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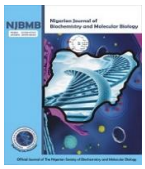

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*Research Article*

# Effect of Ketogenic Diet on 7,12-Dimethylbenz(A)anthracene (Dmba)-Induced Breast Cancer in Female Wistar Rats

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## ABSTRACT

Cancer of the breast is known to affect women worldwide. Dietary patterns have been associated with increased risk of breast cancer and modification of diet may have a positive effect on the progression of cancer. The study investigated the effect of a ketogenic diet on the progression of 7,12-dimethylbenz(a) anthracene (DMBA)-induced mammary cancer in female Wistar rats. Three different ketogenic diets were prepared using white rice, catfish and sunflower oil. Ketogenic diets A, 10% carbohydrate (CHO), B (20% CHO) and C (30% CHO) were fed to rats and after the confirmation of breast cancer. DMBA (15 mg/kg bw) was used to induce breast cancer in experimental rats. Administration of DMBA resulted in a significant ($P \leqslant 0.05$) increase in the levels of cancer antigen 15-3 (CA15-3), carcinoembryonic antigen (CEA), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), bilirubin, total cholesterol (TC), triglyceride (TG) and low density lipoprotein-cholesterol (LDL-c) and a decrease in the levels of albumin, total protein, high density lipoprotein-cholesterol (HDL-c), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and platelet (PLT) compared to normal rats. Ketogenic diets at different concentrations significantly reduced the biochemical parameters assayed in a dose-dependent manner with diet A group showing significant ($p \leqslant 0.05$) reduction in the levels of CA15-3, CEA, ALT, AST and ALP as well as bilirubin, TC, TG and LDL-c and significant ($P \leqslant 0.05$) increase in HDL-c, SOD, GR, GPx, RBC. Therefore, the results suggest that the administration of the ketogenic diet (sunflower oil, catfish and rice) containing 10% carbohydrate could be beneficial in reducing the progression of breast cancer.

**Keywords**: *Breast cancer, Dimethylbenz-(A)-anthracene, Ketogenic diet, Tumour markers*

## INTRODUCTION

Cancer is a disease condition due to uncontrolled cell division in the body to form mass of tissues. These cells (cancer) can infiltrate adjacent or surrounding structures, spread to distant sites and proliferate uncontrollably within the body causing death (Brown *et al*., 2023). In 2020, WHO rated cancer as the leading cause of death globally accounting for nearly 10 million deaths or nearly one in six deaths. According to the WHO, the most common form of cancers are breast, lungs, colon, rectum and prostate. Several efforts have been put in place to combat cancer by the world health organization, but cancer remains a serious threat to health worldwide (WHO, 2022). Meanwhile, the numbers of cases of certain types of cancer, such as liver, prostate cancer, colon cancer, cervical cancer and gastrointestinal cancer have declined while others such as breast cancer are increasing (Joshua *et al*., 2023). Breast cancer occurs in women more than any

form of cancer (WHO, 2023). According to report, breast cancer is the leading cause of death in women worldwide accounting for about 23% of all new cases of cancer in Nigeria (Sade, 2023). Nigerian women die of breast cancer than women from other parts of Africa (Agodirin *et al*., 2023). Breast cancer accounted for 14,274 (18.1%) of all cancer deaths in Nigeria, making it the most common cause of cancer-related death (IARC, 2020). According to a recent multinational study conducted across sub-Saharan Africa (SSA), the three-year survival rate for breast cancer patients in Nigeria was the lowest (McCormack *et al*., 2020). Breast cancer risk factors have largely remained difficult to change or alter them unlike dietary pattern and obesity (Xio *et al*., 2019).

The 7,12-dimethylbenz(a) anthracene (DMBA), is a procarcinogen specifically used to induce breast cancer in experimental female Wistar rats, it undergoes metabolic activation to carcinogen dihydrodiolepoxide (Liu *et al*., 2016). The carcinogen and mutagenic activity of DMBA require metabolic activation by mixed-function oxidases located in rat liver microsomes. The dihydrodiolepoxide binds with adenine residues of deoxyribonucleic acid, resulting in mutagenesis and carcinogenesis (Liu *et al.,* 2016).

Ketogenic diet (KD) is composed of high-fat, moderate-protein and low-carbohydrate (Masood *et al*., 2023). The diet mimics physiological fasting, where fats are utilized which may be beneficial in the treatment of cancer. During fasting, the body converts ketones bodies, including acetoacetate, acetone and B-hydroxybutyrate, which can be used for energy by the various cells, including muscles cells and neutrons. And for those cells that require sugar, gluconeogenesis can generate sugar *de novo* from the glycerol that is produced during lipolysis and thus, the levels of sugar in the blood stay within the normal range during ketosis (Dhillon and Gupta, 2023). Recent studies indicate that, ketogenic diet (KD) significantly decreased tumour volume and increased the survival time in mouse model for prostate cancer, this effect was observed without restricting total calories and the mouse did not lose the body weight (Li *et al*., 2021). Ketogenic diet had been demonstrated to be therapeutically useful for the treatment of epilepsy and cardiovascular diseases (Imdad *et al*., 2022). It has also been found to be effective as an adjuvant therapy for other types of cancer such as glioblastoma, stomach and colon cancer (Martin-McGill *et al*., 2020). Hence the need to study the effects of ketogenic diet on breast cancer. This study therefore, is aimed at studying the anticancer potential of ketogenic diet as a dietary intervention for delaying the progression and possible treatment of breast cancer.

