histology laboratory of Faculty of medicine, Department of Anatomy, Ahmadu Bello University, Zaria (ABU), using Automatic tissue processor.

Data Analysis

Multiple comparisons of the mean value was carried out using one way analysis of variance (ANOVA), using SPSS software version 23.0. The results were presented using tables and figures.

Data Availability statement

Data available on request from the authors:The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Presentation of Results

Phase I Lethal dose (LD_{50}) study

The lethal dose study of ethanolic extract of *A. Sativum* phase 1 is presented in Table I, in this table, the lethal studies shows 9 rats distributed in to 3 groups of 3rats each, with 10mg,100mg and 1000mg doses of ethanolic extract *A. Sativum* respectively with no recorded abnormal behavioural changes from each.

TABLE I. LETHAL DOSE (LD_{50}) STUDY OF ETHANOLIC EXTRACT OF *A. Sativum* (PHASE I)

Groups	No	Dosage (mg/kg b.w)	Observational period (behavioural changes (24 hrs).
1	3	10mg	0/3
2	3	100mg	0/3
3	3	1000mg	0/3

Key: no. =Number of rats, hrs = Hours, mg/kg. b.w = milligram per kilogram body weight, 0/3 = none of the animal died out of the three Albino rats in a group.

Phase II Lethal dose (LD_{50}) study

The lethal dose study of ethanolic extract of *A. Sativum* phase 2 is presented in Table II. In this Table, the lethal studies shows 9 rats distributed in to 3 groups of 1rat each, with 1600mg, 2900mg and 5000mg doses of ethanolic extract *A. Sativum* respectively with no recorded abnormal behavioural changes from each group.

TABLE II. LETHAL DOSE (LD_{50}) STUDY OF ETHANOLIC EXTRACT OF *A. Sativum* phase II

Groups	No	Dosage (mg/kg b.w)	Observational period (Behavioural changes 24 hrs).
1	1	1600mg	0/1
2	1	2900mg	0/1
3	1	5000mg	0/1

Key: no. =Number of animal, hrs = Hours, mg/kg.b.w = milligram per kilogram body weight, 0/1 = none of the animal died out of the three Albino rats in a group.

Effect of ethanolic extract of A. Sativum on serum IL-33

The effect of ethanolic extract of *A. Sativum* on serum IL-33 concentrationin (DMBA)-induced breast cancer in female wistar albino rats is presented in Table III. In this Table, five groups of 5 rats each shows the doses of DMBA, extract and ASA (mg/kg B.W) with the concentration of serum IL-33 (pg/ml) respectively. In the Table, the F-value and the P- value is also presented.

TABLE III. EFFECT OF ETHANOLIC EXTRACT OF *A. Sativum* ON SERUM IL-33

Groups	N	Dose (mg/kg b.w)	Il-33 (pg/ml)
1	5	H_2O only	7.92 ± 0.16
2	5	65mg/kg DMBA	10.67 ± 0.32^{c}
3	5	65mg/kg+500mg/kg ASA	$8.99 \pm 0.30^{a, c}$
4	5	500mg/kg extract+ 65mg/kg	8.60 ± 0.18^{c}
5	5	65mg/kg DMBA + 500mg/kg	$8.88 \pm 0.16^{c, b}$
P value	0.00		
F value	19.77		

Values expressed as mean ± SEM, n= number of rats, mg/kg = milligram per kilogram, b.w = body weight, DMBA = Dimethylbenzene (a) anthracene, ASA = acetylsalicylic acid, pg/ml = pictogram per mil. Values with superscript differ significantly at $^{a}p \leq 0.05$ and $^{b}p \leq 0.01$ and $^{c}p \leq 0.001$using Turkey's HSD Post Hoc Multiple Comparison Test.

