**Pharmacology and Alternate Medicine Academic Journal**
Vol.1, No. 4, October-December-2022;
ISSN (7392 – 5820);
p –ISSN 7880 – 7492
Impact factor: 3.72

# PHARMACOLOGICAL POTENTIAL OF DHFCOP-A, DERIVATIVE OF OIL PALM EMPTY BUNCH ON TRIPLE NEGATIVE BREAST CANCER PATIENT-DERIVED XENOGRAFT MICE MODELS.

**Uzoamaka Adaobi Okoli, Michael Tochukwu Okafor, Chioli Pascal Chijoke and Iroka Joseph Udeinya**

University of Nigeria Nsukka, Department of Pharmacology and Therapeutics, Enugu Campus, Enugu, Nigeria.
Basic and Translation Cancer Research Group, College of Medicine, University of Nigeria, Nsukka
Department of Pharmacology and Therapeutics, College of Medicine, Enugu State University of Science and Technology, Enugu State, Nigeria

**Abstract**: **Introduction:** Triple-Negative Breast Cancer (TNBC) is clinically aggressive. There is growing evidence that the tumour microenvironment's low pH (acidity) tends to boost cancer aggressiveness. The acidity of the tumour microenvironment tends to correlate with high TNBC chemo-resistance. Strategic manipulation to raise the tumour microenvironment's pH may help discover new anti-cancer therapies. An anhydrous chemical, DFHCOP-A has high pH of 10.5 and is remarkably non-corrosive. However, no known study has established the effect of DFHCOP-A in altering tumour microenvironment pH.

**Material and Methods:** Ethical clearance was given. Patient-Derived Xenograft (PDX) model was derived from a consenting Nigerian woman with TNBC who had undergone mastectomy. 100mg/kg, 300mg/kg and vehicle of DFHCOP-A were administered to mice models after 1-week xenograft and 6-weeks xenograft in a time/dose-dependent manner.

**Results and Discussions:** 100mg/kg DFHCOP-A treatment inhibited cancer growth while 300mg/kg DFHCOP-A had no significant effect on cancer growth compared to vehicle control after 1-week xenograft. Paradoxically, there was a significant elevation of serum pH and ionic calcium depletion in the 100mg/kg treated group compared to the vehicle control and 300mg/kg-treated groups.

**Conclusion:** This study provides preliminary evidence that DFHCOP-A at a low dose may increase the pH of the tumour microenvironment to potentiate cancer drugs.

**Keywords:** Hormesis; Triple Negative Breast Cancer; pH; Tumour microenvironment; patient-derived xenograft; Nigerian woman; DFHCOP-A, ionic calcium, Lactate, Anion Gap, Serum Electrolytes, serum pH

## Introduction

Breast cancer is the leading cancer in women in the developing world. Epidemiological study shows that women of African descent have lower incidence and higher mortality rates than their Caucasian – counterpart [1]. This disparity between both races may be due to the late onset of aggressive metastases and genetic factors in the former [2–4]. Triple Negative Breast Cancer (TNBC) is a subtype of breast cancer that cannot be treated with targeted therapeutics owing to the lack of progesterone, oestrogen and human epithelial hormone receptors [5]. TNBC are clinically aggressive. There is

**Pharmacology and Alternate Medicine Academic Journal**
Vol.1, No. 4, October-December-2022;
ISSN (7392 – 5820);
p –ISSN 7880 – 7492
Impact factor: 3.72

mice after pre-treatment of the tumour with an acidic medium injected through the tail [11]. Their study revealed induction of proteinases production such as matrix metalloproteinase (MMP) and proangiogenic factors such as IL-8 and VEGFA) leading to metastasis. Another study illustrated an increase in tumour microenvironment pH by injection of sodium bicarbonate, which inhibited spontaneous metastasis [12]. These studies show that the tumour microenvironment's low or elevated pH either promotes or inhibits experimental metastasis.

An anhydrous chemical DFHCOP – A, derived from an empty palm bunch (*Elaeis genesis*), has a remarkably high pH of 10.5 and is non-corrosive. Empty palm bunch ash filtrate is used as an ingredient for saponification of palm oil to make a sauce base for a food delicacy known as Abacha (processed from cassava) in Nigeria, West Africa [13]. The physiochemical properties, anion and cation composition of DFHCOP-A have been elucidated [14]. Also, the LD50 value of DFHCOP- A in mice is known [13]. However, no known study has established the effect of DFHCOP-A in biological systems concerning altering the pH of the tumour microenvironment. Therefore, we hypothesize that DFHCOP-A will strategically manipulate the pH of the tumour microenvironment. This study aims to investigate the effect of DFHCOP-A administration on TNBC tumour xenograft growth and progression.

## 1. Results

### *1.1 Chemical composition and Physiochemical properties of DFHCOP-A*

DFHCOP-A is an anhydrous chemicals fractionated from the de- seeded fruit head of oil palm (Figure 1b), with pH remarkable high pH of 10 – 11. The chemical composition and physiochemical properties of DFHCOP-A has been previously determined The total dissolved solids (ppm) - TDS, total organic carbon (%) - TOC and, total organic matter (%) TOM, were 10540 ±538, 4.28±0.2, and 7.39±0.3 respectively (Table 1). DFHCOP-A is composed of anions including

growing evidence that the extracellular tumour microenvironment acidity tends to boost cancer aggressiveness. The low pH of the extracellular microenvironment tends to correlate with the high proportion of cancer cells being relatively resistant to some cancer therapeutics and may impede the immune rejection of tumour cells [6].

