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Effete of cyclophosphamide on normal and cancerous cel*ls*

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Breast cancer, cyclophosphamide, MN Test, Cell line

ABSTRACT
It is a infirmity in which cells in the breast develop abnormally. It's can be cured with early diagnosis and non -metastatic disease .breast cancer stays the second procuring reason of cancer passing in women. In our research, we investigated the levels of cytogenecity of breast cancer patients undergoing different treatment regimens, as well as a control group. The elevated mean was spotted in the chemotherapy category , followed by the hormonal therapy group.The lowest mean was found in the chemotherapy-hormonal treatment group and The control group which was lower than most of the treatment groups. Genomic instability can be offered as a marker of cancer and its examination is crucial in treatments, especially with chromosomal alterations. finding pointed which these antineoplastic components regarded as possible cytotoxic operator and rise harm of the immune system or body of an organism once come in connection to these components.

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INTRODUCTION

Breast cancer is a widespread tumors amongst females in the world. It is the second most repeatedly occurring type of cancer. There are several factors that affect the development of cancer, such as a high-fat diet, spirit , lack of exercise, obesity, and sex (3). Early diagnosis of cancer can increase the chances of survival, and it is the procureing reason of dying amongst females in the world (2). It is a disease in which breast cells develop abnormally and form tumors. It is a molecularly heterogeneous disease due to different genetic changes in breast cells (1). Genetic preparedness accounts for 5-10% of breast cancer statutes . A digit of genes related to breast cancer hazard have lately been specified , inclusive BRCA1, BRCA2, p53, and the Cowden disease gene PTEN/MMAC1.

These genes are thought to act as tumour elimination, but some populace have a bearer repeatedly till 1% to certain BRCA1 and BRCA2 alterations. Genes can give important pridence into the pathogenesis of humane carcinoma.

The clinical implementations of these molecular disclosures lifts dialectical matters related presymptomatic testing of infirms supposed to having cancer-predisposing mutations (14). Breast cancer tumor markers are proteins generated straight by cells. Malignant cells or others in response to a specific tumor, as some tumor signals are related to a specific organ tumor and are tools to easily detect and measure cancer in the body, such as serum or plasma (11). Biomarkers of breast tumors inclusive cytokines that mediate and regulate immunity and inflammation (12).

Several chemical, physical, or biological operators may rise cell demise. Yet, there's a group of agents that don't put cells to death but destroy their genetic material. These agents are called genotoxins. There are several methods for repairing DNA damage , but they sometimes fail, which may lead to stabilization of the damage, if the destroyed cell remains, and then to its transmission to subsequent genesis, if the destroyed cell splits. Exposure to various genotoxic operators exist in the desecrated ambience. Therefore trials are required to demonstrated the rate of exposition and healthiness risks. Albeit several trials are accessible, the micronucleus (MN) test is one of the best and most prominent trials [15,16]. It is also used to test genotoxicity in the laboratory (16,17) although, it must be confirmed which the connotation of MN covers several divergent mechanics which can be utilized in specified and specific statuses (17).

Objective: to evaluate the cytogenecity level in MN cytogenetic indicator and cell lines.

MATERIAL

Micronucleus Test:

Materials and Devices :

Material and devices used in the test

	Company	Origin
Digital microscope	Leica microsystems	Germany
May granwald stain	Central drug house	India
Giemsa stain	Jourilabs	Europe

Sample collection :

Correct preparation The following steps were followed to prepare the studied samples.

1.Washing the eyes of impurities stuck to the lining of the mouth Using a tongue cover, the cells were taken from the side of the lining of the mouth

2.The cells were spread on sterilized and cleaned slides using previous steps

3.The slides were left at room temperature for drying

4.The cells were fixed with a methanol solution of high concentration

He was transferred to the cell and genetics laboratory.

Procedure:

1- Fixing the samples with ethanol

2- Dilutions of the dyes were made:

• May Grunwald dye was diluted by adding 1 ml of the dye with 3 ml of distilled water

• Giemsa dye was diluted by adding 1 ml of the dye with 9 ml of distilled water

3- Staining was done The slide was stained with May Grunwald dye first for 20 minutes, then washed with distilled water.

4- Then stained with Giemsa dye for 3-5 minutes, then washed with distilled water.

5- We wait for the slide to dry, then examine it under a microscope at 40x power.