Effect of ethanolic extract of A. Sativum on serum TNF-α

Theeffect of ethanolic extract of *A. Sativum* on serum TNF–*α* concentrationin (DMBA)-induced breast tumor/cancer in female wistar albino rats is presented in Table IV. In this Table, five groups of 5 rats each shows the doses of DMBA, extract and ASA (mg/kg B.W) with the concentration of serum TNF-*α* (pg/ml) respectively. In the Table, the F-value and the P- value is also presented.

TABLE IV. EFFECT OF ETHANOLIC EXTRACT OF *A. Sativum* ON SERUM TNF-a

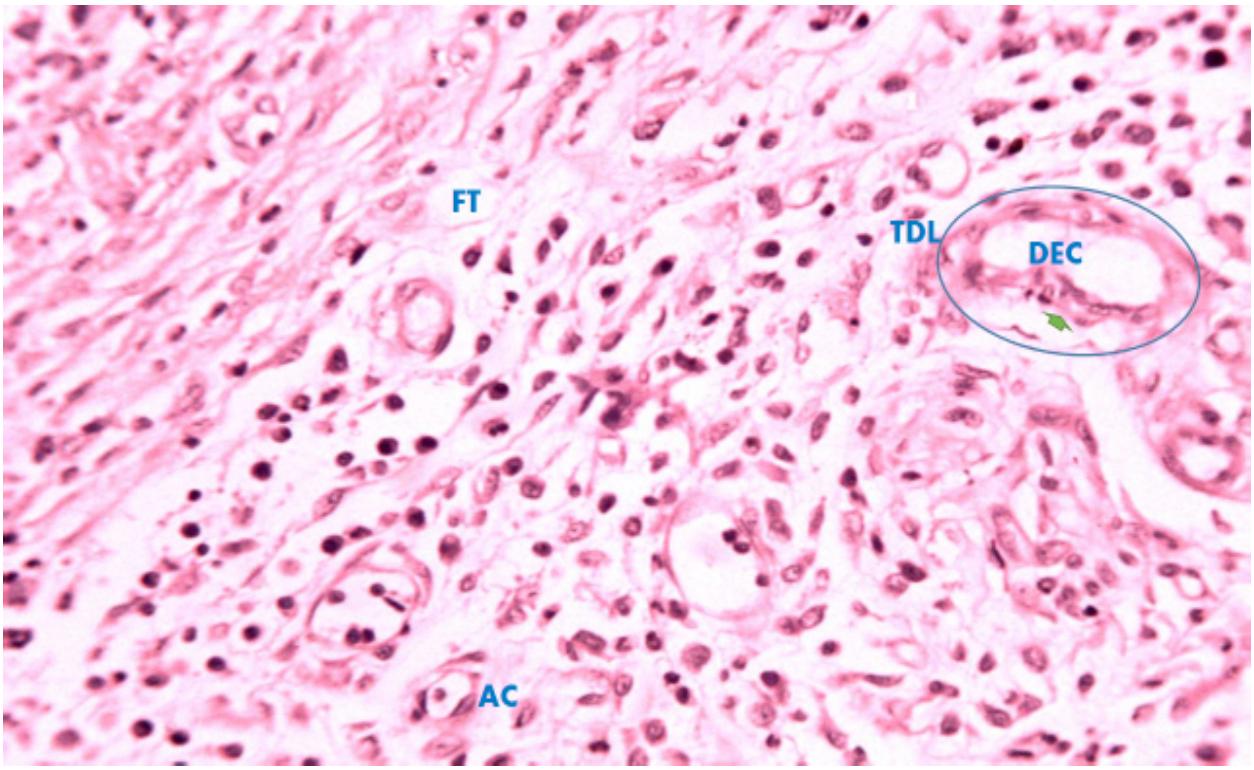
Groups	N	Dose (mg/kg b.w)	TNF- α (pg/ml)
1	5	H_2O only	20.60 ± 0.20
2	5	65mg/kg DMBA	26.16 ± 0.33^{c}
3	5	65mg/kg+500mg/kg ASA	24.60 ± 0.30^{c}
4	5	500mg/kg extract+ 65mg/kg	$22.80 \pm 0.57^{a,b,}$
5	5	65mg/kg DMBA + 500mg/kg	$23.42 \pm 0.43^{a, c}$
P value	0.00		
F value	29.32		

