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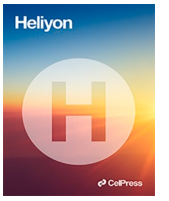

Research article

Oligandrin from *Croton oligandrus* (Euphorbiaceae) exhibits anti-breast cancer activity through immune-boosting mechanisms: *In vitro* and *in vivo* study

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ABSTRACT

Aim: Recent developments in cancer research indicate that cancer is a manifestation of immune system dysfunction. Many natural anticancer agents developed recently possess immune-modulatory properties. In our ongoing pursuit of anticancer alternatives, we evaluated the immune-modulatory potential of oligandrin, an ent-pimarane type diterpenoid from *Croton oligatrus*.

Methods: we assessed on Breast cancer patients' peripheral blood mononuclear cells (PBMCs) were isolated to assess the effect of oligandrin (0.5, 1, 10, 100, 200 mg/mL) *in vitro* using the Ficoll-histopaque density centrifugation method. The parameters that were assessed included, PBMC viability and cytokine (IL-6, IL-12, IL-10, EGF, TNF-α, INF-γ) production. *In vivo*, we chemically induced breast cancer using DMBA (50 mg/kg BW) in Wistar rats, then treated them with oligandrin (1 mg/kg BW) or standards (tamoxifen 3.3 mg/kg; letrozole 1 mg/kg) for 20 weeks. The parameters that were evaluated included, tumor burden, volume, incidence, histopathology, antioxidant, and inflammatory status.

Results: Oligandrin (1, 10, 100 and 200 μg/mL) significantly increased ($p < 0.05$) PBMC cell number 24 h after incubation. *In vivo*, it induced 62.5 % tumor incidence reduction compared to DMBA rats (100 %). Oligandrin significantly protected ($p < 0.001$) rats against increased tumor burden, mass and volume, which was accompanied by a significant antioxidant effect [increment

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of GSH ($p < 0.01$) and SOD ($p < 0.001$)]. Oligandrin prevented high-grade adenocarcinomas according to SBR stratification and significantly reduced pro-inflammatory cytokine levels (IL-6, IL-12) while increasing anti-inflammatory cytokine levels (INF-γ).
Conclusion: Oligandrin is reported for the first time to protect against breast cancer onset and this effect seems to be at least in part attributable to its immune-boosting capacity.

1. Background

Cancer-related deaths are approximately 10 million each year [1]. Breast cancer is the most diagnosed cancer in women and the leading cause of cancer mortality worldwide with an estimated 2,308,897 new cases and 665684 deaths in 2020 [2]. This increased incidence is correlated with human development, excess body weight, physical inactivity, alcohol intake, availability and utilization of breast cancer screening methods, the age of primary menarche and exposure to hormone therapy [3,4]. The tumor microenvironment (TME) is an inflammatory environment containing various inflammatory and regulatory mediators such as cytokines (tumor necrosis factor (TNF), interleukins (IL)-1β, IL-6 and IL-10), as well as chemokines and reactive oxygen species (ROS) [5].

Advancement in medical technologies and modern cancer therapies, including chemotherapy, immunotherapy and radiotherapy, have achieved successful clinical outcomes in the management of certain cancer types [6]. However, these different treatment options have side effects on overall patient health. For example, chemotherapeutic drugs that act by damaging mitotic apparatus or DNA damage, kill cancer cells and also destroy normal tissues with high mitotic index. As a result, anemia, myelosuppression, alopecia and diarrhea are observed [7]. On the other hand, radiotherapy causes tiredness, weakness, skin problems and swelling of the breast [8,9]. It is thus of great importance to search for compounds with fewer or no side effects and/or toxicity to normal cells for the management of this disease.

In contrast to conventional treatment options, the therapeutic efficacy of targeted anticancer agents is mediated by immunostimulatory or immunosuppressive effects [10]. Similarly, targeted therapies that trigger cancer cells' molecular signature can greatly influence therapeutic response due to a diverse range of heterogeneity expressed based on cancer types and among individual patients. In fact, the positive or negative influence of targeted anticancer agents showing therapeutic efficacy over the past three decades is mediated indeed by their immunostimulatory or immunosuppressive effects [10]. Tumors develop and progress by a process referred to as three-phase immune editing: elimination, equilibrium and escape [11]. Effective immunomodulatory substances act by interacting with cancer cells, but they also have the capacity to interact with immune cells in these three phases in such a way as to awaken them or reinforce their potency [6,10].

Natural compounds (polyphenols, alkaloids, terpenoids) have been known as anticancer agents for decades. Their effects encompass, anti-inflammatory, antioxidant, cytotoxic, anti-angiogenic and immunomodulatory properties, for which there is a growing interest [1]. Resveratrol, curcumin and the polyphenol EGC have been studied widely for their anticancer and immunomodulatory properties [12,13]. Resveratrol's immunomodulatory effect is accomplished through the decrease in TGF-β and an increase in IFN-γ which result in suppression in the production of cytokines (IL-10 and TGF-β) and an increase in the production of NK cells [14–16]. Curcumin on the other hand exerts its immunomodulatory property through the suppression of T-reg function, preventing the depletion of T cells by tumors and promoting the expansion of central and effector memory T cell phenotypes [17].

Natural compounds with immunomodulatory potential are in demand as an alternative to chemotherapy, given that the pathogenesis of cancer involves the activation of host defense mechanisms [18]. They can be administered in combination with other treatments, thereby attenuating their side effects and/or helping the immune system to eliminate foreign agents. It is on this basis that the present study was undertaken to assess the immunomodulatory effects of oligandrin *in vivo* in a model of DMBA-induced breast cancer and *in vitro* on PBMC isolated from breast cancer patients. Oligandrin is an ent-pimarane-type diterpenoid isolated from the leaf extract of *Croton oligandrus* Pierre ex Hutch (Euphorbiaceae) which has shown improved enzyme inhibition and antioxidant activities [19]. Popular traditional uses of Croton species include their use in the treatment of cancer, constipation, diabetes, fever, hypercholesterolemia, hypertension, inflammation, malaria, pain and weight-loss [20]. *Croton oligandrus* Pierre ex Hutch is a tree (5–10 m high), commonly found in Western and Central African forests, especially in Cameroon and Gabon [21]. Clerodane diterpenes are widely distributed within the genus Croton, and are known to possess antifeedant, cytotoxic and antiprotozoal properties [22]. A study of *Croton oligandrus* harvested in Cameroon revealed the presence of clerodane-type diterpenes as the main class of metabolites and some of these compounds displayed a moderate cytotoxic effect against PC3 (prostate) and MCF-7 (breast) cancer cells [23]. To the best of our knowledge, no *in vitro* and *in vivo* study has yet been conducted on the potential of oligadrin from *Croton oligandrus* to prevent breast cancer. Therefore, this study was aimed at assessing the immune-modulatory potential of oligandrin on peripheral blood mononuclear cells (PBMCs) isolated from breast cancer patients and to assess antioxidative status, breast cancer biomarker CA 15-3, total proteins, hematology, and histopathology of the mammary glands in DMBA-induced breast cancer in rats.

2. Methods

2.1. Substances and reagents

Oligandrin (Table 1) was obtained from the leaf extract of *Croton oligandrus* Pierre ex Hutch (Euphorbiaceae) as described by Fongang et al. [19]. Sigma-Aldrich (Stanford, Germany) provided the breast carcinogen 7,12-dimethylbenz(a)anthracene (DMBA,

purity 98 %), phytohemagglutinin (PHA) and cell culture necessities (RPMI-1640, L-glutamine, HERPES, FBS, penicillin/streptomycin), while Salutas Pharma GmbH (Barbelen, Germany) provided tamoxifen citrate (Norvatis Access®) and Letrozole (Norvatis Access®). Diazepam (Valium® 10 mg/2 mL) and ketamine (Ketamine hypochloride® 50 mg/mL) were acquired from Roche (Fontenay-sous-bois, France) and Rotex Medica (Tritau, Germany). Milipore (R&D systems, Inc, Minneapolis, USA) provided the cytokine assay kit MILIPLEX® for luminex® Xmap® Technology. Monobind Inc® (California, USA) provided the cancer antigen 15-3 (CA 15-3) ELISA kit. Fortress Diagnostics Limited® (Muckamore, United Kingdom) provided the ALT and creatinine reagent kits.

