Effects of Active Materials in Alcoholic Extract of Iraqi Propolis on Growth of Some Cancer Lines in The Laboratory and Cancer of Mammary Gland in Mice

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Abstract: The present study was designed to Extract and investigate the chemical composition, antitumor activity of Iraqi propolis ethanolic extract (PE), which has not been studied previously. Nine compounds were identified by TLC and HPLC chromatography. Total phenolics were determined to be 29.29, 21.56, 21.90 $mg.g^{-1}$ by using soxhlet, maceration 7 days, maceration 10 days respectively. Phenolic acids and flavonoids are present in medicinal plants and propolis in high concentration. A water-soluble extract of propolis (PE) was investigated for direct antitumor activity *in vivo* and *in vitro*. The local presence PE in the tissue caused a significant delay in tumor formation and increased life span 54.50 to 75.80%, respectively. PE was found to show very potent cytotoxicity against four neoplastic cancer cells: SF-295 (central nervous system), HCT-8 (colon), MDAMB-435 (breast) and HL-60 (leukaemia), with IC_{50} below 1 μg/mL. Their cytotoxicities were compared to doxorubicin as a positive control. Based on these results, we postulate that the antitumor activity of Iraqi PE compounds includes direct cytotoxic effects on tumor cells. [Report and Opinion 2010;2(5):76-85]. (ISSN:1553-9873).

Key words: antitumor activity; cytotoxic effects; propolis

1. Introduction

Bees and bee products have long been recognized for their medicinal properties, often being sold as nutritional supplements and health products. Recently, there has been renewed interest in the medicinal properties of honey bee products which include antibacterial, antifungal, cytostatic, wound healing, anti-tumor effects and anti-inflammatory effects [1-4].

Propolis (bee glue) is the generic name for the resinous substance collected by honeybees from various plant sources. It is rich in biochemical constituents, including mostly a mixture of polyphenols, flavonoid aglycones, phenolic and ketones. Experimental evidence suggests that polyphenolic compounds can exhibit anti-tumour effects in murine tumor models [5-8]. Reports also describe the potential use of synthetic flavonoids, such as flavone acetic acid, in the protection of mice from certain solid tumours[9]. Moreover, caffeic acid and their esters, caffeic acid phenethyl ester and quercetin, are powerful antioxidants and can arrest the growth of cancer cells in vitro [9,10].

Propolis has attracted researchers' interest in the last decades because of several biological and pharmacological properties, such as immunomodulatory, antitumor, antimicrobial, antiinflammatory, antioxidant, among others. Besides, propolis-containing products have been intensely marketed by the pharmaceutical industry and health-food stores [11]. The ethnopharmacological approach, combined with chemical and biological methods, may provide useful pharmacological leads. Propolis is in no way a new discovery. The use of propolis goes back to ancient times, at least to 300 BC, and it has been used as a medicine in local and popular medicine in many parts of the world, both internally and externally. Egyptians, Greeks and Romans reported the use of propolis for its general healing qualities and for the cure of some lesions of the skin. Propolis has always been reputed as an anti-inflammatory agent and to heal sores and ulcers. Ancient Egyptians used it to embalm their dead, and more recently it was used during the War for healing wounds and tissue regeneration [12]. However, its use continues today in remedies and personal products, and the list of preparations and uses is endless. It is still one of the most frequently used remedies in the Balkan States [13], and it has only been in the last decades that scientists have investigated its constituents and biological properties.

Propolis is a resinous material collected by bees from bud and exudates of the plants, which is transformed in the presence of bee enzymes. Its color varies from green, red to dark brown. Propolis has a characteristic smell and shows adhesive properties because it strongly interacts with oils and proteins of the skin. In general, propolis *in nature* is composed of 30% wax, 50% resin and vegetable balsam, 10% essential and aromatic oils, 5% pollen, and other substances [14].

