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Original Research Article

Effect of Ketogenic Diet on the Progression of 7, 12-Dimethylbenz(A) Anthracene –Induced Mammary Cancer in Female Wistar Rats

Hussaini Joshua[1*], Hauwa A. Umaru[1], Daniel Dahiru[1], Mela Yoro[2]

[1]Department of Biochemistry Modibbo Adama University of Technology, Yola Adamawa State, P.M.B 2076, Nigeria
[2]Department of Chemical Sciences, Faculty of Science, Federal University of Kashere, Gombe, Nigeria

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***Corresponding author:** Hussaini Joshua
Department of Biochemistry Modibbo Adama University of Technology, Yola Adamawa State, P.M.B 2076, Nigeria

Abstract

In the present research, the level of cancer antigen 125 (CA125) significantly increased in DMBA experimental groups (group II) compared to normal control group. However the level of CA125 decreased in dose dependent manner with 10% carbohydrate ketogenic diet having the most significant effect. Also, the 7,12-Dimethylbenz(α) anthracene (DMBA) caused significant increase in liver enzymes (Alanine amino transferase, Aspartate amino transferase and Bilirubin) of 41±1.06, 40±1.16, 20.80 ±1.47 and 25±1.34, 27±143, 12.17±1.08 compared to normal control at P<0.05 respectively. Furthermore, with the group treatment 10%, 20% and 30% carbohydrate ketogenic diets also elevated the liver enzymes. More so, a significant decrease was observed in total protein, albumin, RBC, Hb, PCV, MCH superoxide dismutase, glutathione reductase and glutathione peroxidase with a corresponding increase in white blood cells. Interestingly, ketogenic diets significantly increase total protein, albumin, superoxide dismutase, glutathione reductase, and glutathione peroxidase with a decrease in white blood cells, total cholesterol, triglyceride, low density lipoprotein bilirubin concentration. It can therefore be concluded that, the administration of ketogenic diets slows the progression of breast cancer through inhibiting the downstream of insulin receptors of both mammalian target of rapamycin signal cascade and motogen activator protein kinase pathways.

Keywords: Ketogenic diets, 7,12-dimethylbenz(α) Anthracene (DMBA), Wistar Rats, mammary cancer.

1. INTRODUCTION

Medically speaking, breast cancer is considered the most frequent malignant tumor that begins in the breast cells and spread over other parts of the body. Breast cancer is a global disease of significant burden and its incidence continue to rise globally, it accounts for most of mortality rate in women (Jemal M, *et al.*, 2021). The number of new cases and deaths as a result of breast cancer in United State are estimated to be 268,600 and 41,760 respectively asserted American cancer society (Clement R J, *et al.*, 2020; DeSantis C.E, *et al.*, 2019). The incidence of breast cancer in black women are lower than that of white women and the deaths rate in black women is higher than that of white women, possibly due to diets and lifestyle (DeSantis, C.E, *et al.*, 2019). The breast cancer in Nigeria had risen over that of cervical cancer and maybe attributed to delay in first child birth, shorter duration of breast feeding, family with history of breast cancer and late menopause as reported by previous literature (**Morounke S.G, et al., 2017)** The 7,12-dimethylbenz(α) anthracene (DMBA), is a procarcinogen specifically used to induced breast cancer in experimental rats and it undergoes metabolic activation to carcinogen dihydrodiolepoxide. The carcinogen and mutagenic activity of DMBA require metabolic activation by mixed function oxidases located in rat's liver microsomes. The dihydrodiolepoxide binds with adenine residues of deoxyribonucleic acid, resulting to mutagenesis and carcinogenesis (Jayakumar J.K, *et al.*, 2016). On the other hand, ketogenic diet (KD) is a high level of fat, very low-carbohydrate diets and moderate protein levels. This composition of diet changes the body metabolism toward the burning of fat rather than carbohydrates. In the liver, the ketogenic diets are oxidized to ketone bodies (B-hydroxibutyrate, acetoacetate and acetone) and are transported to various tissue in the body where they are converted to Acetyl-CoA as documented in the earlier study (Allen B.G, *et*

al., 2014). Recent studies indicated that ketogenic diet (KD) significantly decreased tumor 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 loss the body weight (Freedland S.J, *et al.,* 2008). A ketogenic diet had been demonstrated to be therapeutically useful for the treatment of epilepsy and cardiovascular diseases (Ruskin and Masino, 2012). This has also been found to be effective as an adjuvant therapy for other types of cancer such as glioblastoma, stomach and colon cancer (Ruskin and Masino, 2012; Reitman and Frankel, 1957). The present study evaluates the effect of ketogenic diet on the progression of 7,12-dimethylbenz(α) anthracene (DMBA) induce mammary cancer in rats.

