Original Research

Journal of Apitherapy
www.japitherapy.com
DOI: 10.5455/ja.20170203075953

Evaluate the cytotoxic activity of honey, propolis, and bee venom from different localities in Egypt against liver, breast, and colorectal cancer

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Received: January 09, 2017
Accepted: January 23, 2017
Published: February 04, 2017

ABSTRACT

Introduction: Cancer is a large group of disorders characterized by uncontrolled cellular proliferation. It is one of the most devastating diseases all over the world. Recently, there is an increased interest in the clinical use of natural products as a safe, efficient, and economic therapeutic alternative. Honey bee products therapy, apitherapy, was used to control various diseases including cancer. **Objectives:** The primary objective of this study was to screen potential cytotoxic effects of honey bee products against different cancer types. **Materials and Methods:** Samples of honey, venom and propolis were collected from different Egyptian localities with different techniques and tested by MTT cell-based assay against liver (HeP-G2), breast (MCF-7), and colorectal (Caco-2) cancer cell lines. **Results:** The results showed that, Italian dissected method-bee venom was the most active among all the tested samples against Hep-G2 (93.92%), Caco-2 (93.92%) and MCF-7 (90.17%). However, Libyan propolis proved to be most active among all tested propolis samples. On the other hand, all tested honey samples showed non-remarkable activities except Asyut-clover honey (49.84% against Caco-2 cell line). **Conclusion:** The results showed that, bee venom, propolis and honey are interesting agents that have valuable activities against Hep-G2, Caco-2 and MCF-7 with bee venom being the most effective agent that might be incorporated in cancer remedy regimens after further studies.

KEY WORDS: Apitherapy, bee venom, Caco-2, Hep-G2, honey, MCF-7, propolis

INTRODUCTION

Cancer is a large group of disorders characterized by uncontrolled cellular proliferation. Cancer cells are also capable of metastasizing to other regions causing a number of devastating outcomes [1]. Nearly, all body organs are vulnerable to cancer with liver, colon, and breast being the most common ones. Hepatic cancers are the third leading cause of cancer-associated deaths worldwide, and currently, the frequent cause of deaths in cirrhotic patients [2]. Colon cancer has an estimated incidence of over one million new cases annually worldwide [3]. Almost one of three patients with colon cancer dies from the disease. Colon cancer also more often affects people of well-developed countries in comparison to less developed countries [4]. Breast cancer is the leading cause of female mortality, more than a half a million deaths were reported in 2012. It continues to represent the most frequently diagnosed cancer in females with more than 1.7 million new cases diagnosed in 2012; it represents 25% of all new cancer cases diagnosed in women [5].

Does this mean that we are helpless against cancer? The answer to this question was presented in 1994 in Cancer letters by Badria [6], whereas 62 Egyptian food and medicinal preparations were extensively examined for antimutagenic/anticarcinogenic activity using short-term and host-mediated assays. The antimutagenic activity of the substances examined was ranked as follows: 13 (strong), 7 (mild), and 5 (weak) after metabolic activation. Metabolic activation seems to be necessary for most antimutagenic substances in this study, e.g., radish inhibits 29% of mutagenicity produced in direct antimutagenic assay and inhibits 89% of mutagenicity induced in host-mediated assay. Hence, there is an urgent need for discovery of new regimen for hepatocellular carcinoma (HCC) treatment. Recently, many antitumor compounds with new structural features and mechanism of action have been isolated from natural products. Natural products serve as a good and affordable source for new drug entities. Different vaccines and biologics have been inspired from natural products structure. Such as betulinic acid and its analogous had an inhibitory activity against topoisomerase [7]. Oleogum resin of *Boswellia carterii* showed antiproliferative activity on T-lymphocyte culture [8]. Moreover, the combination of *Boswellia serrata*, licorice root (*Glycyrrhiza glabra*), and turmeric root (*Curcuma longa*) was used in the control of bronchial asthma because of their leukotriene inhibition, anti-inflammatory, and antioxidant activity, respectively [9]. These combinations of terpenoids were also used in the treatment of knee osteoarthritis and hepatitis C [10]. Cucurbitacin proved to have potent *in vitro* and *in vivo* activities toward HCC [11]. Recently, cucurbitacin B used as antitumor activity against ovarian cancer cell line (A2780) and as a chemosensitizer for cisplatin cytotoxicity in cisplatin-resistant ovarian cancer cell line (A2780CP) in two-dimentional and three-dimentional culture model [12]. Recently, many antitumor compounds with new structural features and mechanism of action have been isolated from natural products. Natural products serve as a good and affordable source for new drug entities [13-19].

