

## ***In vitro* Study of Inhibition Effect of Propolis on Growth of Hepatocellular carcinoma cell lines (HepG 2)**

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**Abstract:** This study was primarily concerned with gauging the *in-vitro* inhibitory effect of Propolis on the growth of liver cancer cell lines, compared to the standard anti-neoplastic agent, Dacarbazine. The results of treatment with three (3) concentrations of Propolis (50,100 and 200 µg /ml), treatment with Dacarbazine and combined treatment with Propolis and Dacarbazine demonstrate the morphological closeness of the inhibitory impact exerted by each treatment on the growth of liver cancer cell lines (HepG2), after incubation for 48 and 72 hours. Comparison of the inhibitory impact of various treatments demonstrated remarkable, concentration-proportional improvements as a result of the treatments under study. Upon calculation of the lethal inhibitory concentration that inhibits 50% of the cells (IC<sub>50</sub>) and 90% of the cells (IC<sub>90</sub>), the best result was posted by treatment with Dacarbazine, followed by the combined treatment with both Dacarbazine and Propolis, followed by treatment with Propolis per se, respectively. Based on the foregoing, we propose to use Propolis in conjunction with chemotherapeutic agents in the treatment of cancer patients, being a strong anti-neoplastic agent and because of the *in-vitro* prophylactic impact exerted by its components, which is attributable to its anti-oxidant and anti-neoplastic properties.

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**Key Words:** Propolis, Dacarbazine, Liver cancer cell lines, Apoptosis

### **1. Introduction**

#### **Liver cancer or Hepatocellular carcinoma (HCC):**

HCC is one of the most common tumors, represents the fifth common tumor in the world and the third cause of death among cancers.

The estimated number of cases per year is about 600000 case 55% of them are only in china, total average age for patients with hepatocellular carcinoma is about five years for 8.9% of them, At present the treatment of hepatocellular carcinoma is represented by surgery and chemotherapy. But the therapeutic effects of the currently used chemotherapy drugs including HCC drugs are not very effective with the appearance of many side effects.

So it was important to find new strategies and more efficient antitumor drugs to treat hepatocellular carcinoma. It was found that chemoprophylaxis and using natural or artificial chemicals to suppress and prevent cancer is considered as an important means to face hepatocellular carcinoma, as many natural chemicals have protective properties against diseases.

(Stagos *et al.*,2012; Lee *et al.* , 2015).

Dietary flavonoids have been associated with decreasing the risk of cancer diseases, including Hepatocellular carcinoma (HCC).

When studying the effect of Quercetin compound on stimulating apoptosis (programmed cell death) in Hepatocellular carcinoma, it was

observed after 18 hours of incubation after treatment that the incidence of apoptosis depends on dose and time, as it stops the cell cycle in the stage of DNA replication (S-Phase), in addition to its powerful ability to inhibit topoisomerase I enzyme in DNA, without causing any negative impact on normal cells indicating that Quercetin compound is considered as antagonist for Hepatocellular carcinoma, has anti-proliferative activities and antioxidant (Granado *et al.*, 2006; Wang *et al.* , 2012 ; Casella *et al.* , 2014 Sudan and Rupasinghe ., 2014).

And as the propolis is a Bee glue collected by bees from leaves buds and cracks of the bark from different plants, which composed of 50% of flavonoids linked with phenolic acids, 30% wax, 10% essential oils, 5% pollen grains and 5% different organic compounds.( Pietta *et al.*,2002).

It has been used widely in medicine since ancient times as an antioxidant and anti-inflammatory, and strengthens the immune and has anti-cancer activity of different parts at different cancer cell lines. (Ozkul *et al.*, 2005; Watanabe *et al.*,2011 ;Turan *et al.*,2015).

So propolis compound was selected to estimate the cytotoxic effect of Dacarbazine drug as one of the chemical treatments that shows the scientific miracles in the therapeutic capability set by God Almighty in one of bees' products.

## 2. Materials & Methods

### Cell lines:

Hepatocellular carcinoma cell lines HepG 2 (ATCC®HB-8065TM) were obtained from king Fahad research center at king Abdul-Aziz University.

### Dacarbazine(DTIC)

Dacarbazine is a drug used in chemotherapy for the treatment of cancer patients and known commercially as DETICENE, it was obtained from King Abdul-Aziz hospital in Jeddah, And purchased from (Medac, Germany).

### Propolis:

Bee glue (propolis) is a substance collected by bees from the buds of trees, it has multiple benefits and was obtained from Wild honey company Saudi Arabia, Riyadh.

### Experimental Design:

#### SRB Cells Cytotoxicity Assay:

Hepatocellular carcinoma cell lines have been used in this test, and it was divided into four essential groups as following:

1. First group: represents the Non-treated control group.
2. Second group: represents the treated group with concentrations (50µg/ml, 100µg/ml, 200µg/ml) of bee glue (propolis) ( Xuan *et al.*,2014).
3. Third group: represents the treated group with concentrations (50µg/ml, 100µg/ml, 200µg/ml) of Dacarbazine drug.
4. Fourth group: represents the double treatment with bee glue and Dacarbazine drug with concentrations of (50µg/ml, 100µg/ml, 200µg/ml).

The method of (Houghton *et al.*,2007) was followed in the preparation and installation and dye the cancerous cells to apply the test of SRB Cells Cytotoxicity Assay.

The ratio of growth inhibition (IC<sub>50</sub>) and (IC<sub>90</sub>) was calculated as following:

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

### Statistical Analysis:

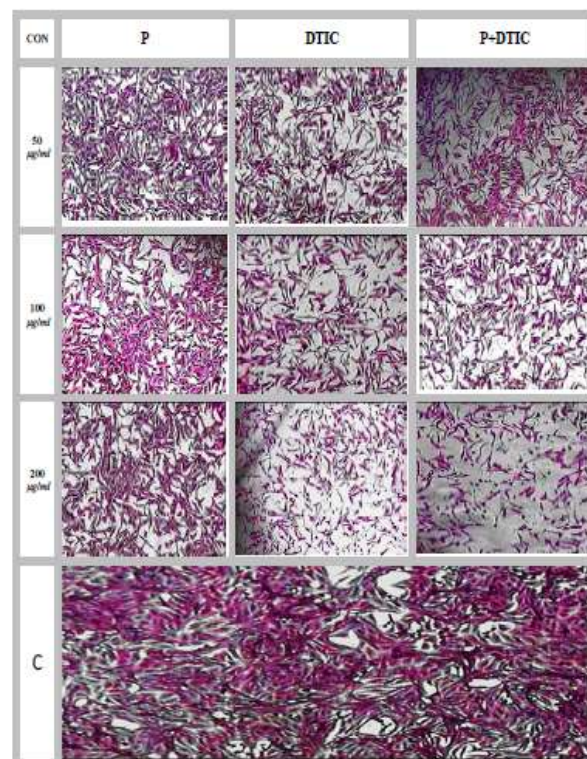
Student "t" test and variance analysis Anova were applied both to calculate the significance of obtained results from test being studied statistically.

## 3. Results

**Treatments with propolis, treatment with Dacarbazine drug and the double treatment with both propolis and Dacarbazine effect on the value of (IC<sub>50</sub>) and (IC<sub>90</sub>) after 48 hours.**

With microscopic examination *in vitro* for Hepatocellular cell lines (HepG2) after incubation for 48 hours it became possible to identify the morphological effects and stimulate apoptosis represented in: increasing the size of cells and rupture

of their membranes, cytoplasm enlargement, and nucleus fragmentation due to multiple treatments compared with control sample (Fig:1), the inhibitory effect that kills the half of cells (IC<sub>50</sub>) was calculated for each treatment with propolis, Dacarbazine drug and double treatment with both propolis and Dacarbazine and it was equal to 335, 159,330,µg/ml respectively, while the value of killing concentration for 90% of (IC<sub>90</sub>) cells for multiple treatments was 735, 359,730µg/ml respectively (Fig:9).



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 200(µg/ml)

**Fig ( 1 ) :** Morphological and cytological features of liver cancer cells of the lines HepG 2 Treatment with Different concentrations of Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine after 48 h . (X1000)

**Treatments with propolis, treatment with Dacarbazine drug and the double treatment with both propolis and Dacarbazine effect on the appearance mean for Hepatocellular carcinoma cell lines (HepG2) after 48 hours on concentration of (50µg/ml):**

The results obtained from (Table:1) shows that the treatment with propolis, Dacarbazine drug and the double treatment with propolis and Dacarbazine caused a significant decrease in the appearance mean for Hepatocellular carcinoma cell

lines (HepG2) so their values were (0.209±0.217 , 0.186±0.163 ,0.201±0.007) respectively compared with control sample mean (0.355±0.009), as the mean treatment with drug and the mean double treatment were close, while it was slightly increased when treated with propolis (Fig: 7), when calculating the inhibition ratio of cancer cells (HepG2) as a result of multiple treatments it was 41.25% , 47.74% ,43.50% respectively. Pointing out that the highest value recorded was for drug treatment then double treatment and then treatment with propolis (Fig: 11). And the inhibition ratio was inversely proportional to the rate of absorbance and vitality, as the rate of absorbance and vitality increased the rate of inhibition decreased. (Figs: 3& 5).