The extracellular pH of the tumour cell microenvironment is acidic owing to the production of lactate, an acidic metabolite by an anaerobic glycolytic pathway in hypoxia. This process is also known as the Warburg effect [7,8]. Lactate inhibits monocyte migration and cytokine release, enhances the motility of tumour cells, and increases migration and invasiveness in vitro by acidic pH [6]. Energy production in cancer cells relies on a combination of factors including oxidative phosphorylation, glycolysis, and other metabolic pathways. Oxidative phosphorylation and glycolysis generate acidic waste products from CO2 and H2, respectively. Redox potential increases due to increased concentration of hydrogen ions (H+) and decreased pH. A shift in redox balance occurs driven by intracellular reactive oxidative substances (ROS) due to the high metabolic rate of TNBC cells. Thus, intracellular accumulation of H+ from metabolism can negatively control metabolic activity and may limit cancer cell proliferation and survival. [9]. Similarly, accumulation of lactate can cause product inhibition of glycolytic activity. In effect, accelerated monocarboxylate transporter (MCT)-mediated export of lactate allows tumours defective in oxidative phosphorylation to proliferate much faster.

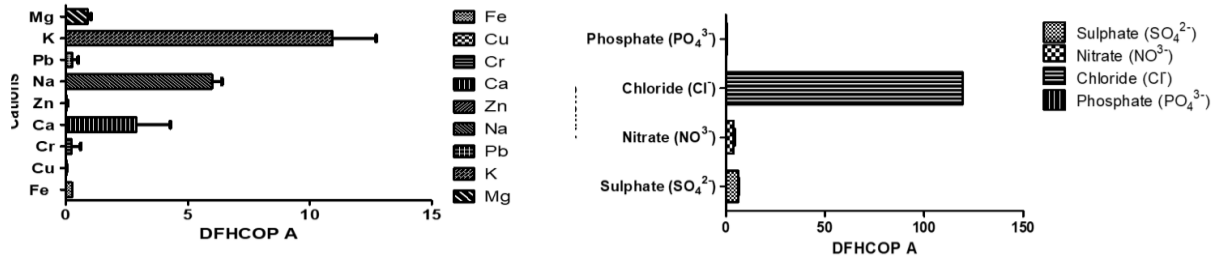
Defective oxidative phosphorylation will produce ROS, which may boost cell transformation and ultimately lead to tumorigenesis. TNBC is dependent on glucose metabolism, with the highest expression of glycolytic marker - MCT4 expression occurrence when compared to the other breast cancer subtypes [10]. Strategic manipulation to raise the tumour cell microenvironment's pH may help discover new anti-cancer drugs. Previous studies have demonstrated that an acidic tumour microenvironment promoted experimental metastasis in nude

**Pharmacology and Alternate Medicine Academic Journal**
Vol.1, No. 4, October-December-2022;
ISSN (7392 – 5820);
p –ISSN 7880 – 7492
Impact factor: 3.72

Magnesium, Potassium, Sodium, Calcium and cations including Chloride very little amount of sulphate and nitrate.

**Table 1. The Physicochemical properties of DFHCOP-A**

| pH | Conductivity (µS/cm) | TDS (ppm) | TOC (%) | TOM (%) | Melting Point ($^0$C) | Salinity mg/l |
|---|---|---|---|---|---|---|
| 10.5±0.5 | 111400±111.9 | 10540±537.9 | 4.28 ± 0.16 | 7.39 ± 0.27 | 330±0 | 2223.00 ±115.10 |




(a)


(b)

*Figure1: Chemical compostion of DFHCOP-A. 1a) Depictes the anions and cation contained in DFHCOP-A. 1b) Illustrates DFHCOP-A as a derivative of Oil Palm (Elaeis guineensis*).

300mg/kg. These doses formed the treatment conditions and including water as the vehicle control.

The TNBC PDX Female mice models were treated with vehicle control, 100 mg/kg and 300mg/kg of DFHCOP -A after one week of xenograft in groups of five each. Other groups were treated using the same treatment conditions but after 6 weeks of xenograft, at this stage the

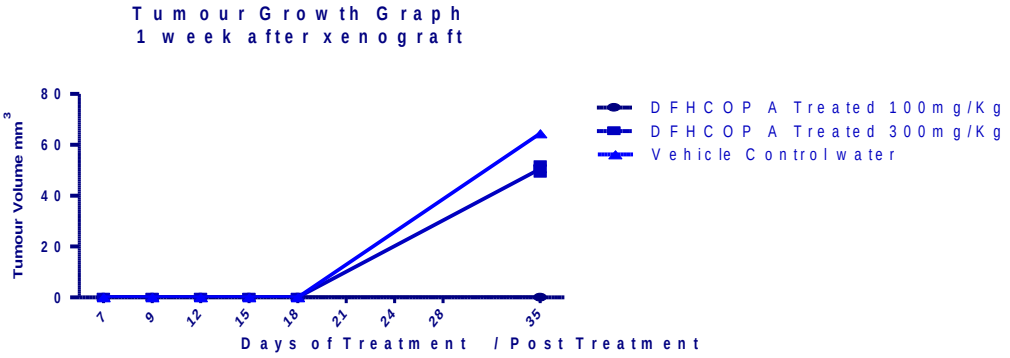
### *2.2. Effect of DFHCOP-A treatment on tumour progression in TNBC PDX Model*

Considering that DFHCOP -A has high pH and is remarkably non-corrosive, a low dose and high dose of DFHCOP-A was deduced from $LD_{50}$ of 3000mg/kg. Low dose was deduced as 100mg/kg while high dose at

