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Evaluation of the cytotoxic effects of coconut juice, coconut oil and methotrexate on human breast cancer cell line MCF-7

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Abstract: The study aimed to evaluate the cytological toxicity for each Coconut juice, oil and mixture of them with Methotrexate on breast cancer cells MCF-7, using Sulforhodamine B (SRB) assay, along with assessment of possible their anti-cancer effects in comparison with Methotrexate alone. The experiment sample was divided into 6 groups; control, Methotrexate alone, Coconut juice alone, Virgin Coconut oil alone and mixing Coconut juice, Oil with Methotrexate at concentrations [0.1,0.3, 1.0, 3.0,10,30,100 µg/ml]. The main findings were no difference on average breast cancer cells MCF7 for most experiment groups at low concentrations 0.1 and 0.3 µg/ml, while at higher concentrations 10, 30 and 100 µg/ml, Coconut juice with Methotrexate had the superior effect on suppressing cancer cells, then juice, then mixture of Virgin Coconut Oil and Methotrexate, then Virgin Coconut Oil and lastly Methotrexate alone. The study concluded that the mixing Coconut juice with Methotrexate had a significant regressing treatment effect on growing (apoptosis) and spreading of cancer cells in vitro. We recommend to do further studies aiming to combine Coconut juice, oil as an adjuvant to chemotherapeutic agents in treating cancer patients.

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Keywords: MCF-7, Coconut juice, Coconut oil, Breast cancer cell line, apoptosis, cytotoxicity, SRB

1. Introduction:

Cancer is one of the most prevalent diseases, and is a major cause of mortality Globally, nearly 1 in 6 deaths is due to cancer, Regarding Saudi Arabia, breast cancer is the most common cancer among females. https://www.moh.gov.sa

Chemotherapy is so tough, causing serious side effects and some are carcinogenic themselves, so many researchers in cancer nowadays are committed to produce new generations of cancer medications more specific on cancer cells, to improve quality of life and minimizing the hazards of those medications. (Lindgren et al.,2006)

Although chemotherapy is so effective, there are many challenges regarding resistance and serious side effects. (Song et al.,2014)

Methotrexate is an antimetabolite drug, that is widely used as anticancer, and in some autoimmune diseases like severe psoriasis and rheumatoid arthritis. This medication is also antifolate, which facilitates apoptosis in highly potential cancer cells by suppressing enzymes thymidylate synthase and dihydrofolate reductase. (Famurewa et al.,2017).

On the other side, Virgin Coconut oil (Cocos Nucifera) is a powerful antioxidant that minimizes the side effects of chemotherapy without interfering with its medical efficiency. Studies show that regular consumption of virgin Coconut oil improves overall health, because of its high phenols substances like; Protocatechuic, Vanillic, Ferulic, and p-coumaric acids. (Famurewa et al.,2017)

Studies revealed that using Coconut oil during chemotherapy improves overall health status in breast cancer patients and reduce side effects, as well as its chemical components; vitamins, minerals, and medium-chain fatty acids play an important role in that. (Yong et al.2009; Law et al. 2014)

2. Materials & Methods:

2.1 Cell Lines

This study was implemented on breast cancer cells MCF-7 (ATCC® HTB22™) from king Fahd Medical Research Center in Jeddah.

2.2 The drug used

We used Methotrexate (Cytotrex), which is a chemotherapeutic agent. This was obtained from Dr. Erfan Hospital in Jeddah city after imported from KOÇAK Farma.

2.3 Natural Materials

Virgin Coconut oil had been prepared by the wet process from fresh grated mature coconut.

Coconut juice obtained from Coconut imported from India by NKLEPORT company.

2.4 Cytological Study:

2.4.1 Experimental Design:

We used Sulforhodamine B (SRB) Cells Cytotoxicity Assay in this study on MCF-7 breast cancer cell line, from king Fahd Medical Research Center in Jeddah, and test groups were divided as follows:

First: Control group.

Second: Methotrexate drug at concentrations (0.1,0.3,1.0,3.0,10,30,100µg/ml).

Third: Virgin Coconut juice group at concentrations (0.1,0.3,1.0,3.0,10,30,100µg/ml).

Fourth: Coconut oil group at concentrations (0.1,0.3,1.0,3.0,10,30,100µg/ml).

Fifth: giving Virgin Coconut juice with Methotrexate at concentrations (0.1,0.3,1.0,3.0,10,30,100 µg/ml).

Sixth: giving Coconut oil with Methotrexate at concentrations (0.1,0.3,1.0,3.0,10,30,100µg/ml).