Etymologically, the Greek word propolis means *pro*, for or in defence, and *polis*, the city, that is "defence of the hive". Bees use it to seal holes in their

honeycombs, smooth out internal walls as well as to cover carcasses of intruders who died inside the hive in order to avoid their decomposition. Propolis also protects the colony from diseases because of its antiseptic efficacy and antimicrobial properties [15].

After its administration to mice or to humans propolis does not seem to have side effects [16]. According to Burdock[14] propolis is non-toxic, and its DL_{50} ranges from 2 to 7.3 g/kg in mice. This author suggested that the safe concentration for humans could be 1.4 mg/kg per day, or approximately 70 mg/day. After treatment of rats with different concentrations of propolis (1, 3 and 6 mg/kg/day), different extracts (water or ethanol) and varying the time of administration (30, 90 and 150 days) no significant alterations in total lipids, triglycerides, cholesterol, HDL-cholesterol concentrations, nor in AST and LDH specific activities were observed [17]. The body weight of rats was measured in all these protocols, and propolis administration did not induce alterations in their weight. Cuesta *et al*.[18] have not observed either mortality or growth rate alteration after daily intake of propolis in the diet during 6 weeks.

Propolis extraction methods may influence its activity, since different solvents solubilization and extract different compounds. The most common extracts used in biological assays are ethanol, methanol and water in different concentrations [19]. Its chemical composition is very complex: more than 300 components have already been identified, and its composition is dependent upon the source plant and local flora. Moreover, propolis composition is completely variable creating a problem for the medical use and standardization [20].

1.2 Propolis' antitumor activity

Cancer is the second leading cause of death worldwide after cardiovascular diseases. Considerable insight has been gained into the mechanisms by which some chemicals affect cellular growth and how this knowledge has been used the design of new chemotherapeutic drugs[21] providing more selectivity toward cancer cells than to normal cells leading to lower side effects.

Several researchers have reported the antitumoral property of propolis *in vivo* and *in vitro*. Propolis antiproliferative activity on tumor cells has been demonstrated and some responsible compounds were isolated [22,]. Matsuno [23] isolated an active substance from Brazilian propolis and characterized it as a new clerodane diterpenoid (namely PMS-1), which inhibited the growth of hepatoma cells and arrested the tumor cells at S phase. Matsuno et al. [24] isolated a compound (PRF-1) from a water extract of propolis, which showed antioxidant activity and was cytotoxic to human hepatocellular carcinoma, HeLa and human lung carcinoma HLC-2 cells. Their group also isolated a tumoricidal compound identical to artepillin C, described as a constituent from *Baccharis* species, and its cytoxicity seemed to be partly attributable to apoptosis-like DNA fragmentation [25]. Kimoto *et al.*[1] investigated the effects of artepillin C *in vitro*, verifying suppression of tumor growth, and *in vivo* there was an increase in the ratio of CD4/CD8 T cells, indicating that this compound activated the immune system. Liao *et al.* [26] demonstrated the inhibitory effect of CAPE on angiogenesis, tumor invasion and pulmonary metastatic capacity of CT26 cells. CAPE also prolonged the survival of mice implanted with CT26 cells, suggesting its potential as an antimetastatic agent.

The purpose of the present study was to examine whether the antitumor activity of Iraqi Prpolis Extract (PE) may be the result of direct cytotoxic activity on tumor cells.

2. MATERIALS AND METHODS

2.1. Propolis origin

Propolis sample had been collected from colonies of honeybees located in different areas of Baghdad by using plastic bags.

2.2 Preparation of propolis extracts (PE)[19]

Extraction procedures

Maceration. Finely ground propolis was extracted by maceration at room temperature, with occasional shaking, in the proportion of 20 g of propolis to 100 mL of solvent. Extracts were obtained after 7 and 10 days of maceration,and filtered. solvents 100% absolute ethanol alcohol (Merck) was used.

Soxhlet. Finely ground propolis was extracted in a Soxhlet extractor for 24 hours at a maximum temperature of 60 °C, in the proportion of 20 g of propolis to 400 mL of absolute alcohol. In the case where distilled water was used as a solvent, in the same proportions, the temperature was of 100 °C.