**Treatments with propolis, treatment with Dacarbazine drug and the double treatment with both propolis and Dacarbazine effect on the appearance mean for Hepatocellular carcinoma cell lines (HepG2) after 48 hours on concentration of (100 µg/ml):**

The results obtained from (Table:1) shows that the treatment with propolis, Dacarbazine drug and the double treatment with propolis and Dacarbazine caused a significant decrease in the appearance mean for Hepatocellular carcinoma cell lines (HepG2) so their values were (0.217±0.005 , 0.193±0.006 , 0.190±0.106) respectively compared with control sample mean (0.355±0.009), as the best treatment due to its impact in reducing the appearance mean of HepG2 was double treatment then Dacarbazine treatment and then propolis treatment (Fig: 7), when calculating the inhibition ratio of cancer cells (HepG2) as a result of multiple treatments it was 38.70% ,45.48%, 46.33% respectively. The highest value was for the double treatment then Dacarbazine treatment and then propolis treatment (Fig: 11). And the inhibition ratio was inversely proportional to the rate of absorbance and vitality (Figs: 3& 5).

**Treatments with propolis, treatment with Dacarbazine drug and the double treatment with both propolis and Dacarbazine effect on the appearance mean for Hepatocellular carcinoma cell lines (HepG2) after 48 hours on concentration of (200 µg/ml):**

The results obtained from (Table:1) shows that the treatment with propolis, Dacarbazine caused a significant decrease in the appearance mean for Hepatocellular carcinoma cell lines (HepG2) so their values were (0.217±0.006 , 0.178±0.003 , 0.182±0.006) respectively compared with control sample mean (0.355±0.009), as the best treatment due to its impact in reducing the appearance mean of HepG2 was Dacarbazine treatment then double treatment and then propolis treatment (Fig: 7), when calculating

the inhibition ratio of cancer cells (HepG2) as a result of multiple treatments it was 38.70% , 50.00% , 48.59% respectively. The highest value was for the Dacarbazine treatment then propolis treatment and then double treatment (Fig: 11). ). And the inhibition ratio was inversely proportional to the rate of absorbance and vitality (Figs: 3& 5).

**Treatments with propolis, treatment with Dacarbazine drug and the double treatment with both propolis and Dacarbazine effect on the appearance mean for Hepatocellular carcinoma cell lines (HepG2) after 48 hours on concentrations of (50,100,200 µg/ml) using variance analysis and the least significant difference (LSD):**

The results obtained from (Table:3) shows significant difference ( $P \leq 0.001$ ) in appearance mean for Hepatocellular carcinoma cell lines (HepG2) on different concentrations between treatment with propolis or treatment with Dacarbazine drug and double treatment with both propolis and Dacarbazine which was equal to ( $F = 28.842$ ) on concentration of 50µg/ml, ( $F = 96.102$ ) on concentration of 100µg/ml, and ( $F = 175.112$ ) on concentration of 200µg/ml compared with control sample.

And by comparison test through using the least significant difference (LSD) the significant difference ( $P \leq 0.001$ ) appeared in the appearance mean for Hepatocellular carcinoma cell lines (HepG2) due to treatment with propolis or with drug and double treatment with propolis and drug on concentrations of 50,100,200µg/ml.(Fig:13).

Hence, the order of treatments in terms of its high impact in lowering the appearance mean for Hepatocellular carcinoma cell lines (HepG2) on concentrations of 50,200 µg/ml is as follows:

Dacarbazine treatment > double treatment > propolis treatment

While, the order of treatments in terms of its high impact in lowering the appearance mean for Hepatocellular carcinoma cell lines (HepG2) on concentration of 100 µg/ml as follows:

Double treatment > Dacarbazine treatment > propolis treatment

**Treatments with propolis, treatment with Dacarbazine drug and the double treatment with both propolis and Dacarbazine effect on the value of (IC<sub>50</sub>) and (IC<sub>90</sub>) after 72 hours.**

With microscopic examination *in vitro* for Hepatocellular cell lines (HepG2) after incubation for 72 hours it became possible to identify the morphological effects (Fig:2). As the indicators of apoptosis raised by increasing the morphological changes in terms of size increasing , contents egress due to rupture of their membranes, nuclear condensation and cytoplasm shrinkage also the count



and vitality of cancerous cells decreased compared to cells morphology when incubated for 48 hours and also compared to control sample, the inhibitory effect that kills the half of cells ( $IC_{50}$ ) was calculated for each treatment with propolis, Dacarbazine drug and double treatment with both propolis and Dacarbazine and it was equal to 246,213, 239 $\mu$ g/ml respectively, while the value of killing concentration for 90% of ( $IC_{90}$ ) cells for multiple treatments was 646,613,639  $\mu$ g/ml respectively (Fig: 10).

**Treatments with propolis, treatment with Dacarbazine drug and the double treatment with both propolis and Dacarbazine effect on the appearance mean for Hepatocellular carcinoma cell lines (HepG2) after 72 hours on concentration of (50  $\mu$ g/ml):**

The results obtained from (Table:2) shows that the treatment with propolis, Dacarbazine drug and the double treatment with propolis and Dacarbazine caused a significant decrease in the appearance mean for Hepatocellular carcinoma cell lines (HepG2) so their values were (0.224 $\pm$ 0.007, 0.191 $\pm$ 0.006, 0.199 $\pm$ 0.010) respectively compared with control sample mean (0.551 $\pm$ 0.009), as the mean treatment with drug and the mean double treatment were close, while it was slightly increased when treated with propolis (Fig: 8), when calculating the inhibition ratio of cancer cells (HepG2) as a result of multiple treatments it was 59.27%, 65.46%, 64.00% respectively. Pointing out that the highest value recorded was for drug treatment then double treatment and then treatment with propolis (Fig: 12). And the inhibition ratio was inversely proportional to the rate of absorbance and vitality, as the rate of absorbance and vitality increased the rate of inhibition decreased. (Figs: 4& 6).

**Treatments with propolis, treatment with Dacarbazine drug and the double treatment with both propolis and Dacarbazine effect on the appearance mean for Hepatocellular carcinoma cell lines (HepG2) after 72 hours on concentration of (100  $\mu$ g/ml):**

The results obtained from (Table:2) shows that the treatment with propolis, Dacarbazine drug and the double treatment with propolis and Dacarbazine caused a significant decrease in the appearance mean for Hepatocellular carcinoma cell lines (HepG2) so their values were (0.254 $\pm$ 0.152, 0.196 $\pm$ 0.152, 0.230 $\pm$ 0.142) respectively compared with control sample mean (0.551 $\pm$ 0.009), as the best treatment due to its impact in reducing the appearance mean of HepG2 was Dacarbazine treatment the double treatment and then propolis treatment (Fig: 8), when calculating the inhibition ratio of cancer cells (HepG2) as a result of multiple treatments it was 54.00%, 64.55%, 58.36%

respectively. The highest value was for Dacarbazine treatment then double treatment and then propolis treatment (Fig: 12). And the inhibition ratio was inversely proportional to the rate of absorbance and vitality (Figs: 4& 6)

**Treatments with propolis, treatment with Dacarbazine drug and the double treatment with both propolis and Dacarbazine effect on the appearance mean for Hepatocellular carcinoma cell lines (HepG2) after 72 hours on concentration of (200  $\mu$ g/ml):**

The results obtained from (Table:2) shows that the treatment with propolis, Dacarbazine caused a significant decrease in the appearance mean for Hepatocellular carcinoma cell lines (HepG2) so their values were (0.299 $\pm$ 0.023, 0.264 $\pm$ 0.006, 0.242 $\pm$ 0.017) respectively compared with control sample mean (0.551 $\pm$ 0.009), as the best treatment due to its impact in reducing the appearance mean of HepG2 was double treatment then Dacarbazine treatment and then propolis treatment (Fig: 8), when calculating the inhibition ratio of cancer cells (HepG2) as a result of multiple treatments it was 45.65%, 52.00%, 56.00% respectively. The highest value was for double treatment then Dacarbazine treatment and then propolis treatment (Fig: 12). And the inhibition ratio was inversely proportional to the rate of absorbance and vitality (Figs: 4& 6).

**Treatments with propolis, treatment with Dacarbazine drug and the double treatment with both propolis and Dacarbazine effect on the appearance mean for Hepatocellular carcinoma cell lines (HepG2) after 72 hours on concentrations of (50,100,200  $\mu$ g/ml) using variance analysis and the least significant difference (LSD):**

The results obtained from (Table:4) shows significant difference ( $P \leq 0.001$ ) in appearance mean for Hepatocellular carcinoma cell lines (HepG2) on different concentrations between treatment with propolis or treatment with Dacarbazine drug and double treatment with both propolis and Dacarbazine which was equal to ( $F=494.79$ ) on concentration of 50 $\mu$ g/ml, ( $F=144.41$ ) on concentration of 100 $\mu$ g/ml, and ( $F=88.82$ ) on concentration of 200  $\mu$ g/ml compared with control sample.

