Cytotoxicity effect of 5-fluorouracil and bee products on the MCF-7 Human Breast Cancer Cell Line *in vitro*

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ABSTRACT

Background: Cancer is a devastative disease in the world and uncontrolled cellular proliferation. Breast cancer is one of the most common causes of morbidity and 2nd cancer leading death among women worldwide. Current therapy available for cancer treatment is associated with the number of side effects. However, natural products offer an alternative route for the treatment of tumors. Objectives: The primary objective of our research to assess chemotherapeutic activities of 5-fluorouracil, natural bee products and combination doses against human breast adenocarcinoma cell line MCF-7 and screen cytotoxic effects of honey bee products against MCF-7 breast cancer cell lines. Materials and Methods: Samples of honey, Royal jelly, Pollen grains bee and their combinations without or with 5-FU, and tested by sulforhodamine B (SRB) cell-based assay against breast (MCF-7), cancer cell lines.Results: Treatment of *in vitro* MCF-7 breast cancer cell lines cultured cells with 5-FU alone resulted in an elevation in IC50 value (7.65uM/ml,), compared with one or more of bee products (IC50>100uM/ml). Contrary, treated by 5-Fu and with one or more of Honey (RJ, H, PG&Mix), led to a decrease in IC50(7.65; 4.95; 23.45 and 4.26 uM/ml). Combination with honey products such as RJ, H, PG led to a deficiency of cell viability and capability of 5-FU to effect MCF-7 growth of breast cancer *in vitro* only during treatment supplementation with bee products. The high dose-dependent effect in cell viability tests. Conclusion, Combinations of one dose of Royall jelly, honey, pollen grains or its combinations with 5-FU five suppressive actions on the viability of MCF-7 breast cancer cell lines in contrast to 5-FU (same dose). Bee products (RJ, H&PG) have synergistic cytotoxic effects with 5-FU in MCF-7 breast cancer cell lines *in vitro*.

Key Words: MCF-7, breast cancer, cytotoxicity, Natural honey products.

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INTRODUCTION

Cancer is a group of more than two hundreds neoplastic diseases [1], considered as a major cause of death worldwide irrespective of human population development, and is characterized by uncontrolled cellular proliferation or growth and the probability to spread of abnormal cells to other parts of the body [2]. Spearing abnormity cells led to death [3].

Globally, Cancer has become a major cause of mortality, about 18 millions as new cancer cases and more 9.5 million deaths were predictable. Breast cancer is common in women (12%) [3, 4], 231,840 new cases and 40,290 deaths due to breast cancer (USA / 2015).

Cancer mainly is variations occurred in DNA sequence for a normal cell. Breast cancer, in particular, developed from mutation genes [5]. This mutation is influenced by exposure to radiation, hazardous chemicals or genetic factors [6].

Tumor inhibiting mechanism present in structures of normal cells and distinguishes among normal cells and abnormal growing cancer, Tumor inhibited genes and constraining by many environmental agents such as radiation, pollution, etc. or habits such as tobacco [7-10].

Breast cancers that progress in the breast tissue are carcinomas that start in epithelial cells existing in the

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mammary gland due to the mutation of some genes. Breast tumors (10–15%) are hereditary**.** Other etiological factors that contribute to elevating the frequency of breast cancer are food contamination, high exposure to radiations, chemicals, physical inactivity, late gestation (after 30 years of age), oral contraceptives, hormone therapy after menopause, and high intake of alcohol and tobacco [11].

Mammary gland tumors had progesterone receptors as PR-positive (or PR+) tumors, and which have estrogen receptors (ER) were generally termed ER-positive (or ER+) tumors and those which have growth-promoting protein termed HER2/neu are identified as HER2-positive [12].

Several anticancer materials are naturally formed by an extensive collection of different organisms such as microorganisms, animals, and plants.

