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Effect of Cucumis Callosus Fruit Extract on the Kidney Function of DMBA-Induced Mammary Cancer in Female Albino Wistar Rats

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Abstract

Breast cancer is currently one of the most prevalently diagnosed cancers and the 5th cause of cancer-related deaths with an estimated number of 2.3 million new cases worldwide according to the GLOBOCAN 2020 data. Treatment and management therapies for cancer are expensive and hardly affordable by patients in developing and underdeveloped countries. This study aimed at investigating the effect of *Cucumis callosus* fruit extract on the kidney function of DMBA-induced mammary cancer in female albino Wistar rats. The study utilized the extract of mature, fresh, healthy pulp of *Cucumis Callosus* fruits. Thirty-six (36) healthy Adult female Wistar Albino rats weighing about 150 to 200g were organized into 5 groups: The groups in which the rats were categorized into are as follows: Group 1: Standard diet + water only; Group 2: Induced intravenously with 0.01% DMBA without treatment; Group 3: Induced with 0.01% DMBA + treatment with standard drug; Group 4: Induced with 0.01% DMBA + 100mg/b.wt *Cucumis callosus* fruit extract; Group 5: Induced with 0.01% DMBA + 200mg/b.wt *Cucumis callosus* fruit extract. Rats administered a single dose of DMBA orally per body weight. The serum samples of the rats were analyzed for various kidney function parameters including creatinine, bicarbonate, urea, sodium, potassium, and chloride using a UV/ visible spectrophotometer.

The data obtained were analyzed statistically using One-Way Analysis of Variance (ANOVA). The significance between means was determined at a p-value of less than 0.05 ($p<0.05$). The results for each treatment were presented as mean ± standard deviation. The result of serum creatinine concentrations obtained in this study showed a significant ($P<0.05$) reduction in Group 5 rats (21.1 mmol/L) upon administering 200mg/b.wt *Cucumis callosus* fruit extract to the rats induced with 0.01% DMBA compared to the group of experimental rats induced with cancer without treatment. Serum urea concentration was highest in Group 2 (36.35 mmol/L) which represents the set of rats induced 0.01% DMBA without treatment. However, serum urea concentration was significantly ($p<0.05$) reduced in Group 5 rats (16.89 mmol/L) upon administering 200mg/b.wt *Cucumis callosus* fruit extract to the rats induced with 0.01% DMBA compared to all other groups besides the control group. In this study, administration of *Cucumis callosus* fruit extract significantly lowered serum sodium concentration across the groups ($P<0.05$) compared to Group 2 experimental rats which had high concentration of sodium possibly caused by cancer induction. Findings from this study revealed that *Cucumis callosus* fruit extract therapy did not provide statistically significant improvements in serum chloride, potassium, and bicarbonate levels. However, *Cucumis callosus* fruit extract therapy was found effective in ameliorating the poor renal function of the cancer-induced female Wistar rats.

Keywords: Breast Cancer; 7, 12-Dimethylbenz[a]anthracene (DMBA); *Cucumis callosus*; Wistar rats; Kidney

Introduction

The number of cancer related deaths is disturbingly increasing every year ranking them as one of the major causes of death worldwide [1]. Even though a significant number of cancers do not always need to result in death, they significantly lower the quality of life and require larger costs in general [2]. Breast cancer is currently one of the most prevalently diagnosed cancers and the 5th cause of cancer-related deaths with an estimated number of 2.3 million new cases worldwide according to the GLOBOCAN 2020 data [3]. Deaths due to breast cancer are more prevalently reported (an incidence rate approximately 88% higher) in transitioning countries like Melanesia, Western Africa, Micronesia/Polynesia, and the Caribbean compared to the transitioned ones like Australia/New Zealand, Western Europe, Northern America, and Northern Europe. Several procedures such as preventive behaviors in general as well as screening programs are crucial regarding a possible minimization of breast cancer incidence rate and the implementation of early treatment. Breast cancer is a major public health concern in Nigeria [4].

The incidence of breast cancer in Nigeria is 52 per 100,000 people, which is three times higher than it was four decades ago. Unfortunately, breast cancer incidence is projected to continue to increase in the coming years. In Nigeria, access to breast cancer screening and treatment can be challenging due to limited resources, lack of awareness, and cultural barriers [5]. However, there are ongoing efforts to improve access to breast cancer services in the country. For example, some organizations are working to increase awareness about breast cancer and promote early detection through screening programs. There are also initiatives to improve access to treatment, including the provision of free or subsidized cancer drugs and the training of healthcare workers in breast cancer management. The treatment of breast cancer in Nigeria consists of surgery, chemotherapy, radiation therapy, and hormone therapy. However, the availability and quality of these treatments can vary depending on the location and resources available (Vallon and Thomson, 2020. Surgery is generally available in most parts of Nigeria, but specialized procedures like breast conserving or reconstructive surgery may be limited. Chemotherapy facilities are available in many hospitals, but availability of certain drugs may be limited due to cost or supply issues. Radiation therapy is less widely available, and hormone therapy is available in some parts of Nigeria, but access may be limited due to cost or availability of drugs [6].