2.4.2 Cells Preparation

We followed (Houghton et. Al. 2007) method in preparation, fixation and staining for application of SRB assay. The steps were:

- Promoting the growth of cells in flasks containing 10 ml of nutritional medium RRMI-1640 with Glutamine Bicarbonate and 10 ml of FBS.
- We had been changing the medium every 48 hours, and separation of cells by 0.25% using trypsin EDTA solution.
- The cells were transferred into cell culture plates, and the cells were incubated for 72 hours at 37 C° in presence of 5% of CO2.

2.4.3 Cell Fixation

The cells were washed by a buffer solution before their fixation to remove the serum that causes cell division and losing. The cells were fixed by adding 50% of Trichloroacetic acid (TCA) solution and kept at 4 C°. Staining was the next step.

2.4.4 Staining

According to (Skehan et al., 1990), Sulforhodamine B stain was used in our study as 0.4% SRB in 1% acetic acid to remove the extra stain. Then drying in air, and melted with 10 ml of tries optimizer for 5 minutes along with shaking. Optical density (OD) was read in all cell lines using the Automated Spectrophotometric Plate Reader device at wavelength 490 nm.

The rate of growth inhibition (IC50) AND (IC90) was calculated as following:

(OD) control wells/ (OD)control wells – (OD) treated wells

2.5 Statistical Analysis:

We implemented Computerized methods for statistical analysis for study results (0.24 version) of SPSS software, which works in Windows. Using this software, Analysis of Student's t-test, variance ANOVA, then calculating the least significant difference LSD, and Excel 2017 was used for diagrams.

3. Results:

3.1 Effect of treatment with Methotrexate, Coconut juice, Coconut oil, and their individual mixing with Methotrexate drugon the values of IC50 and IC90 after 72 hours:

In Vitro microscopic examination of breast cancer cell lines MCF-7 demonstrated the easily observed morphologic impact of various treatments after incubation for 48 hours, further supported by evidence of Apoptosis e.g. nuclear condensation, increase the size of some cells dramatically, loss of cell membranes, inflation cytoplasm, bursting of cells and extrusion of contents, compared to the control group. The values of inhibited proliferation of 50% of cells (IC50) were then calculated for treatments with either Methotrexate, Coconut juice, Coconut oil, and their individual mixing with Methotrexate drug and it was 77.85, 39.089, 67.750, 27.89 and 54.123 µg/ml respectively (figure 1), whereas the values of inhibited proliferation of 90% of cells (IC90) with various treatments were 144.516, 90.37, 134.166, 76.67 and 108.917 µg/ml respectively (figure 2).

3.2 Impact of Methotrexate, Coconut juice, Coconut oil, and their individual mixing with Methotrexate drug on the average appearance of breast cancer cells line MCF-7 after 72 hours on concentration 0.1 µg/ml:

The results in table (1) revealed that the Coconut juice impact made significant decrease ($P \leq 0.001$) on average appearance of breast cancer cells line MCF-7 with a value (1.840±0.032), while the results of mixture Methotrexate with Coconut juice showed a significant decrease ($P \leq 0.01$) with value (1.881±0.031). There was no significant difference for the Methotrexate, Coconut oil and mixture of Methotrexate and Coconut oil on average appearance of breast cancer cells line MCF-7, with a values of (2.104±0.024), (2.078±0.019) and (2.108±0.001) respectively, in comparison to the control sample average (2.112±0.003). the results of average Inhibition of cancer cells were: 0.378%, 12.878%, 1.609%, 10.937%, and 0.189% respectively. Later results showed that the highest value was for Coconut juice, then the mixture of Methotrexate with Coconut juice. (Figure 4), and the percentage of Inhibition inversely proportional to the average absorption and vitality; means the more average absorption and

vitality, the Inhibition rate decreases. (Table 1) & (figure 5).

3.2 Impact of Methotrexate, Coconut juice, Coconut oil, and their individual mixing with Methotrexate drug on the average appearance of breast cancer cells line MCF-7 after 72 hours on concentration 0.3 µg/ml:

The results shown in table 1 revealed that Coconut juice and its mixture with Methotrexate had a significant decrease (P≤ 0.001) on the average appearance of breast cancer cells lines MCF-7, of a value (1.681±0.34), (1.740±0.032) respectively. The best was Coconut juice in suppressing the cancer cells MCF-7, while individual Methotrexate, Coconut oil and mixing the oil with Methotrexate didn't show any significant difference in cancer cell average appearance, of values (2.101±0.025, 2.075±0.018, 2.104±0.002) respectively, in comparison to control sample (2.112±0.003). The values of average Inhibition of the cancer cells line MCF-7 are: 1.751%, 20.407%, 0.520%, 0.378%, 17.631% respectively, as the highest values were for Coconut juice and then mixing juice with Methotrexate (figure 4), and the percentage of Inhibition inversely proportional with the average absorption and vitality. (Table 1) (figure5).