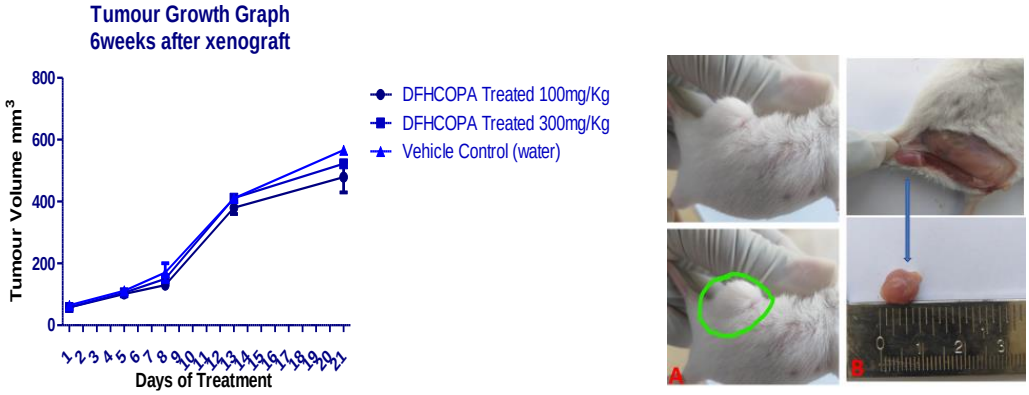
**Pharmacology and Alternate Medicine Academic Journal**
Vol.1, No. 4, October-December-2022;
ISSN (7392 – 5820);
p –ISSN 7880 – 7492
Impact factor: 3.72

DFHCOP-A had no significant effect on cancer growth when compared to vehicle control after one-week Xenograft (Figure 2a). The 100mg/kg and 300mg/kg DFHCOP-A treatment had no significant effect on cancer growth when compared to vehicle control after a 6-weeks Xenograft (Figure 2b)

orthotopic implanted tumours were palpable at 70mm$^{2.}$ After one week xenograft the mice were left up to 35 days, to monitor tumour growth. However ,100mg/kg DHFCOP-A inhibited growth while 300mg/kg DFHCOP-A had no significant effect on tumour growth when compared to normal. The 100mg/kg DFHCOP-A treatment inhibited cancer growth while 300mg/kg




(a)




(b) (c)

Figure 2: Effect of DHFCOP-A treatment on tumour progression.

from the control using two-way ANOVA/Bonferroni Post-test (Graph Pad Prism 5.0) P > 0.05) 2c.) A representative of tumour collected from the PDX model after 6-week Xenograft

**2.1. Effect of DFHCOP-A treatment on Serum electrolytes and chemical analysis including Lactate and oxidative stress.**

The serum calcium and electrolytes including potassium, sodium bicarbonate and chloride help maintain acid- base balance. The alkalinity (high pH) or acidity (low pH) of

2a.) The 100mg/kg DFHCOP-A treatment inhibited cancer growth while 300mg/kg DFHCOP-A had no significant effect on cancer growth when compared to vehicle control after 1-week Xenograft; 2b.) The 100mg/kg DFHCOP-A treatment and 300mg/kg DFHCOP-A had no significant effect on cancer growth when compared to vehicle control after a 6-week Xenograft (P<0.05). All graphs represent the mean and SEM of 5 PDX models' independent determinations of each group; data were analyzed for significant differences

**Pharmacology and Alternate Medicine Academic Journal**
Vol.1, No. 4, October-December-2022;
ISSN (7392 – 5820);
p –ISSN 7880 – 7492
Impact factor: 3.72

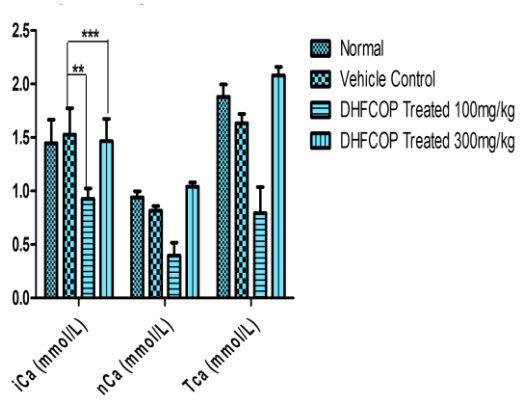
The anion gap (AG) was determined as the difference between sodium and the sum of chloride and bicarbonate. AG was determined for the treated and normal conditions. The AG values were calculated using the average serum electrolyte concentrations. The values of the treated were compared with the normal. The AG values for Normal, Vehicle Control, 100mg/kg and 300mg/Kg DFHCOP-A treated are 7, 13, 2 and 12 respectively. The vehicle control had a 85% increase; 300mg/kg DFHCOP-A treated had a 64% increase; while the 100mg/kg had a 75% decrease. Serum AG is useful in evaluating metabolic acidosis in patients. For reference, the normal range of AG in human is 4 to 12 mmol/L. To deduce if the tumor microenvironment of TNBC was manipulated, the serum pH , lactate dehydrogenase (LDH) and oxidative stress was analyzed. The serum pH values were elevated above normal value 7.3 in both the 100mg/ml and 300mg/ml DFHCOP-A treated when compared to the normal and vehicle control (Figure 2c). The serum bicarbonate (Figure 2d) result proportional to the serum pH results. Lipid peroxidase was significantly elevated ($P<0.05$) in 300mg/kg DFHCOP-A treated group when compared to the normal group indicating increased oxidative stress activity (Figure 2e). LDH is significantly elevated in the vehicle control, 100mg/kg and 300mg/kg DFHCOP-A treated groups when compared to the normal ($P<0.05$) (Figure 2f).

the blood can be indicated on the pH Scale. Hence the serum electrolytes of the treated TNBC PDX mice models after 6 weeks – xenograft blood serum were analysed.