Apitherapy (Apis is a Latin word means bee) is the practice of using bee products such as honey, bee and venom propolis for disease prevention or treatment. It can be also described as the science (and art) of using honey-bee products, to maintain health and assist the individual in regaining health [20]. Bee venom (BV) has been used to treat diverse disorders. The main component of BV, which constitutes approximately 50% of its dry matter, is melittin (MEL) [21-24]. MEL is 26 amino acids long peptide. Modern pharmacological studies showed that MEL exerts various antitumor effects by inhibiting tumor cell growth [25,26], promoting tumor cell apoptosis [27,28], and inhibiting angiogenesis [29] and migration [30,31]. It is also reported to have strong hemolytic activity [32]. Propolis is the resinous mixture that honey bees collect from different sources to use it as a sealant for unwanted open spaces in hives. Propolis as anti-inflammatory, antioxidant, anti-infective, and anticancer agents has been studied [33]. Honey is a natural substance formed from nectar by honeybees. Honey constituents have been reported to exert anti-inflammatory, antioxidant, antiproliferative, antimetastatic, and anticancer effects [30-34]. Apitherapy could be used as cancer therapeutic agent or to complement conventional cancer treatments.

MATERIALS AND METHODS

About 10 honey bees colony same equal powers and number of comb almost were selected and divided into two groups. In each of them five colonies, Group A has Queen of Carniolan hybrid, second Group B has headed by the Queen of Italian hybrid.

Materials

Honeybee products

Honey

About 15 honey samples were collected from different localities in Egypt depending on geographic area as well as nectar producing plants in each area. Honey samples were kept in airtight, plastic containers at 4°C until treatment Figure 1a.

Propolis

Propolis samples were obtained from three different sources. Two honey bee hybrid strains colonies propolis were collected (Carniolan - Italian). Scraping propolis from the hive monthly at the end of each month throughout the season study. The weight of the amount of propolis obtained monthly for a year of study. Compared between the amount of propolis obtained from the two hybrid strains (Carniolan - Italian), the amounts of propolis obtained in different seasons (winter - autumn - spring - summer).

Figure 1b shows Bulgarian, Libyan, and Egyptian propolis.

BV

Powdered BV was obtained via lyophilization (freeze-drying) after collection from diverse honey bee strains by two different collection methods; Figure 1c shows BV (Craniolian - dissected, Craniolian - electric shock, Italian - dissected, and Italian - electric shock).

Materials used in the biological assays

Cell lines; Hep-G2, Caco-2 and MCF-7 (Holding Company for Biological Products and Vaccines, VACSERA, Agouza,

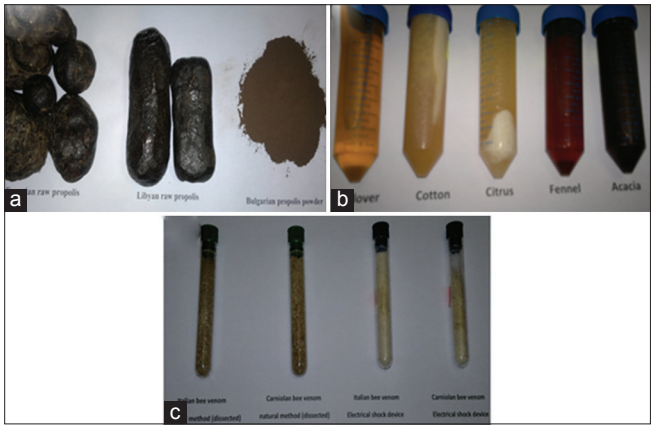

Figure 1: (a) Honeys of different types of vegetable sources and geographical locations, (b) Propolis (Egyptian, Libyan, Bulgarian), (c) bee venom (Craniolian - dissected, Craniolian - electric shock, Italian - dissected, Italian - electric shock)