## MATERIALS AND METHODS

### Chemicals and reagents

7,12-dimethylbenz-(a)-anthracene (DMBA) and 2,2-Diphenyl-1-picylhydryl (Sigma Aldrich, Germany). Biochemical kits for the assay of ALT, AST, γ-GT, BIL, TP, CHOL, TG, HDL, LDL, and α-fetoprotein were purchased from Randox Ransod Ltd, UK. All other chemicals and reagents used were of analytical grade.

### Experimental animals

Eighty-four virgin adults female Wistar rats weighing 160 ± 20 g were purchased from the National Veterinary Research Institute Vom, Jos, Nigeria. The rats were maintained under standard laboratory conditions (12h light and dark cycles, 22 ± 2°C and relative humidity of 50 - 65%), fed with Vital Feed, standard rodent pellet diet (Grand Cereal and Oil Mills, Ltd., Jos) and tap water supplied *ad libitum*. The rats were treated in accordance with the universally accepted guidelines for animal experimentation.

### Preparation of ketogenic diets

The ketogenic diet was prepared according to the method of Healy *et al*., (2015) with modifications to macronutrients (carbohydrate, proteins and fats) contents. The keto diet **A** contained 10% carbohydrate, 70% fat and 20% protein, keto diet **B** contained 20% carbohydrate, 60% fat and 20% protein and keto diet **C** contained 30% carbohydrate, 50% fat and 20% protein. The source of carbohydrates was white rice, the source of protein was dried catfish while the source of fat was sunflower oil.

### Induction of breast cancer

The 7, 12-dimethylbenz(a) anthracene (DMBA) was dissolved in sunflower oil (0.75ml) and physiological saline (0.25ml). Breast cancer was induced with DMBA subcutaneously at a dosage of 15 mg/kg body weight in the mammary region once a week for a period of four (4) weeks (Jaykumar *et al*., 2021).

### Confirmation of breast cancer using cancer antigen 15-3 test and CEA

The CA 15-3 and CEA ELISA kit is a solid-phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The CA 15-3 and CEA ELISA tests were used to confirm the presence of breast cancer (Welander, 1992).

### Experimental design

A total of eighty-four (84) virgin female Wistar rats were used, they were divided into six groups (n = 14). Group I served as normal control. Breast carcinogenesis was induced in groups II, III, IV, V and VI through the injection of DMBA at a dosage of 15 mg/kg body weight in the mammary region once a week for a period of four (4) weeks subcutaneously. Group II served as experimental control while Group III was treated with the standard drug vincristine at a dose of 500 µg/kg intraperitoneally weekly for 12 consecutive weeks. Groups IV, V and VI were fed

with the ketogenic diet containing 10%, 20% and 30% carbohydrates respectively. The ketogenic diet was given to the rats *ad libitum* for a period of 12 weeks.

**Collection of blood and tissue samples**

The administration of the ketogenic diet lasted for a period of 12 weeks after which the animals were sacrificed twelve hours after the last administration in accordance with guidelines of the European Convention for the Protection of Vertebrate Animals and other scientific purposes (ETS-123). Rats were sacrificed by cardiac puncture under anaesthesia. Blood was collected in two separate sample bottles, a plain sample bottle for biochemical parameters and EDTA bottle for evaluation of haematological parameters. Serum was separated from the blood by centrifuging the blood sample at 10,000 rpm for 5 minutes and stored at −20°C for analysis. Breasts were carefully removed and collected for histopathology analysis.

**Biochemical estimation**

**Cancer biomarkers test**

The cancer biomarkers (CA-15-3 and CEA) were determined according to the method described by Welander (1992).

**Liver marker test**

**Determination of aspartate aminotransferase (AST)**

The AST activity was determined using commercially prepared kits according to the method described by Reitman and Frankel, (1957).

**Determination of alanine transaminase (ALT)**

The activity of the enzyme was also estimated according to the method described by Reitman and Frankel (1957) using kits.

**Determination of alkaline phosphatese (ALP)**

The activity of ALP was determined according to the method described by Plummer (1978) using commercially prepared kits.

**Determination of total protein**

The method described by Kroll, (1998) was used to determine the concentration of total protein. Briefly, 20 microlitre of serum was added to 1mL of biuret reagent. It was mixed thoroughly and incubated for 10 minutes. After ten minutes of incubation, the blue colour formed was read using a photometer at 640 nm.