Cell lines:

Whole natural and cancer cell lines subculture were collected from the laboratories of the Biotechnology Research Center, Al-Nahrain University. Cancer cell lines WRL68 ,MCF 7 and MDA: Three kinds of cell line WRL68 ,MCF 7 and MDA were utilized in this research. They were collected genially from laboratories of Biotechnology Research Center, Al- Nahrain University. Cells kept in RPMI – 1640 consist of 10% bovine calf serum. Whilst the in vitro cells culture shaped a monolayer. cells were treated with divergent concentricity of Cyclophosphamide drug.

Statistical Analysis:

The Statistical Analysis System- SAS (2018) program was utilized to identify the influence of divergent operators on research parameters. The lowest considerable divergence-LSD was utilized to considerably contrast amidst means (ANOVA/ One way) in this study.

RESULTS

In our research, we investigated the levels of cytogenecity of breast cancer patients undergoing different treatment regimens, as well as a control group The treatment groups included chemotherapy, hormonal treatment, chemotherapy-hormonal treatment,The control group consisted of healthy individuals.

The elevated mean was spotted in the chemotherapy category , followed by the hormonal therapy group.The lowest mean

was found in the chemotherapy-hormonal treatment group and The control group which was lower than most of the treatment groups.

Table 1: Comparison between different groups in the Micronucleus test

Group	Broken egg	Karyo-lytic	Bi-nucleated	M.N-3	M.N-2	M.N-1	P.C	Total
Control	0 c	30 d	18 d	0	1 b	1 b	1550 b	1600 b
Horm-ochemo	7 b	63 b	112 c	0	0 b	3 a	1130 c	1315 c
Hormonal	19 a	143 a	152 b	0	2 b	3 a	1170 c	1732 b
Chemo	17 a	48 c	195 a	0	5 a	2 ab	2687 a	2953 a
LSD	5.027**	16.95**	24.74**	0.0 NS	2.54 *	1.72 *	176.43 **	256.4 **
P- value	0.0001	0.0001	0.0001	-	0.0387	0.049	0.0001	0.0001

Mean with different letters in the same column differed significantly.
* (P≤0.05), ** (P≤0.01).

