HORMETIC LOW DOSE EFFECT OF DFHCOP-A DEPLETES IONIC CALCIUM AND ELEVATES SERUM pH TO INHIBIT CANCER IN NIGERIAN-TRIPLE NEGATIVE BREAST CANCER PDX MODEL

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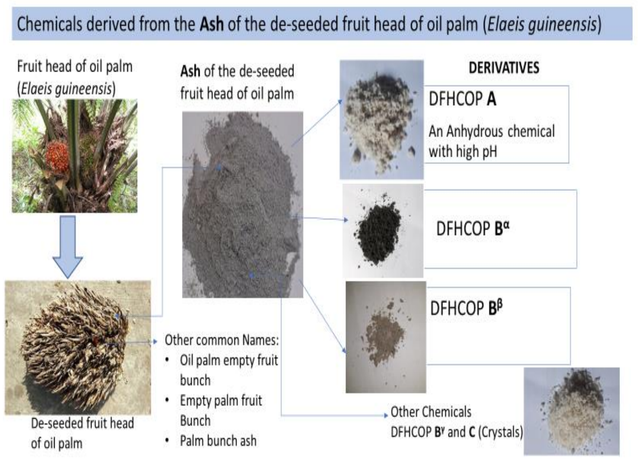
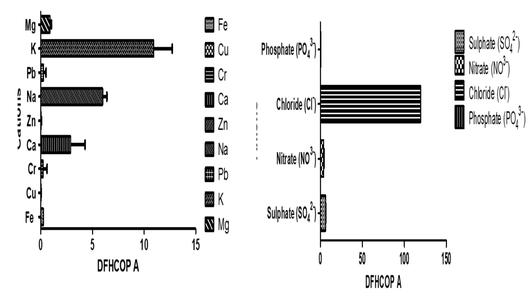
• INTRODUCTION

Triple Negative Breast Cancer (TNBC) cannot be treated with targeted therapeutics and are clinically aggressive. There is growing evidence that low pH (acidity) of the tumour microenvironment tends to boost cancer aggressiveness. The acidity of extracellular microenvironment tends to correlate with the high proportion of cancer cells being relatively resistant to some cancer therapeutics. Strategic manipulation to raise pH of tumour cell micro-environment may help in discovery of new anti - cancer therapies.

The de-seeded fruit head of the fruit head of the oil palm (DFHCOP) is usually incinerated, and the ash filtrate used as ingredient of common food dishes such as 'Abacha' (made from cassava) in Nigeria. Anhydrous chemical derived from DFHCOP (- A) has a high pH of 10.5 and is remarkably non-corrosive, although no known study has established the effect of DFHCOP-A in biological systems concerning altering pH of the tumour microenvironment.





pH	Conductivity	TDS	TOC	TOM	Melting point	Salinity
10.5 ± 0.05	11392 ± 2	11738 ± 11	4 ± 0.04	6.8 ± 0.08	330 ± 0	196 ±0.5