2.2. In vitro experimental design

2.2.1. Sampling blood of breast cancer patients and healthy controls

Joint Institutional Review Board for Animal and Human Bioethics (Ref: BTC-JIRB2021-010) issued authorization for human blood usage in this study. Five (5) patients with breast cancer and 5 healthy control women aged 50 years (average onset age) who went to the Yaoundé General Hospital and agreed to take part in the study, were included. All breast cancer patients undergoing any treatment or positive for HIV, hepatitis or diabetes were excluded. Once informed consent had been obtained from each individual, blood was drawn into EDTA tubes.

2.2.2. Isolation of peripheral blood mononuclear cells (PBMC)

PBMCs were isolated using the Ficoll-histopaque density centrifugation method as described by Boudjeko et al. [24]. This process separates mononuclear cells from whole blood based on their low density. PBMCs are found at the interface between plasma and the density gradient medium. Briefly, 5 mL of EDTA-anticoagulated whole blood from donors was diluted with 5 mL of Phosphate-Buffered Saline +2 % fetal bovine serum (FBS) and mixed gently. In SepMate TM tubes, 3.5 mL of Ficoll-histopaque was added through the central hole of the insert (a device used to minimize mixing of the sample and density gradient medium). Holding the tube vertically, the diluted sample was carefully added down the side of the tube to settle on the density gradient medium. Tubes were subsequently centrifuged at 1200 g for 10 min. The top layers containing PBMC were transferred to sterile tubes and washed twice for 8 min at 300 g. The density gradient medium, erythrocytes and granulocytes were retained under the insert. Pellets were resuspended in 1 × PBS (500 μL) before counting by trypan blue exclusion method.

2.2.3. Peripheral blood mononuclear cell (PBMC) culture assay

Cells were centrifuged for 5 min at 300 *g* and the pellet was resuspended in culture medium consisting of RPMI1640 supplemented with: L-glutamine plus Herpes, 10 % FBS, 0.05 mM 2β Mercapto-ethanol, 1 % pen/strep and 0.11 g/L sodium pyruvate. Cell suspensions were adjusted to 1×10^6 cells/mL. Then, 200 μL were dispensed into each well from one suspension to another in a 96-well plate. Two different plates were used to inoculate PBMC from healthy controls and patients. The first wells of each plate served as untreated control cells, receiving only cell suspension. The second well served as positive control loaded with 20 μL of the mitogen phytohemagglutinin (PHA) whose concentration was 1 μg/mL addition to the cell suspension and the remaining wells received 20 μL of oligandrin (0.5, 1, 10, 100 and 200 μg/mL) in addition to the cell suspension. Lids were the used to seal plates before incubation for 24 h at 37 °C, in 5 % CO_2 and 95 % oxygen. The experiment was carried out in triplicate and repeated three times.

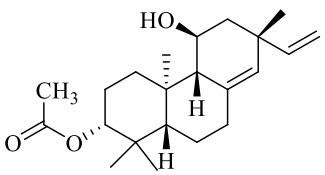
2.2.4. Assessment of the immunomodulatory activities of oligandrin of PBMC

The immunomodulatory activity of oligandrin was assayed by assessing PBMC growth and cytokine production.

2.2.4.1. Assessment of PBMC growth. The viability and number of PBMC were performed through the Trypan Blue exclusion method. Cell suspension (10 μL) was mixed with Trypan blue (90 μL, 0.4 %). This mixture (10 μL) was then loaded into a Neubauer counting chamber and observed under a microscope with a 10 × objective. Using a hand-held counter, cells were counted on the four large squares and averaged. The number of viable cells/mL was obtained using the following formula

$$\textit{Number of viable cells per mL} = \textit{number of cells counted} \times \textit{dilution factor} \times 10000 \times \textit{initial volume of cells}$$

Table 1
General information on oligandrin isolated from *Croton oligandrus*.

Code and names	Crystal color	Structure, molecular weight and formula
Oligandrin	White	 Molecular weight = 346.51 M Molecular formula = $C_{22}H_{34}O_3$

2.2.4.2. Assessment of cytokine production by PBMC. The immune-boosting potential of oligandrin was determined by assessing its ability to stimulate PBMCs to synthesize cytokines. After 24 h of incubation (5 % CO_2 and 95 % oxygen at 37 °C) with different treatment substances, the contents of the 96-microtiter plates were centrifuged for 5 min at 300 *g*. The supernatant for each sample was collected in separate tubes and stored at −20 °C for subsequent cytokine assays. The experiment was performed in triplicate and repeated three times.

2.3. In vivo experimental design

2.3.1. Animals

Animal groups were composed of 40 healthy female Wistar rats (*Rattus norvegicus*) of 5 weeks old and weighing between 55 and 65 g. They were obtained from the Animal Physiology Laboratory of the University of Yaoundé I and maintained in plastic cages with adequate ventilation at room temperature (~25 °C) and with a natural light cycle. They were given a normal soy-free rat chow consisting of the following ingredients: corn (42 %), bone meal (3 %), wheat flour (22 %), fish meal (19 %), crushed palm kernel meal (4 %), sodium chloride (0.75 %), peanuts (9 %), multivitamin complex (Olivitazol® 0.5 %) and water *ad libitum*. Housing and animal treatments were approved by the Faculty of Science's Joint Institutional Review Board for Animal and Human Bioethics (Ref: BTC-JIRB2021-010), which followed the European Union's rules on both human and animal welfare (EEC Council 86/609).

2.3.2. Chemopreventive effect of oligandrin on DMBA induced-breast cancer in rats

Forty (40), 45-days-old Wistar rats were acclimatized for 10 days before being randomly divided into five groups of eight rats (n = 8) each. Both the normal control (NOR) and the negative control (DMBA) groups received 3 % ethanol as a vehicle. Group III received tamoxifen at 3.3 mg/kg BW and served as the positive control. Groups IV and V were given oligandrin at doses of 0.5 and 1 mg/kg BW respectively [23]. Treatments began 10 days before malignancy was induced, were administered orally (intra-gastric gavage) and lasted 20 weeks. Rats were subcutaneously injected with 50 mg/kg BW of DMBA (diluted in 0.2 mL of olive oil) into the fat pad of the right inguinal mammary gland to induce breast cancer, while the control group received olive oil only. Following the 20 weeks of treatment, rats were sacrificed and blood was collected by intra-cardiac puncture into EDTA and dry tubes. The blood in EDTA tubes was used for hematological analysis. The latter were further centrifuged at 3000 rpm for 15 min and stored at −20 °C for biochemical studies. Tumors and mammary glands, liver, spleen, lungs, and kidneys were excised, weighed, and promptly fixed in 10 % formalin for histological investigation. Breast tumor volume was determined using the method of Faustino Rocha et al. [25].

The relative organ weights were computed as follows:

$$\textit{Relative organ weight}\left(\frac{mg}{kg}\right) = \frac{\textit{organ weight}\ (mg)}{\textit{animal body weight}\ (kg)} \times 10^6$$

The tumor incidence was computed as follows:

$$\textit{Tumor incidence}\ (\%) = \frac{\textit{number of rats with tumors}}{\textit{total number of rats}} \times 100$$

The tumor load was determined in the following way:

$$\textit{Tumor burden} = \sum \textit{tumor mass in a group}$$

Tumor burden reduction was computed as follows: $\%\ \textit{tumor burden inhibition} = \frac{\textit{tumor burden in DMBA} - \textit{tumor burden in the test group}}{\textit{tumor burden in DMBA}} \times 100.$

2.3.3. Histopathologic analyses

Once the organs had been fixed, the basic histological processes were trimming, dehydration, and inclusion. Hematoxylin and eosin stain was used to stain 5-μm slices of paraffin-embedded tissues mounted in resin. Photomicrographs were taken with a light Axioskop 40 × microscope equipped with a digital Celestro-44421 camera and connected to a computer, where the images were transmitted and analyzed using Image J software. Tumors were investigated using Russo et al. [26] histopathologic criteria at the Department of Morphological Sciences and Pathological Anatomy, Faculty of Medicine and Biomedical Sciences.

