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Antiviral effect of Camelyn (honey extract) against SARS-CoV-2 virus

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SUMMARY

The purpose of this study was to evaluate the potential antiviral activity of a honey extract called "Camelyn" against coronavirus 2 causing severe a c u t e respiratory syndrome (SARS-CoV-2). A cell line from the kidney of a newborn hamster (BHK-21), hematopoietic stem cells (HSCs) derived from bone marrow, and spleen cells were used in the cytotoxicity test of the Camelyn product. After isolation procedures, cell viability was assessed after trypan blue staining under a microscope using a hemocytometer. The in vitro cell proliferation rate was assessed using the Cell Counting Kit 8 (CCK-8) assay. Cells were seeded in growth media with different concentrations of Camelyn product (35 µg, 50 µg, 70 µg, 100 µg, 150 µg, and 200 µg). Absorbance at 450 nm was determined using a multi-plate reader. In order to determine the susceptibility of SARS-CoV- virus.
2 per drug antiviral activity was evaluated using a baldness reduction assay. Serial dilutions of the selected compounds were preincubated with 40 to 100 SARS-CoV-2 virus baldness-forming units (PFUs). The mixture of Camelyn product and SARS-CoV-2 virus after preincubation was then added to Vero E6 cells forming a confluent layer. After incubation, the cells were fixed and stained, and PFUs were counted under an inverted microscope, after which presented results in form chart in relation to concentrations of the agent antiviral presented in logarithmic form. Our study showed that Camelyn's product is not cytotoxic, has a cell proliferation-stimulating effect, and has an inhibitory effect on SARS-CoV-2 virus, with EC50 (half-maximal effective concentration) values ranging from 85.7 µg/ml to 192.4 µg/ml, depending on the product concentration and viral baldness per cell.

INTRODUCTION

In view of the current pandemic, there is a great need for all possible treatments and prevention methods against COVID-19 disease, including existing natural products. Various organic compounds, including bee honey, propolis, royal jelly, curcumin, resveratrol, have been widely studied and used as potential therapeutic options for treating various infections [1]. Despite criticism of modern medicine in recent years, honey has received considerable attention due to its wide range of therapeutic properties, including antibacterial, anti-inflammatory and antiviral effects [2, 3]. Researchers have described various phytochemicals such as hydrogen peroxide, volatile organic acids, lysozyme, glucose oxidase and catalase as effective antimicrobial agents [4]. Beeswax, pollen and propolis are important chemical compounds responsible for honey's antimicrobial properties [5, 6]. Honey also contains small amounts of oligosaccharides that inhibit the growth of various microorganisms, such as intestinal bacteria [7]. Phenolic compounds present in honey, propolis and royal jelly, including flavonoids, are among the biologically active compounds that exhibit antimicrobial activity [8, 9]. These physical and chemical factors give honey unique properties. It has been established that honey eliminates wound infections, reduces scarring, inhibits inflammation, and stimulates angiogenesis and epithelial growth [10]. The anti-inflammatory effect of honey has been shown to be associated with inhibition of cytokine expression [11]. It is known that honey can enhance the proliferation of T and B lymphocytes, and it also stimulates phagocytosis and regulates the production of cytokines by monocytes, such as tumor necrosis factor (TNF),

interleukin 1 beta (IL-1β) and IL-6 [12]. Honey and several of its components have been found to block the cell cycle of colorectal cancer cell lines at the G0/G1 stage [13, 14].

In vitro studies have demonstrated honey's antiviral activity against various types of viruses [15-17]. The antiviral effect of honey is attributed to its various components, for example, copper, which is among the trace elements contained in honey and has the ability to inactivate viruses. Phenolic compounds (such as flavonoids), ascorbic acid or hydrogen peroxide, inhibit viral growth by interfering with virus transcription, translation and replication [1], [18], [19]. To elucidate the possible effect of honey, a study by Watanabe et al [20] used viral baldness reduction assays. According to available reports, manuka honey effectively inhibits influenza virus replication (EC50 = 3.6 ± 1.2 mg/ml), which is associated with its antiviral activity. In the presence of manuka honey at a concentration of 3.13 mg/ml, the EC50 value for zanamivir or oseltamivir dropped to nearly 1/1000th of that for a single administration of these drugs. The results showed that the honey exhibited a strong inhibitory effect on the influenza virus, and also demonstrated the possible therapeutic value of honey.