Natural products serve as a good and inexpensive source for novel drug entities [13, 14] and have confirmed to be the most consistent solitary origin of novel and effective antitumor substances [15-18] and have an influence on cellular signaling and gene expression [19].

Natural photochemical diversity was used as treatments for many illnesses comprising cancer [18, 20-23]. Natural physiochemical was establishing as efficient methods for many tumor types and also induce effectiveness via alternations to tumor commencement, development, and progression, in addition to interrupting several mechanisms [24-26]. Several growth factors and its receptors, anti-apoptotic, tumor suppressors and transcription were altered in tumors and could be used as therapeutical factors [27, 28].

Recent investigations revealed that in antitumor therapy, the researchers can use various natural substances affecting embryonic developmental pathways or programmed cell death mechanisms to repair normal tissue homeostasis [29-31].

Natural products 5-Flourouracil (5-FU) is one of the greatest chemotherapeutic substances used for the treatment of a great variety of tumors [32]. Due to its structure, which is similar to the pyrimidine molecules of DNA and RNA, 5-FU interferes with nucleoside metabolism and can be incorporated into RNA and DNA, and led to death [33]**.**

Honey is a useful food substance and has many important components as minute quantities of pigments, flavor and aroma substances, phenolic compounds and minerals [34].

Generally, honey products were applied in ancient medicine to treat various disorders and illnesses. It was applied externally in case of wounds, acne, and burns, besides internally for treatment of seasonal allergies, anti-inflammatory, antioxidant to augment immunity, cough, bronchial asthma, urinary tract infections, nausea, diarrhea, anti-bacterial, anti-malarial and anticancer, antimetastatic antiproliferative, properties [35-38]**.**

The researchers attributed the biological properties of honey to the small number of phenolic compounds. Phenolic compounds are classified according to the presence of the chemical group into flavonoids, phenolic acids, flavones, flavanones, flavonols, etc.

Greek honey had a weak osteogenic effect in minimal concentration against the MCF-7 cells [39]**.** Concerning the Egyptian crude honey samples, it carries both the anti-cancer and antimycotic activity against the HTB-26 breast cancer cell line. *Ziziphus* honey played a major role in suppressing cell proliferation Mervat and El-Gendy, [40].

Indian honey retarded the growth of MCF-7breast cancer cells with an increase in concentration along with an increase in cells at the sub-G1 phase and induction of apoptosis in breast cancer cells [41].

Tualang honey (TH) was found to be efficient in inducing apoptosis in both breast cancer cell lines (MCF-7 and MDA-MB-231), where TH promoted mitochondrial-dependent apoptosis induced by tamoxifen in both MCF-7 and MDA-MB-231 breast cancer cell lines [42] and reduce mitochondrion membrane [42, 43].

At *in vivo* conditions, the growth of cancer cells was destructed by TH treatment and the pre-clinical study determines that varied actions on DMBA-induced breast cancers were employed by TH [44]. Suppressing breast cancer developing when orally taken (2 g/kg) [45].

Apigenin (one of the constituents of phenolic phytochemicals) retarded the growth of MCF-7 and MDA-MB-468 breast carcinoma cells lines through cell cycle regulatory molecules, where Apigenin caused degradation of HER2/neu protein and Rb phosphorylation and induced apoptosis in breast cancer cells (MCF-7 cells) in time and dose-dependent manner [46]. Furthermore, apigenin up-regulated the concentration of *p*27 protein whereas reducing cyclin D1, D3 and CDK4 in breast cancer cells [47].

MATERIAL AND METHODS:

Reagents and chemicals:

The reagents and chemicals 5-fluorouracil and sulpharodamine-B (SRB) were bought from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Media, fetal bovine serum (FBS) and other cell culture materials were purchased from Gibco™, Thermo Fisher Scientific (Grand Island, NY, USA)

Bee products (honey, royal jelly, bee pollen) were obtained from the company of wild honey (Riyadh, Saudi Arabia kingdom).

