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Breast histopathological examination of *Rattus norvegicus* induced with 7,12-dimethylbenz[a]anthracene and treated with a blend of *Zingiber officinale* and *Allium sativum* ethanol extracts

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Abstract

Breast cancer is a disease in which abnormal breast cells grow out of control and form tumors. Cancer cells can spread into nearby breast tissues; a condition known as invasion. This creates tumors that cause lumps or thickening. In 2022, there were 2.3 million women diagnosed with breast cancer and 670,000 deaths were recorded globally. Treatment combines surgery, radiation therapy, and medications. Attention has been diverted to herbal drugs due to toxicities of the orthodox anticancer drugs. This present study evaluated the potentials of *Zingiber officinale* and *Allium sativum* to treat breast cancer after inducement with 7,12-dimethylbenz[a]anthracene (DMBA) through histopathological examinations. The ratio/proportion of the two herbs that gave the best results was also established using *Rattus norvegicus* breast cancer models. Female virgin Wister *Rattus norvegicus* aged 7 – 8 weeks old were used for this study. Fresh *Z. officinale* and *A. sativum* were purchased from the market, washed, dried, pulverized separately, and macerated in one liter of ethanol for 48 hours to extract the active components. Phytochemical analysis, acute toxicity, and histopathology studies were done using standard methods. Photomicrographs of the stained slides were taken to analyze the morphological and pathological changes for all the groups. All the treated groups including the control doxorubicin group reduced the histological deformities which was caused by cancer inducement to the minimum. Group 8 which was treated with 6;4 = ZO:AS had the greatest impact on the cancerous cells and showed only mild carcinoma with islands of uniform tumor cells.

Keywords: *Allium sativum*; Cancer inducement; Doxorubicin; Histopathology; *Zingiber officinale*

1. Introduction

Breast cancer is a disease in which abnormal breast cells grow out of control and form tumors. If left untreated, the tumors can spread throughout the body and become fatal. Breast cancer cells begin inside the milk ducts or the milk-producing lobules of the breast or both. The earliest form is not life-threatening and can be detected in early stages. Cancer cells can spread into nearby breast tissues (invasion) to create tumors that cause lumps or thickening. Treatment combines surgery, radiation therapy, and medications. In 2022, there were 2.3 million women diagnosed with breast cancer and 670,000 deaths were recorded globally. Breast cancer occurs in every country of the world in women at any age after puberty but with increasing rates in later life. Global estimates showed that there were conspicuous

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inequalities in the breast cancer burden according to human development. For instance, in countries with a very high Human Development Index (HDI), 1 in 12 women will be diagnosed with breast cancer in their lifetime and 1 in 71 women die of it [1]. In contrast, in countries with a low HDI; while only 1 in 27 women is diagnosed with breast cancer in their lifetime, 1 in 48 women will die from it [1]. In adolescents and young adult females (AYA) aged 15-39 years, breast cancer is linked with different patterns of invasiveness, as well as diverse psychosocial and economic implications. This fact was studied in Nigeria and the researchers highlighted the burden of breast cancer on AYAs and its consequences through a retrospective review of data from cancer registries in Nigeria between 2009 and 2016 [2]. They further observed high proportion of AYA with breast cancer and suggested that urgent actions are required to ensure early detection and improved breast cancer care among this age group [2]. In experimental animals, breast cancer can be induced using 7,12-dimethylbenz[a]anthracene (DMBA). Another study tested an experimental model of chemical mammary carcinogenesis induction in rats using young virgin Sprague-Dawley female rats, aged 47 days and administering 20 mg of 7,12-dimethylbenz(a)anthracene (DMBA) intragastrically by gavage. At 8 and 13 weeks post inducement, their mammary glands were examined. All the rats developed breast carcinomas that were confirmed with histopathology analysis. They concluded that the experimental animal model of chemical-induced mammary carcinogenesis is feasible and can be used in further experiments on the role of tumorigenic biomodulator substances [3].

