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Cytotoxicity, Toxicity and Anticancer Activity of Manuka Honey, Saudi's Honey and *Peganum harmala* Plant against Cancer Cells

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Abstract

Cancer occurs when the body's cells grow beyond the usual control. Normal body cells multiply in a controlled manner and undergo apoptosis when the body no longer needs them. Different types of cancers exist, and the common ones include breast, cervical, prostate, lung, colon, and skin cancers. Several factors have been associated with cancers, and these factors include poor dieting, exposure to harmful chemicals and radiation, weak immune system, and genetics. Cancer presents an enormous health threat in the modern world and thus the need to identify an effective treatment. The conventional treatments used in the management of cancer include chemotherapy and physiotherapy. These forms of cancer treatments usually have enormous side effects on the subjects. In this respect, an alternative form of treatment would be effective in managing cancer patients. A substantial number of natural products have been observed to be effective in the management of cancer. These natural products include plants and other natural substances such as honey. This study focuses on the efficiency of natural products in the treatment of cancer. Also, the anticancer effects of *Peganum harmala*, Manuka honey, and Saudi honey will be analyzed. Bee honey and *Peganum harmala* have been traditionally used in the treatment of cancer. The extracts from *Peganum harmala* plant have also been shown to exhibit divers' antitumor effects similar to the mode of action of a vast number of anticancer agents. These established hypotheses thus give the rationale for this study. In this experiment, extracts were obtained from *Peganum harmala* leaves and exposed to cervical, lung, and prostate cancer cells. Similarly, solutions of Manuka honey and Saudi honey were exposed to the cervical, lung, and prostate can-

cer cells. The experiment duration was 24 hours, which obtained results were recorded and analyzed. *Peganum harmala* extracts inhibit cancer cell growth at different and achievable concentrations. Manuka honey highly inhibits the growth of HeLa cancer cells while Saudi honey highly inhibits the growth of A549 cells. *Peganum harmala* can form an effective treatment for managing several types of cancers. Manuka Honey can be applied as an effective treatment for managing cervical cancer while Saudi honey can form an effective treatment for managing lung cancers.

Keywords

Peganum harmala, Manuka Honey, Saudi Honey, Anticancer, Apoptosis, Natural Products

1. Introduction

Cancer can be defined as an ailment that occurs when the cells in a particular body part start to grow out of control. The body consists of trillions of cells. It is a dangerous disease which is brought about mainly by environmental factors. These factors make the genes mutate and encode critical cell regulatory proteins. It can also be described as a genetic disease that is principally caused by environmental factors. Cancer causing agents are found in foods, drinks, air, sunlight or chemical. People come from these agents daily. The precise etiology of cancer in an individual is typically indeterminable, making it challenging to ascertain the specific factors that contribute to its development. However, studies have demonstrated that specific risk factors can elevate an individual's likelihood of developing cancer. Exposure to chemicals or other substances, along with specific habits, are considered risk factors for cancer. In addition, they include factors beyond individuals' control, such as age and genealogy [1] [2]. Cancer is the second leading disease after heart affiliated diseases in the United States. It is estimated that about one-half of all men and one-third of all women in the States will develop cancer in their lifetime [1]. The history of cancer stretches far back. 80 million years ago, fossilized dinosaur bones showed possible evidence of cancerous cells. The word cancer is from the Greek word karkinos that meant carcinoma tumors. As per the American Cancer Society's description of the history of cancer treatments, In 1600 BC, stomach cancer was treated using boiled barley mixed with dates [3] [4]. On the other hand, the cancer of the uterus was treated using a concoction of fresh dates with a mixture of pig brains introduced in the vagina. Radiotherapy was not developed until 1900 AD. Chemotherapy was then later introduced in 1945 AD as a treatment [3]. Approximately 30% of cancers can be attributed to tobacco use and diet. This means that much of cancer could be prevented if only drastic measures were implemented, and people followed the latter. Due to demographic changes, more than 30% of cancer deaths have been predicted in developed countries while 70% has been predicted

in developing countries [5]. In a research article by Ahmed & Othman (2013), the possibility of using natural products in the prevention and treatment of cancer was demonstrated. Among the target natural treatments, honey was identified as the most common and extensively researched product. According to this study, honey was identified as a constituent of various sugars, enzymes, flavonoids, amino acids, phenolic acids, and miscellaneous compounds, which potentially induce anticancer effects [6] [7].