2.3.4. Assessment of oxidative stress markers

The primary oxidative stress markers and total protein levels were evaluated in mammary glands from normal (NOR) and treated groups. The level of reduced glutathione (GSH) was measured using modified Sehirli et al. [27] method. Catalase and superoxide dismutase (SOD) activities were assessed using the Misra [28] and Sinha [29] techniques, respectively. Wilbur et al. [30] methodology was used to quantify malondialdehyde (MDA), a biomarker of lipid membrane peroxidation. The Gonal et al. [31] method was used to determine total protein levels in samples.

2.3.5. Assessment of chemokines/cytokine levels

Interferon gamma (IFNγ), tumor necrosis factor-alpha (TNF-α), epidermal growth factor (EGF), interleukin-6 (IL-6), interleukin-12 (IL-12), and fractalkine levels in serum and supernatant of PBMC treatment were measured using magnetic luminex screening test. This assay was carried out using a MILIPLEX® Kit (Millipore, Minneapolis, USA) according to the manufacturer's instructions. In brief, 96-microplates were shaken horizontally with 50 L/well of previously diluted samples and standard cytokines, as well as 50 μL of

magnetic, premixed, microparticle cocktail containing antibodies specific for each cytokine. The microplates were then cleaned with a magnetic plate separator before being treated with a 50 μL/well cocktail of biotin-antibodies specific for each cytokine. Streptavidin-PE was used to expose the antibody-cytokine complexes, which were then examined in a Luminex MAGPix Analyzer (XMAP Technology, SN, USA), and the results were expressed as median fluorescence intensity (MFI). The MFI was translated into cytokine relative concentrations using a cytokine-specific standard curve. Each cytokine had a minimum detectable concentration of 5.2 pg/mL (IFNγ), 1.9 pg/mL (TNFα), 0.3 pg/mL (EGF), 0.7 pg/mL (fractaklin), 0.2 pg/mL (IL-6), and 0.4 pg/mL (IL-12).

2.4. Statistical analysis

Unpaired student's *t*-test was used to compare differences between the negative control group (DMBA) and the normal control group (NOR). Analysis of variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons was used for the various statistical analyses involving 3 or more groups using GraphPad Prism software version 5.03. Data were expressed as mean ± standard error of the mean (SEM). Significance was set at $p < 0.05$.

3. Results

3.1. Effect of oligandrin on the level of PBMC

The influence of oligandrin on peripheral blood mononuclear cells (PBMC) isolated from both healthy individuals and breast cancer patients is depicted in Table 2. In cells from healthy individuals, the positive control wells (PHA) exhibited a significant increase ($p < 0.05$) in cell count compared to normal wells. However, no significant increase in PBMC count was observed in test wells treated with various concentrations of oligandrin. In cells from breast cancer patients, PHA-treated wells equally displayed a significant increase ($p < 0.05$) in PBMC count compared to normal wells. Interestingly, wells treated with oligandrin showed a significant increase in PBMC count at 1 μg/mL ($p < 0.05$), 10 μg/mL ($p < 0.01$), 100 μg/mL ($p < 0.01$), and 200 μg/mL ($p < 0.01$) compared to control.

3.2. Effect of oligandrin on cytokine levels

Fig. 1A and B illustrate the effects of various treatments on cytokine levels in samples obtained from healthy individuals following a 24-h culture. PHA induced a significant increase ($p < 0.05$) in INF-γ levels but did not significantly alter TNF-α levels compared to the normal control. Conversely, in wells treated with oligandrin, a significant increase ($p < 0.001$) in IFN-γ levels was observed at 0.5, 1, 10, and 100 μg/mL concentrations.

In the case of samples from breast cancer patients, Fig. 1C and D depict the effects of different treatments on cytokine levels after a 24-h culture. PHA induced a significant increase ($p < 0.05$) in INF-γ levels and, concurrently, a significant decrease ($p < 0.001$) in TNF-α levels compared to control. However, in wells treated with oligandrin at various concentrations, significant increases in IFN-γ levels were noted at concentrations of 1, 10, 100, and 200 μg/mL ($p < 0.05$) and ($p < 0.01$). Additionally, TNF-α levels significant increased at 0.5 μg/mL ($p < 0.05$), 100 μg/mL ($p < 0.01$), and 200 μg/mL ($p < 0.01$) compared to control.

3.3. Chemoprotective effects of oligandrin on DMBA-induced breast cancer in rats

3.3.1. Effect of oligandrin on body weight

Fig. 2A below depicts the weight progression of animals over 20 weeks of experimentation. There was no significant difference in the weights of the animals in all groups. In fact, all the animals showed continuous growth during the experiment.

3.3.2. Effect of oligandrin on death rate

During the 20-week experiment, the lowest death rates were observed in the normal group, with 87.5 % animal survival, in the oligandrin group, a 75 % survival rate was observed. Animals in the negative control group (DMBA) and those treated with tamoxifene showed 62.5 % survival. The highest death rate was recorded in the oligandrin 0.5 mg/kg -treated group, with a survival rate of 37.5 %. However, it should be noted that the first deaths were recorded in the DMBA group (Fig. 2B).

Table 2
Effect of oligandrin on PMBC levels.

	Control	PHA	0.5	1	10	100	200
NOR	0.85 ± 0.05	2.18 ± 0.23[a]	1.865 ± 0.03	2.01 ± 0.18	2.33 ± 0.02	2.385 ± 0.05	2.24 ± 0.2
CANCER	0.80 ± 0.04	1.35 ± 0.05[a]	1.13 ± 0.02	2.05 ± 0.05[b]	2.2 ± 0.2[c]	2.09 ± 0.01[c]	2.1 ± 0.15[c]

NOR = cell suspension; PHA = cell suspension + phytohemagglutinin (1 μg/mL); Oligandrin = cell suspension + Oligandrin at different concentrations.

[a] $p < 0.05$ compared with normal.

[b] $p < 0.05$.

[c] $p < 0.01$ compared with control cells.

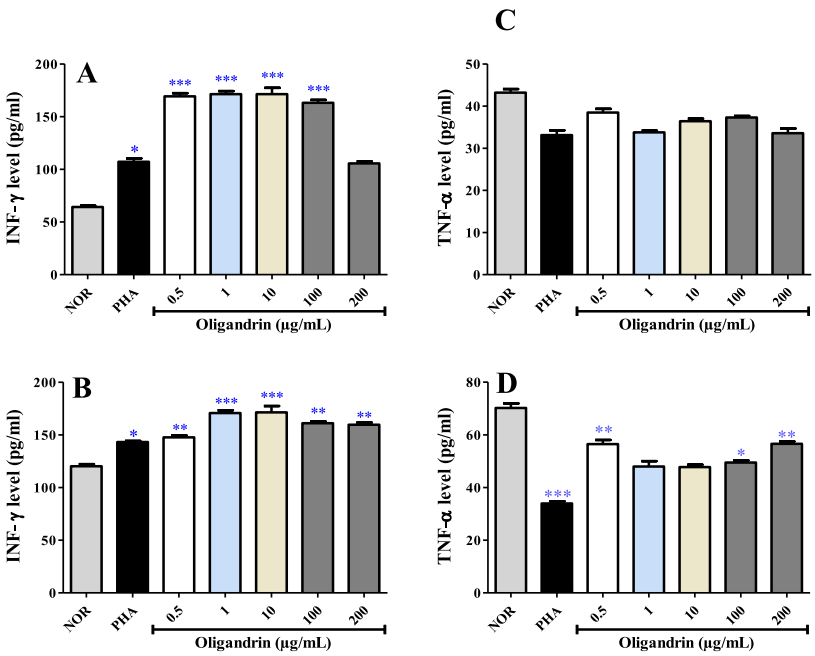


Fig. 1. Effects of oligandrin on INF-γ and TNF-α in healthy individuals (A, B) and breast cancer patients (C, D), respectively. NOR = cell suspension; HAP = cell suspension + HAP (1 µg/mL); oligandrin = cell suspension + Oligandrin at different concentrations. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with normal.