2. MATERIALS AND METHOD

2.1 Materials

The materials used during this work include but not limited to; Female albino rats, red meat (protein), white corn (carbohydrate), vegetable oil and animal fat (groundnut oil and cow fat), propylene cages, Vital Feeds, water, *libitum*, most of which were obtained from Jimeta main market, Adamawa state, Nigeria.

2.2 Methods

2.2.1 Preparation of Ketogenic Diets

Locally available food materials including red meat (protein), white corn (carbohydrate), vegetable oil and animal fat (groundnut oil and cow fat) were used to constitute the ketogenic diets as presented in Table 1 below.

Table 1: Dietary Composition of Normal and Ketogenic Diet

Dietary constituent	Normal control	10%carb KD	20%carb KD	30%carb KD
Carbohydrate				
Maize	65	10	20	30
Protein				
Meat	20	20	20	20
Fat				
Cow fat	10	35	30	25
Groundnut oil	5	35	30	25
Total	100	100	100	100

Key: KD = Ketogenic diet

2.2.2 Animal Collection and Maintenance Prior to the commencement of the experiment

Female albino rats weighing between (120 ± 20g) was obtained from National Veterinary Research Institute (NVRI) Plateau State, Nigeria. They were housed in propylene cages and were given standard grower diet (Vital Feeds) and water and libitum. They were maintained under laboratory conditions of temperature (28EC) and 12 h of light and dark cycle for seven days to allow them to acclimatize before the commencement of the experiment.

2.2.3 Experimental Designs

The rats were randomly divided into six equal groups of four rats each. Group I serve as normal control, that is, no inducement. Breast carcinogen (DMBA) was induced at dose of 15mg kgG^{1} b.wt in group II, III, IV, V, and VI. Group II served as experimental control while group III served as the standard drug control. That is, after inducing the cancer they were treated with a standard drug Vincristine sulfate 500ug kgG^{1} b.wt intraperitoneal every week for four (4) consecutive weeks. The group IV, V and VI were fed with 10%, 20% and 30% carbohydrate ketogenic diet respectively. The ketogenic diets were administered to the rats through oral gavages for a period of four (4) weeks.

2.2.4 Biochemical estimation

At the end of the experimental period, rats in all groups were sacrificed under chloroform as an anesthesia. Whole blood was collected into EDTA anti-coagulated specimen bottles for hematological analysis and some was collected into plane specimen containers for biochemical assays. The specimen collected in plane bottles were centrifuged at 10,000 rpm for 5 min and serum was collected for biochemical assays. About 10g of mammary gland biopsy was collected into containers containing 10 % neutral formalin solution for histological investigations.

2.2.5 Determination of Aspartate Transaminase (AST)

The AST activity was determined according to the method of Reitman and Frankel, (1957) using commercially prepared kits. This enzyme formally known as Glutamate Oxaloacetate Transferase (GOT) will have its level increased in serum if there is an injury in liver, skeletal muscles, kidney, erythrocytes or heart. It can also be increased when liver function is impaired in case of necrosis or cell damage. The enzyme activity is expressed in IU LG^{1}.

2.2.6 Determination of Alanine Transaminase (ALT)

This enzyme ALT formally referred to as Glutamate Pyruvate Transaminase (GPT) was also estimated using the method described by the earlier

report (Reitman and Frankel, 1957). The level increases in serum if there is an injury in liver, skeletal muscles, kidney, erythrocytes or heart. It can also be increased when liver function is impaired in case of necrosis or cell damage.

Total bilirubin: The Bilirubin level in serum was determined using the method of Ou *et al*., 1984. Total serum bilirubin consists of the conjugated and unconjugated form.

Total protein: For estimating total protein, 20 micro liter of serum was added to 1mL of biuret reagent. This was mixed thoroughly and incubated for 10 min. After ten min incubation, the blue colour formed was read using a photometer at 640 nm. The level of protein was expressed as g LG^{1} of serum. The total protein concentrations in serum were determined using the methods described in the literature (Kroll.M, 1999).