The apoptotic activity of honey was illustrated by reviewing the apoptotic pathways of human cells. Within the review of this study, cancer cells were marked to exhibit uncontrolled proliferation and inadequate apoptotic turnover. The conventional medications used for the treatment of cancer were also described as apoptotic inducers. Based on the three phases of apoptosis; (induction, effector, and degradation phases), the changes in the standard cascade were marked controllable through the use of an effective therapy. Honey was observed to elevate the activation of caspase 3 and the cleavage of the poly (ADP-ribose) polymerase in human colon cancer cell lines. This observation was attributed to the high phenolic and tryptophan content. Honey was also identified as a generator of reactive oxygen species (ROS), responsible for the activation of p53; a component, which potentially modulates the expression of anti-apoptotic and pro-apoptotic proteins such as Bcl-2 and Bax [6] [8]. In research by Moloudizargari, Mikaili, Aghajanshakeri, Asghari, & Shayegh (2013), *Peganum harmala* and its alkaloids were observed to exhibit a wide array of pharmacological and therapeutic effects. This study revealed that quinazoline and beta-carboline alkaloids as the active compounds of *Peganum harmala*. Also demonstrated to suppress the expression of different pro-angiogenic factors, which include pro-inflammatory cytokines, NO, and endothelial growth factor [9] [10] [11]. The active alkaloids of *P. harmala* seeds were identified to be Mono Amine Oxidase Inhibitor (MAOI-A) compounds. The compounds included "harmine, harmane, harmaline, tetrahydroharmine, harmalol, and vaccine" [12]. Honey is produced by bees following regurgitative techniques. In this respect, this natural product contains a vast array of medicinal effects such as; antibacterial, anti-inflammatory, and anti-cytotoxic [13]. Honey enhances the repair of damaged mucosa, reduces inflammation, and promotes the growth of new tissues. Raw honey has been established to contain a large amount of health-essential compounds, such as polyphenols and flavonoids [13] [14]. Manuka honey is categorized as monofloral honey, derived exclusively from the nectar of flowers belonging to a single plant species (the manuka plant); Saudi honey is considered polyfloral honey due to its derivation from numerous floral sources [15]. As Mandal *et al*. (2011) further highlight, the Manuka enhances wound healing by stimulating monocytes (macrophages precursors), to secrete TNFα. TNFα is an immune compound that induces wound repairing cascade. Manuka Honey also reduces the release of reactive intermediaries and thus preventing extensive damage to the injured tissue during wound healing. Deficient wound healing

mechanisms have been associated with some tumors. In this regard, utilization of the therapeutic effects of honey may prevent chronic-wound related tumors [14] [15].

Main Objectives of This Study

1) Determine the growth inhibitory effect of *Peganum Harmala* leaves extracts on HeLa cervical cancer cell lines, A549 lung cancer cell lines and PC3 Prostate cancer cell lines.

2) Determine the growth inhibitory effect of Manuka honey extracts on HeLa cervical cancer cell lines, A549 lung cancer cells lines and PC3 Prostate cancer cell lines.

3) Determine the growth inhibitory effect of Saudi honey extracts on HeLa cervical cancer cell lines, A549 lung cancer cells lines and PC3 Prostate cancer cell lines.

4) Using infrared spectroscopy (IR) for the analysis of Saudi honey, Manuka honey, and *Peganun harmala* plant.

2. Materials and Methods

2.1. Cell Culture

The proliferation and cytotoxic activity of cancerous cell lines were evaluated using screening systems that are based on human cells. The cell lines for the study represented some common types of cancers, and they were selected as given in Table 1. These samples were obtained from the American Type Culture in Collection; Rockville, MD. The study cells were sustained in RPMI-1640 augmented with 10% heat attenuated FBS, with the exception of 1% penicillin-streptomycin and 2 mM l-glutamine. MCF-7 was cultured in DMEM; a low glucose (Gibco) culture media that contains 1% penicillin-streptomycin, 2 mM L-glutamine, and non-essential amino acids. The DMEM was also supplemented with 10% fetal calf serum (Atlanta Biologicals, 1mM sodium pyruvate, and 0.01 mg/ml insulin. The cells were incubated at 370C in a 5% CO_2 humidified incubator and sub-cultured twice every week after attaining an 80% confluence. The dilutions for the subcultures were done at the ratios of 1:2/1:4 for a maximum of 40 times.

2.2. Trypan Blue Cell Viability Test

Testing the integrity of the cell membrane is one of the essential ways of ensuring the viability and the cytotoxic effects of a cell. In this event, cells were plated

Table 1. Cancer cell culture.

Cell Line	ATCC Number	Organ	Oncogenes	Culture Media
A549	CCL-185	Lung	FRA-1	DMEM M-F12
HeLa	CCL-2	Cervix	E6/E7	EMEM
PC3	CRL-1435	Prostates	c-myb	DMEM

in a 12- well plate or 24-well plate. Approximately 1×10^5 cells were plated in each well of the 12-well plate or 5×10^4 for the 24-well plate. 2ml medium was used for the 12-well plate while 1 ml was used for the 24-well plate. The inoculated plate was incubated at 37˚C in a 5% CO_2 incubator. The plate was removed from the incubator after 24 hours; the cells were then harvested from the culture medium and washed using phosphate buffered saline. The washed cells were exposed to 0.25% trypsin-EDTA solution for 2 - 3 minutes at 37˚C. The treated cells were introduced to trypan blue stain and the live cells counted. Eventually, the cells were titrated to a final concentration of 10^3 cells per 20 µl.

