

Effect of Citrus limon juice and Tamoxifen on the Hematological Indices of MCF-7 Cell Induced Breast Cancer in Sprague Dawley Rats

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Abstract

Original Research Article

In search of possible, safe and toxic anticancer drug for the treatment of breast cancer, molecular pathological study of the activities of *Citrus limon* juice on MCF-7 cell line induced breast cancer in Sprague Dawley rats compared to Tamoxifen was carried, over one hundred and twenty Sprague dawley rats of 40 days old, average body weight 180-220g were divided into ten (10) containing of 12 animals per group, group 1 was control, fed only with rat chow and water, group 2 was MCF-7 cell line induced rat alone (BCIR only), group 3 Citrus limon juice (CLJ) at 8.88%, Group 4 Citrus limon juice (CLJ) at 17.32%, group 5 Citrus limon juice (CLJ) at 25.98%, group 6 was given 0.2mg/kg of Tamoxifen alone, group 7 (BCIR+CLJ at 8.88%), group 8 (BCIR+CLJ at 17.32%), group 9 (BCIR+CLJ at 25.98%) and group (BCIR+ 0.2mg/kg of Tamoxifen). At the end of the administration, animals were sacrificed, fresh blood were taken into the heparinized container for hematological analysis such as RBC, MCV, MCHC, PCV, WBC, Hb, Eosinophil, Basophil, Lymphocyte, Monocyte and Platelet counts, while the other part of the blood were centrifuged at 3000 rpm, serum collected and stored appropriately for Biochemical studies, Hematological profile revealed significant reduction in the red cell indices, Breast cancer induced rat group at $p < 0.001$ compared to the Breast cancer induced rat treated with *Citrus limon* and Breast cancer induced rats group treated with Tamoxifen while other non-Breast cancer induced group were not significant. There was significant increase in WBC cells and differential respectively in the Breast cancer induced group at $p > 0.001$ when compared to all the Breast cancer induced post treated with *Citrus limon* and Breast cancer induced group post-treated with Tamoxifen simultaneously. In conclusion, this study showed that *Citrus limon* juice exhibit anticancer activities reverting the abnormal blood indices thereby preventing possible pathological conditions on the body during and after usage, hence it could be used as alternative therapy in the treatment of Breast cancer owing to its tolerance activities and reversibility effects on the hematological indices in similar manner to Tamoxifen.

Keywords: MCF-7 Cell lines, Breast cancer, Citrus Limon Juice, Tamoxifen, Molecular Pathology, Sprague Dawley Rats.

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INTRODUCTION

Blood cells in Breast cancer patients in many studies have shown significant reduction and this has revealed the relevances between blood and Breast cancer. Cancer or tumour cells migrate via blood flow with and outside adjacent organs and this has contributed greatly as the major reason why the tumour cells proliferate and swim round the body [1]. Blood carry oxygen to different part of tissues for respiratory activities and supply of nutrients to places where there is need for one element or substances or the other. Some part of the blood mainly white cells such as leucocytes fight against this tumour cells and probably reduces their effect depending on the immune status of

the patients while some blood components are full responsible for detecting allergy in case the toxins are released from the tumour into the body [2].

Breast cancer usually claim life of victim, one out of every ten will die of in live time, especially when it resulted into bleeding and eventually give rise to anaemia and some clothing disorders, it has been the common malignancy resulting into high mortality rate when compared to other form of cancer affecting women. It is also uncommonly found in men, though association between the ABO Blood group and Breast cancer has been documented according to Sujatha B and Sherry Jenilin G [8].

Breast cancer are usually form from cancer cells or normal cells of the body that grow out of control within the body as a result of direct or indirect assault on the cell, there cells may eventually form tumor cells based on the lost of the normal function of the precursor lining cells in the tissue, tumor cells found in the breast may travel to other part of the body to become cancer cells taking their derivative from the tissues organ of their new location, Aggarwal BB and Gehlot P [5].

