

The Assessment of the Cytotoxic Activity of propolis and dacarbazine against HCT116 cells with the SRB Assay.

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Abstract: A study was conducted on the *in vitro* cytotoxic impact of Propolis, a natural bee product, and Dacarbazine, a standard chemotherapeutic agent used in the treatment of malignant tumors, on colon cancer cell lines (HCT116) at concentrations of 50, 100 and 200 µg /ml after an incubation period of 48/72 hours. Results demonstrated that both treatment with Propolis per se and combined treatment with Dacarbazine exerted a high and almost equally inhibitory impact on colon cancer cell lines as treatment with Dacarbazine per se with a significant difference at $P \leq 0.001$ at all concentrations. The highest percentile inhibitory effect on cancer cell lines after 48 hours of exposure was exhibited at 200 µg/ml concentration, while the highest percentile inhibitory effect on cancer cell lines after 72 hours of exposure was exhibited at 100 µg/ml concentration. Results of this study shed a much needed light on the effective role played by Propolis in the treatment of cancer, through its inhibitory impact on cancer cells.

[Lina Abdul-Fattah Kurdi. **The Assessment of the Cytotoxic Activity of propolis and dacarbazine against HCT116 cells with the SRB Assay.** *Cancer Biology* 2016;6(2):113-129]. ISSN: 2150-1041 (print); ISSN: 2150-105X (online).<http://www.cancerbio.net>. 14. doi:[10.7537/marscbj060216.14](https://doi.org/10.7537/marscbj060216.14).

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

1. Introduction

Colon Cancer is a global health problem and one of the leading causes of death, chemotherapy may be not effective against some cancer cells, and the effectiveness of these drugs may decline due to cancer cells' drug resistance.

That is why researchers focused on using natural compounds as complementary supplements in treating cancer because of the ineffectiveness of the available drugs, or because of its adverse effects

propolis (Bee glue) is a resin formed by bees from leaves' buds and bark's cracks of different trees, more than 300 components had been identified in propolis till now, including polyphenols (Flavonoids + phenolic acid), aldehydes, phenolic aldehydes, ketones and volatile oils, aromatic acids, and some of the essential elements such as magnesium, calcium, sodium, iron, nickel, zinc, copper and some vitamins, as E,C,B1,B2,B6. (Pietta *et al.*, 2002; Elmazoudy *et al.*, 2011; El Sayed and Ahmad., 2012 ;chan *et al.*, 2013)

Many studies inquired treating with propolis as an anticancer from different regions on various cancer cell lines. (Turan *et al.*, 2015)

Propolis has been used widely in medicine long ago, it was found that it has no toxic effect when used in large doses (Burdock,1998),it has an effect in protecting DNA from damage caused by gamma irradiation that is because its ability to get rid of harmful free radicals (Montoro *et al.*, 2005), also it

has an anticancer effect (Ozkul *et al.*, 2005), a positive effect on the immune system against microbes and against the aggravation of tumor growth and, in particular, Natural killer cells concerned in killing cancer cells and preventing cancer cells; division and growth (Sforcin,2007), as well as its effects in lowering cholesterol levels and preventing precipitation of platelets on each other (Martos *et al.*, 2008), and due to propolis' biological characters as an antimicrobial, and its effects as an antioxidant, anticancer and anti-inflammatory, (Ozkul *et al.*, 2005; Watanabe *et al.*, 2011), it has been selected to evaluate the cellular toxic effect of Dacarbazine as one of the chemical drugs on one cancer cell lines in order to demonstrate scientific miracles of the therapeutic ability in this natural material Allah created.

2. Materials & Methods

Cell lines

Colorectal cancer cell lines HCT116 (ATCC®CCL-247™) were obtained from king Fahad research center at king Abdulaziz university.

Dacarbazine(DTIC)

Dacarbazine is chemotherapy used in cancer patients; its trade name is known as DETICENE, obtained from king Abdulaziz hospital in Jeddah.

Propolis

Bee glue, a material collected by bees from leaves' buds and has numerous benefits, and was obtained from Wild Honey Company in Saudi Arabia, Riyadh.

Experimental Design

SRB analysis of cytotoxicity of cells

Colorectal cancer cell lines are used

Divided into four main groups:

1. First group: control untreated group.
2. Second group: treated with propolis (bee glue) at the concentrations (50µg/ml, 100µg/ml, 200µg/ml) (Xuan *et al.*,2014).
3. Third group: treated with Dacarbazine at the concentrations (50µg/ml, 100µg/ml, 200µg/ml).
4. Fourth group: dual-therapy by propolis and Dacarbazine, at the concentrations (50µg/ml, 100µg/ml, 200µg/ml).

The method used in preparing, fixing and dying cancer cells was that of (Houghton *et al.*, 2007) to apply SRB Cells Cytotoxicity Assay.

Rate of growth inhibition was calculated as (IC₅₀) and (IC₉₀) as the following:

$$\text{OD control cells} - (\text{OD treated cells} / (\text{OD control cells} - \text{OD treated cells})) \times 100$$

Statistical Analysis:

Statistical analysis was carried out by applying both Student "t" test and analysis of variance – ANOVA, to calculate significant results obtained from the test under study.

3. Results

The effect of treatment by propolis ,treatment by Dacarbazine , and the dual-therapy by propolis and Dacarbazine calculated at the value (IC₅₀) and (IC₉₀) after 48 hours.

In vitro microscopic examination of colon cancer cell lines HCT116 after being incubated for 48 hours revealed the facility of determination of the morphological changes and apoptosis, in terms of: nuclear fragmentation, morphological changes of the cells and disruption of membranes, the results of different treatments and compared with control sample were calculated (Fig:1), and value of Inhibiting Cellular Proliferation by 50% to kill half of cells (IC₅₀) for each one, treated with propolis, treated with Dacarbazine, and that treated with dual-therapy by propolis and Dacarbazine, and it was 331· 157 · 309µg/ml respectively, while concentration of Inhibiting Cellular Proliferation by 90% of cells (IC₉₀) for different treatments was 731,357, 709 µg/ml respectively (Fig:9).

The effect of treatment by propolis ,treatment by Dacarbazine , and the dual-therapy by propolis and Dacarbazine on median emergence of cancer colon cell lines HCT116 after 48 hours at a concentration 50 µg / ml:

Tabulated results obtained from table 1 revealed that treatment by propolis, treatment by Dacarbazine, and the dual-therapy by Dacarbazine and propolis caused significant ($P \leq 0.001$) decline in median emergence of colon cancer cell lines, its value was (0.297±0.004 , 0.262±0.003 ,0.263±0.004) respectively compared to median of control sample (0.525±0.008).

Where median of that treated with the drug approximately equals that treated with the dual-therapy, while slightly higher when treated with propolis (Fig:7), and when calculating rate of cancer cells growth inhibition HCT116, results of different treatments was at a value of 43.32 % , 50.00%, 49.81% respectively, noting that the highest value recorded was by the drug treatment, then the dual-therapy, then by propolis (Fig:11), and the rate of inhibition is inversely proportional to absorbance and vitality rate, the more the absorbance rate and vitality the less the inhibition (Figs:3&5).

The effect of treatment by propolis ,treatment by Dacarbazine , and the dual-therapy by propolis and Dacarbazine on median emergence of colon cancer cell lines HCT116 after 48 hours at a concentration 100 µg/ml:

Tabulated results obtained from table 1 revealed that treatment by propolis, treatment by Dacarbazine, and the dual-therapy by propolis and Dacarbazine caused significant ($P \leq 0.001$) decline in median emergence of colon cancer cell lines HCT116, its value was (0.288±0.006 , 0.264±0.004 , 0.253±0.005) respectively compared to median of control sample (0.525±0.008), dual therapy showed to be the best in reducing median emergence of HCT116, then treatment with Dacarbazine, then treatment with propolis (Fig: 7), and when calculating rate of cancer cells growth inhibition HCT116, results of different treatments was at a value of 45.04 % , 49.81% ,51.72 % respectively, noting that the highest value recorded was by the dual-therapy, then the treatment by the drug, then by propolis (Fig: 11), and the rate of inhibition is inversely proportional to absorbance and vitality rate, the more the absorbance rate and vitality the less the inhibition (Figs: 3&5).