Different types of honey from eight floral sources were analyzed to evaluate their anti-HIV-1 activity, as well as their effects on lymphocyte proliferation. A quantitative polymerase chain reaction (PCR) method was used to evaluate the anti-HIV-1 activity of eight different types of honey. The study showed that single-flower honeys (from the same plant species) had anti-HIV-1 activity that depended on the plant sources and the amount of methylglyoxal in the biomass of these plants [21].

A study by Abedi et al [22] provided some evidence for the potential effects of honey and the compounds it contains on coronaviruses due to their ability to regulate the attachment and entry of the virus into host cells and the replication of its RNA. Honey and its components may also regulate cellular signaling pathways, including oxidative stress, inflammatory processes and apoptosis. One mechanism of antiviral action is inhibition of viral proteins necessary for viral adherence and entry into host cells [23]. It has been reported that honey can affect the disulfide bonds of hemagglutinin (HA) receptors, which prevents the influenza virus from binding to the host cell surface. The coronavirus spike protein belongs to the same class of proteins [24]. According to available reports [25,26], compounds present in honey, such as quercetin, chrysin, kemferol, galangin and caffeic acid, show antiviral activity against COVID-19 virus through binding with strong affinity to the main protease and [inhibiting] virus replication. The main compounds in honey, such as kemferol, galangin and caffeic acid, can inhibit virus adsorption, invasion and replication. Chrysin can prevent viruses from entering host cells and replicating. Quercetin can inhibit viral sheathing, invasion and replication [27-29]. Recent studies and a review article on the potential pharmacological activities of honey [30] indicate that honey and its main components may find applications in the prevention and treatment of coronavirus infections, which includes COVID-19 disease.

Although honey's antimicrobial activity has been well studied against many bacteria and fungi [31] [32], its antiviral activities still require extensive research so that it can be used in the prevention and treatment of various viral infections. The purpose of this study was to evaluate the antiviral activity of a honey extract called "Camelyn" against coronavirus 2 causing severe acute respiratory syndrome (SARS-CoV-2).

MATERIAL AND METHODOLOGY

The commercial product Camelyn in ampoules, obtained from the company JSC "Silicon Biotechnology", is produced from selected honey extract. The contents of the ampoule consist of 35% Camelyn product and 65% water for injection. Camelyn product contains ketones, ethers, bioorganic acids, phenols, aldehydes and furfural. For cytotoxicity and antiviral activity tests, Camelyn product was diluted to final concentrations ranging from 10 μg/ml to 2,500 μg/ml.

Experimental animals

Six-week-old BALB/c mice (n=3) were bred and housed in a breeding facility at the State Research Institute Centre for Innovative Medicine (Lithuania). All procedures were carried out in accordance with the institutional guidelines of the European Union and were approved by the Lithuanian Ethical Commission for the Use of Laboratory Animals under the State Veterinary Service, Resolution No. G2-124 (2019.07.11). Animals were maintained in a temperature-controlled environment (23 ± 1°C). *Ad libitum* food and water were provided.

Cell preparation

A cell line from the kidney of a newborn hamster (BHK-21) was obtained from Vilnius University Life Sciences Center (Lithuania). Parental BHK-21 cells were seeded in Dulbecco's Modified Eagle Medium (DMEM) with a high glucose concentration (4.5 g/l) (Life Technologies, USA) containing 10% FBS (Lonza, Switzerland) and 1% antibiotics (penicillin and streptomycin 10,000 U) (Lonza, Switzerland). Cultures were maintained at 37°C and in an atmosphere of 5% CO_2. The cell monolayer was dispersed using a mixture of 0.25% trypsin - EDTA (Lonza, Switzerland).

Bone marrow-derived hematopoietic stem cells (HSCs) were isolated by flushing the femurs and tibias of BALB/c mice, following the methodology previously presented in Juppperi et al.
[33] with some modifications. Splenic cells were isolated by stratifying tissues under low pressure with PBS and then passed through a sterile cell filter with a pore diameter of 70 μm. The suspensions of the collected

HSCs and spleen cells were washed with PBS and then fractionated in a density gradient using Lympholyte M medium (Cedarlane, USA) according to the manufacturer's instructions. Isolated HSCs and spleen cells were washed three times with RPMI-1640 solution containing 10% FBS (Lonza, Switzerland) and 1% antibiotics (penicillin and streptomycin 10,000 U (Lonza, Switzerland), centrifuged for 10 minutes at an acceleration of 300 x g, resuspended and counted. After isolation procedures, cell viability was assessed by trypan blue (0.4%, w/v) staining under a Nikon ECLIPSE 50i microscope (Nikon, Japan) using a hemocytometer.