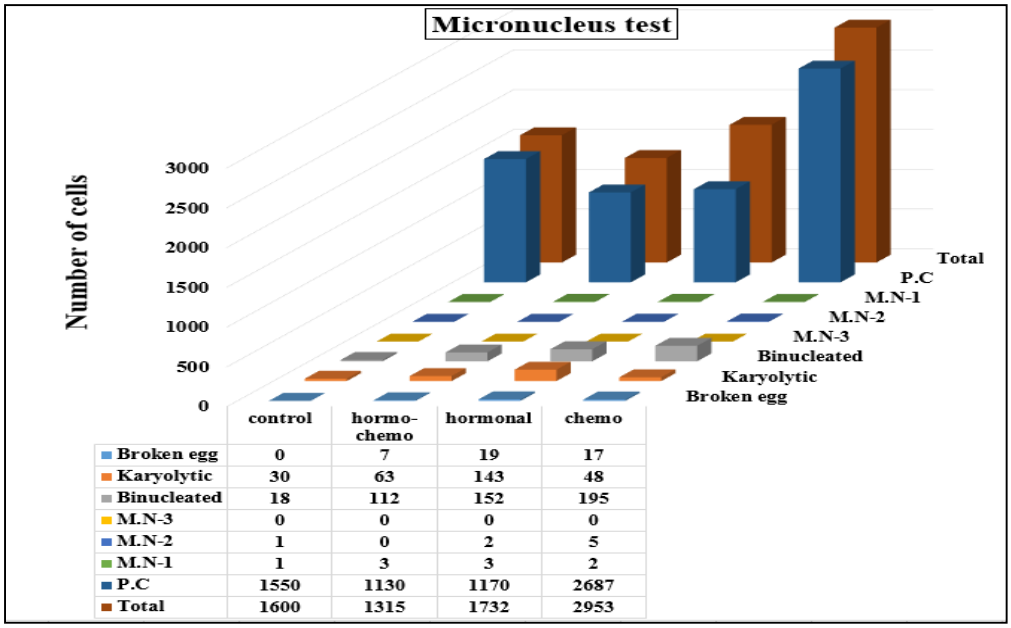


Figure 1 : Micronucleus test groups analyze

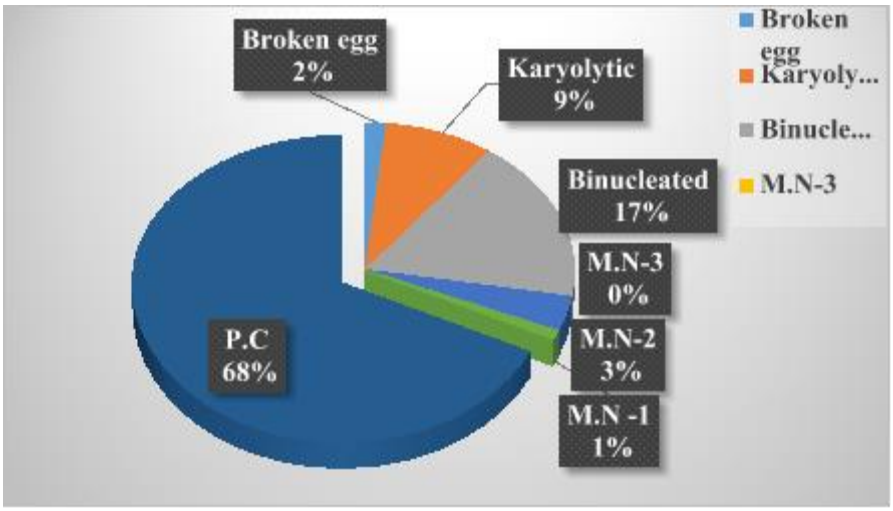


Figure 2 : chemotherapy group analyze

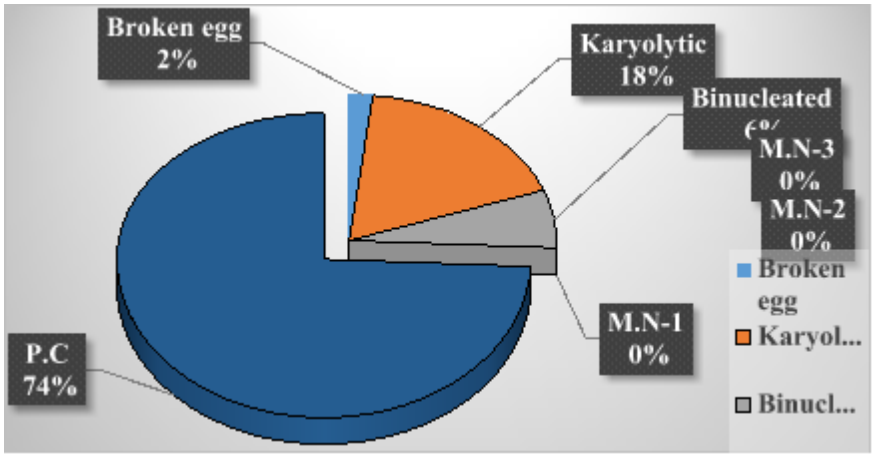


Figure 3 : hormonal therapy group analyze

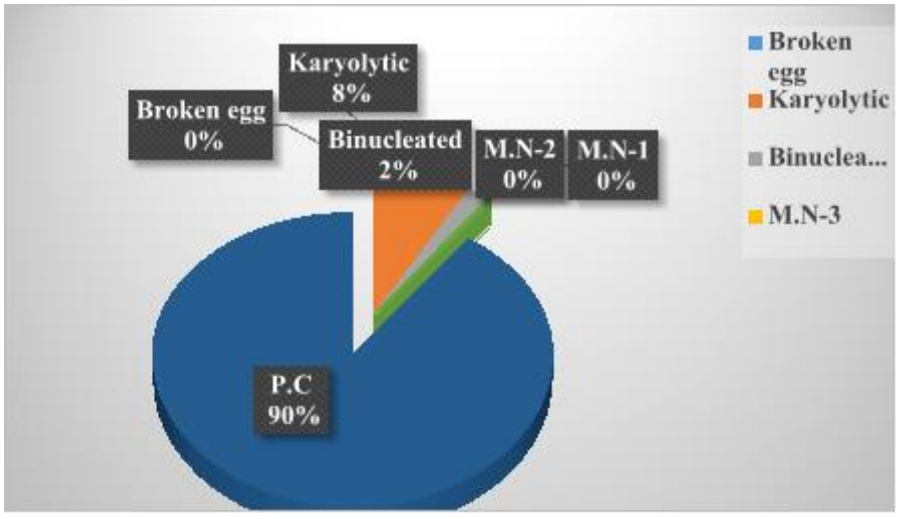


Figure 4 : hormono-chemotherapy group analyze

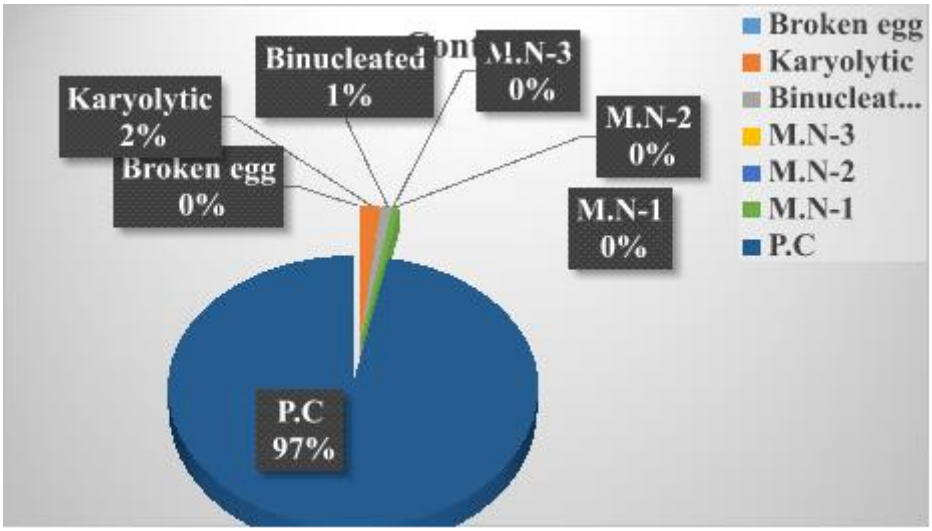
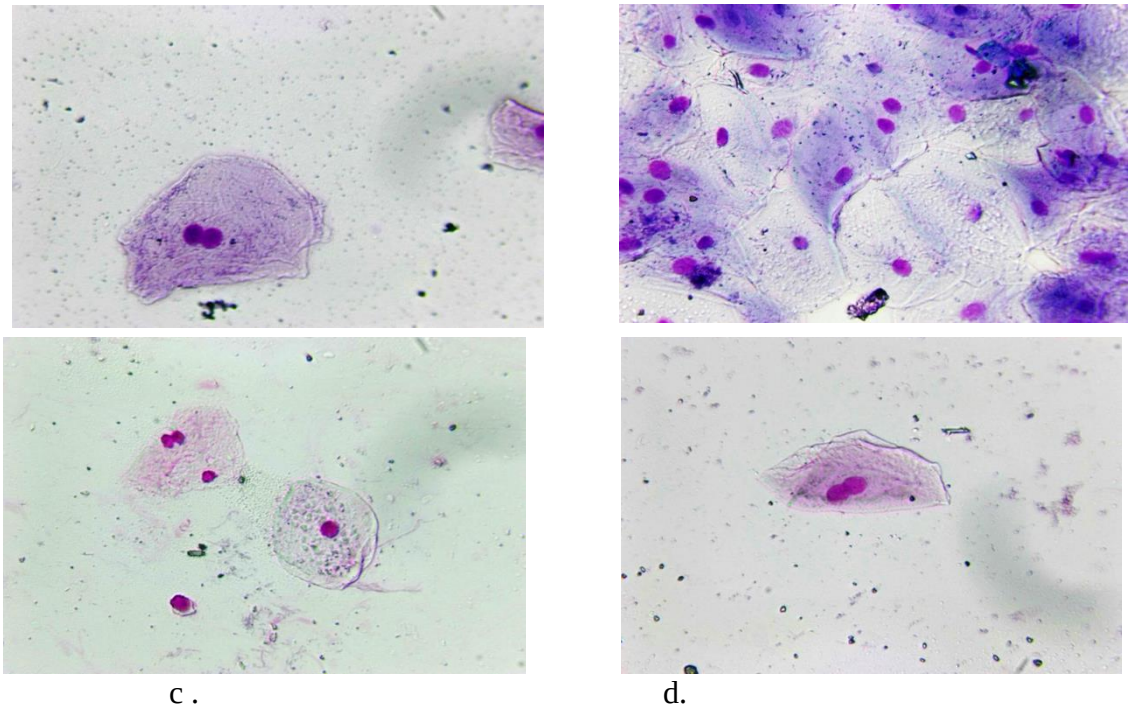


Figure 5 : control group analyze

The results of the running research offered that chemotherapy brought-about considerable increase in the digit of immature red blood cells (PCEs) containing micronuclei (Figure 1) in a greater percentage among those exposed to hormonal treatment and those exposed to chemotherapy-hormonal treatment and control. Table (1) shows the response relation by dose for smaller nuclei.

a. b.



c . d.