Serum albumin: Serum Albumin (ALB) concentration was measured using the method described by Doumas B.T, *et al.*, 1971. The measurement of ALB is based on its quantitative binding to the indicator, 3, 3', 5, 5'-tetrabromo-m cresol sulphonephthalein (Bromocresol Green, BCG).

Total cholesterol (TC): the serum cholesterol was measured using the method described by Allain *et al*., 1974 as follows: Cholesterol reagent, 500 ul each was dispensed into clean test tubes labeled blank, standard, test and control. To standard, test and control samples, 10 microliters each was added respectively and the content of the tubes were mixed and incubated at room temperature for 10 minutes. After incubation, 1 ml of deionized water was added to each test tube and these were measured spectrophotometrically at 546 nm.

Triglycerides: the serum tryglyceride was measured using the method described by Fossati P., (1982): Triglycerides reagent, 1 ml each was dispensed into a clean test tubes labeled blank, standard, test and control. To standard, test and control samples 20 ul each was added respectively. The contents of the tubes were mixed and incubated for 10 minutes at room temperature. After incubation, 1 ml of deionized water was added to each tube and measured spectrophotometrically at 546 nm.

High Density Lipoprotein (HDL): the high density lipoprotein was measured using the method described by Friedwald *et al*., (1972): High density lipoprotein (HDL) reagent, 1000 ul of each was dispensed into clean test tubes labeled blank, standard, test and control. To standard, test and control samples 200 ul each was added respectively and was mixed and incubated at room temperature for 10 min. it was centrifuged at 4000 rpm for 10 minutes. Cholesterol reagent, 1 ml of each was dispensed into clean test tubes labeled blank, standard, test and control. Standard, test, control supernatant 100 ul was added to each respectively. The tubes were incubated at room temperature for 10 minutes. Exactly 1 ml of deionized water was added to each and was measured spectrophotometrically at 546 nm.

Low Density Lipoprotein: Serum LDL cholesterol level was determined by Formulae outlined in the previous report (Friedwald *et al.*, 1972).

$$\text{LDL (mg/dl)} = \frac{\text{TC} - (\text{TG}) + \text{HDL}}{5}$$

Glutathione Peroxidase (Gpx): For the determination of Glutathione peroxidase in plasma about 0.02 mL of heparinised blood was treated with 0.1 mL of 5 mM GSH, 0.1 mL of 1. 25 mM H_2O_2, 0.1 mL of 25 mM NaN_3 and phosphate buffer (0.05 mM, pH 7.0) in a total volume of 2.5 mL at 37EC for 10 min. The reaction was stopped by adding 2 mL of 1.65 % HPO32- and the reaction mixture was centrifuged at 1500 rpm for 10 min. About 2 mL of the supernatant was mixed with 2 mL 0.4 M Na_2HPO_4 and 1 mL of 1 mM dithio nitrobenzene (DTNB). The absorbance of the yellow coloured complex was measured at 412nm after incubation for 10 min 37EC against distilled water. A sample without the processed blood in the same way was kept as the non- enzymatic reaction. One unit of enzyme activity was defined as decrease in log GSH by 0.001 L $minG^{1}$ after subtraction of the decrease in log GSH per minute for the nonenzymatic reaction and is expressed as U mgG^{1} protein. The result was expressed as U gG^{1} Hb (Ilio C.D, *et al*., 1983)

Glutathione Reductase (GR): Glutathione reductase activity was determined by the method reported previously (Ilio C. D, *et al*., 1983). Glutathione reductase was assayed by following the oxidation of NADPH at 340 nm at 37EC. Glutathione reductase activity was expressed as mmol NADPH oxidized/min/mg protein.

Superoxide Dismutase (SOD): In SOD estimation, the procedure that was adopted is that described by Woolliams, J.A. (1983). The reaction mixture contains, 1.9 mL of phosphate buffer (pH 7.8), 1×10.2 M methionine, 16.8×10.5 M NBT and 1.17×10.6 M riboflavin, with suitably diluted erythrocyte hemolysate in a total volume of 3 mL. Illumination of the solution taken in 10 mL beaker was carried out in an aluminum foil lined box, with a 15 W fluorescent lamp for 10 min. Control without the enzyme source was always included. The absorbance was measured at 560 nm. The values were expressed in U gG^{1} Hb (Sun Y, *et al.*, 1988).