Cucumis Callosus, also known as the horned cucumber or kiwano, is a tropical fruit native to Africa, belonging to the Cucurbitaceae family. Its spiky exterior and vibrant orange-yellow skin make it a refreshing and tangy fruit [4] reported that the premature fruit contains carbohydrate (9.31%), protein (0.48%), lipid (0.29%), and ash (0.93%). The plant has been reported to possess many bioactive components, such as flavonoids, alkaloids, steroids, terpenoids etc., which are secondary metabolites, possess healing activity and are used to cure several diseases [4]. The plant has been used in traditional medicine to treat ailments like fevers, urinary tract infections, and stomach problems. Recent research has explored their potential therapeutic properties, including anti-inflammatory, antimicrobial, and anticancer effects. *Cucumis Callosus*, can be used as a spasmolytic and anesthetic agent due to the presence of alkaloid content, whereas appreciable amounts of saponins are helpful to boost the immune system and lower the risks of various degenerative diseases. Total phenolic content has been reported to be between 56 and 72 µg GAE/mg extract in *Cucumis Callosus* [4].

7,12-dimethylbenz[a]anthracene (DMBA), a potent chemical carcinogen, is commonly used in experimental studies to induce mammary tumors in laboratory animals. DMBA's carcinogenic effects are primarily induced through metabolic activation by cytochrome P450 enzymes in the liver, forming reactive intermediates that bind covalently to DNA, causing 3 mutations and DNA damage, particularly in critical oncogenes or tumor suppressor genes [4]. Mutations in key regulatory genes involved in cell proliferation, apoptosis, and DNA repair pathways can lead to mammary tumorigenesis [6]. DMBA is widely used in laboratory animal models, particularly rodents like rats and mice, to induce mammary tumors that closely resemble human breast cancer. These tumors exhibit features such as cellular heterogeneity, increased cell proliferation, angiogenesis, invasion into surrounding tissues, and metastatic potential, mirroring the hallmarks of human breast cancer [7]. However, caution must be exercised when extrapolating findings to human breast cancer, as DMBA-induced mammary tumors represent a simplified model focusing primarily on the effects of a single carcinogen [8]. Research using DMBA-induced mammary tumors has provided valuable insights into the molecular mechanisms underlying breast cancer development, progression, and metastasis, and has been instrumental in evaluating the efficacy of novel therapeutic agents in preclinical settings. This study therefore investigated the possibility of using an alternative treatment, specifically, plant-based treatment for breast cancer considering that it is cheaper and probably more effective.

Materials and Method

Study Area

The study was conducted at the Central Research Laboratory, Federal University Wukari, Taraba state, Nigeria, from October 2023 to March 2024.

Sample Collection and Extract Preparation

The study utilized mature, fresh, healthy pulp of *Cucumis Callosus* fruits. The fresh *Cucumis Callosus* fruits were collected chopped into pieces and then dried at room temperature for 3 weeks. The completely dried fruit pulps were ground into powder by using a mortar and pestle then stored. 10 g of the dried fruit powder was successively extracted with 100 ml of methanol for 48hrs. Afterwards it was filtered with white mesh and then with

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Whatmann No 1 filter paper. The filtration was concentrated using a rotatory evaporator. The concentrated extract was stored in small vials and used for further analysis.

Experimental Animals

Thirty-six (36) healthy Adult female Wistar Albino rats weighing about 150 to 200g were purchased. These rats were housed in polypropylene cages under the standard laboratory condition (25 ± 2°C, humidity 60-70 %, 12 hours light/dark cycles). The animals were fed with commercial rat pellet diet and water was provided ad libitum. The rats were then acclimatized to laboratory conditions for one week prior to the commencement of the experiment.

Treatment of Animals

Thirty-six (36) healthy Adult female Wistar Albino rats weighing about 150 to 200g were organized into 5 groups: The groups in which the rats were categorized into are as follows:

Group 1: Standard diet + water only;

Group 2: Induced intravenously with 0.01% DMBA without treatment;

Group 3: Induced with 0.01% DMBA + treatment with standard drug;

Group 4: Induced with 0.01% DMBA + 100mg/b.wt *Cucumis callosus* fruit extract;

Group 5: Induced with 0.01% DMBA + 200mg/b.wt *Cucumis callosus* fruit extract.