**Determination of serum bilirubin**

The concentration of Serum Albumin (ALB) was determined using the method described by Busher, (1990).

**Liquid profile**

**Determination of total cholesterol (TC)**

Determination of total cholesterol was based on CHOD-PAP enzyme colorimetric method described by Meiattini (1978).

**Determination of triglyceride (TG)**

Determination of serum triglycerides was based on the GPO-PAP enzyme colorimetric method which involves lipase-catalysed hydrolysis (Tietz, 1995).

**Determination of high-density lipoprotein (HDL)**

Estimation of HDL was done according to the direct measurement method as described by Warnick *et al*., (2001).

**Determination of low-density lipoprotein (LDL)**

The estimation of LDL was done according to the method described by Belcher *et al*., (1991).

**Antioxidant markers**

**Determination of superoxide dismutase (SOD)**

The total SOD activity was determined using the method of Misra and Fridovich (1972).

**Determination of glutathione peroxidase (GPx)**

Glutathione peroxidase activity was determined using the method of Flohe and Gunzler (1984).

**Determination of glutathione reductase (GR)**

Glutathione reductase activity was determined according to the method described by Dillio *et al*., (1983) based on the oxidation of NADPH at 340 nm.

**Determination of haematological parameters**

Complete blood count was analysed using automated coagulating Sysmex apparatus of the type 8999. The parameters analysed include: haemoglobin (Hb), red blood cells count (RBCs), white blood cells count (WBCs), platelets (PLT), and packed cell volume (PCV) (Merghani, 2010).

**Histological examination**

Haematoxylin and Eosin staining techniques was employed for studying the tissue histology. Specimens of breasts were collected and placed in a solution of 10% formaldehyde to fix. Sections were taken and processed using the automatic tissue processing machine for 24hrs. It underwent microtomy and thin sections of about 0.5micron were obtained. This was then stained in prepared Haematoxylin and Eosin staining solution, using standard staining procedure. It was mounted and examined under a microscope (Akshatha *et al*., 2018).

### Statistical analysis

Results were expressed as mean ± SEM (Standard Error of Mean). Test for significant difference between two means was done using one-way analysis of variance (ANOVA) and post hoc test using Duncan Multiple Range Test. Significant differences was considered at p< 0.05. Statistical package for Social Scientist (SPSS) version 23 (USA) was used for the analysis.

## RESULTS

Table 1 below shows the effect of ketogenic diet on cancer markers. The levels of CA15-3 and CEA were significantly increased (P<0.05) in the negative control group (group II) following administration of DMBA compared to normal control group. The administration of ketogenic diets at variable concentrations significantly decreased (P<0.05) in a dose dependent manner the levels of CA15-3 and CEA when compared with group II, with the highest reduction observed in group administered keto diet A.

**Table 1**. Effect of Ketogenic Diet on Cancer Antigen (CA 15-3) and Carcinoembryonic Antigen (CEA) in DMBA-Induced Mammary Cancer

| Group | CA 15-3 (U/mL) | CEA (ng/mL) |
|---|---|---|
| Normal control | 24.00 ± 1.55 | 1.66 ± 0.22 |
| Negative control | 59.73 ± 2.78$^{a}$ | 15.75 ± 1.56$^{a}$ |
| Positive control (Vincristine 500µg/kg bw) | 29.74 ± 0.40$^{b}$ | 4.75 ± 0.63$^{b}$ |
| Ketogenic diet A (10% CHO, 20% protein 70% Fat) | 30.19 ± 1.72$^{b}$ | 5.58 ± 0.42$^{b}$ |
| Ketogenic diet B (20% CHO, 20% protein 60% Fat) | 36.99 ± 1.02$^{c}$ | 7.53 ±0.59$^{c}$ |
| Ketogenic diet C (30% CHO, 20% Protein 50% Fat) | 44.11 ± 1.67$^{d}$ | 10. 85 ±0.18$^{d}$ |

Values are Mean ± SEM, n = 7. Mean values with different superscripts in the same column are significantly different (p < 0.05).

The effect of ketogenic diet on liver function marker-enzymes in DMBA-induced mammary cancer is shown in Table 2. The activities of ALT, AST and ALP significantly increased (P>0.05) in the negative control group (group II) following administration of DMBA compared to normal control group. However, the administration of ketogenic diets at variable concentrations significantly reduced the levels of ALT, AST and ALP (P<0.05) in a dose dependent manner when compared with group II (59.16 ±1.73 IU/L, 42.45±0.92 IU/L and 69.86 ±0.88 IU/L respectively) with the highest reduction seen in the keto diet A group (22.93 ± 0.89 IU/L, 24. 89 ±0.83 IU/L and 39.15 ±1.59 IU/L, respectively).