3.3.3. *Effect of oligandrin on tumoral parameters*

The results reported in Table 3 show that animals in the normal control group did not develop any tumors but all the rats in the negative control group (DMBA) developed tumors thus giving a tumor incidence of 100 %. Tamoxifen significantly ($p < 0.001$) decreased the total tumor burden as well as tumor incidence (25 % and 37.5 %) compared to the DMBA group. Oligandrin at the doses of 0.5 and 1 mg/kg significantly ($p < 0.001$) reduced the total tumor burden as well as tumor incidence (62.5 %) compared to the DMBA group.

3.3.4. *Macroscopic/morphologic effect of oligandrin on tumors*

Fig. 2C, D & E, depict the macroscopic architecture of tumors. Rats from the negative control group (DMBA) developed larger tumors than animals in the other groups. Tamoxifen significantly reduced ($p < 0.001$) tumor mass (3.07 mg ± 0.02 in the tamoxifen group compared to 13.40 mg ± 1.7 in the DMBA group) and tumor volume (1417.32 cm^3 ± 320.63 in the tamoxifen group in comparison to 7190.1 cm^3 ± 825.1 in the DMBA group). Animals treated with oligandrin (1 mg/kg) displayed a significant ($p < 0.001$) decrement in tumor mass (1.5 mg ± 0.02) and tumor volume (541.30 cm^3 ± 106.78) compared with animals in the DMBA group. The tumor volume was 0.32 mg ± 0.03 in the oligandrin group (0.5 mg/kg) compared to 13.40 mg ± 1.7 in the DMBA group and a tumor volume of 244.84 cm^3 ± 30.14 in comparison to 7190.1 cm^3 ± 825.1 in the DMBA group.

3.3.5. *Effect of oligandrin on some oxidative stress parameters*

Table 4 shows the effect of oligandrin on certain markers of oxidative stress after 20 weeks of experimentation. A significant increase ($p < 0.001$) in total protein levels was observed in the DMBA group compared with the normal group. With the treated groups, a significant decrease in total protein level was observed with the tamoxifen ($p < 0.05$) and oligandrin 0.5 and 1 mg/kg ($p < 0.001$) treated animals compared to those in received DMBA.

With regards to catalase activity, a significant decrease ($p < 0.01$) was noted in animals in the DMBA group compared to those in the normal group. A significant increase in catalase activity was observed in groups treated with oligandrin 0.5 mg/kg ($p < 0.05$) and 10 mg/kg ($p < 0.001$) compared to the DMBA group. Those treated with tamoxifen showed a non-significant increase of this antioxidant marker.

In comparison to animals in the normal group, a significant increase ($p < 0.01$) in MDA levels was noted in animals in the DMBA group. On the other hand, a significant decrease was observed in groups treated with tamoxifen ($p < 0.001$) and oligandrin (0.5 mg/kg ($p < 0.05$) and 10 mg/kg ($p < 0.05$)) compared to those in the DMBA group.

SOD activity was significantly increased ($p < 0.01$) in animals in the DMBA group compared to those in the normal group. In addition, it increased significantly ($p < 0.001$) both in animals treated with tamoxifen and oligandrin in comparison to the DMBA group.

Glutathione (GSH) level was significantly decreased ($p < 0.01$) in animals of the DMBA group compared to those in the normal

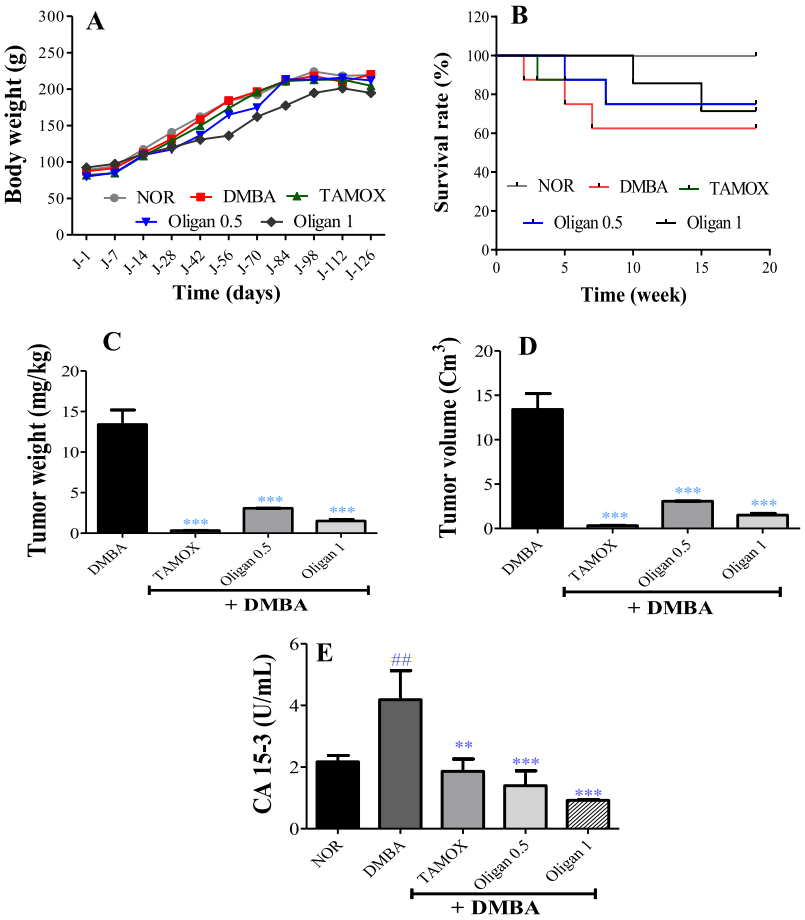


Fig. 2. Effects of oligandrin on animal weight gain (A), animal survival rate (B), tumour morphology (C), mass (D) and volume (E). NOR = Normal control animals given distilled water; DMBA = Negative control animals given distilled water; TAMOX + DMBA = Positive control animals given tamoxifen 3.3 mg/kg; Oligandrin + DMBA = Animals given oligandrin at 0.5 and 1 mg/kg. All groups, with the exception of the NOR group, received a single dose of DMBA (50 mg/kg).

Table 3
Effect of oligandrin on some tumoral parameters.

		Rats with tumor	Incidence of tumor (%)	Tumor burden (mg)	% Inhibition of tumor
+ **DMBA**	**NOR**	0/8	–	–	–
	DMBA	8/8	100	9.73	–
	TAMOX	2/8	25	2.95	77.09
	OLIGAN 0.5	3/8	37.5	3.65	97.58
	OLIGAN 1	3/8	37.5	4.32	88.77

NOR = Normal control animals receiving distilled water; DMBA = Negative control animals receiving distilled water; Tamox + DMBA = Positive control animal 1 receiving tamoxifen at a dose of 3.3 mg/kg; Oligandrin + DMBA = Animals receiving Oligandrin at a dose of 0.5 and 1 mg/kg.

group. An increase although not significant was noted in animals treated with tamoxifen and oligandrin. A non-significant decrease of this marker was observed in animals treated with oligandrin 0.5 mg/kg in comparison with the DMBA group.

3.3.6. Effect of oligandrin on mammary glands microarchitecture

The microarchitectures of mammary glands from animals in the normal group (Fig. 3A) showed normal mammary parenchyma with mammary lobules containing mononuclear acinar cells surrounded by abundant adipocytes. Animals in the DMBA group (Fig. 3B) all had high-grade ductal carcinoma (SBR III) with low lymphocytic infiltration (<10 %) and around 30 % comedonecrosis. Animals treated with tamoxifen (Fig. 3C) showed low-grade fibrosarcoma (SBRI) without lymphocytic infiltration and comedonecrosis. Those treated with oligandrin (0.5 mg/kg) (Fig. 3D) showed low-grade fibrosarcoma (SBR I) or fibrous degeneration, no lymphocytic

Table 4
Effects of Oligandrin on oxidative stress parameters.