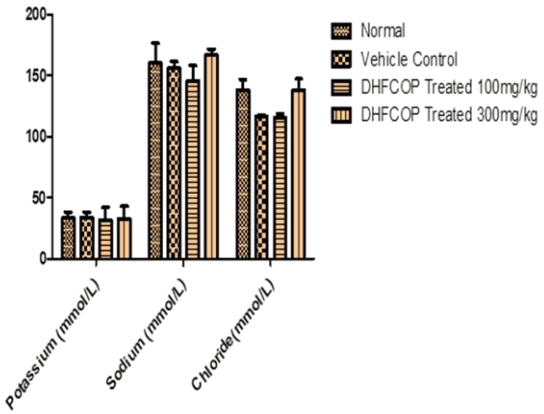
The serum calcium was analysed as ionic calcium (iCa), non-ionic (nCa) and total calcium(tCa). iCa was significantly reduced by 30% as well as nCa and tCa in 100mg/kg DFHCOP-A treated group compared to the normal, vehicle control groups and 300mg/kg treated. The serum potassium of mice treated with 100mg/kg and 300mg/kg DFHCOP-A when compared to the normal (without xenograft) and vehicle control (with xenograft) had no significant difference (p-value 0.05). The serum potassium value ranged from 40 to 45mmol/L.(Figure 2a)

The serum sodium indicated a slight decrease in the 100mg/kg DFHCOP-A treated which although not statistically significant ($\leq 0.05$) when compared to the normal, vehicle control and 300mg/kg DFHCOP-A treated. (Figure 2b)

The serum chloride value of the 300mg/kg DFHCOP-A treated was equivalent to the vehicle control. While the 100mg/kg DFHCOP-A treated was equivalent to the vehicle control. Both the vehicle control and 100mg/kg DFHCOP-A treated were slightly decreased when compared to both the normal and the 300mg/kg treated with non-significant decrease ($p \geq 0.05$).(Figure 2b)




(**a**)




(**b**)

**Pharmacology and Alternate Medicine Academic Journal**
Vol.1, No. 4, October-December-2022;
ISSN (7392 – 5820);
p –ISSN 7880 – 7492
Impact factor: 3.72

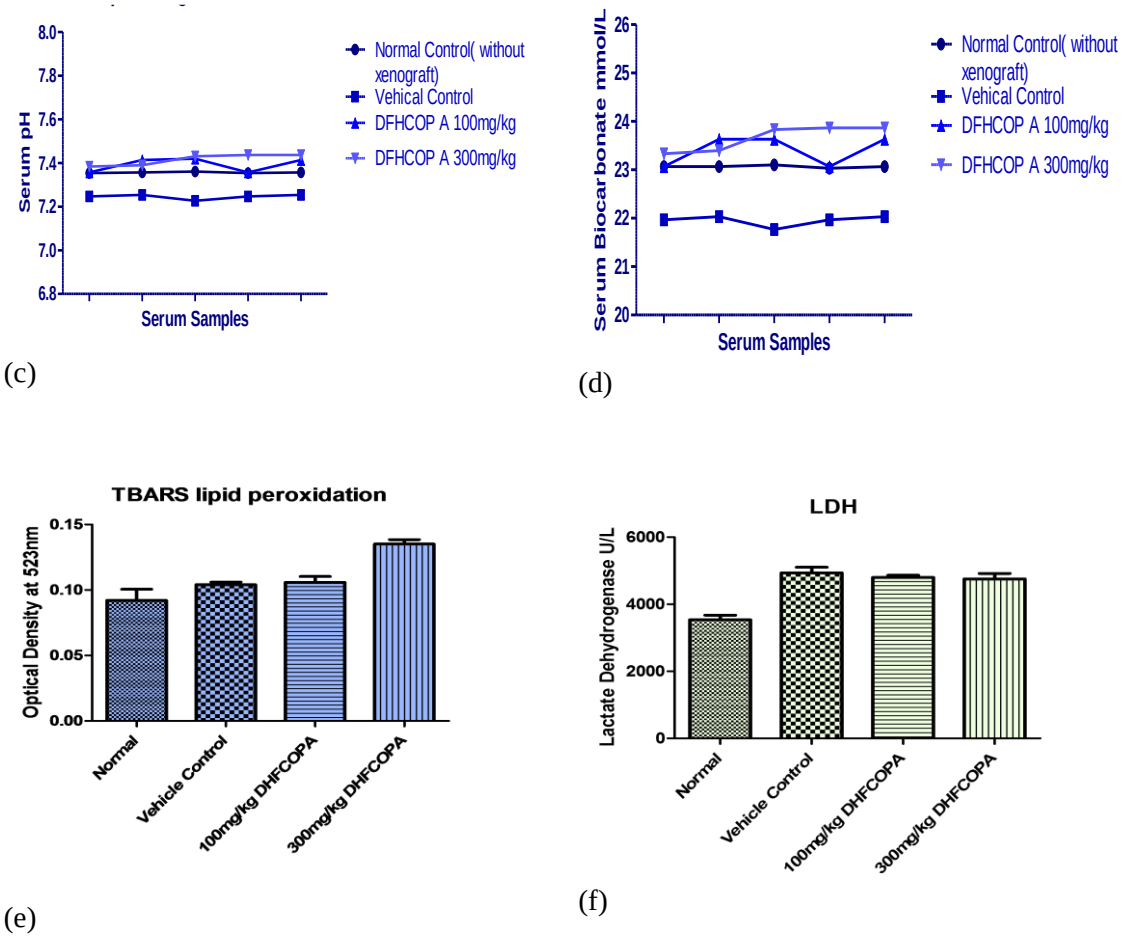



**Figure 3: Serum chemistry of TNBC PDX mice models after 6 weeks of Xenograft.**

compared to the normal group indicating increased oxidative stress activity; f) Lactate dehydrogenase (LDH) is significantly elevated in the vehicle control, 100mg/kg and 300mg/kg DFHCOP-A treated groups when compared to the normal. (P<0.05). All graphs represent the mean and SEM of 5 independent determinations, data were analysed for significant differences from the control using two-way ANOVA/Bonferroni Post-test (Graph Pad Prism 5.0) P > 0.05.