Cell culture:

Human breast adenocarcinoma cancer cell line MCF-7 (ATCC®HTB-22™) was obtained from King Fahd center for medical research (Jeddah, Saudi Arabia) and cultured in RPMI 1640 supplemented with 10%FBS, 100 U/ml penicillin and 100 µg/ml streptomycin and incubated in a humidified with 5% CO2 and 95% air (37°C).

Subculture of MCF-7 cell Lines:-

Cell lines subculture was performed as described by [48]. The cell monolayer was trypsinized by trypsin/EDTA solution, the excess amount of trypsin was decanted and the flask kept in an incubator for 1-5 minutes at 37 °C. The cells were examined using an inverted microscope to ensure that all cells are detached and rounded. The cells were resuspended in a small volume of fresh growth medium to inactivate the trypsin biodegradable activity. One hundred µl of cell suspension was taken to perform a cell count.

Evaluation of IC50 using sulforhodamine B (SRB) assay:

Survived cells were evaluated using SRB assay according to [49]. Cytotoxicity of 5-FU combinations was tested against MCF-7 cells by sulforhodamine B (SRB) assay. cells were collected using 0.25% Trypsin-EDTA and seeded in 96-well plates at 1000–2000 cells/well.

Treatment of MCF-7 cell lines with 5-FU and bee products:

The monolayer cells were trypsinized and re-suspended in growth media (90 % media + 10 % FBS). An equal number of cells were seeded in 6 well tissue culture plate incubating (37°C /24 hrs). The spent medium was removed 24 hours post culture and fresh medium containing IC_{50} value of honey products and 5-FU in solely and in combination were added to each well.

Data analysis.

The dose-response curves of drugs under investigation were analyzed using Emax model in the following formula:

$$\%\ Cell\ Viability = (100 - R) \times \left(1 - \frac{[D]^m}{K_d^m + [D]^m}\right)$$

where "R" is the residual unaffected fraction (the resistance fraction); "[D]" is the drug concentration used; "Kd" is the drug concentration that produces a 50% reduction of the maximum inhibition rate and m is a Hill-type coefficient. "IC_{50}" Drug concentration (i.e., Kd=IC50 when R=0 and E_{max}=100−R). Combination index (CI) was calculated from the formula:

$$CI = \frac{IC_{50}\ of\ drug\ (x) comblnallon}{IC_{50}\ of\ drug\ (x) alone} + \frac{IC_{50}\ of\ drug\ (y) comblnallon}{IC_{50}\ of\ drug\ (y) alone}$$

The nature of drug interaction is to defend as synergism if CI1.2; and additive if CI ranges from 0.8–1.2.

Statistical analysis.

Data are presented as mean ± SD using Prism® for Windows, ver. 5.00 (GraphPad Software Inc., La Jolla, CA, USA). (ANOVA test with LSD post hoc test used for significance using SPSS® version 17.0.0. $p < 0.05$.

RESULTS

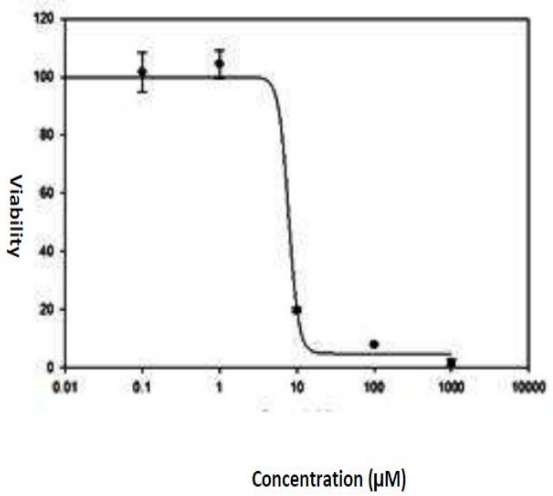
Table (1), showing The chemo modulatory effect of (Royal jelly, honey, bee pollen and combination of them) on the cytotoxicity of 5-FU in MCF-7 breast cancer cell lines and honey products (**Fig.1 A**, **B, C, D, E**, **F, G, H, R**). Following overnight incubation, MCF-7breast cancer cells were then treated with either (A)5-FU (control positive group), (B)Royall Jelly, (C)Honey, (D)Pollen grains bee, (E)combinations of bee products and (F-R)treated with 5-FU with a single treatment of RJ, H or PG or their combinations, respectively. The percentage of viable cells was calculated in triplicate (presented as mean±SD, n=3). After 72 hours, a SRB-assay was performed. Cells were exposed to serial dilution of 5-FU, bee products or their combination for 72h.