The effect of treatment by propolis ,treatment by Dacarbazine , and the dual-therapy by propolis and Dacarbazine on median emergence of colon cancer cell lines HCT116 after 48 hours at a concentration 200 µg/ml:

Tabulated results obtained from table 1 revealed that treatment by propolis, treatment by Dacarbazine, and the dual-therapy by propolis and Dacarbazine caused significant ($P \leq 0.001$) decline in median emergence of colon cancer cell lines HCT116, its value was (0.277±0.003 , 0.225±0.006, 0.231±0.005) respectively, compared to median of

control sample (0.525 ± 0.008), treatment with the drug showed to be the best in reducing median emergence of HCT116, then the dual-therapy, then treatment with propolis (Fig: 7), and when calculating rate of cancer cells growth inhibition HCT116, results of different treatments was at a value of $47.33\% \cdot 57.06\% \cdot 55.92\%$ respectively, noting that the highest value recorded was by treatment with the drug, then the dual-therapy, then by propolis (Fig : 11), and the rate of inhibition is inversely proportional to absorbance and vitality rate (Figs: 3&5).

The effect of treatment by propolis ,treatment by Dacarbazine , and the dual-therapy by propolis and Dacarbazine on median emergence of colon cancer cell lines HCT116 after 48 hours at concentration 50,100,200 µg/ml using analysis of variance and least significant difference LSD:

Tabulated results obtained from table 3 revealed significant change ($P \leq 0.001$) in median emergence of colon cancer cell lines HCT116, at different concentrations between treatment with propolis , treatment with Dacarbazine ,and the dual-therapy by propolis and Dacarbazine and its value was ($F = 580.71$) at a concentration $50 \mu\text{g} / \text{ml}$ and was ($F = 462.47$) at a concentration of $100 \mu\text{g} / \text{ml}$, while the value was ($F = 601.20$) at $200 \mu\text{g} / \text{ml}$ concentration compared to the control sample.

least significant difference LSD revealed significant change ($P \leq 0.001$) in median emergence of colon cancer cell lines HCT116 due to treatment with propolis, treatment with Dacarbazine and the dual-therapy by propolis and Dacarbazine at concentrations of 50, 100, 200 µg/ml (Fig : 13). Therefore, arranging treatments in terms of their effects in lowering median emergence of colon cancer cell lines HCT116, at concentrations 50,200 µg / ml as follows:

Drug treatment >dual-therapy > propolis treatment

While arranging treatments in terms of its effect in lowering the median emergence of colon cancer cell lines HCT116, at concentration $100 \mu\text{g}/\text{ml}$ as follows:

Dual-therapy > drug treatment > propolis treatment

The effect of treatment by propolis ,treatment by Dacarbazine , and the dual-therapy by propolis and Dacarbazine on (IC_{50}) and (IC_{90}) after 72 hours:

In vitro microscopic examination of colon cancer cell lines HCT116 after being incubated for 72 hours and determining the morphological effect of different treatments (Fig: 2), apoptosis indicators increased and cancer cell lysis as well, moreover number and vitality of cancer cells decreased compared to morphology of the cells after being incubated for 48 hours and to the control sample, and by calculating the value of Inhibiting Cellular

Proliferation by 50% to kill half of cells (IC_{50}) for each treatment with propolis, treatment with Dacarbazine, and dual-therapy by propolis and Dacarbazine, was $135.50, 115 \cdot 224 \mu\text{g}/\text{ml}$ respectively, while the value of the concentration for killing 90% of cells (IC_{90}) for different treatments $335.50, 315, 624.00 \mu\text{g}/\text{ml}$ respectively (fig:10).

The effect of treatment by propolis ,treatment by Dacarbazine , and the dual-therapy by propolis and Dacarbazine on median emergence of colon cancer cell lines HCT116 after 72 hours at concentration 50 µg/ml:

Tabulated results obtained from table 2 revealed that treatment by propolis, treatment by Dacarbazine, and the dual-therapy by propolis and Dacarbazine caused significant ($P \leq 0.001$) decline in median emergence of colon cancer cell lines HCT116, its value was ($0.349 \pm 0.006, 0.273 \pm 0.005, 0.266 \pm 0.008$) respectively, compared to median of control sample (0.811 ± 0.012) (Fig : 8), and when calculating rate of cancer cells growth inhibition HCT116, results of different treatments was at a value $57.09\% \cdot 66.34\% \cdot 67.20\%$ respectively, noting that the highest value of recorded was by the dual-therapy, then the drug treatment, then by propolis (Fig: 12), and the rate of inhibition is inversely proportional to absorbance and vitality rate, the more the absorbance rate and vitality the less the inhibition (Figs: 4&6).

The effect of treatment by propolis ,treatment by Dacarbazine , and the dual-therapy by propolis and Dacarbazine on median emergence of colon cancer cell lines HCT116 after 72 hours at concentration 100 µg/ml:

Tabulated results obtained from table 2 revealed that treatment by propolis, treatment by Dacarbazine, and the dual-therapy by propolis and Dacarbazine caused significant ($P \leq 0.001$) decline in median emergence of colon cancer cell lines HCT116, its value was ($0.313 \pm 0.008, 0.245 \pm 0.006, 0.247 \pm 0.007$) respectively, compared to median of control sample (0.811 ± 0.012), treatment with the drug showed to be the best in reducing median emergence of colon cancer cell lines HCT116 then the dual-therapy then treatment with propolis, and when calculating rate of cancer cells growth inhibition HCT116, results of different treatments was at a value of $61.53\% , 69.91\% , 69.67\%$ noting that the value recorded by the dual-therapy was equal to that by drug treatment, then by propolis (Fig : 12), and the rate of inhibition is inversely proportional to absorbance and vitality rate (Figs: 4&6).

The effect of treatment by propolis ,treatment by Dacarbazine , and the dual-therapy by propolis and Dacarbazine on median emergence of colon cancer cell lines HCT116 after 72 hours at concentration 200 µg/ml:

Tabulated results obtained from table 2 revealed that treatment by propolis, treatment by Dacarbazine, and the dual-therapy by propolis and Dacarbazine caused significant ($P \leq 0.001$) decline in median emergence of colon cancer cell lines HCT116, its value was (0.311 ± 0.014 , 0.261 ± 0.015 , 0.273 ± 0.016) respectively, compared to median of control sample (0.811 ± 0.012), treatment with Dacarbazine showed to be the best in reducing median emergence of colon cancer cell lines HCT116, the the dual-therapy then treatment with propolis (fig: 8), and when calculating rate of cancer cells growth inhibition HCT116, results of different treatments was at a value of 61.65 % , 67.82%, 66.34% respectively, noting that the value recorded by the dual-therapy was close to that by treatment with the drug then that by treatment with propolis, (Fig : 12), and the rate of inhibition is inversely proportional to absorbance and vitality rate (Figs: 4&6).

The effect of treatment by propolis ,treatment by Dacarbazine , and the dual-therapy by propolis and Dacarbazine on median emergence of colon cancer cell lines HCT116 after 72 hours at concentration 50,100,200 $\mu\text{g}/\text{ml}$ using analysis of variance and least significant difference LSD:

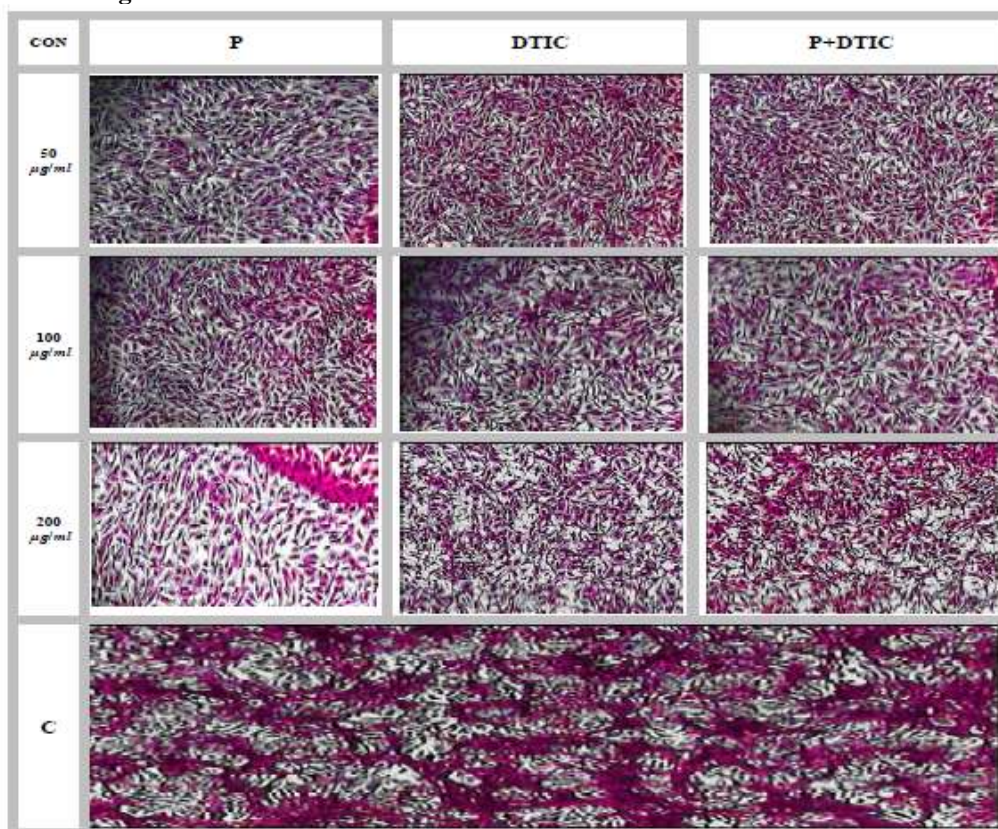
Tabulated results obtained from table 4 revealed significant change ($P \leq 0.001$) in median emergence of colon cancer cell lines HCT116, at different concentrations between treatment with propolis, treatment with Dacarbazine, and the dual-therapy by propolis and Dacarbazine and its value was ($F = 1007.92$) at a concentration 50 $\mu\text{g}/\text{ml}$ and was ($F = 1001.98$) at a concentration of 100 $\mu\text{g}/\text{ml}$, while the value was ($F = 340.55$) at 200 $\mu\text{g}/\text{ml}$ concentration compared to the control sample.