Hematological parameter: Electrical impedance method used with Sysmex Hematology analyzer was used in the analysis of complete blood count of the test samples. Parameters studied includes, Hematocrit

(HTC), Hemoglobin content, White Blood Cell count (WBC), Red Blood Cell count (RBC) and Platelets count (Ike S.O, *et al.*, 2010).

Histological investigation: The mammary gland biopsies were excised from the experimental animal of each group after collecting the blood sample. The biopsies were fixed in 10% neutral formalin solution. The sections were processed in alcohol xylene series. They were then embedded in paraffin and sections of 0.45 microns were cut using microtome techniques. After microtomy, they were stained with hematoxylin and eosin. The different sections were examined microscopically for the evaluation of histopathological changes (Akshatha G.M, 2018).

Data analysis: All data were presented as mean value ± standard error of mean (SEM).Oneway ANOVA was used for multiple comparisons of groups followed by Duncan's Multiple Range Test (MRT) for the post-hoc treatment.

3. RESULTS AND DISCUSSION

3.1 Effects of Ketogenic Diets on Cancer Antigen 125 (CA 125)

The Cancer antigen 125 (CA 125) and carcino embryonic antigen (CEA) are two major glycoprotein which a mostly found on the cell surface membrane and are easily released into the surrounding fluid. The CA 125 and CEA are known to be tumour marker with good sensitivity for breast cancer. In the present study the level of CA125 was significant high in experimental group (group II) compared to normal control group. Upon the administration of ketogenic diets their level were significantly reduced in dosed depending manner when compared with group II. This may be due to ability of ketogenic diets to reduce the activities of insulin like growth factor-1 (IGF-1) and mTOR signaling pathways which are strongly linked with the growth of breast cancer (Vijayakanth *et al.*, 2014).

Table 2: Effect of Ketogenic Diet on Cancer Antigen 125 (CA 125) in DMBA Induced Mammary Cancer at Four Weeks Treatment

Group	CA 125 (IU/L)
Normal control	17.00 ±1.29
Negative control	43.50±1.93[ab]
Standard drug control	27.27 ±1.25[a]
Keto diet 10% CHO	29.50 ±1.32[a]
Keto diet 20% CHO	36.50 ±0.65[ab]
Keto diet 30% CHO	41.50±1.55[ab]

Values are Mean ± SEM (n = 4).
[a]Significantly increased ($p<0.05$) compared to normal control
[b]Significantly increased ($p<0.05$) compared to standard drug control

3.2 Effect of Ketogenic Diets on Liver Enzymes

The effect of ketogenic diets on liver function parameters in DMBA induced mammary cancer and normal control rats was shown in Table 3. The result showed the activities of ALT and AST are significantly ($p<0.05$) increased in experimental group (41±1.06 and 40 ±1.16 IU LG^1) compared to normal control group (25±1.34 and 27±1.43 IU LG^1) respectively.

Biochemical enzymes marker is extensively used to screen diseases particularly cancer condition for differential diagnosis and monitoring the progressive response to therapy (Thomas, J.O, 2000). These enzymes are more unique and change in their activities reflect the effect of proliferation of cells growth and their activities is shown to be a good correlation with the number of transformed cell in cancer condition. The present study shows that administration of DMBA in rats led to significant elevation of alanine amino transferase (ALT) and aspartate amino transferase (AST) as it is shown in Table 3. These could be as the result of tissue damage (DMBA) that led to shed of these enzymes markers into the plasma (Mitani, H., 2003) Also the groups treated with 10%, 20% and 30% carbohydrate ketogenic diets appeared to increase the level of ALT and AST. This in line with previous studies that shown that intake of ketogenic diet has a long time and short time effect, dyslipidemia, kidney stone, carnithine deficiency are some of long time effect (Cervenaka, M.C, *et al.*, 2016). Ketogenic diets as shown to alters the serum level of liver enzyme and increased the level of ALT and AST in rats, also mice randomly assigned to ketogenic diets had a significant high level of AST and ALT compared to those in the normal control group suggesting that increased may be due to fat content (saturated Fat), these complications maybe reversed by aerobic exercise combined with ketogenic diets (Arslan, N, *et al.*, 2016; Zhanng, Q, *et al.*, 2018). Their finding is similar to the current study as ALT and AST level of the rats increased significantly in all the group administered with ketogenic diet.