1.1. Some medicinal plants with anti-breast cancer activities

A lot of medicinal herbs have been confirmed to be very effective in breast cancer chemotherapy. Some Nigerian medicinal plants numbering 51 were reported to exhibit anticancer activities of the prostate, cervices, lung, skin, colon, esophagus, blood, ovary, central nervous system/brain, breast, stomach, pancreas, larynx, and kidney [4]. According to the researchers, the major classes of bioactive compounds indicated to be responsible for the anticancer activity included: polyphenols, flavonoids, alkaloids, saponins, triterpenes, tannins, and quinones. These plants exhibited their major anticancer pharmacological actions via antiproliferative, cytotoxic, cytostatic, antimetastatic, apoptotic, and antioxidative as well as provoked cell cycle arrest, inhibition of angiogenesis, and reduction of cancer cell viability [4]. Some of the plants comprised: *A. sativum* (Amaryllidaceae) which repressed the proliferations of skin, colon, lung, prostate, leukemia, and breast cancer cells in vitro [5]. The methanol leaf extract of *Boerhaavia diffusa* was established to have cytotoxic activity against breast cancer (MCF-7) cell lines [6]. The ethanolic leaf extract of *Cajanus cajan* repressed CaCo-2 (colorectal), MCF-7 (breast), and HeLa (cervical) cancer cell lines [7]. According to a certain study, aqueous extract of *Cassia occidentalis* inhibited the proliferation of SW-620 (colon cancer), HCT-15 (colon cancer), OVCAR-5 (ovarian cancer), SiHa (cervical cancer), MCF-7 (breast cancer), and PC-3 (prostate cancer) cell lines [8]. The aqueous extract of *Glycyrrhiza glabra* (Fabaceae) exerted anticancer activity against breast cancer (MCF-7) and colon cancer (HT-29) cell lines [9]. *Goniothalamus macrophyllus* (Annonaceae) was also found effective. Its methanolic root and stem extracts were highly cytotoxic against breast MCF-7 and colon LS-174T cancer cell lines [10]. *Mangifera indica* (Anacardiaceae) popularly called mango, has shown inhibitory effects against the proliferation of cancer cells following the administration of the methanol stem-bark extract. *M. indica* suppressed the proliferation of MDA-MB-435 (breast cancer), UACC-257 (melanoma), TK-10 (renal cancer), SK-OV-3 (ovarian cancer), and KM 12 (colon cancer) cell lines [4]. Caffeic acid (3, 4-dihydroxycinnamic acid) and oleanolic acid from *Ocimum gratissimum* (Labiatae) exhibited promising antibreast cancer activity. Among other anticancer activities, oleanolic acid from ethanol leaf extract of *O. gratissimum* repressed breast carcinoma, prostate adenocarcinoma, lung carcinoma, colon adenocarcinoma, pancreatic carcinoma, and renal carcinoma in humans [11]. Studies also indicated that *Vernonia amygdalina* (Compositae) extracts inhibited the proliferation of BT-549 and MCF-7 breast cancer cell lines [12]. The anticancer bioactive compounds in *Z. officinale* (Zingiberaceae), namely, 10-gingerol, 10-shogaol, 6-gingerol, and 6-shogaol hindered the proliferation of PC-3 prostate cancer cell line, human hepatocellular carcinoma) cell lines proliferation of MCF-7 breast, HepG2 liver, and KYSE-150 esophageal cancer cell lines 13, 14]. This present study therefore evaluated the potentials of two of these herbs, *Z. officinale* and *A. sativum*, to treat breast cancer after inducement with 7,12-dimethylbenz[a]anthracene (DMBA); as indicated by the breasts histopathology photomicrographs. The ratio/proportion of the two herbs that gave the best results was also established using rat breast cancer models.