Camelyn product cytotoxicity test

The in vitro cell proliferation rate was determined using the CCK-8 cell counting kit (Dojindo Laboratories, Japan) according to the manufacturer's recommendations. 2 x 10^5BHK-21 and HSC cells and 5

x 10^5spleen cells were seeded into growth medium placed in 96-well plates and incubated for 72 hours with control solution and Camelyn product solutions of different concentrations (35 µg, 50 µg, 70 µg, 100 µg,150 µg and 200 µg) at 37°C in a 5% CO_2 atmosphere. Absorbance at 450 nm was determined using a Sunrise multi-plate reader (Tecan, Austria). The viability of Camelyn-treated cells was compared with that of control (untreated) and DMSO-treated (positive control) cells. All tests were performed in three independent experiments.

Baldness reduction test for SARS-CoV-2 virus

A baldness reduction assay was performed to determine the drug susceptibility of SARS-CoV-2 virus (Quebec City/21697/2020). Selected compounds were evaluated in the baldness reduction assay, which is a phenotypic method that is the gold standard for assessing the drug susceptibility of SARS-CoV-2 virus. Briefly, Vero E6 cells in the confluent stage were seeded into 6 well plates at 1 x 10^5cells per well. Serial dilutions of the selected compounds were pre-incubated with 40 to 100 hairpin forming units (PFUs) of SARS-CoV-2 virus for 60 minutes at 37°C in a 5% CO_2 atmosphere. The mixture of the respective compound and SARS-CoV-2 virus after preincubation was then added to Vero E6 cells and incubated for 60 minutes at 37°C in an atmosphere of 5% CO_2. The inoculum was then removed, and the infected cells were incubated for three days (without the compound in question) in Minimum Essential Medium (MEM) (Merck, Germany) with 2% fetal bovine serum (Thermo Fisher Scientific, USA) containing 0.6% SeaPlaque agarose (Lonza, Switzerland). Cells were fixed and stained, and PFUs were counted under an inverted microscope, after which the result was plotted against the logarithm of antiviral concentrations. EC50 values were then calculated. In parallel, a baldness reduction assay for the antiviral drugs flavipiravir and remdesivir was performed according to the standard protocol (without preincubating the virus with the drugs) to determine the susceptibility of the SARS- CoV-2 virus to these drugs.

Statistical analysis

Statistical analyses were performed using Microsoft Excel and IBM SPSS Statistics 25. Spearman's rank correlation was used to calculate relationships between variables. A probability level of $P < 0.05$ was considered statistically significant.

RESULTS

Cytotoxicity test

The cytotoxicity assay is a quantitative determination of the difference between cell death and proliferation rates, which was used in our experiments to evaluate the possible effects of the tested honey product on cell growth and proliferation. Each ampoule of Camelyn product (honey extract) contained 2 ml of amber-colored solution for injection. The content of active compounds was 0.035 g/ml. None of the tested concentrations of Camelyn product (35 µg, 50 µg, 70 µg, 100 µg,150 µg and 200 µg) showed harmful cytotoxic effects (Figure 1a, 1b, 1c). Higher concentrations of Camelyn significantly increased the number of hematopoietic stem cells. Compared to the control sample, the number of HSCs ranged from 114%, at a Camelyn product concentration of 70 µg/ml, to 206% when the product concentration reached 200 µg/ml. Similar trends were observed in the BKH-21 cell line assay (Figure 1a, 1b). The number of cells increased from 116% to 203% when the product concentration increased from 70 µg/ml to 200 µg/ml. The stimulatory effect of the Camelyn product was even more pronounced with respect to spleen cell growth. Increased spleen cell proliferation began at a Camelyn product concentration of 50 µg/ml, and at the end of the experiment the average number of cells treated with the product was more than three times higher than in the control sample (Figure 1c.).

