Acacia Honey Modulates Cell Cycle Progression, Pro-inflammatory Cytokines and Calcium Ions Secretion in PC-3 Cell Line

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Abstract

Background: Prostate cancer is the most common malignancy in men and the second leading cause of cancer-related deaths. Honey has been proven to have a potentiality of being among the functional foods by both in vivo and in vitro studies.

Aim: This study was primarily designed to provide evidence on the apoptotic role of acacia honey on PC-3 cell line.

Methods: MTT and mitotic index assay were used to determine the anti-proliferative effects. Cell cycle analysis was conducted on flow cytometer using propidium iodide. Expression of pro-inflammatory cytokines [TNF α and IL-1β] and prostate specific antigen were determined by ELISA kits. Calcium ion level was analyzed by colorimetric method.

Results: Our results show the anti-proliferative effects of acacia honey on NIH/3T3 and PC-3 cell lines with the IC50 of 3.7 and 1.9% [v/v] respectively with simultaneous increase in the secretion of calcium ion and down regulation of prostate specific antigen. Significant [p < 0.05] dose-dependent modulation of GO/G1 phase was observed as compared with the control. However, there was an inverse relationship between TNF-α and IL-1β with latter being up regulated. Mitotic index of PC-3 cells decreased as the concentration of honey increases.

Conclusion: Apoptotic role of acacia honey on PC-3 cell line may be due to modulation of GO/G1 phase, pro-inflammatory cytokines, calcium ions secretion and down regulation of prostate specific antigen in vitro.

Keywords: Acacia honey; PC-3 cells; Apoptosis; Cytokines; Calcium ion

Introduction

Cancer is a disease of multidimensional processes that affects most of the populace in both developed and under developed countries. It is an abnormal cell growth that is characterized by the fact that the cells keep replicating when they are actually suppose to be differentiated. It has been established to be a complex disease caused by abnormal cellular proliferation associated with resistance to normal growth inhibitory signals, uncontrolled activation of growth signals, impairment of apoptosis, promotion of angiogenesis (the development of new blood vessels to support tumor growth), invasion of surrounding tissues and metastasis i.e. spread of tumor cells to distant tissues [1,2].

Prostate cancer is the most common malignancy in men and the second leading cause of cancer-related deaths [3]. The majority of the prostate cancer cells are classified as adenocarcinomas characterized by an absence of basal cells, and uncontrolled proliferation of malignant tumor cells with features of luminal differentiation including glandular formation and the expression of Androgen Receptor (AR) and prostate-specific antigen [4,5].

Cytokines are hydrosoluble extracellular polypeptides or glycoproteins ranging from 8 to 30 kDa [6]. They are produced by several types of cells, at the site of injury, and immune cells, through mitogen-activated protein kinases. Some cytokines can have a pro- (Th1) or anti-inflammatory (Th2) actions, according to the microenvironment in which they are located [6]. Example of pro-inflammatory cytokine are interleukins [IL] 1, 2, 6, 7, and TNF [tumor necrosis factor]. While anti-inflammatory cytokines include IL-4, IL-10, IL-13, and TGFβ [transforming growth factor]. Interestingly, most of the pro-inflammatory cytokines tends to play a dual role of apoptosis and carcinogenesis especially at the level of cancer progression. The divalent calcium cation Ca2+ is used as a major signaling molecule during cell signal transduction to regulate energy output, cellular metabolism, and phenotype as well as apoptosis via direct and indirect mechanisms [7]. Apoptosis, also called programmed cell death or physiological cell death, is an essential and evolutionarily conserved property involved in the maintenance of multicellular organisms and found in all species from worm to man. It is required in many fundamental biological processes including embryonic development, metamorphosis, hormone-dependent atrophy and chemically-induced cell death [8,9].