1.2. Importance of histological examination

Histopathological examination is crucial in pharmacological research because it provides a microscopic view of tissue and cell changes, allowing researchers to understand drug effects on various organs and tissues. Histopathology allows researchers to examine how a drug interacts with cells and tissues at a microscopic level. This includes observing changes in cell morphology, tissue structure, and the presence of inflammation or damage. Histopathological examination also serves an important role in non-clinical toxicological evaluation. Particularly, it is pivotal for obtaining data on organ-specific toxicity and carcinogenicity [15].

Histopathological evaluation is a cornerstone in cancer diagnosis and treatment, providing critical insights into tumor biology, grading, and staging hence it is indispensable for effective cancer management, ensuring precise diagnoses, appropriate treatments, and improved patient outcomes [16]. In an earlier study, high-quality histopathology was noted to be essential for the success of clinical trials as it is essential throughout every stage of research and clinical trials, from concept development and study design to trial delivery, analysis and dissemination of results [17]. This present study therefore used histopathological examination to evaluate the potentials of different ratios of a blend of *Z. officinale* and *A. sativum* in correcting the morphorlogical changes that resulted from cancer inducement with DMBA.

2. Material and methods

2.1. Animals

Female virgin Wister rats aged 7 – 8 weeks old were used for this study. To reduce individual susceptibility to chemical-induced cancer and variation in drug response, the animals were bred in the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology under ideal conditions of temperature, humidity, and light. The animals were fed with pelletized feed (Vital Feeds, Nigeria) and had access to filtered water *ad libitum*. The animals can live an average of 3 years, starting their reproductive function at 50 to 60 days of age which lasts for about 1 year. All animal experiments were conducted in compliance with NIH guides for the care and use of laboratory animals (National Institute of Health (NIH) (2011) Pub No: 85-23). Ethical approval was obtained from the Animal Care and Ethics Committee of Enugu State University of Science and Technology (ESUT) with the approval number ESUT/AEC/0431/AP397. Ethical approval was also obtained from the Animal Research Ethics Committee of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria with approval number (NAU/AREC/2023/00021).

2.2. Plant materials

The plants used in this research were *Z. officinale* rhizome and *A. sativum* bulb. These plants were procured in a market in Enugu state, Nigeria.

2.3. Extraction of the active components

The plant sources, fresh *Z. officinale* rhizome, and *A. sativum* bulb, after being purchased from the market, were washed and dried. After drying, they were pulverized separately and 200 g of each pulverized plant parts were macerated in one liter of ethanol for 48 hours. The filtrates were collected by sieving through a muslin cloth, concentrated in a water bath at 50 °C, and stored in the refrigerator until used.

2.4. Phytochemical analysis of *Z. officinale* and *A. sativum* separately

The qualitative phytochemical analysis of the extracts was carried out using standard methods described by Odoh *et al*., [18].

2.5. Test for alkaloids

Each plant extracts weighing 0.2 g was heated in 20 mL of 2% hydrochloric acid (HCL) solution in a water bath for about 2 minutes. The resulting solutions were allowed to cool. They were filtered and 5 mL of the filtrates were used for the following tests:

- Dragendorff's test: To each labeled test tube, 5 mL of the sample was added. This was followed by the addition of 1 mL of Dragendorff's reagent. The formation of orange or red precipitates indicated the presence of alkaloids.
- Hager's test: The samples (5 mL) were placed in labeled test tubes and a few drops of Hager's reagent (saturated picric acid solution) were added. The formation of yellow precipitates indicated the presence of alkaloids.
- Wagner's test: The samples (5 mL) were placed in labeled test tubes and a few drops of Wagner's reagent (solution of iodine and potassium iodide) were added. Reddish brown precipitates indicated the presence of alkaloids.
- Mayer's test: A quantity of 5 mL of each samples was placed in labeled test tubes and a few drops of Mayer's reagent (potassium mercuric iodide solution) were added. The formation of cream-colored precipitates indicated the presence of alkaloids.

2.6. Test for glycosides

The samples were extracted with 1% H_2SO_4 solution in a hot water bath for about 2 minutes. The resulting solutions were filtered and made distinctly alkaline by adding 4 drops of 20% potassium hydroxide (KOH) and confirmed with litmus paper. One milliliter of Fehling's solution (equal volume of A and B) was added to each filtrate and heated on a hot water bath for 2 minutes. Brick red precipitates indicated the presence of glycosides.