And by comparison test through using the least significant difference (LSD) the significant difference ( $P \leq 0.001$ ) appeared in the appearance mean for Hepatocellular carcinoma cell lines (HepG2) due to treatment with propolis or with drug and double treatment with propolis and drug on concentrations of 50,100,200  $\mu$ g/ml.(Fig:14).

Hence, the order of treatments in terms of its high impact in lowering the appearance mean for

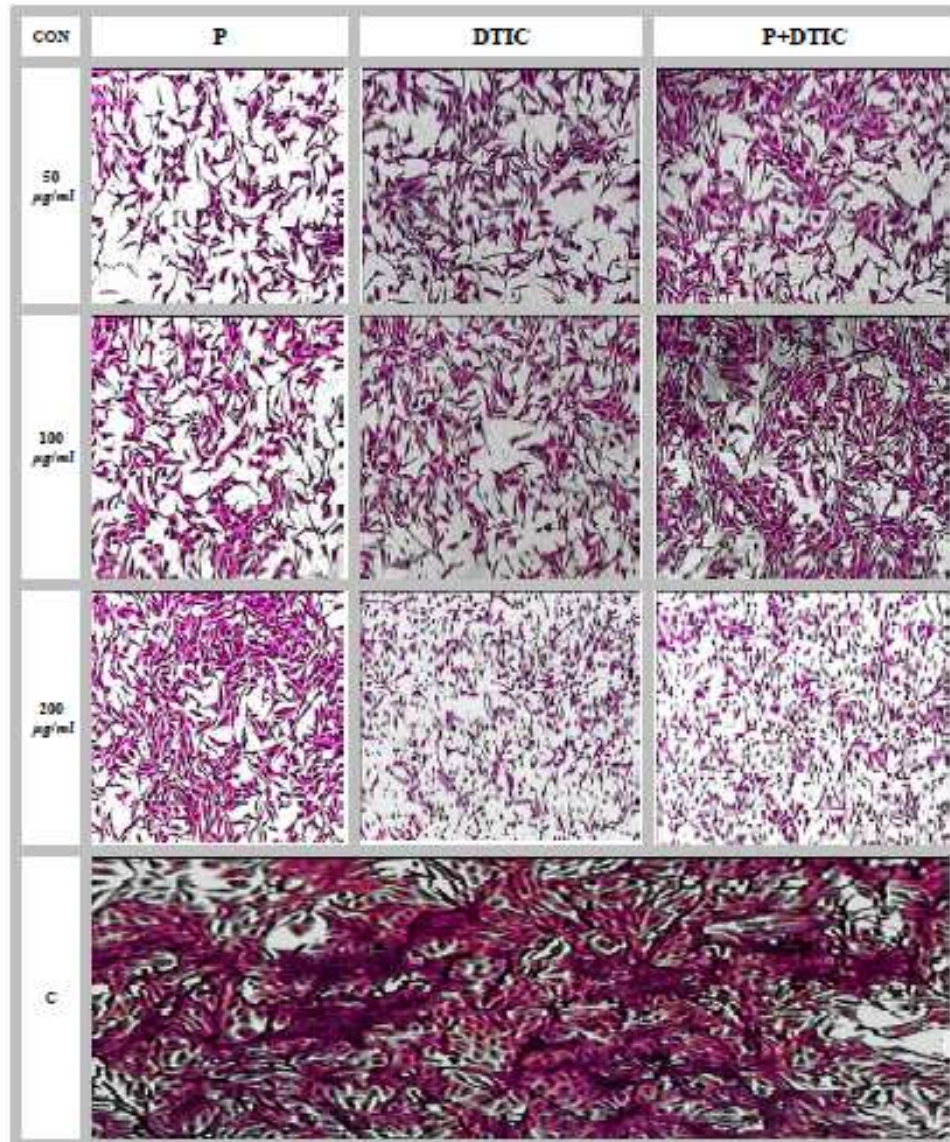
Hepatocellular carcinoma cell lines (HepG2) on concentrations of 50,100  $\mu\text{g/ml}$  is as follows:

Dacarbazine treatment>Double treatment>Propolis treatment

While, the order of treatments in terms of its high impaction in lowering the appearance mean for

Hepatocellular carcinoma cell lines (HepG2) on concentration of 200  $\mu\text{g/ml}$  is as follows:

Double treatment>Dacarbazine treatment > propolis treatment



**Fig ( 2 ) :** Morphological and cytological features of liver cancer cells of the lines HepG 2 Treatment with Different concentrations of Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine after 72 (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 liver cancer cells of the lines HepG 2 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	0.355 $\pm$ 0.009	0.354	1	
	P	1 2 3 4 5 6 Mean $\pm$ Std.Error	**** <sup>a</sup> 0.209 $\pm$ 0.217	0.208	0.588	41.249
	D	1 2 3 4 5 6 Mean $\pm$ Std.Error	**** <sup>a</sup> 0.186 $\pm$ 0.163	0.185	0.523	47.740
	P+D	1 2 3 4 5 6 Mean $\pm$ Std.Error	**** <sup>a</sup> 0.201 $\pm$ 0.007	0.200	0.565	43.503
100	C	1 2 3 4 5 6 Mean $\pm$ Std.Error	0.355 $\pm$ 0.009	0.354	1	
	P	1 2 3 4 5 6 Mean $\pm$ Std.Error	**** <sup>a</sup> 0.217 $\pm$ 0.005	0.217	0.613	38.701
	D	1 2 3 4 5 6 Mean $\pm$ Std.Error	**** <sup>a</sup> 0.193 $\pm$ 0.006	0.193	0.545	45.480
	P+D	1 2 3 4 5 6 Mean $\pm$ Std.Error	**** <sup>a</sup> 0.190 $\pm$ 0.106	0.190	0.537	46.328
200	C	1 2 3 4 5 6 Mean $\pm$ Std.Error	0.355 $\pm$ 0.009	0.354	1	
	P	1 2 3 4 5 6 Mean $\pm$ Std.Error	**** <sup>a</sup> 0.217 $\pm$ 0.006	0.217	0.613	38.701
	D	1 2 3 4 5 6 Mean $\pm$ Std.Error	**** <sup>a</sup> 0.178 $\pm$ 0.003	0.177	0.500	50.000
	P+D	1 2 3 4 5 6 Mean $\pm$ Std.Error	**** <sup>a</sup> 0.182 $\pm$ 0.006	0.182	0.514	48.588

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 liver cancer cells of the lines HepG 2 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	0.551 ± 0.009	0.550	1	
	P	1 2 3 4 5 6 Mean ± Std.Error	0.224 ± 0.007 *** <sup>a</sup>	0.224	0.407	59.273
	D	1 2 3 4 5 6 Mean ± Std.Error	0.191 ± 0.006 *** <sup>a</sup>	0.190	0.345	65.455
	P+D	1 2 3 4 5 6 Mean ± Std.Error	0.199 ± 0.010 *** <sup>a</sup>	0.198	0.360	64.000
100	C	1 2 3 4 5 6 Mean ± Std.Error	0.551 ± 0.009	0.550	1	
	P	1 2 3 4 5 6 Mean ± Std.Error	0.254 ± 0.152 *** <sup>a</sup>	0.253	0.460	54.000
	D	1 2 3 4 5 6 Mean ± Std.Error	0.196 ± 0.152 *** <sup>a</sup>	0.195	0.355	64.546
	P+D	1 2 3 4 5 6 Mean ± Std.Error	0.230 ± 0.142 *** <sup>a</sup>	0.229	0.416	58.364
200	C	1 2 3 4 5 6 Mean ± Std.Error	0.551 ± 0.009	0.550	1	
	P	1 2 3 4 5 6 Mean ± Std.Error	0.299 ± 0.023 *** <sup>a</sup>	0.299	0.544	45.636
	D	1 2 3 4 5 6 Mean ± Std.Error	0.264 ± 0.006 *** <sup>a</sup>	0.264	0.480	52.000
	P+D	1 2 3 4 5 6 Mean ± Std.Error	0.242 ± 0.017 *** <sup>a</sup>	0.242	0.440	56.000

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 liver cancer cells of the lines HepG 2 after 48 h .

