Evaluation of dacarbazine cytological effects by using bee glue (propolis) *in vitro* to manifest the scientific miracles in the Quran

Lina Abdul-Fattah Kurdi and Fatimah Aliyan Aljeddani

Department of Biology " Zoology", Faculty of Sciences - Al Faisaliah- King Abdul Aziz University,
P.O. Box. 4938, Jeddah 21412, KSA.
dr.lina_kurdi@hotmail.com

Abstract: The aimed of the present study to assess the potential therapeutic effect of propolis treatment Which is a natural bee products and it has a healing properties compared to treatment with a dacarbazine(DTIC) as one of the drugs used in the treatment of many tumors cells *in vitro* . Breast cancer cell lines were used *in vitro* studies, they were divided into four main groups, control. Propolis treated group, that farther divided into three sub-groups and received propolis in a concentrations of 50µg/ml , 100µg/ml , 200µg/ml. Dacarbazine treated group, that divided into three sub- groups and received dacarbazine in a concentrations of 50µg/ml , 100µg/ml , 200µg/ml. The 4th group that received the dual treatment of dacarbazine and propolis in the same concentrations as the 2nd and 3rd groups. All groups were investigated after inculpation for 48 and 72 hours.. It could be concluded the protective effects of propolis against the adverse effects of dacarbazine. It could be recommended to use the propolis as adjuvant with chemotherapeutic agents.

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Key Words: Propolis, Dacarbazine, Breast cancer cell lines, Apoptosis

1. Introduction

Breast cancer classified as one of the commonest malignant tumors that affect women in all the world (Wang *et al.*, 2010)

Cancer, being a lethal disease, has triggered many efforts to cure it, using various substances, natural and synthetic. Chemoprevention aims at inhibiting carcinogenesis or arresting it in its early stages, to avoid the development of a tumor capable of invading surrounding tissues and spread .However, undesirable side effects associated with chemotherapy as well as drug resistance have brought forward complementary medicine as an alternative solution. Over the last few years, many scientists all over the world have conducted extensive research to find a compound that inhibits the proliferation of cancer cells.(Lotfy ., 2006 ; Yemis *et al* ., 2008 ;Khan *et al* ., 2008 Szliazka *et al* ., 2011)

Natural/organic food systems have long been known for their positive and useful impact on human health. Recently, natural products have proven to be a promising source for the production of new drugs; a trend that Allah has already revealed in his holy book, uncovering some of the secrets of natural cure as represented by bees, which produce from its tiny bellies 5 different substances, namely Honey, Royal jelly, Bee glue (Propolis), beeswax and even Bee poison, that bring cure to disease-stricken humanity!

God Almighty has spoken the truth when he said in his holy book“Then eat all the fruits and follow the ways of your Lord laid down [for you]." There emerges from their bellies a drink, varying in colors, in which there is healing for people. Indeed in that is a sign for a people who give thought..”Sura An-Nahl, verse 69.

Many studies have proven that Propolis anticancer activity can effectively impact various cancer cell lines (Turan *et al*., 2015).

Treatment with propolis has been proven to play an effective role in inhibiting cancer cell lines, the toxic effect it exerts on the variouscancer cell lines, Be through DNA fragmenting by the efficiency of apoptosis and eliminating most free radicals (Suzuki *et al* ., 2002 ;Chen *et al* ., 2003)

Efficacy of chemotherapeutic agents used in the treatment of breast cancer could be enhanced by biologically active natural substances that increase the susceptibility of cancer cells to medication

It has been proven that many chemical substances of plant origin, including Propolis, counter the viability of cancer cells. (Stojko *et al* ., 2015).

Recent reports indicate that Propolis possesses various biological activities, of which an anti-cancer property. (Xuan *et al*., 2014)

This anti-cancer activity of propolis exerted through its cellular toxicity to breast cancer cells is achieved by treatment with Propolis concentrations of 25, 50, 100and 200µg/ml, respectively (Xuan *et al*., 2014).

It appears that the impact of propolis on cancer cells after incubation for 24-48h cause apoptosis clearly. It concluded that propolis has an anti-tumor effect in breast cancer cells, suggesting the

possibility of its use as alternative therapeutic agent of breast cancer(Rzepecka-Stojko *et al* ., 2015).

From this point of propolis, it was selected to evaluate the anti-cancer activity of dacarbazine as one of the drugs of chemotherapy on a cancer cell lines to manifest the scientific miracles in the therapeutic potential that god has placed in this natural material.

2. Materials & Methods

Cell lines

Experiments were conducted on Breast cancer cell lines MCF-7 (ATCC®HTB-22TM), obtained from King Fahd research center at King Abdul-Aziz university.

Dacarbazine(DTIC)

Dacarbazine, a drug used in chemotherapy for cancer patients and is known commercially as DETICENE, obtained from king Abdul Aziz hospital in Jeddah.

Propolis

Bee glue (propolis) substance collected by bees from the buds of trees and have multiple benefits have been obtained from the wild honey company in Riyadh.

Experimental Design

SRB Cells Cytotoxicity Assay

Breast cancer cell lines, used in this experiment were divided into 4 principal groups as follows:

1-The first Group: the control group represents non-treatment.

2-The Second Group: represents the treatment of Propolis, with concentrations (50, 100 and 200 µg/ml) (Xuan *et al*.,2014).

3-The Third Group: medical treatment of Dacarbazine, with concentrations (50, 100 and 200 µg/ml)

4-The Fourth Group: combination treatment Propolis with Dacarbazine, with concentrations (50, 100 and 200 µg/ml).

The method, established by Houghton *et al*., 2007,to prepare and install dye cancer cell lines for the application of SRB cells cytotoxicity assay.

It calculated the percentage inhibition of growth (IC_{50}) and (IC_{90}) as follows:

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

Statistical Analysis:

Statistical Analysis performed by applying both the student 't' test and ANOVA test to calculate the significant results obtained from the test under study.

3. Results:

Effect of treatment with Propolis and treatment with Dacarbazine, as well as combined treatment with both Propolis and Dacarbazine on the values of IC_{50} and IC_{90} after 48 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 (Fig.1), further supported by evidence of Apoptosis e.g. nuclear condensation, increase the size of some cells dramatically , inflation cytoplasm , loss of cell membranes, bursting of cells and extrusion of contents, compared to the control group. The values of inhibited proliferation of 50% of cells (IC_{50}) were then calculated for treatments with either Propolis or Dacarbazine, as well as for combined treatment with both Propolis and Dacarbazine and it was 285.46, 248.9 and 659.1 µg/ml respectively, whereas the values of inhibited proliferation of 90% of cells (IC_{90}) with various treatments were 552.13, 484.23and 1325.8µg/ml respectively (Fig 9)

The impact of treatment with Propolis and treatment with Dacarbazine, as well as combined treatment with both Propolis and Dacarbazine on the mean appearance of breast cancer cell lines MCF-7 after 48 hours at a concentration of 50 µg/ml:

The results obtained from table (1) indicate that treatment with Propolis, and Dacarbazine, as well as with combined treatment of the two caused a highly significant ($P \leq 0.001$) reduction in mean appearance of breast cancer lines MCF-7, valued at 0.274±0.006, 0.268±0.010 and 0.268±0.012 respectively, compared with the control sample mean of 0.354±0.00, where the chemotherapeutic treatment mean equaled the combined treatment mean, While slightly higher when treatment with propolis(Fig. 7).

Inhibited proliferation rates of MCF-7 breast cancer cell lines, as calculated with various treatments, were 22.88%, 24.29% and 24.58% respectively, indicating that the highest percentage was posted by the combined treatment followed by treatment with Dacarbazine and treatment with Propolis (Fig.11)

The inhibited cellular proliferation rate was inversely proportional to the rates of absorbability and viability whenever the higher the rates of viability and absorbability the lower the inhibition rate (Figs. 3, 5)

The impact of treatment with Propolis and treatment with Dacarbazine, as well as combined treatment with both Propolis and Dacarbazine on the mean appearance of breast cancer cell lines MCF-7 after 48 hours at a concentration of 100 µg/ml:

The results obtained from table (1) indicate that treatment with Propolis, and Dacarbazine, as

well as with combined treatment of the two caused a highly significant ($P \leq 0.001$) reduction in mean appearance of breast cancer lines MCF-7, valued at (0.268±0.014 , 0.253±0.007 , 0.246±0.007) respectively, compared with the control sample mean of 0.354±0.009, indicating that the best treatment in terms of reduction of mean appearance of MCF-7is the combined treatment, followed by chemotherapeutic treatment and treatment with Propolis respectively(Fig. 7).