Values expressed as mean ± SEM, n= number of rats, mg/kg = milligram per kilogram, b.w = body weight, DMBA = Dimethylbenzene (a) anthracene, ASA = acetylsalicylic acid, pg/ml = pictogram per mil. Values with superscript differ significantly at $^{a}p \leq 0.05$ and $^{b}p \leq 0.01$ and $^{c}p \leq 0.001$using Turkey's HSD Post Hoc Multiple Comparison Test.

Histological Evaluation

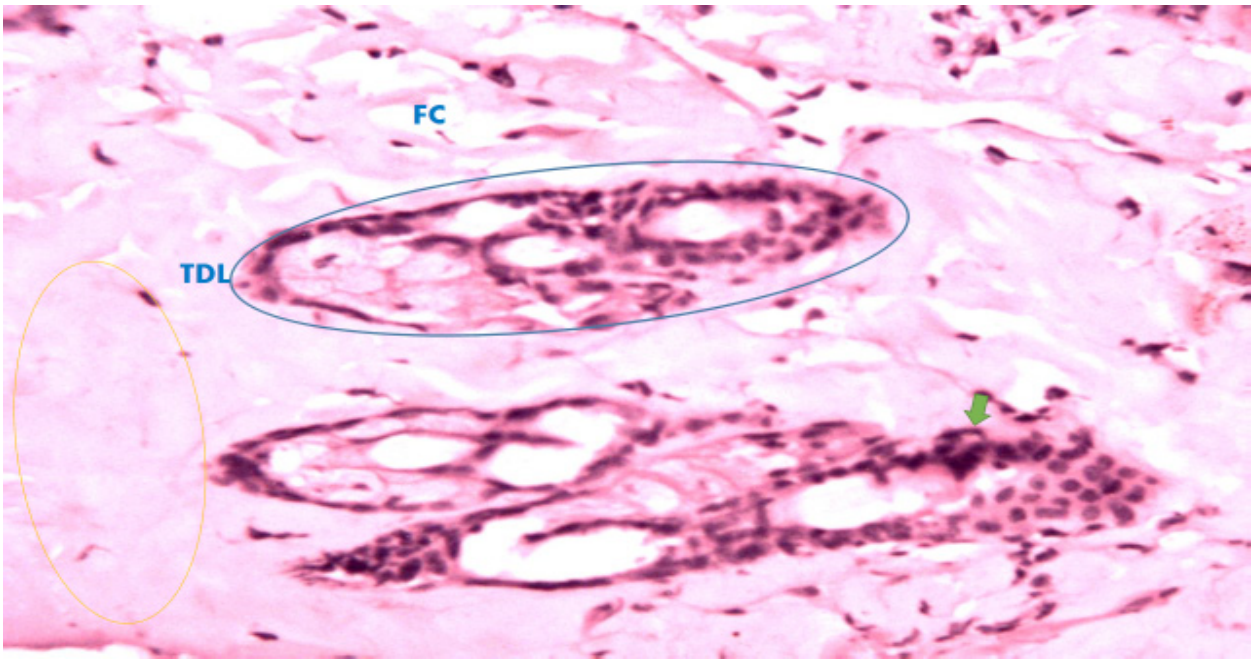
Effects of ethanolic extract of A. Sativum on breast tissue (H&E x250)