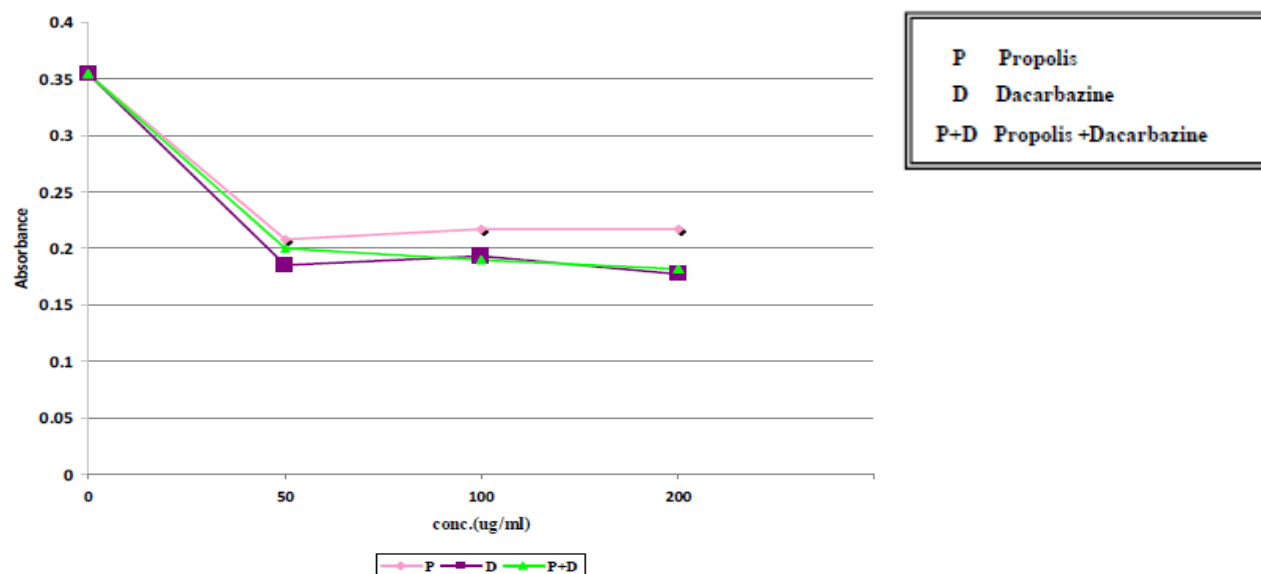
		(ANOVA)		(LSD)		
		(F)	(Sig)	Groups Treatment	Mean Difference	Sig)(
50	Control (C)	28.842	***	P	0.146	***
				D	0.169	***
				P+D	0.154	***
100	Control (C)	102.96	***	P	0.138	***
				D	0.161	***
				P+D	0.164	***
200	Control (C)	112.175	***	P	0.138	***
				D	0.177	***
				P+D	0.172	***

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 liver cancer cells of the lines HepG 2 after 72 h .

		(ANOVA)		(LSD)		
		(F)	(Sig)	Groups Treatment	Mean Difference	Sig)(
50	Control (C)	790.494	***	P	0.327	***
				D	0.360	***
				P+D	0.352	***
100	Control (C)	409.144	***	P	0.297	***
				D	0.355	***
				P+D	0.321	***
200	Control (C)	819.88	***	P	0.252	***
				D	0.287	***
				P+D	0.309	***

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 liver cancer cells of the lines HepG 2 after 48 h .



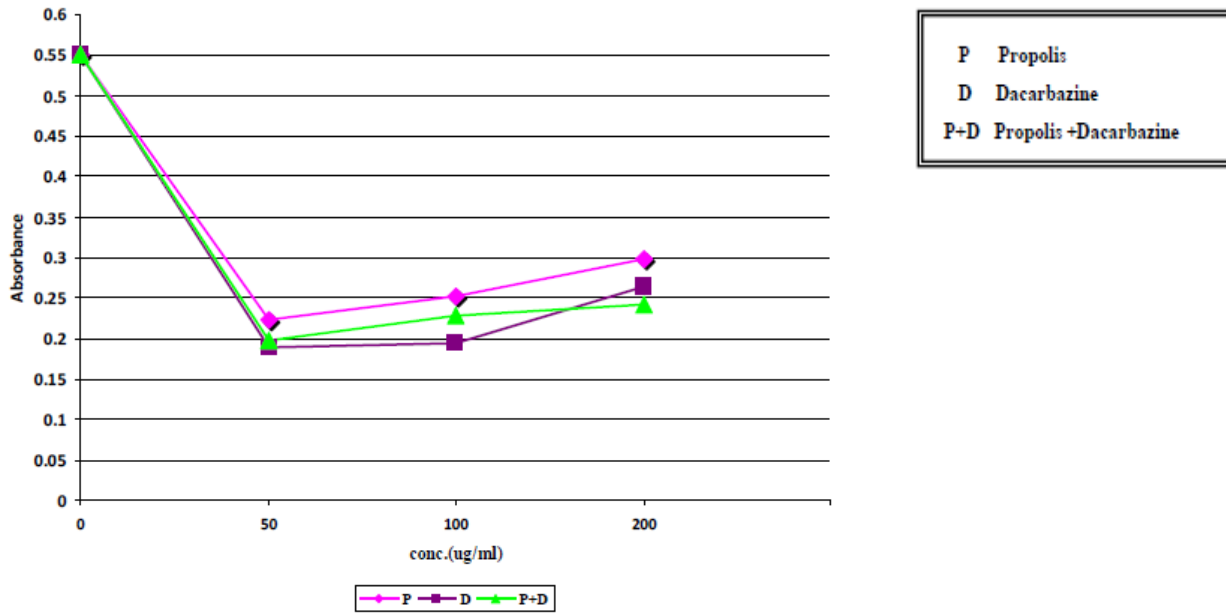


Fig ( 4 ) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Absorbance values of liver cancer cells of the lines HepG 2 after 72 h .

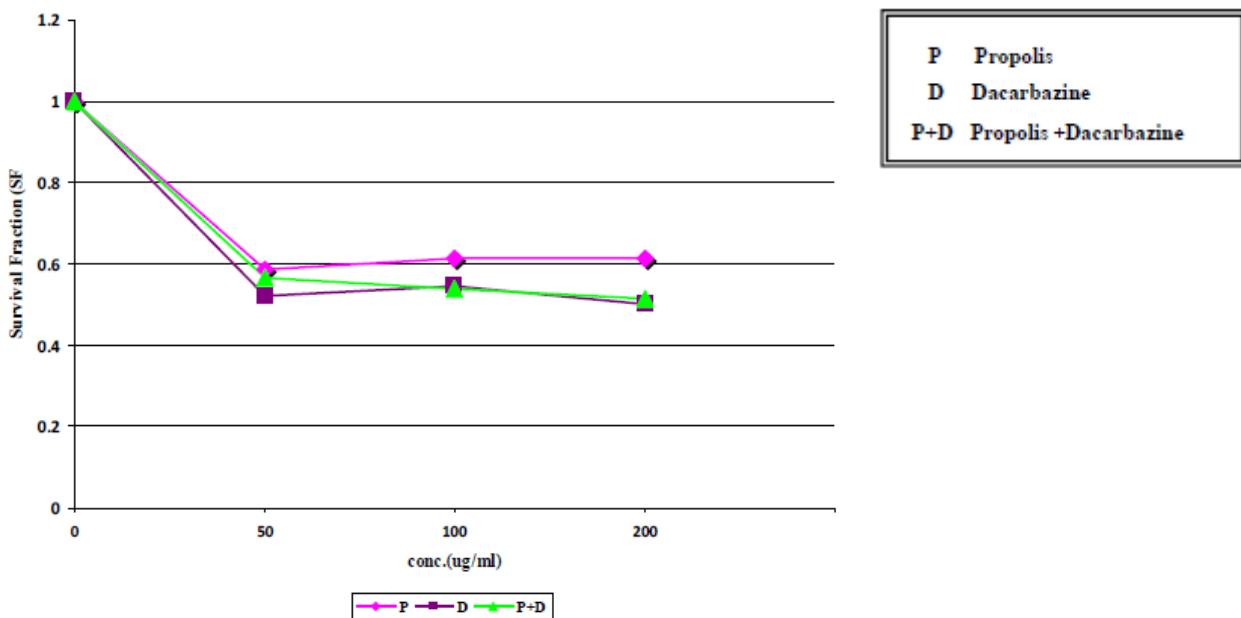


Fig ( 5 ) : Effects of Different concentrations of Treatment with Propolis, Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Survival Fraction (SF) values of liver cancer cells of the lines HepG 2 after 48 h .