least significant difference LSD revealed significant change ($P \leq 0.001$) in median emergence of cancer colon cell lines HCT116 due to treatment with propolis, treatment with Dacarbazine and the dual-therapy by propolis and Dacarbazine at concentrations of 50, 100, 200 $\mu\text{g}/\text{ml}$ (Fig :14). Therefore, arranging treatments in terms of their effects in lowering median emergence of cancer colon cell lines HCT116, at concentrations 100, 200 $\mu\text{g}/\text{ml}$ as follows:

drug treatment > dual-therapy > propolis treatment

While arranging treatments in terms of its effect in lowering the median emergence of colon cancer cell lines HCT116, at concentration 50 $\mu\text{g}/\text{ml}$ as follows:

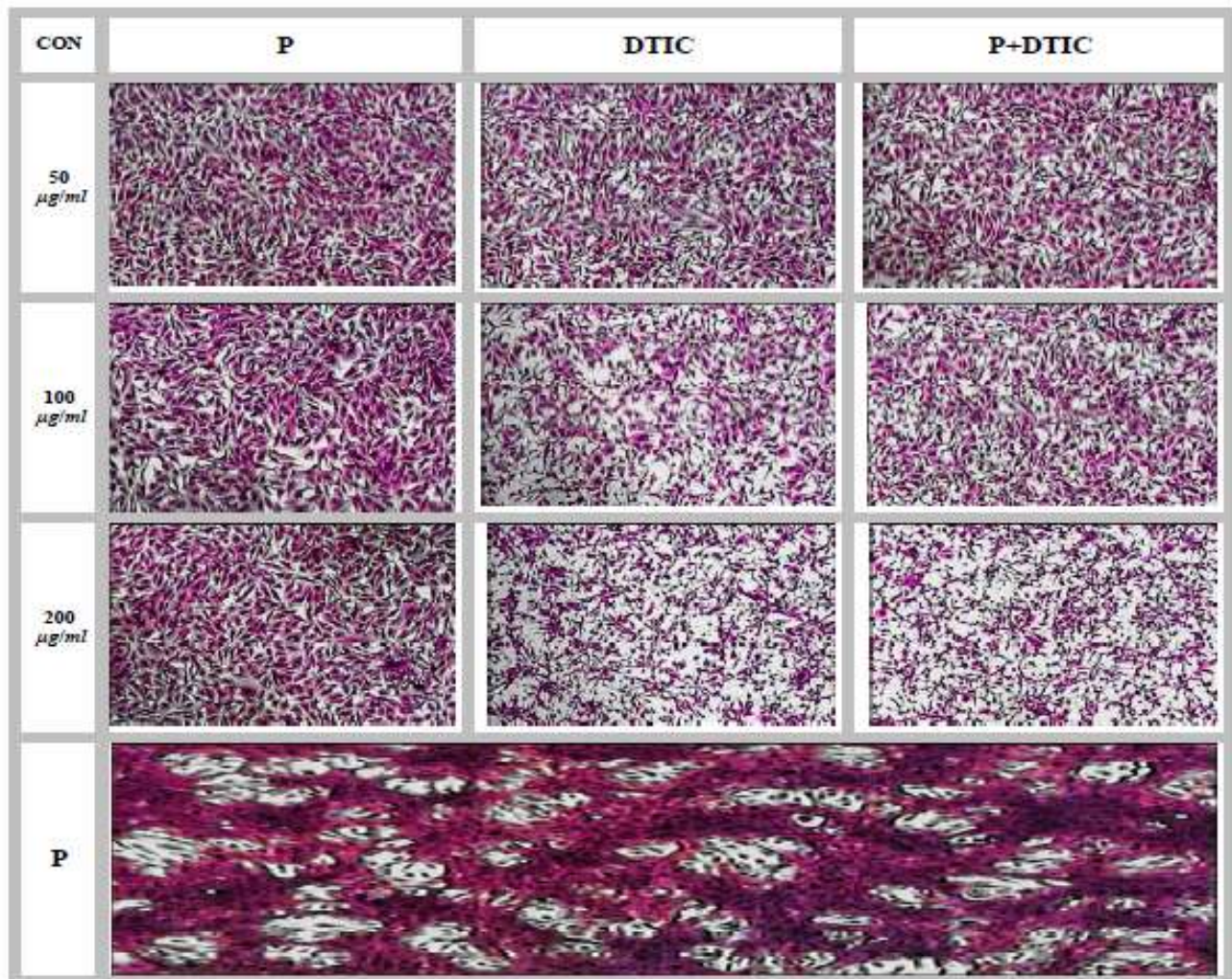
Dual-therapy > drug treatment > propolis treatment



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

1: concentration of 50($\mu\text{g}/\text{ml}$), 2: concentration of 100($\mu\text{g}/\text{ml}$), 3: concentration of 2000($\mu\text{g}/\text{ml}$)

Fig (1) : Morphological and cytological features of Colon cancer cells of the lines HCT116 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($\mu\text{g/ml}$), 2: concentration of 100($\mu\text{g/ml}$), 3: concentration of 2000($\mu\text{g/ml}$)

Fig (2): Morphological and cytological features of Colon cancer cells of the lines HCT116 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 Colon cancer cells of the lines HCT116 after 48 h .

Con. (ug/ml)	Groups Treatment	No. cell line	Mean \pm Std.Error	Absorbance	Survival Fraction (SF)	% Inhibition
50	C	1 2 3 4 5 6 Mean \pm Std.Error	0.525 \pm 0.008	0.524	1	
	P	1 2 3 4 5 6 Mean \pm Std.Error	0.297 \pm 0.004 *** ^a	0.297	0.567	43.321
	D	1 2 3 4 5 6 Mean \pm Std.Error	0.262 \pm 0.003 *** ^a	0.262	0.500	50.000
	P+D	1 2 3 4 5 6 Mean \pm Std.Error	0.263 \pm 0.004 *** ^a	0.263	0.502	49.809
100	C	1 2 3 4 5 6 Mean \pm Std.Error	0.525 \pm 0.008	0.524	1	
	P	1 2 3 4 5 6 Mean \pm Std.Error	0.288 \pm 0.006 *** ^a	0.288	0.550	45.038
	D	1 2 3 4 5 6 Mean \pm Std.Error	0.264 \pm 0.004 *** ^a	0.263	0.502	49.809
	P+D	1 2 3 4 5 6 Mean \pm Std.Error	0.253 \pm 0.005 *** ^a	0.253	0.483	51.718
200	C	1 2 3 4 5 6 Mean \pm Std.Error	0.525 \pm 0.008	0.524	1	
	P	1 2 3 4 5 6 Mean \pm Std.Error	0.277 \pm 0.003 *** ^a	0.276	0.527	47.328
	D	1 2 3 4 5 6 Mean \pm Std.Error	0.225 \pm 0.006 *** ^a	0.225	0.429	57.061
	P+D	1 2 3 4 5 6 Mean \pm Std.Error	0.231 \pm 0.005 *** ^a	0.231	0.441	55.916