**Table 2**. Effect of Ketogenic Diet on Liver Function Maeker-Enzymes in DMBA-Induced Mammary Cancer

| Group | ALT (IU/L) | AST(IU/L) | ALP (IU/L) |
|---|---|---|---|
| Normal control | 20.78 ± 1.34 | 24.01 ± 0.78 | 37.60 ± 1.09 |
| Negative control | 59.16 ± 1.73$^{a}$ | 42.45± 0.92$^{a}$ | 69.86 ± 0.88$^{a}$ |
| Standard drug control | 21.29 ± 1.11$^{b}$ | 23.32 ± 0.97$^{b}$ | 37.31 ± 0.55$^{b}$ |
| Keto diet A | 22.93 ± 0.89$^{b}$ | 24. 89 ± 0.83$^{b}$ | 39.15 ± 1.59$^{b}$ |
| Keto diet B | 29.49 ± 0.81$^{c}$ | 29.67 ± 1.90$^{c}$ | 44.02 ± 1.03$^{c}$ |
| Keto diet C | 37.04 ± 1.34$^{d}$ | 36.15± 0.84$^{d}$ | 48.67 ± 0.93$^{d}$ |

Values are Mean ± SEM, n = 7. Mean values with different superscripts in the same column are significantly different (p < 0.05).

Results in Table 3 reveal the effect of the ketogenic diet on non-enzyme markers of liver. The concentrations of albumin and total protein were significantly decreased (P<0.05) while that of bilirubin was significantly increased in the negative control group (group II) following administration of DMBA compared to normal control group. Upon the administration of ketogenic diets at variable concentrations, their levels (albumin and total protein) were significantly increased (P < 0.05) while bilirubin decreased in a dose dependent manner when compared with group II, with the highest increase/reduction seen in the keto diet A group. The decrease was found to be comparable to group administered standard drug (group III).

**Table 3**. Effect of Ketogenic Diet on Non-Enzymes Markers of Liver in DMBA-Induced Mammary Cancer

| Group | Albumin (g/L) | Total Protein (g/L) | Bilirubin (umol/L) |
|---|---|---|---|
| Normal control | 31.04 ± 1.11 | 70.48 ± 0.87 | 0.37 ± 0.08 |
| Negative control | 19.31 ± 0.49$^{a}$ | 39.02 ± 1.49$^{a}$ | 2.95 ± 0.78$^{a}$ |
| Standard drug control | 30.91 ± 0.97$^{b}$ | 69.29 ± 0.99$^{b}$ | 0.57 ± 0.03$^{b}$ |
| Ketogenic diet A | 29.78 ± 1.08$^{b}$ | 68.02 ± 0.78$^{b}$ | 0.54 ± 0.05$^{b}$ |
| Ketogenic diet B | 25.62 ± 0.81$^{c}$ | 63.29 ± 0.94$^{c}$ | 1.29 ± 0.08$^{c}$ |
| Ketogenic diet C | 21.70 ± 0.86$^{d}$ | 54.12 ±1.09$^{d}$ | 1.47 ± 0.32$^{d}$ |

Values are Mean ± SEM, n = 7. Mean values with different superscripts in the same column are significantly different (p < 0.05).

Table 4 shows the effect of the ketogenic diet on lipid profile parameters in DMBA-induced mammary cancer. The administration of DMBA significantly increased (P < 0.05) the levels of TC, TG and LDL in the negative control group (group II) while that of HDL was significantly decreased in the negative control group (group II). However, the administration of ketogenic diets at variable concentrations significantly decreased (P<0.05) their levels (TC, TG and LDL) while HDL level was increased in a dose dependent manner. Reduction in the lipid profile parameters in Keto group A was comparable to the standard drug (Vincristine) group.

**Table 4**. Effect of Ketogenic Diet on Lipid Profile Parameters in DMBA-Induced Mammary Cancer in mg/dL

| Group | Total Cholesterol | Triglyceride | HDL-Cholesterol | LDL-Cholesterol |
|---|---|---|---|---|
| Normal control | 93. 07 ± 1.89 | 75.15 ± 2.41 | 68.68 ± 0.98 | 59.23 ± 1.03 |
| Negative control | 245. 45 ± 1.77$^{a}$ | 179.08 ± 1.73$^{a}$ | 24.21 ± 1.28$^{a}$ | 169.09 ± 1.05$^{a}$ |
| Standard drug | 97.89 ± 1.09$^{b}$ | 77.39 ± 1.97$^{b}$ | 64.72 ± 1.95$^{b}$ | 83.34 ± 1.38$^{b}$ |
| Ketogenic diet A | 98.23 ± 0.68$^{b}$ | 78.38 ± 1.87$^{b}$ | 65.09 ± 1.82$^{b}$ | 89.90 ± 0.91$^{b}$ |
| Ketogenic diet B | 106.08 ± 0.99$^{c}$ | 89.43 ± 1.78$^{c}$ | 56.19 ± 1.04$^{c}$ | 136.03 ± 1.99$^{c}$ |
| Ketogenic diet C | 113.32 ± 1.45$^{d}$ | 107.08 ± 2.11$^{d}$ | 40.30 ± 0.72$^{d}$ | 148.29 ± 1.09$^{d}$ |

Values are Mean ± SEM, n = 7. Mean values with different superscripts in the same column are significantly different (p < 0.05).