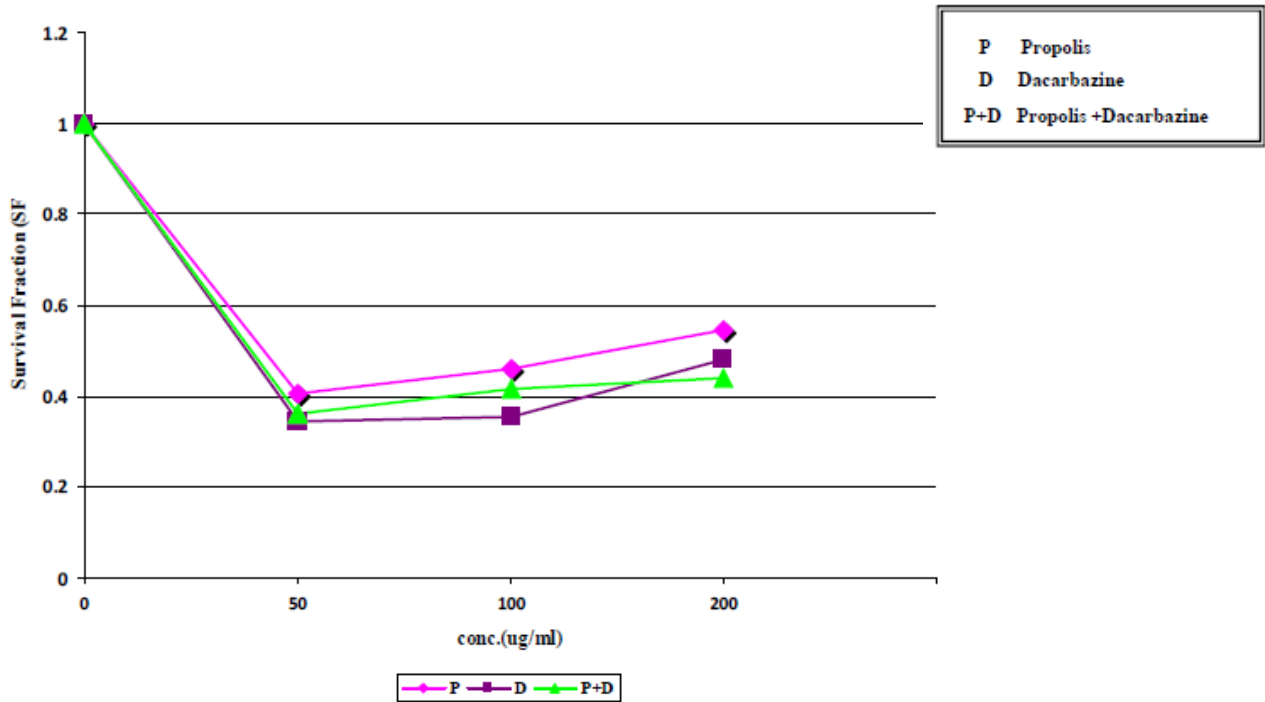


Fig ( 6 ) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Survival Fraction (SF) values of liver cancer cells of the lines HepG 2 after 72 h .

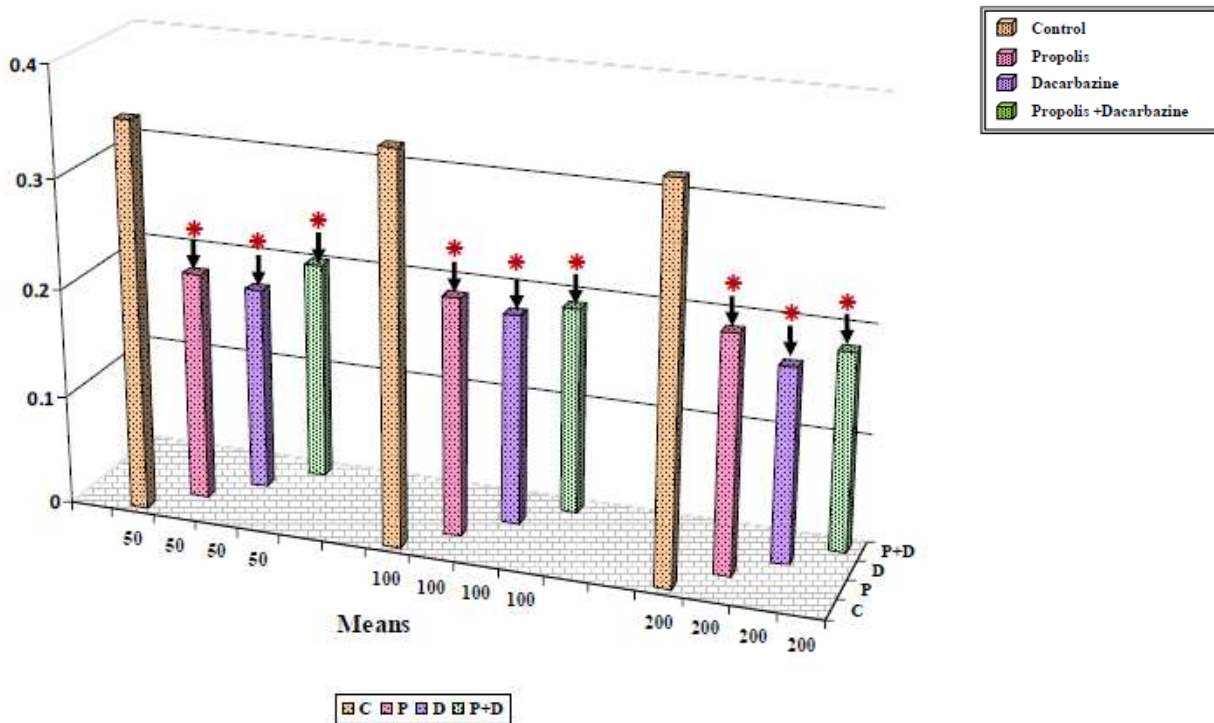


Fig ( 7 ) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Means of liver cancer cells of the lines HepG 2 after 48 h .

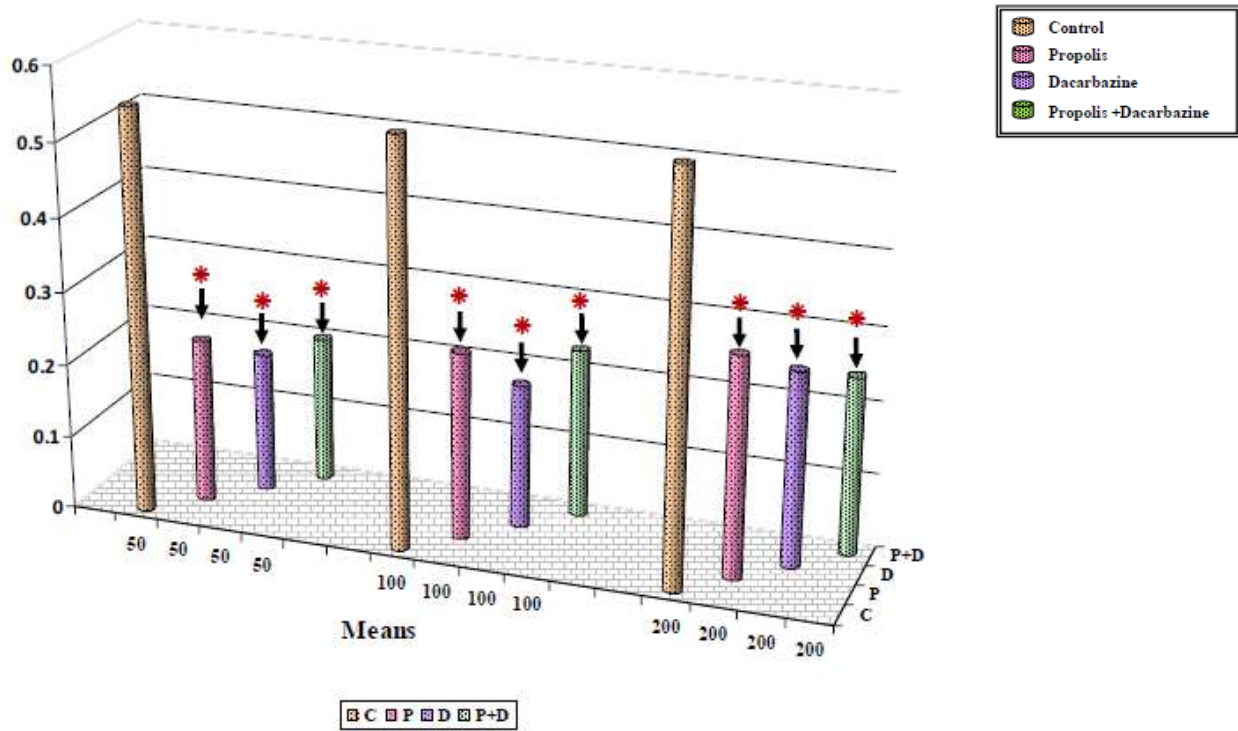


Fig ( 8 ) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Means of liver cancer cells of the lines HepG 2 after 72 h .