3.3 Impact of Methotrexate, Coconut juice, Coconut oil, and their individual mixing with Methotrexate drug on the average appearance of breast cancer cells line MCF-7 after 72 hours on concentration 1 µg/ml:

Table 1 shows that the individual Coconut juice, Coconut oil, and mixing of juice, oil with Methotrexate causing significant decrease (P≤ 0.001) in average appearance of breast cancer cells of (1.707±0.001, 1.968±0.015, 1.614±0.001, 1.905±0.002) respectively, in comparison with control sample (2.112±0.003), as the best in suppressing the appearance of cancer cells MCF-7 was the mixing Methotrexate with Coconut juice, then Coconut juice, then mixing Coconut oil with Methotrexate and lastly the Coconut oil alone. The values were: (0.331%, 19.176%, 6.818%, 23.579%, 9.801%) respectively. (figure4), and the percentage of Inhibition inversely proportional with the average absorption and vitality. (Table 1) (figure5).

3.4 Impact of Methotrexate, Coconut juice, Coconut oil, and their individual mixing with Methotrexate drug on the average appearance of breast cancer cells line MCF-7 after 72 hours on concentration 3 µg/ml:

In table 1, Methotrexate, Coconut juice, Coconut oil and the mixture of juice, oil with Methotrexate impacting a significant decrease (P≤ 0.001) in the average appearance of breast cancer cells MCF-7, in the values (1.942±0.004, 1.664±0.010, 1.744±0.019, 1.255±0.014, 1.621±0.009) respectively, in comparison to control sample (2.112±0.003), as the best impact on suppressing cancer cells was mixture juice with Methotrexate, then oil with Methotrexate, then juice, next was oil and lastly Methotrexate. The values were: 8.049%, 21.212%, 17.424%, 40.577%, 23.248% respectively. (figure4), and the percentage of Inhibition inversely proportional with the average absorption and vitality. (Table 1) (figure5).

3.5 Impact of Methotrexate, Coconut juice, Coconut oil, and their individual mixing with Methotrexate drug on the average appearance of breast cancer cells line MCF-7 after 72 hours on concentration 10 µg/ml:

The results shown in table 1 revealed a significant decrease (P≤ 0.001) in average appearance of breast cancer cells line MCF-7 by Methotrexate, Coconut juice, Coconut oil, mixing Methotrexate with oil or juice, the values were: (1.832±0.006, 1.154±0.015, 1.460±0.023, 1.855±0.021, 1.445+0.023) respectively, in comparison with control sample (2.112±0.003). Again the mixture of Methotrexate and Coconut juice showed the best suppressing results on cancer cells appearance, then Coconut juice, then Methotrexate with oil, then oil and lastly Methotrexate alone. The Inhibition rates were: (13.257%, 45.359%, 30.871%, 59.517%, 31.871%) respectively. (figure4), and the percentage of Inhibition inversely proportional with the average absorption and vitality. (Table 1) (figure5).

3.6 Impact of Methotrexate, Coconut juice, Coconut oil, and their individual mixing with Methotrexate drug on the average appearance of breast cancer cells line MCF-7 after 72 hours on concentration 30µg/ml:

In table 1, Methotrexate, Coconut juice, Coconut oil and the mixture of juice, oil with Methotrexate impacting a significant decrease (P≤ 0.001) in the average appearance of breast cancer cells MCF-7, in the values (1.487±0.022, 0.871±0.019, 1.261±0.019, 1,265±0.002, 1,131±0.010) respectively, in comparison to control sample (2.112±0.003), as the best impact on suppressing cancer cells was mixture juice with Methotrexate, then juice, then oil with Methotrexate, then oil, and lastly Methotrexate. The values were: (29.592%, 58.759%, 40.293%, 87.452% and 46.448%) respectively. (figure4), and the percentage of Inhibition inversely proportional with the average absorption and vitality. (Table 1) (figure5).

3.7 Impact of Methotrexate, Coconut juice, Coconut oil, and their individual mixing with Methotrexate drug on the average appearance of breast cancer cells line MCF-7 after 72 hours on concentration 100µg/ml:

The results shown in table 1 revealed a significant decrease (P≤ 0.001) in average appearance of breast cancer cells line MCF-7 by Methotrexate, Coconut juice, Coconut oil, mixing Methotrexate with oil or juice, the values were: (0.835±0.029, 0.186±0.012, 0.751±0.024, 0.091±0.006, 0.477±0.060) respectively, in comparison with control sample (2.112±0.003). Again the mixture of Methotrexate and Coconut juice showed the best suppressing results on cancer cells appearance, then Coconut juice, then Methotrexate with oil, then oil and lastly Methotrexate alone. The Inhibition rates were: (60.464%, 91.193%, 64.441%, 95.691%, 77.414%) respectively. (figure4), and the percentage of Inhibition inversely proportional with the average absorption and vitality. (Table 1) (figure5).