**3. Discussion**

Discussion

The strategic manipulation of tumour microenvironment pH is essential to develop therapies that may target the acidic nature of TNBC. The acidic tumour environment

a.) This illustrates that ionic calcium was significantly reduced by 30% as well as non-ionic (nCa)and total calcium(tCa) in 100mg/kg DFHCOP-A treated group compared to the normal, vehicle control groups and 300mg/kg treated group, (** P value <0.01; *** P value < 0.05); b.) All groups had no significant change in the potassium, sodium, and chloride serum electrolytes, (P > 0.05); c.)The 100mg/kg DFHCOP-A and 300mg/kg DFHCOP-A treatment elevated serum pH when compared to serum pH of normal mice while vehicle control serum pH remains below serum pH of normal mice; d.) These findings in fig c. also corelate with serum bicarbonate; e.) Lipid peroxidase was significantly elevated (P<0.05) in 300mg/kg DFHCOP-A treated group when

**Pharmacology and Alternate Medicine Academic Journal**
Vol.1, No. 4, October-December-2022;
ISSN (7392 – 5820);
p –ISSN 7880 – 7492
Impact factor: 3.72

clinical benefit and may reduce toxicity caused by anti-tumour drugs.

There is little or no literature on the effect of DFHCOP-A on breast cancer. Owing to the chemical composition of DFHCOP- A, it could be the combination of salts that may have caused the inhibition of tumour progression at one week of xenograft at a low dose. A variety of some of these salts has been demonstrated to show beneficial or stimulatory effects on breast cancer [21]. One of the limitations of this study is the unavailability of In vivo imaging system (IVIS) to monitor the tumour progression of the different PDX models' treatment conditions. However, this study showed that all treated and untreated cancer groups had significantly elevated LDH compared to the normal. According to Warburg in 1924, the extracellular pH of the tumour microenvironment is acidic. This phenomenon is due to the production of lactate by an anaerobic glycolytic pathway in hypoxia, also known as the Warburg effect. [7] Lactate production is by converting pyruvate to lactate and back, which the enzyme LDH catalyzes. In another study the authors found LDH to contribute to TNBC cells being glycolysis - dependent and LDH knockdown inhibited tumour growth in xenograft mice [22]. Due to the avid production of lactate, most tumours exhibit mild acidic extracellular pH[7,17]. Some researchers postulate that the intracellular pH of the tumour microenvironment is acidic, but certain studies have found the pH to be neutral or mildly alkaline [15]. Although tumour microenvironment pH was not measured using techniques such as pH-sensitive electrodes, MRI, PET, optics or MRS, the results of the serum pH of the PDX models validated their findings. The serum pH provides a potential measure since blood bathes the tumour and harbours molecular elements, including circulating tumour DNA (ctDNA). The serum pH of normal mice is in tandem with the physiological value of 7.4 (Silva et al., 2009). The vehicle control exhibited mild acidity lower than pH 7.4, while the treated groups were elevated slightly above normal pH but maintained mild alkalinity. The serum bicarbonate level, to some degree, may increase the subnormal pH without significant regulates the proliferation, apoptosis, and metastasis, which modulate the angiogenesis and immune response to tumour cells [15,16]. Direct oral administration of small alkaline molecules such as sodium bicarbonate may manipulate the tumour microenvironment pH [11]. This strategy has been demonstrated in mice and inhibits tumour proliferation and metastasis ([17]

In this study, DFHCOP-A was orally administered to TNBC PDX mice models to determine its effect on tumour progression. The PDX model has been established and characterized to ensure that the established tumour originates from primary tumours and not any random tumours from mice. Hence, the primary outcome is cancer or no cancer growth in treated and non-treated mice. Furthermore, the secondary outcome measures treatment time and dose dependency with a low dose (100mg/kg) compared to a high dose (300mg/kg) one week and six weeks after xenograft. DFHCOP-A at a low dose inhibited tumour growth after one week of xenograft, while the high dose did not affect tumour growth compared to vehicle control. At six weeks of xenograft, both high and low dose of DFHCOP-A has no significant effect on tumour growth. DFHCOP-A treatment low dose treatment for one week exhibited potential prophylactic properties. Therefore, one would expect the high dose to demonstrate a more beneficial effect than the low dose. However, the opposite effect of low and high DFHCOP-A treatment is in line with hormesis.

Hormesis is a biphasic response to exposure to an increasing amount of chemical substance, where low doses cause a stimulatory or beneficial response, and high doses cause inhibition or toxicity [18]. Some antitumour drugs exhibit a hormetic effect, e.g. rapamycin has demonstrated immune enhancement and antitumour development at a lower dose [19] A study that treated patient-derived organoids from prostate cancer with a low dose of a preclinical multikinase inhibitor AUM 302 demonstrates hormesis. AUM302 treatment elicited dose-dependent inhibition of proliferation, with significantly lower IC50s [20]. In this context, a low dose can be of

**Pharmacology and Alternate Medicine Academic Journal**
Vol.1, No. 4, October-December-2022;
ISSN (7392 – 5820);
p –ISSN 7880 – 7492
Impact factor: 3.72

detected in various cancers, promoting many aspects of tumour development and progression ([27].