Table 3: Effect of Ketogenic Diet on Liver Enzymes in DMBA Induced Mammary Cancer at Four Weeks Treatment

Group	ALT (IU/L)	AST(IU/L)
Normal control	25 ±1.34	27 ±1.43
Negative control	41 ±1.06*[b]	40±1.16*[b]
Standard drug control	26 ±0.20[a]	28 ±0.24[a]
Keto diet 10% CHO	27 ±1.62*[a]	30 ±1.86*[a]
Keto diet 20% CHO	28 ±1.93*[a]	30 ±1.62*[a]
Keto diet 30% CHO	29 ±2.70*[ab]	31±1.73*[ab]

Values are Mean ± SEM (n = 4). *Significantly increased ($p<0.05$) compared to normal control, [a]Significantly decreased ($p<0.05$) compared to negative control, [b]Significantly increased ($p<0.05$) compared to standard drug control. Key: AST = Aspartate aminotransferase, ALT = Alanine aminotransferase.

3.2 Effects of Ketogenic Diets on Non-Liver Enzymes

Decreased in albumin and total protein are characteristic of liver damage. The current findings (Table 4) shows decrease in albumin and total protein in group administered DMBA compared to normal control group, this may be due to hepatoxicity of DMBA which leads to hepatic damage which in turn cause defective protein biosynthesis in liver (Arirudran A, *et al*., 2014). Bilirubin concentration increased in DMBA induced rats, these maybe due to the disturbance in the transport function of the hepatocytes as the result of the hepatic injury causing the leakage of enzymes from cells due to altered permeability of membrane (Ramakrishna S, *et al.,* 2013) In the present study, albumin concentration and total protein significant decreased in all groups administered with ketogenic diet compared to normal control group, this is similar with previous finding that shows decreased in the albumin and total protein concentration in rats fed with different ketogenic diets, and the liver is the only site for protein (albumin) synthesis hence, anything that affects the liver may affects its synthesis (Eiya and Aikpitany-iduitua, 2020; Marjolain, R, *et al.,* 2006). Interestingly post treatment of ketogenic diets reduced the serum bilirubin to near normal compared to normal control.

Table 4: Effect of Ketogenic Diet on Some Non-Enzymes Biochemical Markers of Liver Disease in DMBA Induced Mammary Cancer at Four Weeks Treatment

Group	Albumin (g/L)	Total Protein (g/L)	Bilirubin (umol/L)
Normal control	26.67 ±0.49	44.48 ±0.99	12.17 ±1.08
Negative control	15.55 ±1.55*	29.02 ±2.80*	20.80 ±1.47[ab]
Standard drug control	25.44 ±1.41[a]	42.42 ±1.40*[a]	12.75 ±0.78
Keto diet 10% CHO	23.51 ±1.61*[a]	41.86 ±1.40*[a]	13.94 ±0.95
Keto diet 20% CHO	23.62 ±0.81*[a]	40.54 ±0.58*[a]	13.67 ±1.00
Keto diet 30% CHO	22.70 ±0.86*[a]	40.82 ±1.60*[a]	13.30 ±1.22

Values are Mean ± SEM (n = 4). *Significantly decreased ($p<0.05$) compared to normal control
[a]Significantly increased ($p<0.05$) compared to negative control,[b]Significantly increased ($p<0.05$) compared to standard drug control

3.3 Effect of Ketogenic Diets on Lipid Profile

Total cholesterol (TC) triglyceride (TG) and low- density lipoprotein (LDL) increased with DMBA administration compared to normal control (table 5). The increased may be due to increased utilization by neoplastic cells for new membranes biogenesis (Chaudhari, S.C, *et al.,* 2012). Significant decrease in total cholesterol, and increase in HDL in all group treated with ketogenic diet. This is in agreement with the previous findings that shows a significant reduction in total cholesterol and an increased in high density lipoprotein levels (Paoli, A, *et al.,* 2013) Elevation of HDL- cholesterol concentration is very vital and helps in the reduction of coronary heart diseases (Erel O, 2004). No significant difference was observed in TG and LDL in group administered with 10% and 20% carbohydrate ketogenic diets compared to normal control group. The low carbohydrate diets have also shown to particularly effect the level of blood triglycerides. The increased in HDL and decreased in LDL in rats fed 10% and 20% carbohydrate ketogenic diet shows that the use of this diet may not predispose individual to the risk of cardiovascular disease, since this diet show normal level of LDL-cholesterol, but elevation of LDL predispose individual to cardiovascular diseases (Borge and Nordestgaard, 2014).