3.8 Impact of Methotrexate, Coconut juice, Coconut oil, and their individual mixing with Methotrexate drug on the average appearance of breast cancer cells line MCF-7 after 72 hours on concentrations 0.1,0.3,1,3,10,30,100 µg/ml using variance analysis and least significant difference LSD:

The results from table 2 showed a significant difference (P≤ 0.001) in the average appearance of breast cancer cells MCF-7 in the different concentrations between Methotrexate drug, or Coconut juice, or Coconut oil and the mixing of juice or oil with Methotrexate, the value was (F=30.763) at 0.1 µg/ml, (F= 75.673) at 0.3 µg/ml, (F=310.213) at 1 µg/ml, (F= 636.111) at 3 µg/ml, (F= 779.788) at 10 µg/ml, (F=2208.233) at 30 µg/ml, (F= 599.436) at 100 µg/ml, in comparison of the control sample.

From variety test using LSD there was no significant difference in average breast cancer cells appearance with Methotrexate, oil, and mixing oil and Methotrexate at concentrations 0.1 and 0,3 µg/ml, while the significant difference (P≤ 0.001) appeared only with juice alone or mixing juice with Methotrexate. The juice had the most impaction on the average appearance of breast cancer cells MCF-7.

On other hand, using LSD at concentrations 1,10,30, and 100 µg/ml, no significant difference at concentration of 1 µg/ml with Methotrexate, and there was a significant difference (P≤ 0.001) at concentrations (10,30, and 100 µg/ml) with Methotrexate, Juice, oil, oil with Methotrexate, and juice with methotrexate, and the increasing arrangement of them according to their effect on breast cancer cell lines MSF-7 as following:

(CJ+ MTX) > CJ (VCO+ MTX) > VCO > MTX

When variability test using LSD applied at the concentration of 3 µg/ml, there was a significant difference (P≤ 0.001) with Methotrexate, Juice, oil, juice with Methotrexate, oil with Methotrexate, and the increasing arrangement of them according to their effect on breast cancer cell lines MSF-7 as following:

(CJ+ MTX) > (VCO+ MTX) > CJ > VCO > MTX

Table (1): Impact of Methotrexate, Coconut juice, Virgin Coconut oil alone and the mixture of juice or oil with Methotrexate on the average appearance of breast cancer cell lines after 72 hours

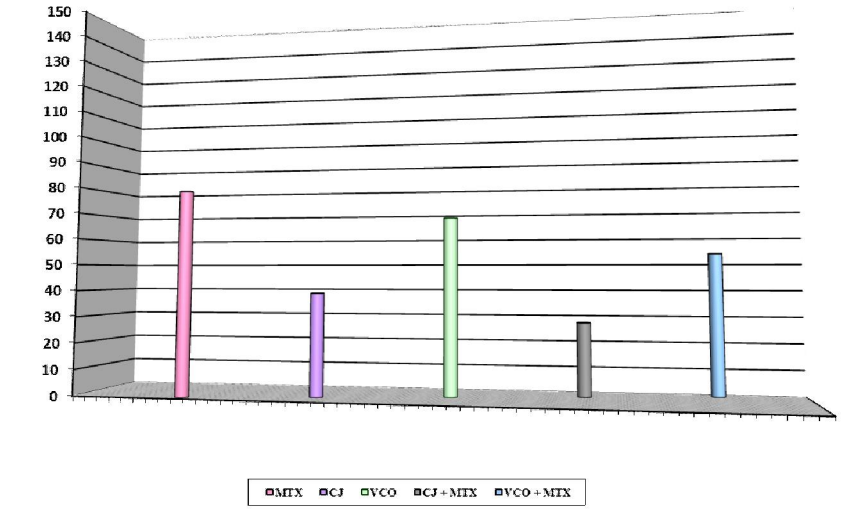
Category	Average ± Standard Error	Absorbance	Survival Fraction (SF)	% Inhibition rate	Concentration (µg/ml)
C	2.112±0.003	2.112	1	___	0.1
MTX	2.104±0.024	2.104	0.996	0.378	
CJ	*** [a]1.840±0.032	1.840	0.871	12.878 ↑	
VCO	2.078±0.019	2.078	0.983	1.609	
CJ+MTX	** [a]1.881±0.031	1.881	0.890	10.937 ↑	
VCO+MTX	2.108±0.001	2.108	0.998	0.189	
C	2.112±0.003	2.112	1	___	0.3
MTX	2.101±0.025	2.101	0.994	0.520	
CJ	*** [a]1.681±0.034	1.681	0.795	20.407 ↑	
VCO	2.075±0.018	2.075	0.982	1.751	
CJ+MTX	*** [a]1.740±0.032	1.740	0.823	17.613 ↑	
VCO+MTX	2.104±0.002	2.104	0.996	0.378	