Photomicrograph of mammary gland (Fig.I) from Group I (control group), representing healthy group of experimental animal reveals normal histological architecture of terminal duct-lobule unit and fatty tissue each terminal duct-lobule contain terminal ducts composed of ductal epithelial cells surrounded by thick basement membrane. The acini were lined by a single layer of cells with occasional myoepithelial cells. Mammary gland from Group II (positive control) (Fig.II), representing the cancerous control of experimental animals inducted with (65mg/kg b.w) of DMBA shows histopathological changes in the breast tissue. The terminal duct-lobule appeared dilated with degenerative atypical cells of the ductal epithelium while some of the cells shows the appearance of fibro adenocarcinoma. Mammary gland from Group III treated with (ASA) after induction with (65mg/kg b.w) of DMBA(Fig.III), shows terminal duct-lobule (TDL) units and fatty tissue with infiltrative and proliferative cells in a mixed with normal and some disorderly pattern. Mammary gland from Group IV representing the preventive control of experimental animal treated (500mg/kg/day) of ethanolic extract of *Allium sativum* before induction of DMBA (65mg/kg b.w)(FIg.IV), shows mild histopathological changes in the breast tissue. The terminal duct-lobule, acini cells, fatty tissues and ductal epithelium appeared relatively normal. Mammary gland from Group V administered (500mg/kg/day) after treated with (65mg/kg b.w) DMBA (Fig.V), shows a well-circumscribed nodular aggregation of terminal duct-lobule units. The fatty tissue (FC) and the acini appears normal. H&E x250



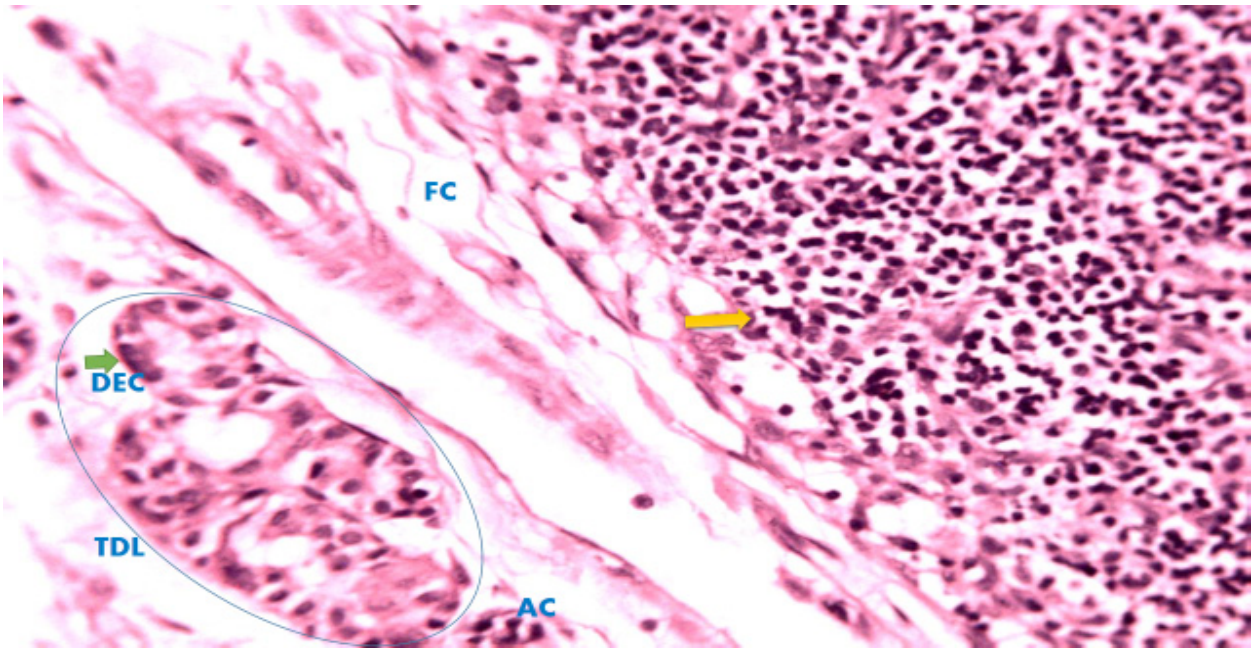
FigI. *Photomicrograph of the mammary gland. Group I (H&E x250)*

The Photomicrograph of mammary gland from Group I (control group), presenting the negative control rats, animals fed orally with distilled water only till termination of practical period (28 days) showing normal histological architecture of terminal duct-lobule (TDL) (blue circle) units and fatty tissue (FT); each TDL unit contain terminal ducts composed of ductal epithelial cells (DEC) surrounded by thick basement membrane (green arrow head). The acini (AC) were lined by a single layer of cells with occasional myoepithelial cells.