MCF-7 inhibited proliferation rates posted 24.29% ,28.53% and 30.79% with various treatments, with the highest percentage posted by the combined treatment, followed by the treatment with dacarbazine and lastly by the treatment with Propolis (Fig.11). The inhibition ratio is inversely proportional rate absorbency and vitality (Figs. 3, 5)

The impact of treatment with Propolis and treatment with Dacarbazine, as well as combined treatment with both Propolis and Dacarbazine on the mean appearance of breast cancer cell lines MCF-7 after 48 hours at a concentration of 200 µg/ml:

The results obtained from table (1) indicate that treatment with Propolis, and Dacarbazine, as well as with combined treatment of the two caused a highly significant ($P \leq 0.001$) reduction in mean appearance of breast cancer lines MCF-7, valued at 0.234±0.004 , 0.218±0.005, respectively,While combined treatment recorded a highly significant ($P \leq 0.01$) decrease its value 0.256±0.018compared with the control sample mean of 0.354±0.009, indicating that the best treatment in terms of reduction of mean appearance of MCF-7is the treatment with Dacarbazine, treatment with Propolis the combined treatment with both Propolis and Dacarbazine respectively(Fig. 7).

MCF-7 inhibited proliferation rates posted 34.18% ,38.7% and 27.68% for treatment with the highest percentage posted by the chemotherapeutic agent treatment, followed by the treatment with Propolis and the combined treatment respectively (Fig.11).The inhibition ratio is inversely proportional rate absorbency and vitality(Figs. 3, 5)

The impact of treatment with Propolis and treatment with Dacarbazine, as well as combined treatment with both Propolis and Dacarbazine on the mean appearance of breast cancer cell lines MCF-7 after 48 hours at a concentration of 50,100&200 µg/ml using analysis of variance and the least significant difference (LSD):

The results obtained from table 3 posted a highest significant difference ($P \leq 0.001$) in the mean appearance of breast cancer cell lines MCF-7 at various concentrations, whether under treatment Propolis, under treatment with Dacarbazine, or under combined treatment with both Propolis and Dacarbazine, measuring (F=670.19) at 50µg/ml concentration, (F=838.20) at 100µg/ml concentration and (F=450.33) at concentration of 200 µg/ml compared to the control sample.

The comparison test using the least significant difference LSD showed a highest significant difference of $P \leq 0.001$ in the mean appearance of breast cancer cell lines MCF-7 as a result of treatment with Propolis, Dacarbazine and combined treatment with Propolis and Dacarbazine at concentrations 50, 100 and 200 µg/ml (Fig.13) theorder treatments terms their effect in reducing the average higher emergence MCF-7 at concentrations50,100µg/ml as follows:

Treatment with Propolis< treatment with Dacarbazine<Combined treatment

Treatments ranked in order of highest impact on the reduction of the mean appearance of MCF-7 breast cell lines at 200µg/ml were as follows:

Combined treatment < treatment with Propolis< treatment with Dacarbazine

The impact of treatment with Propolis and treatment with Dacarbazine, as well as combined treatment with both Propolis and Dacarbazine on the values of IC50 and IC90 after 72 hours:

In Vitro microscopic examination of morphologic changes undergone by breast cancer cell lines MCF-7 under the impact of various treatments after incubation for 72 hours (Fig.2) as indicated by evidence of Apoptosis e.g. nuclear condensation, most increased size of the cells greatly,cytoplasmic shrinkage, loss of cell membranes, bursting of cells and extrusion of contents, as well as by a marked reduction in the number and viability of cancer cells compared with both cellular morphology after a 48 hour incubation and compared with the control sample.

Inhibited cellular proliferation by 50% (IC_{50}%), calculated for treatment with Propolis, Treatment with Dacarbazine and combined treatment with both Propolis and Dacarbazine were 208.50، 154.45 and 298.30µg/ml respectively, while value of the deadly concentrationby 90 %(IC_{90}%), of various treatments, were 443.88 , 472.63 and 622.66 µg/ml respectively (Fig.10).

The impact of treatment with Propolis and treatment with Dacarbazine, as well as combined treatment with both Propolis and Dacarbazine on the mean appearance of breast cancer cell lines MCF-7 after 72 hours at a concentration of 50 µg/ml:

The results obtained from table (2) indicate that treatment with Propolis, and Dacarbazine, as well as with combined treatment of the two caused a highly significant ($P \leq 0.001$) reduction in mean appearance of breast cancer lines MCF-7, valued at

0.306±0.003, 0.283±0.004 and 0.282±0.007 respectively, compared with the control sample mean of 0.488±0.005, Equal the average dacarbazine treatment with the average treatment almost combined while slightly higher when the treatment propolis(Fig. 8).

MCF-7 inhibited proliferation rates posted 37.17%, 42.09% ,and 42.30%respectively, with the highest percentage posted the combined treatment, followed by treatment with Dacarbazine and treatment with Propolis (Fig.12).The inhibition ratio is inversely proportional to the rate of absorbance and vitality whenever the rate of absorbance and vitality increased proportion of the inhibition(Figs. 4, 6)

The impact of treatment with Propolis and treatment with Dacarbazine, as well as combined treatment with both Propolis and Dacarbazine on the mean appearance of breast cancer cell lines MCF-7 after 72 hours at a concentration of 100 µg/ml:

The results obtained from table (2) indicate that treatment with Propolis, and Dacarbazine, as well as with combined treatment of the two caused a highly significant ($P \leq 0.001$) reduction in mean appearance of MCF-7 breast cancer cell lines, valued at (0.298±0.005, 0.254±0.004, 0.263±0.009) respectively, compared with the control sample mean of 0.488±0.005, indicating that the best treatment in terms of reduction of mean appearance of breast cancer cell lines MCF-7 is chemotherapeutic treatment followed by the combined treatment, followed by treatment with Propolis respectively(Fig. 8). MCF-7 inhibited proliferation rates posted 38.81%, 47.84% and 45.99%, with the highest percentage posted by the treatment with Dacarbazine, followed by the combined treatment and the treatment with Propolis, respectively (Fig.12)

The inhibition ratio is inversely proportional to the rate of absorbance and vitality(Figs. 4, 6)

The impact of treatment with Propolis and treatment with Dacarbazine, as well as combined treatment with both Propolis and Dacarbazine on the mean appearance of breast cancer cell lines MCF-7 after 72 hours at a concentration of 200 µg/ml:

The results obtained from table (2) indicate that treatment with Propolis, and Dacarbazine, as well as with combined treatment of the two caused a highly significant ($P \leq 0.001$) reduction in mean appearance of breast cancer lines MCF-7, valued at (0.290±0.004, 0.234±0.000,0.224±0.003) respectively, compared with the control sample mean of 0.488±0.005, indicating that the best treatment in terms of reduction of mean appearance of MCF-7breast cancer cell lines is the combined treatment, followed by treatment with Dacarbazine and treatment with Propolis (Fig. 8).

MCF-7 inhibited proliferation rates posted 40.45%, 51.95%, and 54.21% respectively, with the highest percentage posted by the combined treatment, followed by treatment with Dacarbazine and treatment with Propolis (Fig.12).