Category	Average ± Standard Error	Absorbance	Survival Fraction (SF)	% Inhibition rate	Concentration (µg/ml)
C	2.112±0.003	2.112	1	___	1
MTX	2.094±0.023	2.094	0.991	0.331	
CJ	*** [a] 1.707±0.001	1. 707	0.808	19.176 ↑	
VCO	*** [a] 1.968±0.015	1.968	0.931	0.931	
CJ+MTX	*** [a] 1.614±0.001	1.614	0.764	23.579 ↑	
VCO+MTX	*** [a] 1.905±0.002	1.905	0.901	9.801	
C	2.112±0.003	2.112	1	___	3
MTX	*** [a] 1.942±0.004	1.942	0.919	8.049	
CJ	*** [a] 1.664±0.010	1.664	0.787	21.212 ↑	
VCO	*** [a] 1.744±0.019	1.744	0.825	17.424 ↑	
CJ+MTX	*** [a] 1.255±0.014	1.255	0.594	40.577 ↑	
VCO+MTX	*** [a] 1.621±0.009	1.621	0.767	23.248 ↑	

Category	Average ± Standard Error	Absorbance	Survival Fraction (SF)	% l ibition rate	Concentration (µg/ml)
C	2.112±0.003	2.112	1	___	10
MTX	*** [a] 1.832±0.006	1.832	0.867	13.257	
CJ	*** [a] 1.154±0.015	1.154	0.546	45.359 ↑	
VCO	*** [a] 1.460±0.023	1.460	0.691	30.871 ↑	
CJ+MTX	*** [a] 0.855±0.021	0.855	0.404	59.517 ↑	
VCO+MTX	*** [a] 1.445±0.023	1.445	0.684	31.871 ↑	
C	2.112±0.003	2.112	1	___	30
MTX	*** [a] 1.487±0.022	1.487	0.704	29.592	
CJ	*** [a] 0.871±0.001	0.871	0.412	58.759 ↑	
VCO	*** [a] 1.261±0.019	1.261	0.597	40.293 ↑	
CJ+MTX	*** [a] 0.265±0.002	0.265	0.125	87.452 ↑	
VCO+MTX	*** [a] 1.131±0.010	1.131	0.535	46.448 ↑	

.0.0	Average ± Standard Error	Absorbance	Survival Fraction (SF)	% Inhibition rate	Concentration (µg/ml)
C	2.112±0.003	2.112	1		
MTX	*** a 0.835±0.029	0.835	0.395	60.464	
	*** a			↑	
				↑	
				↑	
				↑	

C MTX CJ VCO CJ+MTX VCO+MTX



] juice or oil with
]

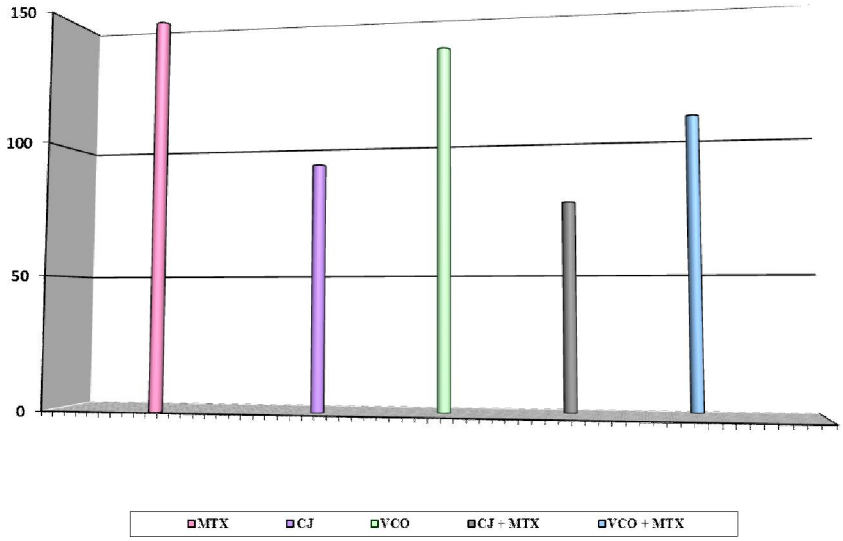


Figure (2): Impact of Methotrexate, Coconut juice, Virgin Coconut oil alone and the mixture of juice or oil with Methotrexate on the value IC90 of breast cancer cell lines after 72 hours