	NOR	DMBA	TAMOX	Oligandrin 0.5	Oligandrin 1
Proteins	0.033 ± 0.005	0.092 ± 0.007[b]	0.056 ± 0.01[c]	0.025 ± 0.002[d]	0.029 ± 0.01[d]
Catalase	92 ± 9.5	35.8 ± 8.9[a]	51.2 ± 4.7	118.7 ± 14.4[c]	298.3 ± 51.8[d]
MDA	2.1 ± 3.7	7.91 ± 1.1[a]	2.1 ± 2.4[d]	2.2 ± 4.3[c]	2.3 ± 3.2[c]
GSH	25.3 ± 2.8	11.3 ± 1.6[a]	25.8 ± 2.4	2.4 ± 0.1	22.1 ± 1.5
SOD	28.7 ± 3.3	45.2 ± 3.5[a]	75 ± 7[d]	50.1 ± 3.5	85 ± 61.1[d]

NOR = Normal control animals receiving distilled water; DMBA = Negative control animals receiving distilled water; Tamox + DMBA = Positive control animal 1 receiving tamoxifen at a dose of 3.3 mg/kg; Oligandrin + DMBA = Animals receiving Oligandrin at a dose of 0.5 and 1 mg/kg. All groups except NOR received a single 50 mg/kg dose of DMBA.

**$p < 0.01$.

[a] $p < 0.01$.

[b] $p < 0.001$ compared to normal group.

[c] $p < 0.05$.

[d] $p < 0.001$ compared to DMBA group.

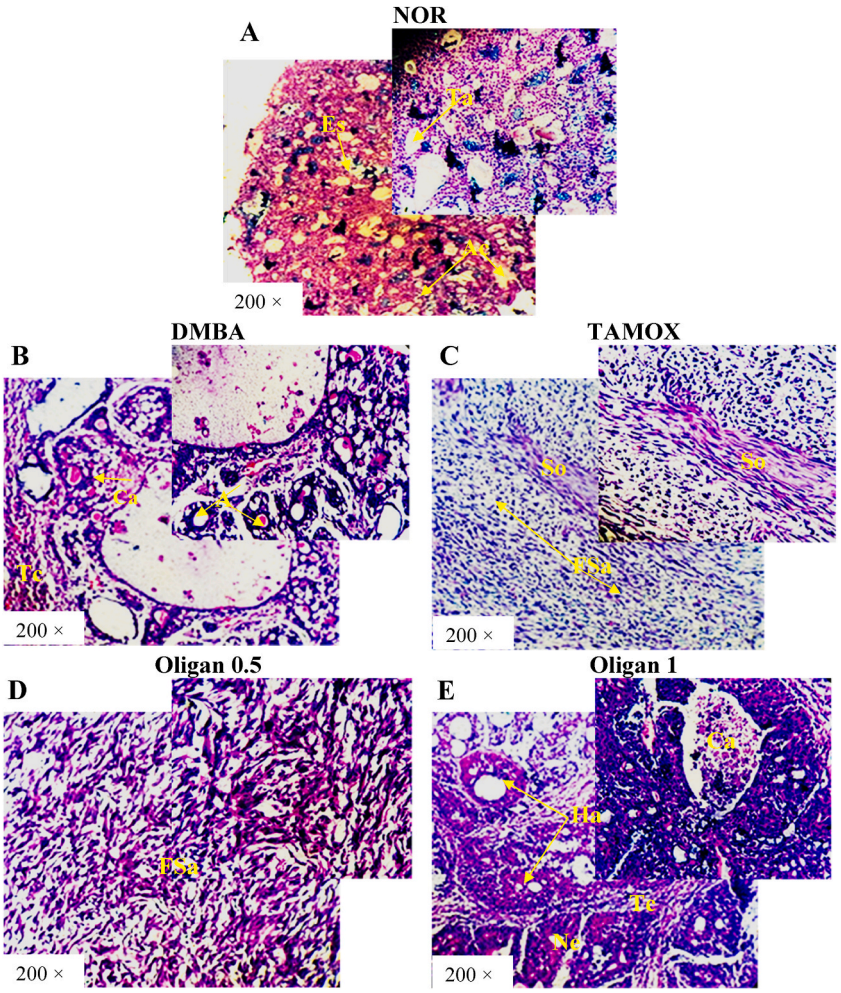


Fig. 3. Histopathology of mammary glands and mammary tumors. NOR = normal control receiving distilled water (**A**); DMBA = negative control receiving distilled water (**B**); TAMOX + DMBA = animals receiving tamoxifen (**C**); Oligandrin + DMBA = animals receiving Oligandrin at 0.5 (**D**) and 1 mg/kg (**E**). All groups, with the exception of the NOR group, received a single dose of DMBA (50 mg/kg).

infiltration and around 20 % comedonecrosis. Animals treated with oligandrin (1 mg/kg) (Fig. 3E) showed SBR grade II fibrosarcoma with no lymphocytic infiltration and 15 % comedonecrosis (Fig. 3).

3.3.7. Effect of oligandrin on cytokine levels

Fig. 4 shows the effect of different treatments on serum cytokine levels. DMBA induced a significant increase in the levels of TNF-α ($p < 0.001$), IL-6 ($p < 0.001$), IL-12 ($p < 0.001$) and EGF ($p < 0.05$), but did not significantly change the levels of IFN-γ and fractalkine compared with the normal group (Fig. 4A–F). Tamoxifen and oligandrin (0.5 mg/kg) treatments significantly ($p < 0.001$) decreased TNF-α, IL-6, IL-12 and EGF serum levels with a significant ($p < 0.01$) increment in IFN-γ levels compared to the DMBA group (Fig. 4A–F). In animals treated with oligandrin, there was a significant increase in serum IFN-γ levels (Fig. 4B). There was also a significant decrease in serum levels of IL-6 ($p < 0.05$), IL-12 ($p < 0.001$), EGF ($p < 0.01$) and TNF-α compared to the DMBA group (Fig. 4A, C and 4E-4F). Fractalkine levels revealed no significant changes.

3.3.8. Effect of oligandrin on the relative mass of organs

Table 5 shows a significant decrement ($p < 0.001$) of thymus relative mass and a significant increment ($p < 0.01$) of the liver, lungs and kidneys ($p < 0.05$) in the DMBA group animals compared with the normal group. Tamoxifen significantly decreased the relative mass of the liver ($p < 0.01$), lungs ($p < 0.001$) and spleen ($p < 0.01$) and increased that of the thymus ($p < 0.001$). For animals treated with oligandrin (0.5 mg/kg), a significant increase in the relative mass of the thymus ($p < 0.01$) and a significant decrease in that of the liver ($p < 0.001$), kidneys ($p < 0.05$), lungs ($p < 0.001$) and spleen ($p < 0.001$) were also observed compared with the DMBA group.

3.3.9. Effect of oligandrin on some haematological and biochemical parameters

The results presented in Table 6 show a significant increase ($p < 0.001$) in the number of granulocytes and a significant decrease in