2.3. Treatment with Alarm Blue Cell Viability Reagent

Alamar Blue reagent functions to determine the viability of a cell by employing the reducing ability of a living cell. The reducing ability of the subject cell directly relates to the quantitative measure of the proliferation activity. In this respect, the determination of the reducing capacity of a given cell will establish the relative cytotoxic activity of the cell. This observation is based on the idea cells maintain a reducing environment inside their cytosol while they are alive. Alamar Blue reagent contains resazurin as the active ingredient. Resazurin is a virtually non-fluorescent and non-toxic cell-permeable compound with a blue consistency. Resazurin is reduced to resorufin (**Figure 1**); upon entering Alamar Blue subjected cells. Resorufin is a red-colored and highly fluorescent compound. Viable cells actively convert resazurin to resorufin, and this event leads to an overall increase in fluorescence and the intensity of the color of media surrounded cells. Alamar Blue reagent is employed in the cell viability assessment via a simple procedure. The 10X ready-to-use solution is added to the study cell (mammalian or bacterial), in a culture media. This process does not call for the aspiration of media from the cell cultures or placing the cells in a minimal media dilution. As a result, Alamar Blue reagent can just be employed in a microtiter plate format; in a "no-wash" procedure, or by use of a single tube.

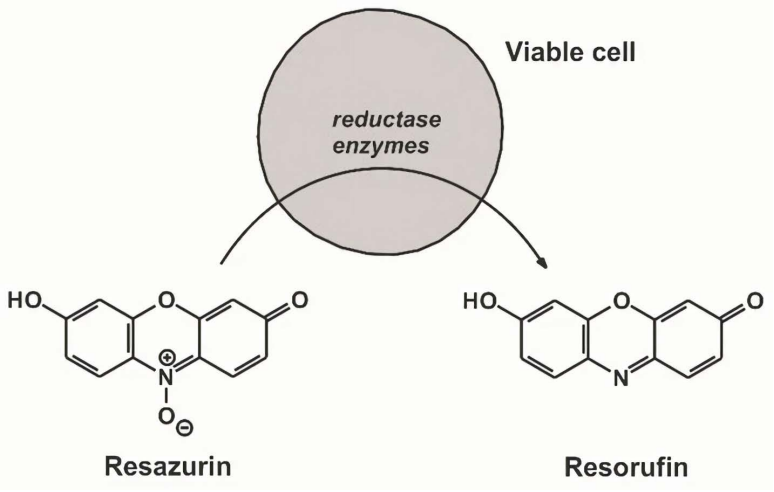

Figure 1. Resazurin is reduced to Resorufin.

This simple process involves the addition of 10% Alamar Blue reagent to a 100 μL volume sample. The resultant solution is then incubated at 37˚C for 1 - 4 hours. Extended incubation period would enhance increased sensitivity without jeopardizing the viability of the cell. The resulting fluorescence is quantified using a fluorescence spectrophotometer or read on a plate reader. Alternatively, a spectrophotometer can be applied to read the Alamar Blue reagent's absorbance.

Ultimately, the obtained results are analyzed through an absorbance (or fluorescence) against a compound concentration graph (Invitrogen).

2.4. Extraction of Crude Organic Compounds

Crude organic compounds were extracted from plants using an evaporator, a Lyophilizer, and a Soxhlet. The dried plant leaves were obtained from plant vegetation. The collected sample of plant leaves were placed in separate 15-millimeter chemical resistant centrifuge tubes and frozen in liquid nitrogen for twenty-four hours. The frozen leave samples were then removed from the nitrogen and grounded separately using a pestle and mortar. Methanol was then poured to infuse the crude extract, and the infusion was distilled for 6 hours using Soxhlet. The methanol was evaporated from the infusion using a rotary evaporator. The obtained crude extracts were weighed and added to dimethyl sulfoxide in order to dissolve. The organic extracts were placed in freezer vials and stored at −20˚C.

2.5. Dimethyl Sulfoxide (DMSO)

Dimethyl Sulfoxide is an essential polar aprotic solvent. In this study, Dimethyl Sulfoxide was obtained from Sigma-Aldrich. Dimethyl Sulfoxide (DMSO) is an organic compound, which contains sulfur. DMSO readily dissociates into a vast array of compounds. DMSO is a colorless hydroscopic organic liquid with C2H6OS as the molecular formula. Irrespective of being an organic compound, DMSO is also a hydrophilic compound and thus forming a control for this study.

2.6. Tamoxifen

Tamoxifen used in this experiment was obtained from Sigma-Aldrich. This non-steroidal compound exhibit anti-cancer properties. As a result, tamoxifen is employed in the management of advanced estrogen-dependent breast cancers. In this study, tamoxifen was used as a supplementary toxin to compare the inhibition of the Echinacea Angustifolia extracts with the excerpts from Podophyllum peltatum. A portion of the tamoxifen's stock solution was dissolved in 1ml of Dimethyl Sulfoxide.