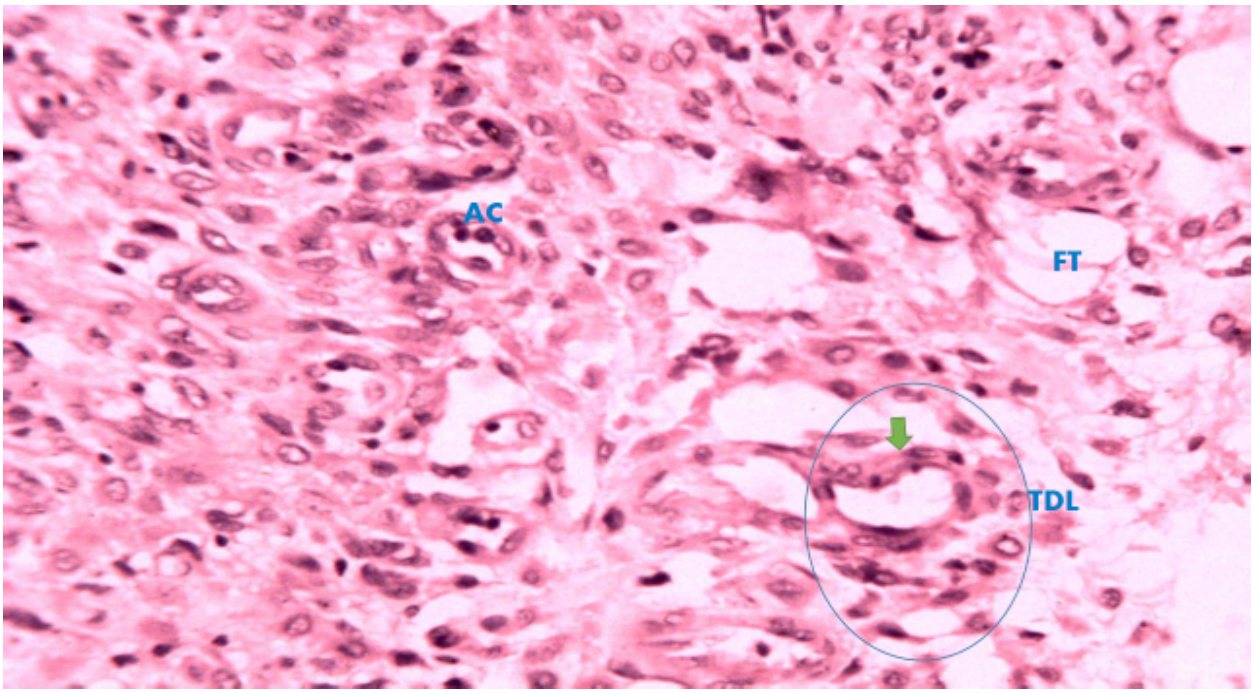
FigII. *Photomicrograph of the mammary gland. Group II (H&E x250)*

Photomicrograph of mammary gland from Group II representing positive control, treated with (65mg/kg b.w) DMBA for 28days shows histopathological changes in the breast tissue. The terminal duct-lobule (TDL) appeared dilated with degenerative atypical cells of the ductal epithelium and the acini (blue circle and green arrow). Some of the cells in the yellow circle presents an appearance of fibro adenocarcinoma.