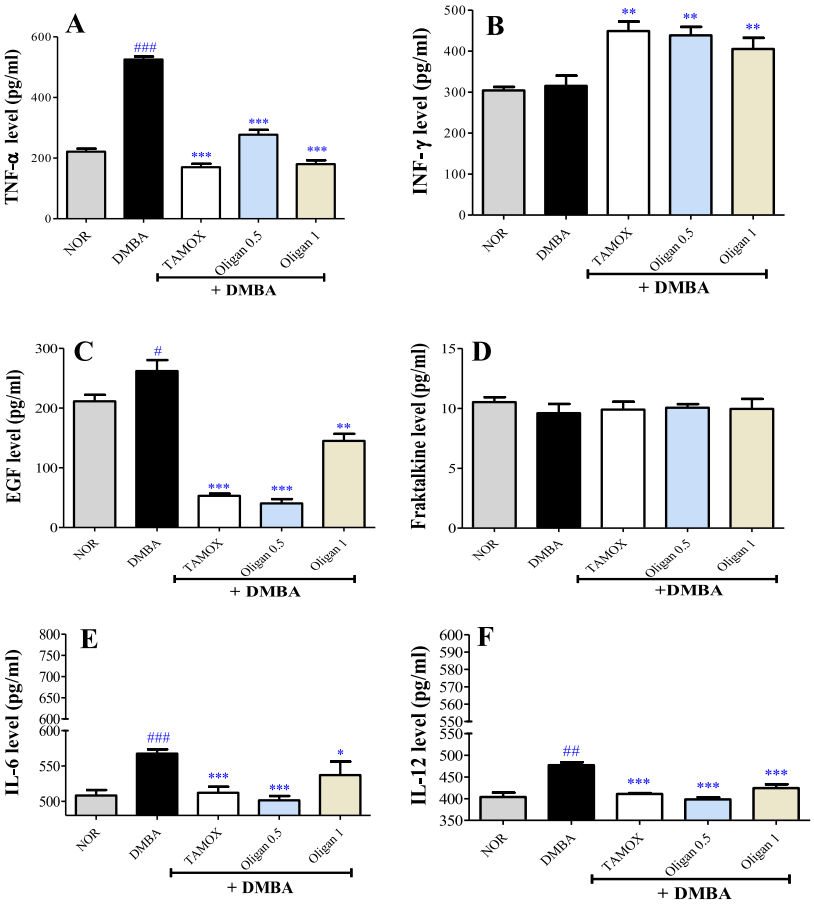


Fig. 4. Effects of Oligandrin on some cytokines levels. NOR = normal control receiving distilled water; DMBA = negative control receiving distilled water; Tamox + DMBA = animals receiving tamoxifen at 3.3 mg/kg; Oligandrin + DMBA = animals receiving oligandrin at 0.5 and 1 mg/kg. All groups, with the exception of the NOR group, received a single dose of DMBA (50 mg/kg). #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ compared to normal group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the DMBA group.

Table 5
Effects of oligandrin on organs relative mass.

	NOR	DMBA	TAMOX	Oligandrin 0.5	Oligandrin 1
Thymus	1483.1 ± 4.3	767.9 ± 40.3[c]	1423.6 ± 20.9[f]	976.4 ± 42.9[e]	1516.7 ± 101.4[f]
Liver	30380.9 ± 624.6	35214.6 ± 1331.6[b]	30922.6 ± 235.4[e]	29049.1 ± 488.4[f]	27812.2 ± 546.6[f]
Lungs	6481.7 ± 190.2	7284.8 ± 180.92[b]	5886.9 ± 49.2[f]	5381.2 ± 139.6[f]	6488.4 ± 180.9[e]
Kidney	5547.3 ± 96.6	6154.6 ± 193.2[a]	6402.1 ± 83.6	4851.5 ± 103.1[d]	5459.6 ± 280.9[d]
Spleen	5999.9 ± 85.5	5357.2 ± 344.2	4335.3 ± 44.5[e]	3055.7 ± 19.9[f]	3348.9 ± 111.9[f]

NOR = Normal control animals receiving distilled water; DMBA = Negative control animals receiving distilled water; Tamox + DMBA = Positive control animals 1 receiving tamoxifen at a dose of 3.3 mg/kg; Oligandrin + DMBA = Animals receiving Oligandrin at a dose of 0.5 and 1 mg/kg.
[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$ compared to normal group.
[d] $p < 0.05$.
[e] $p < 0.01$.
[f] $p < 0.001$ compared with the DMBA group.

the number of red blood cells ($p < 0.01$) and leukocytes ($p < 0.001$) in the DMBA group compared to the normal group. A significant decrease ($p < 0.001$) in monocytes and granulocytes number and a significant increase ($p < 0.001$) in leukocytes, lymphocytes and red blood cells count was observed in oligandrin-treated animals compared to the DMBA group. In the tamoxifen-treated animals, a significant decrease in the number of leukocytes ($p < 0.001$) and a significant increase in the number of lymphocytes ($p < 0.05$), monocytes ($p < 0.001$) and red blood cells ($p < 0.001$) were observed compared with those in the DMBA group. On the other hand, a significant decrease in the number of granulocytes and monocytes ($p < 0.001$) with a significant increase in the number of lymphocytes ($p < 0.001$) and red blood cells ($p < 0.01$) were also observed in the group treated with oligandrin (0.5 mg/kg) compared with those in the DMBA group.

With regard to transaminase activity, a significant increase in serum ALT and creatinine ($p < 0.05$) was observed in animals of the DMBA group compared with animals in the normal group. Tamoxifen and oligandrin (0.5 mg/kg) significantly decreased serum creatinine ($p < 0.05$) and non-significantly decreased ALT activity compared to the DMBA group. Equally, treatment with oligandrin significantly decreased ($p < 0.05$) serum creatinine and ALT activities compared with the DMBA group.

3.3.10. Effect of oligandrin on breast cancer metastasis to other organs

Fig. 5 shows the effects of oligandrin on the microarchitecture of the kidney, spleen, liver, lungs and thymus. It can be seen that DMBA caused renal leukocyte infiltration. Animals in the DMBA group also showed disorganization of the white pulp in the spleen and leukocyte infiltration of the lungs and liver. The sections also showed a decrease in the density of cortical lymphocytes in the thymus and a thickening of the interlobular septum in the DMBA group compared with the normal control group. The normal control group had normal kidney structures (with well-differentiated urinary space and glomerulus), normal spleen (with well-differentiated white and red pulp), and normal liver and lungs. Animals treated with tamoxifen and oligandrin prevented this decrease in cortical lymphocyte density and leukocyte infiltration of the kidney, liver and lungs, as well as disorganization of the white pulp of the spleen.

4. Discussion

Despite improvements in the diagnosis and treatment of breast cancer, it remains a major health problem for women [32]. Existing therapies are limited due to their high cost and adverse effects. Consequently, the search for new accessible compounds with little or no

Table 6
Effect of oligandrin on haematological and biochemical parameters.

	NOR	DMBA	TAMOX	Oligandrin 0.5	Oligandrin 1
RBC	7.12 ± 0.01	5.35 ± 0.61[b]	7.28 ± 0.15[e]	6.85 ± 0.55[e]	7.66 ± 0.25[f]
WBC	12.46 ± 0.62	15.42 ± 0.58[b]	12.8 ± 0.7	6.9 ± 0.6	18.44 ± 1.16[f]
GRA%	18.4 ± 1.74	27.65 ± 1.34[c]	24.25 ± 0.90	12.7 ± 0.63[f]	11.98 ± 1.63[f]
LYM%	68.4 ± 1.89	66 ± 0.5	72.6 ± 3.62[d]	84.25 ± 1.13[f]	86.85 ± 2.54[f]
MONO%	5.2 ± 0.47	6.35 ± 1.04	9.7 ± 0.41[f]	2.55 ± 0.24[f]	2.5 ± 0.04[f]
ALAT	155.1 ± 47.7	302.1 ± 1[a]	235.6 ± 23.2	233.9 ± 24.2	149.9 ± 41.4[d]
CREA	0.33 ± 0.02	0.44 ± 0.01[a]	0.33 ± 0.03[d]	0.35 ± 0.01[d]	0.36 ± 0.01[d]

NOR = Normal control animals receiving distilled water; DMBA = Negative control animals receiving distilled water; Tamox + DMBA = Positive control animals 1 receiving tamoxifen at a dose of 3.3 mg/kg; Oligandrin + DMBA = Animals receiving Oligandrin at a dose of 0.5 and 1 mg/kg.
[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$ compared to normal group.
[d] $p < 0.05$.
[e] $p < 0.01$.
[f] $p < 0.001$ compared with the DMBA group.