The activities of SOD, GR and GPx as shown in Table 5 were significantly reduced (P < 0.05) in all the groups following the administration of DMBA compared to the normal group. The administration of ketogenic diets at different concentrations significantly increased (P < 0.05) the activities of the enzymes in a dose-dependent manner when compared with group II, with the highest increase seen in the group administered 10% carbohydrate (keto diet A group).

**Table 5**. Effect of Ketogenic Diet on Antioxidant Markers in DMBA-Induced Mammary Cancer

| Group | SOD (U/ml) | GR (U/L) | GPx (U/mL) |
|---|---|---|---|
| Normal control | 4.99 ± 0.05 | 49.24 ± 1.57 | 46.03 ± 2.97 |
| Negative control | 1.31 ± 0.05$^{a}$ | 20.34 ± 1.45$^{a}$ | 19.43 ± 0.60$^{a}$ |
| Standard drug | 3.93 ± 0.05$^{b}$ | 37.18 ± 1.03$^{b}$ | 35.44 ± 0.49$^{b}$ |
| Ketogenic diet A | 3.79 ± 0.03$^{b}$ | 38.21 ± 1.89$^{b}$ | 36.08 ± 0.33$^{b}$ |
| Ketogenic diet B | 2.63 ± 0.04$^{c}$ | 30.51 ± 1.32$^{c}$ | 29.99 ± 0.37$^{c}$ |
| Ketogenic diet C | 2.39 ± 0.06$^{c}$ | 26.43 ± 1.48$^{d}$ | 25.02 ± 0.66$^{d}$ |

Values are Mean ± SEM, n = 7. Mean values with different superscripts in the same column are significantly different (p < 0.05).

Table 6 shows the levels of WBC, RBC, Hb, PCV, MCH and PLT in DMBA Induced Mammary Cancer. All the levels of the haematological parameters significantly decreased (P < 0.05) except that of WBC which significantly increased in all the groups following administration of DMBA compared to normal control group. The administration of ketogenic diets at variable concentrations significantly increased (P < 0.05) their levels (RBC, Hb, PCV, MCH and PLT) in a dose dependent manner while WBC decreased in a similar manner when compared with group II, with the highest increase and decrease observed in group administered keto diet A.

**Table 6.** Effect of Ketogenic Diet on Haematological Parameters in DMBA Induced Mammary Cancer

| Group | WBC ($x10^3$/µL) | RBC ($x10^6$/µL) | Hb (g/dL) | PCV (%) | MCH (pg/cell) | PLT ($x10^3$/µL) |
|---|---|---|---|---|---|---|
| Normal control | 6.66 ± 1.06 | 7.11 ± 0.29 | 13.28 ± 0.63 | 43.30 ± 0.21 | 45.20 ± 0.19 | 491.89 ± 0.34 |
| Negative control | 28.06 ± 1.23$^{a}$ | 3.08 ± 1.10$^{a}$ | 8.11 ± 1.33$^{a}$ | 26.93 ± 1.14$^{a}$ | 28.09 ± 1.03$^{a}$ | 420.34 ± 0.05$^{a}$ |
| Standard drug control | 7.31 ± 1.07$^{b}$ | 7.47 ± 0.76$^{b}$ | 13.03 ± 0.17$^{b}$ | 42.93 ± 0.52$^{b}$ | 44.20 ± 0.98$^{b}$ | 489.39 ± 0.19$^{b}$ |
| Ketogenic diet A | 8.52 ± 1.11$^{b}$ | 6.99 ± 1.24$^{b}$ | 12.73 ± 0.75$^{b}$ | 42.30 ± 1.02$^{b}$ | 43.11 ± 1.08$^{b}$ | 488.13 ± 0.07$^{b}$ |
| Ketogenic diet B | 15.23 ± 1.90$^{c}$ | 5.41 ± 1.09$^{b}$ | 12.02 ± 1.39$^{b}$ | 36.31 ± 1.66$^{c}$ | 37.29 ± 1.23$^{c}$ | 476.90 ± 0.12$^{c}$ |
| Ketogenic diet C | 20.04 ± 1.43$^{d}$ | 4.35 ± 1.23$^{ab}$ | 10.14 ± 1.09$^{c}$ | 30.62 ± 1.43$^{d}$ | 31.36 ± 0.97$^{d}$ | 462.02 ± 0.89$^{d}$ |

Values are Mean ± SEM, 7. Mean values with different superscript in the same column are significantly different (P < 0.05).