Giza, Egypt), Dulbecco's Modified Eagle's medium (DMEM), fetal bovine serum (FBS), an antibiotic/antimycotic solution containing 1000 U/ml penicillin, 1000 μg/ml streptomycin and 25 μg/ml fungizone, phosphate buffer saline, dimethylsulfoxide (DMSO) and 3-(4,5-dimethyl-thiazoyl)-2,5-diphenyl-tetrazolium bromide (MTT) (Sigma-Aldrich, St Louis, MO, USA), 96-well plates, tissue culture treated polystyrene (#3512, Corning Inc., NY, USA), ELISA BioTek Lx800 microplate reader (BioTek, Bedfordshire, UK) were used for cell-based cytotoxicity assay.

Methods

Collecting honeybee products

Honey

Honey samples collection from colonies from different plant nectar sources [Table 1] depending on different geographical locations was conducted.

BV

two honeybee hybrid strains venom were collect (Carniolan - Italian) by dissected method (manual) and BV electric shock device (automated) obtained from the Department of Bee Research Qalyubia - Agricultural Research Center - Egypt.

Dissected method (manual way method) according to the method of; Pence (1981) [35] with one exception that the author was replaced heat by cold. 500 random worker bees from every strain in the month middle of each month throughout the duration of the study, which extends to 12 months collected, then placed in the refrigerator for drugging the worker, removed the stinging with venom sac and placed in a 10 ml distilled water in a Petri dish. So placed in a centrifuge tubes device under cooling at a temperature of −15°C for 15 min the number of laps 5000 laps/min. Then, we grinding output to bring out all the venom, the amount of liquid with venom takes, and put it in the drying device (lypholizer) under cooling for a period of eight hours, after the drying scraping venom by scalpel and then weighed Figure 2.

Propolis

Three honeybee colonies sources, Egyptian, Libyan and Bulgarian, were used to collect and prepare the propolis samples. They were obtained by scraping propolis from the hive at the end of each month throughout the season.

The scraped resinous material was shade dried, ground in a tooth miller. Each propolis powder was then extracted by 70% MeOH under sonication for one hour at 60°C until exhaustion. After filtration, the solvent was removed by rotary evaporation under reduced pressure at temperatures below 45°C. Crude extracts were refrigerated at 0°C until used in the assay. DMEM medium was used to prepare serial dilutions from DMSO stock solutions, DMSO limit 0.02% v/v.

Cytotoxicity assay using MTT

Activity as cytotoxic agents against hepatic, colorectal, and breast cancers was tested in a cell-based assay using Hep-G2, Caco-2 and MCF-7 cell lines, respectively, by MTT assay. Cell lines were cultured in complete growth DMEM media containing 10% FBS, 1% penicillin/streptomycin, and incubated at 37°C with 5% CO_2 and 90% relative humidity. All cell passages used were between passages 30 and 40. 5-FU or cisplatin was used as standard cytotoxic agent. The compounds were dissolved in DMSO-free media (water-soluble components) or DMSO/Media vehicle so that the DMSO limit does not exceed 0.05%.

The viability of cells was measured colorimetrically using MTT assay which indicates mitochondrial metabolic activity. This assay depends on measuring the activity of mitochondrial