Honey, a natural sweet substance produced by bees through
regurgitation mechanisms has been proven to have a potentiality of being among the functional foods by both in vivo and in vitro studies. It is a promising antitumor agent with pronounced anti-metastatic and anti-angiogenic effects [10], antibacterial, anti-inflammatory, immune-stimulant, antiulcer and wound/burn healing effects [11]. Various signaling pathways, including stimulation of tumor necrosis factor-alpha (TNF-α) release, inhibition of cell proliferation, induction of apoptosis and cell cycle arrest, as well as inhibition of lipoprotein oxidation, mediate the beneficial effects exerted by honey and its major components such as chrysin and other flavonoids [12-16]. However, chronic consumption of honey that was mixed with rat pellets has been shown to have a positive impact on the architecture and integrity of hepatocytes in vivo [17]. Its protective role against the kidney dysfunctions induced by sodium nitrite, a known food additives, hepatoprotective, hypoglycemic, reproductive, anti hypertensive and of course antioxidant effects has also been reported [18,19]. Honey is produced from many different floral sources and its biochemical and pharmacological activities vary depending on its origin and processing. Honey contains a variety of biologically active compounds such as flavonoids, vitamins, antioxidants as well as hydrogen peroxides [20]. Daily consumption of honey has been shown to have both positive and negative effect on male Wister albino rat [21]. Acacia honey and chrysin the active principle has reduced proliferation of melanoma cells through alterations in cell cycle progression in vitro [22]. To the best of our knowledge, so far no study has so far being carryout to establish the links between anticancer activity of acacia honey with modulation of cytokines and possible secretion of calcium ion as a mediator of apoptosis. Therefore, this study was undertaken to provide an evidence on the apoptotic role of acacia honey on PC-3 cell line through the modulation of pro-inflammatory cytokines (TNFα and IL1β) and secretion of calcium ion in an attempt to justify its effects on the expression of prostate specific antigen, mitotic index and anticancer properties in vitro (Chart 1).

Materials and Methods

Sample collection

Honey, produced by Apis mellifera was collected from the northwest frontier of Pakistan during spring season of 2012 from Acacia modesta flower and maintained at 4°C until analysis at the industrial analytical centre, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan.

Cell cultures and treatments

PC-3 Human prostate cancer cell line and NIH/3T3 normal cell lines were generously provided by Dr. Ahmed M. Mesaik of Molecular Immunology Laboratory, Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan. The cells were grown in DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS). 1% (v/v) L-glutamine, 100 U penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere with 5% CO2. The cells were passage twice weekly. Acacia honey was added to cells in complete medium after 24 h of growth. Honey was diluted to a final concentration of 0.5, 1.0, 2.0, 4.0 and 8.0 % (v/v) for cancer cell line and 2.0, 4.0, 6.0, 8.0 and 10 % (v/v) for normal cell line. The experiments were in triplicates/duplicates and repeated at least three times along with untreated cells serving as control.

MTT assay

Anticancer/cytotoxicity of acacia honey on PC-3 and NIH/3T3 cells was evaluated in 96-well flat-bottomed micro plates by using standard MTT (3-(4,5-dimethyl thiazole-2-yl)-2, 5-diphenyl-tetrazolium bromide) colorimetric assay [23]. Exponentially growing cells were harvested, counted with hemocytometer and diluted with a medium. Cell culture with the concentration of 1×105 cells/mL were prepared and introduced (100 μL/well) into 96-well plates. After overnight incubation, medium was removed and 200μL of fresh medium was added with different concentrations of Honey. After 48 hrs, 200 μL MTT (2 mg/mL) was added to each well after aspiration of media and incubated further for 4 hrs. Subsequently, 100 μL of DMSO was added to each well after aspiration of media. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a micro plate reader (spectra 340, softmax PRO 4.3.1.LS, Molecular device, USA). The same procedure was followed checking the anticancer activity of honey on PC-3 cells after 24 hrs incubation with honey and another plates 24 hrs after the replacement of honey with just the medium. The cytotoxic effect was recorded as concentration causing 50% inhibition (IC50) for PC-3 and NIH/3T3 cells. The percent inhibition was calculated using the following formula:

\[
\% \text{ inhibition} = 100 - \frac{\text{mean of Absorbance of sample}}{\text{mean of Absorbance of control}} \times 100
\]