Wax extraction. The extracts obtained through both Soxhlet and maceration procedures were left in a freezer overnight to induce the crystallization of dissolved waxes and then filtered at a temperature of approximately 0 °C to remove waxes from the extract. These waxes were not analyzed in the present study as their composition has already been studied.[13, 14]

2.3 Analytical procedures[19]

Yield of the propolis extracts. All extracts were evaporated to dry and weighed to obtain the yield. The results were given as a percentage of the original weight of crude propolis.

Total phenolic content. The procedure used was based on the methods outlined by Folin-Ciocalteau[19] .The method was based on an oxidation-reduction reaction in alkaline conditions, where the phenolate ion was oxidized while Folin's reagent was reduced, turning the solution blue. Many of the active components in propolis, such as phenolic acids and flavonoids, have a phenolic nucleus and can be evaluated by this method. A calibration curve was built using standard aqueous solutions of phenol containing between 2 and 12 µg mL^{-1}. One mL of each solution was added to 250 µL of sodium carbonate-tartarate buffer and 25 µL of the Folin–Ciocalteau reagent in a test tube, homogenized and allowed to react for 30 minutes at a temperature of 20 °C. Absorbance was measured at 700 nm on a Shimadzu 160 spectrophotometer and the calibration curve calculated by the minimal squares method. The dry extracts of propolis were dissolved in absolute alcohol to a concentration of 20% (w/v), one mL of this ethanolic solution was further diluted in 1000 mL of distilled water and homogenized. One mL of this final solution was prepared and analyzed in the same way as the standards. The results are given as a percentage of the dry extract in weight.

2.4TLC analysis[27]

The analysis was performed on precoated 20X 20 cm (0.25 mm thick) TLC plates K6F silica gel 60 A purchased from Whatman, USA. 10 µL of each standard solution (concentration 0.1 mg ml^{-1}) was applied as spots onto TLC sheets. The mobile phase was n-hexane:ethylacetate:acetic acid,31:14:5 to establish the R_f value for every standard (all solvents were of analytical grade). The plates were developed at room temperature in a vertical separating chamber to the height of approximately 16 cm from the start. The chamber was previously saturated with the mobile phase (saturation time was 1 hour). After drying, visualization was performed in spraying with 1 % sulphric vanillin reagent; 1% solution of vanillin in conc. H_2SO_4 heated for 5 minute at 105C^o.

2.5 HPLC analysis of flavonoids and phenolic acids

The authentic standards: 95% naringenin, 98% quercetin, 90% kaempferol, 99% acacetin, 97% CAPE, 99% caffeic acid, 98% p-coumaric acid, 98% ferulic acid were obtained from Sigma ; 95% galangin, 96% chrysin, 99% trans-cinnamic acid from Aldrich and 98% pinocembrin and 98% pinostrobin from Indofine Chemical. AWaters HPLC system comprised of a 600E multi-solvent delivery pump, a 486 UV–VIS detector was employed to analyze the propolis extract. The sample was filtered through a 0.45 mm PVDF membrane (Millipore Corp., USA) before each analysis. A reversed phase column (250 mm -4.6 mm i.d., RP-C8 5U, Macherey-Nagel,Germany)was used to partition flavonoids and tow phenolic acids in the extract. The mobile phase consists of 0.1% phosphoric acid aqueous solution (A) and methanol (B). The gradient program initially set at 65% A, linearly decreased to 50% A within 15 min, then held at 50% A for 20 min, and finally decreased to 35% Awithin 15 min. The flow ratewas 1 mL/min; the injection volume was 10 mL; the UV detector was set at a wavelength of 280 nm, and the column temperature was held at 308 K. The correlation coefficient (R^2) exceeded 0.996 for each linear calibration curve from 10 to 400 mg/g solution for flavonoids and from 10 to 500 mg/g solution for phenolic acids. The minimum detection limit was in the range of 90–150 ng/g solution.