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*** extremely 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 Colon cancer cells of the lines HCT116 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	0.811 ± 0.012	0.811	1	
50	P	1				
		2				
		3				
		4				
		5				
		6				
		Mean ± Std.Error	0.349 ± 0.006	0.348	0.429	57.090
50	D	1				
		2				
		3				
		4				
		5				
		6				
		Mean ± Std.Error	0.273 ± 0.005	0.273	0.337	66.338
50	P+D	1				
		2				
		3				
		4				
		5				
		6				
		Mean ± Std.Error	0.266 ± 0.008	0.266	0.328	67.201
100	C	1				
		2				
		3				
		4				
		5				
		6				
		Mean ± Std.Error	0.811 ± 0.012	0.811	1	
100	P	1				
		2				
		3				
		4				
		5				
		6				
		Mean ± Std.Error	0.313 ± 0.008	0.312	0.385	61.529
100	D	1				
		2				
		3				
		4				
		5				
		6				
		Mean ± Std.Error	0.245 ± 0.006	0.244	0.301	69.914
100	P+D	1				
		2				
		3				
		4				
		5				
		6				
		Mean ± Std.Error	0.247 ± 0.007	0.246	0.303	69.667
200	C	1				
		2				
		3				
		4				
		5				
		6				
		Mean ± Std.Error	0.811 ± 0.012	0.811	1	
200	P	1				
		2				
		3				
		4				
		5				
		6				
		Mean ± Std.Error	0.311 ± 0.014	0.311	0.383	61.652
200	D	1				
		2				
		3				
		4				
		5				
		6				
		Mean ± Std.Error	0.261 ± 0.015	0.261	0.322	67.818
200	P+D	1				
		2				
		3				
		4				
		5				
		6				
		Mean ± Std.Error	0.273 ± 0.016	0.273	0.337	66.338

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*** extremely 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 Colon cancer cells of the lines HCT116 after 48 h .

		(ANOVA)		(LSD)		
		(F)	(Sig)	Groups Treatment	Mean Difference	(Sig)
50	Control (C)	580.710	***	P	0.228	***
				D	0.263	***
				P+D	0.262	***
100	Control (C)	462.466	***	P	0.237	***
				D	0.261	***
				P+D	0.272	***
200	Control (C)	601.204	***	P	0.248	***
				D	0.300	***
				P+D	0.294	***

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

p* significant<0.05

p** highly significant<0.01

p*** extremely 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 Colon cancer cells of the lines HCT116 after 72 h .

		(ANOVA)		(LSD)		
		(F)	(Sig)	Groups Treatment	Mean Difference	(Sig)
50	Control (C)	1007.918	***	P	0.463	***
				D	0.538	***
				P+D	0.545	***
100	Control (C)	1001.977	***	P	0.499	***
				D	0.567	***
				P+D	0.565	***
200	Control (C)	340.547	***	P	0.501	***
				D	0.550	***
				P+D	0.539	***

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

p* significant<0.05

p** highly significant<0.01

p*** extremely significant<0.001

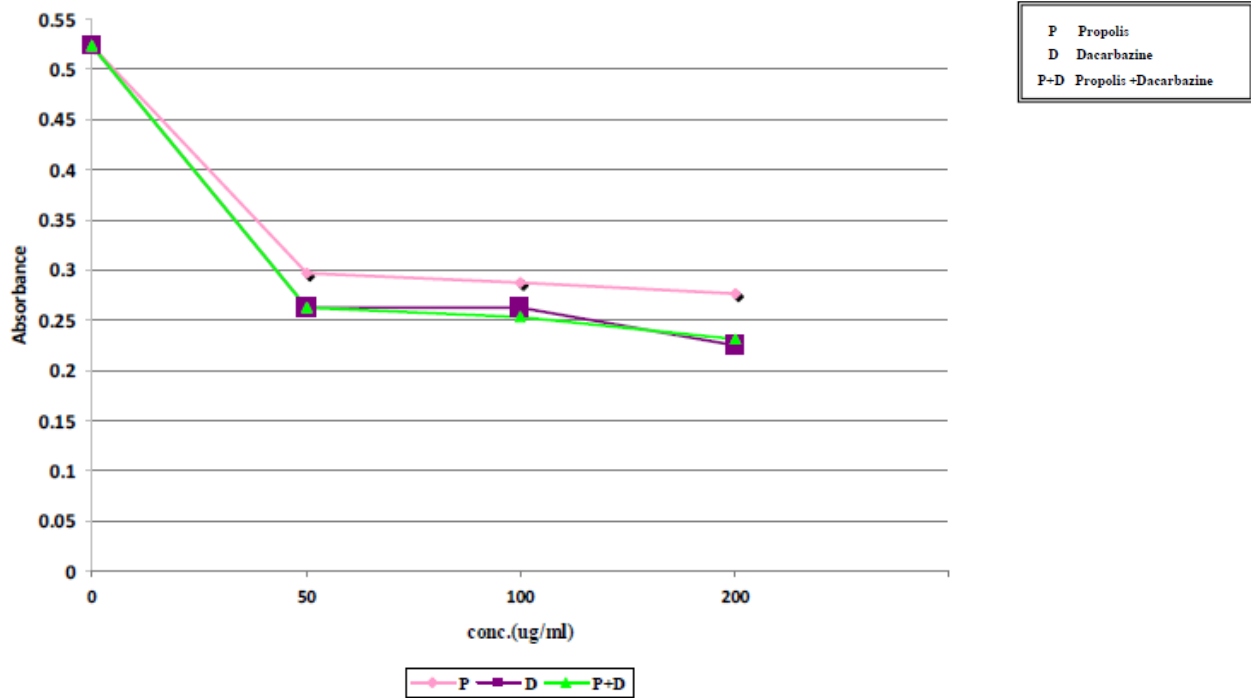


Fig (3) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Absorbance values of Colon cancer cells of the lines HCT116 after 48 h .

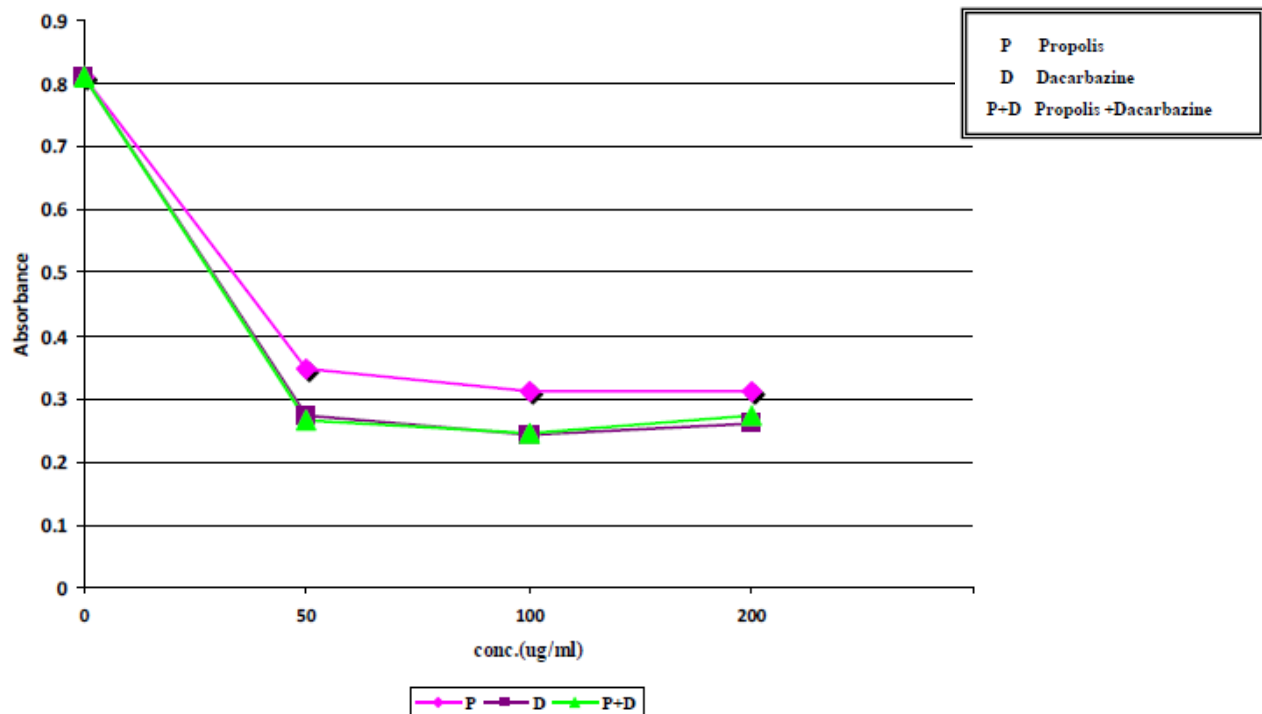


Fig (4) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Absorbance values of Colon cancer cells of the lines HCT116 after 72 h .