Determination of cytokines (TNFα and IL1β) from the cultured supernatant of PC-3 cells

The experiment was conducted for 24 hrs and 48 hrs incubation period. ELISA kits for specific cytokines were used (R&D systems, USA and Canada) according to the manufacturer’s protocol. Each sample was tested in duplicate in each of three or more replicate experiments. After development of the colorimetric reaction, the absorbance at 450 nm was quantitated by using a micro plate reader (spectra 340, softmax PRO 4.3.1.LS, Molecular device, USA), and the absorbance readings were converted to pg/ml based upon standard curves obtained with recombinant cytokine in each assay.

Determination of calcium ion from the cultured supernatant of PC-3 cells

The experiment was conducted for 24 hrs and 48 hrs incubation period as well as 24 hrs after the replacement of honey with just the medium. The calcium ion level in cultured supernatant was quantified using the Cobas kits and Roche/Hitachi 912 automatic analyzer.
according to manufacturer’s protocols. The principle was based on the complex formed between calcium ion and o-cresolphthalein complexone in alkaline solution. The absorbance was read at 450nm. Each sample was tested in duplicate in each of three or more replicate experiments.

**Determination of prostate specific antigen (PSA) from the cultured supernatant of PC-3 cells**

The experiment was conducted for 48 hrs incubation period. ELISA kits specific for PSA (Bio Check, Inc, Vintage Foster City) was used to quantify PSA level in the cultured supernatant according to the manufacturer’s protocol. Each sample was tested in duplicate in each of three or more replicate experiments. After development of the colorimetric reaction, the absorbance at 450 nm was quantitated by using a micro plate reader (spectra 340, softmax PRO 4.3.1.LS, Molecular device, USA), and the absorbance readings were converted to pg/ml based upon standard curves.

**Mitotic index assay on PC-3 cells**

The experiment was conducted for 48 hrs incubation period. Exponentially growing cells were harvested, counted with hemocytometer and diluted with a medium. Cell culture with the concentration of 1x10^5 cells/ml was prepared and introduced 1ml/well. 24 hrs after, the various concentrations of the honey were added to the wells except the control that was given only the medium in order to normalized the condition. After 70 hrs of incubation, a 0.1 ml colcemid solution (10 μg/mL) was added to each culture tube and mixed by shaking gently. After 72 hr of incubation, cells were harvested by centrifugation, by hypotonic treatment (0.075 M KCl) and fixing in a fresh fixative solution (methanol: acetic acid, 3:1). The fixation step was repeated three times. Slides were air-dried and stained with 5% Giemsa [24]. Mitotic Index was calculated as the proportion of metaphase for 1000 cells as shown below:

\[
\text{Percent mitotic index} = \frac{\text{Number of metaphase}}{\text{Total number of cells}} \times 100 
\]

**Cell cycle analysis by FACS (fluorescence-activated cell sorting) analysis**

PC-3 cells were seeded at density of 1 x 10^6 cells/well in 6-well plates. After 24 h cells were treated with honey as previously described. After 48 hrs treatment and incubation at 37°C and 5% CO₂, cells were washed with PBS harvested with trypsin and centrifuged, fixed with ice-cold 100 % ethanol overnight. They fixed cells were centrifuged for 1 hr at 300 xg and washed with PBS twice. The cells were then treated with 125 μL of 500 U/ml RNase A and incubated at 37°C for 15 minutes and thereafter, 125 μL of 5 mg /100 ml Propidium iodide in 1.12% sodium citrate was added and allowed to stand at room temperature for 30 minutes. Cell cycle distribution was analyzed by flow cytometry on a FACSC alibur (Becton Dickinson, Canada, Inc). PI was excited at 488 nm, and fluorescence analyzed at 620 nm. A total of 10,000 events in each sample were acquired. Using CellquestPro software, the percentages of cells at different phases of the cell cycle were determined.