2.7. Alamar Blue

Alamar Blue is employed in the treatment of cells in order to ascertain their viability. A bright red of the cells following exposure to Alamar Blue reagent indicates the viability of the cells while a dark blue consistency indicates that the subject cells are dead. Alamar Blue is indicative of the rate of metabolism and viability of the study cell.

2.8. PC-3 Cells Line

PC-3 Cell Line is the cell line responsible for the prostate cancer (PC). PC-3 cells were purchased from the American Type Culture Collection; Rockville, MD. The PC-3 cells were sustained in the DMEM media augmented with 10% FBS, L-glutamine, penicillin-streptomycin, and pyruvate sodium. These cells were incubated at 37˚C in 5% CO_2. Equal number of cells were planted in the individual wells of 96-well plate and maintained with the ordinary medium supplemented with charcoal-stripped FBS or regular FBS. A growth curve was obtained based on the observations on the assay from day 1 to day 7. This study was conducted in quadruplets. PC3 cells were used to obtained Xenograft tumor cells. The study cells were then washed with cold PBS and rinsed with trypsin, at the confluence of 80%. The cells were washed again while in the media, diluted to a concentration of 2×10^6 cells per 50 µl, and introduced to an equal volume of culture. The tumor cells were harvested, formalin-fixed and embedded in paraffin.

2.9. A549 Cell Lines

The A549 Cell Lines are responsible for the lung cancer. This tumor cells metastasis to the trachea and even the heart [16]. In this study, The A549 Cell Lines were obtained from the American Type Culture Collection; Rockville, MD. These cells were dipped into a cold Hanks' balanced salt solution and immediately on ice. The A549 cells were washed and cultured in 0.1% protease prepared in magnesium/calcium free Hanks' balanced salt solution (HBSS). The culture was then incubated throughout the night at 4˚C. The A549 cells were then isolated by rinsing the luminal side with Hanks' balanced salt solution supplemented with 10% heat attenuated fetal calf serum; FCS. The harvested cells were centrifuged for 10 minutes (at 270 g) and re-suspended in Hanks' balanced salt solution impregnated with 10% FCS. The resultant solution was filtered through a 100 µm sterile mesh and centrifuged again. The obtained pellet was re-immersed in serum-free hormone infused Ham's F12 medium. F12 complete medium contained 100 μml_{-1} penicillin, 100 $\mu g{\cdot}ml_{-1}$ streptomycin, 2100 $\mu g{\cdot}ml_{-1}$ amphotericin B, and 2 m/M L-glutamine. The F12 complete medium was also supplemented with 5 $\mu g{\cdot}ml_{-1}$ insulin, 5 $\mu g{\cdot}ml_{-1}$ transferrin, 25 $\mu g{\cdot}ml_{-1}$ epidermal growth factor, 15 $\mu g{\cdot}ml_{-1}$ cell growth supplement, 0.1 µM retinoic acid, and 1 µM hydrocortisone. The cell suspension was re-centrifuged again and re-suspended in the complete F12 medium. The cell counts were performed using a Neubauer hemocytometer, and the results analyzed graphically.

2.10. HeLa Cells Lines

HeLa Cells lines are the most commonly used cancer cell lines in the world.

These cell lines proliferate rapidly and efficiently. HeLa Cells also share some fundamental characteristic of the normal cells. In the light of this fundamental similarity, HeLa Cells produce proteins, communicate with one another, are susceptible to infections and express and regulate proteins [17]. In this study,

HeLa Cell Lines were obtained from the American Type Culture Collection; Rockville, MD. The obtained cells were maintained in RPMI-1640 augmented with 10% heat attenuated FBS, with the exception of 1% penicillin-streptomycin and 2 mM l-glutamine. HeLa Cells were cultured in DMEM; a low glucose culture media augmented with 2 mM l-glutamine, 1% penicillin-streptomycin, and non-essential amino acids. The DMEM was also augmented with 10 % fetal calf serum. The plated cells were then incubated in at 37˚C in a 5% CO_2 humidified incubator and sub-cultured two times in a week after attaining an 80% confluence. The dilutions for the subcultures were done at the ratios of 1:2/1:4 for up to 40 times. The obtained cell titers were recorded and analyzed graphically.

2.11. Infrared Spectroscopy

Infrared Spectroscopy (IR) is the analysis of the infrared light molecule that interacts with the subject sample. IR analysis can involve measuring the emission, reflection, or absorption. IR is used to determine the functional groups of a substance. The general principle with the use of IR is that light atoms will vibrate at far-stretching frequency. Conversely, strongly bonded atoms will absorb more light than the loosely-bonded atoms [18]. With respect to this study, infrared spectroscopy was used in the analysis of Saudi honey, Manuka honey, and *Peganun harmala* plants.