The inhibition ratio is inversely proportional to the rate of absorbance and vitality (Figs. 4, 6)

The impact of treatment with Propolis and treatment with Dacarbazine, as well as combined treatment with both Propolis and Dacarbazine on the mean appearance of breast cancer cell lines MCF-7 after 72 hours at a concentration of 50,100&200 µg/ml using analysis of variance and the least significant difference (LSD):

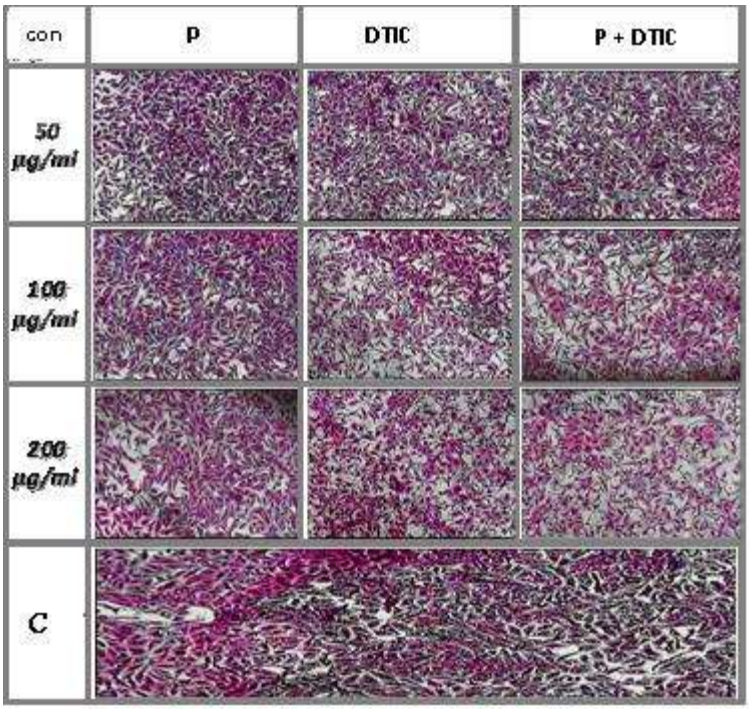
The results obtained from table 4 posted a highest significant difference ($P \leq 0.001$) in the mean appearance of breast cancer cell lines MCF-7 at various concentrations, whether under treatment with Propolis, under treatment with Dacarbazine, or under combined treatment with both Propolis and Dacarbazine, measuring (F=733.401) at 50µg/ml concentration, (F=890.315) at 100µg/ml concentration and (F=260.864) at concentration of 200 µg/ml compared to the control sample.

The comparison test using the least significant difference LSD showed a highest significant difference of $P \leq 0.001$ in the mean appearance of breast cancer cell lines MCF-7 as a result of treatment with Propolis, Dacarbazine and combined treatment with Propolis and Dacarbazine at concentrations 50, 100 and 200 µg/ml (Fig. 14) the order treatments terms their effect in reducing the average higher emergence MCF-7 at concentrations50, 200µg/ml as follows:

Treatment with Propolis< treatment with Dacarbazine<Combined treatment

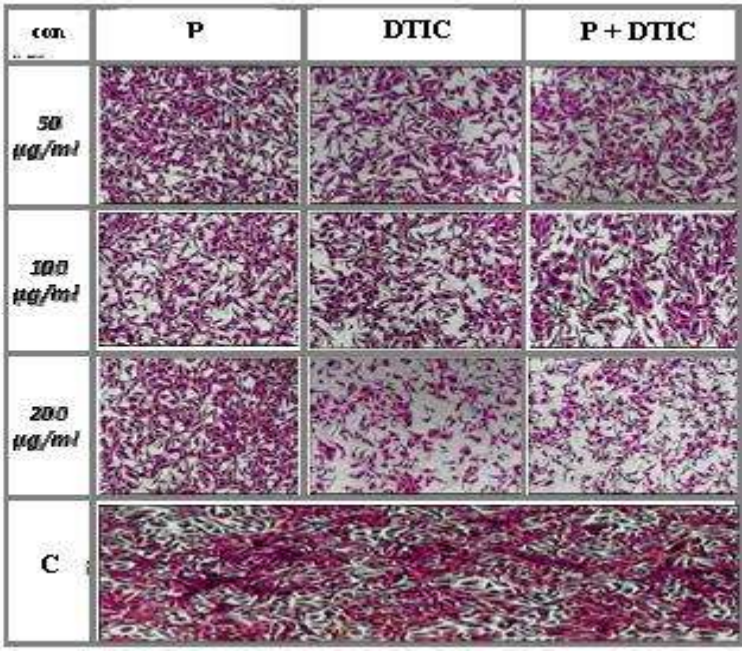
Treatments ranked in order of highest impact on the reduction of the mean appearance of MCF-7 breast cell lines at 100µg/ml were as follows:

Treatment with Propolis< Combined treatment < treatment with Dacarbazine



C:Control , P: Propolis ,D: Dacarbazine, P+D:: Propolis + Dacarbazine
1: concentration of 50(µg/ml), 2: concentration of 100(µg/ml), 3: concentration of 2000(µg/ml)

Fig (1): Morphological and cytological features of breast cancer cells of the lines MCF-7 Treatment with Different concentrations of Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine after 48 h . (X1000)



C: Control, P: Propolis ،D: Dacarbazine, P+D: Propolis + Dacarbazine
1: concentration of 50(µg/ml), 2: concentration of 100(µg/ml), 3: concentration of 2000(µg/ml)

Fig (2): Morphological and cytological features of breast cancer cells of the lines MCF-7 Treatment with Different concentrations of Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine after 72 h . (X1000)

Table (1) : The Effects of Different concentrations of Treatment by Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the mean of breast cancer cells of the lines MCF-7 after 48 h .

Con. (ug/ml)	Groups Treatment	No. cell line	Mean ± Std.Error	Absorbance	Survival Fraction (SF)	% Inhibition
50	C	1 2 3 4 5 6 Mean ± Std.Error	0.354 ± 0.009	0.354	1	___
	P	1 2 3 4 5 6 Mean ± Std.Error	***a 0.274 ± 0.006	0.273	0.771	22.881
	D	1 2 3 4 5 6 Mean ± Std.Error	***a 0.268±0.010	0.268	0.757	24.293
	P+D	1 2 3 4 5 6 Mean ± Std.Error	***a 0.268 ± 0.012	0.267	0.754	24.576
100	C	1 2 3 4 5 6 Mean ± Std.Error	0.354 ± 0.009	0.354	1	___
	P	1 2 3 4 5 6 Mean ± Std.Error	***a 0.268 ± 0.014	0.268	0.757	24.293
	D	1 2 3 4 5 6 Mean ± Std.Error	***a 0.253 ± 0.007	0.253	0.715	28.531
	P+D	1 2 3 4 5 6 Mean ± Std.Error	*** a 0.246 ± 0.007	0.245	0.692	30.790
200	C	1 2 3 4 5 6 Mean ± Std.Error	0.354 ± 0.009	0.354	1	___
	P	1 2 3 4 5 6 Mean ± Std.Error	***a 0.234 ± 0.004	0.233	0.658	34.180
	D	1 2 3 4 5 6 Mean ± Std.Error	***a 0.218 ± 0.005	0.217	0.613	38.700
	P+D	1 2 3 4 5 6 Mean ± Std.Error	** a 0.256 ± 0.018	0.256	0.723	27.683

C:Control , P: Propolis , D: Dacarbazine, P+D : Propolis +Dacarbazine a: Comparison with C , b: Comparison with D

p* significant<0.05 **p**** highly significant<0.01 **p***** extremly significant<0.001

Table (2) : : The Effects of Different concentrations of Treatment by Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the mean of breast cancer cells of the lines MCF-7 after 72 h .

Con. (ug/ml)	Groups Treatment	No. cell line	Mean ± Std.Error	Absorbance	Survival Fraction (SF)	% Inhibition
50	C	1 2 3 4 5 6 Mean ± Std.Error	0.488 ± 0.005	0.487	1	___
	P	1 2 3 4 5 6 Mean ± Std.Error	***[a] 0.306 ± 0.003	0.306	0.628	37.166
	D	1 2 3 4 5 6 Mean ± Std.Error	***[a] 0.283 ± 0.004	0.282	0.579	42.094
	P+D	1 2 3 4 5 6 Mean ± Std.Error	*** [a] 0.282 ± 0.007	0.281	0.577	42.299
100	C	1 2 3 4 5 6 Mean ± Std.Error	0.488 ± 0.005	0.487	1	___
	P	1 2 3 4 5 6 Mean ± Std.Error	***[a] 0.298 ± 0.005	0.298	0.612	38.809
	D	1 2 3 4 5 6 Mean ± Std.Error	***[a] 0.254 ± 0.004	0.254	0.522	47.84
	P+D	1 2 3 4 5 6 Mean ± Std.Error	*** [a] 0.263 ± 0.009	0.263	0.540	45.995
200	C	1 2 3 4 5 6 Mean ± Std.Error	0.488 ± 0.005	0.487	1	___
	P	1 2 3 4 5 6 Mean ± Std.Error	***[a] 0.290 ± 0.004	0.29	0.595	40.45
	D	1 2 3 4 5 6 Mean ± Std.Error	***[a] 0.234 ± 0.005	0.234	0.480	51.95
	P+D	1 2 3 4 5 6 Mean ± Std.Error	*** [a] 0.224 ± 0.003	0.223	0.458	54.209

C:Control , P: Propolis , D: Dacarbazine, P+D : Propolis +Dacarbazine a: Comparison with C , b: Comparison with D

p* significant<0.05 **p**** highly significant<0.01 **p***** extremly significant<0.001

Table (3) : ANOVA and LSD between The Effects of Different concentrations of Treatment by Propolis ,Dacarbazine,and The Dual Treatment with Propolis and Dacarbazine on the mean of breast cancer cells of the lines MCF-7 after 48 h .