**Statistical analysis**

To address the biological variability and stability of the samples, each and every experiment was repeated at least three times and the results were expressed as mean ± Standard deviation. Differences between the groups were analyzed by one-way analysis of variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS) software, SPSS Inc, Chicago, Standard version 10.0.1. P-values < 0.05 were considered statistically significant for differences in mean using the least of significance difference (LSD).

**Results**

As seen in figure 1, honey was able to significantly (p < 0.05) inhibit the growth of 3T3 cell line in a concentration dependent manner with an IC₅₀ of 3.7 % (v/v). We used 2, 4, 6, 8 and 10 % (v/v) in order to evaluate the cytotoxicity of honey on normal cell line and from our results; it indicates that the honey is cytotoxic at about 4 % (v/v). Probably, this might be as a result of the generation of hydrogen peroxide.
peroxide due to oxidation of monosaccharides present in honey which has been implicated in initiating apoptosis.

Figure 2 shows the anticancer activity of honey on PC-3 cell line with an IC_{50} of 1.9 % (v/v). This indicates that it is more cytotoxic to cancer cells than the normal ones. This might be attributed to the fact that in a disease condition/non-diseases conditions there seem to be different mode of response towards treatments as their metabolisms differ. However, in figure 3, acacia honey was only able to confer its anticancer activity after 24 hrs incubation with an IC_{50} of 4.43% (v/v). On replacement of honey with a just a medium, there was a reverse of anticancer activity with an IC_{50} >8.0% (v/v) which was the highest concentration used. By implication, acacia honey seems to be more active against PC-3 cells after long time incubation.

As depicted in figure 4, there was a significant (p < 0.05) increase in calcium ion level from 24 to 48 hrs incubation periods with honey. However, the level declined after replacement of the honey with the medium. This indicates that there is a calcium ion involvement on the anticancer activity of acacia honey providing an evidence of apoptotic role since calcium ion is a mediator of programmed cell death.

In figure 5, TNF-α level increased significantly (p < 0.05) after 24 hrs incubation at the lowest concentration. However, the level declined with increase in concentration of honey. After 48 hrs incubation, the level was drastically reduced as compared to 24 hrs. This suggests that acacia honey was able to modulate TNF-α expression by acting as an anti-inflammatory agent. This means that more TNF α is being utilized for apoptotic pathway rather than inflammation which is a key factor for the survival of cancer cells.

Furthermore, from figure 6, IL-1β level increases significantly (p < 0.05) at the lowest concentration and start declining with increase in concentration after 24 hrs incubation indicating anti-inflammatory role. Surprisingly, the level elevated after 48 hrs incubation with increase in concentration of honey. This might be as a result of the fact that much of TNF α has been utilized in the production of IL-1β which also believed to play a key role in the release of calcium ion from endoplasmic reticulum. The sudden increased observed in control might be as a result of the cytokine’s role in keeping the cancer cells alive. Therefore, we propose that acacia honey modulates this cytokine towards apoptotic rather than inflammatory role by virtue of the increased in calcium ion level.

From figure 7, the level of prostate specific antigen decreased from 2-8% (v/v) of honey indicating an effect on its expression. Similarly in figure 8, mitotic index of PC-3 cell line decreases significantly (p < 0.05) in a concentration dependent manner. In fact we could not detect appreciable number of cells at 8% (v/v). This is not surprising because the IC_{50} was 1.9% (v/v).

Arrest of cell cycle phases is one of the important steps towards anticancer activity. Our results (Figure 9 a,b,c) indicate that acacia honey was able to modulate G0/G1 phase significantly (p < 0.05) by altering its progression to other phases in a concentration dependent with concomitant decreased in % S phase and G2/M phase as compared with control. This means that honey was able to arrest the cancer cell proliferation at G0/G1 phase due to the observed % DNA contents.
Discussion