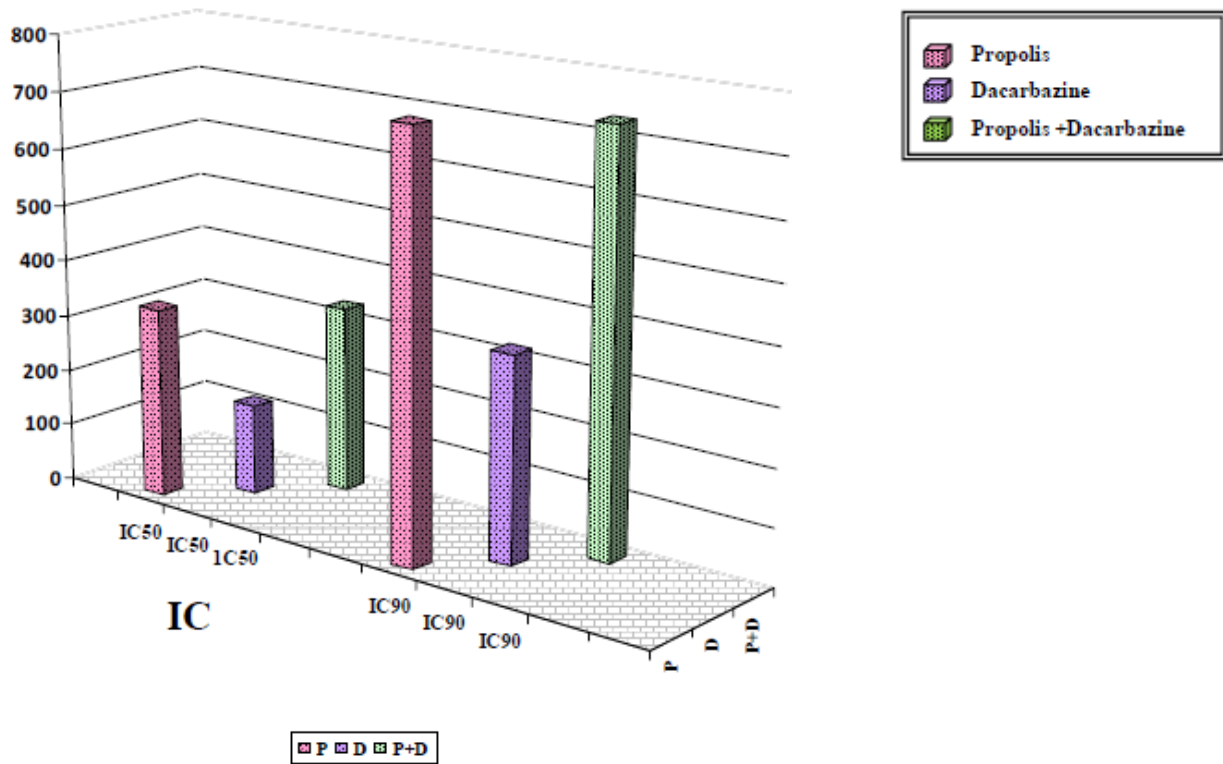


Fig ( 9 ) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the IC50 and IC90 values of liver cancer cells of the lines HepG 2 after 48 h .

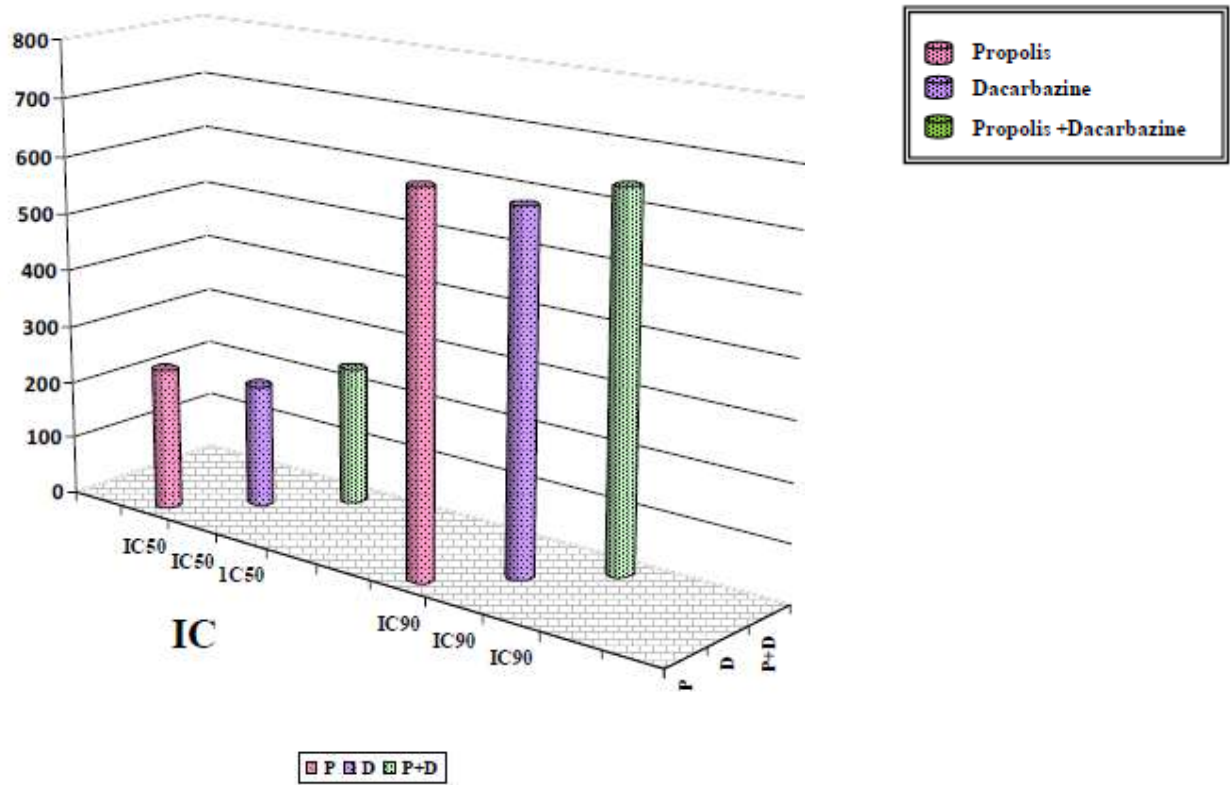


Fig ( 10) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the IC50 and IC90 values of liver cancer cells of the lines HepG 2 after 72 h .

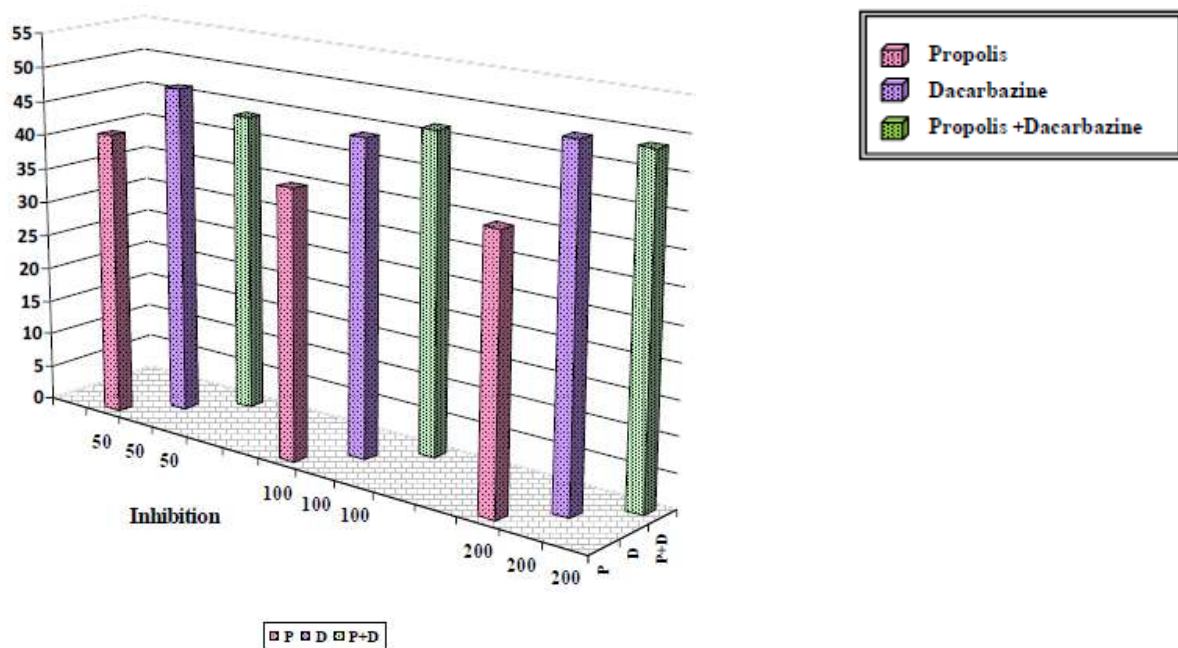


Fig ( 11) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Inhibition rate of liver cancer cells of the lines HepG 2 after 48 h .