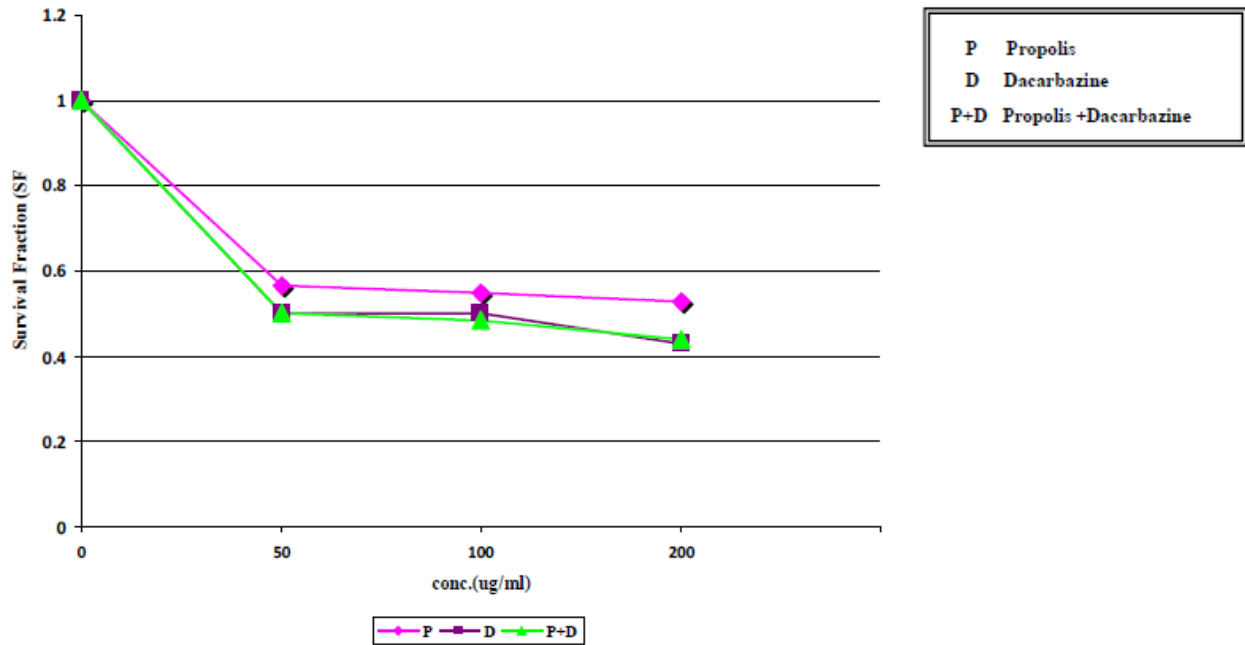


Fig (5) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Survival Fraction (SF) values of Colon cancer cells of the lines HCT116 after 48 h .

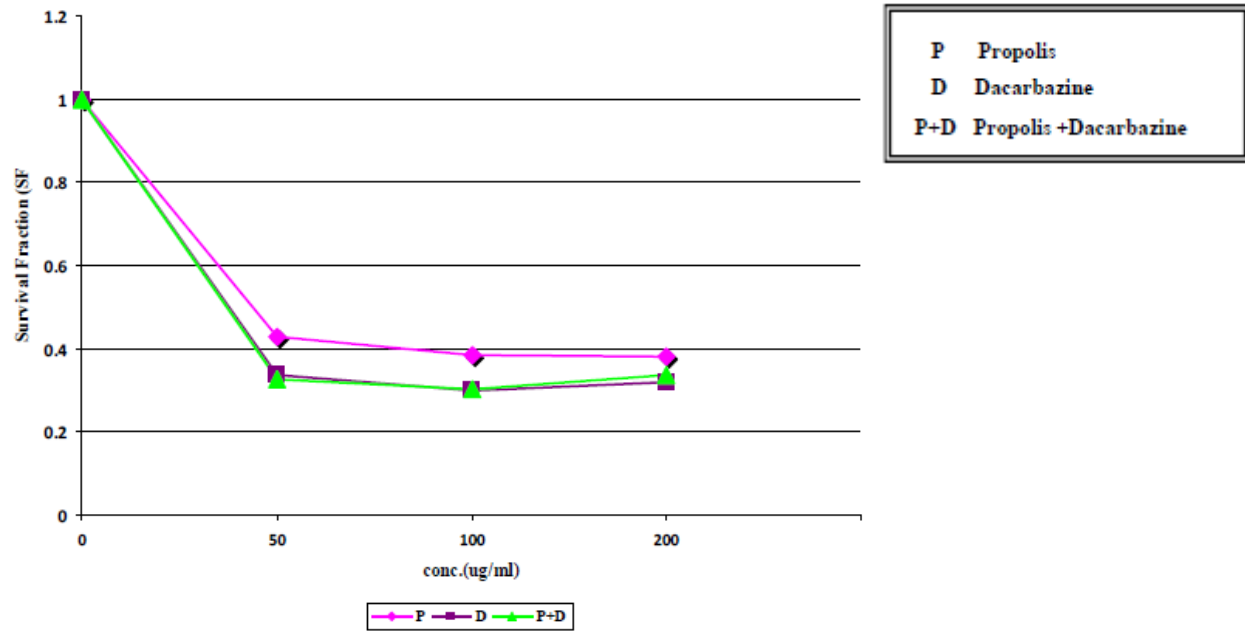


Fig (6) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Survival Fraction (SF) values of Colon cancer cells of the lines HCT116 after 72 h .

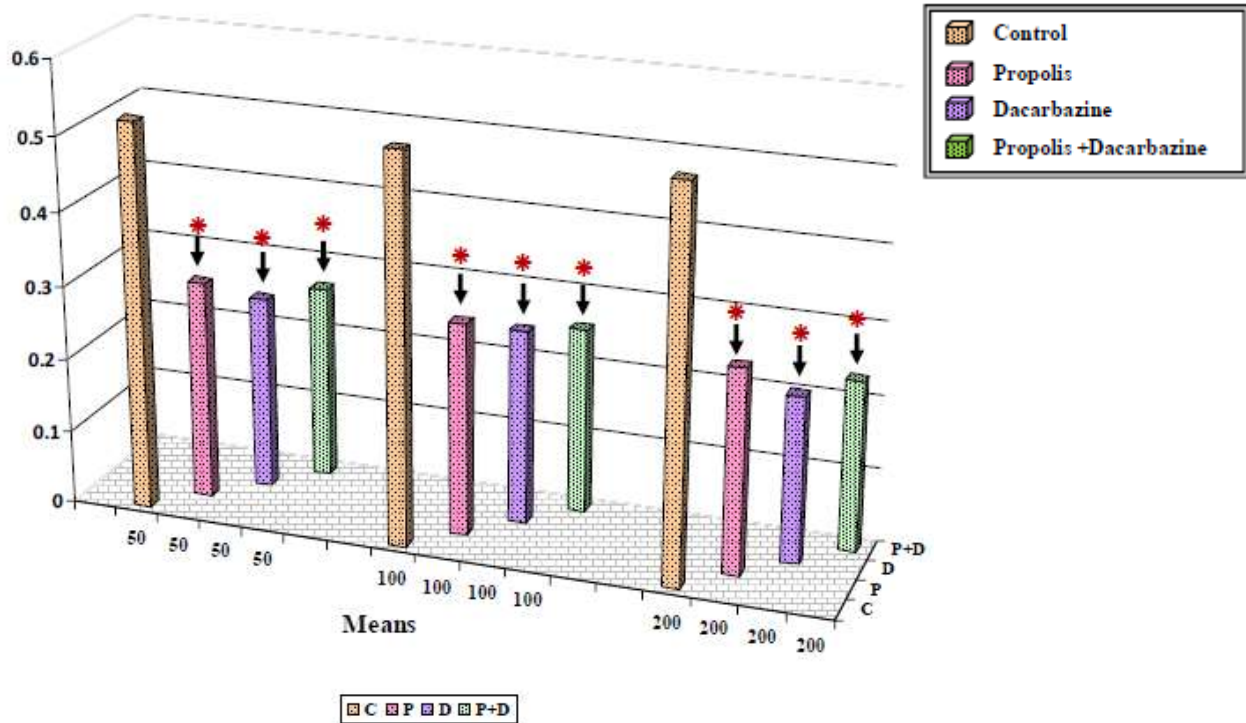


Fig (7) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Means of Colon cancer cells of the lines HCT116 after 48 h .