Antioxidants activity of honey has been found to be as a result of the synergistic effects of wide range of compounds present in it [12]. The diversity and concentration of these components is dependent on the botanical and geographical origin of honey. Moreover, it is also influenced by the variation in climate and availability of plant sources for the honeybees to harvest nectar within a specific region [25]. Therefore, honey originating from different floral sources differs in their chemical compositions. Our findings show that acacia honey was able to act as an anti-proliferative agent by its ability to inhibit the growth of PC-3 cell line in vitro. This is in support with reports that acacia honey reduces the proliferation of other cell lines like melanoma cells [22]. Basically, various polyphenols are reported in honey. Some of which are Caffeic acid, Caffeic acid phenyl ester, Chrysin, Galangin, Quercetin, Acacetin, Kaempferol, Pinocembrin, Pinobanksin and Apigenin. These have evolved as promising pharmacological agents in treatment of cancer [26]. Characterization of acacia honey revealed three (3) phenolic acids (p-hydroxybenzoic, ferulic and t-cinnamic acid), abscisic acid and five (5) free flavonoids (pinobanksin, apigenin, pinocembrin, crysin and acacetin) [27]. Polyphenols and phenolics have been reported to inhibit cancer-related pathways and processes including prostate cancer [28,29]. High phenolic and hydroxymethylfurfural (HMF) contents of honey resulted in the growth inhibitory effects of cancer cells [30], and flavonoids have been reported to induce cell death of various cancer cell lines [31]. It has previously been reported that a Japanese pure unfractonated honey significantly reduced the proliferation of the human bladder cancer cells, T24 [32]. It has been reported that other type honey shows no significant cytotoxic effect on normal human cell lines [33]. The cytotoxic effect of honey may be attributed to the fact that it may contain pyrrolizidine alkaloids which are considered as toxic compounds [34].

Inhibition of apoptosis can be seen as the basis of abnormal cell growth and calcium ion has been implicated to play a vital role via direct and indirect apoptotic pathways. The involvement of Ca\(^{2+}\) in cell death has been recognized very early in the history of programmed cell death, with the demonstration in vitro that Ca\(^{2+}\) ionophores, which are the molecules capable of transporting Ca\(^{2+}\) across membranes down its electrochemical gradient, are highly toxic to cells [35]. In the present study we demonstrate that anti-proliferative effects of acacia honey is being accompanied by stimulation of calcium ion and thus, implicating its anticancer activity in relation to the induction of apoptosis via the calcium ion dependent pathway.

Pro-inflammatory cytokines are one of the major key players during chronic inflammation which favored the survival of cancer cells. Of such type are TNF α and IL 1β. It has been reported that pro-inflammatory cytokines are capable of promoting proliferation, invasion and angiogenesis of prostate cancer, and of several other solid tumors [36-38]. Our results show the anti-inflammatory role of acacia honey by its ability to down regulate the level of TNF α in PC-3 cell lines [33]. The cytotoxic effect of honey may be attributed to the fact that it may contain pyrrolizidine alkaloids which are considered as toxic compounds [34].

Figure 8: % mitotic index of PC-3 cell lines after 48 hrs treatment with different concentration of honey.

Figure 9a: Results of cell cycle analysis on PC-3 cell line in the presence of control.

Figure 9b: Results of cell cycle analysis on PC-3 cell line in the presence of 0.5% acacia honey.

Figure 9c: Results of cell cycle analysis on PC-3 cell line in the presence of 1% acacia honey.

Figure 9d: Results of cell cycle analysis on PC-3 cell line in the presence of 2% acacia honey.

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ERK has been reported to stimulate the generation of reactive oxygen species (ROS) and produce pro-angiogenic and anti-angiogenic growth factors [45]. It has also been shown to play a significant role in prostate tumor growth by regulating various processes such as cell proliferation, survival, and metastasis [46].