2.6 In vivo study

Animal Studies: Animal studies were carried out according to the Care and Use of Laboratory Animals. Male and female CBA inbred mice from our conventional mouse colony were used. In all experiments, mice were of the same sex and were approximately 3 months old at the initiation of the study. The animals were housed not more than 5 per a cage and were maintained on a pellet diet and water. Experimental groups were composed of 7-10 mice each.

Tumor: A transplantable mammary carcinoma (MCa) of spontaneous origin in a CBA mouse was used. The tumor is weakly immunogenic for syngeneic recipients as shown by different methods *in vivo*[28]**.**

Tumor-Cell Suspension: Single-cell suspensions were prepared by digestion of tumor tissue with trypsin, which contained no visible regions of necrosis or hemorrhage[29]. Each suspension was passed through a stainless steel mash (200 wires/inch), centrifuged three times at 24 ***g*** for 5 min in saline and then resuspended in medium RPMI-1640 .supplemented with 5% serum from normal syngeneic mice. Viable cells were counted in a hemocytometer. Viability determined by observing the ability of intact cells to exclude Trypan blue dye was found to be greater than 95%.

Production of Subcutaneous Tumor in the Leg: Tumors in the hind leg were generated by subcutaneous injection of 10^5 tumor cells. The growth of tumor at the site of tumor cell inoculation was checked every day thereafter. During the experimental period mice were weighed every 5 d.

Survival Analysis: For the survival analysis, CBA mice were inoculated with 10^5 MCa cells at exact site of subcutaneous injection of different doses of PE at doses of 50 or 150 mg kg^{-1}. The endpoint of experiments was determined by spontaneous death of the animals. Results are expressed as percentage of mean survival time of treated animals over mean survival time of the

control group (treated *versus* control, T/C %). The percentage of increased lifespan (ILS%) was calculated according the formula: ILS%=(T-C)/C X 100 where T represents mean survival time of treated animals and C represents mean survival time of the control group. By UN National Cancer Institute criteria, T/C exceeding 125% and ILS exceeding 25% indicate that the drug has significant antitumor activity[30].

Cell Lines**:** The experiments were performed using human cervical carcinoma cells. The average doubling time in log phase was about 20 h for HeLa cells. Cells were grown in monolayer cultures in plastic disposable Petri dishes (Falcon) in minimal essential medium (MEM) with 10% bovine calf serum. Cell cultures were incubated at 37 °C in a humid atmosphere containing 5% CO_2 in air.

2.7 In vitro study[31]

Cytotoxicity against cancer cell lines: (PE) were tested for cytotoxic activity against four cancer cell lines: SF-295 (Central Nervous System), HCT-8 (colon), MDAMB-435 (breast) and HL-60 (leukemia) all from Cancer Researchs Center, Baghdad. All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37C° with 5% CO_2. PE was dissolved water to obtain a concentration of 1 mg/mL. They were incubated for 72 h. The negative control received the same amount of water. Doxorubicin (0.1-0.58 μg/mL) was used as a positive control. The cell viability was determined by reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2, 5-diphenyl-2H-tetrazolium as described by Mosmann[31].

3. RESULTS

3.1Propolis extract

Propolis was extracted using only absolute ethanol alcohol by soxhlet and by maceration at room temperature for 7 and 10 days in order to observe how these procedures affected the yield and total phenolic content of the extract (table 1). The use of the Soxhlet resulted in higher yields. No significant difference was observed between the yields of samples of the three extracts.

Table1. Iraqi PE yield and phenolic content of samples treated with absolute alcohol by Soxhlet and maceration.

Extraction Method	% yield (PE)	Total phenolicsa(mg/g)
Soxhlet	31.25 ± s.d. 1.52	29.29 ± s.d. 1.03
Maceration 7 days	25.67 ± s.d. 2.12	21.56 ± s.d. 2.65
Maceration 10 days	27.03 ± s.d. 0.91	21.90 ± s.d. 1.12

(a) Total phenolics were determined by Folin-Ciocalteau[15] method.

