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Original Research Article

Effect of Citrus Limon Juice and Tamoxifen on the Tumour growth mass Indices, Cell Proliferation, Cell Viability and Cytogenetic (Mitotic Index) of Sprague Dawley Rats Induced MCF-7 Breast Cancer Cells

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Abstract

Possible Effect of *Citrus Limon* Juice and Tamoxifen on the Tumour growth mass Indices, Cell Proliferation, Cell Viability and Cytogenetic (Mitotic Index) of Sprague Dawley Rats Induced MCF-7 Breast Cancer Cells was carried using, over one hundred and twenty Sprague dawley rats of 40 days old with average body weight 180-220g were divided into Ten (10) groups with 12 animals per group, group 1 was control, fed only with rat chow and water, group 2 was MCF-7 cell line induced Breast cancer rat alone (BCIR only), group 3 *Citrus limon* juice (CLJ) at 8.88%, Group 4 *Citrus limon* juicen (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). Acute and sub – acute toxicity were carried out after the establishment of safety dose following determination of LD50, on the fresh C*itrus limon* juice, Tumour growth mass Indices were carrying out on weekly basis on the experimental rats, on completion of the administration of the treatments, animals were sacrificed, Blood was collected into Plain and heparinized bottles simultaneously for Cytogenetic (Mitotic Index) analysis, Tumour tissues were also extracted, passaged and cells were cultured and analysed for Cell Proliferation and Cell Viability assays. Microscopically, it was revealed that DMSO at 1.5% was highly toxic to the MCF-7 cells and other components of media, meanwhile the *Citrus limon* juice at 8.88%, 17.32% and 25.98% showed minimum cell growth inhibition of MCF-7 cell lines and permit cell viability at 40%, 25% and 10% respectively without any effect on the media components. In conclusion, this study showed that C*itrus limon* juice posses anticancer activities and does not pose pathological conditions on the body during and after usage, hence it could be used as alternative therapy in the treatment of Breast cancer that are hormonal dependant in similar manner to Tamoxifen.

Keywords: MCF-7 Cell lines, Breast cancer, *Citrus limon* Juice, Tamoxifen, Molecular Pathology, Sprawgue Dawley Rats.

INTRODUCTION

It has been known that over 12.5% of death occur as a result of Breast cancer in Nigeria given more death estimation than Human Immunodeficiency virus and Acquired Immuno deficiency syndrome, Breast cancer in Nigeria are much younger than Caucasian females (less than 35 years old), it is usually diagnosed at a more advanced stage due to the gender factor and culture of silence secrecy. The Stigma - disclosure would jeopardize social standing and marriage for the family members, the fear of mastectomy which leads in the disfigurement, physical disability and the need to keep the body intact insufficient access to quality health care providers such as physicians, screening scientists due to economical issues and most of all, the lack of awareness on the importance of screening and usage of anticancer drugs [1].

Breast cncer usually claim life of victim, one out of every ten will die of in live time, it has been the common malignancy resulting into high mortality rate when compared to other form of cancer affecting

women. It is also found in men but not very common [2].

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 insult on the cell, there cells may eventually form tumor cells based on the lost of the normal function within the precursor lining in the tissue surrounding cells normal this 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.

Spread of cancer cells may occur by the lymph and lymphoid system when cells are transferred to other part of the body. Sarcomas and lymphomas are other form of cancer that usually starts from the breast tissue [3].

Breast cancer cells may spread from the lymph nodes under the arm and migrate to many part of the body, they may spread by the lymph node in the collar bones 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.

Elson *et al.,* [4] illustrated 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. Modern anti-cancer therapies revealed positive response and survival, but the side or adverse effects and high cost frequently leads to discontinuation. Therefore, there is an imperative need of highly effective compounds with tolerable adverse effects that are affordable and at the patient's disposal.

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 theraupeutic form of drugs [5].

Due to ongoing progress of the chemotherapeutic agents in cancer management, it still remains to be chemopreventive drugs for breast cancer such as Tamoxifen. Yet, there is limited finding or no scientific backing on the curative effect.