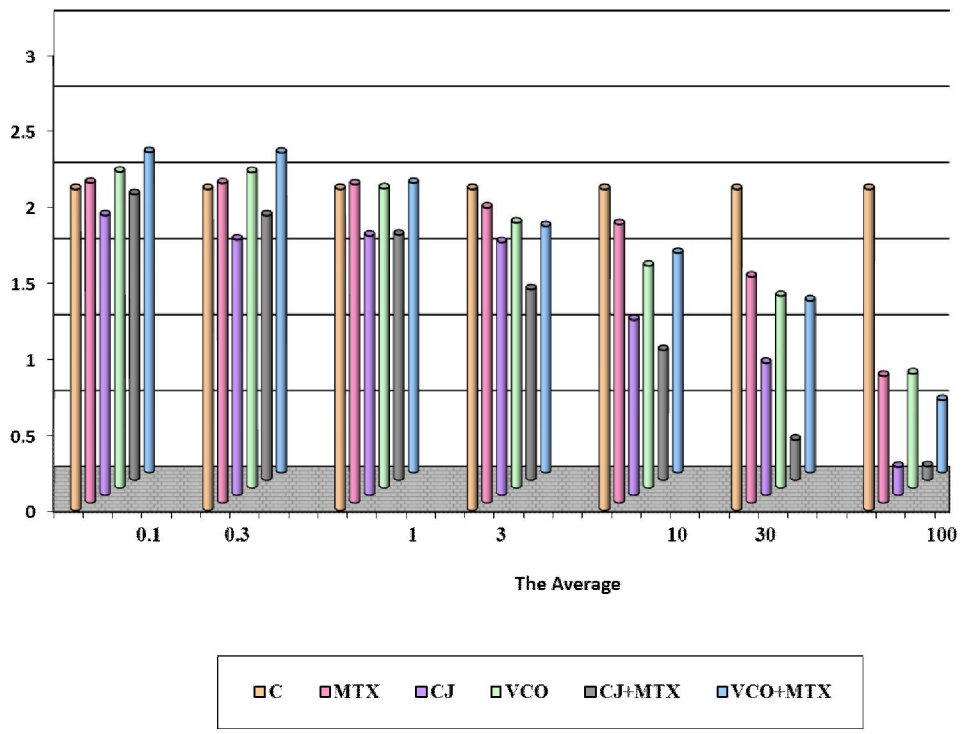


Figure (3): Impact of Methotrexate, Coconut juice, Virgin Coconut oil alone and the mixture of juice or oil with Methotrexate on the average appearance of breast cancer cell lines after 72 hours

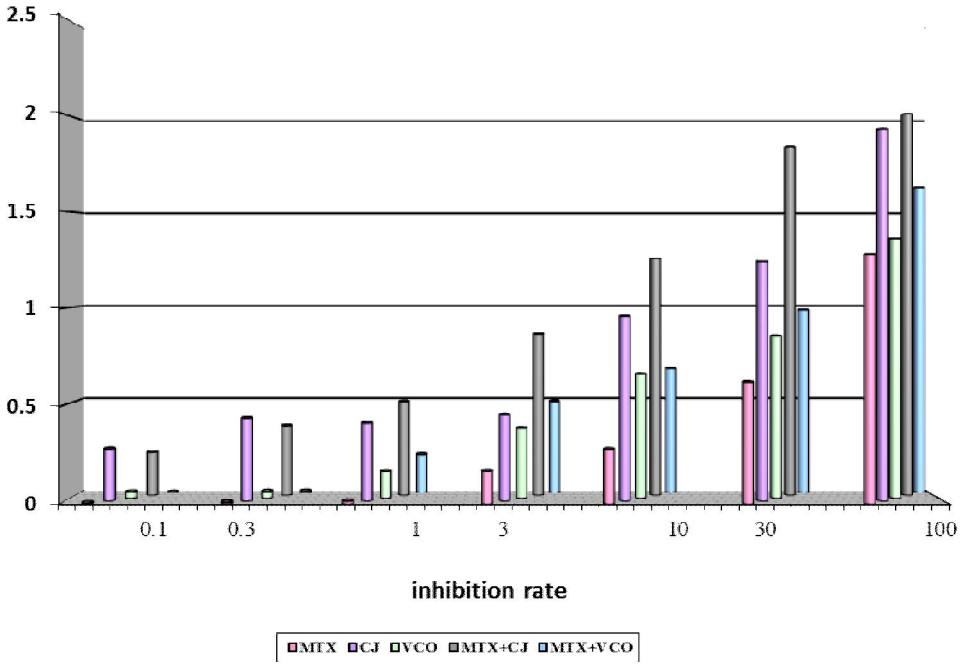


Figure (4): Impact of Methotrexate, Coconut juice, Virgin Coconut oil alone and the mixture of juice or oil with Methotrexate on rate of inhibition of breast cancer cell lines after 72 hours