Histopathological analysis of breast tissues from various groups are represented as plates. Plate 1 is the photomicrograph of normal breast showing normal morphology as seen by the presence of connective tissue interspersed by lobules and ducts. The lobules show intact nuclei, surrounded by intact cytoplasmic components. Plate 2 is photomicrograph of breast tissue from negative group, showing massive lobular proliferation as seen by increased lobular masses, congestion and massive reduction in intercellular spaces. Haemorrhage is evident due to the massive presence of red blood cells within the tissue. There is nuclei hypertrophy as seen by lobules presenting

increased nuclei sizes with stromal atrophy evident by the reduction in the stroma of the tissue. Few fat cells are seen within the stroma. Plate 3 shows breast tissue of positive control rats with normal morphology, mild stromal atrophy as evident by the reduction in stroma. The lobules are present with intact nuclei. Plate 4 is photomicrograph of breast of keto diet A Group rats showing normal morphology. Lobules, presenting with intact nuclei, ducts and stroma. Plate 5 is breast tissue of rats fed keto diet (B Group), showing a characteristic interpose of lobules and stroma. The blood vessels are seen within the tissue some of which contain red blood cells. Plate 6 shows photomicrograph of breast of rats fed keto diet C, showing massive proliferation of lobules and stromal atrophy evident by the reduction in the stroma. Congestion is due to presence of red blood cells within the tissue. The lobules present with hypertrophied nuclei.

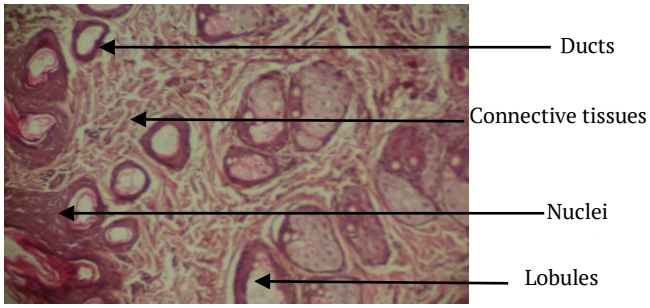


**Plate 1:** Photomicrograph of Breast of Normal Control Rats. H&E: X100

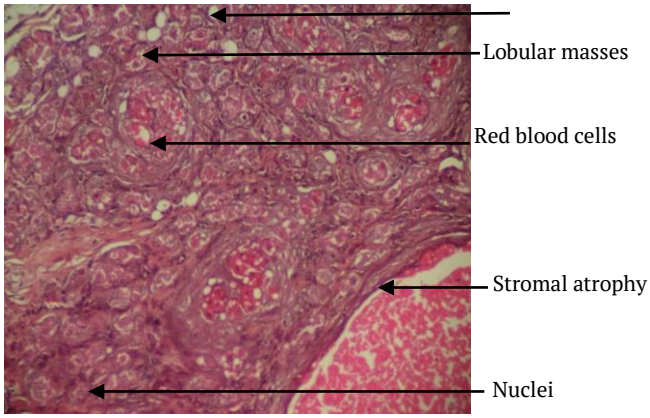


**Plate 2:** Photomicrograph of Breast of Negative Control Rats. H&E: X100

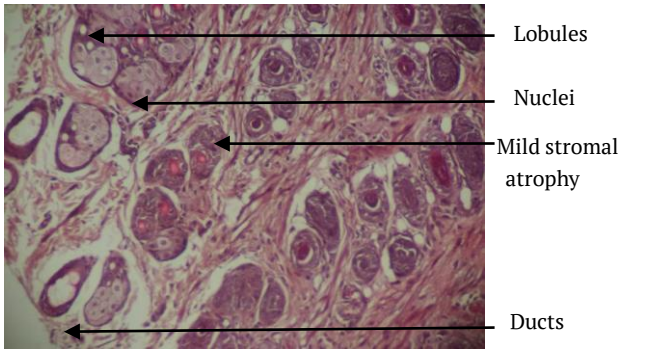


**Plate 3:** Photomicrograph of Breast of Positive Control Rats. H&E: X100

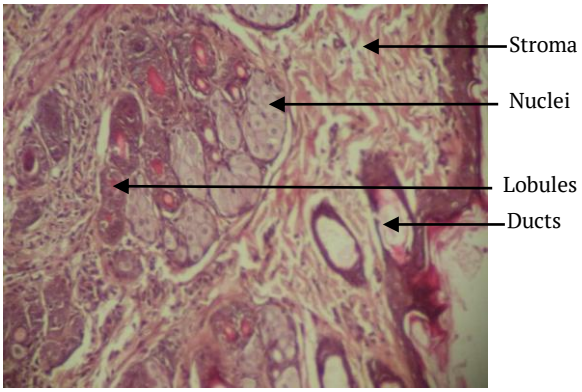


Plate 4: Photomicrograph of Breast of Keto Diet A Group. H&E: X100.