The following figure (Fig.1.) shows the standard graph that was used to determine total phenolics as described in analytical procedure (item 2.3). The figure also shows the regression equation, correlation index (r) and standard deviation (s.d.) for the curve.

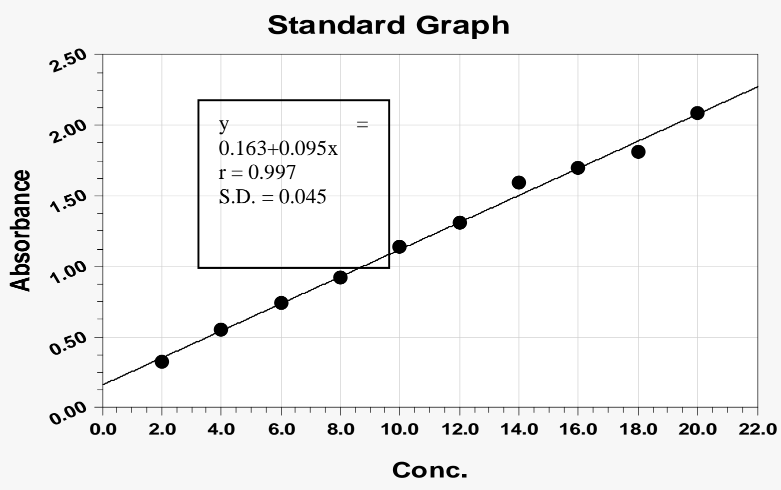

Figure 1. Standard graph for determination total phenolics by Folin-Ciocalteau method

3.2TLC analysis

The following table (table 2.) summarizes the TLC analysis results ,and indicates the R_f Values tabulated and observed for each analyte ,Furthermore ,Molecular weight (MW), General Class and the Chemical structure for phenolics (flavonoids and phenolic acids) for Iraqi Propolis constituents were shown.

Table 2.TLC analysis results; R_f values, Molecular Weight (MW), General Class and Chemical structure for Iraqi propolis constituents.

Standard	R_f value Tabulated[36]	R_f value Observed	MW (g/mole)	Class	Structure
Chrysin	0.68	0.68	254.237	flavonoid	
Galangin	0.72	0.71	270.236	flavonoid	
Naringenin	0.52	0.53	272.252	flavonoid	
Caffeic acid	0.43	0.41	180.157	Phenolic acid	
p-Cumaric acid	0.69	0.69	164.158	Phenolic acid	
Pinocembrin	0.60	0.58	256.253	flavonoid	

3.3 HPLC analysis

Some of the samples extracts were analyzed by HPLC (Table 3). The composition of the extracts, the relative concentration of the components, which is in agreement with the yield of PE of the phenolic compounds identified in the propolis extracts, several

have been mentioned in other papers studying the antibiotic[32-33] antioxidant[34] anti-inflammatory[35] cytotoxic[36] and antiparasitic[32] effects of propolis.

Table 3. Composition and quantification by HPLC of selected extracts of Iraqi PE obtained by maceration, results in mg g^{-1} dry extract

Compounds	*Weight mg /g extract*
Chrysin	***2.63***
Galangin	***2.21***
Naringenin	***2.35***
Caffeic acid	***2.09***
p-Cumaric acid	***16.11***
Pinocembrin	***3.12***
Total phenolics	***28.51***