The TNBC microenvironment pH in mice was successfully manipulated with DFHCOP-A. Further invitro studies are needed to investigate the potential use of DFHCOP-A to potentiate antitumor drugs. This may involve the use of different breast cancer organoids and ex vivo breast cancer tissues, to investigate apoptotic response of DFHCOP-A treatment or in combination with antitumor drugs. A spatial transcriptomics analysis of the cultured treated ex vivo explants may reveal new genes that can be targeted and mechanism of action of DFHCOP-A in combination with antitumor drugs.

## 4. Materials and Methods

### Animals

4-6week old nulliparous female albino were used for the study as per institutional guidelines. The mice were maintained in laboratory mouse cages with a 12-h light/12-h dark cycle. Sterile food pellets and autoclaved water were provided ad libitum.

### Pharmacologically Induced Immunosuppression in Mice.

A modified immunosuppression protocol [[28] was used to induce immunosuppression in mice. Briefly, mice were administered with 35mg/kg cyclosporin A ( Tocris, Biotechne, UK) intraperitoneally and 10mg/kg ketoconazole (Tocris, Biotechne, UK) by gavage for 5days. On day 6 and day 8, 200mg/kg and 100mg/kg cyclophosphamide (Tocris, Biotechne,UK) were administered by subcutaneous injection, respectively. On day 9, thawed frozen breast cancer tissue fragments derived from a consenting Nigerian woman[29] was surgical implanted into immunosuppressed mice.

### TNBC PDX models

TNBC xenograft has been characterised and established in immunosuppressed mice [30]. Breast cancer fragments 2- 3mm$^3$ in size, from the second passage were dipped in cultrex BME type 3,Pathclear purchased from R&D systems to further enhance tumour take up rate before orthotopic implantation into immunosuppressed mice as previously described[31,32].

interference with normal blood pH. An alkalemia pH increases calcium binding, hence a decrease in ionized calcium fraction [23]The ionized calcium (iCa) was notably decreased by 30%, likewise the non-ionized calcium (nCa) and total calcium (tCa), in the low dose DFHCOP-A treatment which corroborates their findings. Literature reveals that once cells choose to use high-energy phosphate compounds as metabolic currency, they encounter tremendous evolutionary pressure to maintain a low intracellular Ca2+ concentration ([Ca2+]i < 100 nM). Otherwise, salts of phosphate and calcium would precipitate, turning the cytosol into a bone-like solid. Accordingly, unique homeostatic mechanisms such as calcium pumps evolved. In turn, the low [Ca2+]i made it possible to produce a significant change in concentration with only a tiny Ca2+ flux across the cell membrane; this kind of leverage for signaling is not shared by sodium or potassium ions[9]. Again, our serum sodium and serum potassium results agree with their findings. An increase in AG as an indicator of acidosis has been postulated to be due to a number of factors including glycolysis, leading to high level of lactate in the blood (hyperglycemia). In our study, the high dose of DFHCOP-A exhibited high AG when compared to the normal. While the Low dose exhibited low level of AG both with elevated LDH. Although high dose AG is neither specific nor sensitive for detection of acidosis, it can be used as an adjunctive test [24]. High AG has been correlated with increase in cancer mortality[25]. As seen during treatment after one week xenograft, 300mg/kg did not inhibit tumour growth and has high AG. The notable thing here is that the low AG associated with low dose of DFHCOP-A corroborates the finding of decrease in ionic calcium demonstrating hormetic low dose effect of DFHCOP-A. Entry from extracellular influx initiates calcium signaling as indicated by the low dose of DFHCOP-A. Calcium influx plays an essential role in breast cancer [[26]]. A high dose of DFHCOP-A stimulated increased oxidative stress activity, while the low dose did not affect oxidative stress level. Elevated production of reactive oxygen species has been

**Pharmacology and Alternate Medicine Academic Journal**
Vol.1, No. 4, October-December-2022;
ISSN (7392 – 5820);
p –ISSN 7880 – 7492
Impact factor: 3.72

vehicle control- (with xenograft) for Groups A,B,C&D. Vehicle was water and DFHCOP-A was kindly provided by Prof Iroka Udeinya. Size of tumour of the treated and untreated xenograft models were assessed.

The tumour volumes were measured using a Vernier calliper when tumour growth became palpable. Tumour sizes were measured twice a week (length and width), for the duration of treatment. Tumour volume was calculated as (3.14 × length × width × width/6)[33].

Biochemical analysis after six weeks of treatment with DHFCOP-A effects on lactate dehydrogenase (LDH), serum pH and electrolytes, lipid peroxidase (oxidative stress indicator) were measured on the treated and vehicle control.

**Dose and time relationship of DFHCOP-A in breast cancer progression in mice.**

Following successful tumour xenografts in 30 mice and 10 normal (no xenograft), weighing 24 ± 5 g and 8–12 weeks of age the mice were divided into four groups of treatment conditions, A, B, C and D (n = 5). Two of the groups A & B were treated using low dose of 100mg/kg and high dose 300mg/kg DFHCOP-A, respectively after one week of xenograft to determine dose relationship.