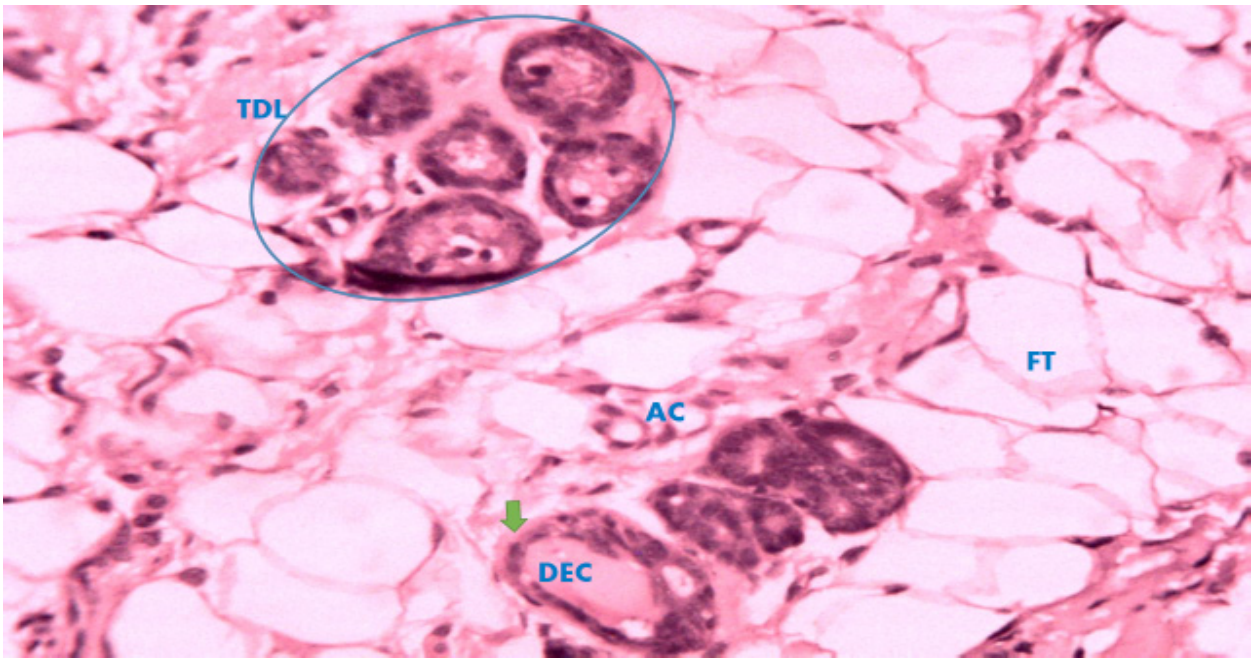
FigIII. *Photomicrograph of the mammary gland. Group III (H&E x250)*

Photomicrograph of mammary gland from Group III treated with (ASA) after induction with (65mg/kg b.w) of DMBA for 14days, shows terminal duct-lobule (TDL) units (green arrow in blue circle) and fatty tissue (FC); the presents infiltrative and proliferative cells in a mixed with normal and some disorderly pattern (yellow arrow).



FigIV. *Photomicrograph of the mammary gland. Group IV (H&E x250)*

Photomicrograph of mammary gland from Group IV treated (500mg/kg/day) of ethanolic extract of *A. sativum* before induction of DMBA (65mg/kg b.w) showing mild histopathological changes in the breast tissue. The terminal duct-lobule (TDL), acini cells (AC), fatty tissues (FT) and ductal epithelium (green arrow) appeared relatively normal.



FigV. *Photomicrograph of the mammary gland. Group V (H&E x250)*

Photomicrograph of mammary gland from Group V treated with (500mg/kg/day) of ethanolic extract of *A. sativum* after induction with (65mg/kg b.w) DMBA, showing a well-circumscribed nodular aggregation of terminal duct-lobule (TDL) units (blue circle). The fatty tissue (FC) and the acini appears normal.