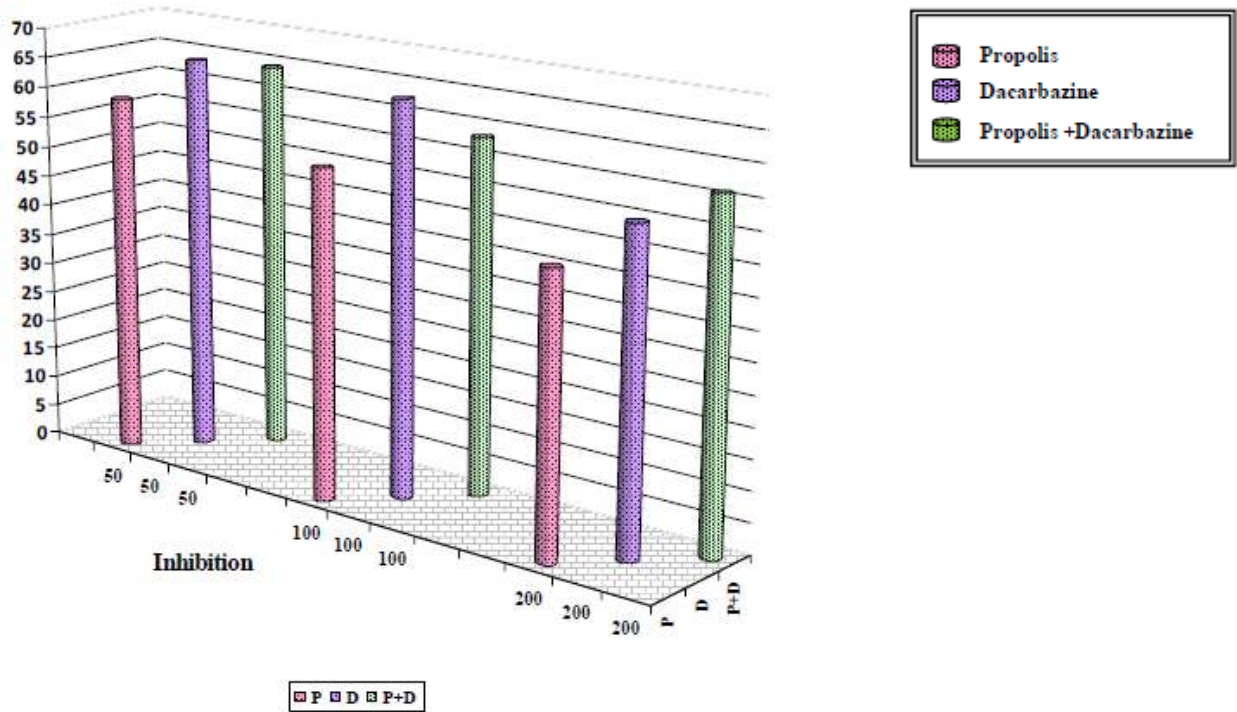


Fig ( 12 ) : Effects of Different concentrations of Treatment with Propolis ,Dacarbazine, and The Dual Treatment with Propolis and Dacarbazine on the Inhibition rate of liver cancer cells of the lines HepG 2 after 72 h .

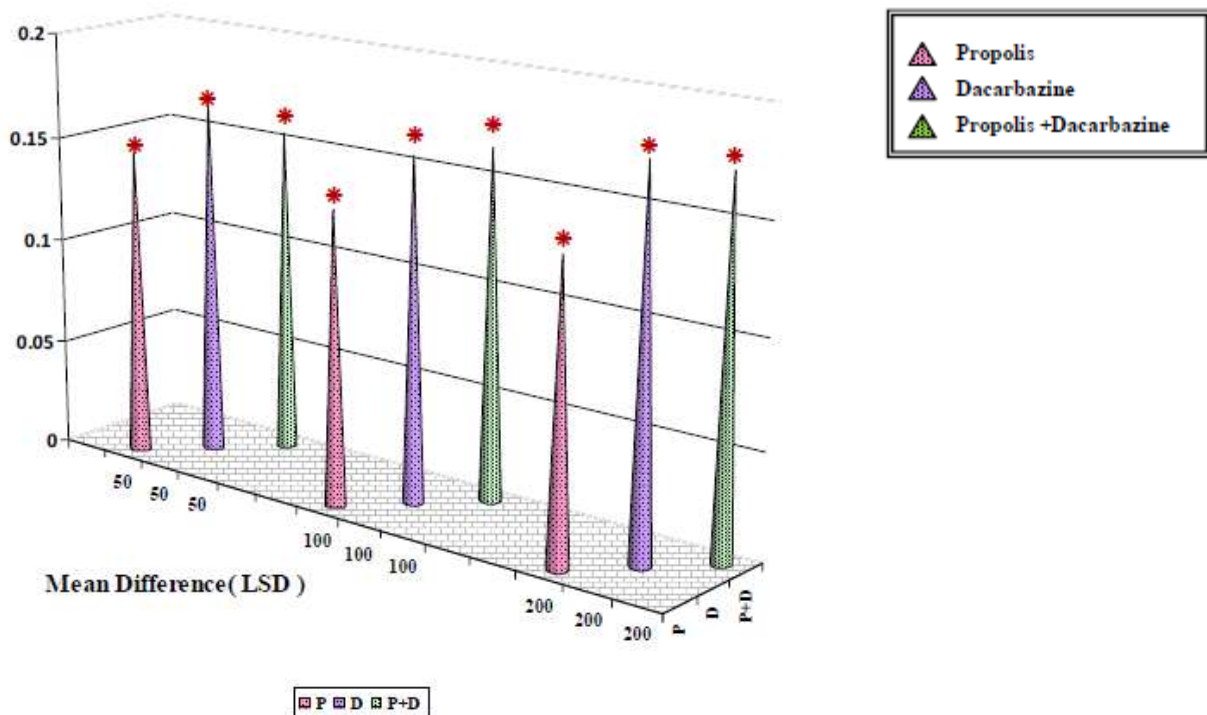


Fig ( 13 ) : Comparison between The Effect of Treatment of Propolis, Dacarbazine and The Dual Treatment with Propolis and Dacarbazine on the mean of liver cancer cells of the lines HepG 2 after 48 h .

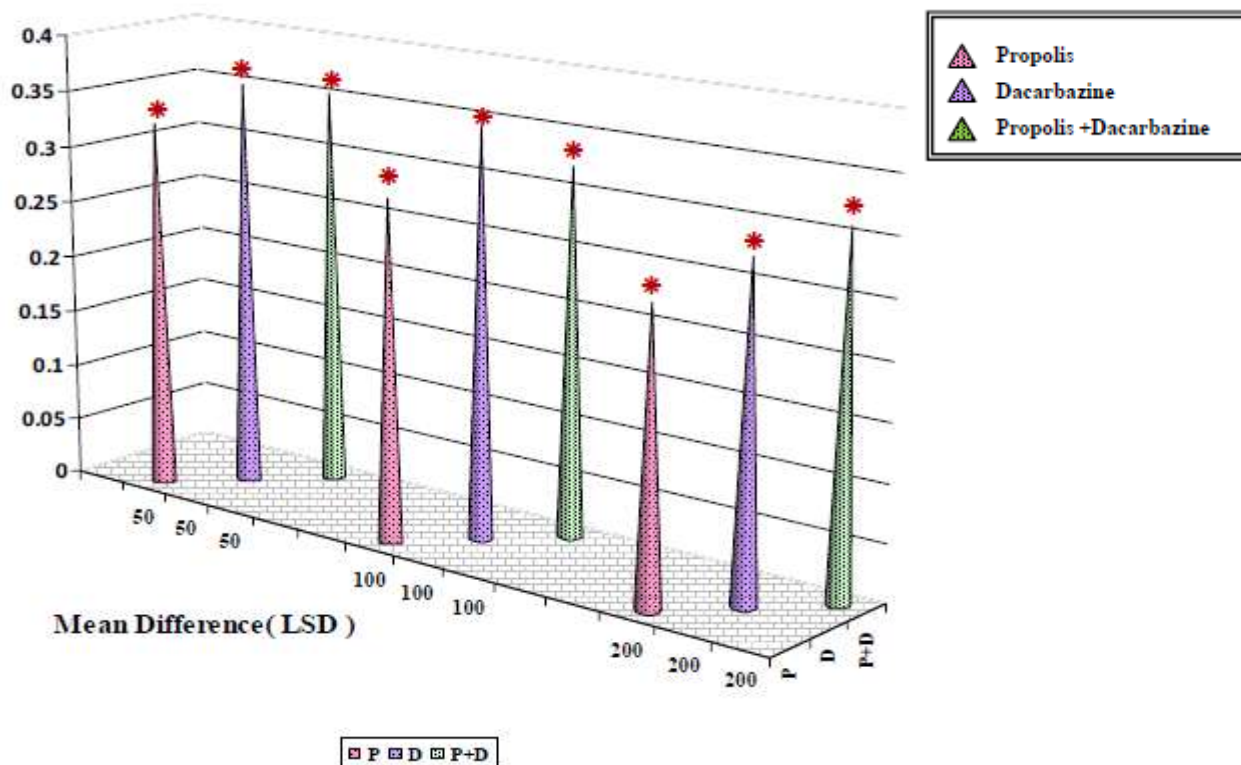


Fig ( 14) : Comparison between The Effect of Treatment of Propolis, Dacarbazine and The Dual Treatment with Propolis and Dacarbazine on the mean of liver cancer cells of the lines HepG 2 after 72 h .