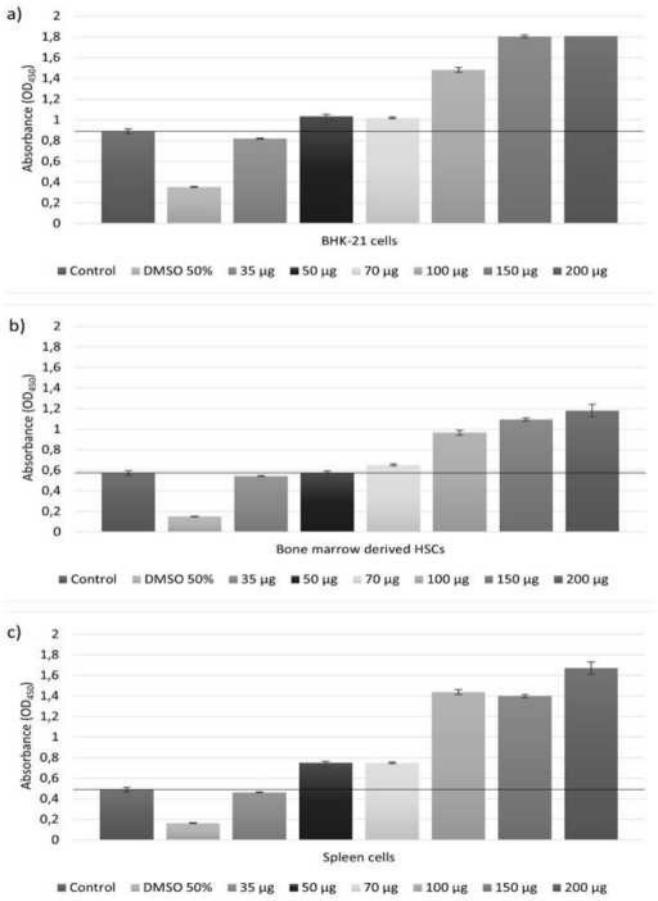

Figure 1. Effect of different concentrations of Camelyn product (honey extract) on the growth of neonatal hamster kidney cells line 21 (BHK- 21) (a), hematopoietic stem cells (HSC) (b) and spleen cells (c); black horizontal line - control optical density (OD) value.

Baldness reduction test for SARS-CoV-2 virus

To evaluate the antiviral activity of Camelyn against SARS-CoV-2 virus, its half-maximal effective concentration (EC50) was determined. Vero E6 cells at the sink stage were seeded in 6-well plates. Two-fold serial dilutions of the Camelyn product were preincubated with approximately 50-100 Psoriasis Forming Units (PFUs) of SARS-CoV-2 virus (Quebec City/21697/2020) for 60 minutes, and then used to infect the cells. After 3 days of incubation (without Camelyn product), cells were fixed and stained with crystal violet. PFUs were counted under an inverted microscope, and the result was plotted against the logarithm of antiviral product concentrations to determine the EC50 value.

Concentrations of Camelyn (honey extract) ranging from 9.08 µg/ml to 72.6 µg/ml showed an indistinguishable effect on viral baldness reduction when cells were infected with 100 PFU. The number of viral alkaloids was reduced by 4.5-13.5%. The higher concentration of 145.3 µg/ml reduced the number of viral alkaloids to 53.85% (Figure 2). The EC50 value was 192.4 µg/ml.

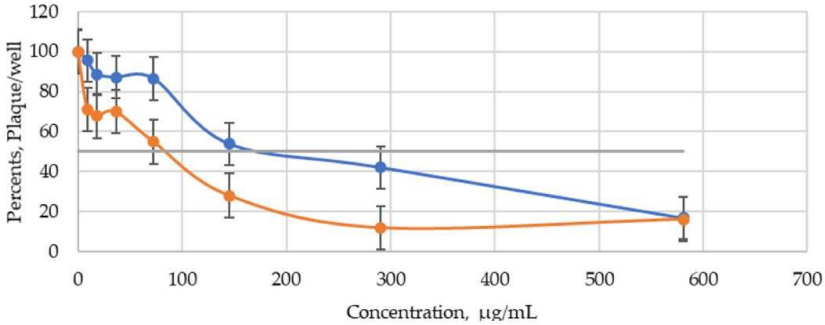

Figure 2. Effect of different concentrations of Camelyn product on the reduction of viral baldness caused by SARS-CoV-2 virus in VERO E6 cells: blue line - the average number of viral alveoli per well was 100 (Spearman's rank correlation between the number of viral alveoli per well and concentration was rs1 = -1.000 and was significant at the 0.01 level (by 2-tailed test)); Red line - the average number of viral alkaloids per well was 30 (Spearman's rank correlation between the number of viral alkaloids per well and the concentration was rs1 = -0.952 and was significant at the 0.01 level (in a 2 - w a y test)); gray line - half-maximal effective concentration (EC50).