		ANOVA		LSD		
		F	Sig	Groups Treatment	Mean Difference	Sig
50	Control C	670.19	***	P	0.082	***
				D	0.086	***
				P+D	0.087	***
100	Control C	838.26	***	P	0.086	***
				D	0.101	***
				P+D	0.109	***
200	Control C	450.33	***	P	0.121	***
				D	0.137	***
				P+D	0.098	***

C: Control, P: Propolis ,D: Dacarbazine, P+D:: Propolis + Dacarbazine

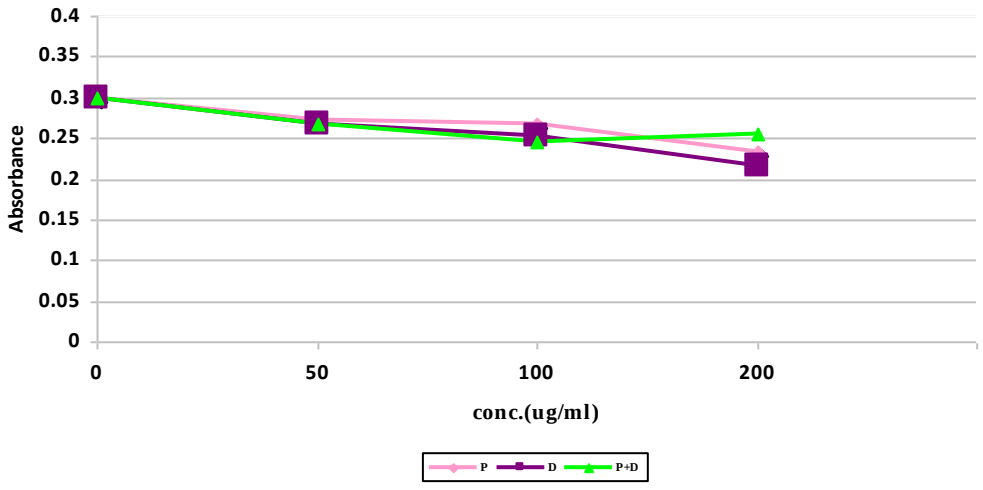
p* significant<0.05 **p**** highly significant<0.01 **p***** extremly significant<0.001

Table (4) : ANOVA and LSD between The Effects of Different concentrations of Treatment by Propolis ,Dacarbazine,and The Dual Treatment with Propolis and Dacarbazine on the mean of breast cancer cells of the lines MCF-7 after 72 h .

		ANOVA		LSD		
		F	(Sig)	Groups Treatment	Mean Difference	Sig
50	Control C	733.401	***	P	0.182	***
				D	0.205	***
				P+D	0.206	***
100	Control C	890.315	***	P	0.190	***
				D	0.234	***
				P+D	0.224	***
200	Control C	260.864	***	P	0.198	***
				D	0.253	***
				P+D	0.264	***

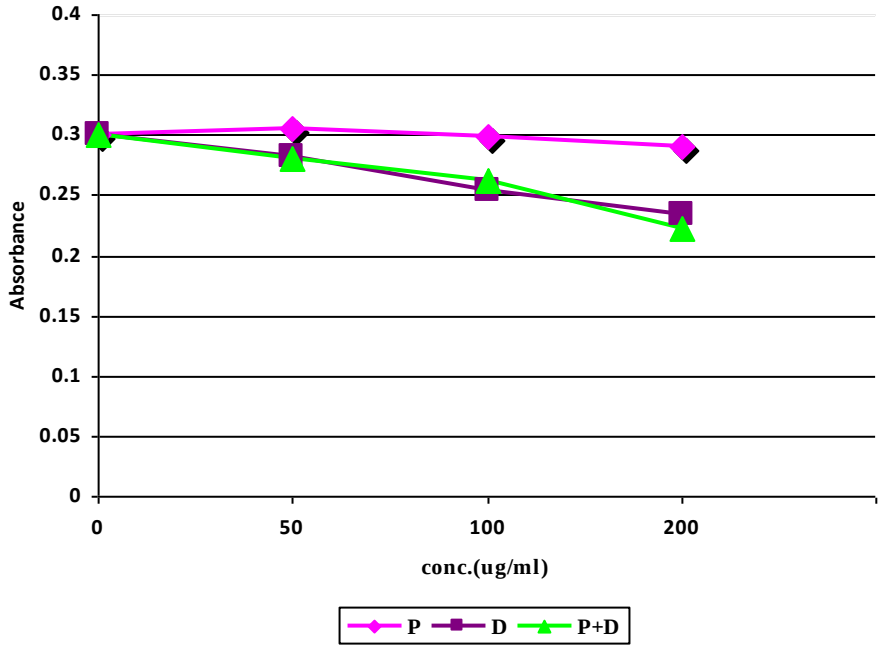
C:Control , P: Propolis ,D: Dacarbazine, P+D:: Propolis + Dacarbazine

p* significant<0.05 **p**** highly significant<0.01 **p***** extremly significant<0.001



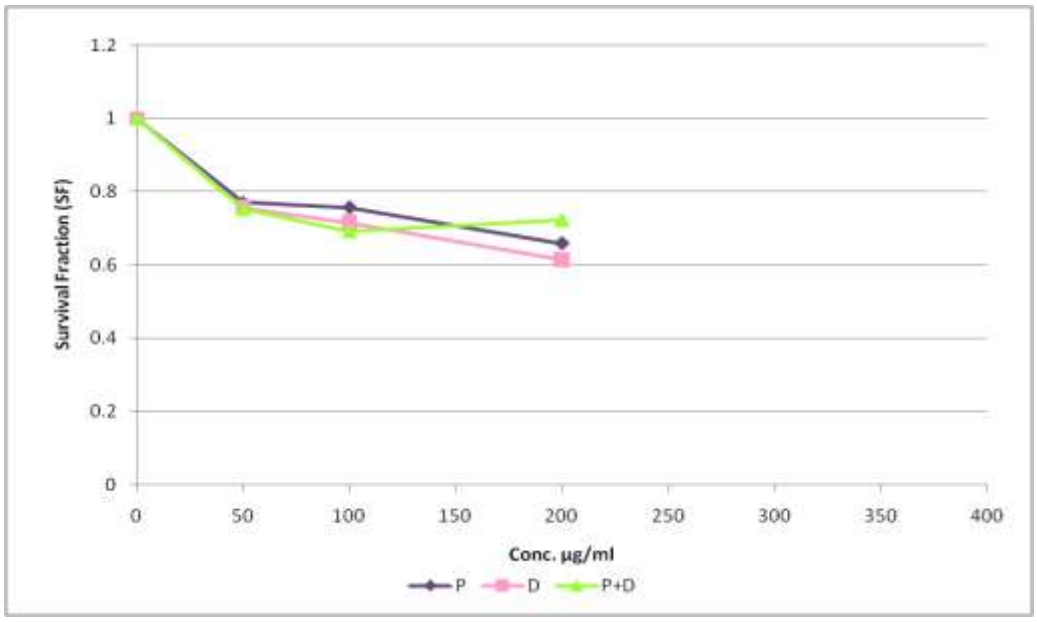
P Propolis D Dacarbazine P+D Propolis +Dacarbazine

Fig (3) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Absorbance values of breast cancer cells of the lines MCF-7 after 48 h .