Spread of cancer cells may occur by the lymph and lymphoid system when cells are transferred to other part of the body. Breast cancer cells may spread from the lymph nodes under the arm and migrate too many part of the body, they may spread by the lymph node in the collarbones and spread as well or they may spread by the help of the internal mammary lymph nodes, which are usually found within the breast chest.

Breast cancer that spread from the lymph node may usually give rise to metastasis but not all the metastasis are originated by the spread of cancer cells from the lymph nodes.

Calomme et al. [6], have attributed *Citrus limon* fruit as juice to posses antioxidant and anti-cancer properties, this may be due to its fruit constituent such as essential oil or d-limonene, citric acid which could be nonamal, decamal, dodecal, yarcuyl, linanyl, citronelyl flavonoids, neohesperidine, rutin, erioatin anthronil acid, limonins and methyl ester, in alternative and ayurvedic medicine it is considered as liver tonifier and purifier, digestive, immune and intergumentary system tonic, while lemon essential contain vapour which has been experimented to ameliorate effect of stress in mice study [7].

The administration of Fresh juice of *Citrus limon*, is reported effective at reducing the sizes of tumour, quality of live of breast cancer patients, it is well tolerated in the body without any side effect such as vomiting, alopecia, diorhea and lost of weight as common side effect known by other chemotherapies when comparad to the other active pharmacological and chemotherapeutic obtained from plants which proved to be useful in clinical trials as therapy. *Citrus limon* has historical use as treatment for breast diseases, yet there is little or no scientific evidence which shows chemotherapeutic potential towards breast tumours. Preparation and analysis of fresh juice from *Citrus Limon* may reveal novel chemotherapeutic agents that can be used towards targeting cancer cells, understanding the activities will explore the basic underlying ingredients in finding possible solution to breast cancer.

The use of Tamoxifen as drug of choice in Breast cancer therapy, has generated good discovery and helped in the treatment of Breast can cer that are

hormone dependant based on Tamoxifen properties as an antagonist wich act against the estrogen to prevent cancer cell from utilizing this estrogen for their metabolic activities resulting into death of the tumour cell death.

Despite the usage of Tamoxifen for the treatment of Breast cancer, it has been implicated in the many conditions that have practically made it not suitable as reliable therapeutic form of drugs.

Due to ongoing progress of the chemotherapeutic agents in cancer management, it remains to be chemopreventive drugs for breast cancer such as Tamoxifen. Heamatological assesement of the Blood could be of great significance in evaluating and monitoring the progress of Breast cancer patients underging therapy. Despite these activities, there is limited or no scientific based evidence on Heamatological indices on anticancer activaties of the Citrus limon juice on-vivo wise using animal model and the need for toxicity study using animal induced model is also encouraged in this study.

MATERIALS AND METHOD

Chemicals, Reagents and Equipments

All the chemicals, reagents, and equipments used in this study are of international standard organization grade stardandardised by ISO and analytical grade without any form of impurities.

Citrus Limon fruit Collection

The Citrus limon fruit samples of the same species and varieties were collected from a the local farm in Uyo, Akwa Ibom, Nigeria within the month of October and December 2016 in sterilized conditions from the same set of trees in sterilized polythene bags, stored at 4oC in a refrigerator until use, they were authenticated by Botanist at the Agricultural Biotechnology unit, Derindam Research Institute of Biotechnology

Toxicity Study of *Citrus limon* (L) juice

Acute toxicity study of *Citrus limon* (L) juice was carried out based on Lorke's method (Lorke, 1983), LD₅₀ values of the *Citrus Limon* (L) at 10%, 20% and 30% were 8.88%, 17.32% and 25.98% and they were considered and used as Low, Middle and High dose respectively.

Drugs

Tamoxifen citrate tablets (Cipla Ltd., Goa 403 722 India).

Animals and management

Experimental rats used were approved by Animal Care and Use Committee (IAUC) of Derindam Research Institute of Biotechnology, Nigeria based on the rules guiding the use of laboratory.120 virgin-female Sprague-Dawley (SD) rats (40 days old) with

weight of 180-220g were obtained from the Animal house of the Institute, DRIB. The animals were divided into Ten (10) groups of 12 rats per group. Animals are housed two rats per plastic cages and allowed to acclimatize in standard conditions (under a 12 hours light and dark reaction, free access to distilled water and commercialized food throughout the experiment.