Figure 6 : a,b,c,d Binucleated cells in therapy groups.

As well as offered that hormonal therapy caused significant increase in the digit of immature red blood cells (PCEs) containing micronuclei (Figure 2) in a greater percentage among those exposed to chemo- hormono therapy and control.

a. b.

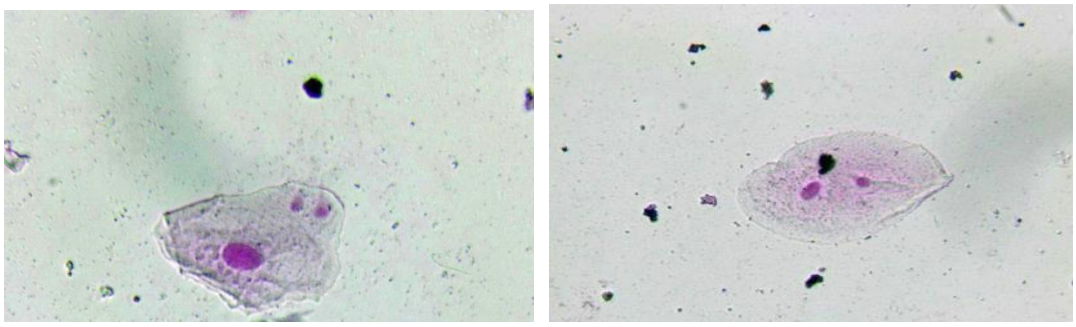
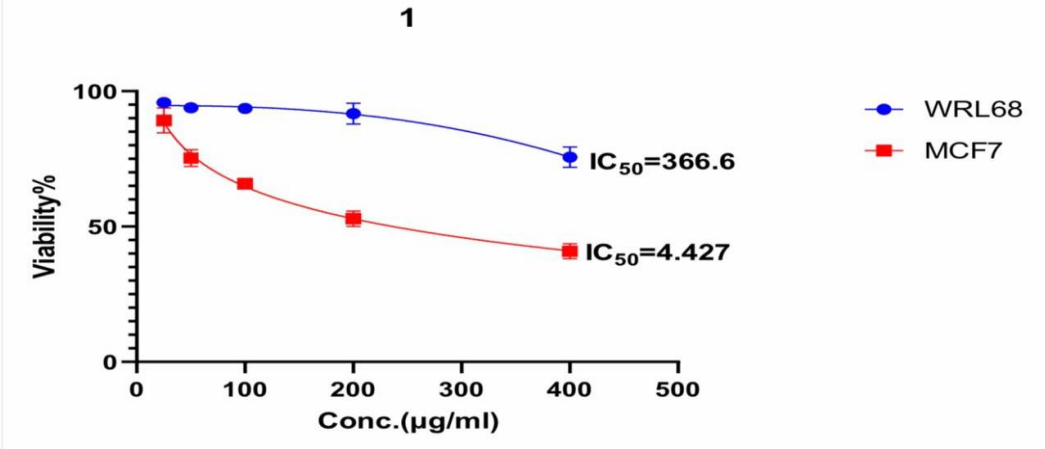


Figure 7 : micronuclus and broken egg in therapy groups.

Cell lines:

Table 2 : cyclophosphomide concentrations on WRL68 , MCF and MDA.

Conc.	WRL68		MCF7		MDA	
	Mean	SD	Mean	SD	Mean	SD
400	74.52733	2.628936	39.74733	1.86678	30.23422	2.57018
200	90.605	2.717863	51.755	1.784268	38.329	0.816801
100	92.53323	0.648052	64.717	0.027389	51.03367	1.084578
50	92.80322	0.110981	74.20867	2.03806	62.63467	2.569003
25	94.64633	1.726114	88.09767	3.514273	76.832	1.468805