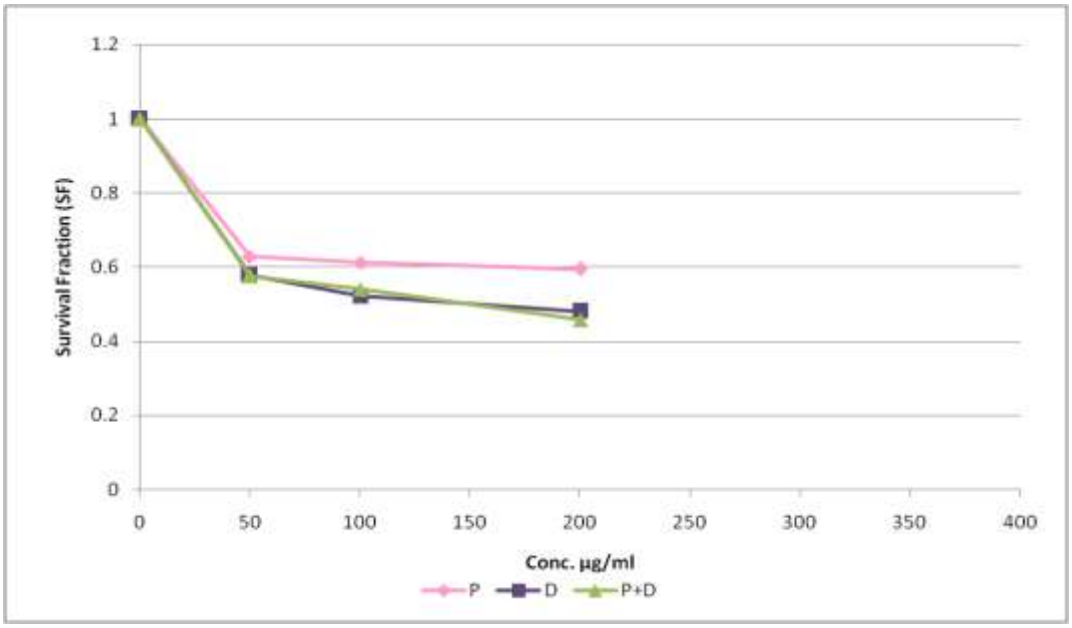
P Propolis D Dacarbazine P+D Propolis +Dacarbazine

Fig (4) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Absorbance values of breastcancer cells of the lines MCF-7 after 72 h .



P Propolis D Dacarbazine P+D Propolis +Dacarbazine

Fig (5) :Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Survival Fraction (SF) values of breast cancer cells of the lines MCF-7 after 48 h .



P Propolis D Dacarbazine P+D Propolis +Dacarbazine

Fig (6) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Survival Fraction (SF) values of breast cancer cells of the lines MCF-7 after 72 h .

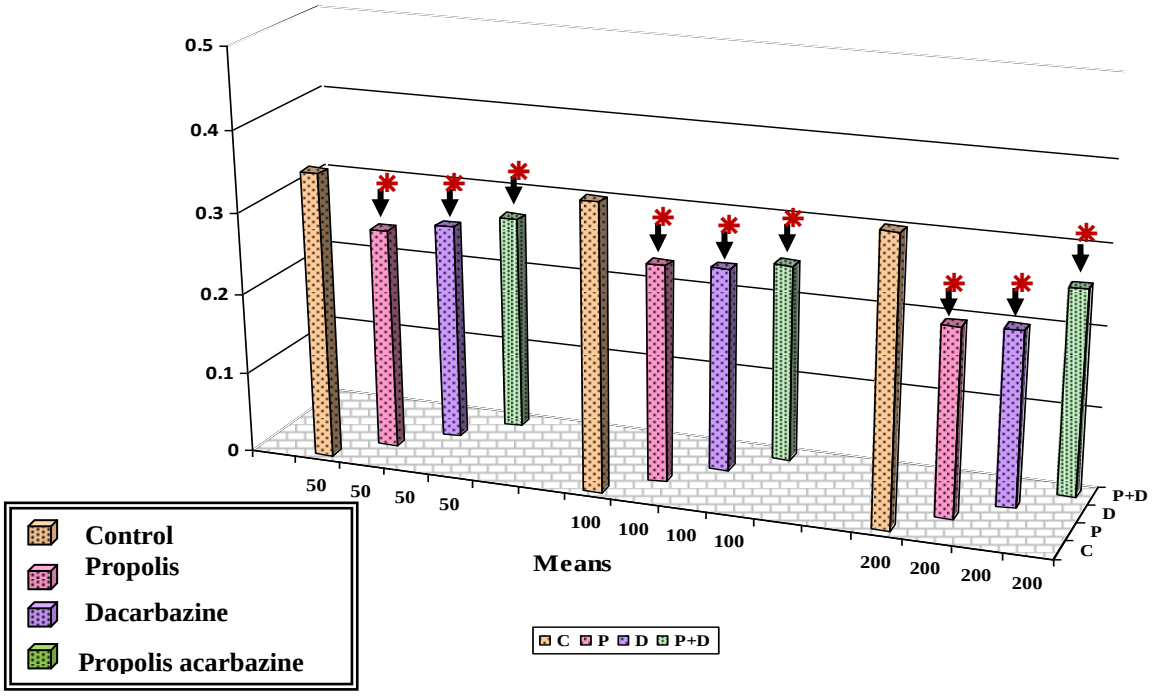


Fig (7) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatmen with Propolis and Dacarbazine on the Means of breast cancer cells of the lines MCF-7 after 48 h .

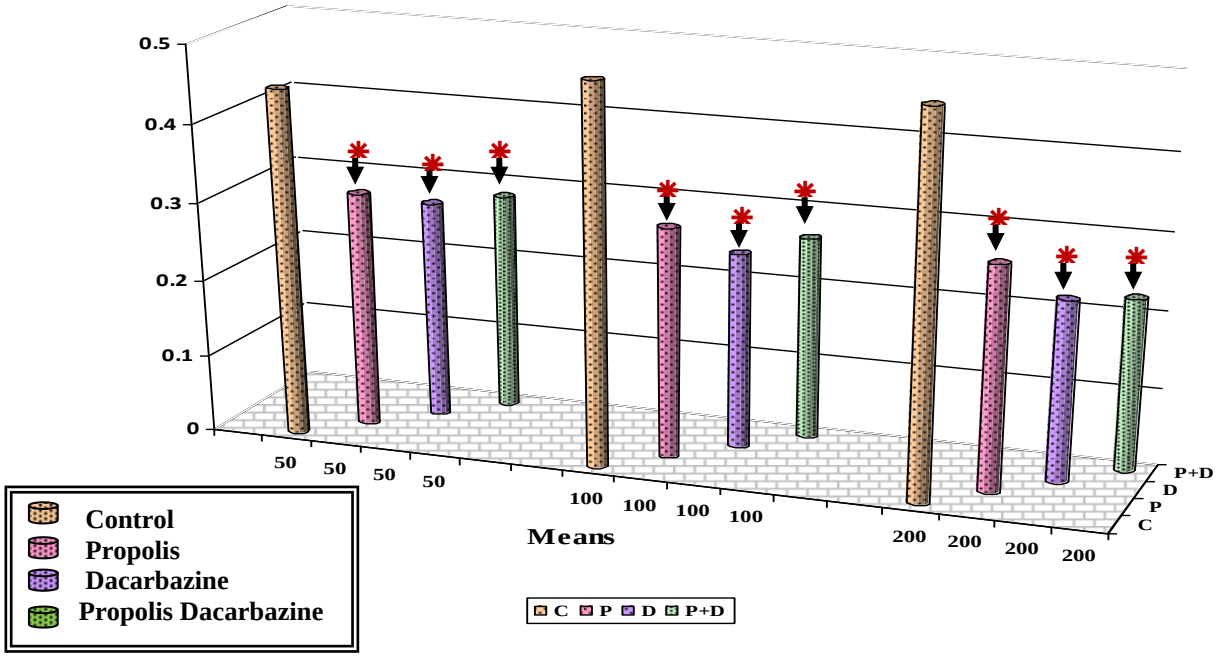


Fig (8) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatme with Propolis and Dacarbazine on the Means of breast cancer cells of the lines MCF-7 after 72 h .