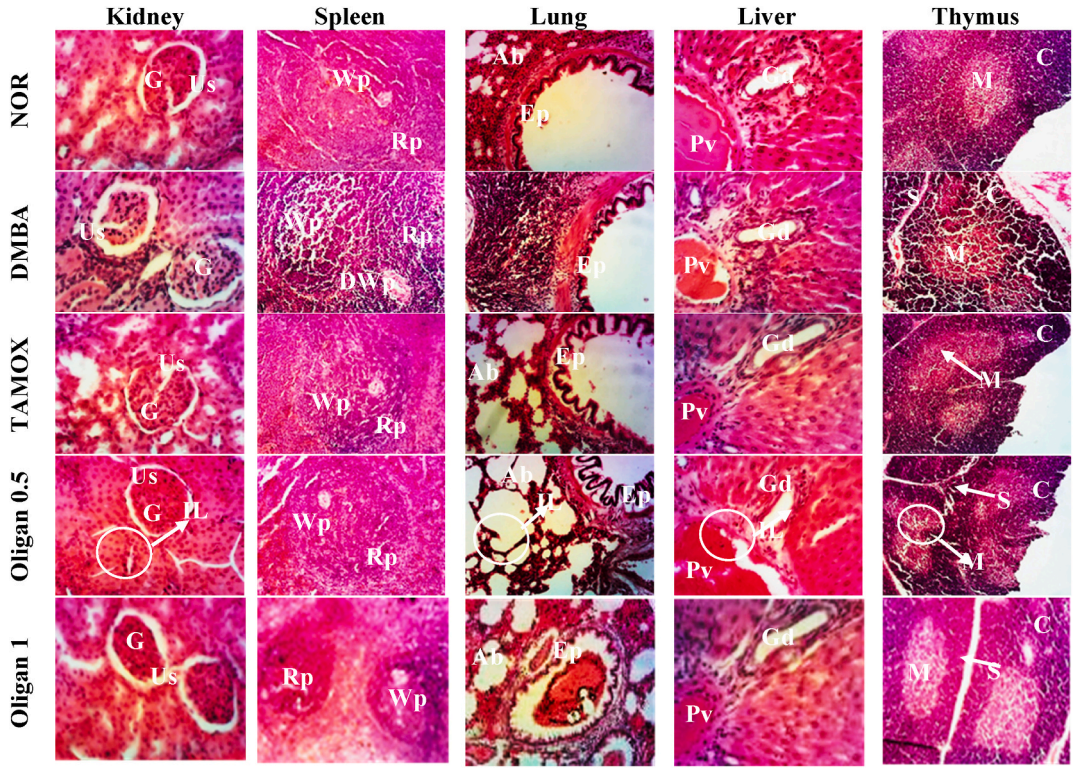


Fig. 5. Histological sections of metastasis organs stained with hematoxylin-eosin. NOR = normal control animals receiving distilled water; DMBA = negative control animals receiving distilled water; TAMOX + DMBA = positive control animals receiving tamoxifen, COL + DMBA = animals receiving the oligandrin at 1 mg/kg. All groups, with the exception of the NOR group, received a single dose of DMBA (50 mg/kg). Kidney: Us = Urinary space, G = Glomerulus, IL = Leukocyte infiltration; Spleen: Wp = White pulp, Rp = Red pulp, DWb = Disorganization of the white pulp; Lung: LE = Lung epithelium, As = Alveolar sac, LI = Leukocyte infiltration; Liver: Pv = Portal vein, Gd = Gall duct, Thymus: M = Medullar layer, C = Cortex, S = Sinusoid.

side effects is warranted in this field. According to literature reports, more than 65 % of synthetic drug molecules are derived from nature, and most of the natural active drugs, especially from plant sources, constitute 75 % of anti-cancer drugs [33]. This is made possible through bioactive secondary metabolites such as polyphenols, alkaloids and terpenoids present in plants. Recently, new diterpenes among which is oligandrin, was isolated from *Croton oligandrus* Pierre ex Hutch (Euphorbiaceae) leaves extract [19]. Oligandrin was selected in this study for its improved enzyme inhibitory effect and antioxidant activities with an IC_{50} value of 39.4 μM [19]. Taking into consideration that many antioxidant compounds are endowed with immunomodulatory potential, the present study was aimed at assessing the immunostimulatory properties of oligandrin both *in vitro* (on PBMC cells isolated from breast cancer patients) and *in vivo* (on a DMBA-induced breast cancer model in rats). This is due to the fact that compounds with immunomodulatory potential can provide an alternative to conventional chemotherapy for a variety of diseases, especially when the host defense mechanism has to be activated under conditions of impaired immune response like cancers [18].

Numerous studies have shown that DMBA induces mammary tumors in rats, which is characterized by hemorrhagic lesions in some organs or immunotoxicity in lymphoid organs [34,35]. This would explain the higher percentage of deaths observed in the negative control group (DMBA). The deaths observed in the positive control groups could be attributed to the anorexic action of tamoxifen [36]. Animals treated with oligandrin showed a lower percentage of death suggesting that this compound protects against DMBA-induced toxicity. DMBA is a well-characterized and known carcinogen that induces mammary tumors closely resembling human mammary cancer [26]. It has a high genotoxic activity, which would justify the presence of tumors in animals that received it [37]. It acts by altering the normal process of mammary gland differentiation, and covalently binding to the cell's DNA to induce cancer-causing mutations [38]. This mechanism of action could be responsible for the 100 % tumor incidence observed in the negative control group (DMBA). Just like reference medicine such as tamoxifen used in this study, oligandrin significantly protected the animals against the adverse effects of DMBA. A 37.5 % tumor incidence with 88.77 % tumor inhibition and a significant reduction ($p < 0.001$) in tumor mass and volume was observed in animals treated with oligandrin. Similarly, animals treated with tamoxifen showed low-grade fibrosarcoma (SBR I) without lymphocytic infiltration or comedonecrosis, confirming their protective effect against breast cancer. Rats treated with oligandrin (1 mg/kg) showed grade SBR II fibrosarcoma with no lymphocytic infiltration and 15 % comedonecrosis compared to those in the negative control group, which showed a grade SBR III cribriform ductal carcinoma with approximately 10 % lymphocytic infiltration and 30 % comedonecrosis. In addition, no breast cancer metastases were identified target organs' (lungs and liver) tissues examined in this study. These suggests the protective effects of oligandrin on breast tumorigenesis. This anticancer effect

could be attributed to the ability of oligandrin to induce apoptosis or cause cell cycle arrest by inducing DNA damage and the expression of repair-associated genes. Just like the diterpene triptolide, oligandrin could be involved in the down-regulation of multiple cellular signaling pathways such as the Wnt/β-catenin signaling pathway, which is involved in the proliferation, migration, tissue polarity and organogenesis of many cancers [39–41].

Several studies have demonstrated the undeniable role of oxidative stress in human breast carcinogenesis. DMBA-induced breast carcinoma also acts partly through the production of reactive oxygen species (ROS) which damage DNA. Various enzymatic and non-enzymatic antioxidant systems exist to combat this oxidative stress. In the present study, oligandrin showed antioxidant effect by inducing a significant increase ($p < 0.001$) in SOD and catalase activities and a significant decrease ($p < 0.05$) in MDA compared to the DMBA group. MDA, being one of the derivatives of lipid peroxidation and a biomarker of oxidative stress, increases in breast cancer patients [42]. These results suggest an antioxidant potential of oligandrin, thus, explaining its anticancer effects.

The hematopoietic system is one of the most sensitive targets of xenobiotics and is therefore, an important indicator of the physiological and pathophysiological status of an organism [43]. In the present study, we observed a significant increase in the number of white blood cells and a significant decrease in the number of red blood cells in animals in the DMBA group compared to animals in the normal (NOR) group. These observations may be due to possible hematotoxicity of DMBA which caused changes in the lipid composition of RBC membranes, thus, resulting in morphologically abnormal erythrocytes with decreased life span [44]. The fact that oligandrin significantly ($p < 0.001$) protected animals against the drop in red blood cell count and white blood cell count is evident of the beneficial effects of this compound in preventing DMBA-induced anemia immunomodulatory. This could be due to its ability to stabilize cell membranes by breaking down radical chain reactions and scavenging free radicals, thereby reducing oxidative stress and related damages [45].