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, dodecamal, yarcuyl, linanyl, citronelyl flavonoids, neohesperidine, rutin, erioatin anthronil acid, limonins and methyl ester. Though, the use of the Citrus juice has been attributed to posses giardiacidal activity, antioxidant activity, pediculicidal activities, and antibacterial activities. *Citrus Limon* can also be used for douching in sexually active women, while the protective activity in rat urolithiasis model had been proved as well. MCF-7 cell line make use of estrogen in the production of estradiol and to process through estrogen receptors in the cell cytoplasm and estrogen receptor ER positive control cell lines, it has been useful for in-vitro studies of breast cancer with many characteristics particular to the mammary epithelium (www,altogen,com). However, the need to investigate the proliferative and cytotoxicity effect of *Citrus limon* juice on Breast cancer cell line (MCF-7) and microscopical cytogenetic cytoxicity evaluation is highly considered.

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, Voucher specimen number DRIB-ABU-005.11 was created for the fruit and deposited in the herbarium

Acute Toxicity Study of Citrus*limon* (L) juice

Acute toxicity study of C*itrus limon* (L) juice was carried out based on Lorke's method [7], LD_{50} 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% CO_2 incubator and the media were changed regularly throught the experiment. 90% of the cells were confluent, the medium was removed and the cells washed with Phosphate buffer solution, dead cells was 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 were extracted 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 anesthesized 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 1cc 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 continouesly for 30 seconds to generate warnt as this facilitate theier 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 [8] 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:

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

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 measuremt of Length and Breast and witdth 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 mammallry pad and aneasthesised 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.

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% CO_2 Incubator for 24 hours.

Microscopical Cytotoxic Analysis of Citrus *limon* juice

Cytotoxicity test was carried out to check the *Citrus limon* juice inhibition activity to on the MCF-7 cells, MCF-7 cell line were maintained in DMED (Dulbecco's Modified Eagle's Medium) containing 1% penicillin-streptomycin at ration 1:1, 0.2 % gentamycin and 10% Fetal bovine serum (FBS). MCF-7 cell suspension were seeded into a 24 well cell culture plates at a concentration of 10000 /400ul/well and incubated at 37oc and 5% CO_2 for 24 hours, 100ul of *Citrus limon* at 8.88%, 17,32% and 25.98% concentrations were added, control was also made using 1.5% of DMSO in each well and 18.5% of Distilled water to each well in duplicate, the plate was incubated at 37^{o}C and 5% CO_2 for another 48 hours, the cultured cells were observed under microscope viability and morphological changes.

Determination of Cell Viability detection with Cell Counting Kit 8 (CCK8)

Cell counting kit 8 contain non-radioactive s substances employed in colometric analysis used for detection and counting of living cell or viable cells before proliderative activity occour, it is usually carried out in combination with WST8 undergoing reduction to produce formazon colour reaction on that are released by the dehydrogenase in cells, the formazon produced are soluble in Tissue culture medium as the number of cells that are alive in the culture medium. 100 microliter per well were inoculated in a 96 well plate, incubated at 37 degree celciusin a humidified 5% CO_2 incubator, 10 microliter of CCK8 was added and incubated for 1-4 hours in incubator, Absorbance were measured at 450nm wavelength using microplate reader.

Determination of Cell Proliferation and Cytotoxicity

This is an advance and modified method of cell counting kit 8(CCK-8) method, it involve the use of the same procedure with the readings taken sphectrophotometrically using microplate reader at different interval, 6, 12, 24 and 48 hours to determine the proliferative and cytotoxic cells that are non viable rather than involving in the proliferation, combination of this non-viable cell with WTS-8 are being reduced by the action of dehydrogenase in the cells to produce a formazon reaction that is soluble in cell and tissue culture medium, the amount of dye released in each 100ul in each microlitre well plate is proportional to the non-viable proliferative and cytotoxic cells present in the samples measured at 450nm at room temperature using microplate reader, concentration of the proliferating cells is extrapolated to obtain the standard curve for deduction of proliferative or cytotoxic cells were deduced.

Cytogenetic Analysis

Cytogenetic Study was carried on all the experimental animals. 10 flasks containing the samples from group to group 10 were incubated at 37°C for 48 hrs. The chromosomes were prepared by evaluation of MI in every one thousand cells viewed and counted under the microscope. In these cases the slides were examined fewer than 10 X magnifications, and observed chromosomes or chromatides aberrations under 100X magnification. The M.I % was determined as a ratio of the mitotic cells to the cells in interphase in 1000 calculated cells. M. I. % = (No. of dividing cells / No. of dividing cells + No. of nondividing cells) X 100.