The results showed that the IC_{50} was averaged 7.79uM (R value=4.86%) for 5-FU, while it averaged >100uM/ml(R=5.25 %); >100uM/ml (R-value=3.714%); 98.09uM /ml (R=2.13%)and6.13 uM/ml(R=5.91%) for treatment with Honey, Royal jelly, PG and their combinations, respectively. Treatment with 5-FU, followed by treatment with H; RJ; PG and their combinations (H+RJ+PG)resulted in the decrease in the value of IC_{50} which averaged 7.65; 4.95; 23.45 and 4.26uM, respectively in comparison with 5-FU only (7.79uM).

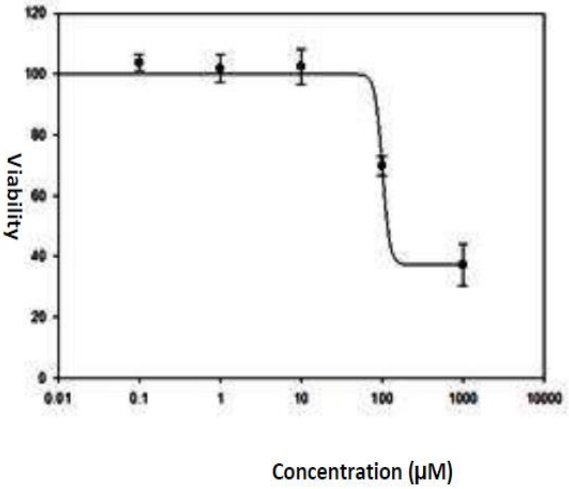
The chemo modulatory effect of (honey, royal jelly, bee pollen and mix of them) on the cytotoxicity of 5-FU onMCF-7 breast cancer cell lines are shown in figure 1(A-R). Cells were exposed to serial dilution of 5-FU, bee products or their combination for 72h.

Table 1.Combination analysis for the cytotoxicity of 5-FU and bee products against MCF-7 breast cancer cell lines. Data is presented as IC_{50}; n = 3.

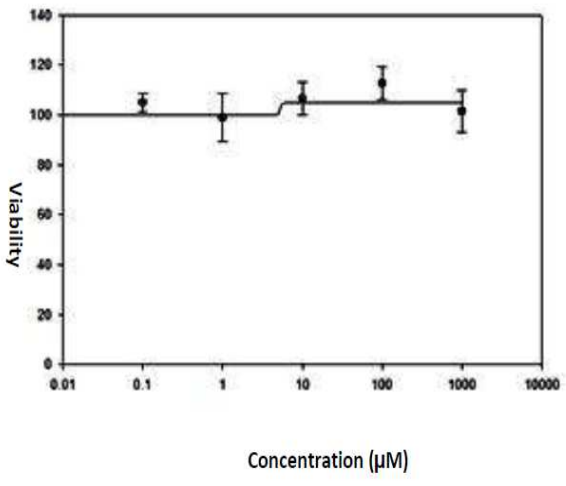
Exposure Time 72h	MCF-7	
	IC_{50} (µM)	R-Value (%)
5-FU	7.79	4.86
H(honey)	>100	5.24
RJ (Royal jelly)	>100	3.714
PG (Pollen grains)	98.09	2.13
(H+RJ+PG)	6.23	5.91
5-FU+H	7.65	5.17
5-FU+RJ	4.95	2.37
5-FU+PG	23.45	2.35
5_FU+(H+RJ+PG)	4.26	2.76