After treatment for 3 weeks, the liver function marker enzymes levels of the rats such as Aspartate transaminase, Alanine transaminase, Alkaline phosphatase, alongside other parameters were analyzed.

Induction of Breast Cancer

The solution induced in the rats was prepared by dissolving 7, 12-Dimethylbenz[a]anthracene (DMBA) in olive oil. The rats were allowed to acclimate to the laboratory environment for at least one week before the experiment. Rats administered a single dose of DMBA orally per body weight.

Assessment of Kidney Function Parameters

The serum samples of the rats were analyzed for various kidney function parameters including creatinine, bicarbonate, urea, sodium, potassium, and chloride using a UV/ visible spectrophotometer.

Assessment of Serum Creatinine Concentration

The creatinine concentration in serum samples were measured using the Creatinine K® commercial kits (Labtest Diagnostica SA, Lagoa Santa, Brazil), which uses a two-point optimized kinetic procedure based on the modified-Jaffe reaction. 18 For dosing purposes, 50 µL of the serum sample was added to 50 µL of alkaline picrate, mixed and aspirated into the automatic analyzer bucket set to zero at 510 nm, and then we measured the absorbance at 30 and 90 seconds. The results were expressed in mg/dL; and for urine they were corrected by the dilution factor.

Assessment of Serum Urea Concentration

For determining serum urea, we used the Liquiform® Urea UV test (Labtest Diagnostica SA, Lagoa Santa, Brazil) which uses an enzymatic system by UV photometry and two-point kinetics. Urea is hydrolyzed by urease, producing ammonia and carbon dioxide. The ammonia reaction reduces absorbance, which is proportional to the urea concentration in the sample. 10 µL of serum were aspirated into the photometer reservoir previously adjusted to 340 nm, and then we measured the absorbance at 30 and 90 seconds. The results were calculated from the absorbance difference between the two time periods and expressed in mg/dL

Determination of Serum Electrolyte Concentration

The method of Nwankpa et al. (2018) [9] was used to determine serum sodium concentration, serum potassium concentration, serum chloride concentration, serum bicarbonate (HCO3-) concentration, as well as serum creatine and serum urea concentration.

Statistical Analysis

The data obtained were analyzed statistically using One-Way Analysis of Variance (ANOVA), and post hoc least-significant difference (LSD) test using Statistical Package for Social Science (SPSS). The significance between means was determined at a p-value of less than 0.05 ($p<0.05$). The results for each treatment were presented as mean ± standard deviation

Results

Effect of *Cucumis Callosus* Fruit Extract on Serum Creatinine Concentration of DMBA-Induced Mammary Cancer in Female Albino Wistar Rats. Figure 1 shows the result of serum creatinine concentration obtained in the five groups of experimental rats. The result projects that serum creatinine concentration was highest in Group 2 (48.14 mmol/L) which represents the set of rats induced 0.01% DMBA without treatment. followed by Group 3 (33.48 mmol/L). Serum creatinine level was significantly ($p<0.05$) reduced in Group 5 rats (21.1 mmol/L) upon administering 200mg/b.wt *Cucumis callosus* fruit extract to the rats induced with 0.01% DMBA compared to all other groups besides the control group. It was also observed that serum creatinine concentration differed significantly ($p<0.05$) across group 1 to group 5 as shown in the order provided: rats fed with standard diet + water only (17.02 mmol/L), rats induced with 0.01% DMBA without treatment (48.14 mmol/L), rats induced with 0.01% DMBA + treatment with standard drug (33.48 mmol/L), rats induced with 0.01% DMBA + 100mg/b.wt *Cucumis callosus* fruit extract (29.00 mmol/L), and rats induced with 0.01% DMBA + 200mg/b.wt *Cucumis callosus* fruit extract (21.1 mmol/L).

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Source: Snapshot

Figure 1: Cucumis Callosus fruits.

Effect of *Cucumis Callosus* Fruit Extract on Serum Urea Concentration of DMBA-Induced Mammary Cancer in Female Albino Wistar Rats

The result of serum urea concentration of DMBA-induced mammary cancer in female albino wistar rats is presented in Figure 2 shown below. The result revealed that serum urea concentration was highest in Group 2 (36.35 mmol/L) which represents the set of rats induced 0.01% DMBA without treatment. followed by Group 3 (28.44 mmol/L). Serum urea level was significantly ($p<0.05$) reduced in Group 5 rats (16.89 mmol/L) upon administering 200mg/b.wt *Cucumis callosus* fruit extract to the rats induced with 0.01% DMBA compared to all other groups besides the control group. It was also observed that serum urea concentration differed significantly ($p<0.05$) across group 1 to group 5 as shown in the order provided: rats fed with standard diet + water only (14.29 mmol/L), rats induced with 0.01% DMBA without treatment (36.35 mmol/L), rats induced with 0.01% DMBA + treatment with standard drug (28.24 mmol/L), rats induced with 0.01% DMBA + 100mg/b.wt *Cucumis callosus* fruit extract (24.88 mmol/L), and rats induced with 0.01% DMBA + 200mg/b.wt *Cucumis callosus* fruit extract (16.39 mmol/L).