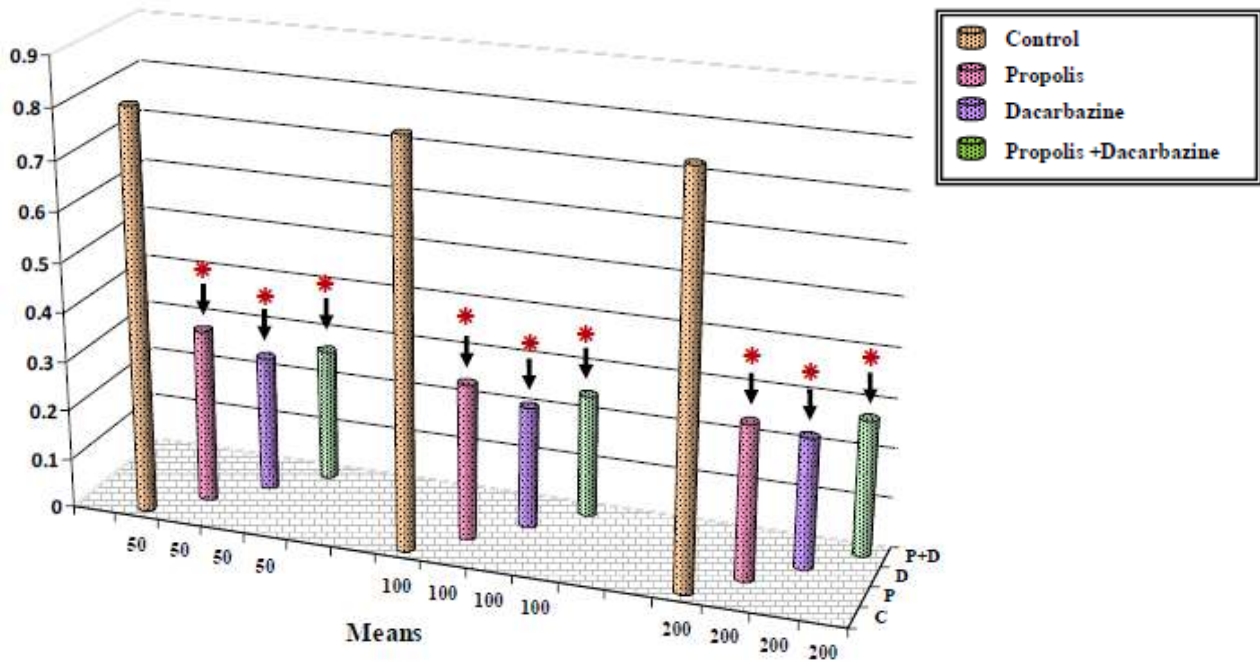


Fig (8) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Means of Colon cancer cells of the lines HCT116 after 72 h .

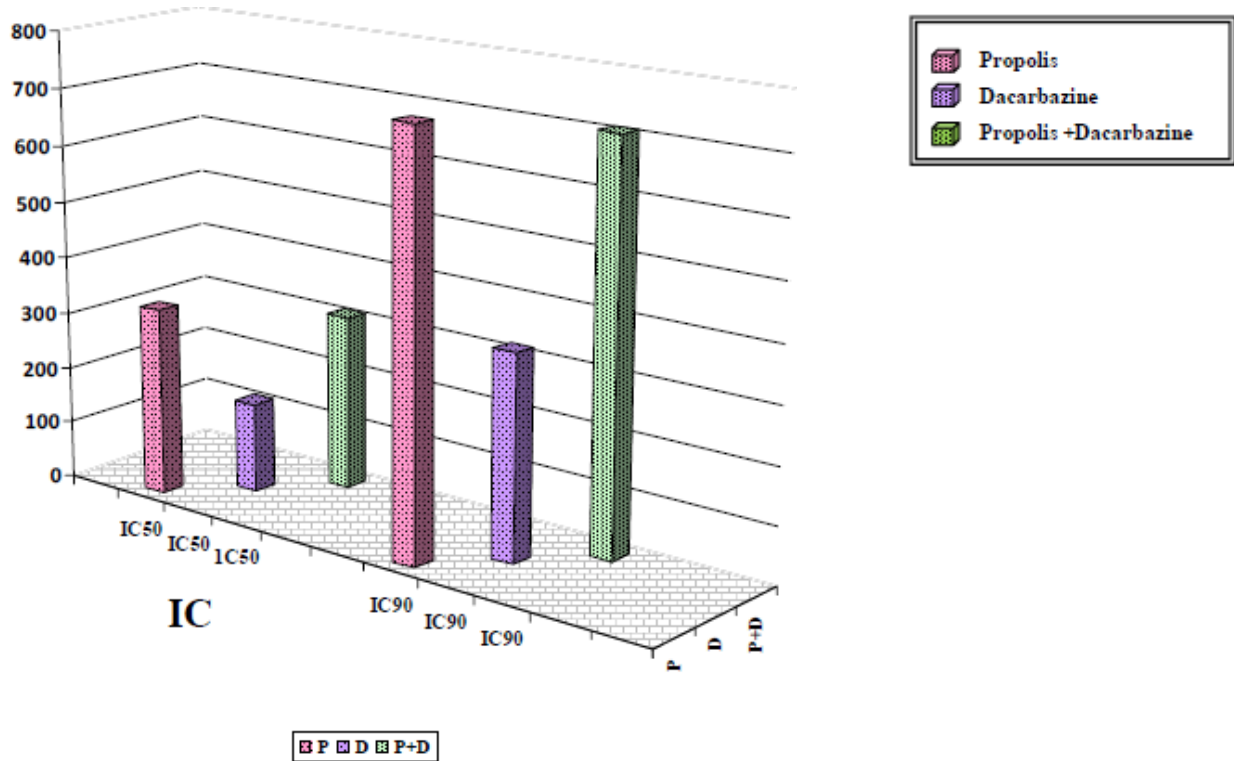


Fig (9) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the IC₅₀ and IC₉₀ values of Colon cancer cells of the lines HCT116 after 48 h .

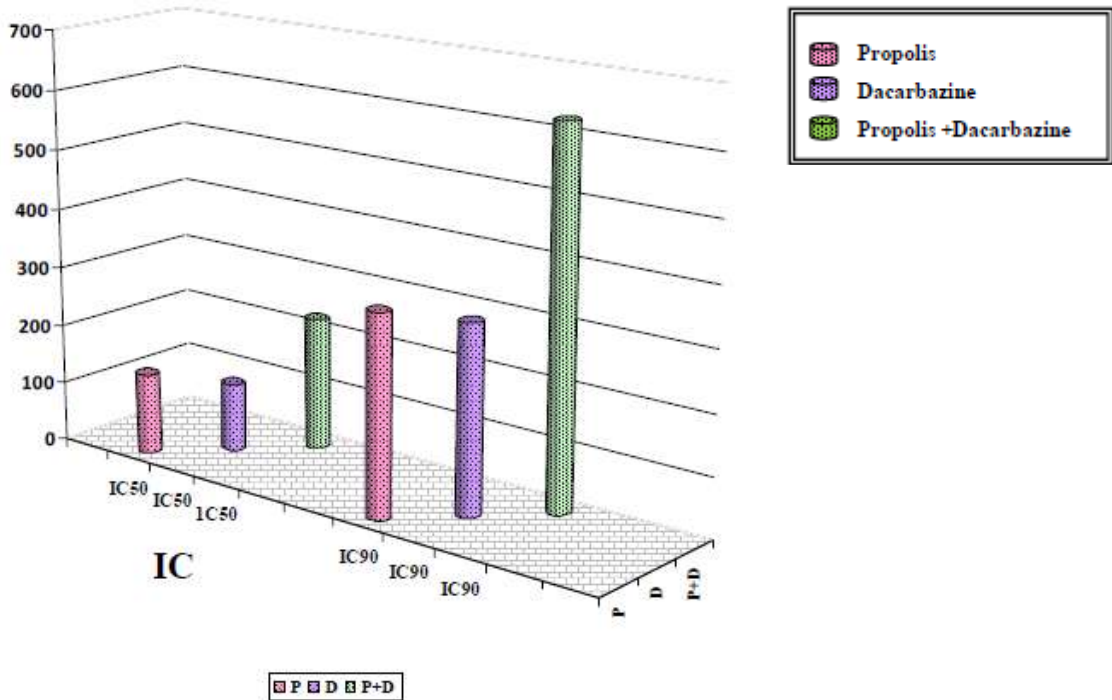


Fig (10) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the IC₅₀ and IC₉₀ values of Colon cancer cells of the lines HCT116 after 72 h .