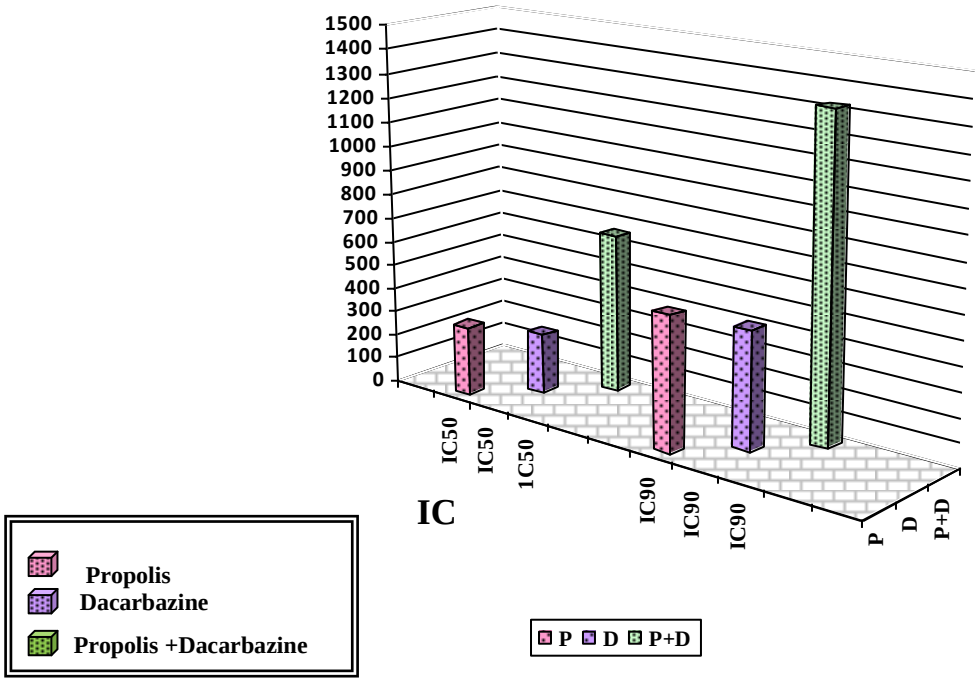


Fig (9) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the IC_{50} and IC_{90} values of breast cancer cells of the lines MCF-7 after 48 h .

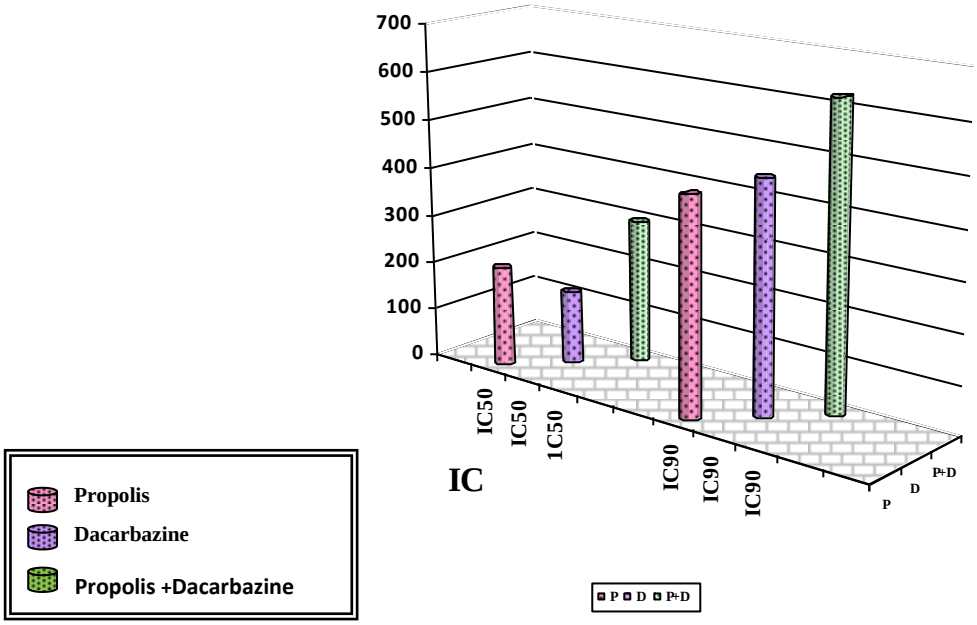


Fig (10) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the IC_{50} and IC_{90} values of breast cancer cells of the lines MCF-7 after 72 h .

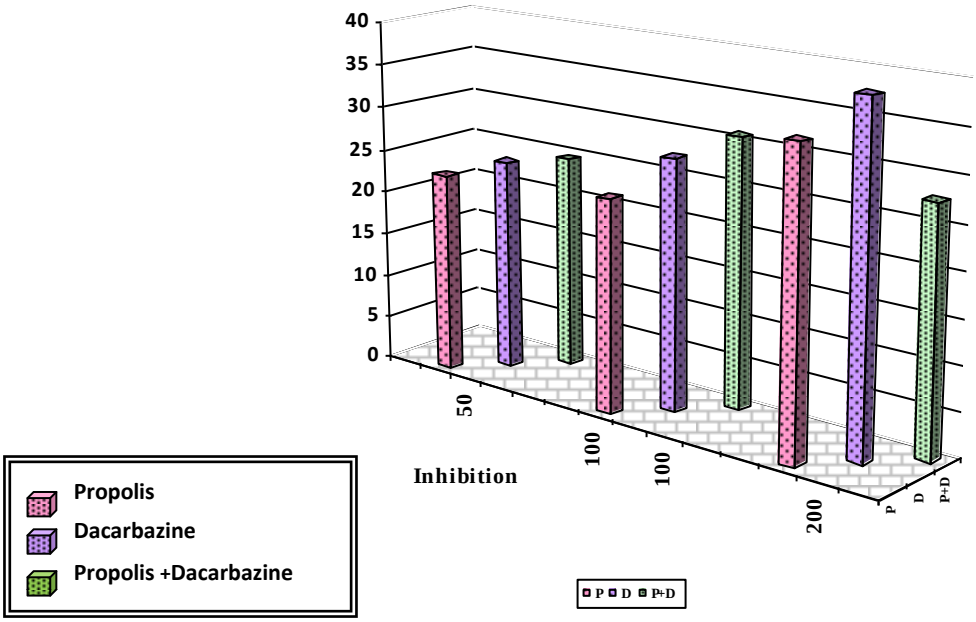


Fig (11) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatme with Propolis and Dacarbazine on the Inhibition rate of breast cancer cells of the lines MCF-7 after 48 h .

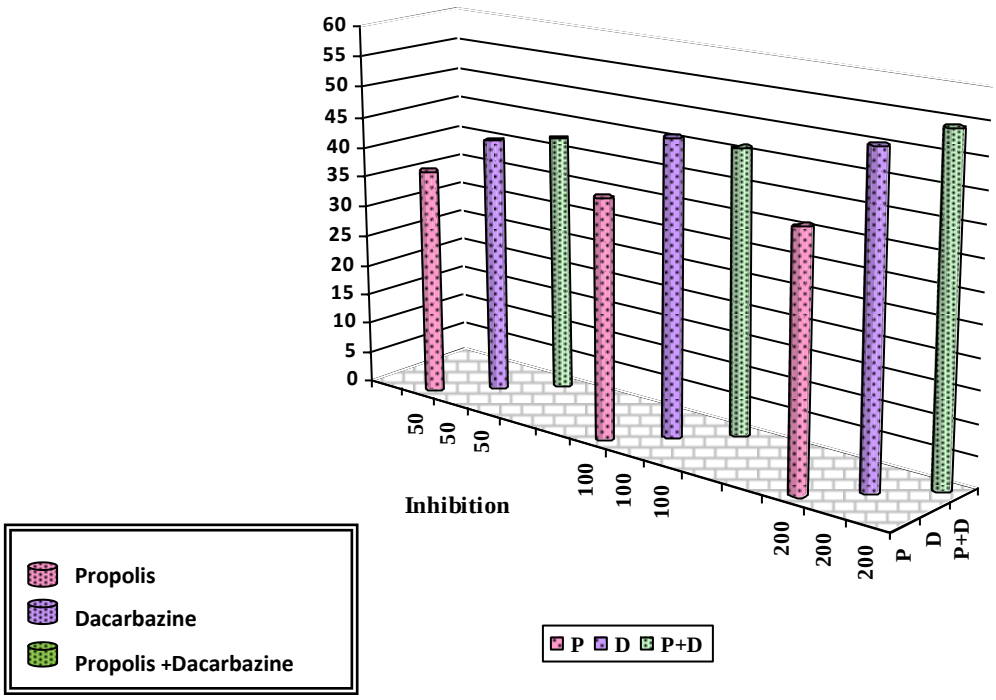


Fig (12) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatme with Propolis and Dacarbazine on the Inhibition rate of breast cancer cells of the lines MCF-7 after 72 h .