Table 1: List of tested honey samples

Honey type samples			Production date	Governorate	Location
Common name	Ripening	Scientific name			
Clover	Ripe	*T. alexandrinum*	June 2014	Kafr El-Sheikh	eshaka
Clover	Unripe	*T. alexandrinum*	June 2014	Kafr El-Sheikh	eshaka
Clover	Ripe	*T. alexandrinum*	June 2015	Kafr El-Sheikh	eshaka
Clover	Unripe	*T. alexandrinum*	June 2015	Kafr El-Sheikh	eshaka
Clover	Ripe	*T. alexandrinum*	June 2014	Assuit	Dirout
Clover (market honey)	Ripe	*T. alexandrinum*	2014	Giza	Giza
Banana	Ripe	*M. acuminate*	October 2014	El-Beheira	Badr
Acasia (somrah honey)	Ripe	*A. tortilis*	July2014	Saudi Arabia	South area
Fennel	Ripe	*F. vulgare*	May 2014	Asyut	Moaabdah village – Abanoub
Sider	Ripe	*Ziziphus* spp.	October 2014	Asyut	Asyut
Brazillian pepper	Ripe	*S. terebinthifolia*	October 2014	Alexandria	Al-Sabahia
Cotton	Ripe	*G. barbadense*	September 2014	Kafr El-Sheikh	Mneat al-ashraf – fwah
Medical plants (Coriander+Black cumin+Anise)	Ripe	*C. rsativum*+*N. sativa*+*P. anisun*	May 2014	El-Minya	Smalot
Citrus	Ripe	*Citrus* spp.	April 2014	El-Beheira	Badr
Sunflower+Sesame	Ripe	*Helianthus annuus*+*S. umindicum*	August 2014	Asyut	Dirout

T. alexandrinum: Trifolium alexandrinum, M. acuminate: Musa acuminate, A. tortilis: Acacia tortilis, F. vulgare: Foeniculim vulgare, S. terebinthifolia: Schinus terebinthifolia, G. barbadense: Gossypium barbadense, C. rsativum: Coriandru rsativum, N. sativa: Nigella sativa, P. anisun: Pimpinella anisun, H. annuus: Helianthus annuus, S. umindicum: Sesam umindicum

nicotinamide adenine dinucleotide phosphate-oxidase dependent reductase that converts the yellow tetrazolium salt, MTT, to a purple formazan product that is water-insoluble. After solubilization of formazan crystals, the purple solution is easily measured quantitatively using ELISA plate reader at wavelength of 540 nm (Hussain *et al*., 1993; Mosmann, 1983) [36,37].

Cell lines were cultured in 96-well plates (1×10^5 cells/mL). After incubation, the medium was removed and the wells were treated with 100 μL of 5 mg/mL MTT and incubated for 4 h at 37°C. Then, 100 μL of solubilizing solution, DMSO, were added to each well and the produced purple solution was quantified colorimetrically at 540 nm.

Statistical analysis

The cytotoxic activity of the test compounds was indicated by the ratio of tested well to negative control and IC_{50} was calculated. Cytotoxic activity was calculated from the following formula:

$$\%\,\text{Activity} = \frac{OD_{\text{-ve control}} - OD_{\text{test}}}{OD_{\text{-ve control}}} \times 100$$

Analysis of data was performed using GraphPad Prism V6.01 (GraphPad Software Inc., San Diego, CA, USA). Mean comparisons were made with significant differences reported at $P < 0.05$, replication $n = 3$.

RESULTS

Craniolian and Italian BV (by dissected method) recorded highest activity on Hep-G2, with 93.92% inhibition, followed by Italian venom (electric shock device) with 93.49%. In Caco-2 cell lines, the dissected Italian venom recorded highest effect, 93.23%, followed by shock device Italian venom, 92.72%. On MCF-7, the dissected Italian venom recorded highest effect, 90.17% followed by shock device Italian 90.21%. Data are summarized in Table 2 and Figure 3. Data in the Table 2 and Figure 4 show that, in Hep-G2 the Libyan propolis recorded highest activity with 84.99% inhibition followed by Bulgarian powdered propolis, 58.97%, while the lowest value was with Egyptian propolis, 7.87%. Cinnamic acid showed 12.52% inhibition. In Caco-2, the Libyan propolis recorded highest inhibition, 79.63%, followed by Bulgarian powder propolis, 51.87%. The lowest value was recorded with cinnamic acid, 22.82%, followed by Egyptian propolis 27.73%. While in MCF-7 the Libyan propolis recorded highest activity, it showed 83.57% inhibition, followed by Bulgarian powder propolis 75.65%. The lowest value recorded was ferulic acid, 14.89%, followed by Cinnamic acid, 27.06% inhibition.