Preparation Breast cancer (MCF-7) Cell lines

MCF-7 (Breast cancer) Cell lines was obtained from NCCS, Pune. Cells were cultured in Duilecco's modified Eagle's medium, 10% Fetal Bovine Serum complete medium supplemented with antibiotics, cells were maintained at 37°C in a 5% CO₂ incubator and the media were changed regularly throught the experiment. 90% of the cells wereconfluent, the medium was removed and the cells washed with Phosphate buffer solution, dead cells were removed by the addition of EDTA to detach the sticked cells. Cells obtained immediately were centrifuged at 1000 rpm for 10 minutes at 4o C, the cells were washed twice with PBS and dispersed

Extractin of Citrus Limon (L) Juice

Fruits were washed with Distilled water, the juice were extracted manually, by cutting the fruits in halves and carefully squeezing to extract juice. Juice was using cloth of muslin of 4 fold into the beakers for the admistration during the exerimal procedures.

Breast Cancer / Mammary Tumor Induction

Experimental animals were anesthetized using 150mg/kg ketamine and 10mg/kg of xylazine mixture by injection via intraperitoneal respectively. The injection site was properly cleaned and sterilized with ethanol. The cell suspension, 600000 cells in 300µl PBS was drawn into 1c or 1ml TB syringes without needles to minimize damage, lysis and death to the cells. The cell suspension was inoculated subcutaneously into the mammary fat pad (right flank) of the Sprauge- Dawley (SD) rats using a TB syringe with #26 gauge needle, cell suspension of 300ul was injected by positioning the needle at 2mm posterioly and 2.5 mm laterally, inserted through the skin and then lowered 5 mm into the mammary fat pad. The beds of rats were supported with suitable heat lamp to avoid loss of body heat during the procedure. The temperature, breathing and heart rated of animals were monitored closely. The rat were were swang backward and over continously for 30 seconds to generate warnt as this facilitate their breathing rate and they became stabilized shortly after this procedure.

Experimental Design for in - vivo Anticancer Study

One hundred and Twenty Sprague –Dawley rats,40 days old, average body weight 180 -220g were dividedinto ten (10) groups (labeled as group 1-10) containing Ten (12) animals per group. Cancer was induced using MCF-7 cell lines (Roghayeh et al., 2010)

in groups 2,7,8,9,10,11 and 12, after twenty one days of development of Breast cancer, animals were treated with various concentrations of *Citrus limon* juice and Tamoxifen respectively for Twelve (12) weeks as indicated below:

- Group 1: Control animal fed with feed and water only.
- Group 2: MCF-7 Cell line Induced Breast cancer rats only
- Group 3: *Citrus limon* Juice, 8.88% (Low dose)
- Group 4: *Citrus limon* Juice, 17.32% (Middle dose)
- Group 5: *Citrus limon* Juice, 25.98% (High dose)
- Group 6: Tamoxifen 20mg/kg only
- Group 7: MCF-7 Cell line Induced Breast cancer rats + *Citrus limon* Juice, 8.88% (Low dose)
- Group 8: MCF-7 Cell line Induced Breast cancer rats + *Citrus limon* Juice, 17.32% (Middle dose)
- Group 9: MCF-7 Cell line Induced Breast cancer rats + *Citrus limon* Juice, 25.98% (High dose)
- Group 10: MCF-7 Cell line Induced Breast cancer rats + Tamoxifen 0.2mg/kg

Preparation of Tamoxifen doses for Administration

Tamoxifen was prepared using the formulae stated below:

$$\frac{\text{Rat weight (kg)} \times \text{Dosage (mg/kg)}}{\text{Concentration of Tamoxifen (mg/ml)}} = \text{Tamoxifen Administered (ml)}$$

Tumor Study

Following the Breast cancer induction, all the animals were monitored on daily baisis for any form of tumour growth and development, areas found to be of abnormal groth in Tumour mass were measured using the formulae of Carisson: $V = (ab^2)/2$ to calculate the following following the measurement of Length and Breast and width of the area of the tumour, where 'a' and 'b'dennotes Length and Breath tumour distance covered or measured by use of the caliper.