Infrared spectra of substances are obtained by irradiating molecules with infrared radiation of varying frequencies to achieve a resonance that matches the vibration of the molecules. IR is commonly used in chemistry, since vibrational information is specific to the chemical bonds. Therefore, they provide a fingerprint by which the molecule can be identified.

Fourier Transform Infrared (FTIR) measurements were carried out at room temperature on a Thermo Scientific Nicolet iS10 equipped with a Diamond attenuated total reflectance (ATR) accessory. The spectra were recorded between 4000 and 600 cm^{-1} at 4 cm^{-1} resolution and 16 scans were accumulated. Routine smoothing and normalization were applied to all the infrared spectra.

2.12. Procedure of the Experiment

Two sets of 7 different tubes (1.5 ml) were labeled 0-6 while the remaining tube was labeled blank. 15 µl was obtained from each of the serially diluted stock concentrations. The obtained samples were then put into the corresponding 1.5 ml tube. 525 µl of the media was added into each of the 1.5 ml tubes and the 60 µl of Alamar Blue introduced into each tube. The lids of the tubes were closed and the contents were vortex for 5 seconds. 180 µl of media was added into 20 µl of Alamar Blue in the 1.5 ml blank tube.

A 96-well plate was obtained and a sample of 190 µl was picked from the blank and put in the well labeled #A1. 3 different (190 µl) samples of 0 concentrations were taken and put in wells F2, G2 and H2. This step was repeated by taking 3 different 190 µl samples of concentration 1 and placing in wells F3, G3 and

H3. It was noted that it was necessary to vortex each1.5µl tube before being placed in the 96-well plate. This procedure was repeated for the remaining concentrations.

The next step was performed with the addition of the cells. 7 different tubes (1.5 ml) were labeled 0-6 while the remaining tube was labeled blank. The obtained samples were then put into the corresponding 1.5 ml tube. 625 µl of the media was added into each of the 1.5 ml tubes and the 60 µl of Alamar Blue introduced into each tube. 60 µl of cells (adjusted to 10,000 cells/20 µl) were added into the tubes. Each tube was vortex for 5 seconds. 3 different (190 µl) samples of 0 concentrations were taken and put in wells A2, B2 and C2. This step was repeated by taking 3 different 190 µl samples of concentration 1 and placing in wells A2, B2 and C2. Each tube was vortex for 5 seconds before putting into the 96-well plate. This procedure was repeated for the remaining concentrations. Finally, the 96-well plates were placed in a CO_2 incubator at (37˚C) for 24 hours. The florescence was read using a spectrophotometer.

2.13. Statistical Analysis

The obtained results were expressed as mean ± standard deviation (SD). Paired Student t-test was applied to ascertain the statistical differences between the correlated samples. The correlated samples were noted to be significantly different.

3. Results and Discussion

3.1. Infrared Spectrum Results

3.1.1. Comparative Study of Saudi and Manuka Honeys

The infrared spectra of Saudi honey and Manuka honey were recorded. In general, the two IR spectra are very similar, but a detailed analysis shows that the two types of honey are different.

Region 2500 - 3600 cm^{-1}:

The two honeys contain OH and NH broad bands located at 3300 and 3261 cm^{-1} for Saudi honey and Manuka honey, respectively. The difference of about 40 cm^{-1} is significant and means that the two honeys are different in terms of OH (water) and NH group content. At this point, it is difficult to determine, which one contains more water than the other one. The low frequency at 3261 cm^{-1} indicates stronger H bonds in the network of Manuka honey. The peaks at around 2900 cm^{-1} represent the C-H stretching modes.

Region 900 - 1800 cm^{-1}:

This region seems to be the most interesting one because it allows determining the presence of proteins, lipids (fat) and carbohydrates. These biomolecules have specific signature bands in the 1700 - 1800 cm^{-1} range for lipids, 1600 - 1700 cm^{-1} for proteins, and 900 - 1200 cm^{-1} for carbohydrates. In our work conditions, the two types of honey do not show evidence of the presence of lipids, as there is no peak in the 1700 - 1800 cm^{-1} region. These peaks of lipids may be hidden by the presence of water. It would be good to dry the samples. The presence of proteins is evidenced by the peaks at 1644 and 1646 cm^{-1} for Saudi honey and Manuka honey, respectively. The very intense peaks at 1024 cm^{-1} for both

types of honey indicate the presence of carbohydrates.

3.1.2. Infrared Spectrun of *Peganum harmala* Plant

Region 2500 - 3600 cm^{-1}:

Peganum harmala plant contains a broadband located at 3401 cm^{-1} due to OH and NH stretching modes. The peaks at around 2900 cm^{-1} represent the C-H stretching modes.