The prostate-specific antigen (PSA), a member of the kallikrein protein family, is widely used as a surrogate marker to monitor the progression of prostate cancer [42]. PSA is a serine protease that is released from the prostate gland and enters the circulation where it exists as a mixture of various molecular forms, including free PSA and several PSA protease inhibitor complexes [44]. Our findings indicate that acacia honey was able to decrease the level of PSA in a concentration-dependent manner. PSA has been established to play a significant role in prostate tumor growth by regulating various pro-angiogenic and anti-angiogenic growth factors [45]. It has also been reported to stimulate the generation of reactive oxygen species (ROS) and produce pro-angiogenic and anti-angiogenic growth factors [46].

The inhibition of cell growth could be a result of the induction of apoptosis that may be mediated by cell cycle arrest. Therefore, we hypothesize that acacia honey mediated inhibitory effects of cell growth of PC-3 cells might be due to perturbation in the cell cycle, which may possibly lead to programmed cell death.

Conclusion

We therefore conclude that anticancer activity and apoptotic role of acacia honey on PC-3 cell line may be due to modulation of G0/G1 phase of cell cycle, TNF-α, IL-1β, calcium ions secretion, and downregulation of prostate specific antigens in vitro.

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References

1. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100: 57-70.
2. D'Addario A, Capasso D, Ceruso B, De Vivo C, Di Palma A, et al. (2008) Basic principles of the molecular biology of cancer. J. Surgery 24: 43-47.
3. Cooperberg MR, Park S, Carroll PR (2004) Prostate cancer 2004: insights from national disease registries. Oncology (Williston Park) 18: 1239-1247. 1248-1250, 1256-1258.
4. Sun Y, Liu J, Huang J (2009) Neuroendocrine differentiation in prostate cancer. Am J Transl Res 1: 148-162.
5. Huang J, Wu C, Di Sant'Agnese PA, Yao JL, Cheng L, et al. (2007) Function and molecular mechanisms of neuroendocrine cells in prostate cancer. Anal Quant Cytol Histol 29: 128-138.
6. de Oliveira CM, Sakata RK, Issy AM, Gerola LR, Salomão R (2011) Cytokines and Pain. Rev Bras Anestesiol 61: 255-265, 260-265, 137-142.
7. Kass GE, Orrenius S (1999) Calcium signaling and cytotoxicity. Environ Health Perspect 1: 25-35.
8. Hunot S, Flavell RA (2001) Apoptosis: death of a monopoly? Science 292: 865-866.
9. Meier P, Finch A, Evan G (2000) Apoptosis in development. Nature 407: 796-801.
10. Hanana MR, Shaymaa MMY (2011) Enhancement of the antitumor effect of hormone and some of its extracts using adiponectin hormone. Austral J Basic Appl Sci 5: 100-108.
11. Fiorani M, Accorsi A, Blasa M, Diamantini G, Piatti E (2006) Flavonoids from Italian multifloral honeys reduce the extracellular ferricoyanide in human red blood cells. J Agric Food Chem 54: 8328-8334.
12. Gheldof N, Wang XH, Engeseth NJ (2002) Identification and quantification of antioxidant components of honeys from various floral sources. J Agric Food Chem 50: 5870-5877.
13. Mabrouk GM, Moselhy SS, Zehny SF, Ali EM, Helal TE, et al. (2002) Inhibition of methylthiouracil (MNU) induced oxidative stress and carcinogenesis by orally administered bee honey and Nigella grains in Sprague Dawley rats. J Exp Clin Cancer Res 21: 341-346.
14. Swellam T, Miyanaga N, Onozawa M, Hattori K, Kawai K, et al. (2003) Antineoplastic activity of honey in an experimental bladder cancer implantation model: in vivo and in vitro studies. Int J Urol 10: 213-219.
15. Tonika A, Cooper RA, Price AJ, Molan PC, Jones KP (2001) Stimulation of TNF- 