When the viral inoculum was reduced to 25-30 PFU, Camelyn product at concentrations ranging from 9.08 µg/ml to 36.3 µg/ml reduced the number of viral alkaloids by 30-33% (Figure 2). Starting at a concentration of 72.6 µg/ml, the number of viral alopecia decreased significantly compared to the control sample. This test showed that the Camelyn product (honey extract) has an inhibitory effect, with an EC50 value of 85.7 µg/ml. An additional test was performed using a concentrated product, Camelyn tablets, to check the similarity of the inhibition profile. When Camelyn tablets were used, a similar inhibitory effect on the number of viral alkaloids was obtained. However, dilutions of the concentrated solution of Camelyn tablets showed a stronger inhibitory effect, with an EC50 value of 116.27 ± 73.39 µg/ml when used to infect 100 PFU of virus. Our results showed that Camelyn extract probably exerts an inhibitory effect early in the replication cycle of SARS-CoV-2 virus.

For comparison, the EC50 values for the antiviral drugs favipiravir and remdesivir were found to be 15.71 µg/ml (100 µM) and 0.616 µg/ml (1.16 µM), respectively. Wang et al [34] found that favipiravir has *in vitro* activity against SARS-CoV-2 virus, although a high concentration of the drug is required here compared to remdesivir (EC50 = 61.80 µM). Note that remdesivir strongly blocked viral infection at low micromolar concentration (EC50 = 0.77 µM) [35].

REVIEW

Honey, known for its beneficial medicinal properties, is attracting interest as a natural medicine. A growing number of scientific and clinical reports suggest that honey can be used not only in home treatments, but also in wound healing and tissue repair [36], [37], [38]. The beneficial effect of honey on wound healing was mainly attributed to its antimicrobial effect. The high sugar content that provides high osmotic pressure and low pH value causes bacterial cells to dehydrate and break the cell wall. Studies show that honey's antimicrobial activities are associated with an increase in hydrogen peroxide activity belonging to reactive oxygen species (ROS) [39]. Honey's antioxidant activity correlates with its anti-inflammatory effects and beneficial effects on wound healing [40].

Honey samples can release hydrogen peroxide, which is produced by the enzyme glucose oxidase and which is responsible for antimicrobial activity [39]. The harmful oxidative effect of hydrogen peroxide is not observed in skin cells due to the polyphenols in honey, which can counteract the action of this ROS compound. Some researchers have noted honey's ability to induce stem cell proliferation, stimulate hematopoietic stem cell migration, and mediate healing processes by increasing tissue blood flow. On the other hand, honey showed an inhibitory effect on cell growth by limiting their ability to proliferate, inducing cell apoptosis and inhibiting the cell cycle in a dose-dependent manner [41].

In many cases, honey should be used in its natural form. However, if necessary, honey's active ingredients, such as small peptides, amino acids, polyphenols, sugars or vitamins, can be extracted from honey. Camelyn is the original honey extract, obtained from a special type of honey by a patented extraction method, which is a mixture of sugars, proteins, polyphenols, vitamins, minerals and free amino acids. The data obtained indicate that Camelyn product is not cytotoxic and has a strong stimulating effect on cell proliferation. Due to this property, the Camelyn product would be useful in wound healing as another honey product.

Data from a clinical study conducted by Bakradze et al. on periodontal inflammatory diseases indicate that Camelyn has immunostimulatory and anti-inflammatory properties, activates regenerative processes and has an analgesic effect. The clinical observation included 56 patients with various forms of the disease (gingivitis, periodontitis). The results of the study confirmed the clinical validity of Camelyn in the combined treatment of gingivitis and periodontitis [42]. A study by Chumburidze et al [43] demonstrated the excellent regenerative and healing effects of the Camelyn product on damaged tissues. This study reported on the characteristics of Camelyn in the treatment of various types of infections and tumors, as well as the pharmacokinetics of Camelyn in rat plasma. In a study by Maglakelidze et al, the minimum inhibitory concentration (MIC) of Camelyn was established against certain bacterial and fungal strains [44]. Camelyn product was found to have a strong inhibitory effect (0.012-0.150 µg/ml) against most of the bacteria tested *in vitro*. *In vitro*, Camelyn product showed strong activity against fluconazole-resistant strains of Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis and Candida krusei, and the MIC value for inhibition of 90% of isolates was 0.012 µg/ml.

As far as we know, no published scientific or clinical study has observed the effect of honey on SARS-CoV-2. To date, the efficacy of honey and the active substances it contains in patients with COVID-19 disease has been evaluated in four registered clinical trials (NCT04323345, NCT04345549, NCT04347382, NCT04468139) [45].