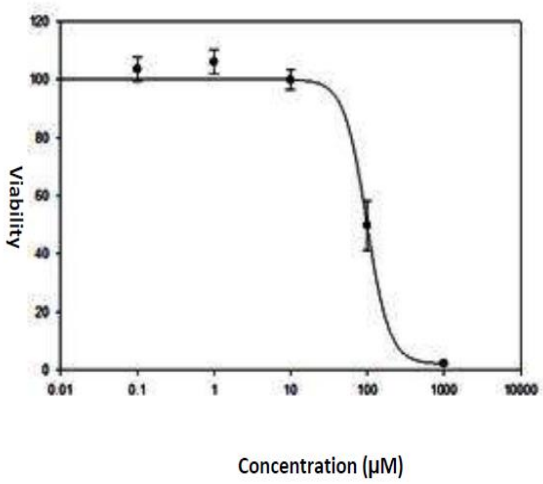
A) Viability of MCF-7breast cancer cells after incubation for 72hr in different concentrations of 5-flurouraciI (5-FU +ve sample).



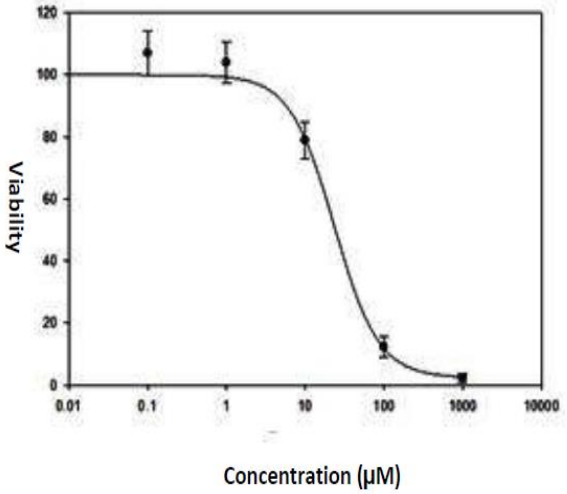
B) Viability of MCF-7 breast cancer cells after incubation for 72hr in different concentrations of Royal jelly.



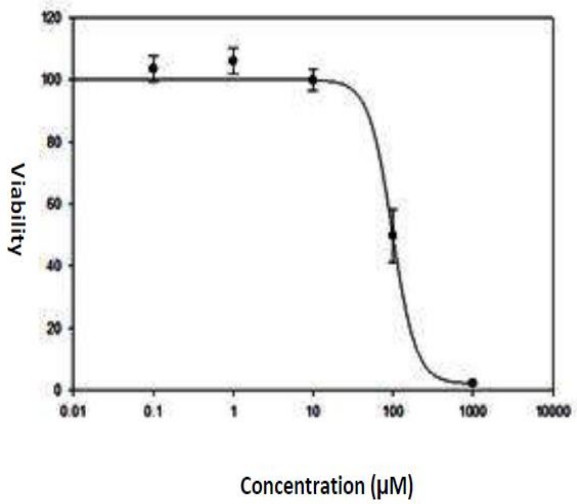
C) Viability of MCF-7breast cancer cells after incubation for 72hr in different concentrations of Honey.