Region 900 - 1800 cm^{-1}:

This region seems to be the most interesting one because it allows determining the presence of proteins, lipids (fat) and carbohydrates. These biomolecules have distinct signature bands in the 1700 - 1800 cm^{-1} range for lipids, 1600 - 1700 cm^{-1} for proteins, and 900 - 1200 cm^{-1} for carbohydrates. *Peganum harmala* plant IR spectrum shows a peak at 1730 cm^{-1} due to carbonyl groups of lipids. The obtained peaks of 1660 cm^{-1} evidence the presences of proteins. The position of that peak at 1660 cm^{-1} reveals that the structure of the protein in *Peganum harmala* plant is α-helix. Proteins containing this structure, display a peak in the 1648 - 1665 cm^{-1} region. The very intense peak at 1024 cm^{-1} for both honeys indicates the presence of carbohydrates. The peak at 1436 cm^{-1} is suggesting the presence of phenol.

3.2. Cytotoxic Results

3.2.1. The Cytotoxic Activity of *Peganum harmala* on Cancer Cell Line PC3

The growth analysis of Cancer Cell Line PC3 following an exposure to *Peganum harmala* for 24 hour was recorded and illustrated in **Figure 2**. The paired Student t-test indicated an inhibition of the growth of the PC3 prostate cancer cell line after 24 hours of exposure to *Peganum harmala* leaf extracts at all concentrations: 164, 82, 41, 20.5, 10.3, 5.1, 2.6 µg/µl. The rate of inhibition of the growth of PC3 Cancer Cell Line increased with an increase in the concentration of the *Peganum harmala* solution. An optimum inhibition was observed at 82 µg/µl. Using the obtained findings, it can be said that *Peganum harmala* leaf extracts inhibit PC3cell viability significantly at achievable concentration.

3.2.2. The Cytotoxic Activity of *Peganum harmala* on Cancer Cell Line HeLa

The growth analysis of Cancer Cell Line HeLa after an exposure to *Peganum harmala* for 24 hour was recorded and illustrated in **Figure 3**. As given by the paired Student t-test, exposure of the growth of the HeLa cancer cell line to *Peganum harmala* affected the growth of these cancerous cells after 24 hours. The observed inhibition was effective at all concentrations: 164, 82, 41, 20.5, 10.3 and 5.1 µg/µl, except 2.6 µg/µl, of leave extracts. The rate of HeLa cancer cell line inhibition increased with the increase in the concentration of the *Peganum harmala* leaf extract. In this regard, *Peganum harmala* leaf extracts significantly inhibited HeLa cell viability at the attainable concentrations.

3.2.3. The Cytotoxic Activity of *Peganum harmala* on Cancer Cell Line A549

The anticancer effects of *Peganum harmala* on Cancer Cell Line A549 were rec-

orded in Figure 4. As analyzed by the paired Student t-test, a growth of the A549 Lung cancer cell line was given to be affected after 24 hours of exposure to *Peganum harmala* leaf extracts at all concentrations: 164, 82, 41, 20.5, 10.3, 5.1, 2.6 µg/µl. In this respect, *Peganum harmala* leaf extracts significantly inhibited A549 cell viability at most of the concentrations. The rate of inhibition is also shown to increase with the increase in the concentration of *Peganum harmala* leaf extracts.

3.3. Cytotoxic Effects of Manuka Honey on Cancer Cells Lines HeLa, A549 and PC3

As given in Figure 5. According to the paired Student t-test a growth of the HeLa, A549, and PC3 cancer cell line were affected after 24 hours of exposure to 50% Manuka Honey. Manuka Honey extracts inhibited HeLa, A549 and PC3 cell viability by (46, 26 and 51 percent) respectively at 50% concentration.

As given in Figure 6, the paired student t-test analysis indicated a growth of the HeLa, A549, and PC3 cancer cell line were affected after 24 hours of exposure to 50, 25 and 12.5% Manuka Honey. In this respect, Manuka Honey extracts inhibited HeLa and PC3 cell viability at all concentrations. The level of growth was significantly high for A549 cells, moderate for PC3 cells and low for HeLa cells.

3.4. Cytotoxic Effects of Saudi Honey on Exposure to A549, HeLa, and PC3

Effects of Saudi Honey on exposure to A549, HeLa, and PC3 Cancer Cell Line were recorded in Figure 7. Following the analysis using the paired Student t-test, the growth of the HeLa, A549, and PC3 cancer cell line were shown to be affected after 24 hours of exposure to 50% Saudi Honey. The level of inhibition was significantly high for A549 cells, and low for PC3 cells and HeLa cells. In this respect, Saudi Honey extracts can be deduced to inhibit A549cell viability highly at 50% concentrations.