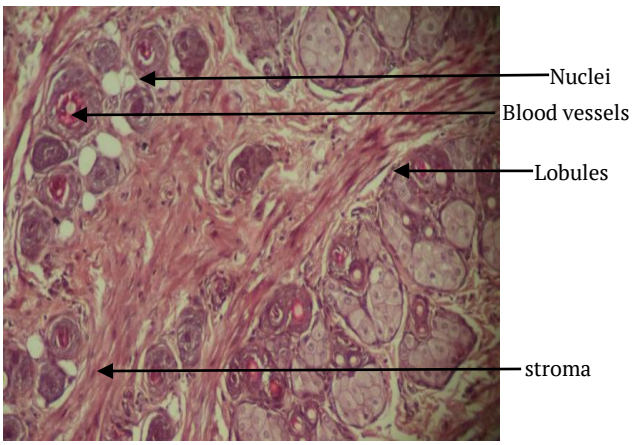


**Plate 5:** Photomicrograph of Breast of Keto Diet B Group Rats. H&E: X100

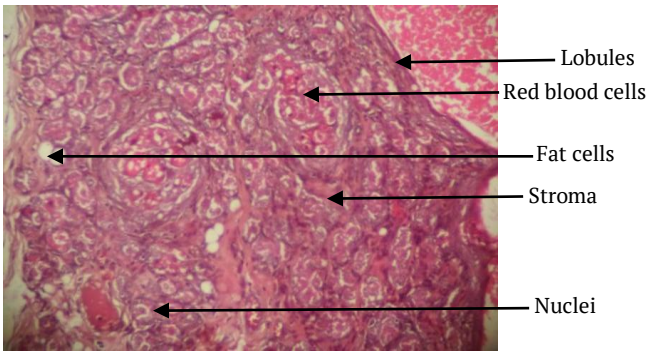


**Plate 6:** Photomicrograph of Breast of Keto Diet C Group Rats. H&E: X100

## DISCUSSION

This study was aimed at investigating the efficacy of a ketogenic diet supplemented with sunflower oil, catfish and white rice in reducing the progression of breast cancer using a DMBA-induced rat model. Our results demonstrate that the administration of DMBA resulted in several biochemical and morphological changes in rats, including an increase in the level of CA15-3 and CEA. Some authors were of the opinion that carcinoembryonic antigen (CEA) and carbohydrate antigen 15–3 (CA15-3) can be used to monitor and analyse radiological response in metastatic breast cancer. Thus, these markers are used for monitoring the

therapeutic response of metastatic breast cancer patients. The majority of tumour markers are used for early diagnosis, determining prognosis, monitoring therapeutic efficacy and follow-up after therapy (Khushk *et al*., 2021). CA15-3 (also known as mucin 1) is overexpressed in >90% of human breast cancers and in their subsequent metastases. CA15-3 promotes tumour invasion and metastasis through activation of the mitogen-activated protein kinase signalling pathway and downregulation of E-cadherin. Thus, elevated levels of CA15-3 predict a poor prognosis with an increased risk of metastasis (Yang *et al.,* 2017). Similarly, CEA has also been observed to correlate with treatment response (Lee *et al.,* 2013). Results of our study are consistent with previous studies that link CA15-3 and CEA levels with breast cancer prognosis Thus results from these studies indicate that elevated levels of CEA and CA15-3 in the negative control group may be predictive of increased tumour burden in breast cancer of the rats. The keto diet A group, in particular, showed the highest reduction of CA15-3 and CEA levels comparable to the standard drug vincristine.

Enzyme activities reflect the proliferation of cells growth and their activities are shown to have a good correlation with the number of transformed cells in cancer conditions. Serum enzyme and non-enzyme assessments are a useful tool in clinical diagnosis (Klein *et al*., 2020). The rise in serum alkaline phosphatase activity may be a sign of liver injury since it is most likely caused by increased cell membrane permeability, which allows the enzymes to seep out of the tissues and into the serum (Klein *et al*., 2020). The two main enzymes that are known to be linked to liver parenchymal cells are alanine transaminase (ALT) and aspartate transaminase (AST). The activity of these enzymes increase as liver injury increases in severity (Lala *et al*., 2023). The decrease in ALT, AST and ALP activities upon administration of the ketogenic diets suggests that these diets could prevent liver damage resulting from DMBA exposure. Similarly, the improvement observed in the concentrations of albumin and total protein suggests a reversal of the negative effects of DMBA on liver function. The exact mechanism for this improvement is not known, however, it could be attributed to the ability of ketogenic diets to reduce oxidative stress and inflammation (Lu e*t al*., 2018).