Changes in relative organ mass are sensitive indicators of chemically induced organ damage [46]. A significant increase ($p < 0.01$) in liver relative mass of animals in the negative control group (DMBA) was observed in this study compared to the normal group. This goes in line with the significant increase ($p < 0.05$) in the transaminase ALT specific to hepatocytes and widely known as a marker of cell lysis and hepatotoxicity [47]. Creatinine is the product of spontaneous dehydration of creatine phosphate in muscles, it is eliminated entirely by the kidneys and is, therefore, a good indicator of renal function [48]. Proteins are the main constituents of cells and tissues and are largely manufactured by the liver. In this study, a significant reduction ($p < 0.05$) in serum creatinine and total protein levels was noted in groups tamoxifen and oligandrin-treated animals with compared with the DMBA animals. These observations suggest that the latter exhibit low nephrotoxicity and hepatotoxicity compared with the carcinogen DMBA.

Inflammation, particularly chronic inflammation, has an important role in tumor progression facilitation and treatment resistance, with cytokines being their mediators [49]. These mediators often intervene in the process of inflammation, immune function, cell damage, angiogenesis, metastasis, and other cellular processes crucial to tumor survival [50]. Furthermore, DMBA exposure leads to increased tumor mutational burden, up-regulation of chemokine (C–C motif) ligand 21 (CCL21) in cancer cells, heightened antigen presentation, and infiltration of $CD8^+$ T cells into breast cancers [51]. Additionally, DMBA-induced breast tumors showed enhanced expression of inflammatory markers like NF-κBp65, COX-2, and iNOS, along with increase in proliferation markers like PCNA and Cyclin D1, and overexpression of the antiapoptotic marker Bcl-2 [52]. To elucidate the immunological and antineoplastic mechanisms of oligandrin, cytokine levels were measured. Generally, cytokines are divided into two groups: pro-inflammatory mediators and anti-inflammatory mediators. High levels of pro-inflammatory cytokines suppress anti-tumor immunity and provide direct tumor-promoting signals through migration, enhancing tumor cell survival and inducing resistance to chemotherapy [53,54]. Inhibition of these cytokines could prevent tumor growth, migration, malignant cell survival and chemotherapy resistance [55]. Oligandrin significantly reduced serum levels of certain pro-inflammatory cytokines such as TNF-α, IL-6 and EGF, involved in tumor growth, progression and resistance to chemotherapy. On the other hand, oligandrin significantly increased serum levels of certain anti-inflammatory factors with anti-tumor effects, namely IL-12 and INF-γ, through their ability to induce interferon production, stimulate cytotoxic lymphocytes, induce apoptotic cell death in cancer cells and inhibit tumor angiogenesis [56–58]. No significant variation was observed in fractalkine (CX3CL1) despite its importance in recruiting and activating immune cells to elicit an antitumor immune response [59]. These modulatory effects of oligandrin suggest that it possesses immunostimulant properties, which are beneficial in breast cancer management.

The immunostimulatory effects observed *in vivo*, led us to assess the ability of oligandrin to stimulate immunological cells *in vitro*. Peripheral blood mononuclear cells (PBMCs) isolated from breast cancer patients and healthy individuals were used for this purpose. PBMC are particularly useful because they allow a higher density of useable cells, thus reducing the number of samples needed for the experiment. Additionally, they play a crucial role in determining drug efficacy and are of paramount importance in eliminating any risk when developing new drug therapies. After a 24-h treatment with oligandrin, an increase in the number of PBMC was seen (although not significant) at different concentrations in samples from healthy subjects. However, in samples from breast cancer patients, a significant increase in the number of PBMC was observed in wells treated with oligandrin at different concentrations of 1 μg/mL ($p < 0.05$) and 10, 100 and 200 μg/mL ($p < 0.01$), demonstrating the immunostimulatory effect of this compound. To better understand this result, the immunostimulatory mechanism of oligandrin was further evaluated by measuring the levels of some inflammatory cytokines in the post-treatment cell supernatant. Two of the cytokines previously measured *in vivo*, TNF-α and INF-γ, were of particular interest. Breast cancer upregulates TNF which is a pro-inflammatory cytokine [60]. Some studies have reported that TNF-α increases the proportion of breast tumor cells [61]. Inhibition of this cytokine could prevent tumor growth and the migration and survival of malignant cells [53]. Treatments targeting tumor necrosis factor-alpha (anti-TNF treatments) were among the first targeted immunotherapies available in therapeutics [59]. Regarding IFN-γ, it has always been considered a central player in antitumor immunity [62]. It has specific antitumor effects such as the inhibition of proliferation, tumor angiogenesis and induction of cell death by apoptosis in several types of cancer cells [55,56]. As observed in this study, oligandrin (0.5, 1, 10 and 100 μg/mL) induced a

significant increase ($p < 0.001$) in INF-γ levels in healthy subjects' samples. In those obtained from cancer patients, oligandrin (0.5 and 200 μg/mL) significantly decreased ($p < 0.01$) TNF-α levels, with a significant increase ($p < 0.01$) in INF-γ levels at concentrations of 1, 100 and 200 μg/mL. These results show that oligandrin possesses immunostimulatory potential more effective in subjects with breast cancer and could exert its effect through a decrease in the K63-linked ubiquitination of NF-κB-essential modulator IKKγ and mRNA expression of IL-1β, IL-6, TNF-α, and IL-1β/IL-18 maturation) [63].

5. Limitations and future suggestions

A larger number of human samples would have been a better sample size. In the near future, it would be wise to elucidate the underlying mechanism of action of oligandrin, particularly its interaction with natural killer cells, dendritic cells, and macrophages, or with members of adaptive immunity such as T cells and B cells.

6. Conclusion

This study reports for the first time the anticancer activity of oligandrin, an ent-pimarane type diterpenoid from *Croton oligatrus*. It significantly increases (at least $p < 0.05$) PBMC at 1, 10, 100 and 200 μg/mL concentrations of after 24 h of incubation. *In vivo*, it reduced tumor incidence to 62.5 % compared to the DMBA rats (100 %). It significantly protected ($p < 0.001$) rats against the increased tumor burden, tumor mass and tumor volume, which were accompanied by a significant antioxidant effect [increment of SOD ($p < 0.001$) and GSH ($p < 0.01$)]. Oligandrin prevented high adenocarcinoma grade tumors according to SBR stratification and significantly reduced the levels of pro-inflammatory cytokines (IL-6; IL-12) while increasing anti-inflammatory cytokines (INF-γ). This study opens the way to better investigate the mechanism of action of oligandrin.

Ethics approval and consent to participate

Housing and animal treatments were approved by the Faculty of Science's Joint Institutional Review Board for Animal and Human Bioethics (Ref: BTC-JIRB2021-010), which followed the European Union's rules on both human and animal welfare (EEC Council 86/609).

Availability of data and materials

The data and materials used in this study are available upon request from the corresponding author (Stéphane Zingue, stephane.zingue@fmsb-uy1.cm).

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Consent to publish

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CRediT authorship contribution statement

Stéphane Zingue: Writing – original draft, Validation, Supervision, Investigation, Data curation, Conceptualization. **Yannick Stéphane Fotsing Fongang:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Eric Roger Ossomba:** Methodology, Investigation. **Vanneck Tatsinda:** Investigation. **Kevine Kamga Silihe:** Writing – original draft, Investigation, Data curation, Conceptualization. **William Defo Mbou:** Investigation. **Balotin Fogang:** Methodology, Investigation. **René Essomba:** Writing – review & editing, Methodology. **Jean Rodolphe Chouna:** Methodology, Investigation. **Dieudonné Njamen:** Writing – review & editing, Supervision. **Lawrence Ayong:** Writing – review & editing, Validation, Supervision, Conceptualization.

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.

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Abbreviations

ALT	Alanine transaminase
DMBA,	7,12-dimethylbenz(a)anthracene
EGF	Epidermal growth factor
FBS	Fetal bovine serum
GSH	Glutathione
IC_{50}	Inhibitory concentration
IFNγ	Interferon gamma
IL-10	Interleukin 10
IL-6	Interleukin 6
IL-1β	Interleukin
MDA	Malondialdehyde;
MFI	Median fluorescence intensity
PBMC	Peripheral blood mononuclear cells
PHA	Phytohemagglutinin
ROS	Reactive oxygen species
SOD	Superoxide dismutase;
TME	Tumor microenvironment
TNF	Tumor necrosis factor
TNF-α	Tumor necrosis factor-alpha
SEM	Standard error on the mean

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