Effect of *Cucumis Callosus* Fruit Extract on Serum Chloride Concentration of DMBA-Induced Mammary Cancer in Female Albino Wistar Rats

The result of serum chloride concentration of DMBA-induced mammary cancer in female albino wistar rats is presented in Figure 3 shown below. The result revealed that serum urea concentration was highest in Group 4 (6.47 mmol/L) which represents the set of rats induced with 0.01% DMBA + 100mg/b.wt *Cucumis callosus* fruit extract. The lowest concentration of serum chloride was associated with Group 3 rats, representing the set of rats induced with 0.01% DMBA + treatment with standard drug (5.11 mmol/L). It was also observed that serum chloride concentration did not differ significantly ($p<0.05$) across group 1 to group 5 as shown in the order provided: rats fed with standard diet + water only (5.35 mmol/L), rats induced with 0.01% DMBA without treatment (5.94 mmol/L), rats induced with 0.01% DMBA + treatment with standard drug (5.11 mmol/L), rats induced with 0.01% DMBA + 100mg/b.wt *Cucumis callosus* fruit extract (6.47 mmol/L), and rats induced with 0.01% DMBA + 200mg/b.wt *Cucumis callosus* fruit extract (5.85 mmol/L).

Effect of *Cucumis Callosus* Fruit extract on Serum Potassium Concentration of DMBA-Induced Mammary Cancer in Female Albino Wistar Rats

Figure 4 shows the result of serum potassium concentration obtained in the five groups of experimental rats. The result projects that serum creatinine concentration was highest in Group 5 (9.48 mmol/L) which represents the set of rats induced 0.01% DMBA without treatment. induced with 0.01% DMBA + 200mg/b.wt *Cucumis callosus* fruit extract, followed by the control group (9.13 mmol/L) which represents the set of rats fed with standard diet + water only. The lowest concentration of potassium was associated with Group 2 rats, representing the set of rats induced with 0.01% DMBA without treatment (6.85 mmol/L). It was also observed that