In honey samples as shown in Table 2 and Figures 1 and 5, the sunflower honey (*Helianthus annuus*) and sesame honey (*Sesamum indicum*) recorded highest activities on Hep-G2, 32.92%, followed by Brazilian pepper (*Schinus terebinthifolia*), 32.68%. While the lowest value recorded in clover (market honey) (*Trifolium alexandrinum*), it was 1.75% followed by ripe clover Kafer Elsheik 2015 (*T. alexandrinum*), 2.07%. In

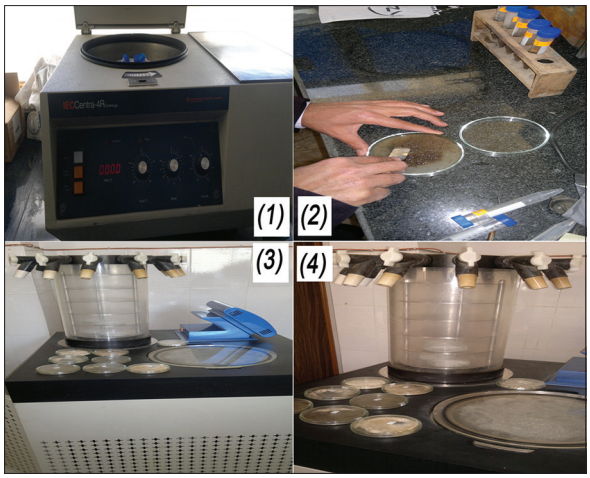

Figure 2: Be venom preparation

Table 2: % cytotoxicity of different honey bee products, with respect to their sources as well as their collection methods, in Hep-G2, Caco-2 and MCF-7 cell lines

Honey bee products*	% inhibition activity		
	Hep-G2	Caco-2	MCF-7
Control			
5-flouro uracil*	12.92	69.93	0.00
Cis-platin*	85.30	90.24	86.23
Bee venom			
Craniolian-dissected	93.92	92.50	88.86
Craniolian-electric shock	91.85	91.76	86.28
Italian-dissected	93.92	93.23	90.17
Italian-electric shock	93.50	92.72	90.21
Propolis			
Bulgarian powder propolis	58.97	51.87	75.65
Egyptian propolis	7.87	27.73	73.92
Libyan propolis	84.99	79.63	83.57
Propolis's main components			
Caffeic acid	0.00	0.00	0.00
Cinnamic acid	12.52	22.82	27.06
Ferulic acid	15.11	30.32	14.89
Honey			
Acasia	0.00	30.77	2.53
Banana	21.03	33.48	21.63
Brazilian piper	32.68	31.11	27.76
Citrus	32.47	35.68	52.53
Clover-Asyut	13.92	49.84	19.90
Clover (Ripe - Kafer Elsheik 2014)	13.56	30.94	28.14
Clover (Ripe - Kafer Elsheik 2015)	2.07	40.08	22.24
Clover (Unripe - Kafer Elsheik 2014)	24.84	34.22	25.56
Clover (Unripe - Kafer Elsheik 2015)	16.39	41.38	15.45
Clover (Market honey)	1.75	44.48	27.76
Cotton	20.08	29.25	45.93
Fennel	11.75	25.53	32.91
Medicinal plants (coriander/black cumin/anise)	8.25	36.64	39.28
Sider	15.94	34.22	29.87
Sunflower/sesame	32.92	26.37	8.47

*All compounds were used in concentration of 100 μg/ml

Caco-2, the Asyut clover honey (*T. alexandrinum*) recorded highest effect, 49.84%, followed by market Clover honey (*T. alexandrinum*), 44.48%. While the lowest value was recorded in fennel honey 25.53 followed by sunflower (*H. annuus*) and sesame honey (*S. indicum*), 26.37%. in MCF-7, the *Citrus*

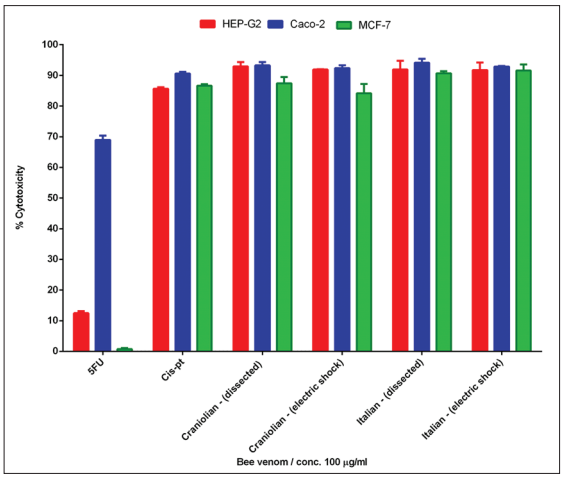

Figure 3: % cytotoxicity of different bee venoms at 100 µg/ml against Hep-G2, Caco-2 and MCF-7 cell lines, using 5-FU and Cis-pt as positive controls