2.7. Test for saponins

The plant extracts weighing 0.2 g each were dissolved in methanol individually and the resulting solutions were used for the following test:

- Frothing test: The samples (5 mL) were placed in labeled test tubes and 5 mL of distilled water was added in each tube and the mixtures were shaken vigorously. The test tubes were observed for the presence of persistent froth.

2.8. Test for tannins

The plant extracts weighing 0.2 g each were dissolved in methanol and the resulting solutions were used for the test. To 3 mL of each of the samples, a few drops of 1% Ferric chloride were added and observed for brownish-green or blue-black colorations.

2.9. Test for flavonoids

Using methanol, 0.2 g of each plant extracts was dissolved and the resulting solutions were used for the following test:

- Ammonium hydroxide test: A quantity of 2 mL of 10% ammonia solution was added to a portion of each samples and allowed to stand for 2 minutes. Yellow coloration at the lower ammoniacal layer indicated the presence of flavonoids.
- Sodium hydroxide solution test: A quantity of 10 mL of 10% sodium hydroxide solution was\ added to a portion of each samples and observed for color changes in the lower alkaline layer. Yellow color indicates flavones, blue to violet color indicated anthocyanins, and yellow to orange color indicates flavonones.
- Concentrated sulphuric acid test: A portion of each samples were mixed gently with concentrated Sulphuric acid and observed for color changes. Yellowish-orange color indicates anthocyanins, yellow to orange color indicates flavones, and orange to crimson indicates flavonones.

2.10. Test for steroids and terpenoids

- Salkowski test: The plant extracts were dissolved in methanol and the resulting solutions were used for the test. The 5 mL of each samples was mixed in 2 mL of chloroform and concentrated. Sulphuric acid (H_2SO_4) was carefully added to form a layer. A reddish brown coloration at the interface indicated a positive test.
- Liebermann-Burchard test: Acetic anhydride (2 mL) was added to 0.5 g of each of the methanol extracts and concentrated H_2SO_4 (2 mL) was carefully added to the resulting mixture and observed for color change from violet to blue or green.

2.11. Acute toxicity studies (LD_{50}) of *Z. officinale* and *A. sativum* ethanol extracts

The actual median lethal dose (LD_{50}) estimations of the *Zingiber officinale* and *Allium sativum* ethanol extracts were conducted with the method described by Lorke, [19] with modifications according to the process defined by Ihekwereme *et al*., [20].

2.12. Experimental design

The rats were grouped into 10 and each group comprised 10 animals. Group 1: Non-induced control (Naïve; received 5 ml/kg body weight of distilled water). Group 2: DMBA control (5 ml/kg body weight of distilled water). Group 3: *Z. officinale* treatment 530 mg/kg body weight. Group 4: *A. sativum* treatment 530 mg/kg body weight. Group 5: *Z. officinale* and *A. sativum* combination 2:8 (106: 424 mg/kg body weight). Group 6: *Z. officinale* and *A. sativum* combination 4:6 (212: 318 mg/kg body weight). Group 7: *Z. officinale* and *A. sativum* combination 5:5 (265: 265 mg/kg body weight). Group 8: *Z. officinale* and *A. sativum* combination 6:4 (318: 212 mg/kg body weight). Group 9: *Z. officinale* and *A. sativum* combination 8:2 (424: 106 mg/kg body weight). Group 10: Reference standard 5 mg/kg doxorubicin.