Groups C & D were treated using 100mg/kg and 300mg/kg for 21 days, respectively after six weeks tumour xenograft were palpable and have reached 70mm$^3$. To determine time relationship (Figure 3) . Group C&D were compared to Group A & B. Group E served as

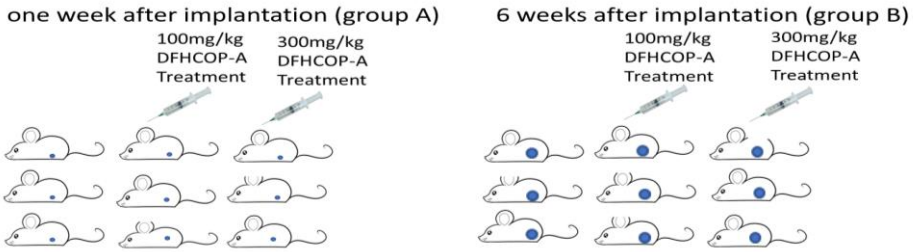



Figure 3 illustrates dose and time relationship of DFHCOP-A in breast cancer progression in mice.

Anion gap = $[Na^+]$ – ($[Cl^-]$ + $[HCO3^-]$) which is serum sodium level (mmol/L) minus the sum of serum chloride level (mmol/L) and serum bicarbonate level(mmol/L). Where $[Na^+]$ is sodium concentration, $[Cl^-]$ is Chloride concentration and $[HCO3^-]$) is bicarbonate concentration.

**Data Analysis**

Two- way/ANOVA/ Bonferroni Post-test (Graph Pad Prism 5.0) was used for statistical analysis.

**5. Conclusions**

This study provides preliminary evidence that DFHCOP-A at low dose may increase pH of tumour microenvironment to potentiate cancer drug.

**Author Contributions:** Individual contribution of authors are as follows, "Conceptualization, UAO and IJU, methodology, UAO and MTO.; formal analysis, UAO investigation, UAO and MTO.; resources, UAO and IJU, data curation, UAO and MTO writing—original draft preparation, UAO; writing—review and editing, UAO,

**To analyse locate dehydrogenase, pH, electrolytes, lipid peroxidase and in DFHCOP-A treated PDX models**

Lactose dehydrogenase (LDH), serum pH, serum electrolytes, lipid peroxidase in DFHCOP-A treated PDX models. After day 21 of Group B treatment, blood was collected by cardiac puncture. Mice were euthanized by chloroform inhalation and tumour collected for storage by cryopreservation and some fixed in paraffin wax for future further analysis. Blood was centrifuged and serum collected for serum pH and electrolyte analysis by Ion Selective Electrode Method using pH Electrolyte Analyser (Perlong) Ghaudong ,China. LDH analysis was done using Cobas c111, Roche, Germany. Lipid Peroxidase was assessed using TBARS Elisa kit (Elabscience) according to Manufacturer's protocol.

**Anion Gap Determination**

AG was calculated using the formula,

MTO, CPC and IJU supervision IJU and CPC.; funding acquisition, UAO. All authors have read and agreed to the published version of the manuscript

**Funding:** This research was part funded by African Research League, grant number ARL 2019 cycle

**Institutional Review Board Statement:** The study involving a Nigerian woman and animal study protocol was conducted in accordance with the Declaration of Helsinki and approved by the College of Medicine Research ethics committee, UNIVERSITY OF NIGERIA (065/03/2019) and approved on 21/02/2019.

**Informed Consent Statement:** Informed consent was obtained from a consenting Nigerian woman with TNBC who had undergone mastectomy and neoadjuvant chemotherapy for use of breast cancer tissue.

Kato Y, Ozawa S, Miyamoto C, et al. Acidic extracellular microenvironment and cancer. *Cancer Cell Int*. 2013;13(1):89. doi:10.1186/1475-2867-13-89

Liberti M v., Locasale JW. The Warburg Effect: How Does it Benefit Cancer Cells? *Trends Biochem Sci*. 2016;41(3):211. doi:10.1016/J.TIBS.2015.12.001

Liu C, Jin Y, Fan Z. The Mechanism of Warburg Effect-Induced Chemoresistance in Cancer. *Front Oncol*. 2021;11:3408. doi:10.3389/FONC.2021.698023/XML/NLM

Boedtkjer E, Pedersen SF. The Acidic Tumor Microenvironment as a Driver of Cancer. *https://doi.org/101146/annurev-physiol-021119-034627*. 2020;82:103-126. doi:10.1146/ANNUREV-PHYSIOL-021119-034627

Putney LK, Denker SP, Barber DL. The changing face of the Na+/H+ exchanger, NHE1: structure, regulation, and cellular actions. *Annu Rev Pharmacol Toxicol*. 2002;42:527-552. doi:10.1146/ANNUREV.PHARMTOX.42.092001.143801

Hao G, Xu ZP, Li L. Manipulating extracellular tumour pH: an effective target for cancer therapy. *RSC Adv*. 2018;8(39):22182-22192. doi:10.1039/C8RA02095G

Robey IF, Baggett BK, Kirkpatrick ND, et al. Bicarbonate increases tumor pH and inhibits spontaneous metastases. *Cancer Res*.

**Acknowledgments:** This study was presented as a poster presentation at the MDPI 7th International Electronic Conference on Medicinal Chemistry session Fighting cancers https://doi.org/10.3390/ECMC2021-11529

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

**References**

Newman LA, Kaljee LM. Health disparities and triple-negative breast cancer in african American women a review. *JAMA Surg*. 2017;152(5):485-493.