Table 5: Effect of Ketogenic Diet on Lipid Profile in DMBA Induced Mammary Cancer at Four Weeks Treatment

Group	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-Cholesterol (mg/dL)	LDL-Cholesterol (mg/dL)
Normal control	153 ±1.53	106 ±2.41	68 ±1.08	59 ±1.23
Negative control	185 ±2.40*[b]	163 ±1.73*[b]	51 ±1.38[a]	67 ±1.95*[b]
Standard drug control	154±1.38[a]	104 ±3.65[a]	67 ±1.45	60 ±1.44[a]
Keto diet 10% CHO	143 ±1.16[a]	105 ±2.77[a]	71 ±1.14*[b]	53 ±1.61[a]
Keto diet 20% CHO	146 ±1.72[a]	106 ±1.82[a]	71 ±1.47*[b]	54 ±1.43[a]
Keto diet 30% CHO	148 ±2.30[a]	122 ±3.09*[b]	70 ±1.94*[b]	61 ±3.18*[b]

Values are Mean ± SEM (n = 4). *Significantly increased ($p<0.05$) compared to normal control [a]Significantly decreased ($p<0.05$) compared to negative control, [b]Significantly increased ($p<0.05$) compared to standard drug control. Key: HDL= High Density Lipoprotein, LDL= Low Density Lipoprotein.

Effects of Ketogenic Diets on Antioxidant Enzymes

Antioxidants are substance that protects living cells from the damages cause by unstable molecules known as free radicals. The antioxidant activities play a vital role in absorbing and neutralizing free radical, buffering singlet and triplet oxygen, or decomposing peroxide (Thirumal M, *et al.*, 2012) The present study shows reduction in the level of antioxidants in rats induced with DMBA (group 2) compared to normal control group. DMBA is one of the common and highly toxic chemical with a strong immunosuppressed activity, the metabolic activities of DMBA can lead to the production of procarcinogen (trans-3-4 dihydrodiol-1,2epoxide) in the body that hampers reactive oxygen species (ROS) antioxidant balance by overproduction of free radicals and the body in turn reacts by modulating activities of antioxidant enzymes to curb the damaging effect of ROS causing decreased in the levels of antioxidant enzymes (Roblin, D.W, *et al.*, 2011) Superoxide dismutase, glutathione reductase and glutathione peroxidase are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydrogen radical and protect the cellular constituents from oxidative stress[35]. In this study significant improvement in the level of antioxidant enzymes in groups treated with 10% and 20% carbohydrate ketogenic diet are shown in Table 6. These enzymes activities were found to have no significant difference with the standard drug. This is similar to previous study that ketone metabolism protects normal cells from oxidative damage by decreasing mitochondrial reactive oxygen species (ROS) production and enhancing endogenous antioxidant defenses, this may be due to the presences of omega -3 fatty acid and vitamin E in the ketogenic diets (Aryadi, A, *et al.*, 2020). Previous studies also reported that ketone metabolism protects the cell from oxidative stress. However, Significant declined in antioxidant enzymes in group treated with 30% carbohydrate ketogenic diet may be due to increase in calories (DeSantis, C.E., 2019).

Table 6: Effect of Ketogenic Diet on Antioxidant Enzymes in DMBA Induced Mammary Cancer at four Weeks Treatment

Group	SOD (%)	GTR (U/L)	GPx (mmole/mg/L)
Normal control	41.32 ±1.20	42.64 ±1.93	2.98 ±0.22
Negative control	23.74 ±1.39*	22.09 ±1.20*	0.45 ±0.28*
Standard drug control	40.83 ±1.79[a]	39.04 ±1.90[a]	2.51 ±0.14[a]
Keto diet 10% CHO	41.56 ±1.06[a]	41.78 ±1.94[a]	2.48 ±0.27[a]
Keto diet 20% CHO	40.41 ±1.21[a]	41.07 ±1.55[a]	2.18 ±0.20[a]
Keto diet 30% CHO	36.71 ±2.22*[a]	38.85 ±2.88*[a]	1.02 ±0.27*

Values are Mean ± SEM (n = 4). *Significantly decreased ($p<0.05$) compared to normal control [a]Significantly increased ($p<0.05$) compared to negative control. [b]Significantly increased ($p<0.05$) compared to standard drug control. Key: SOD= Superoxide Dismutase, GTR= Gluthatione Reductase, GPx=Gluthatione Peroxidase.