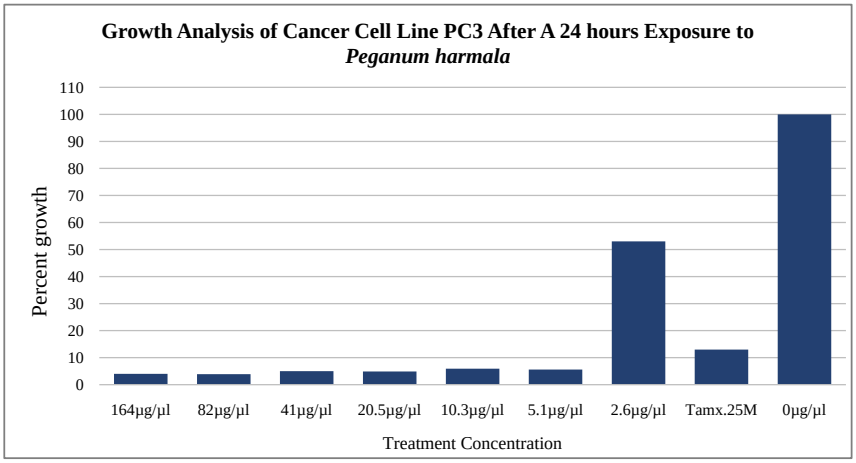

Figure 2. According to the paired student t-test a growth of the PC3 prostate cancer cell line was affected after 24 hours of exposure to *Peganum harmala* leaf extracts at all concentrations: 164, 82, 41, 20.5, 10.3, 5.1, 2.6 µg/µl. This Figure shows that *Peganum harmala* leaf extracts significantly inhibited PC3 cell viability at most of the concentrations.

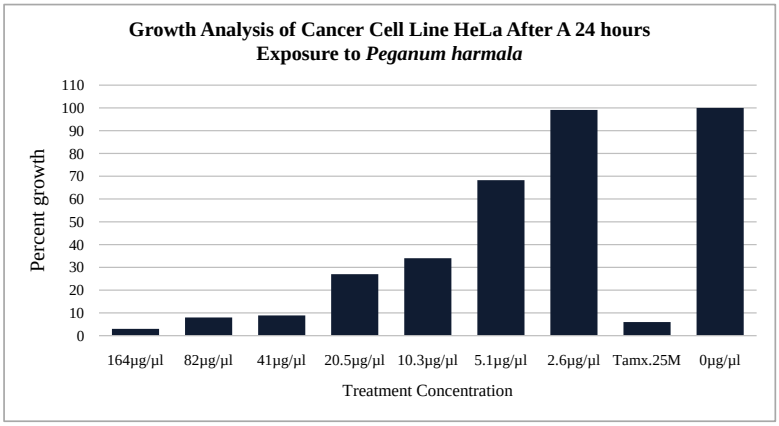

Figure 3. According to the paired student t-test a growth of the HeLa cancer cell line was affected after 24 hours of exposure to *Peganum harmala* leaf extracts at all concentrations: 164, 82, 41, 20.5, 10.3, 5.1, 2.6 µg/µl. This Figure shows that *Peganum harmala* leaf extracts significantly inhibited HeLa cell viability at most of the concentrations.

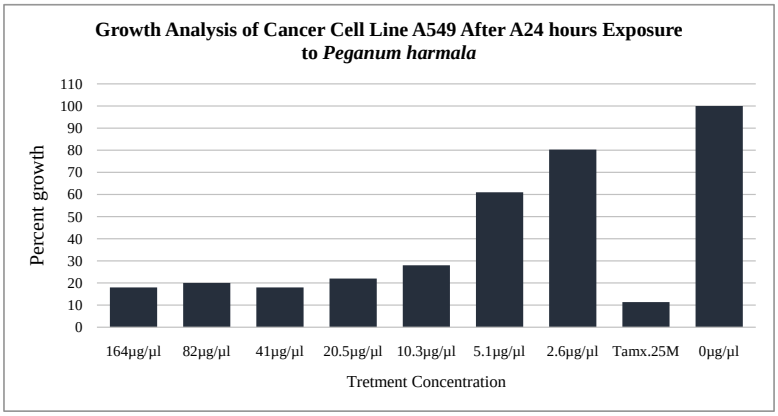

Figure 4. According to the paired student t-test a growth of the A549 Lung cancer cell line was affected after 24 hours of exposure to *Peganum harmala* leaf extracts at all concentrations: 164, 82, 41, 20.5, 10.3, 5.1, 2.6 µg/µl. This Figure shows that *Peganum harmala* leaf extracts significantly inhibited A549 cell viability at most of the concentrations.

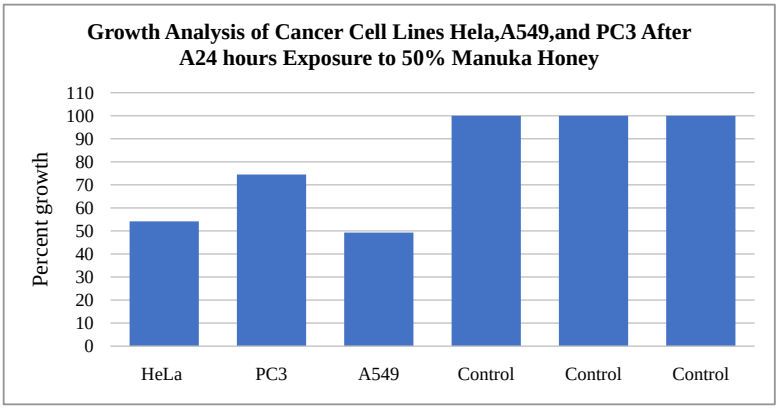

Figure 5. According to the paired student t-test a growth of the HeLa, A549 and PC3 cancer cell line were affected after 24 hours of exposure to 50% Manuka Honey. This Figure shows that Manuka Honey extracts inhibited HeLa, A549 and PC3 cell viability at 50% concentrations.