Discussion

Determination of lethal dose (LD_{50}) was carried out as describe by lorke[20]. From this study, no death or behavioural changes were observed in the entire group, both in phase I and II. All the rats appeared normal and none of them showed any visible signs of toxicity with all the dose of extract administered. This is an indication that LD_{50} of ethanolic extracts of *Allium sativum* is practically safe and non-toxic(Table I, II). This findings is in agreement with a study by Moses *et al* (2017)[21], where 21 albino mice were used (3 mice per dosage) for the experiment. First administered dosage was 400 mg/kg, and was steadily increased by 500 mg/kg after 2 days.The highest administered dosage was 700 mg/kg [21]. In another study, it was shown that *Allium sativum* extracts administered daily for the period of 38days, was found to be safe and non toxic, using Wistar albino rats strain[22].

In this study, Mammary gland assessment, at7 days after DMBA induction revealed that two or more rats developed at least 1 mammary tumor (80%), while at 14th day, all rats developed one or two tumor at the mammary glands (100%). This finding is in agreement with previous studies by Moayad *et al.,(*2006)[23]. Twenty young virgin Sprague-Dawley female rats, aged 47 days, received 20mg of 7,12-dimethylbenzene-(a)anthracene (DMBA) intragastrically by gavage. Eight weeks after DMBA injection, 16 rats presented at least 1 breast tumor (80%). After 13 weeks, all of them (100%) developed breast carcinomasthat were confirmed by histopathological analysis. In group IV, which was first treated with *A. sativum* prior to DMBA induction, only few rats shows signs of tumor initiation, this could be due to the preventive effects of phytochemical constituents present in *A. Sativum.* This finding is in agreement with a previous study by Moayad *et al.,* (2006)[24] designed to investigate the pre and post chemoprotection of garlic on DMBA induced mammary cancer in female albino rats. The result of these findings indicate that A. sativum posses both preventive and therapeutic effects on tumor-induced experimental rats[23]

Table III, represent the effects of ethanolic extracts of A. sativum on serum IL-33, the group 1 of this table is the normal control with 7.92 ± 0.16pg/ml of IL-33, when compared with the positive controls(group 2), there is an upregulations in the level of IL-33 (10.67±0.32). But after treatment of group 3 and 5 with 500mg/kg of acetylsalicyclic acid(ASA) and extract of A. sativum respectively, downregulations of IL-33 was observed in both groups($p < 0.001$), when compared to the positive control. *The chemoprotective activities of A. sativum was also demonstrated in group 4, where 500mg/kg of the extracts was infused into the rats before DMBA induction. The result from this group showed a much downregulation of the level of IL-33 (*8.60 ± 0.18), when compared to the positive control. This is agreement with the work of Ghanzanfariet et al.,(2012)[24], where similar concentrations of fresh garlic juice was used. From this study, the down regulation of IL-33 is in agreement with other studies which demonstrated the treatment of whole blood with 10 μg/L LPS led to a strong liberation of IL-33, IL-1β, (15.7 ± 5.1 μg/L) and TNF-α (8.8 ± 2.4 μg/L). Co-treatment with 100 mg/L garlic powder extract significantly reduced liberated IL-33 (6.2 ± 1.2 μg/L). Similarly, the amount of liberated IL-33 and TNF-α was significantly impaired (3.9 ± 0.8 μg/L) compared with human whole blood treated with LPS only (8.8 ± 2.4 μg/L)[25]

In this study, It was also found that the concentration of TNF–*α* differ significantly in treated group of ethanolic extract of *A. sativum* (500mg/kg) with ($p < 0.00$) compared with positive control group(Table IV). This is in agreement with a previous study conducted to investigate the possible therapeutic effects of garlic in the treatment of patients with IBD. A report in another literature noted association between garlic consumption and decreased incidence of distal colon cancer in women in a cohort study[26]. Among the test samples, the best result was obtained with ethanolic extract of *A.sativum* from treated group before DMBA induction at optimum dose of 500mg/kg/day was potentially effective as compared to inducted before treatment group as proven by multiple comparison with Poat Hoc of stoical analysis of variance ANOVA with ($p<0.01$). From our findings, it's practically proven that ethanolic extract of *A.sativum* posses both preventive, therapeuticas as well as immunodulating effects on inflammatory cytokines (IL-33, and TNF-α).