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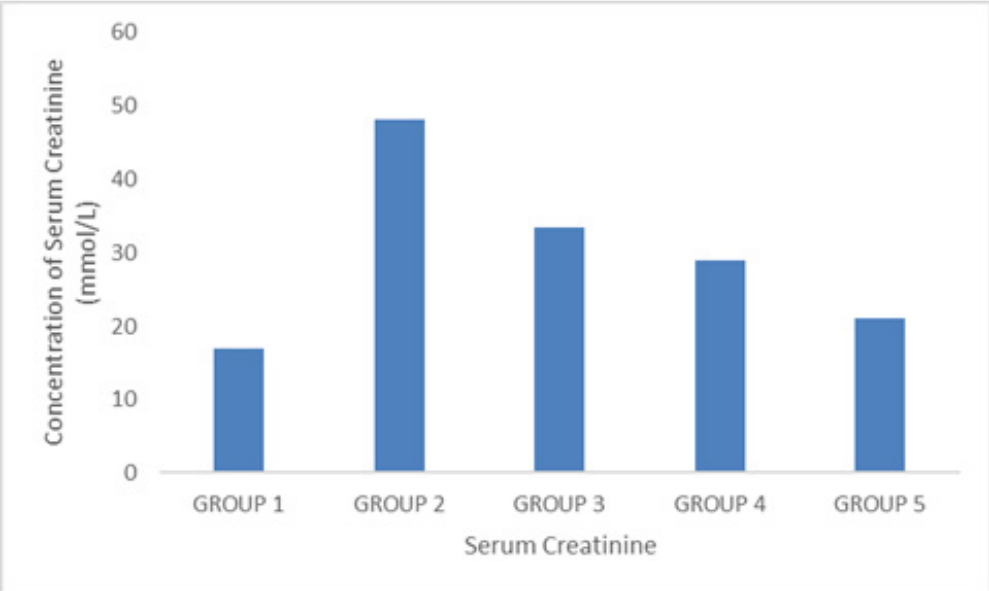
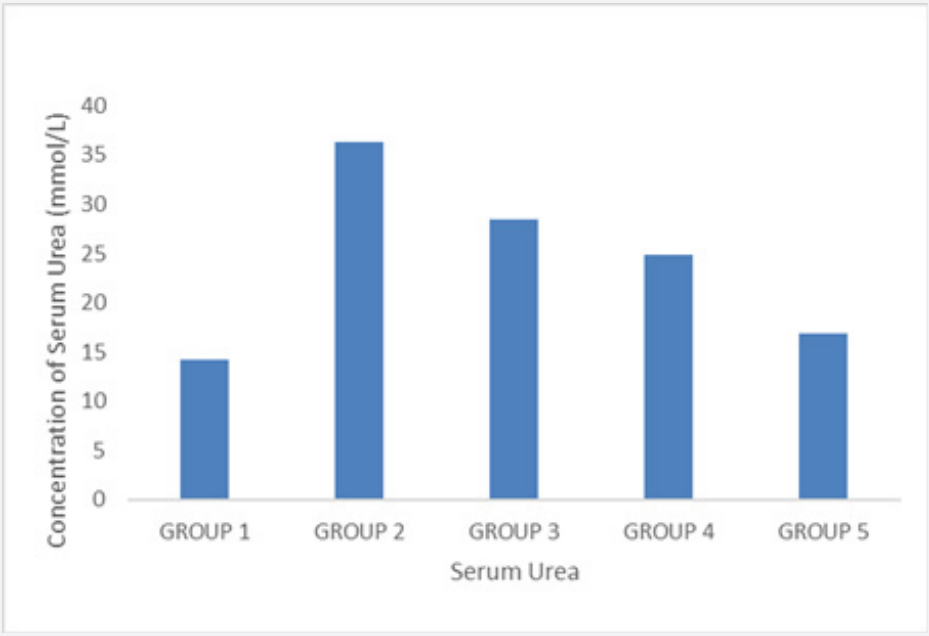
serum chloride concentration did not differ significantly ($p<0.05$) across group 1 to group 5 as shown in the order provided: rats fed with standard diet + water only (9.13 mmol/L), rats induced with 0.01% DMBA without treatment (6.85 mmol/L), rats induced with 0.01% DMBA + treatment with standard drug (8.28 mmol/L), rats induced with 0.01% DMBA + 100mg/b.wt *Cucumis callosus* fruit extract (8.87 mmol/L), and rats induced with 0.01% DMBA + 200mg/b.wt *Cucumis callosus* fruit extract (9.48 mmol/L).



*Results represent mean ± standard deviation of group results obtained (n = 5).

Figure 1: Effect of Cucumis Callosus fruit extract on serum creatinine concentration of DMBA-induced mammary cancer in female Albino Wistar rats.



*Results represent mean ± standard deviation of group results obtained (n = 5).

Figure 2: Effect of Cucumis Callosus fruit extract on serum urea concentration of DMBA-induced mammary cancer in female Albino Wistar rats