Yedjou CG, Sims JN, Miele L, et al. Health and Racial Disparity in Breast Cancer. *Adv Exp Med Biol*. 2019;1152:31. doi:10.1007/978-3-030-20301-6_3

Feng Y, Spezia M, Huang S, et al. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes Dis*. 2018;5(2):77. doi:10.1016/J.GENDIS.2018.05.001

Spratt DE, Chan T, Waldron L, et al. Racial/Ethnic Disparities in Genomic Sequencing. *JAMA Oncol*. 2016;2(8):1070-1074. doi:10.1001/jamaoncol.2016.1854

Yao H, He G, Yan S, et al. Triple-negative breast cancer: is there a treatment on the horizon? *Oncotarget*. 2017;8(1):1913. doi:10.18632/ONCOTARGET.12284

Published online 2010:1-13. doi:10.1007/978-1-60761-495-1_1

Gaya A, Akle CA, Mudan S, Grange J. The Concept of Hormesis in Cancer Therapy – Is Less More? *Cureus*. 2015;7(4). doi:10.7759/CUREUS.261

Luszczak S, Simpson BS, Stopka-Farooqui U, et al. Co-targeting PIM and PI3K/mTOR using multikinase inhibitor AUM302 and a combination of AZD-1208 and BEZ235 in prostate cancer. *Sci Rep*. 2020;10(1). doi:10.1038/S41598-020-71263-9

Lappano R, Malaguarnera R, Belfiore A, Maggiolini M. Recent advances on the stimulatory effects of metals in breast cancer. *Mol Cell Endocrinol*. 2017;457:49-56. doi:10.1016/J.MCE.2016.10.017

Jhan JR, Andrechek ER. Triple-negative breast cancer and the potential for targeted therapy. *Pharmacogenomics*. 2017;18(17):1595. doi:10.2217/PGS-2017-0117

Goldstein DA. Serum Calcium. *THE JOURNAL OF INFECTIOUS DISEASES* •. 1990;130(5):677-679. Accessed October 30, 2021. https://www.ncbi.nlm.nih.gov/books/NBK250/

Salem MM, Mujais SK. Gaps in the Anion Gap. *Arch Intern Med*. 1992;152(8):1625-1629.

2009;69(6):2260-2268. doi:10.1158/0008-5472.CAN-07-5575

Okoli UA, Okafor MT, Nubila NI, Okpe AC, Chijioke CP, Udeniya IJ. Toxicity study of DHFCOP-A, chemical derived from empty oil palm bunch ash in mice. *West Afr J Pharmacol Drug Res*. 2021;34(1):36-42. Accessed September 1, 2022. https://www.ajol.info/index.php/wajpdr/article/view/218473

Udeinya IJ, Nubila NI, Okoli UA, et al. Oil Palm Deseeded Fruit Head Ash: Fractionation and Characterization. *Adv Mat Res*. 2021;1160:75-82. doi:10.4028/WWW.SCIENTIFIC.NET/AMR.1160.75

Justus CR, Dong L, Yang L v. Acidic tumor microenvironment and pH-sensing G protein-coupled receptors. *Front Physiol*. 2013;4. doi:10.3389/FPHYS.2013.00354

Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nature Reviews Cancer 2004 4:11*. 2004;4(11):891-899. doi:10.1038/nrc1478

McCarty MF, Whitaker J. Manipulating tumor acidification as a cancer treatment strategy. *Alternative Medicine Review*. 2010;15(3):264-272.

Mattson MP, Calabrese EJ. Hormesis: What it is and why it matters. *Hormesis: A Revolution in Biology, Toxicology and Medicine*.

study. *Cryobiology*. 2020;97:179-184. doi:10.1016/J.CRYOBIOL.2020.05.006

Okoli UA, Okafor MT, Agu KA, et al. Abstract 1679: Characterization and establishment of triple negative breast cancer patient derived xenograft derived from a Nigerian woman. *Cancer Res*. 2020;80(16_Supplement):1679-1679. doi:10.1158/1538-7445.AM2020-1679

Andreasson A, Kiss NB, Juhlin CC, Höög A. Long-term storage of endocrine tissues at-80 C does not adversely affect rna quality or overall histomorphology. *Biopreserv Biobank*. 2013;11(6):366-370. doi:10.1089/bio.2013.0038

Derose YS, Gligorich KM, Wang G, et al. Patient-derived Models of Human Breast Cancer: Protocols for In vitro and In vivo Applications in Tumor Biology and Translational Medicine. *Curr protoc pharmacol*. 2014;(801):1-52. doi:10.1002/0471141755.ph1423s60.Patient-derived

Liu H, Murphy CJ, Karreth FA, et al. Identifying and targeting sporadic oncogenic genetic aberrations in mouse models of triple-negative breast cancer. *Cancer Discov*. 2018;8(3):354-369. doi:10.1158/2159-8290.CD-17-0679

doi:10.1001/ARCHINTE.1992.00400200063011

Verma A, Qayyum R. Anion gap and cancer mortality: Insight from NHANES database. *https://doi.org/101200/JCO20173515_suppl.e13068*. 2017;35(15_suppl):e13068-e13068. doi:10.1200/JCO.2017.35.15_SUPPL.E13068

Azimi I, Roberts-Thomson SJ, Monteith GR. Calcium influx pathways in breast cancer: opportunities for pharmacological intervention. *Br J Pharmacol*. 2014;171(4):945. doi:10.1111/BPH.12486

Liou GY, Storz P. Reactive oxygen species in cancer. *Free Radic Res*. 2010;44(5):479-496. doi:10.3109/10715761003667554

Jivrajani M, Shaikh Mv, Shrivastava N, Nivsarkar M. An Improved and Versatile Immunosuppression Protocol for the Development of Tumor Xenograft in Mice. *Anticancer Res*. 2014;34(12).

Okoli UA, Okafor michael T, Agu KA, et al. Methodology for processing mastectomy and cryopreservation of breast cancer tissue in a resource- poor setting: A pilot