Several studies have been conducted that predicted the affinity of polyphenolic compounds present in manuka honey for SARS-CoV-2 virus proteins. Most of these studies evaluated the possible antiviral activity of polyphenols based on predicted binding to the major protease (Mpro) of SARS-CoV-2 virus [28] [46]. Hashem's study [28] evaluated the screening bioactivity of six compounds present in bee honey and propolis against COVID-19 virus. The study showed that four compounds bind to COVID-19 virus with high affinity and can inhibit its replication. These bioactive compounds include polyphenols, which are currently being evaluated in phase III clinical trials for the treatment of patients with COVID-19 disease [46]. The results of a study by Watanabe et al [20] showed that honey, particularly manuka honey, exhibited strong inhibitory effects on the influenza virus, indicating its potential therapeutic value. Manuka honey effectively inhibited influenza virus replication (EC50 = 3.6 ± 1.2 mg/ml). Compared to the inhibitory effect of the Camelyn product, this concentration is 10-20 times higher.

Based on a review of scientific studies, Hossain et al [30] concluded that honey may be useful in patients with COVID-19 disease through several main mechanisms: direct antiviral properties, regulation/enhancement of host immune signaling pathways, and treatment and/or mitigation of comorbidities. The use of drugs faces several problems, such as multidrug resistance in bacteria and possible side effects. This makes it necessary to think about new therapeutic alternatives, such as honey and honey-based products.

CONCLUSIONS

The study showed that the honey extract, Camelyn product, has no cytotoxic effect, is safe and has antiviral properties against the SARS-CoV-2 virus. However, further detailed molecular studies on the effect of Camelyn product on virus replication or the immune system are needed in the future. There is no doubt that Camelyn does not work in the same way as other investigational drugs currently used to treat COVID 19 disease, however, given the current emergency caused by the COVID-19 pandemic and limited therapeutic options, Camelyn is presented as a promising and suitable therapeutic option that is safe, easy to administer orally and readily available as a natural supplement.

CONTRIBUTION OF INDIVIDUAL AUTHORS

Concept, L.K.; methodology, M.B., L.K., and G.B.; formal analysis, I.G.; research, M.B. and A.L.; writing the article-preparing the original draft, L.K.; writing the article-review and editing, M.B, A.L., N.J.; visualization, I.G.; supervision, R.B.; obtaining funding, P. J. All authors read and approved the published version of the manuscript.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

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[1] Ali AM, Kunugi H. Propolis, Bee Honey, and Their Components Protect against Coronavirus Disease 2019 (COVID-19): A Review of In Silico, In Vitro, and Clinical Studies. Molecules 2021;26:1231. https://doi.org/10.3390/molecules26051232.

[2] Molan P. Why honey is effective as a medicine: 2. The scientific explanation of its effects. Bee World 2001;82:22–40. https://doi.org/10.1080/0005772X.2001.11099498.

[3] Olaitan PB, Adeleke OE, Ola IO. Honey: a reservoir for microorganisms and an inhibitory agent for microbes. Afr Health Sci 2007;7:159–65.

[4] Bansal V, Medhi B, Pandhi P. Honey - A remedy rediscovered and its therapeutic utility. Kathmandu Univ Med J (KUMJ) 2005;3:305–9.

[5] Estevinho L, Pereira AP, Moreira L, Dias LG, Pereira E. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food Chem Toxicol 2008;46:3774–9. https://doi.org/10.1016/j.fct.2008.09.062.

[6] Al-Hatamleh MAI, Boer JC, Wilson KL, Plebanski M, Mohamud R, Mustafa MZ. Antioxidant-Based Medicinal Properties of Stingless Bee Products: Recent Progress and Future Directions. Biomolecules 2020;10:1–28. https://doi.org/10.3390/biom10060923.

[7] Shin HS, Ustunol Z. Carbohydrate composition of honey from different floral sources and their influence on growth of selected intestinal bacteria: An in vitro comparison. Food Research International 2005;38:721–8. https://doi.org/10.1016/j.foodres.2005.01.007.

[8] Cianciosi D, Forbes-Hernández TY, Afrin S, Gasparrini M, Reboredo-Rodriguez P, Manna PP, et al. Phenolic compounds in honey and their associated health benefits: A review. Molecules 2018;23:1–20. https://doi.org/10.3390/molecules23092322.