Effects of Ketogenic Diet on Haematological Parameters

The significant decrease of hematological indices RBC, Hb, PCV and MCH in DMBA experimental group (group II), as compared to normal control group (group 1) as shown in table 7. This is consistence with the finding of who observed decrease in HTC, Hb, RBC and platelet count as a result of necrosis factors alpha blunting the physiological effect of erythropoietin and interfering with the abilities of the body to store iron (Angiolo, G, *et al*., 2010). Significant decreased in RBC and Hb were observed in group treated with 10%, 20% and 30% carbohydrate ketogenic diets respectively, This is similar to the previous study of who stated that prolong used of ketogenic diets may likely cause anemia, which may be due to dietary restriction (carbonhydrate) leading to copper deficiency (Rashidian, M, *et al*., 2017). Suggesting that this complications of the ketogenic diet can be managed with supplementation (Poff A.M, *et al*., 2014).

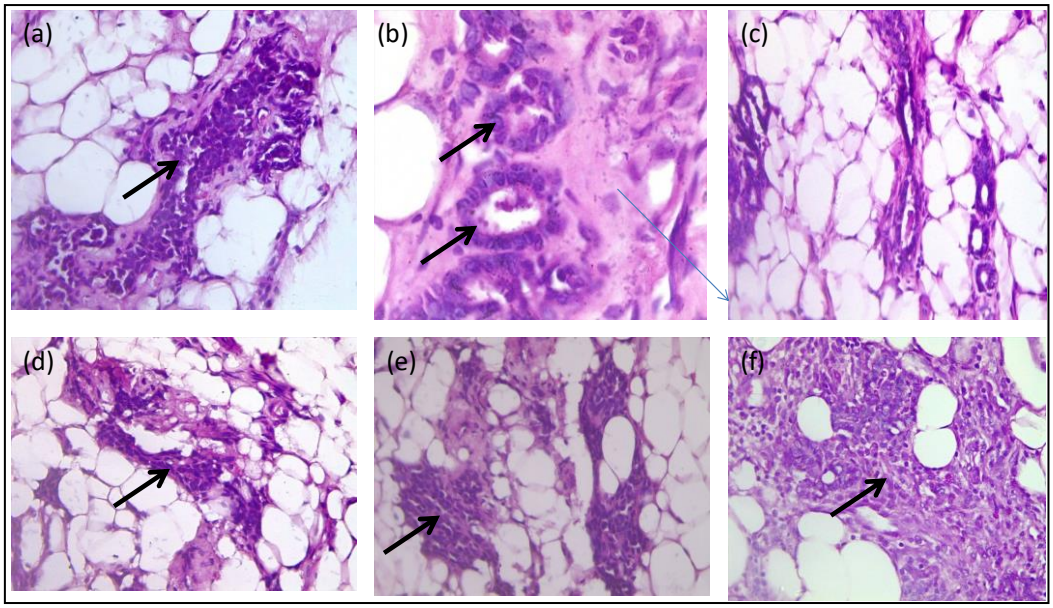

Fig 1(a-f): Photomicrograph of the mammary gland histological sections shown at 10x magnification; (a) Normal control showed normal architecture of mammary gland with terminal duct lobules (TDL) and fatty tissue. (b) DMBA induced group and untreated section showed gross damage with inflamed terminal duct lobules (c) section treated with standard drug showing less mammary gland cell damage (d) Section of rats treated with 10% carbohydrate ketogenic diets. This section showed preserved mammary gland architecture (arrow) nearly, similar to the control group. (e) Mammary gland section of the rats treated with 20% carbohydrate ketogenic diets showed improved mammary gland tissue with some fatty tissues mixed in myoepithelial cell (f) Mammary gland section treated with 30% carbohydrate ketogenic diets showed marked destruction of mammary tissue and fibrosis

Table 7: Effect of Ketogenic Diet on Heamatological Parameters in DMBA Induced Mammary Cancer at Four Weeks Treatment