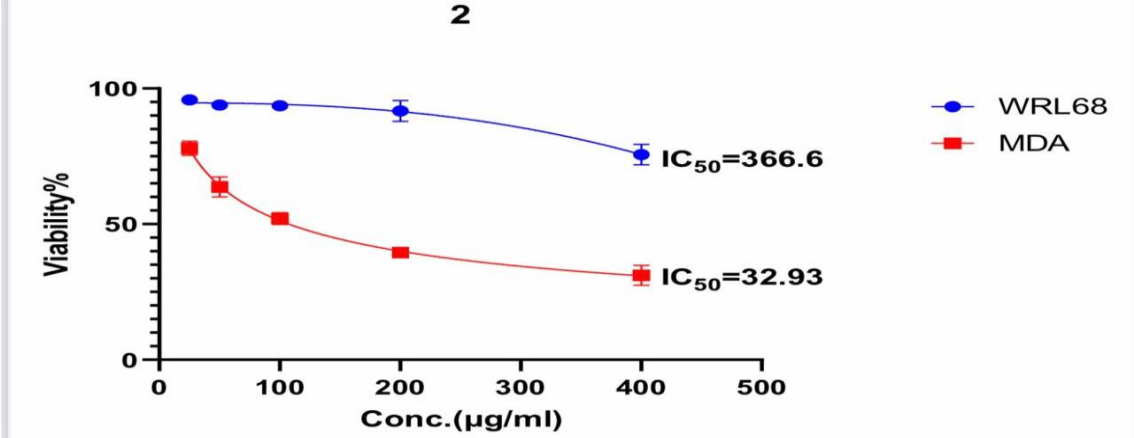


Figure 8 : shows the Lcd50 in normal cells and cancerous cells treated with cyclophosphamide.

Mean for WRL68 (74.5) , MCF (39.7) , MDA (30.23) .Using the drug cyclophosphomide on cancerous and normal cells led to a greate decrease in the number of cancer cells than normal cells, as we can see in the figure (8) : MTT for WRL68 IC is equal to (366.6) in cancer cells and for MDA IC is equal to (4.427). As for the second figure, MTT for WRL68 IC is equal to (366.6). MDA IC equals (32.93) and this shows the effect of the drug on cancer cells.

The table (2) and figure (8) indicate that there are clear significant differences in the response of cancer cells to the drug cyclophosphomide , while the response towards normal cells was small compared to cancer cells.

DISCUSSION

The results appeared that the recurrence of micronuclei in BC infirms treated with chemotherapy and hormonal chemotherapy was greater than that in the control group. Thus, the research results are consistent with some studies that reported a greater recurrence of micronuclei in cancer infirms compared to normal individuals. They performed micronuclei scoring as a biomarker for breast cancer and confirmed that a higher frequency of MN is associated with breast cancer.(10). Genetic alterations, such as telomere harm, chromosomal deviation and amplifications, and epigenetic modulations, are first act in the process of carcinogenesis and tumour development (9). Genetic instability can be offered as an important marker of cancer and monitored in therapy, specially to chromosomal alterations (13).(7) demonstrated a rise recurrence of Micronucleus in lymphocytes of 45 females with untreated invasive or in situ breast carcinoma. (8) Analysis of peripheral blood lymphocytes from 1650 diseases-free appeared powerful prognostic rates for Micronucleus recurrence associated with the hazard of carcinoma passing. Another researches have also indicated DNA harm in infirms with breast carcinoma exposed to radiance therapy through a considerable raise in the incidence of breast carcinoma

amid carcinoma therapy. MN are markers that are routinely screened in cytogenetic preparedness, insuring reliability in the estimation of cytogenetic harm in subjects exposed to mutagenic and carcinogenic operators (5). Because they result from attacks on genetic material, they appear a possible hazard for the outset of carcinoma (4). It has-been noted which the recurrence of Micronucleus out coming from exposition to infrared radiance depends on dose..(6). The concentricity of the treatment appeared a stronger effect compared to the lower concentricity. The outcomes also showed that the treatment had an effect on normal cells, but to a lesser extent. Research has appeared which cyclophosphamide, etoposide and paclitaxel are cytotoxic to RAW cells while their cytotoxicity varies accordingly. The outcomes indicated that these antitumor compounds are a possible cytotoxic operator and raise harm to the immune system or body of the organism when in contact with these compounds. (18). The study demonstrates that Prosigna is a better prognosticator than cyclophosphamide-depend adjuvant chemotherapy in premenopausal infirms with rise-hazard breast carcinoma. These outcomes provided furthermore indication that rise-hazard fundamental subtypes of breast carcinoma have extremely differentiation reactions to cyclophosphamide-based chemotherapy techniques, with a lucid gain in basal-like and luminal B subtypes (19).

CONCLUSION

In this research particular concern was the recognition of stress as a contributor to increment scales of chromosomal insecurity , since this factor has the prospect to be modulated (reduced) through intervention. Given that stress-related chromosomal insecurity has been revealed to kept (and even accumulate) for years.

CONFLICT OF INTEREST

No conflict of interest

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No.

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Abbreviations:

BC	Breast cancer
MN	Micronucleus
PCEs	immature red blood cells