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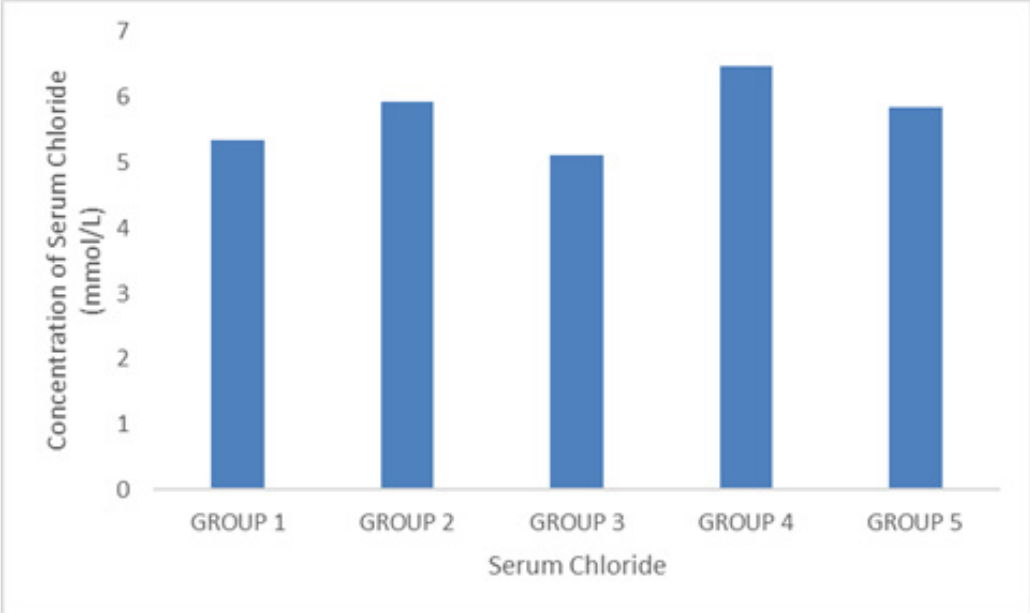
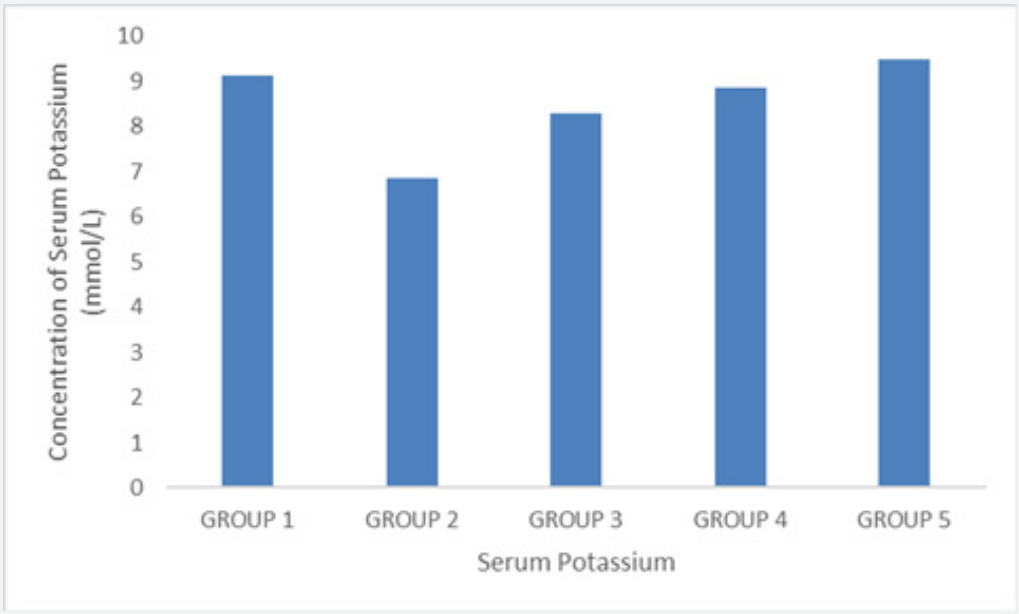


*Results represent mean ± standard deviation of group results obtained (n = 5).

Figure 3: Effect of Cucumis Callosus fruit extract on serum chloride concentration of DMBA-induced mammary cancer in female Albino Wistar rats.



*Results represent mean ± standard deviation of group results obtained (n = 5).

Figure 4: Effect of Cucumis Callosus fruit extract on serum potassium concentration of DMBA-induced mammary cancer in female Albino Wistar rats.

Effect of *Cucumis Callosus* Fruit Extract on Serum Bicarbonate Concentration of DMBA-Induced Mammary Cancer in Female Albino Wistar Rats

The result of serum bicarbonate concentration of DMBA-induced mammary cancer in female albino wistar rats is presented in Figure 5 shown below. The result revealed that serum bicarbonate concentration was highest in the control group (3.78 mmol/L) which represents the set of rats fed with standard diet + water only. The lowest concentration of serum bicarbonate was associated with Group 2 rats, representing the set of rats induced

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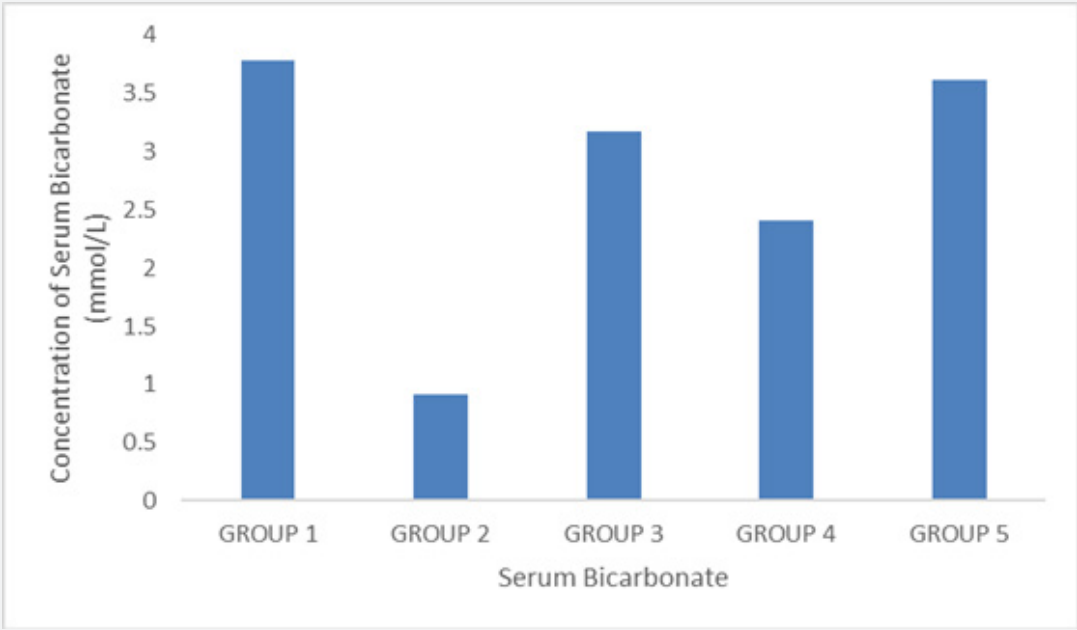
with 0.01% DMBA without treatment (0.91 mmol/L). It was also observed that serum bicarbonate concentration did not differ significantly ($p<0.05$) across the groups except for Group 2. as shown in the order provided: rats fed with standard diet + water only (3.78 mmol/L), rats induced with 0.01% DMBA without treatment (0.91 mmol/L), rats induced with 0.01% DMBA + treatment with standard drug (3.17 mmol/L), rats induced with 0.01% DMBA + 100mg/b.wt *Cucumis callosus* fruit extract (2.41 mmol/L), and rats induced with 0.01% DMBA + 200mg/b.wt *Cucumis callosus* fruit extract (3.62 mmol/L).