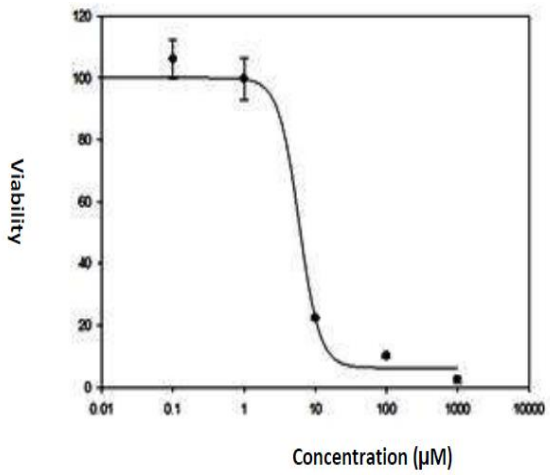
D) Viability of MCF-7breast cancer cells after incubation for 72hr in different concentrations of pollen grain bee (PG).



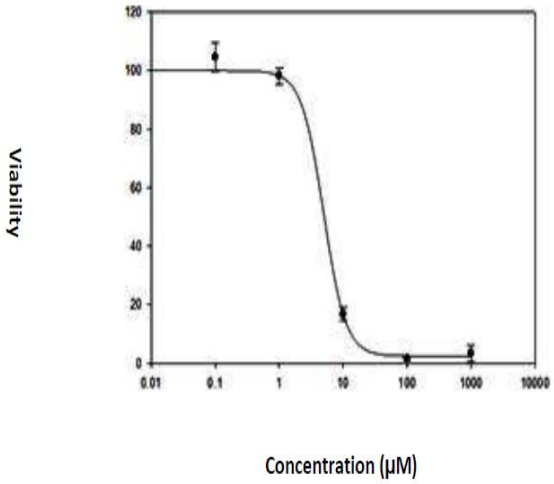
E) Viability of MCF-7breast cancer cells after incubation for 72hr in different concentrations of combinations of Royal jelly, Honey and pollen grains bee.



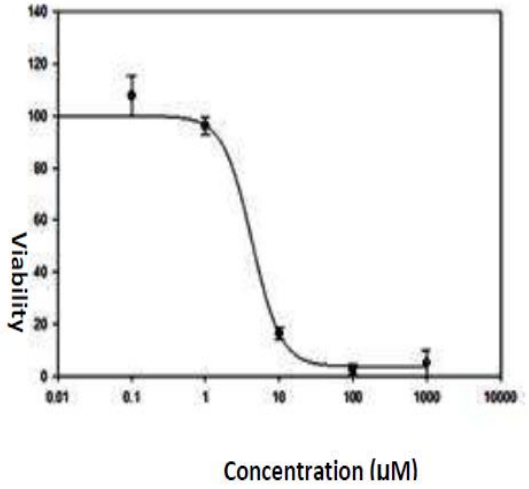
F) Viability of MCF-7breast cancer cells after incubation for72hr in different concentrations of 5-FU and Royall jelly.



G) Viability of MCF-7breast cancer cells after incubation for 72hr in different concentrations of 5-FU and Honey.



H) Viability of MCF-7breast cancer cells after incubation for72hr in different concentrations of 5-FU and pollen grains bee.



R) Viability of MCF-7breast cancer cells after incubation for 72hr in different concentrations of 5-FU and combinations of Royal Jelly, Honey and pollen grains bee

N.B.: Each point in the diagrams represents the mean of the results of three independent experiments. Error bars represent means ± S.E.M. of the three independent experiments.

Figure 1. The chemo modulatory effect of (honey, royal jelly, bee pollen and mix of them) on the cytotoxicity of 5-FUin HTC-116 (A, B, C, D, E, F, G, H, R) breast cancer cell lines. Cells were exposed to serial dilution of 5-FU, bee products or their combination for 72h.