X-ray Imaging

Experimental animals were shved toward the area of mammally pad and aneastesised following the induction of tumour, the of Xray machine a 44 kilvolts for 3 miiliseconds made in Taiwan the advanced radiographs was used to observe the photographs to the prensence tumour formed and the location of the Tumour as a confirmatory test the precence of Mammary tumour induced.

Preparation of Cell lines

MCF-7 Breast cancer cell lines were cultured in DMEM 10% FBS complete medium. 10% heated inactivated fetal bovine serum was added, antibiotics, and the cells were maintained in 5% CO₂ incubator at 37 degree celcius and the media were changed regularly through out the experiment.

Sterility Test of Breast cancer (MCF-7) Cell lines

Sterility test was carried out from the onset to verify the *Citrus limon* juice capacity of contamination free, 35 mm culture dish was plated with MCF-7 cell suspension in 2ml of DMEM media, cells were allowed to adhere, the *Citrus limon* juice were added into the culture dishes and incubated in 5% CO₂ Incubator for 24 hours.

Heamatological analysis

On the last day of the administration, the animals were anaesthetized with chloroform and sacrificed. Blood was collected by cardiac puncture into EDTA bottles and used for assay of haematological parameters.

Blood was also collected into plain bottles centrifuged at 3000 revolution per minute using centrifuge machine, the supernatant were taken using pipette and stored in well labeled plain bottles for biochemical and immunoassay parameters analysis.

Heamoglobin Estimation [8]

Heamoglobin (Hb) concentration was determined using the haemoglobin cyanide method formerly called cyanmethaemoglobin method. The haemoglobin in the blood was oxidized to haemoglobincyanide by the action of potassium ferrocyanide.

Red Blood Cell Count Estimation [8]

Formal citrate solution was prepared by mixing 10ml of formalin with one litre of trisodium citrate solution. 31.3g per litre is used as diluents for visual red blood cell count. The blood was taken into a positive displacement pipette and 40ml of diluents prepared to give a final dilution of 1 in 20 litre. The diluents sample was mixed and loaded into the counting chamber. The erythrocyte was counted using a haemocytometer in mm³ via the formulae stated below:

$$\text{Total Red Blood cells} = \frac{\text{Number of RBC counted} \times \text{Dilution factor} \times 10,000}{\text{No. of chambers counted}} \text{ cells/mm}^3$$

Packed cell volume Evaluation

According to Ochei and Kolhakaar [8], the wintrobe microhaematocrit tube was filled with blood by capillary action up to 2/3. The samples were spun for

5 minutes at 10,000rpm and the PCV was read as a percentage using the designed scale reader.

Mean Corpuscular Haemoglobin Evaluation

Using Ochei and Kolhakaar [8] method indicates the weight of haemoglobin in a single red blood cell and is expressed in pictograms where 1pg is equivalent to 10⁻¹²g.

$$\text{MCH} = \frac{\text{Haemoglobin (g/dl)} \times 10}{\text{RBC (10}^{12}/\text{L)}}$$

Mean Corpuscular Haemoglobin Concentration Estimation

Ochei and Kolhakaar [8] method denotes the haemoglobin concentration per 100 ml of packed red blood cells and is related to the colour of the red cells. This is expressed as percentage of packed cells.

$$\text{MCHC} = \frac{\text{Haemoglobin (g/dl)}}{\text{PCV\%}} \times 100$$

White Blood Cell (WBC) Estimation

Ochei and Kolhakaar [8] procedure for total white cell count preceded exactly the same manner as that described for red cell count except for a different dilution factor (1:20 dilution) and this method utilized glacial acetic acid to breakdown the erythrocytes to enable easy counting of Leucocytes.