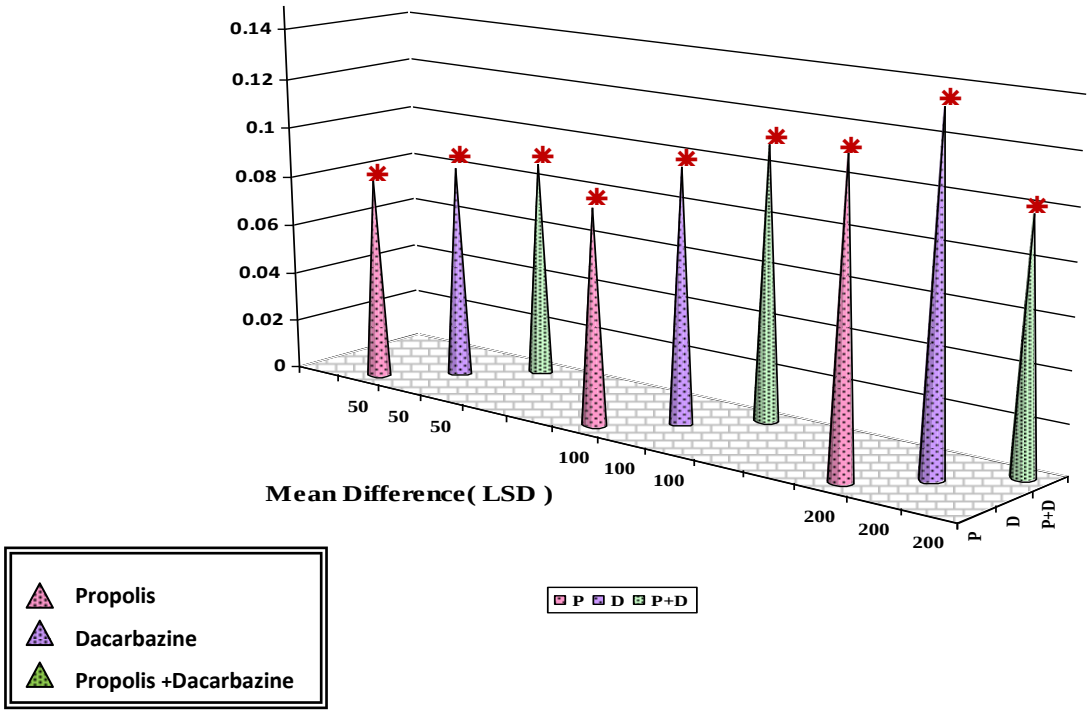


Fig (13) : Comparison between The Effect of Treatment of Propolis, Dacarbazine and The Dual Treatment wi Propolis and Dacarbazine on the mean of breast cancer cells of the lines MCF-7 after 48 h .

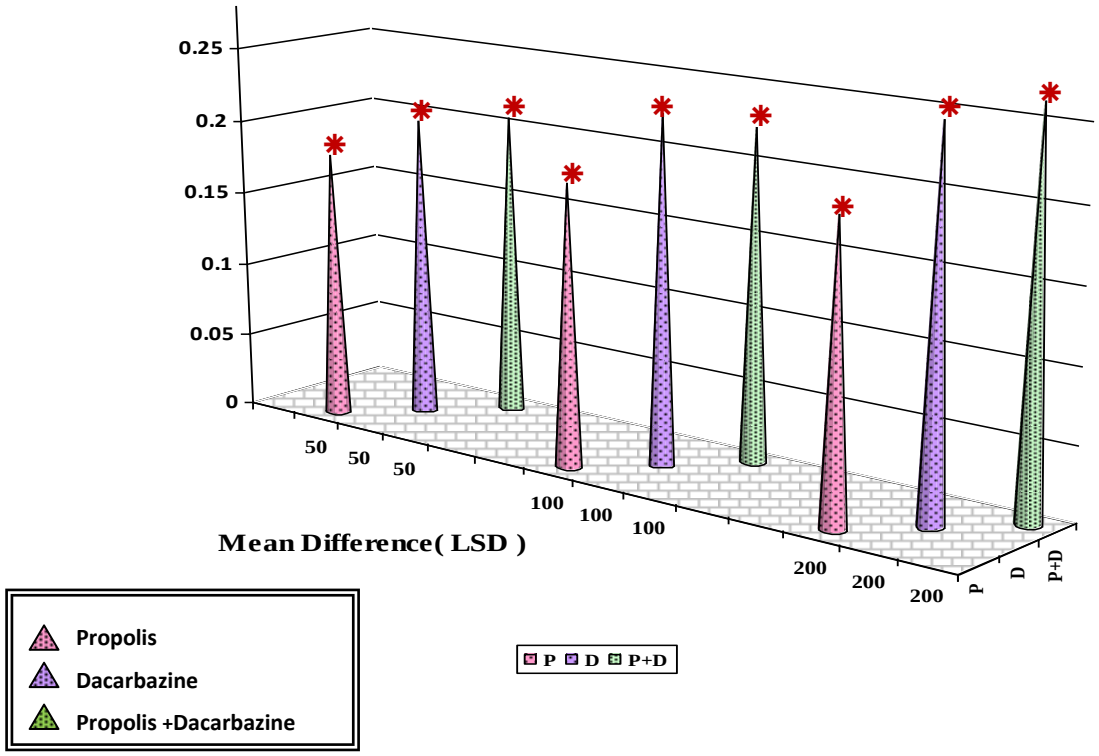


Fig (14) : Comparison between The Effect of Treatment of Propolis, Dacarbazine and The Dual Treatment with Propolis and Dacarbazine on the mean of breast cancer cells of the lines MCF-7 after 72 h .

4. Discussion:

Breast Cancer patients, in particular, are the most common users of alternative medicine, compared to other cancer patients.

To be more specific, breast cancer patients who have used at least one type of alternative medicine range from 63 to 83 % (Wu *et al*., 2011).

This study, therefore, aims at evaluating the potential therapeutic cellular impact of treatment with Propolis, which is a bee product with many natural and therapeutic properties and with Dacarbazine, as well as with a combined treatment of both Propolis and Dacarbazine, as compared with the treatment with Dacarbazine, which is a chemotherapeutic agent used in the treatment of many malignant tumours on breast cancer cell lines MCF-7 and the results of all 3 treatments were examined at 3 concentrations, namely:50,100 and 200 µg/ml and following incubation for 48 and 72 hours.

Morphologic examination in this study demonstrated the inhibitory impact of treatment at 3 concentrations (50, 100 and 200µg/ml) with Propolis, Dacarbazine and a combination of Propolis and Dacarbazine on breast cancer cell lines MCF -7 proliferation after incubation for 48-72 hours, as evidenced by apoptosis with all treatments and at all concentrations, as compared with the control sample.

Such impact was dependent on time and concentrations, therefore, consistent with previous results obtained by many researchers following treatment of breast cancer cell lines MCF -7.

Exposure of breast cancer cells to different doses of Propolis and incubation for 48-72 hours were invariably followed by an observable decrease in cancer cell count, shrinkage of cytoplasm, chromatin condensation and splintering of cancer cells. Such time and dose-dependent impact are attributable to reduction by Propolis of cytotoxic activity of breast cancer cells, indicating that Propolis is a biologically active compound in both chemoprevention and chemotherapy by catalyzing anti-cancer chemotherapeutic agents (Rzepecka-Stojko *et al* ., 2015).

Apoptosis is an important phenomenon in chemotherapy as it results from cancer cell destruction.

Catalyzing apoptosis is, therefore, one of the suggested mechanisms for the therapeutic impact of Propolis (Benguedouar *et al*., 2008; Bufalo *et al*., 2009)

A study of the potential mechanism for apoptosis upon treatment with Propolis attributed such mechanism to fragmenting DNA and catalyzing caspases in breast cancer cells MCF-7 (Vatansever *et al*., 2010) .

Apoptosis of breast cancer cells indicates the potential in concentration 47.45µg/ml of Propolis,Suggesting potential of propolis as an anti-cancer prevents the proliferation of cancer cells and catalyze apoptosis as a pro-apoptotic and anti-cancer agent, preventing extension of cancer cells and catalyzing apoptosis (Sawah and Kav ., 2010).