2.13. Histopathological study

The tissues were processed through a series of ethyl alcohols of ascending strength (70, 80, and 95%) for 1 hour; twice in absolute alcohol (for 1 hour); and twice in xylene (for 1 hour each) as to render the tissue elements transparent. The tissues were then infiltrated with molten paraplast at 58 °C. This was done twice (for 1 hour each). The transparent tissues, after clearing all elements from it, were embedded in a solid mass of paraplast. The blocks were labelled, allowed to cool, and the metal blocks were removed. The solid mass was then trimmed free of excess paraplast, leaving some free margins around the embedded tissues. Three microns thick longitudinal sections were cut with a rotary microtone. The sections were mounted on thoroughly cleaned gelatinized slides and placed on hot plates at 37 °C for 24 hours for proper fixation. The slides were stained by H & E stain according to the prescribed staining method (Bancroft and Stevens, 1990). The stain was prepared by dissolving hematoxylin in absolute alcohol. The mixture was boiled rapidly and mercuric oxide was added. The stain was cool rapidly in a cold water bath; glacial acetic acid was then added and the stain was ready for immediate use. The stained slides, after drying and labelling, were examined microscopically under oil immersion for comparative morphological and pathological changes.

3. Results

3.1. Results of phytochemical analysis

Table 1 Phytochemical analysis of *Z. officinale* and *A. sativum* ethanol extracts

Phytocompounds	***Z. officinale***	***A. sativum***
Alkaloids	+	+
Saponins	-	+
Tannins	+	-
Flavonoids	+	+
Steroids and terpenoids	+	-
Glycosides	-	+

Keys: + = present - = absent

Table 2 Acute toxicity studies for *Z. officinale*

Phase 1 for Z. officinale			
Group	**Number of mice**	**Dose of extract (mg/kg bw)**	**Observation**
1	3	1,000	No mortality, no convulsion, normal fur, skin, respiration and fecal consistency
2	3	2,000	No mortality, no convulsion nor other signs of toxicities
3	3	3,000	No mortality, no convulsion, normal fur, skin, respiration and fecal consistency
Phase 2 for Z. officinale			
Group	**Number of mice**	**Dose of extract (mg/kg bw)**	**Observation**
1	3	3,500	No mortality, no obvious sign of toxicity
2	3	4,000	No mortality, no obvious sign of toxicity
3	3	5,000	Diarrhea observed, no mortality or other obvious signs of toxicity

Phase 3 for Z. officinale			
Group	**Number of mice**	**Dose of extract (mg/kg bw)**	**Observation**
1	3	7,500	Hypoventilation in all the mice which was normalized 6 hours post extract administration
2	3	10,000	Spastic paralysis and hypoventilation in the three Mus musculus which led to death of one.

Actual LD_{50} of *Z. officinale* ethanol extract was calculated as follows: Actual $LD_{50} = (7,500 \times 10,000)^{1/2}$; Actual LD_{50} = 8,660 mg/kg body weight

Table 3 Acute toxicity studies for *A. sativum*

Phase 1 for *A. sativum*			
Group	**Number of mice**	**Dose of extract (mg/kg bw)**	**Observation**
1	3	1,000	No mortality, no obvious sign of toxicity
2	3	2,000	No mortality, loss of appetite for two hours with calmness
3	3	3,000	No mortality, diarrhea, loss of appetite for4 hours
Phase 2 for *A. sativum*			
Group	**Number of mice**	**Dose of extract (mg/kg bw)**	**Observation**
1	3	3,500	Loss of appetite, loss of mechanical activity, diarrhea, no mortality
2	3	4,000	Loss of appetite, fast respiration, diarrhea, partial paralysis, no mortality
3	3	5,000	Loss of appetite, fast respiration, tachycardia, paralysis and death of one *M. musculus* after 6 hours

Actual LD_{50} of *A. sativum* ethanol extract was calculated as follows: Actual $LD_{50} = (4,000 \times 5,000)^{1/2}$; Actual LD_{50} = 4,472 mg/kg body weight

Table 4 Acute toxicity studies of a blend of *Z. officinale (ZO)* and *A. sativum (AS)* ethanol extracts in equal proportion