[9] Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA. Functional properties of honey, propolis, and royal jelly. J Food Sci 2008;73:117–24. https://doi.org/10.1111/j.1750-3841.2008.00966.x.

[10] Molan PC. The evidence supporting the use of honey as a wound dressing. Lower Extremity Wounds 2006;5:40–54. https://doi.org/10.1177/1534734605286014.

[11] Miguel MG, Antunes MD, Faleiro ML. Honey as a complementary medicine. Integr Med Insights 2017;12:1–15. https://doi.org/10.1177/1178633717702869.

[12] Tonks AJ, Cooper RA, Jones KP, Blair S, Parton J, Tonks A. Honey stimulates inflammatory cytokine production from monocytes. Cytokine 2003;21:242–7. https://doi.org/10.1016/S1043-4666(03)00092-9.

[13] Jaganathan SK. Honey constituents and their apoptotic effect in colon cancer cells. J ApiProduct and ApiMed Sci 2009;1:29–36. https://doi.org/10.3896/ibra.4.01.2.02.

[14] Pichichero E, Cicconi R, Mattey M, Muzi MG, Canini A. Acacia honey and chrysin reduce proliferation of melanoma cells through alterations in cell cycle progression. Int J Oncol 2010;37:973–81.

[15] Shahzad A, Cohrs RJ. In vitro antiviral activity of honey against varicella zoster virus (VZV): A translational medicine study for potential remedy for shingles. Transl Biomed 2012;3:2.

[16] Al-Waili NS. Topical honey application vs. acyclovir for the treatment of recurrent herpes simplex lesions. Med Sci Monit 2004;10:94–9.

[17] Berretta AA, Silveira MAD, Cóndor Capcha JM, de Jong D. Propolis and its potential against SARS-CoV-2 infection mechanisms and COVID-19 disease: Running title: Propolis against SARS-CoV-2 infection and COVID-19. Biomed Pharmacother 2020;131. https://doi.org/10.1016/j.biopha.2020.110622.

[18] Kwakman PHS, te Velde AA, de Boer L, Vandenbroucke-Grauls CMJE, Zaat SAJ. Two major medicinal honeys have different mechanisms of bactericidal activity. PLoS ONE 2011;6:3–9. https://doi.org/10.1371/journal.pone.0017709.

[19] Yao L, Jiang Y, D'Arcy B, Singanusong R, Datta N, Caffin N, et al. Quantitative High-Performance Liquid Chromatography Analyses of Flavonoids in Australian Eucalyptus Honeys. J Agric Food Chem 2004;52:210–4. https://doi.org/10.1021/jf034990u.

[20] Watanabe K, Rahmasari R, Matsunaga A, Haruyama T, Kobayashi N. Anti-influenza Viral Effects of Honey In Vitro: Potent High Activity of Manuka Honey. Arch Med Res 2014;45:359–65. https://doi.org/10.1016/j.arcmed.2014.05.006.

[21] Behbahani M, Khan RU. Anti-HIV-1 Activity of Eight Monofloral Iranian Honey Types. PLoS ONE 2014;9:e108195. https://doi.org/10.1371/journal.pone.0108195.

[22] Abedi F, Ghasemi S, Farkhondeh T, Azimi-Nezhad M, Shakibaei M, Samarghandian S. Possible Potential Effects of Honey and Its Main Components Against Covid-19 Infection. Dose-Response 2021;19:1–13. https://doi.org/10.1177/1559325820982423.

[23] Münstedt K. Bee products and the treatment of blister-like lesions around the mouth, skin and genitalia caused by herpes viruses—A systematic review. Complement Ther Med 2019;43:81–4. https://doi.org/10.1016/j.ctim.2019.01.014.

[24] Belouzard S, Millet JK, Licitra BN, Whittaker GR. Mechanisms of Coronavirus Cell Entry Mediated by the Viral Spike Protein. Viruses 2012;4:1011–33. https://doi.org/10.3390/v4061011.