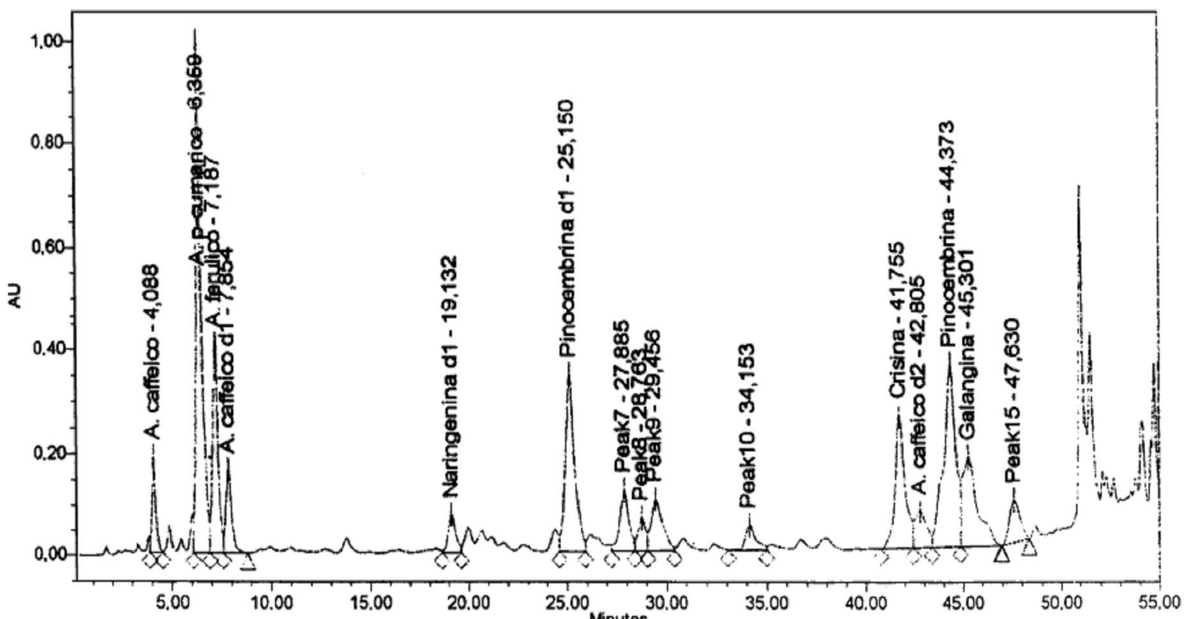

Figure 2.HPLC Chromatogram for Iraqi PE

Figure 2. Shows the HPLC chromatogram obtained from the maceration sample.

3.4In vivo study

Direct Antitumor Activity of PE

Figure 3 and Table 4 summarize whether the presence of PE in the tissue administered locally at the exact site of tumor cell inoculation influenced tumor formation and the survival rate of mice. Test compounds (50 or 150 mg/kg) were injected subcutaneously into the legs. Immediately after injection mice received subcutaneous injection containing 10^5 viable tumor cells. The appearance of tumor was checked daily. Figure 3 shows that tumor formation was dose dependently delayed in mice treated with polyphenolic compounds. While all mice in the control group developed tumors within 22 d after tumor cell inoculation and died between 28 and 40 d thereafter, the development of tumors in treated mice and their survival time were delayed; survival of mice treated with PE was significantly longer. The presence of PE in the tissue of tumor cells inhibited tumor growth and ILS of mice by 56.45 to 75.80% (Table 4).

To compare toxicity profiles of the test components, we administered them subcutaneously at dose of 50 and 150 mg/kg to nontumor-bearing mice. Moreover, in tumor-bearing mice treated with PE, a delay in tumor growth and significant increase in survival without mortality or body weight loss was noted (Table 5).

Table 4. *In Vivo* Antitumor Activity of Iraqi PE[(a)]

Group	Mice per group	Dose mg/kg	Survival time rang (day)	Mean survival time (day)	ILS%	T/C %
Control (normal)	8	-	52-73	62.50	-	-
Control (cancered)	8	-	23-39	31.00		
PE	8	50	45-52	48.50	56.45	156.45
		150	39-70	54.50	75.80	175.80

(a) CBA mice were injected subcutaneously with 10^5 mammary carcinoma cells at the exact site of subcutaneous injection of PE .T/C, treated versus control. ILS% (increased life span %)=(T-C)/C X 100 ;T, mean survival days of treated group ;C, mean survival days of control group.