$$\begin{aligned} \text{Total white blood cell count/mm}^3 &= \frac{\text{Number of WBC counted} \times \text{Dilution factor}}{\text{No. of chambers counted}} \\ &\times 10,000 \text{ cells/mm}^3 \end{aligned}$$

Total WBC count of the given blood sample in cell/mm³

Determination of Platelet Count

Ochei and Kolhakaar [8] method involved whole blood diluted with 1% ammonium oxalate reagent, which lyses the red cells, leaving the white cells and the platelets intact. Platelets are counted microscopically using a ruled counting chamber.

$$\text{Calculation: Total Platelets/mm}^3 = \text{Platelets counted} \times \text{dilution factor} \times \frac{1}{\text{Volume factor}}$$

Estimation of Leucocyte differentials Ochei and Kolhakaar [8] procedure for white blood cell differential consist of neutrophils, lymphocytes, monocytes, eosinophils and Basophils; they were carried out as percentage portion of the white blood cell present in the blood. The combination of polychrome methylene blue and eosin stains has selective staining properties. The differential staining allowed identification of the types of white blood cells on the smear.

Data collection and Analysis

The values of all the morphometric data were analyzed, computed and compared statistically using Graphpad prism 8 version 1.0.2, all data were expressed

as Mean \pm SEM. One-way analysis of variance (ANOVA) was used to test for difference among the groups and t-test was used to compare the significant differences between the means. Values were considered significant at $P < 0.001$.

RESULT

Citrus limon juice and Tamoxifen on the Hematological indices of MCF-7 cell induced breast cancer in sprague dawley rats. Effect of *Citrus limon* and Tamoxifen on Red Cell Indices on MCF-7 cell induced Breast cancer.

In the Breast cancer induced rat group, the Hb concentration, RBC, MCH, MCHC, PCV, MCV and Platelets were significantly reduced compared to the control group, Citrus limon juice at 8.88%, 17.32% and 25.98% respectively and 0.2mg/kg of Tamoxifen alone while in Breast cancer induced rat group treated with Citrus limon juice at 8.88%, Breast cancer induced rat and Citrus limon juice at 17.32%, Breast cancer induced rat and Citrus limon juice 25.98% simultaneously and Breast cancer induced rat treated with 0.2mg/kg of

Tamoxifen, it was significantly increase at $P > 0.0001$ (Table 1.0.0).

Effect of *Citrus limon* juice and Tamoxifen on White Blood Cell indices on MCF-7 induced breast.

Full Blood count including WBC, Monocytes, Lymphocytes and Neutrophil were significantly increased at $p > 0.001$ in Breast cancer induced rat group compared to all the group while the control group was not significantly different from the Citrus limon juice and Tamoxifen alone group, though extremely different from the Breast cancer induced rat treated with Citrus limon juice and Citrus limon juice and Tamoxifen groups. Basophil count was not significant among the groups except the Breast cancer induced rat groups which was extremely different and increased when compared to control, Eosinophil count was insignificant in control, *Citrus limon* juice alone and Tamoxifen group but extremely significantly increased at B Breast cancer induced rat group which was not significantly different from the Breast cancer induced rat post-treated with and *Citrus limon* juice and Breast cancer induced rat and Tamoxifen treated group at $P > 0.001$ (Table 2.0.0).

Table-1.0.0: Effect of *Citrus limon* (L) juice and Tamoxifen on Red Cell Indices on MCF-7 induced breast cancer in Sprague Dawley Rats