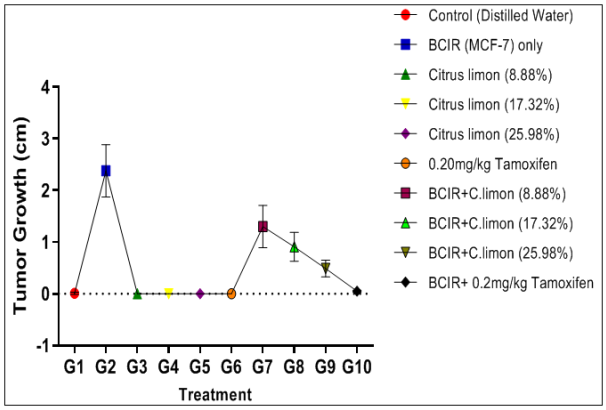
RESULT

Table-1: Showing Tumour growth mass Indices on MCF-7 cell line Induced Breast Cancer in Sprauge Dawley Irats administered with *Citrus limon* (L) Juice and Tamoxifen

Groups	Tumor growth
Control (Distilled water)	0.011±0.010
BCIR (MCF-7) only	2.380±0.227[b]
Citrus limon (8.88%)	0.0±0.0[ns]
Citrus limon (17.32%)	0.0±0.0 [ns]
Citrus limon (25.9%)	0.0±0.0 [ns]
0.20mg/kg Tamoxifen	0.0±0.0 [ns]
BCIR + *C. limon* (8.88%)	1.302±0.183 [c]
BCIR + *C. limon* (17.32%)	0.910±0.125 [c]
BCIR + *C. limon* (25.98%)	0.490±0.072 [c]
BCIR + 0.20mg/kg Tamoxifen	0.050±0.008[c]

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

[a]BCIR Significantly different compared to control group ($p<0.0001$), [c]Significantly different compared to BCIR + CLJ and BCIR + Tamoxifen treated groups ($p<0.001$).



Graph-1: Tumour Growth Mass Indices

Microscopical Cytotoxic Analysis of *Citrus Limon (L)* Juice showing Minimum Cell Inhibition (1C50)

Microscopically, it was revealed that DMSO at 1.5% was highly toxic for the MCF-7 cells and other component by of media, meanwhile the *Citrus limon* juice at 8.88%, 17.32% and 25.98% showed minimum cell growth inhibition of MCF-7 cell lines and permit cell viability at 40%, 25% and 10% respectively without any effect on the media components.

The micrographs of IC50, 50% viability of MCF-7 when 100µl of *Citrus limon* juice and 400 µl of media were added to each well in a 24 plate well revealed the cell-viability, under an inverted microscope after 48 hours of incubationas follow (Figure-1).

Figure-1 showing Microscopical Cytotoxic Analysis of *Citrus Limon (L)* Juice showing Minimum Cell Inhibition (1C50) after 48 Hours.

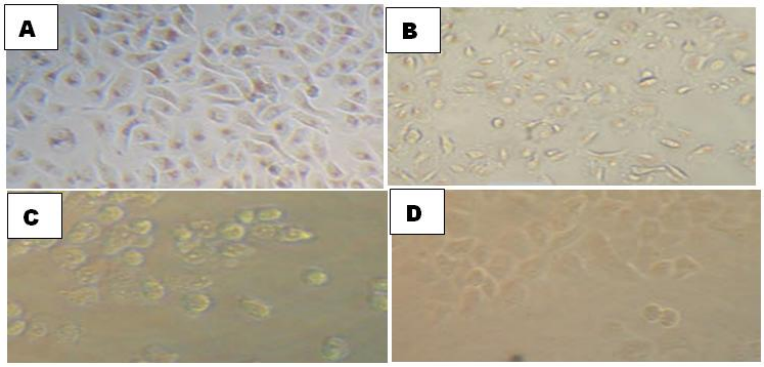


Fig-1: Photomicrographs of IC50, 50% viability of MCF-7 treated with (A) 15% of DMSO, (B) *Citrus limon*, 8.88%, (C) *Citrus limon*, 17.32% and (D) *Citrus limon*, 25.98%. Mag X400.