#### 4. Discussion:

This study aimed to evaluate the potential therapeutic cellular effects for treatment with propolis as one of bee products which has therapeutic properties and treatment with double usage of propolis with Dacarbazine drug in comparison with using Dacarbazine treatment which is as a drug used in chemotherapy for treatment of cancer tumors in one of cancer cells lines which known as Hepatocellular carcinoma cell lines (HepG2), the effect of multiple treatments on the inhibition of Hepatocellular carcinoma cell lines (HepG2) was recorded in terms of the existing decline in the developed cellular density and by measuring the absorbance by (ELISA) device, with wavelength (490 nanometer), as the treatment was done by three concentrations 50,100,200µg/ml for all treatments and with incubation periods of (48,72 h) compared to control sample.

It becomes clear through this study, by morphological examination the treatment by three concentrations( 50,100,200µg/ml) of propolis, Dacarbazine drug and double treatment with drug and propolis can influence on the inhibition of Hepatocellular carcinoma cell lines (HepG2) after incubation for (48,72 h), as the indicators of apoptosis appeared clearly with all treatment at all

concentrations , compared with control sample , also morphological effects on Hepatocellular carcinoma cell lines (HepG2) for double treatment with drug and propolis and results of using drug only treatment were so close. There was a linear relationship between concentrations and effect with time.

Also these treatments showed significant impact on inhibition of Hepatocellular carcinoma cell lines (HepG2) *in vitro* after incubation for (48,72h). Also obtained results clarify that the inhibition of Hepatocellular carcinoma cell lines (HepG2) with these treatments has recorded a significant improvement and elevated by increasing the concentrations, also inhibition ratio was inversely proportional to the rate of absorbance and vitality.

By comparing the means of appearance for Hepatocellular carcinoma cell lines HepG2, all treatments recorded significant decrease ( $P \leq 0.001$ ) compared to control sample and at all concentrations. And to show the effective concentration for treatments and comparing it with control it depended on calculating (IC<sub>50</sub>) and (IC<sub>90</sub>) so the best value was recorded by Drug treatment then double treatment and then propolis treatment respectively. And that was after incubation period equal (48,72h).

These results are compatible with the results obtained by Ex-researchers who treat the

Hepatocellular carcinoma cell lines HepG2 with any of the active components of propolis.

When Hepatocellular carcinoma cell lines treated by any flavonoid compound existing in propolis (Quercetin), it led to decreasing cellular growth and apoptosis according to dose and time. as it stops the cell cycle in the stage of DNA replication (S-Phase), in addition to its powerful ability to inhibit topoisomerase II enzyme in DNA, data showed that Quercetin stimulate apoptosis through direct activation of casapss chain (Mitochondrial path)which inhibit the vital signs of the cells of hepatocellular carcinoma, and by regulating the Specificity protein 1 (SP1) which play an important role in physiological processes such as a regulator for cell cycle, apoptosis and blood vessels. By suppressing apoptosis related proteins, transfer signals linked to cell growth and apoptosis, tumor suppressing genes, cell cycle regulating molecules, carcinogenic genes and blood vessels related factors without causing any negative impact on normal cells.

Also it was found that Quercetin activates the anti- tumor effect resulting from treating with Doxorubicin (DOX) on Hepatocellular carcinoma cell lines with protecting normal hepatic cells, so it was decided that Quercetin is useful with (DOX) treatment to increase the effectiveness of Hepatocellular carcinoma treatment.Suggesting that Quercetin is an antagonist for Hepatocellular carcinoma by stopping the cell cycle and apoptosis. And has a protective role in the early stages of Hepatocellular carcinoma in addition to pro-apoptotic properties and regulate the proteins expression necessary for cell cycle. (Granado *et al.* , 2006; Wang *et al.* ,2012; Casella *et al.* ,2014; Sudan and Rupasinghe ., 2014 ; Lee *et al.* ,2015).

It was found that glangin caused apoptosis in a large scale in Hepatocellular carcinoma cell lines through activation of caspase-8 (Mitochondrial path) in hepatocellular carcinoma cell lines. As it decrease the mitochondrial membrane permeability proportional to dose and time, activates Bax protein in mitochondria which stimulates cytochrome factor C in cytosol, also it prevents the growth of Hepatocellular carcinoma (HCC) by autophagy which reflex its anti-proliferation effect on Hepatocellular carcinoma. When hepatocellular carcinoma cell lines treated with that compound it was found to cause the following:

1. increase the rate of autophagy
2. increase in the level of protein linked to light chain 3
3. increase the vesicles rate in cells and high protein expression rate P53.(Zhang *et al.* ,2012 ; 2013 ; Wen *et al.* ,2012 ;Wang *et al.* ,2014).

Also it was found that apigenin has a protective properties and therapeutic ability against cancer diseases, after treating hepatocellular carcinoma cell lines a decrease in cell vitality was observed, and some morphological changes. Also it significantly inhibits hepatocellular carcinoma cell lines and increases accumulation of protein P53 which is responsible of apoptosis and stopping of cell cycle at stage G2/M, depending on dose and time. Apoptosis by apigenin has been linked to Vimentin protein which has role in cells physiology and regulates expression levels in type one of collagen (Collagen 1) which participate with Vimentin protein in decreasing ions transfer between cancerous cells. So apigenin was considered as a main regulator in blood vessels and ions migration.In addition it causes a significant decrease in mRNA level, catalase activity and glutathione levels inside cancer cells.it can participate with Doxorubicin in treatment of hepatocellular carcinoma by preventing cell growth and development of cancer cells by apoptosis. So it can be used as effective adjuvant to prevent chemical resistance.(Chiang *et al.* ,2006; Kim *et al.* ,2011 ; Valdameri *et al.* ,2011 ; Gao *et al.* , 2013).

Studies showed that Naringenin has toxic effect for multiple types of cancer cells, it works by inhibiting hepatocellular carcinoma metastasis in stages G1,G2,G0 and M from cell cycle as a result of fast accumulation of protein P53, in addition it stimulates apoptosis in hepatocellular carcinoma and inhibits cell growth as it invades the cancer cells and inhibits cytosol production in hepatocellular carcinoma in human and mice, prevents DNA replication by inhibiting active proteins and active nuclear factor , inhibits phosphorylation process outside the cancer cell, inhibits nuclear transition , prevents hepatocellular carcinoma by multiple inhibition of transport signal tracks, also decoding of estrogen receptors link which leads to apoptosis , decreasing the effect of hormones that increases metastasis of Hepatocellular carcinoma cell lines, so it is excellent protective chemical factor for hepatocellular carcinoma (Bulozomi *et al.* ,2010; Arul and Subramanian., 2013; Yen *et al.* ,2015).

By studying the anti-cancer effect of Tricetin (TCN) in hepatocellular carcinoma, which is a type of flavonoid, they found that it kills cancer cells by influencing mitochondria , death receptors 5 (DR5) which cause apoptosis. When it was injected in the intraperitoneal cavity of mice they found that TCN cause the decrease in size of hepatocellular tumor by 60%.

Results of this studying shows that TCN stimulates apoptosis in hepatocellular carcinoma so it is a promising protective factor against cancer( Hsu *et al.* ,2010).

Also the effect of (CAPE) -derived from propolis- appeared effectively in hepatocellular carcinoma in a way that depends in dose, and stopping protein expression for MMP-9 and MMP-2 related to cancer cells, and significantly inhibits nuclear factor kappa B (NF- $\kappa$ B) which stimulate DNA replication in Hepatoceelular carcinoma (Lee *et al.*, 2008).

Accordingly, this studying suggests that the ability of propolis- in decreasing the toxic cellular effects for Dacarbazine drug is due to propolis considered as an strong anti- cancer agent.(Orsolich *et al.*, 2005; Ozkul *et al.*, 2005; Xuan *et al.*, 2014) .

And the activity of anti- cancer propolis can possibly compatible with its active constituents related to anti- cancer activity( Banskota *et al.*, 2002 ; Stan *et al.*, 2011 ), the most important of which is :Flavonoid, it is a group of compounds naturally exist, have low molecular weight , have multiple biological effects like; anti- inflammatory, anti-allergic, antiviral ,anti- cancer, antioxidant, and have protective biological effects *in vivo* and *in vitro* and the most important of it are: quercetin ,chrysin , caffeic acid phenethyl ester (CAPE), naringenin , glangin, acaacetin , apigenin, artepilin C, And its mechanism of work can be summarized as following:

1. direct scavenging of reactive oxygen species (ROS)
2. activation of antioxidant enzymes
3. Chelating metal activity
4. Reducing roots of a-tocopheryl
5. Oxidases inhibition
6. Reducing of oxidative stress resulting from treating with mutagens
7. Increase in antioxidant properties of antioxidant agents with low molecular weight
8. Decrease in regulation of protein mutagens P35
9. Stopping the cell cycle in cancer cells
10. Tyrosine kinase inhibition
11. The ability to link estrogen receptors
12. Inhibit the expression of ROS proteins (Prochazkova *et al.*, 2011 ; kumar and Pandey ., 2013)

Therefore, this study recommends using of propolis in chemotherapy for cancer patients as an alternative treatment, adjuvant or a complementary treatment with other anti- cancer treatments.

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