Groups	Haemoglobin (Hb) (g/dl)	Erythrocytes ($10^6/\text{mm}^3$)	PCV (%)	MCHC (g/dl)	MCH (pg)	Platelets ($10^9/\text{ml}$)
Control (Distilled water)	14.92 \pm 0.485	7.520 \pm 0.301	47.17 \pm 1.138	34.40 \pm 0.510	18.90 \pm 0.508	793.4 \pm 52.49
BCIR (MCF-7) only	8.440 \pm 0.641 ^c	2.640 \pm 0.280 ^b	24.50 \pm 2.592 ^c	18.54 \pm 0.973 ^c	11.00 \pm 0.829 ^c	209.6 \pm 26.38 ^b
<i>Citrus limon</i> (8.88%)	16.44 \pm 0.416 ^{ns}	7.840 \pm 0.256 ^{ns}	50.67 \pm 1.783 ^{ns}	35.00 \pm 0.837 ^{ns}	18.50 \pm 0.253 ^{ns}	853.0 \pm 33.06 ^{ns}
<i>Citrus limon</i> (17.32%)	17.12 \pm 0.097 ^{ns}	7.680 \pm 0.306 ^{ns}	52.33 \pm 0.989 ^{ns}	35.20 \pm 0.735 ^{ns}	18.57 \pm 0.229 ^{ns}	804.8 \pm 50.25 ^{ns}
<i>Citrus limon</i> (25.9%)	16.44 \pm 0.615 ^{ns}	7.920 \pm 0.193 ^{ns}	53.17 \pm 1.740 ^{ns}	34.80 \pm 1.068 ^{ns}	18.37 \pm 0.175 ^{ns}	959.8 \pm 25.71 ^{ns}
0.20mg/kg Tamoxifen	14.80 \pm 0.425 ^{ns}	7.402 \pm 0.160 ^{ns}	51.33 \pm 1.406 ^{ns}	34.00 \pm 1.761 ^{ns}	17.32 \pm 0.178 ^{ns}	293.6 \pm 28.35 ^c
BCIR + <i>C. limon</i> (8.88%)	12.58 \pm 0.707 ^{ns}	5.500 \pm 0.241 ^{ns}	41.50 \pm 0.992 ^{ns}	21.80 \pm 1.744 ^c	13.87 \pm 0.264 ^c	508.4 \pm 28.47 ^{ns}
BCIR + <i>C. limon</i> (17.32%)	13.56 \pm 0.390 ^{ns}	5.600 \pm 0.245 ^{ns}	44.17 \pm 1.249 ^{ns}	24.20 \pm 1.068 ^b	14.32 \pm 0.409 ^b	646.6 \pm 27.98 ^{ns}
BCIR + <i>C. limon</i> (25.98%)	14.14 \pm 0.398 ^{ns}	6.600 \pm 0.192 ^{ns}	45.17 \pm 1.138 ^{ns}	27.00 \pm 2.387 ^{ns}	17.08 \pm 0.285 ^{ns}	640.2 \pm 17.89 ^{ns}
BCIR + 0.20mg/kg Tamoxifen	14.63 \pm 0.164 ^{ns}	7.260 \pm 0.242 ^{ns}	42.33 \pm 0.803 ^{ns}	31.00 \pm 0.316 ^b	17.60 \pm 0.230 ^{ns}	610.0 \pm 22.52 ^{ns}

Values are Mean \pm SEM of 5 rats in a group

^aBCIR Significantly different compared to control group ($p < 0.0001$), ^{ns} Significantly different compared to BCIR + CLJ and BCIR + Tamoxifen treated groups ($p < 0.05$).

RBC = Red blood cell, Hb = Haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, MCH = Mean corpuscular haemoglobin, PCV = Packed cell volume, PLT = Platelet.

Table-2.0.0: Effect of *Citrus limon* (L) juice and Tamoxifen on White Blood Cell and differential Indices on MCF-7 induced breast cancer in Sprague Dawley Rats