*Results represent mean ± standard deviation of group results obtained (n = 5).

Figure 5: Effect of Cucumis Callosus fruit extract on serum bicarbonate concentration of DMBA-induced mammary cancer in female albino wistar rats.

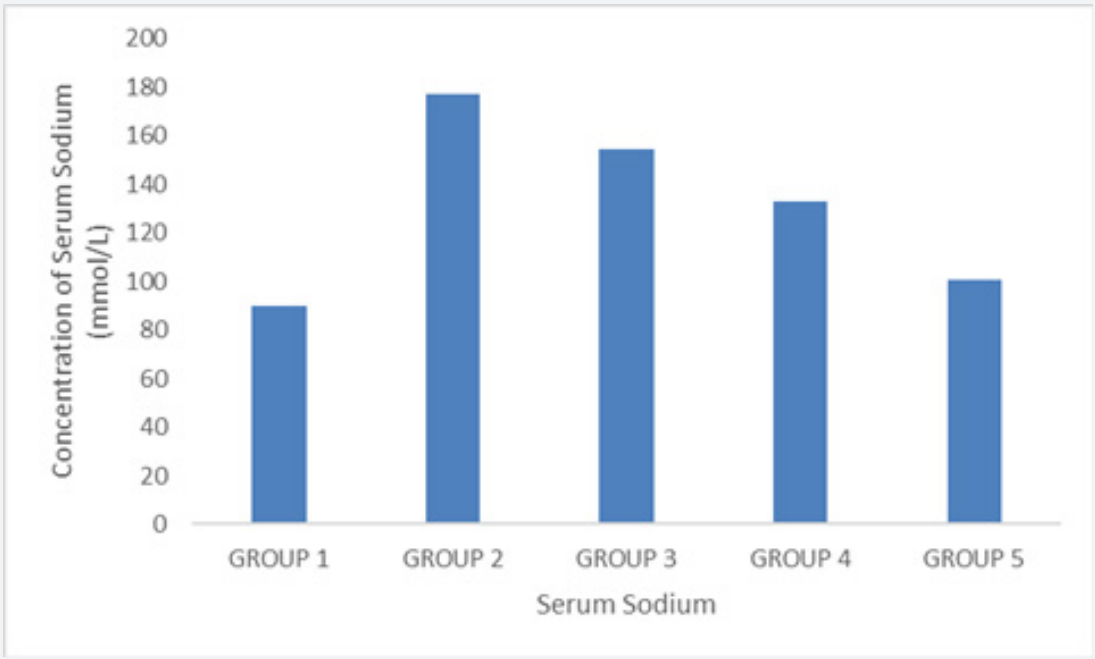


Figure 6: Effect of Cucumis Callosus fruit extract on serum sodium concentration of DMBA-induced mammary cancer in female Albino Wistar rats.