Groups	**Dose of *ZO* + *AS* (mg/kg bw)**	**Number of mice**	**Number of death**
1	1,000 + 1,000	3	Nil
2	2,000 + 2,000	3	Nil
3	3,000 + 3,000	3	Nil
4	4,000 + 4,000	3	Nil
5	5,000 + 5,000	3	Nil
6	6,000 + 6,000	3	1

bw = body weight; Actual LD_{50} of *Z. officinale* and *A. sativum* when given in combination was calculated as follows:*;* Actual $LD_{50} = (5,000 \times 6,000)^{1/2}$*;* Actual LD_{50} = 5,477 mg/kg body weight

3.2. Results of histopathological examination

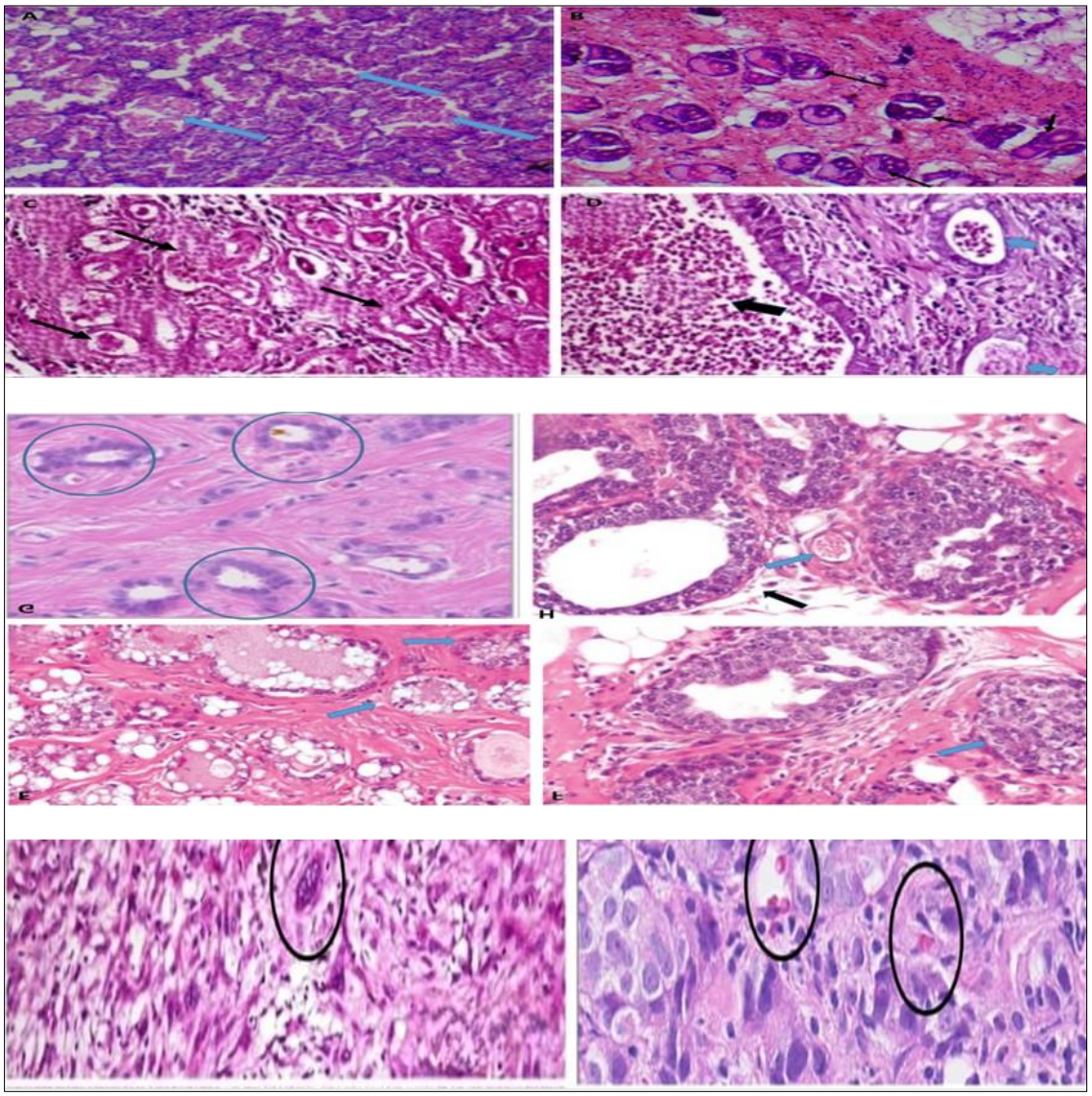

Figure 1 Photomicrographs of groups A and J

4. Discussion

4.1. Phytochemical analysis

The phytochemical compounds present in *Z. officinale* include: alkaloids, tannins, flavonoids, steroids and terpenoids while saponins and glycosides are absent. These present phytochemicals are responsible for the profound pharmacological potentials of *Z. officinale* which include but not limited to its antioxidant, antiinflammatory, anticancer activities. These phytochemicals were recognized to show several pharmacological activities on human health such as anti-cancer, anti-inflammatory, anti-malarial, anti-microbial, anti-hypertensive, anti-diabetic, and anti-oxidant among others; by directly acting on the central nervous system in the human body and also affecting nucleic acid, DNA (Deoxy Ribonucleic acid), RNA (Ribonucleic acid), membrane permeability and proteins [21]. The phytochemical compounds present in *A. sativum* include: alkaloids, saponins, flavonoids, and glycosides while tannins, steroids, and terpenoids were absent. In addition to the components discussed above, saponins and glycosides also play some roles in the Pharmacological activities of herbs, *A. sativum* inclusive. Saponins