(*Citrus* spp.) honey recorded highest activity, 52.53%, followed by Cotton (*Gossypium barbadense)*, 45.93%. While the lowest value recorded in Acasia honey with 2.53% inhibition followed by Sunflower (*H. annuus*) and Sesame *(S. indicum)*, 8.47% inhibition.

DISCUSSION

Honeybee's products (Honey - BV - Propolis) consider as a traditional medicine and dietary natural products and have recently become the focus of attention in the treatment of certain diseases as well as promoting overall health and well-being. There is strong evidence supporting the positive role of natural food and food product on the induction of apoptosis in different tumor cells. In this regard, we investigated the effect of all products against liver (Hep-G2), breast (MCF-7), and colorectal cancer (Caco-2). Honey samples have variable effect against the three cancer types (1.75% in clover [market honey] on Hep-G2 and the highest inhibition activity 52.53% in *citrus* on MCF-7).

We suggested that effects of honey on breast cancer (MCF-7) may be its antagonizes estrogen activity, inhibits cell proliferation, induces apoptosis, reduces mitochondrial membrane potential [38-40]. As liver cancer (Hep-G2) its Inhibits cell proliferation, suppresses angiogenesis, induces apoptosis, protects against mutagen-induced DNA damage [41-46] and colorectal cancer (Caco-2) its Inhibits cell proliferation, induces apoptosis, arrests cell cycle, reduces mitochondrial membrane potential, increases generation of reactive oxygen species, depletes intracellular non-protein thiols, induces DNA damage, and suppresses inflammation [47-49].

The venoms from stinging bees important weapons in defense of the colony or themselves. A single sting will kill other insects instantly but will only cause a transient and bothersome local inflammatory reaction characterized by pain, redness and swelling in humans. Venom has been utilized as pain reliever and as treatment against inflammatory diseases since ancient times. In this regard, we investigated the effect of venom against liver, breast, and colorectal cancer. We found that all venom treatments have effect against liver, breast, and colorectal cancer as showed in Figure 3. The affect was ranged between the lowest inhibition activity 86.28% in Craniolian - electric shock on MCF-7 and the highest inhibition activity 93.92% in Craniolian, Italian - dissected on Hep-G2. Craniolian - electric shock, respectively.

From the obtained results, it could be suggested that BV collected by (dissected method) recorded the highest inhibition toward cancer cell lines, it could be concluded that it might contain more protein content than that obtained by electric shock device and may extract all venom components especially volatile components.