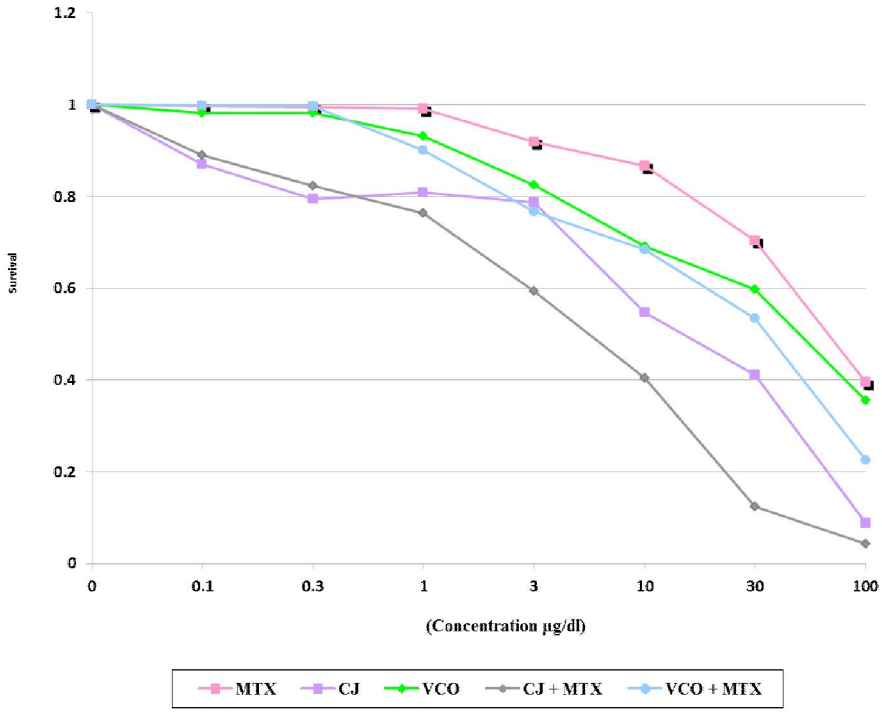


Figure (5): Linear relation of Impaction of Methotrexate, Coconut juice, Virgin Coconut oil alone and the mixture of juice or oil with Methotrexate on the average appearance of breast cancer cell lines after 72 hours

Table (2): Impact of Methotrexate, Coconut juice, Virgin Coconut oil alone and the mixture of juice or oil with Methotrexate on the average appearance of breast cancer cell lines after 72 hours using ANOVA and LSD

Lowest standard deviation test (LSD)			ANOVA analysis			
Sig	Mean difference	Groups treatment	Sig	F		
	0.007	MTX	✳✳✳	30.763	Control (C)	0.1
✳✳✳	0.271	CJ				
	0.033	VCO				
✳✳✳	0.230	CJ+MTX				
	0.003	VCO+MTX				
	0.011	MTX	✳✳✳	75.673	Control (C)	0.3
✳✳✳	0.430	CJ				
	0.036	VCO				
✳✳✳	0.371	CJ+MTX				
	0.008	VCO+MTX				
	0.017	MTX	✳✳✳	310.213	Control (C)	1
✳✳✳	0.405	CJ				
✳✳✳	0.143	VCO				
✳✳✳	0.497	CJ+MTX				
✳✳✳	0.206	VCO+MTX				
Lowest standard deviation test (LSD)			ANOVA analysis			
Sig	Mean difference	Groups treatment	Sig	F		
✳✳✳	0.170	MTX	✳✳✳	636.111	Control (C)	3
✳✳✳	0.447	CJ				
✳✳✳	0.368	VCO				
✳✳✳	0.856	CJ+MTX				
✳✳✳	0.490	VCO+MTX				
✳✳✳	0.280	MTX	✳✳✳	779.788	Control (C)	10
✳✳✳	0.958	CJ				

***	0.652	VCO				
***	1.256	CJ+MTX				
***	0.667	VCO+MTX				
***	0.624	MTX				
***	1.241	CJ				
***	0.850	VCO	***	2208.233	Control (C)	30
***	1.847	CJ+MTX				
***	0.981	VCO+MTX				