RESULTS

- Effect of DFHCOP-A on triple negative breast cancer

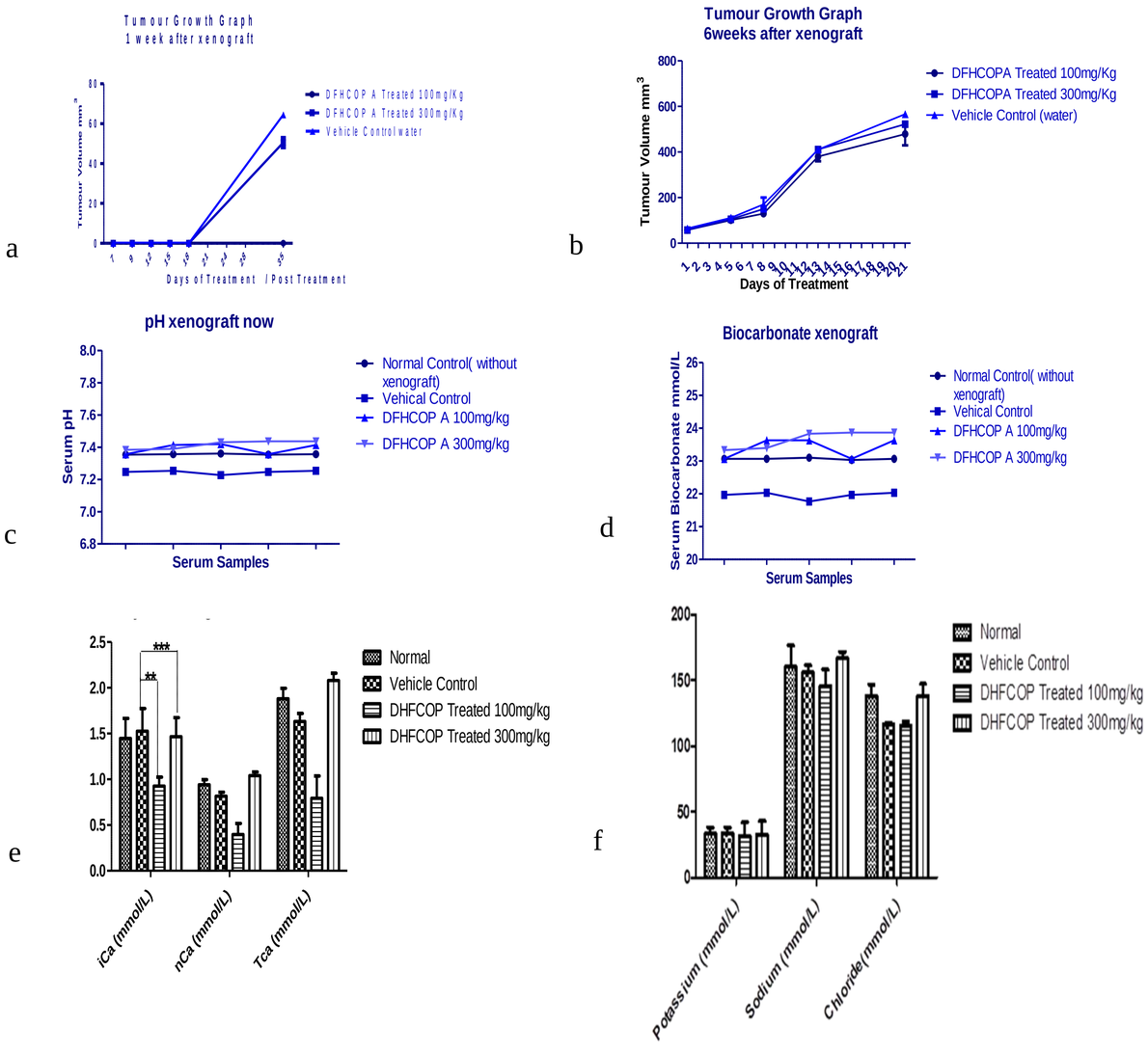


Figure 2: Effect of DHFCOP-A treatment on tumour progression xenograft.

a.) 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; b.) The 100mg/kg DFHCOP-A treatment and 300mg/kg DFHCOP-A had no significant effect on cancer growth when compared to vehicle control after 6-week xenograft; 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.) This illustrates that ionic calcium was significantly reduced by 30% 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); f.) 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; g) 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); h.) All groups had no significant change in the potassium, sodium and chloride serum electrolytes, (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

• MATERIAL AND METHODS

- Ethical clearance was obtained from the College of Medicine Research ethics committee, University of Nigeria (065/03/2019) and PDX model was derived from a consenting Nigerian woman with TNBC who had undergone neoadjuvant chemotherapy. 100mg/kg, 300mg/kg and vehicle of DFHCOP-A were administered to mice models after 1-week xenograft and after 6-weeks xenograft.