Effect of *Citrus Limon* Juice and Tamoxifen on the Cell Proliferation, Cell Viability Cytogenetic (Mitotic Index) of Sprague Dawley Rats Induced MCF-7 Breast Cancer Cell Lines

Effect of C*itrus limon* and Tamoxifen on Cell Viability of MCF-7 cell induced Breast cancer in Sprawgue Dawley rats

There were signifcant reduction in cell viability in BCIR + CLJ at 8.88%, BCIR + CLJ at 17.32%, BCIR + CLJ at 25.98%, and BCIR + 0.2 mg/kg of Tamoxifen group at P>0.0002 meanwhile BCIR group while in the non - BCIR groups there was no the cell viability remain unchanged (Table-2).

Effect of C*itrus limon* and Tamoxifen on Cell Proliferation of MCF-7 cell induced Breast cancer in Sprawgue Dawley rats

It was observed that *Citrus limon* juice and tamoxifen inhibit cell proliferation and exhibit MCF-7 cell cytotoxicity in groups 7, 8, 9 and 10 respectively. There was a significant difference in group 7 (BCIR +CLJ), group 8 (BCIR+CLJ at 17.32%), group 9 (BCIR+CLJ at 25.95%) and group 10 (BCIR + 0.2mg/kg Tamoxifen) when compared to group 2 (BCIR) only at p>0.001showing significant decrease in cell proliferation and cytotoxicity when compared to group 3 (CLJ at 8.88% only), group 4 (CLJ at 17.32%), (CLJ at 25.98%) and group 6 (0.2mg/kg of Tamoxifen alone) at 6, 12, 24 and 48 hours rspectively (Table-2).

Effect of C*itrus limon* and Tamoxifen on Cytogenetic (Mitotic Index) of MCF-7 cell induced Breast cancer in Sprawgue Dawley rats

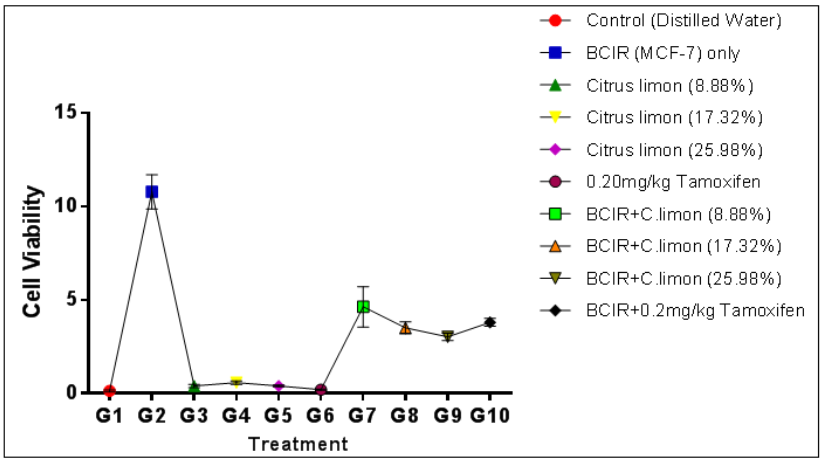
Cytogenetic effect (mitotic index) showed significant decrease in M.I value of BCIR groupcompared to BCIR + CLJ at 8.88%, BCIR + CLJ at 17.32%, BCIR + CLJ 25.98% and BCIR + 0.2mg/kg of Tamoxifen at P>0.0001, while the level of significant was increased when compared to the non BCIR groups; control, CLJ at 8.88%, CLJ at 17.32%, CLJ at 25.98% and 0.2mg/kg of Tamoxifen group alone (Table-2).

Table-2: Showing Cell Viability, Cell Proliferation at 6, 12, 24 and 48 hours and Cytogenetic Cytotoxicity (Mitotic Index)