This study supports the findings of the previous study where first, research conducted by Hamedani *et al.* [50] recommended the antitumorogenic property of BV in the treatment process of cancer which was paralleled with findings of Magnan *et al.* [9,27]. The results of this study revealed that, honey BV affects cell proliferation, MMP-2 activity and interferon beta production in a time and dose-dependent manner. MEL is the main compound found in BV [51]. Modern pharmacology studies have observed that MEL exerts various antitumor effects by inhibiting tumor cell growth [25,26], promoting tumor cell apoptosis [24,27], and inhibiting tumor angiogenesis [23] and migration [34,31]. MEL exerts multiple effects on cellular functions of cancerous cells such as proliferation, apoptosis, metastasis, angiogenesis as well as cell cycle, and the anticancer processes involve diverse signal molecules and regulatory pathways, BV and its main component can inhibit the proliferation of cancer cells through induction of apoptosis, suppression of tumor metastasis, and invasion by regulating the expression of caspases, Ca^{2+} concentration, death receptors (DRs), extracellular matrix degradation enzymes, angiogenesis factors,and a variety of signal pathways [52]. BV and its main component MEL have been reported to affect multiple aspects of the tumor, including inhibiting cancer cell growth and/or proliferations, inducing apoptosis or suppressing tumor metastasis, suggesting that it could be an excellent alternative for the management of cancer [53]. The induction of apoptotic cell death through several cancer cell death mechanisms, including the activation of caspase and matrix metalloproteinases, is important for the MEL induced anticancer effects. The conjugation of cell lytic peptide (MEL) with hormone receptors and gene therapy carrying MEL can be useful as a novel targeted therapy for some types of cancer, such as prostate and breast cancer [29]. There are at least 18 active components in the venom which have some pharmaceutical properties [54]. Among these compounds, MEL, a small linear peptide consisting of 26 amino acids, is the major potent toxin of BV [55], which comprises ~50% of BV [56]. Moreover, several studies have demonstrated that BV and/or MEL have anticancer effects in liver [57,58], breast [59]. Genistein enhanced antitumor effects due to greater reduction in the DNA-binding activity of NF-κB [60]. Natural toxin BV could be useful as an anticancer agent through the overexpression of DR3 and inactivation of NF-κB for the treatment of lung cancer cells and drug-resistant cancer cells [61].

We proposed that effects of BV on breast cancer (MCF-7) may be its Suppressing cell motility and invasion by inhibiting PI3K/Akt/mTOR signaling pathway [62]. As Liver cancer (Hep-G2) its preventing cell metastasis, through inhibition of the Rac1-dependent pathway [31] and colorectal cancer (Caco-2) its inhibition of vascular endothelial growth factor (VEGF)-induced neovascularization [63]. Blocks VEGFR2 and the cyclooxygenase-2 (COX-2) mediated mitogen-activated protein kinase signaling pathway (tumor-promoting inflammation [64] blockade of epidermal growth factor - induced signaling (sustaining proliferative) [65], Immune activation (signaling) [66].

We come to the conclusion that propolis treatments have effect against liver, breast, and colorectal cancer as showed in Figure 4. The affect was ranged between the lowest inhibition activity 7.87% in Egyptian propolis on Hep-G2 and the highest inhibition activity 84.99% in Libyan propolis on Hep-G2. The effects were very clear The IC_{50} for venom at Hep-G2 was 84.99, 58.97, and 7.87 in Libyan propolis, Bulgarian powder propolis, Egyptian propolis, respectively. As the IC_{50} at Caco-2 was 79.63, 51.87, 27.73 in Libyan propolis, Bulgarian powder propolis, Egyptian propolis, respectively. While, The IC_{50} at MCF-7 was 83.57, 75.65, 73.92 in Libyan propolis, Bulgarian powder propolis, Egyptian propolis, respectively.

Propolis showed remarkable effects on MCF-7, HepG2 and Caco-2. It inhibited the growth of the cells. The cytotoxic activity of the different Propolis types can be correlated to their composition. The most active as anticancer were those with higher flavonoids and total phenolics composition content. These results are in agreement with Li *et al.* [67] who discussed the cytotoxicity of 13 cylcoartane-type triterpenes and four prenylated flavanones, isolated from propolis collected in Myanmar against a panel of six different cancer cell lines [68,69]. The hypothesis that breast cancer cells' viability gradually decreases depending on the increasing dose of caffeic acid phenethyl ester (CAPE). The estimated IC50 value amounted to 15 μM for MDA-MB-231 and MCF-7 cell lines by Wu *et al.* [23] was only slightly higher than the results obtained in our experiment, with 14.08 μM and 8.01 μM for MDA-MB-231 and Hs578T, respectively [70]. Propolis and the CAPE substantially inhibit the growth of the cells of triple-negative breast cancer of the lines MDA-MB-231 and Hs578T. The cytotoxic activity of compounds depends on the time of exposure and the concentration of the CAPE and ethanol extract of propolis [71]. Propolis using both anti-inflammatory (tumor necrosis factor-α, COX-1, COX-2) and anticolon cancer (DLD-1 colon cancer cell viability) assays and determined the phenolic compounds responsible for the activity. Propolis tincture solids had very high levels of the dihydroflavonoids pinocembrin and pinobanksin-3-O-acetate, and high levels of the dimethylallyl, benzyl and 3-methyl-3-butenyl caffeates relative to CAPE. It showed good broad spectrum activity in anti-proliferative assays against three other gastrointestinal cancer cell lines; HCT-116 colon carcinoma, KYSE-30 esophageal squamous cancer, and NCI-N87 gastric carcinoma [72].