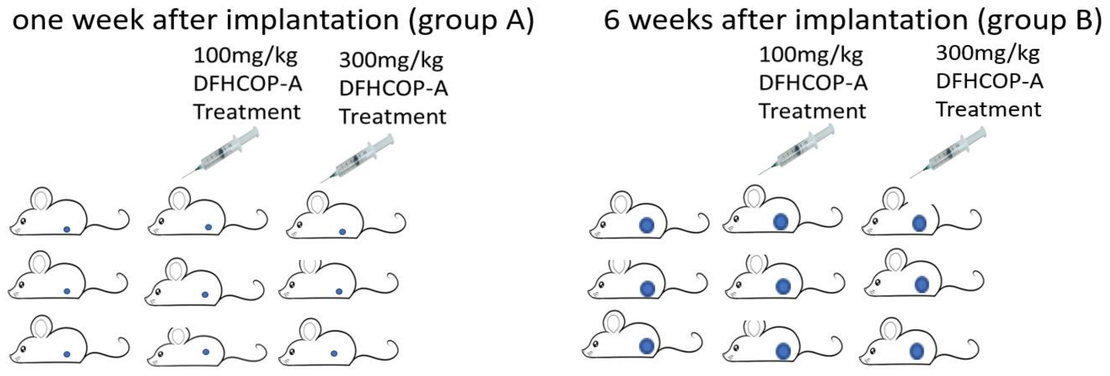


Figure 3: Schematic representation of DFHCOP-A treatment

Primary outcome measures

1) Cancer or no cancer growth in treated and non treated group

Secondary outcome measures

2) Time and dose dependency of treatment with 100mg/kg (low dose) and 300mg/kg (high dose) one week after implantation and 6 weeks after implantation

DISCUSSION and CONCLUSION

- In this study fig showed that all cancer groups both treated and untreated had elevated LDH. According to Warburg in the 1920's the extracellular pH of the microenvironment of tumour cell is acidic owing to the production of lactate by anaerobic glycolytic pathway in hypoxia also known as Warburg effect. (Liberti & Locasale, 2016)
- Lactate is produced by the conversion of pyruvate to lactate and back which is catalysed by the enzyme lactate dehydrogenase (LDH).
- In another study by Jhan et al 2017, LDH was found to contribute to TNBC cells being glycolysis - dependent and LDH knockdown inhibited tumour growth in xenograft mice.
- Owing to the avid production of lactate most tumours exhibit mild acidic extracellular pH (Liberti & Locasale, 2016; McCarty & Whitaker, 2010).
- The serum pH of normal mice in figure 26 is in tandem with the physiological value of 7.4 (Silva et.al,2009).
- Clearly the vehicle control exhibited mild acidity lower than pH 7.4 while the treated groups were elevated to normal. The serum bicarbonate level to some degree may elevate the subnormal pH without notably interference with normal blood pH.
- An alkalemic pH leads to an increase in calcium binding hence a decrease in the fraction of ionized calcium (Goldstein, 1991).The ionic calcium (figure 27)was notably decreased by 30% in the low dose DFHCOP-A treatment.
- Calcium signalling can be initiated by entry from extracellular influx. Calcium influx has been recognized to play a role in breast cancer (Azimi et. al, 2014). Anti cancer drugs like cisplatin has been shown to interfere with intracellular calcium signalling while inducing apoptosis. (Büsselberg & Florea, 2011. Cisplatin or carboplatin which contains metal - platinum, is usually the first line of treatment in TBNC and other cancer types.
- In figure 24, high dose of DFHCOP-A stimulated increased oxidative stress activity while low dose had no effect on oxidative stress level.
- Elevated production of reactive oxygen species have been detected in various cancers, where they promote many aspects of tumour development and progression (Liou & Storz, 2010). As there is little or no literature of the effect of DFHCOP-A on breast cancer, a combination of its chemical components have been demonstrated to show beneficial or stimulatory effects on breast cancer. (Lappano et. al, 2017 and Craig, 1934).
- The opposite effect of low and high DFHCOP-A treatment is in line with hormesis.
- Hormesis can be defined as a biphasic response to exposure to increasing amount of chemical substance, where low dose cause a stimulatory or beneficial response, and high dose cause inhibitory or toxicity. Limitations of this stusy include lack of resources to further assay for hypoxia 1and VEGF
- In conclusion, this study provides preliminary evidence that DFHCOP-A at low dose may be used as an adjunct in TNBC treatment to increase pH of extracellular space and may potentiate cancer drugs.

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