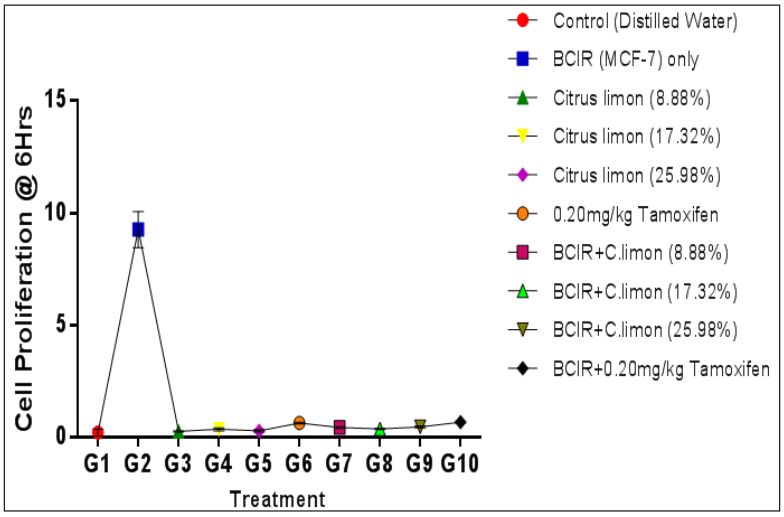
		Cell Proliferation				
Groups	**Cell viability**	**6hours**	**12hours**	**24hours**	**48hours**	**Mitotic Index**
Control (Distilled water)	0.130±0.057	0.216±0.159	0.201±0.087	0.042±0.006	0.114±0.029	0.384±0.119
BCIR (MCF-7) only	10.78±0.918[b]	9.263±0.805[b]	8.694±0.131[a]	8.183±0.369[a]	8.962±0.857[b]	12.65±1.039[b]
Citrus limon (8.88%)	0.392±0.085[ns]	0.267±0.024[ns]	0.179±0.042[ns]	0.714±0.09[c]	0.340±0.026[c]	0.522±0.109[ns]
Citrus limon (17.32%)	0.568±0.098[ns]	0.367±0.069[ns]	0.110±0.025[ns]	0.492±0.027[a]	0.450±0.034[a]	5.300±1.340[ns]
Citrus limon (25.9%)	0.393±0.051[ns]	0.295±0.056[ns]	0.254±0.062[ns]	0.452±0.021[b]	0.367±0.056[ns]	0.400±0.130[ns]
0.2mg/kg Tamoxifen	0.198±0.039[ns]	0.646±0.040[ns]	0.526±0.121[ns]	0.412±0.042[a]	0.687±0.057[c]	0.372±0.093[ns]
BCIR + *C. limon* (8.88%)	4.626±1.081[ns]	0.444±0.036[ns]	3.409±0.322[a]	6.452±0.151[a]	5.716±0.189[a]	6.556±0.404[b]
BCIR + *C. limon* (17.32%)	3.500±0.315[b]	0.386±0.024[ns]	3.748±0.153[a]	5.244±0.194[a]	4.566±0.145[a]	5.526±0.097[a]
BCIR + *C. limon* (25.98%)	3.016±0.202[b]	0.474±0.048[ns]	3.050±0.054[a]	3.666±0.424[b]	3.134±0.248[b]	5.268±0.113[a]
BCIR + 0.2mg/kgTamoxifen	3.788±0.213[a]	0.679±0.037[ns]	3.838±0.205[a]	4.646±0.476[b]	4.472±0.193[a]	4.648±0.249[b]

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

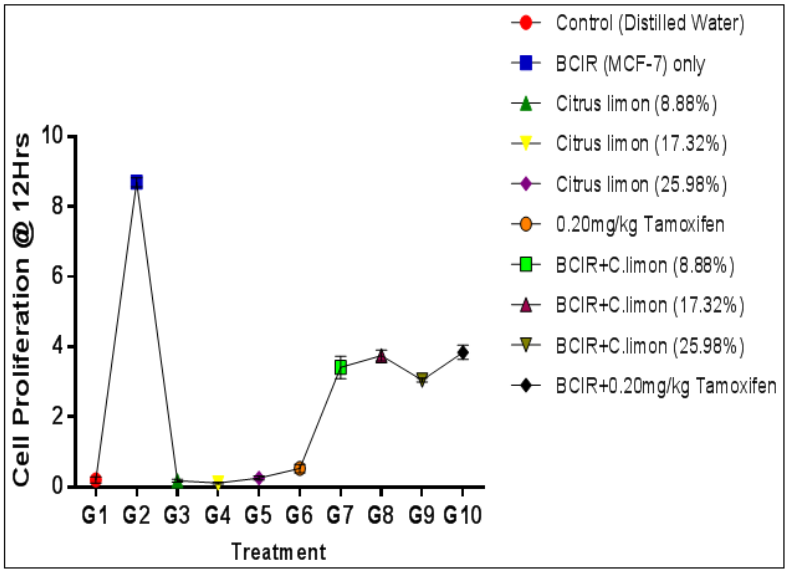
[a]BCIR Significantly different compared to control group (p<0.0001), [abc]Significantly different compared to BCIR + CLJ and BCIR + Tamoxifen treated groups (p<0.001).