[25] Anand K, Ziebuhr J, Wadhwani P, Mesters JR, Hilgenfeld R. Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. Science 2003;300:1763–7.
[26] Hilgenfeld R. From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. FEBS J 2014;281:4085–96. https://doi.org/10.1111/febs.12936.
[27] Choi HJ, Kim JH, Lee CH, Ahn YJ, Song JH, Baek SH, et al. Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus. Antiviral Res 2009;81:77–81. https://doi.org/10.1016/j.antiviral.2008.10.002.
[28] Hashem HE. IN Silico Approach of Some Selected Honey Constituents as SARS-CoV-2 Main Protease (COVID-19) Inhibitors. EJMO 2020;4:196–200. https://doi.org/10.14744/ejmo.2020.36102.
[29] Mehla R, Bivalkar-Mehla S, Chauhan A. A flavonoid, luteolin, cripples HIV-1 by abrogation of Tat function. PLoS ONE 2011;6:e27915. https://doi.org/10.1371/journal.pone.0027915.
[30] Hossain KS, Hossain MG, Moni A, Rahman MM, Rahman UH, Alam M, et al. Prospects of honey in fighting against COVID-19: pharmacological insights and therapeutic promises. Heliyon 2020;6:e05798. https://doi.org/10.1016/j.heliyon.2020.e05798.
[31] Israili Z h. Antimicrobial properties of honey. Am J Ther 2014;21:304–23.
[32] Albaridi NA. Antibacterial potency of honey. Int J Microbiol 2019;1:1–10. https://doi.org/10.1155/2019/2464507.
[33] Juopperi TA, Schuler W, Yuan X, Collector MI, Dang C v., Sharkis SJ. Isolation of bone marrow-derived stem cells using density-gradient separation. Exp Hematol 2007;35:335–41. https://doi.org/10.1016/j.exphem.2006.09.014.
[34] Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020;30:269–71. https://doi.org/10.1038/s41422-020-0282-0.
[35] Coomes EA, Haghbayan H. Favipiravir, an antiviral for COVID-19? J Antimicrob Chemother 2020;75:2013–4. https://doi.org/10.1093/jac/dkaa171.
[36] Saikaly SK, Khachemoune A. Honey and Wound Healing: An Update. Am J Clin Dermatol 2017;18:237–51. https://doi.org/10.1007/s40257-016-0247-8.
[37] Martinotti S, Bucekova M, Juraj Majtan, Ranzato E. An Effective Regenerative Medicine Product in Wound Management. Curr Med Chem 2019;26:5230–40.
[38] Martinotti S, Laforenza U, Patrone M, Moccia F, Ranzato E. Honey-mediated wound healing: H 2 O 2 entry through AQP3 determines extracellular Ca 2+ influx. Int J of Mol Sci 2019;20:764. https://doi.org/10.3390/ijms20030764.
[39] Nooh HZ, Nour-Eldien NM. The dual anti-inflammatory and antioxidant activities of natural honey promote cell proliferation and neural regeneration in a rat model of colitis. Acta Histochem 2016;118:588–95. https://doi.org/10.1016/j.acthis.2016.06.006.
[40] Manjunatha HD, Chua LS. The anti-inflammatory and wound healing properties of honey. Eur Food Res Technol 2014;239:1003–14. https://doi.org/10.1007/s00217-014-2297-6.
[41] Afrin S, Giampieri F, Gasparrini M, Forbes-Hernández TY, Cianciosi D, Reboredo-Rodriguez P, et al. The inhibitory effect of Manuka honey on human colon cancer HCT-116 and LoVo cell growth. Part 1: the suppression of cell proliferation, promotion of apoptosis and arrest of the cell cycle. Food Funct 2018. https://doi.org/10.1039/c8fo00164b.
[42] Bakradze MS, Chantladze VG, Shoniia NO. Using Camelyn in stomatology, results and vistas. Georgian Med News 2009;171:24–7.
[43] Chumburidze TB, Murtazashvili TG, Kunchuliia LS, Nemsitsveridze NG. Analysis of pharmacokinetics of kamelin in rat's blood plasma. Georgian Med News 2009;167:96–8.
[44] Maglakelidze B, Abasshidze G, Dedashidze I, Mshvildadze V, Pichete A. Evaluation of in vitro and in vivo antibacterial and antifungal activity of "CAMELYN M". Science end Technology Against Microbial Pathogens. Proceedings of the International Conference on Antimicrobial Research, 2011, p. 94–9.
[45] Tantawy MA. The Efficacy of Natural Honey in Patients Infected with Novel Coronavirus (COVID-19): A Randomized, Controlled, Single Masked, Investigator Initiated, Multi-center Trial. 2020. https://clinicaltrials.gov/ct2/show/NCT04323345.
[46] Mustafa MZ, Shamsuddin SI I, Sulaiman SA, Abdullah JM. Anti-inflammatory properties of stingless bee honey may reduce the severity of pulmonary manifestations in COVID-19 infections. Malays J Med Sci 2020;27:165–9. https://doi.org/10.21315/mjms2020.27.2.17.