alpha release in monocytes by honey. Cytokine 14: 240-242.
16. Woo KJ, Jeong YJ, Park JW, Kwon TK (2004) Chrysins-induced apoptosis is 
mediated through caspase activation and Akt inactivation in U937 leukemia 
cells. Biochem Biophys Res Commun 325: 1215-1222.
17. Wilson J, George BO, Umukoroko GE (2011) Effects of honey on the histology of 
life in adult Wistar rats. Biol Med 3: 1-5.
18. Hassan HA (2007) The possible protective role of bee honey against hazard 
effects of some synthetic food additives on the kidney functions of male rats. J 
Egypt Soc Toxicol 36: 13-21.
19. Omotayo OE, Sulaiman SA, Wahab MSA (2012) Honey: A novel antioxidant. 
Molecules 17: 4400-4423.
20. Mohammadzadeh S, Sharifatpanahi M, Hamedi M, Amanzadeh Y, Ebrahimi 
SSE, et al. (2007) Antioxidant power of Iranian propolis extract. Food Chem 103: 
729-733.
21. Muhammad A, Oyonenke AO, Solomon EO, Michael AG, Muhammad IC, 
et al. (2012) daily consumption of honey: effects on male wister albino rats. 
International Journal of Food Nutrition and Safety 1: 66-74.
22. Pichichero E, Cicconi R, Mattei M, Muzi MG, Canini A (2010) Acacia honey and 
chrysin reduce proliferation of melanoma cells through alterations in cell cycle 
progression. Int J Oncol 37: 973-981.
23. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: 
application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55- 
63.
24. Ozkuz Y, Silici S, Eroglu E (2005) the anticarcinogenic effect of propolis in 
human lymphocytes culture. Phytomedicine 12: 742-747.
25. Ankram E (1998) A review of the analytical methods to determine the 
geographical and a botanical origin of honey. Food Chem 63: 549-562.
26. Jaganathan SK, Mandal M (2009) Antiproliferative effects of honey and of its 
alkaloids: their occurrence in honey from tansy ragwort (Senecio jacobaea L.). 
Science 195: 497-499.
27. Ricole M, Garcia-Tunon I, Bethencourt FR, Fraile B, Paniagua R, et al. (2004) 
Interleukin-1 (IL-1alpha and IL-1beta) and its receptors (IL-1RI, IL-1RII, and 
IL- 1Ra) in prostate carcinoma. Cancer 100: 1388-1396.
28. Salazar-Onfray F, Lopez MN, Mendoza-Naranjo A (2007) Paradoxical effects 
of cytokines in tumor immune surveillance and tumor immune escape. Cytokine 
Growth Factor Rev 18: 171-182.
29. Lou W, Ni Z, Dyer K, Tweardy DJ, Gao AC (2000) Interleukin-6 induces prostate 
cancer cell growth accompanied by activation of stat3 signaling pathway. 
Prostate 42: 239-242.
30. Ormenius S, Zhivotovsky B, Nicotera P (2003) Regulation of cell death: the 
calcium-apoptosis link. Nat Rev Mol Cell Biol 4: 552-565.
31. Pan MH, Ho CT (2008) Chemopreventive effects of natural dietary compounds 
on cancer development. Chem Soc Rev 37: 2558-2575.
32. Eisenhut M, Wallace H (2011) ion channels in inflammation. Pflugers Arch 461: 
401-421.
33. Diamandis EP (1998) Prostate-specific antigen: its usefulness in clinical 
medicine. Trends Endocrinol Metab 9: 310-316.
34. Armbuster DA (1993) Prostate-specific antigen: biochemistry, analytical 
methods, and clinical application. Clin Chem 39: 181-195.
35. Huang WM, Finne P, Leinonen J, Vesalainen S, Nordling S, et al. (1999) 
Measurement of the complex between prostate-specific antigen and alpha1- 
protease inhibitor in serum. Clin Chem 45: 814-821.
36. Bindukumar B, Schwartz SA, Nair MP, Aalinkeel R, Kawinski E, et al. (2005) 
Prostate-Specific Antigen Modulates the Expression of Genes Involved in 
Prostate Tumor Growth. Neoplasia 7: 241-252.
37. Sun XY, Donald SP, Phang JM (2001) Testosterone and prostate specific 
antigen stimulate generation of reactive oxygen species in prostate cancer 
cells. Carcinogenesis 22: 1775-1780.