Effects of Royal jelly, Honey and pollen grains bee with /or without 5-FU on MCF-7 breast cancer cells viability in vitro:

Figure 1B -1D showed effective growth-inhibitory impacts of Royal jelly (B), Honey (C) and Pollen grains bee (D)honey on MCF-7 breast cancer cells in a dose-dependent manner. A single treatment with one of the following honey products (H; RJ; PG and their mixes) were exhibited a gradual decrease in cell viability as the concentration of honey was increased reaching an IC_{50} of >100uM/ml (R value=5.24%); RJ IC50 was >100uM/ml(R=3.71%); Bee Pollen grains, IC_{50} reached 98.09uM/ml (R=2.13%); whereas, combinations of (H+RJ+PG) IC50, was averaged 6.23uM/ml (R=5.91%). In contrast, supplementation with Bee products (RJ, H, PG) or their combinations with 5FU, showed a much steeper decline with regards to its anti-proliferative ability against MCF-7 breast cancer cells reaching an $IC_{50}$of 7.65; 4.95; 23.45 and 4.26uM/ml, respectively. in comparison with 5-FU alone (7.79uM). This indicates that any of the bee products alone or combined are much more potent in inhibiting the growth of MCF-7 breast cancer cells in comparison with control samples, where it was averaged >100; >100; 98.09 and 6.23uM/ml corresponding to H; RJ; PG and their combinations, respectively.

DISCUSSION

Several medicinal plants are used for the treatment of various diseases comprising tumors that are becoming critical for drug discovery and clinical application and research [18, 20-22]. These natural phytochemicals are established to be efficient toward various kinds of tumors particularly breast. In this time, despite the recent introduced techniques and advancement in the methods of diagnosis and therapies for controlling of breast cancer, yet is still one of the main etiology of elevating the morbidity and mortality rates among patients suffering from cancers, while the available therapies are not sufficient to manage breast cancer metastasis.

Natural products 5-Flourouracil (5-FU) is one of the greatest chemotherapeutic substances used for the treatment of a great variety of tumors [31].

Many works of literature established that the combination of more than one treatment is better than a single drug,

particularly mix from chemical and natural products. In the current study, the effects of different bee products (with and without 5-FU) on MCF-7 breast cancer cell lines have been evaluated *in vitro*. The results revealed that supplementation with Honey plus 5-Fu, inhibited the vitality of MCF-7 breast cancer cell lines in vitro, where IC_{50} reached 7.65 uM/ml vs. 7.79uM/ml with 5-FU alone, also, Royal jelly plus 5-FU, inhibited significantly the growth of MCF-7 breast cancer cells, where IC_{50} was reached 4.95uM vs. 5-FU alone (IC_{50}=7.79uM). Also, supplementation with PG and combinations of bee products (H+RJ+PG) were significantly inhibited the growth or viability of MCF-7 breast cancer cell lines in vitro with values equal to 23.45 and 4.26uM/ml, respectively. Many authors dealing with the influence of Gelam honey (one of the bee products) as a chemoprotective agent due to its contents of phenolic compounds which are rich with anti-oxidants, in addition to their potent action against carcinogenic cells [41, 50]. Honey products were applied in ancient medicine to treat various disorders and illnesses and possess anticancer, antimetastatic antiproliferative effects [36-38]. In the current study, the suppressing effect of honey products on MCF-7 breast cancer cell lines particularly, honey, PG and their combinations when given with 5-FU was in coordinated with many investigators, where Anna *et al.* [39] reported the greek honey to have a weak osteogenic effect against MCF-7 cells. In another study, carried by Mervat and El-Gendy, [40] showed that crude *Ziziphus* honey has important roles for suppressing and proliferation cells. Moreover, Tualang honey increases mitochondrial-dependent apoptosis induced [42, 43]. Also, some authors agree with our finding, they reported that honey significantly suppressed breast cancer cell growth when orally taken (2 g/kg) and control of metastasis of cancer [45]. We can conclude from the current study that honey products represented in hone, Royal jelly, and bee grain pollen can be used to enhance the cytotoxicity of 5-Furacil and consequently reduce the toxicity of 5-furacil for overcoming the breast cancer and their metastasis and their actions may be due to the phytochemical constituents in the honey, particularly phenolic compounds [39, 46].

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