Kaplan-Meier survival estimates, by dose

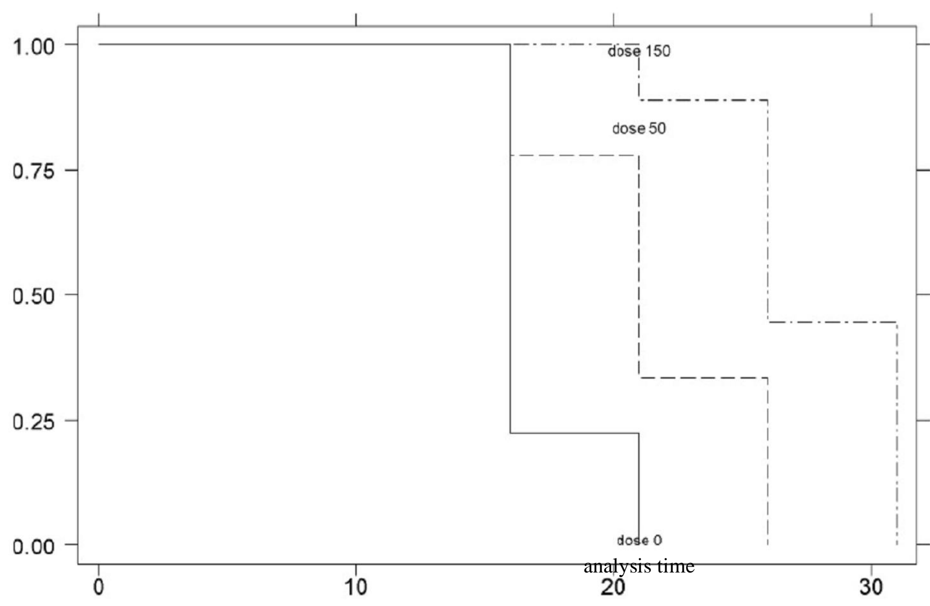

Figure 3. Sequential Observation of Palpable Mammary Tumor in CBA Mice Inoculated with 10^5 Mammary Carcinoma Cells at the Exact Site of Subcutaneous Injection of Propolis Extract.

Tumor cells were introduced immediately after test compound injection. Each group comprised 8 mice. Tumor formation of mice treated with PE was significantly delayed (*p*=0.0127 or *p*=0.000, log-rank test).

Table 5. Change in body weight of mice treated with PE .

Group	Dose (mg/kg)	%weight change per days						No. of toxic death
		0	5	10	15	20	25	
Control (Normal)	-	20.05	+0.19	+1.01	+1.10	+1.03	+1.09	0
Control (Cancered)	-	21.17	+0.11	+0.32	-0.10	-2.1	-2.9	0
PE	50	21.30	+0.21	+0.69	+0.71	+0.98	+1.12	0
	150	20.50	+0.10	+0.92	+0.93	+1.09	+1.10	0

3.5 In vitro study

The Iraqi PE was tested in vitro against four cancer cells in comparison to doxorubicin, the positive control, by using Mosmann assay[31]. The concentrations that induce 50% inhibition of cell growth (IC_{50}) in µg/mL are reported in Table 6. Compounds were classified by their activity as highly active (IC_{50} < 1 µg/mL), moderately active (1 µg/mL < IC_{50} < 10 µg/mL), or inactive (10 µg/ mL > IC_{50})[37]. The great majority of the PEs are strongly cytotoxic against all cancer cell lines with IC50 below 1 µg/ mL, PE showed a higher cytotoxicity for breast cancer (MDA-MB-435) cell line when compared to doxorubicin, a fact that supports their anti-cancer activity.

Table 6. Cytotoxic activity expressed by IC_{50} in µg/mL of PE for cancer cell lines[a]

Test		HL-60	HCT-8	MDA-MB-435	SF-295
Doxorubicin "positive control"	**IC_{50}**	0.02	0.04	0.47	0.25
	95% confidence interval	0.01-0.02	0.03-0.05	0.34-0.65	0.17-0.36
PE	**IC_{50}**	0.33	0.64	0.23	0.50
	95% confidence interval	0.25-0.45	0.53-0.78	0.18-0.30	0.37-0.68

*(a)*Data are presented as IC_{50} values and 95% confidence intervals obtained by nonlinear regression for all cell lines.