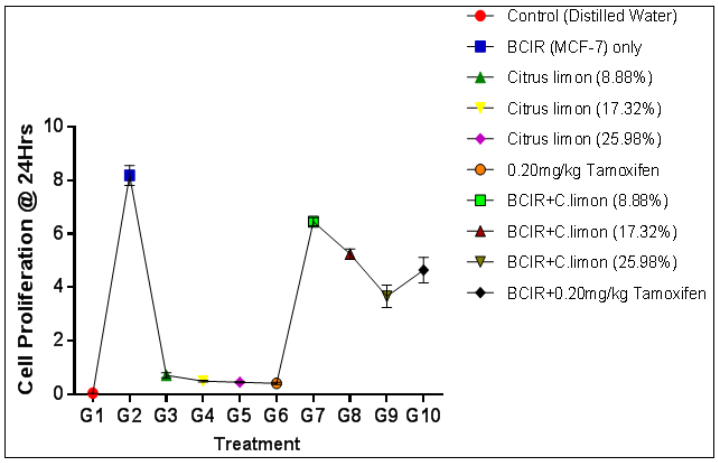
Graph-2: Cell viability



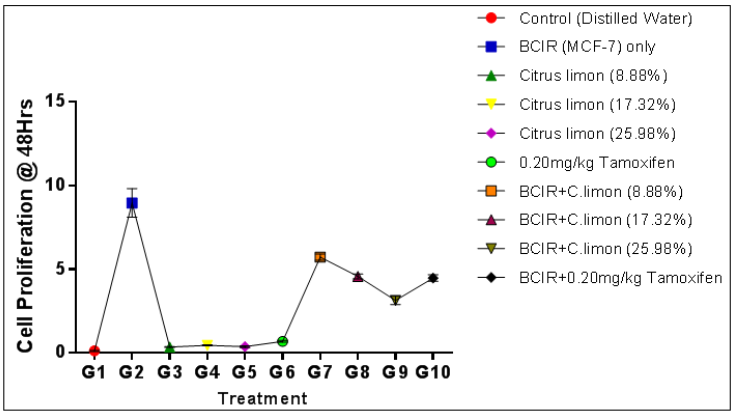
Graph-3: Cell Proliferation at 6 Hhours



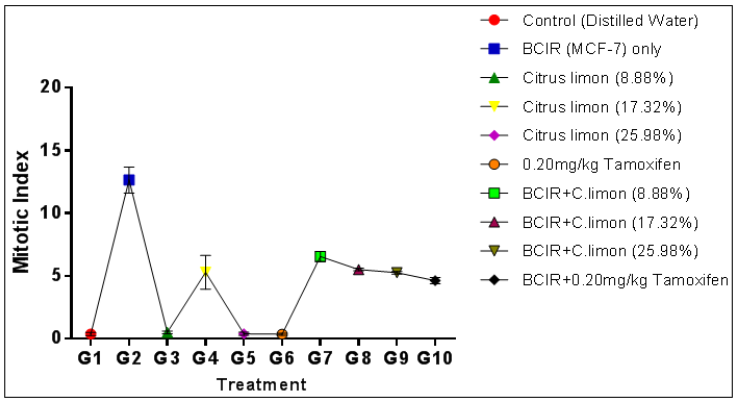
Graph-4: Cellular Proliferation at 12 Hours



Graph-5: Cell Proliferation at 24 Hhours



Graph-6: Cell Proliferation at 48 Hhours



Graph-7: Cytogenetic (Mitotic Index)

DISCUSSION

According to Hsia and Liu [9], 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.

In developing countries like Nigeria, India and many African and Asian countries cancer showed more occurrence than the infectious diseases such as HIV/AIDS, tuberculosis and malaria deaths when combined together and by year 2020, more than 35% causes (10.5 million) of cancer will emerge according to WHO, 2007 since death rate is 16 out of way 1000 death globally, finding possible cure has been an embattle problem over the years which is the major

headache for health care provider and the patients as well.