The decrease in albumin and total protein was probably due to DMBA disruption and dissociation of polyribosomes leading to reduced or defective protein biosynthesis. The increase in bilirubin level may be due to disturbance in the transport functions of the hepatocytes as a result of the hepatic injury causing the leakage of enzymes from cells due to altered permeability of membrane (Ramakrishna *et al*; 2011). The increase in the lipid profile parameters after the administration of DMB may be due to increased production by neoplastic cells for new membrane biogenesis. The decrease in the TC, TG and LDL is in agreement with the findings of Zhang *et al.* (2018). This decrease suggests that these diets could reduce the risk of developing cardiovascular diseases, despite DMBA exposure, and may improve general health. On the other hand, the increase in HDL level, which was observed following the administration of the ketogenic diets, further supports a reduction in cardiovascular disease. A ketogenic diet may promote healthy lipid metabolism by inducing ketosis, where the body shifts from using glucose to ketones as a primary energy source (Maswood *et al*., 2023). During ketosis, fatty acids are converted into ketones in the liver through beta-oxidation (Cantrell and Mohiuddin, 2023). This process enhances mitochondrial function, leading to increased fat oxidation and improved lipid profile, including reduced triglycerides and increased HDL cholesterol (Cantrell and Mohiuddin, 2023).

Studies generally suggest that KD intake benefited cardiac metabolic efficiency and acted as a cardio-protective antioxidant. Various pathways might underlie the effects of ketogenic diets in cardiomyocytes and endothelial cells in different models. In cardiomyocytes, βHB regulates PI3K/Akt pathway and succinate dehydrogenase (SDH) in mitochondria to finally ameliorate cell apoptosis. However, elevated βHB might also act by inhibiting HDAC2 and influencing mitochondrial biogenesis, leading to myocardial fibrosis. Ketone bodies inhibit mTOR pathway to regulate the level of eNOS in endothelial cells, then dilates blood vessels and improves vascular function. Besides, elevated ketone bodies can give rise to mild oxidative/electrophilic stress, activate Nrf2 in the cytoplasm and enhance antioxidant gene expression, which leads to lowered ROS levels and improved vascular functions (Zhang *et al.,* 2021).

The activities of SOD, GR and GPx were significantly reduced in the negative control group after DMBA exposure, consistent with previous studies indicating that oxidative stress could increase in the liver following DMBA exposure (Muqbil *et al*., 2020). Oxidative stress increases due to decreased antioxidants levels thereby increasing the risk of cancer development. They can also induce defects in the cellular signal transformation and the progression and the resistance to treatment (Khalaf *et al.,* 2021).

The administration of the ketogenic diets was found to restore the activities of these enzymes, indicating that these diets could have antioxidative properties. Ketogenic diets are said to exert antioxidant effects by reducing oxidative stress and inflammation (Greco *et al*., 2016). Ketosis induces the production of ketone bodies, particularly beta-hydroxybutyrate (BHB) which acts as a signalling molecule (Newman and Verdin, 2017). BHB enhances antioxidant defence mechanisms by upregulating endogenous antioxidant pathways, such as increasing the expression of antioxidant enzymes. Additionally, ketones reduce the production of reactive oxygen species during energy metabolism, contributing to the overall antioxidant effect of the ketogenic diet (Newman and Verdin, 2017).

Our findings showed that the ketogenic diets reversed the haematological changes caused by DMBA exposure. The increase and decrease in platelet count and white blood cells respectively in the negative control group could lead to haematological disorders, including bleeding and anaemia,

which were not observed in any of the ketogenic diet groups. The observed increases in red blood cell count and haematocrit levels in the ketogenic diet groups suggest an improvement in blood quality. Research on the specific effects of ketogenic diet on haematological changes caused by DMBA exposure is limited. However, ketogenic diets have been associated with anti-inflammatory and antioxidant effects (Greco *et al*., 2016) which might have indirectly contributed to the haematological changes caused by DMBA exposure. The reduction of oxidative stress and inflammation through ketosis is said to positively impact haematological parameters. Ketones, particularly BHB may influence cellular function and mitigate the adverse effects of toxic exposures (Newman and Verdin, 2017). Thus, enhanced antioxidant defences as with ketosis could potentially serve to protect blood cells from damage caused by DMBA.

Finally, the histological changes observed in the negative control group, including the presence of massive lobular proliferation, stromal atrophy and increased nuclei sizes, are consistent with previous reports of breast cancer (Joshua *et al*., 2023). However, the results of our histological analyses showed that the administration of ketogenic diets could prevent these changes, leading to normal breast tissue morphology.

## CONCLUSION

Our findings reveal that the consumption of a ketogenic diet supplemented with sunflower oil, catfish and rice could be beneficial in reducing the progression of breast cancer in the DMBA-induced rat model.

## AUTHORS' CONTRIBUTIONS

The study was designed by HAU, who also prepared the ketogenic diet. DD and MSN supervised the research while MIB and HOA handled the Biochemical analysis. All authors contributed to the development of the manuscript and approved it.

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## CONFLICT OF INTEREST

The authors declare that there is no conflicting interest whatsoever.

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