The Histological analysis of tissues (Mammary gland) was perfomed using Olympus binocular light microscope to observe for adenocarcinoma and

metastasis. In this study, Adenocarcinoma was found in all groups inducted with DMBA (II-IV), however, H&E stain sections of all tissue in negative control group shows normal histological features(Fig.I). In both treated (500mg extract *A. sativum*) before DMBA induction and treated after induction, all the tissue section presented a histological profile almost similar to the normal tissues(Fig.IV-V). The tissue sections from positive control group were histopathological confirmed as differentiated adenocarcinoma in these groups, the mammary gland of the positive control group shows the terminal duct-lobule,which appeares dilated with degenerative atypical cells of the ductal epithelium, while some of the cells shows the appearance of fibro adenocarcinoma(Fig.II), however the treated group before inductuion (500mg/kg/day + DMBA) shows mild histopathological changes in the breast tissue. The terminal duct-lobule, acini cells, fatty tissues and ductal epithelium appeared relatively normal(Fig.IV).While inducted group before treatment (DMBA+500mg/kg/day) shows a well-circumscribed nodular aggregation of terminal duct-lobule units, the fatty tissue (FC) and the acini appears normal(Fig.V). This findings is in agreement with a result from a previous study, where Garlic treatment alters the morphology and inhibits the growth of MCF7 cells[27]. Morphology of control and GE- or BGE-treated cells. MCF7 cells were exposed to garlic extracts for 3 hours, and pictures were taken at 20× magnification under light microscope. When MCF7 cells were exposed to fresh garlic extract (GE), within 1 hour, the cells began to alter their morphology[27]. After 2-4hours, GE-treated MCF7 cells became morphologically distinct, attained mesenchyme like phenotype, and lost cell-to-cell contact[27]. In another study, cancer cells were plated at 15% confluence and grew for 24h at 37°C before the juices (Raw garlic extract) were added to a 1/200 dilution factor of their respective wet weights (5mg/1ml). The cells were co-cultured for another 24h at 37°C before the viable cells in each well were analyzed with CCK-8 kit. RGE was the only one that killed all three lines of cancer cells completely[28].

Conclusion

It could be concluded from the present study that the ethanolic extract A*llium sativum* practically shows an anti-tumour and immunomodulatory effects in tumor/cancer inducted rats. The extract was able to down-regulate the expression of IL-33,and TNF-α, serum cytokines. Thus data obtained from this present study provide a baseline information on the dosage for ethanolic extract of *A. sativum* to be used as management tool for in vivo studies in Wistar albino rats. Therefore, *Allium sativum* may be use or serve as a better preventive and therapeutic options in the management of Breast cancer patients.

Recommendations

Considering the findings of this study, In view of the potentials effects of ethanolic extract of A. sativum, it's recommended that there is a need to carry out clinical trials to see if the same result will be replicated in human. Therefore, further research studies should initiated.

Novelty and Impact Statement

A. Sativum extracts have significantly down-regulated serum IL-33 and TNF-α, and completely restored the normal architecture of breast tissue in the experimental animals. The extracts have also shown chemoprotective potentials in Albino rats, fed daily with A. sativum extracts, before DMBA induction. The laboratory animals showed little-to-no signs of tumor initiations. This research is a pointer to the fact that A. Sativum may be used as an alternative treatment option in breast cancer management.

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Author Contributions

Dr. Alhassan Hussaini Mohd, Dr. Mohammed Haruna Yeldu and Dr. Usman Musa are the supervisors that oversees to the successful completion of this research. They are instrumental in the formulation of research hypothesis and research design and in the analysis of the overall results.

Safiya Yusuf, Isiyaku Adamu and Hamisu Abdullahiare the MSc students, who carried out the experimental research work. Dr. Abdulrazak Nuhu, and Ahmad Hamidu Marafaare responsible for induction of the laboratory animals as well as carrying out the toxicity studies on the laboratory animals.

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