Groups	Basophils (10 ³ /mm ³)	Eosinophils (10 ³ /mm ³)	Neutrophils (10 ³ /mm ³)	Leucocytes (10 ³ /mm ³)	Lymphocytes (10 ³ /mm ³)	Monocytes (10 ³ /mm ³)
Control (Distilled water)	0.0±0.0	0.014±0.003	0.558±0.041	6.140±0.289	5.640±0.496	0.134±0.014
BCIR (MCF-7) only	0.020±0.006 ^{as}	0.352±0.050 ^c	1.364±0.108 ^c	1.720±0.246 ^b	12.86±1.142 ^{as}	0.003±0.001 ^b
<i>Citrus limon</i> (8.88%)	0.0±0.0	0.030±0.003 ^{as}	0.814±0.051 ^{as}	5.900±0.622 ^{as}	6.200±0.311 ^{as}	0.110±0.005 ^{as}
<i>Citrus limon</i> (17.32%)	0.006±0.003 ^{as}	0.028±0.002 ^{as}	0.798±0.025 ^{as}	6.280±0.193 ^{as}	6.320±0.428 ^{as}	0.066±0.005 ^{as}
<i>Citrus limon</i> (25.9%)	0.002±0.002 ^{as}	0.026±0.005 ^{as}	0.740±0.007 ^{as}	6.580±0.199 ^{as}	6.520±0.159 ^{as}	0.076±0.013 ^{as}
0.28mg/kg Tamoxifen	0.001±0.0	0.052±0.007 ^{as}	0.716±0.021 ^{as}	5.560±0.254 ^{as}	6.520±0.466 ^{as}	0.126±0.017 ^{as}
BCIR + <i>C. limon</i> (8.88%)	0.001±0.0	0.054±0.003 ^b	0.728±0.050 ^{as}	3.600±0.221 ^b	12.02±0.710 ^c	0.028±0.006 ^c
BCIR + <i>C. limon</i> (17.32%)	0.001±0.0	0.048±0.002 ^b	0.720±0.046 ^{as}	4.380±0.227 ^{as}	9.700±0.397 ^{as}	0.048±0.005 ^c
BCIR + <i>C. limon</i> (25.98%)	0.001±0.0	0.042±0.006 ^{as}	0.612±0.034 ^{as}	5.120±0.159 ^{as}	8.040±0.368 ^{as}	0.054±0.005 ^c
BCIR + <i>C. limon</i> (25.98%) +0.28mg/kg Tamoxifen	0.001±0.0	0.036±0.004 ^b	0.682±0.034 ^{as}	5.060±0.334 ^{as}	6.880±0.203 ^{as}	0.072±0.007 ^{as}

Values are Mean ± SEM of 5 rats in a group.

^aBCIR Significantly different compared to control group (p<0.0001), ^{as} Significantly different compared to BCIR + CLJ and BCIR + Tamoxifen treated groups (p<0.05).

WBC=White blood cell, Neut = Neutrophil, Lym = Lymphocyte, Mon = Monocyte, Eos = Eosinophil, Baso = Basophil.

DISCUSSION

Obviously, cancer in general appeared to have no definite cure even as at of today in our society, but many drugs have been tried and used but not curing or stopping the growth of the tumour, they rather cause more pain or induced normal cell destruction while destroying the tumor cells.

Series of conventional therapies have been employed and utilized in orthodox, traditional and herbal medicine, yet no clear-cut possible cure have been identified.

Clinical wise, the use of chemotherapy has produced slight significant but the end product still result in dead or irreversible lost of life, even when it has caused the patient huge lost of money fortune and profile lost in the society. Radiotherapy as another form of cure have been employed in many cases, they appeared working initially but at later end causes pain, wasteful and lost of life and general lost in the society.

Finding possible cure for cancer has not been easy and yet the rate of growth of cancer increases on daily basis as there is no clear indication on proper causes and proper treatment. Management of cancer patients too has not been easy and it is quite expensive for the poor especially in developing countries and across the globe.

Surgical method suggested has not given clear evidence of cure, it only ameliorate the immediate pain initially and at the later stage, it inflicts more pain on the victim and make life uncomfortable for the patient and eventually such patients may die and all the money spent lost.

Variety of conventional therapies for cancer based on chemotherapy, radiotherapy and surgery are

limited in efficacy. Most current cancer chemotherapy regimens are normally associated with very high significant levels of toxicity and drug resistance [9].