consisting of carbohydrate and either triterpenoid or steroid aglycone moieties are noted for their multiple biological activities including fungicidal, antimicrobial, antiviral, anti-inflammatory, anticancer, antioxidant and immunomodulatory effects and they have for long been used in herbal and traditional medicines [22].

4.2. Acute toxicity studies

From the results of the acute toxicity studies, *Z. officinale* had an actual LD_{50} of 8,660 mg/kg body weight (practically non-toxic) because it took a very high dose of 10,000 mg/kg body weight to cause mortality of mouse. On the other hand, *A. sativum* had actual LD_{50} of 4,472 mg/kg body weight (slightly toxic). Its ethanol extract kills one mouse at the dose of 5,000 mg/kg body weight. However, when an equal amount of *Z. officinale* and *A. sativum* were blended together, the LD_{50} became 5,477 mg/kg body weight rendering the combination practically non-toxic. These facts implied that *Z. officinale* had more safety profile than *A. sativum* but a blend of the two herbs interacted favorably thus making the blend practically non-toxic.

4.3. Histopathological examination

From the results of the histopathology analysis of the breasts that were treated with different proportions of *Z. officinale* and *A. sativum*, it was obvious from the photomicrographs labelled A – J that each group had an extent to which the treatments were able to cure the cancerous cells. These included: A – Histological section of the breast tissue of DMBA-induced breast cancer in *R. norvegicus* treated with *Z. officinale* alone (530 mg/kg) – blue arrow showing mild to moderate hyperplasia of the ductal epithleial cell with mild damaged basement membrane (HE – 100x).