Breast cancer which is one of the prominent cancer related problem has been known to be more common in female than men and the prevalence is enormous in developing countries. Despite positive indication in the use of chemotherapy, radiotherapy to upstream the survival rate for the illness, the side effect of the chemotherapies, surgery and radiotherapy and high cost frequently leads to discontinuation from purchasing or getting the treatment thus has also generated huge set back in the cure.

Ability of individual, victims, or patient suffering from cancer to lay hands on these treatments is another major factor in the cure.

Manufacturing of drugs employed in the treatment of breast cancer has not been easy as it involve huge capital intensive, man-power and exploration of ideas from scientist, medical expert across the globe.

Finding easily available alternative for the treatment, cure, prevention and management will surely pave ways for targeted different types of cancer and making the treatment easy thereby reducing the mortality rate of cancer in our society. Major challenges to reduce breast cancer burden is to develop highly effective alternative drugs specifically for breast cancer with minimal side effects or no side effect on the patients

This study was carried out to investigate the molecular pathological activities of *Citrus limon*juice on MCF-7 cell line induced breast cancer in Sprague dawley rats.

To minimize the real breast cancer nature of human in animal, MCF-7 cell line was used to establish breast cancer in sprawgue dawley rat and *Citrus limon* juice at safe dose concentration were used as therapeutic treatment compared with Tamoxifen known and established drugs for the treatment of breast cancer that are hormonal dependent. The curiosity to establish possible activities of *Citrus limon* juice as an alternative treatment for the treatment/management of breast cancer has led to this studies in which the main purpose was to compare *Citrus limon* juice activities to inhibit cancer growth in-vivo and in-vitro and to with standard drug (Tamoxifen), the result obtained in this study is expected to add value, contribute to knowledge, to create alternative, easily available, cheaper source or agent of treatment of breast cancer and serve as basis for further studies in searching cheaper, affordable source for the treatment of breast cancer in developing countries.

Acute toxicity of *Citrus limon* was carried out to establish concentration LC50 that is safe for human consumption, it is either carried once singularly within 24 hours of exposure or with multiple exposure with shortest period of time in 24 hours in line with MSDS hyglossary 206 and IUPAC, compendium of clinical technology, 2006, which help to determine the safe dose range in which the juice drug can be used without any harmful effect to animals and in the computing of therapeutic index of drug and chemical in accordance to Rang and Dale [10].

Microscopical cytotoxicity of *Citrus limon* juice thentical wise, 2% of DMSO and 20% of water are usually toxic to animal cells. They were highly toxic with no tolerance for the growth of MCF-7 cells and other all part of the cells by exhibiting plasmolytic activity for the MCF-7 cell lines, meanwhile the C*itrus limon* at 8.88%, 17.32% and 25.98%inhibit the growth of MCF-7 cell line at 25%, 50% and 75% respectively, thus probably may have occurred due to the present of high content of glycoside, saponin, antiquininone, tannin and flavonoid with high capacity of antioxidant activities by limiting the oxidative capacities of the cells which enable the tannin and alcohol to penetrate into the cells, weakened the membrane and inflow of solute for solvent all enable the cell to undergo plasmolysis and death occur as a result of losing the component with the surrounding while on the other hand, the influx of water enable the cell to swell and later burst making the MCF-7 to lose its cytoplasmic constituent which lead to the death of the cells since there is insufficient cellular respiration as a result of depletion in the ATPase level of the MCF-7 cells. In-vitro study, *Citrus limon* juice at 17.32%, 25.98% showed to be more effective in viability study by inhibiting the cell growth in breast cancer induced rat, 8.88%, 17.32% and 25.98% respectively, rate of inhibition was similar to the group of breast cancer induced rat post-treated with 0.2mg/kg of Tamoxifen, there is a strong indication fast the rate of inhibition in the BCIR+CLJ at 25.98% showed similar characteristic inhibition rate with the group of BCIR treated with 0.2mg/kg of Tamoxifen both at 6, 12, 24 and 48 hours.