Group	WBC (cells/L)	RBC (cells/L)	Hb (g/dL)	PCV (%)	MCH (fL)
Normal control	18.10 ±1.90	7.90 ±0.59	14.52 ±0.63	42.55 ±1.87	65.78 ±0.70
Negative control	28.06 ±2.43[b]	4.01 ±2.05*	8.67 ±1.54*	29.18 ±1.27*	58.00 ±1.40*
Standard drug control	19.87 ±1.02[a]	7.34 ±0.47[a]	13.30 ±0.47[a]	41.30 ±0.84[a]	64.97 ±0.52[a]
Keto diet 10% CHO	18.08 ±1.54[a]	5.04 ±1.69*	12.37 ±1.45*[a]	40.10 ±2.51[a]	63.07 ±1.02[a]
Keto diet 20% CHO	19.14 ±1.74[a]	5.41 ±1.53*	12.48 ±1.78*[a]	41.31 ±2.68[a]	64.38 ±1.74[a]
Keto diet 30% CHO	24.70 ±2.65[ab]	5.55 ±2.51*	13.22 ±2.05*[a]	41.42 ±2.83[a]	64.46 ±1.29[a]

Values are Mean ± SEM (n = 4). *Significantly decreased ($p<0.05$) compared to normal control [a]Significantly increased ($p<0.05$) compared to negative control [b]Significantly increased ($p<0.05$) compared to standard drug control. Key: Hb= Heamoglobin, WBC= White Blood Cell, RBC=Red Blood Cell, PCV= package cell volume. MCH= mean corpuscular hemoglobin

The effect of ketogenic diet on lipid profile in DMBA induced mammary cancer and normal rats show significant ($p<0.05$) increased in TC, TG and LDL level (180 ±2.40, 163 ±1.73 and 67 ±1.95 mg/dL) in the experimental group compared to normal control group (153 ±1.53, 106 ±2.41 and 59±1.23 mg/dL) respectively. Significant ($p<0.05$) decrease in HDL in experimental group (51 ±1.38) compare to normal control group (68 ±1.08 mg/dL) (Table 3).

There were significant ($p<0.05$) decrease in SOD (23.74±1.39%), GTR (22.09±1.20 U/L) and GPx (0.45±0.28 mmole/mg/L)) concentration compared to normal control (41.32±1.20 U/L), 42.64±1.93 U/L) and 2.98±0.22 mmole/mg/L) respectively (Table 4).

The effect of ketegenic diets on haematological parameter in normal group and DMBA induced mammary cancer were shown in Table 7. There were significant ($P<0.05$) decreased in RBC (4.01±2.05), Hb (8.67± 1.54), PCV (29.18±2.27) and MCH (58.00±1.40) compared to normal control group (7.90±0.59, 14.52±0.63, 42.55±1.87 and 65.70±0.70 respectively). However, WBC, PCV and MCH shows no significant difference ($p<0.05$) in groups treated with 10% and 20% carbohydrate ketogenic diet when compared with normal control group, more so, with the group treated with standard drug (Vincristine Sulfate) shows no significant difference when compared to normal control group. While WBC was found to be significantly high in negative control group (28.06±2.43) and 30% carbohydrate ketogenic group

(24.70 ±2.65) compared to normal control group (18.92±0.90).

The Photomicrograph of mammary gland section was shown Figure 1. There were mammary gland cells damages following treatment with DMBA as seen in Plate A1. Subsequent treatment with Vincritine sulfate and the 10% and 20% carbohydrate ketogenic diets shows less cancer with a regenerating effect of myoepithelial cell and a near normal architecture of mammary gland (C3, D4 and F5, respectively) compared to normal control group

Effects of Ketogenic Diets on Mammary Gland Cancer

The photomicrograph (Fig 1) DMBA induced mammary tumor in rat section as compared to control group indicated increase in proliferative cells and ducted epithelial cell were bigger (inflammed) when compared to normal control (group 1), the nuclear basophilicity was enhanced and eosinophilic substance were observed (plate II). It's also evidenced the hyperplasia and hypertrophy in line cells of alveoli and increased number of alveoli which indicate damage in a mammary gland as the result of DMBA intoxication. These observations were similar to the finding of Jayakumar *et al.*, (2016) who show that DMBA undergoes metabolic activation of carcinogen dihydrodiolepoxide which bind with adenine residues of deoxy ribonucleic acid resulting to mutagenesis and carcinogenesis in rat mammary gland. The healing effe ct of ketogenic diet is as result of inhibition of the downstream of insulin receptors of both mammalian target of rapamycin signal cascade and motogen activator protein kinase pathways leading to programmed cells deaths (Senapati P, *et al.*, 2019).

CONCLUSION

This study has shown the administration of 10% and 20% carbohydrate ketogenic diets slows the progression of breast cancer and restored the lipid profile and antioxidant enzymes activities respectively, but it may predispose individual to anemia due to reduction in red blood cells concentration.

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