- Histological section of the breast tissue of DMBA-induced breast cancer in *R. norvegicus* treated with *A. sativum* alone (530 mg/kg) – arrow showing mild mucinous carcinoma with extracellular micin surrounding small clusters of tumor cells with different growth patterns and surrounding stromal fibrosis (HE – 100x).
- Histological section of the breast tissue of DMBA-induced breast cancer in *R. norvegicus* treated with a combination of *Z. officinale* and *A. sativum* in the ratio of 8:2 (424: 106 mg/kg) – Black arrow showing lumen of the duct with mild atypical proliferative cells (HE – 100x).
- Histological section of the breast tissue of DMBA-induced breast cancer in *R. norvegicus* treated with combination of *Z. officinale* and *A. sativum* in the ratio of 4:6 (212: 318 mg/kg) – Black arrow showing inflammatory cell infiltration within the ductal lumen and blue arrow showing adenocarcinoma tumor cells arranged into acini (HE – 100x).
- Histological section of the breast tissue of DMBA-induced breast cancer in *R. norvegicus* treated with a combination of *Z. officinale* and *A. sativum* in the ratio of 2:8 (106: 424 mg/kg) – Blue arrow showing atypical ductal hyperplasia with large ductal epithelial cells and increased cytoplasmic-nucleus ratio (HE – 100x).
- Histological section of the breast tissue of DMBA-induced breast cancer in *R. norvegicus* treated with vehicle (5 ml/kg distilled water) – Blue arrow showing large periductal stromal fibrosis and fatty tissues with the presence of immature fibrocytes (HE – 100x).
- Histological section of the breast tissue of DMBA-induced breast cancer in *R. norvegicus* treated with a combination of *Z. officinale* and *A. sativum* in the ratio of 6:4 (318: 212 mg/kg) – Blue circle showing mild carcinoma with an island of uniform tumor cells (HE – 100x).
- Histological section of the breast tissue of DMBA-induced breast cancer in *R. norvegicus* treated with vehicle (5 ml/kg distilled water) – Blue arrow showing mucinous carcinoma with nuclear atypia while-black arrow shows bigger ductal epithelial cells and increased cytoplasm-nucleus ratio (HE – 100x).
- Histological section of the breast tissue of DMBA-induced breast cancer in *R. norvegicus* treated with a combination of *Z. officinale* and *A. sativum* in the ratio of 5:5 (265: 265 mg/kg) – Black circle showing mild tumor necrosis characterized by clusters of degenerating and dead cells with accompanying inflammatory cells (HE – 100x).
- Histological section of the breast tissue of DMBA-induced breast cancer in *R. norvegicus* treated with doxorubicin (5 mg/kg) – Black circle showing mild hemorrhage and inflammatory cell infiltration within a tumor stroma (HE – 100x).

Histopathological examination of *R. norvegicus* mammary glands typically involves assessing tissue samples for structural changes, including normal structures like ducts and acini, as well as any abnormalities like tumors or hyperplasia. Histopathological examination had been a means of identifying morphological changes in breast tissues. The H&E staining of mice mammary tissue showed development of metaplastic triple negative breast cancer thus confirming that DMBA can be used as breast cancer initiator [23]. Histopathology images of mammary glands of DMBA-induced *R. norvegicus* also indicated that the molecular subtype of mammary cancer on *R. norvegicus* induced with 20 mg/kg BW of DMBA showed similarity to human breast cancer [24]. Furthermore, a *R. norvegicus domestica* was presented to the Department of Veterinary Medicine of the University of Bari "Aldo Moro" (Bari, Italy) for the presence of a progressive growing unilateral mass. It was through histopathological examination that the lesion was diagnosed as mammary ductal carcinoma [25]

5. Conclusion

All the treated groups including doxorubicin treated group reduced the histological deformities that were caused by cancer inducement using DMBA but group 8 which were treated with 6;4 = *Z. officinale: A. sativum* had the greatest impact on the cancerous cells; group 8 showed only mild carcinoma with an island of uniform tumor cells.

Compliance with ethical standards

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Disclosure of conflict of interest

- Daniel Ikechukwu Oraekei declared no conflict of interest
- Nnamdi Markben Adione declared no conflict of interest
- Chukwuka Benjamin Uzodinma declared no conflict of interest
- Harrison Odera Abone declared no conflict of interest

Statement of ethical approval

Maintenance and care of all animals were carried out according to EU Directives 2010/63/EU for animal experiments. The guides for care and use of Laboratory Animals, DHHS Publ. # (NIH 86-123) were strictly adhered to. Ethical approval was obtained from the Animal Ethical Committee of the Enugu State University of Science and Technology (Approval number ESUT/AEC/0431/AP397). Also, there was approval by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes (Approval number is NAU/AREC/2023/00021).

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