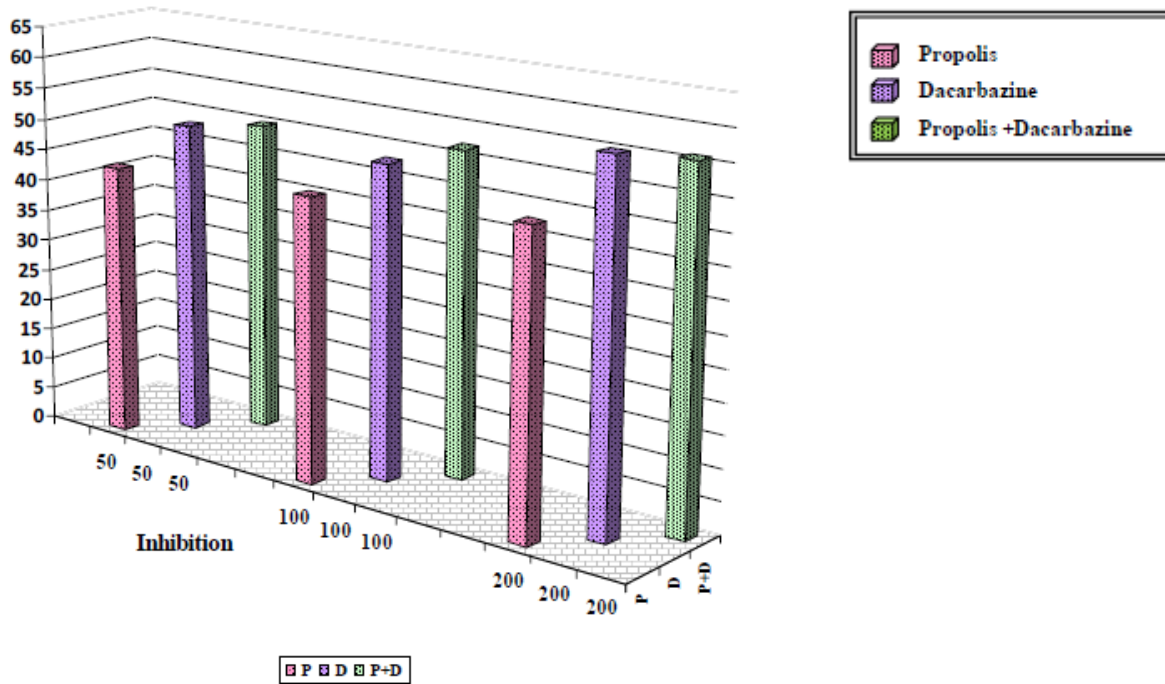


Fig (11) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Inhibition rate of Colon cancer cells of the lines HCT 116 after 48 h .

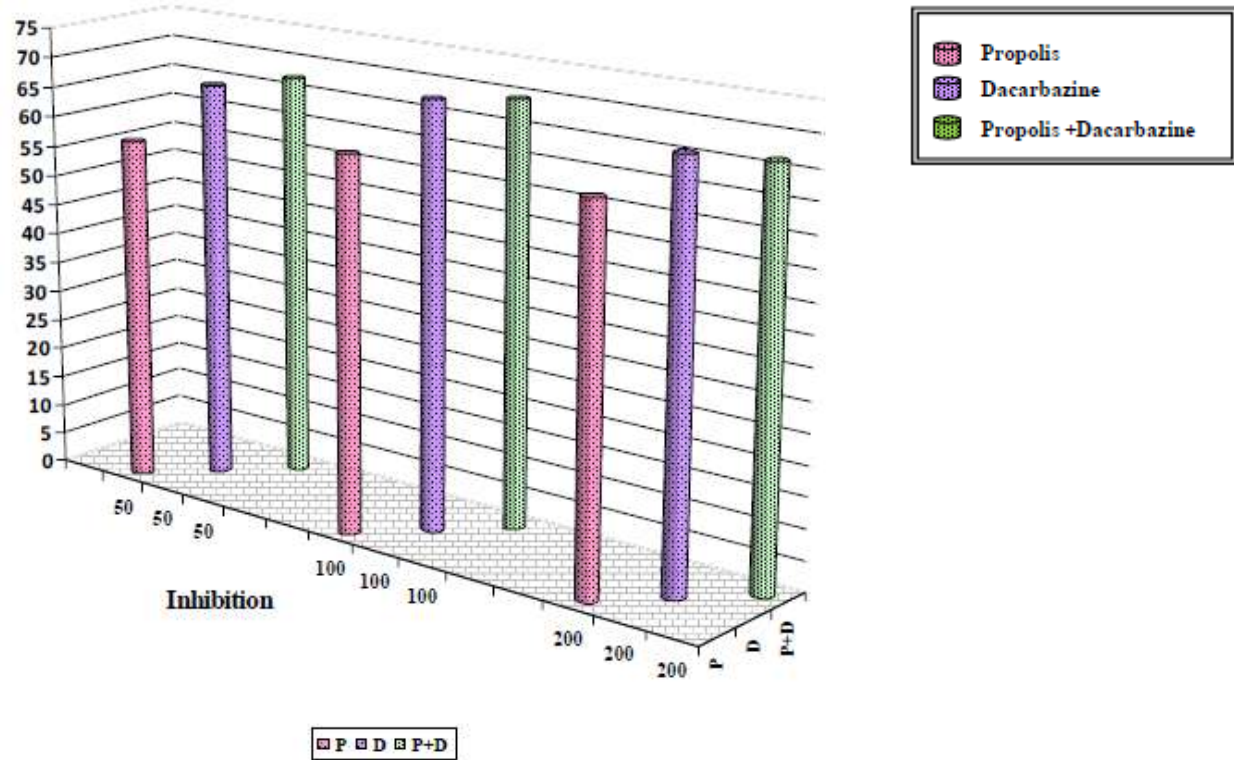


Fig (12) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Inhibition rate of Colon cancer cells of the lines HCT 116 after 72 h .

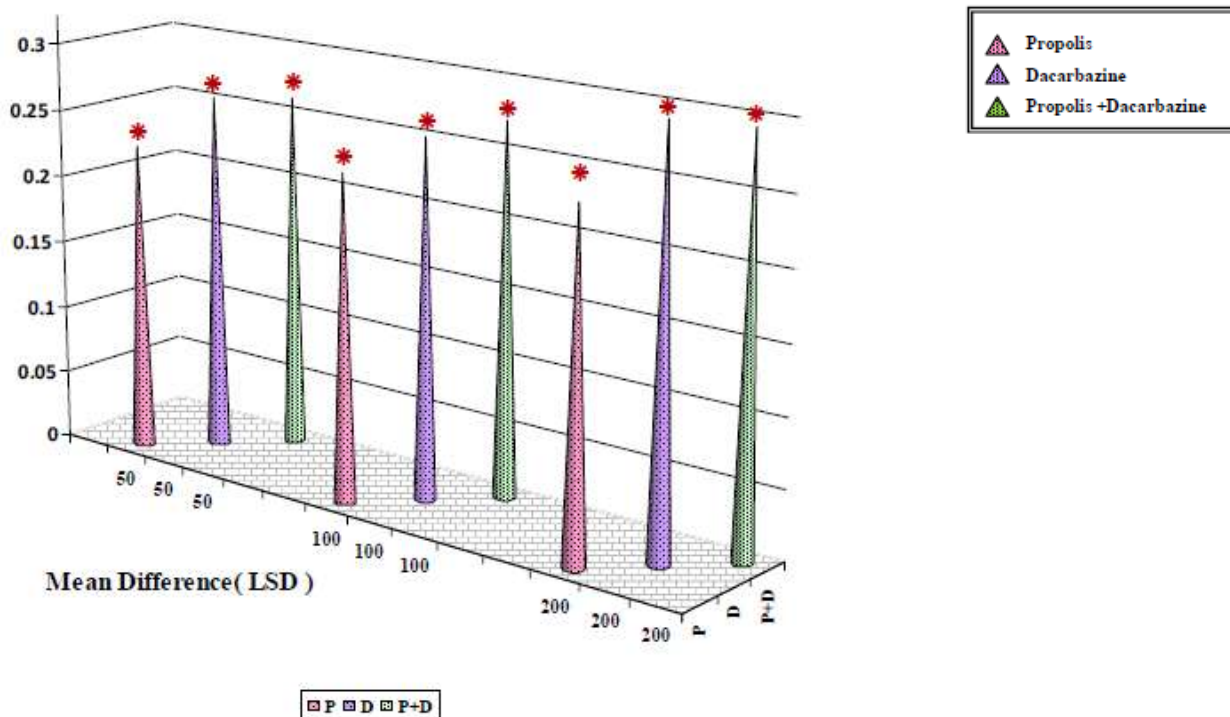


Fig (13): Comparison between The Effect of Treatment of Propolis, Dacarbazine and TheDual Treatment with Propolis and Dacarbazine on the mean of Colon cancer cells of the lines HCT 116 after 48 h .