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Effect of *Cucumis Callosus* Fruit Extract on Serum Sodium Concentration of DMBA-Induced Mammary Cancer in Female Albino Wistar Rat

Figure 6 shows the result of serum sodium concentration obtained in the five groups of experimental rats. The result projects that serum sodium concentration was highest in Group 2 (177.03 mmol/L) which represents the set of rats induced 0.01% DMBA without treatment. followed by Group 3 (154.58 mmol/L). Serum sodium level was significantly ($p<0.05$) reduced in Group 5 rats (100.94 mmol/L) upon administering 200mg/b.wt *Cucumis callosus* fruit extract to the rats induced with 0.01% DMBA compared to all other groups besides the control group. It was also observed that serum creatinine concentration differed significantly ($p<0.05$) across group 1 to group 5 as shown in the order provided: rats fed with standard diet + water only (89.71 mmol/L), rats induced with 0.01% DMBA without treatment (177.03 mmol/L), rats induced with 0.01% DMBA + treatment with standard drug (154.58 mmol/L), rats induced with 0.01% DMBA + 100mg/b.wt *Cucumis callosus* fruit extract (132.78 mmol/L), and rats induced with 0.01% DMBA + 200mg/b.wt *Cucumis callosus* fruit extract (100.94 mmol/L).

Discussion

The treatment of breast cancer in Nigeria consists of surgery, chemotherapy, radiation therapy, and hormone therapy. However, the availability and quality of these treatments can vary depending on the location and resources available [3]. Surgery is generally available in most parts of Nigeria, but specialized procedures like breast conserving or reconstructive surgery may be limited. Chemotherapy facilities are available in many hospitals, but availability of certain drugs may be limited due to cost or supply issues [10]. Radiation therapy is less widely available, and hormone therapy is available in some parts of Nigeria, but access may be limited due to cost or availability of drugs [8]. This study therefore investigated the possibility of using an alternative treatment, specifically, plant-based treatment for breast cancer considering that it is cheaper and probably more effective.

Creatinine clearance in the glomerulus of the kidney is a useful tool to assessing the functionality of the kidney [11,12]. Creatinine is produced endogenously in the muscle by a non-enzymic action on creatine phosphate. The result of serum creatinine concentrations obtained in this study showed a significant ($P<0.05$) reduction in Group 5 rats (21.1 mmol/L) upon administering 200mg/b.wt *Cucumis callosus* fruit extract to the rats induced with 0.01% DMBA compared to the group of experimental rats induced with cancer without treatment. The significant decrease in serum creatinine concentration compared to Group 2 experimental rats induced with cancer may have resulted from amelioration of the kidney from glomerular inflammation and interstitial nephritis, though the exact mechanism was not covered in this study.

The major non-protein nitrogenous catabolite of protein metabolism is urea [13,14]. In this study, serum urea concentration was highest in Group 2 (36.35 mmol/L) which represents the set of rats induced 0.01% DMBA without treatment. However, serum urea concentration was significantly ($p<0.05$) reduced in Group 5 rats (16.89 mmol/L) upon administering 200mg/b.wt *Cucumis callosus* fruit extract to the rats induced with 0.01% DMBA compared to all other groups besides the control group. This suggests either that the urea cycle which may have been affected by cancer induction as seen in Group 2 experimental rats leading to the increase in the production of urea as a result of excess breakdown of proteins may have been ameliorated by *Cucumis callosus* fruit extract as seen in Group 4 and 5. The alkaloids and saponins contained in the plant extract may have been responsible for this redeeming the kidney from systemic toxicity, invariably leading to reduced excretion of urea [15].

Body fluid compartments (both extracellular and intracellular fluids) comprise of inorganic electrolytes which in its dissociated forms help to facilitate the movement of water and electrolytes between the body fluid compartments [16-18]. In this study, administration of *Cucumis callosus* fruit extract significantly lowered serum sodium concentration across the groups ($P<0.05$) compared to Group 2 experimental rats which had high concentration of sodium possibly caused by cancer induction. This most likely conferred the possibility of the hypernatraemic effect to the Na+/H+ exchanger instead of Na+/K+ pump [11]. The membrane-bound aldosterone regulates the absorption of sodium into the cell while Na+/K+ pump may have been impaired as a result of cancer induction which was gradually ameliorated as seen in group 3, 4 and 5 upon administration of the standard drug and the *Cucumis callosus* fruit extract. This is also supported by the significant decrease ($P<0.05$) in bicarbonate, chloride and potassium concentrations observed in this study, suggesting that *Cucumis callosus* fruit extract may have ameliorated the damaged state of the kidney caused by cancer induction.

Conclusion

Findings from this study revealed that *Cucumis callosus* fruit extract therapy did not provide statistically significant improvements in serum chloride, potassium, and bicarbonate levels. However, *Cucumis callosus* fruit extract therapy was found effective in ameliorating the poor renal function of the cancer-induced female Wistar rats. The results of the study also suggest that a higher dose (200 mg/bw.t.) of *Cucumis callosus* fruit extract proved effective in ameliorating the poor renal function of DMBA-induced mammary cancer in the female Wistar rats. However, further research is needed to confirm these findings and to explore their underlying mechanisms.

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Conflict of Interest

The authors declared that there are no conflicts of interest.

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