Studies also attributed the anti-cancer cell potential of Propolis to its catalysis of the inhibition of cancer cell proliferation, resulting in programmed cell death, inhibited proliferation of cancer cells, organized P_{53} protein levels and increased levels of free radicals inside cancer cells and decreased the potential of Mitochondrial membrane.

Propolis was further found to prevent extension of breast cancer cells at concentrations of 25-200µg/ml, thus indicating its potential ability to inhibit proliferation of breast cancer metastases.

P_{53} is protein considered as a tumor suppressor and a principal organizer of cytotoxic stress, thus arresting cancer cell growth or catalyzing apoptosis.

Collective results indicate that Propolis plays a dual role where free radicals are concerned, it is pro-oxidant at high concentrations and antioxidant at low concentrations (Karni-schmidt *et al*., 2008; Xuan *et al*., 2014)

The results of one study demonstrated that in-vivo treatment with propolis for 3-4 weeks reduces the size of breast cancer by 40-60% in mice and further demonstrated that inhibition of tumor growth was directly proportional to increase in Propolis concentration, as it inhibits breast cancer cells during the construction phase S and eliminates it altogether during the G_2 ,M, acting as a pro-apoptotic agent, by releasing cytochrome enzymes from mitochondria into the cytosol, through a series of caspases with pro-apoptotic proteins (Rzepecka-stojko *et al* ., 2015).

Epidemiologic indicators,as well as pre-clinical evidence,indicate that polyphenol compounds and photochemicalcompounds in propolis possess anti-cancer chemo-preventive properties (Orsolic *et al* ., 2007), thus shifting the focus of cancer protection strategies to the use of Propolis as the richest source of Phenol and polyphenol.

Upon treating breast cancer cells with various concentrations of quercetin, a flavonoid element contained by Propolis, it was observed to prevent extension of breast cancer cells MCF-7 by decreasing the viability of breast cancer cells by a mechanism that is dependent on dosage and incubation period. The decrease has also been linked to arresting cellular cycle and apoptosis, as it activates the caspases series, elevates the Bax protein and decreases the anti-apoptotic Bcl-2 protein (Chien *et al*., 2009; Engen 2007; (Ackland *et al*., 2005; Due, *et al*., 2012).

It was also observed by Zhang *et al*. (2012) that 90.6% of breast cancer cells did enter an early stage of apoptosis when treated with quercetin at a dose of 100µg, because of its potent anti-cancer properties, which depend on the high content of free radicals within cancer cells to cause apoptosis.

Two different mechanisms were, therefore, suggested by which quercetin act could inhibit growth of MCF-7 cancer breast cell lines, namely:

1-Inhibition of the progress of cancer cell cycle by accumulating Phase M and arresting phase G_2

2-Acting as a pro-apoptotic agent in cancer cells (Choi *et al*., 2001)

Incubation of breast cancer cells with CAPE, a Propolis extract, arrested the cancer cell cycle at phases G_0 and G_1 in 82% of cases and in phase S in 12%, while increasing remission in the S phase by 41% and decreasing remission in G_0 and G_1 by54%.

4-5 days thereafter, the level of expression of genes responsible for cancer breast dropped by up to 95% (Omene *et al*., 2012)

The most remarkable development was the morphologic and apoptosis impact in breast cancer cells MCF-7, which was more evident and much enhanced with the combined treatment, using both Propolis and Dacarbazine, closely resembling the morphologic impact resulting from treatment with the chemotherapeutic agent. There was a linear relationship between effects of concentrations and with time.

Another study revealed that simultaneous treatment with Propolis and Taxol arrests cell cycle both *in vivo* and *in vitro* and prevents the expression of protein mdr-1, which is a gene that prevents resistance of cancer cells to chemotherapeutic agents and hence the potent inhibition of growth.

The decrease in mdr-1 genes allows the use of Propolis in combination with chemotherapeutic agents.

This study proved that Propolis

*inhibits growth of breast cancer stem cells

*arrests cell cycle and apoptosis

Previous studies revealed that arresting the cell cycle is closely related to the inhibition of tumor growth (Wiliams and Stoeber ., 2012) and neoplastic vascularization (Omene *et al* ., 2013).

Combined treatment with quercetin and Topotecan, as a chemotherapeutic agent, quadruples toxicity of chemotherapy to breast cancer cells. The increase in production of free radicles within breast

cancer cells was observed, indicating that quercetin increases oxidation within breast cancer cells, which plays an important role in supporting Topotecan's toxicity to breast cancer cell lines(Akbas *et al*., 2005)

Treatment with Doxorubicin inflicted considerable damage on the nucleic acid of both cancerous and normal cells.

However, combined treatment with both Doxorubicin and quercetin inflicted damage exclusively on cancer cell nucleic acid, sparing the normal cells, indicating the protective impact of quercetin on healthy cells (Staedler *et al* ., 2011)

The study also involved the impact of various treatments on the inhibition of growth of breast cancer cell lines MCF-7 as determined by the resulting decrease in the density of growing cells and as measured by ELISA at a wavelength of 490 nanometers, following treatments at three concentrations (50, 100 and 200 µg/ml) for all treatments and after an incubation period of 48 and 72 hours, as compared with the control sample.

Treatments undertaken after an incubation period of 48 and 72 hours showed a considerable inhibitory impact on the in-*vitro* growth of cancer cell lines.

Results obtained from this study indicate that these treatments were associated with clear improvement of the inhibitory impact on breast cancer cell lines MCF-7, which is directly proportional to concentrations, posting the best inhibitory impact at a concentration of 200 µg/ml for all treatments and as compared with the control sample.

However, the rate of inhibition was inversely proportional to absorbability and viability.

Also, by comparing the mean appearances of 7breast cancer cell lines MCF-, all treatments posted a highly significant reduction ($P \leq 0.001$) as compared with the control sample and at all concentrations.

Values of IC_{50} and IC_{90} were selected as a measurefor evaluating the most effective concentration of various treatments and comparing with the control sample.

The best value was posted by treatment with the chemotherapeutic agent followed by treatment with Propolis and then by the combined treatment respectively after incubation for 48 hours.

However, the best values for IC_{50} and IC_{90} after incubation for 72 hours were posted by treatment with Propolis followed by treatment with the chemotherapeutic agent and then by the combined treatment respectively.

Through the analysis of (ANOVA) and the LSD comparison test, the previous results showed the highest significant difference $P \leq 0.001$ in the mean appearance of breast cancer cell lines MCF-7 as a result of treatment with Propolis, the chemotherapeutic agent and the combined treatment with Propolis and the chemotherapeutic agent at 50, 100 and 200 µg/ml concentrations after an incubation period of 48 and 72 hours.

Such impact was also reported by previous studies upon treatment with an active component of Propolis e.g. Acacetin.

Acacetin, a Propolis component, was found to impede the growth of 50% (IC_{50})of breast cancer cell lines MCF-7 at 24.4±0.7µm after incubation for more than 24-hours, promoting apoptosis by fragmenting nucleic acid and activating Caspases 7 proteins.

The highest activity of Caspases 7 proteins was observed upon treatment with Acacetin at a concentration of 100µg/ml for a period of 24-hours.

It also limits the expression of Bcl-2 proteins resulting in a corresponding increase in Bax protein and loss of mitochondrial membrane, releasing Cytochrome enzymes and promoting the generation of free radicles within breast cancer cells, ultimately resulting apoptosis (Shim *et al* ., 2007).

Treatment with Apigenin, another Propolis component, markedly inhibits proliferation of breast cancer cells. Such impact is time and dosage-dependent and is associated with an IC_{50} value of 59.44µm at the 24-hour point and 35.15µm at the 72-hour point, resulting in promoted apoptosis, released cytochrome enzymes and activated caspases series (Choi and kim ., 2009)

The fact that Apigenin activates programmed death and self cellular phagocytosis of breast cancer cells indicate that it may play a preventive role (Cao *etal* ., 2013).

The results of this study prove that treatment with Propolis, as well as treatment with a combination of Propolis and Dacarbazine have a therapeutic role to play in growth and proliferation of breast cancer cell lines.

This study, therefore, recommends the administration of Propolis either as an alternative agent or as an adjuvant or complementary agent in anti-cancer treatments.

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