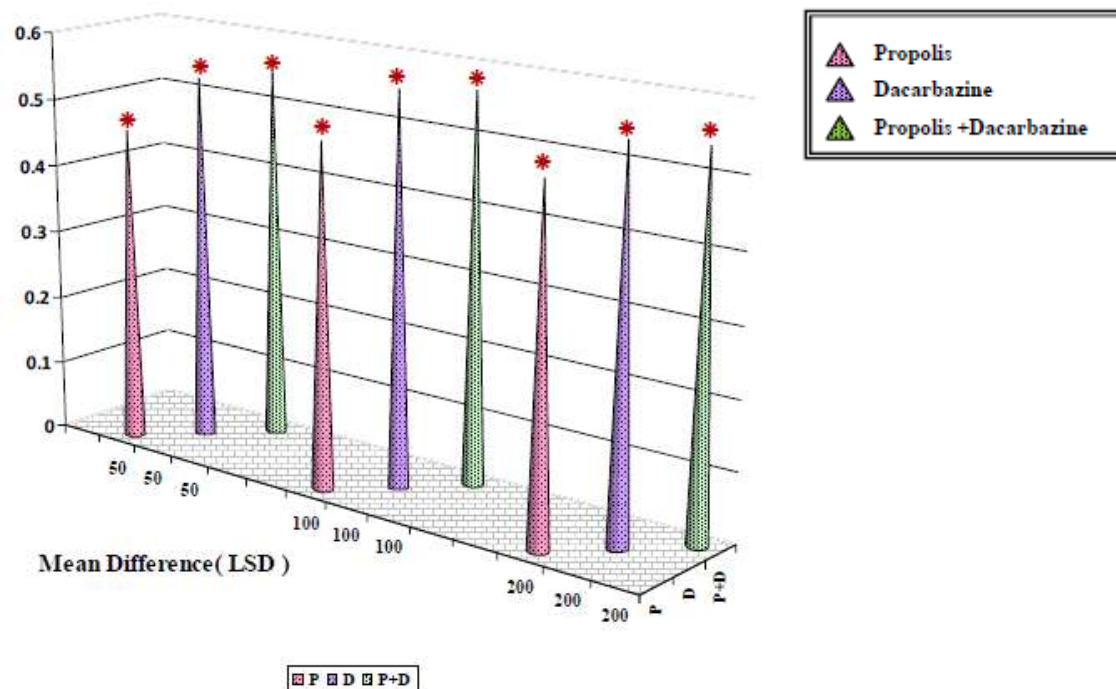


Fig (14): Comparison between The Effect of Treatment of Propolis, Dacarbazine and The Dual Treatment with Propolis and Dacarbazine on the mean of Colon cancer cells of the lines HCT 116 after 72 h .

4. Discussion

This study aimed to evaluate the resulted cellular effects of treatment by Dacarbazine as one of the used chemotherapy in treatment of cancer on

Colon cancer cell lines HCT116 using propolis compound as a bee product who has natural and therapeutic properties, treatment results were tested at

3 different concentrations (50,100,200 µg/ml), and after being incubated for (48,72h).

Microscopic morphological examination revealed the cellular effects of the different treatments at different concentrations (50,100,200 µg/ml), after being incubated for (48,72h), as apoptosis was shown clearly with all treatments and at different concentrations, compared to the control sample, and this effect depended on concentrations and duration.

By comparing the resulted morphological effects of treatments, it was obvious that results of the dual-therapy by the drug and propolis were so close to that of treatment with the drug alone on colon cancer cells HCT116. There was a linear relation between effect with concentrations and duration. Also, these treatments showed obvious effect after being incubated for (48,72h) in the rate of cancer cells growth inhibition HCT116 *in vitro*. The tabulated results indicated that the inhibitory effect to colon cancer cells HCT116 with those treatments had recorded an obvious improvement, was gradually increasing and showed significant improvement with higher concentrations, and the rate of inhibition is inversely proportional to absorbance and vitality rate, all treatments recorded caused highly significant decline in median emergence of colon cancer cell lines HCT116 ($P \leq 0.001$), compared to the control sample at all different concentrations, in order to demonstrate the effective concentration of the treatments and comparing it with the control (IC_{50}) and (IC_{90}) were counted on, the best recorded result was by treatment with the drug then the dual-therapy the treatment with propolis after being incubated for 48 hours, while the best value after being incubated for 72 hours was recorded by the treatment with the drug the treatment by propolis then the dual-therapy respectively, and such resulted are consistent with what many former researchers got as a result of treatment of colon cancer cells HCT116 with propolis compound or one of its active components.

Treating cultured cells with propolis shower significant decline in cancer cells' growth in cancer blood and colon reaching $P \leq 0.05$ compared to control cells and depending on the dose and the incubation duration, to evaluate whether the toxic effects was related to apoptosis, morphological changes were examined after 24 incubation of cancer cells with propolis at a dose of 25 mg/kg where signs of apoptosis such as nuclear condensation, enhanced DNA fragmentation, increased cell size dramatically, and inflated cytoplasm, loss of membrane integrity and cell rupture and exit of their contents were observed. (Sulaiman *et al.*, 2012)

Several studies and laboratory reports recently proved that flavonoid compounds as one of

propolis components, have a great importance in biological activities as an antioxidant, anti-inflammatory, and anticancer, also prevent and inhibit the transcription factor, as well as induce apoptosis by stopping the cell cycle, and increase activation of caspase series proteins (caspase-3 and -9) (mitochondrial pathway) in cancer cells which reduces mitochondrial membrane permeability and kills various types of cancer cells without affecting normal cells as in colon cancer. (Xiang *et al.*, 2006; Wu *et al.*, 2011; Ha *et al.*, 2013; Zizic *et al.*, 2013; Kumazaki *et al.*, 2014; Catchpole *et al.*, 2015; Kubina *et al.*, 2015)

Apoptosis is considered an important phenomenon in chemotherapy factors and that resulted from killing of cancer cell, so inducing apoptosis is one of the proposed mechanisms of propolis therapeutic effects (Benguedouar *et al.*, 2008; Bufalo *et al.*, 2009). Study results concerned with the potential mechanism of apoptosis in treatment with propolis reported that it causes DNA fragmentations, and stimulates caspases in cancer cells, caspase belongs to protease family of cysteine amino acid which is called executioner proteins due to its role in apoptosis, because once activated caspase 8,9 attach and activate caspase 7,3, then attach to nuclear protein stimulating cells to apoptosis (Vatansever *et al.*, 2010; Olsson and Zhivotorsky., 2011).

Suzuki *et al.* (2002) and Chen *et al.* (2003) stated that propolis has an effective role in inhibiting cancer in a variety of cancer cell lines by its phenolic compounds, polyphenol compounds and flavonoids in propolis act as contributing effective and complementary factor to chemotherapy and radiotherapy in treating cancer.

The epidemiological indicators and pre-clinical evidences noted that the polyphenol compounds and phytochemicals in propolis have preventive chemical properties from cancer, which increases emphasis about preventive strategies from cancer in which propolis is used as a rich source of phenol and poly phenol compounds (Orsolich *et al.*, 2007).

Flavonoid compounds in propolis affect nuclear proliferation and apoptosis in cancer cells and can play an important role in chemoprophylaxis against cancer, its anticancer ability may be due to inhibiting DNA synthesis in cancer cells, ability to stimulate apoptosis, activate the phagocytosis process for the production of factors capable of Regulating active chemoprophylaxis in animal models and in cancer cell cultures, high doses of flavonoids are able to reduce DNA oxidative damage, and delay the growth of cancer cells, and hinder cellular signals transfer and cancer cell differentiation, cytogenetic

studies have proved that flavonoids cause genetic instability and poor cancer cell growth by up to 50% (Noel *et al.* ,2006; Engen ., 2007; Orsollae *et al.* , 2007).

Szliszka *et al.* (2011) stated that artepilin C compound found in propolis increases cancer cell sensitivity to protein (TRAIL), this protein is considered as a strong stimulant to apoptosis in cancer cells and an important factor responsible for eliminating growing tumors.

From the foregoing potential therapeutic cellular effects and anticancer effects of propolis and its components can be summarized:

- 1) Stimulation of apoptosis.
- 2) Inhibition of cellular growth and division by blocking cancer cell cycle.
- 3) Stimulation of –proteases- caspases chain.
- 4) Regulation of protein P53 levels which is suppressor for tumors.
- 5) Activation of self cellular phagocytosis in cancer cells.
- 6) Stimulation of genetic instability of tumors.
- 7) Increasing of Bax protein that stimulates for apoptosis and reducing Bcl-2 protein that inhibits apoptosis.
- 8) Increasing cancer cell sensitivity to protein (TRAIL), a strong stimulant to apoptosis in cancer cells.
- 9) A contributing factor or complementary treatment for anti-cancer therapies.

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