Similarly C*itrus limon* juice at 8.88%, 17.32%, and 25.98% and 0.2mg/kg of Tamoxifen also inhibit cell proliferation and cytotoxicity in the breast cancer induced rats by spontaneous arrest in the signaling pathway transporting information between the Go, G1 and cell cycle, Tamoxifen induce multiple disturbance by imitating the unrest action which in turn brings about DNA damages the stress generated by this influence the decision of S-phase, DNA is replicated, differentiation control is inhibited and the progenitor cells falling to response to the variety of stress impair the S-phere forming G1 to represent no return situation leading to the damages of the DNA and cells eventually dies, *Citrus limon* contain antioxidants, natural pigments limonene and citric acid which have the capacity to modulate the impairment of cell cycle is the bring about the cell arrest. Mitotic index activities in the breast cancer induced rat post-treated with *Citrus limon* juice at 8.88%, 17.32% and 25.98% was significantly reduced with that of 0.2mg/kg of Tamoxifen due to the effect of the CLJ and Tamoxifen to inhibit mitosis, in MCF-7 breast cancer induced rats only there were spontaneous breaking of chromosomes as a result of rapid cell division mechanism due to cellular proliferation as differentiation and cell cycle rhythmic spontaneous activities was occurred in breast cancer but the malignant cells were inhibited and the mitotic division was impaired. The acceptable explanation for the C*itrus limon* at 8.88%, 17.32% and 25.98% respectively to reduce the mitotic index can be linked to the chemical component that posses positive effect on the cell cycle progression in which *Citrus limon* posses anticancer activities by blocking cell cycle progression, meanwhile chromosomal aberration, structural or mineral found in MCF-7 cells only and post-treated *Citrus limon* Juice and Tamoxifen showed progressive reduction in the Breast cancer induced rat group by lowering the mitotic index values.

CONCLUSION

From this study, it was discovered that in search for safe and effective drugs for the treatment or cure of breast cancer, the use of plant such as Citrus limon juice obtained from the fruit of the Citrus limon showed possible reversibility effects, the possibility could be as a result of the organic oil components 0f the fruit. However, this study revealed high potency and strong inhibition of Citrus limon juice on cancer cell growth, the cell viability potentials of the animals treated with the juice were significantly improved,

CONFLICT INTEREST

The authors declared that they have no competing interests.

AUTHOR'S CONTRIBUTIONS

All the Authors contributed equally

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REFERENCES

1. American Cancer Society (2007). (www.cancer .org)
2. Crespo-Ortiz, M. P., & Wei, M. Q. (2012). Anti-tumor activity of artemisinin and its derivatives: From a well-known anti-malarial agent to a potential cancer drug. *Journal of Biomedicine and Biotechnology*, 15, 1705-1721.
3. Sariego, J. (2010). Breast cancer in the young patient. *The American surgeon*, *76*(12), 1397-1400.
4. Elston, C. W., & Ellis, I. O. (1991). Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: Experience from a large study with long-term follow-up. *Histopathology*, 19(5): 403-410.
5. Bender, C. M., Sereika, S. M., Brufsky, A. M., Ryan, C. M., Vogel, V. G., Rastogi, P., ... & Berga, S. L. (2007). Memory impairments with adjuvant anastrozole versus tamoxifen in women with early-stage breast cancer. *Menopause (New York, NY)*, *14*(6), 995-998.
6. Calomme, M., Pieters, L., Vlietinck, A., & Berghe, D. V. (1996). Inhibition of bacterial mutagenesis by Citrus flavonoids. *Planta medica*, *62*(03), 222-226.
7. Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of toxicology*, *54*(4), 275-287.
8. Mehdi, K., Roghayeh, E., Farbod, E. F. A., & Sajedi, R. (2010). How Do Medical Students Manage Their Information Needs in Unscheduled and High-Acuity Environments?. *Annals of Behavioral Science and Medical Education*, *16*(2), 20-24.
9. Liu, C. J., Hsia, T. C., Wang, R. F., Tsai, C. W., Chu, C. C., Hang, L. W., ... & Bau, D. T. (2010). Interaction of cyclooxygenase 2 genotype and smoking habit in Taiwanese lung cancer patients. *Anticancer Research*, *30*(4), 1195-1199.
10. Rang, H. P., Dale, M. M., Ritter, J. M., & Moore, P. K. (2001). Farmacologia. ed. *Elsevier Churchill Livingstone. 5ª Ed. Capítulo*, *42*, 713-736.