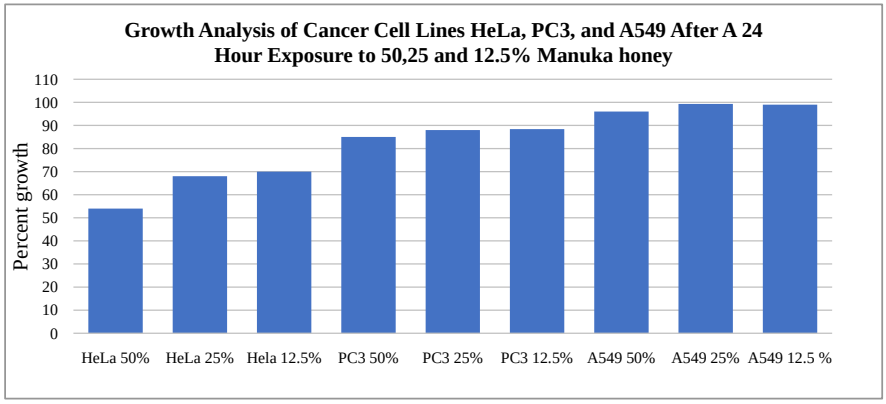

Figure 6. According to the paired student t-test a growth of the HeLa, A549 and PC3 cancer cell line were affected after 24 hours of exposure to 50, 25 and 12.5% Manuka Honey. This Figure shows that Honey extracts inhibited HeLa, A549 and PC3 cell viability at some concentrations.

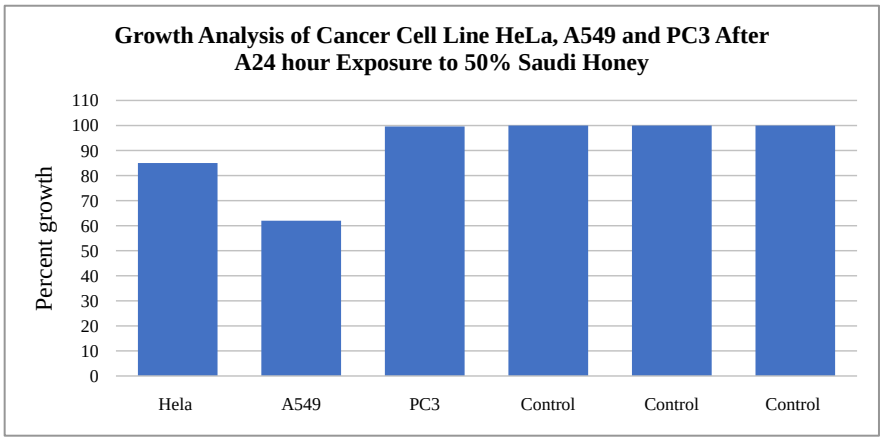

Figure 7. According to the paired student t-test a growth of the HeLa, A549 and PC3 cancer cell line were affected after 24 hours of exposure to 50% Saudi Honey. This Figure shows that Saudi Honey extracts inhibited A549 and HeLa cell viability at 50% concentrations.

4. Conclusion

As given by the observations of this study, *Peganum harmala* seems to be a highly effective plant in inhibiting cancer cell growth. *Peganum harmala* extracts inhibit the growth of cancer cells at achievable concentrations, which can be efficiently tolerated by normal body cells. In this respect, *Peganum harmala* can form an effective treatment for managing several forms of cancer. Manuka Honey and Saudi Honey are also other natural products, which have been observed to be extensively efficient in preventing the growth of cancer cells. Manuka honey inhibits the growth of the HeLa, A549, and PC3 cancer cell lines within 24 hours of exposure. Manuka Honey is highly effective in inhibiting the HeLa cell line. In this respect, Manuka Honey can be applied as an effective treatment for managing cervical cancers. Saudi Honey also showed significant cytotoxicity against A549, HeLa, and PC3 cancer cell lines. The level of inhibition of cancer

cell growth by Saudi honey was significantly high for A549 cells. In this respect, Saudi honey can form an effective treatment for managing lung cancers. In general, the two IR spectra are very similar, but a detailed analysis shows that the two types of honey are different. Also, *Peganum harmala* plant contains a broadband located at 3401 cm-1 due to OH and NH stretching modes.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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