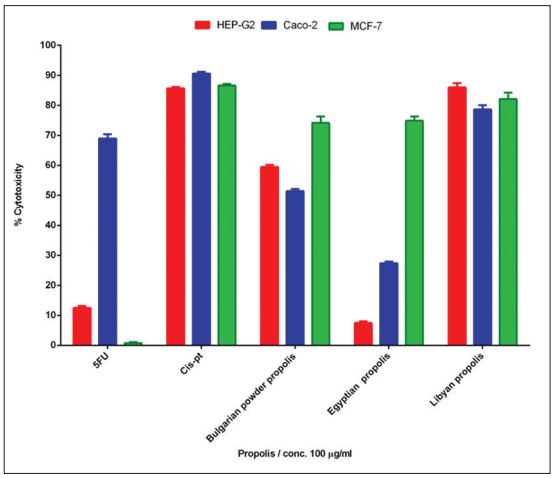

Figure 4: % cytotoxicity of different propolis at 100 µg/ml against Hep-G2, Caco-2 and MCF-7 cell lines, using 5-FU and Cis-pt as positive controls

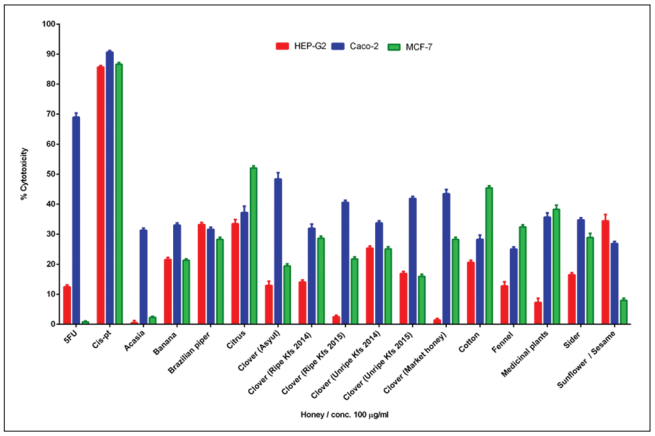

Figure 5: % cytotoxicity of different honey types at 100 µg/ml against Hep-G2, Caco-2 and MCF-7 cell lines, using 5-FU and Cis-pt as positive controls

We suggested that effects of propolis on breast cancer (MCF-70 may be its alters the activity of carcinogen biotransformation enzymes by modulating Phase I and II enzymes [73], inhibits angiogenesis diminished VEGF expression. Suppresses metastatic growth by decreasing hypoxic survival and STAT3 activation [74], inhibits of HDAC8 enzymatic activity [75]. As liver cancer (Hep-G2), its reduces inflammation by decreasing expression of COX-2 and NF-κB p65 levels, induces apoptosis by decreasing the levels of p53, Bax, caspase 3, β-arrestin and Bcl xL [76], attenuation of the canonical Wnt and NF-κB signaling pathways, up-regulation of apoptotic gene expression [77], downregulates the β-catenin expression [78] and colorectal cancer (Caco-2) its inhibits cell proliferation, recovers antioxidant mineral levels, reduces nitrosative stress [79].

Varied honey activities may be attributed to versatility of honey composition that influenced the activity, as osmotic properties of honey [80,81], honey pH [82] or activity of glucose oxidase, hydrogen peroxide [83,84] and non-peroxide substances [85,86].

From the previous results, it could by summarized that all honey bee products under treatments exhibit potential cytotoxic effect on Hep-G2, Caco-2, and MCF-7.

CONCLUSIONS

The results showed that BV, propolis and honey are interesting agents that have valuable activities against Hep-G2, Caco-2 and MCF-7 with BV being the most effective agent that might be incorporated in cancer remedy regimens after further studies.

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Source of Support: Nil, Conflict of Interest: None declared.