Hemoglobin concentration of breast cancer induced rat group was very low, though increased gradually the Breast cancer Induced rats post-treated group with *Citrus limon* juice at 8.88%, 17.32%, 25.98% respectively and 0.2mg/kg Tamoxifen group due to breakdown in cellular activity, and internal bleeding observed in Breast cancer Induced rats only during the experiment, there was loss of blood internally and this brought down the hemoglobin concentration level of the breast cancer induced rat and the hemoglobin concentration level increase gradually in Breast cancer Induced rats treated with Citrus limon juice at 8.88%, 17.32% and 25.98% groups as well as 0.2mg/kg of Tamoxifen group as a result in appetite stimulation the *Citrus limon* juice at impaired on the Breast cancer Induced rats group while the Breast cancer Induced rats group did not have appetite due to degeneration in the cellular level impacted on them by MCF-7 cell lines. Similarly, the red blood cells, mean cell volume, mean cell hemoglobin concentration, packed cell volume and platelets were extremely low in the breast cancer induced group, the low impairment in megakaryoblast induced loss of factors I to VIII, leading deficiency of thrombokinas, thrombin and prothrombin which could have initiate blood clotting formation during bleeding to prevent the loss hemorrhage during the study as illustrated in Table 1.0.0.

While in Table 2.0.0, blood cell indices was grossly increased the cancer induced rat group compared to the breast cancer induced rat plus *Citrus limon* post-treated group at 8.88%, 17.32% and 25.98% and similarly with 0.2mg/kg of Tamoxifen group, increased in white Blood cells (leukocyte) count signifies infection and microbial vulnerability which

indicate lower immunity that encourage infiltration of micro-organism into the body, the study revealed breakdown in the immunological response as a result of diminished nutrient to body as a result of competitive activity of the cells to survive as the hemoglobin concentration reduces, the red blood cells content had reduced and insufficient oxygen was found in the system, their body required more oxygen for metabolic activities and cell dies naturally as a result of starvation, malabsorption and reabsorption of nutrients, the immune system weakened and the influx of micro-organism increased, it was revealed that in the posted treated groups with the *Citrus limon* juice at 8.88%, 17.32% and 25.98% exhibit restoration and the experimental animals begin to have appetite for food at starting from 8th week though at slow rate. Similarly the neutrophil and lymphocyte increase, monocyte decrease, in breast cancer group only but showed sign of increment in the post-treated group with Citrus limon juice at different concentration and 0.2mg/kg of Tamoxifen treated group while the control group and *Citrus limon* juice at 8.88%, 17.32% and 25.98% and 0.2mg/kg of Tamoxifen alone the animal appeared healthy and vibrant, consuming food normally. The basophil count was slightly increased at breast cancer induced rat group alone and not present at all in other group through the experiment [10].

The eosinophil content was very high in the breast cancer group alone suggesting the influx of parasitic and allergic situation of the Breast cancer Induced rats groups, as the immune system breakdown, there is up regulation of the activities of the parasite both of the endosystem and ecosystem into the body. Cancer cells being a competitor of the vital nutrients of the body had succeeded in breaking the defence mechanism which enable the parasites to migrate into the body of the Breast cancer Induced rats group only while in the other post-treated groups with Citrus limon juice at 8.88%, 17.32% and 25.98% and 0.2mg/kg of tamoxifen the eosinophil level which was very high begin to reduced and eventually become very low due to reactivation mechanism of the immune system as a result of the Breast cancer Induced rats responding to treatment and the population of cancer cells becoming reduced significantly which is in line with the work of Williams *et al.* [11] stipulated that analysis of white blood cells and its differentials is frequently required for the evaluation of immune system.

CONCLUSION

From this study, it was discovered that the safety effect of *Citrus limon* as been documented in previous studies were confirmed as it does not inflict any hematological disorder on the experimental animals, the red cell indices maintained their cellular compactments and integrity which is responsible for the transport of oxygen and nutrients to various part of the body, the possibility of anaemia was ruled out while the white cell activities were grossly enhanced based on

their protective activities within normal cellular profile and ranges, the platelets physiology were respected and clothing factors were within the normal reactions providing refined pathways for the prevention of clothing disorders. However effect of *Citrus limon* juice on the Hematological indices provided revealed cellular tolerance of the juice without any adverse or derogative effect on the experimental models.

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