Lowest standard deviation test (LSD)			ANOVA analysis			
Sig	Mean difference	Groups treatment	Sig	F		
***	1.274	MTX				
***	1.925	CJ				
***	1.361	VCO	***	599.436	Control (C)	100
***	2.021	CJ+MTX				
***	1.634	VCO+MTX				

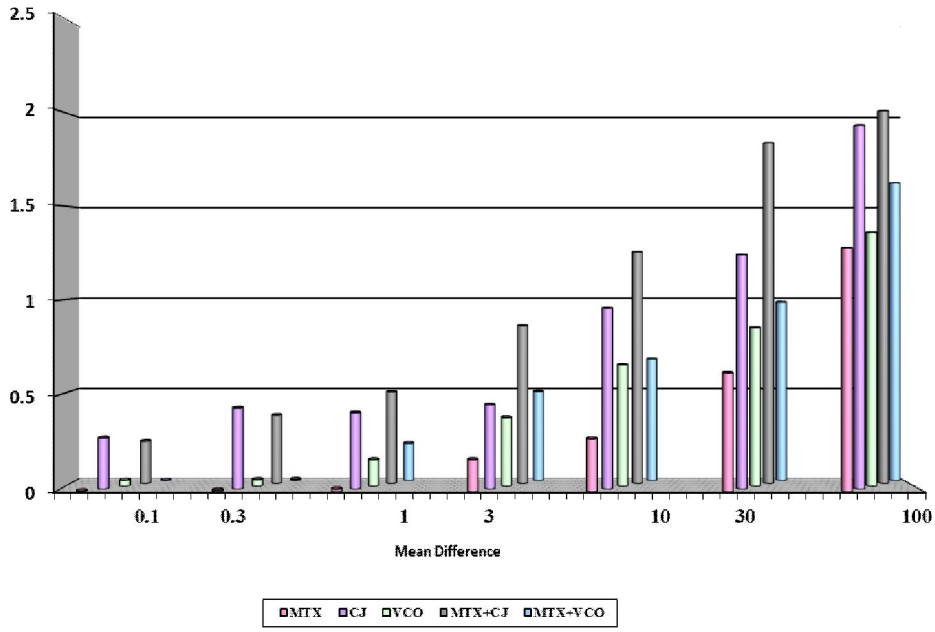


Figure (6): Impact of Methotrexate, Coconut juice, Virgin Coconut oil alone and the mixture of juice or oil with Methotrexate on the average appearance of breast cancer cell lines after 72 hours using ANOVA.

4. Discussion:

Cancer is one of the most leading cause of death worldwide. Chemotherapy which is a principal treatment of cancer patient, has a potential side effects and sometimes it is not tolerated by them. Methotrexate as an anti-folate chemotherapeutic agent used in various clinical situations has significant impacts on bone marrow, hair and mucous membranes of the patients, along with unexpected toxicity effects. Therefore, the aim is to study the preventive roles of natural substances which are: Coconut oil and juice along with Methotrexate at cellular and genetic levels. So, the study evaluates the cellular toxicity levels of Coconut oil, juice and methotrexate alone in comparison with the effect of both Coconut oil and juice with Methotrexate at concentrations of (0.1,0.3,1,3,10,30,100 µg/ml) after 72 hours on MCF-7 breast cancer cells using SRB testing. The cytotoxic effect of Kinetin riboside, which is one of the main substances in Coconut juice, on cancer cells growth in human, plants and animals as antiproliferative and leading to apoptosis, and this was studied on human colon cancer HCT-15 cells which revealed a significant Inhibition of cell's spread by 30%, 65%,

85% at concentrations of 0.5, 1, and 25 µg/ml using SRB assays after 4 days. (2012*et al*., Rajabi). Other studies were implemented to study the preventive effects of Virgin Coconut oil against the cytotoxic effects of Cyclophosphamide chemotherapeutic medication on liver and kidney, and the positive effect was due to linoleic acid and oleic acids which are essential fatty acids found in the oil. (Nair *et al*.,2016). According to many literatures, the study hypothesis was to assess the preventive and possible treating effects of Coconut juice and oil against the cellular and genetic effects of Methotrexate in vitro and vivo. Results obtained from this study at higher concentrations 10, 30 and 100 µg/ml, Coconut juice with Methotrexate had the superior effect on suppressing cancer cells, then mixture of Virgin Coconut Oil, juice and Methotrexate, then Virgin Coconut Oil and lastly Methotrexate alone. The study concluded that the mixing Coconut juice with Methotrexate had a significant regressing treatment effect on growing (apoptosis) and spreading of cancer cells in vitro. We recommend to do further studies aiming to combine Coconut juice, oil as an adjuvant to chemotherapeutic agents in treating cancer patients.

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