4. Discussion

The life span of mice treated with PE before tumor cell inoculation was significantly prolonged compared with controls[38]. To determine direct effects on tumor growth, we injected the test compounds locally and immediately thereafter at the site of tumor cell inoculation. The data shown in Table 4 and Fig. 3 indicate that the presence of PE in the tissue of tumor cells inhibited tumor growth and increased the survival life span percent of treated animals by 56.45to 75.80%, respectively. Moreover, the local presence of PE in the tissue caused a significant delay in tumor formation (p=0.0127 or p=0.000, log-rank test); delay of tumor formation could be the main reason for the ILS of mice treated with PE. It was shown that animals treated with immunostimulants resist, to various degrees, subsequent inoculation of tumor cells as evidenced by the reduced "tumor take," slowed growth of the tumors, and prolonged survival of recipients[1,6,5,23].Scheller *et al*[5] reported that the ethanolic extract of propolis is capable of increasing survival of mice bearing Ehrlich carcinoma and suggested that immunostimulant activity of propolis may be associated with macrophage activation and enhancement of macrophage phagocytic activity. Matsuno[23] reported that various components of propolis have potent anti-inflammatory and antitumor activity. In addition, Hayashi *et al.*[6] showed that quercetin chalcone and modified citrus pectin reduced the growth of solid colon-25 primary tumor when given to mice. So far, no experiment has been performed on the local treatment of solid MCa with polyphenolic compounds. Kimoto *et al.*[1] reported that artepilin C (a component of propolis) has cytostatic and cytotoxic effects on various malignant tumor cells *in vitro* and *in vivo* and that it activates the immune system, especially by increasing the number of macrophages and their phagocytic activity as well as the number of lymphocytes, and has direct antitumor activity. This paper presents data showing that direct cytotoxic effects of polyphenolic compounds are likely to be the most important mechanism of antitumor activity of the test compounds *in vivo* and *in vitro*. Propolis and its compounds stimulate macrophages and reduce the number of MCa metastases in CBA mice[38]. Polyphenolic compounds also delayed tumor formation and increased survival when administered locally at the exact site of tumor cell inoculation (Table 4, Fig. 3). These findings suggest that Iraqi PE suppress MCa growth *via* another mechanism(s) different from those mentioned above. These mechanisms include the ability of PE to inhibit DNA synthesis in tumor cell cultures and the induction of apoptosis of tumor

cells[39,40] Furthermore, the ability of PE to induce apoptosis suggests their potential use in clinical trials as therapeutic anticancer agents. It is likely that the antitumor activity of PE is highly dependent on dose; higher doses exhibited more potent antitumor activity. It has been demonstrated[40] that higher doses of phenolics interfered with cell processes such as enzyme and glutathione levels; this may induce cells death or apoptosis[39,40] and exhibit cytotoxicity in oral cancer cells[41]. Differential cytotoxicity toward tumor cells has been demonstrated through modulation of the cellular redox state[42]. A study by Coffey *et al*.[43] showed that thiol depletion can effectively activate caspase 3 and subsequently induce cancer cell apoptosis. Treatment with phenolics caused rapid activation of caspase 3 after 4 h, downregulation of Bcl-2 expression after 6 h, and upregulation of Bax expression after 16 h in human leukemic HL-60 cells[44].

5. Conclsions

Iraqi Propolis extract (PE) can prepare by soxhlet method with excellent yield and high phenolics (phenolic acids and Flavonoids) content and can be utilize safely as an antitumor drug. The present results show that the antitumor activity of Iraqi PE is a direct cytotoxicity to tumor cells and affect tumor growth through the inhibition of DNA synthesis and exert direct antitumor effects by close contact with tumor cells.

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