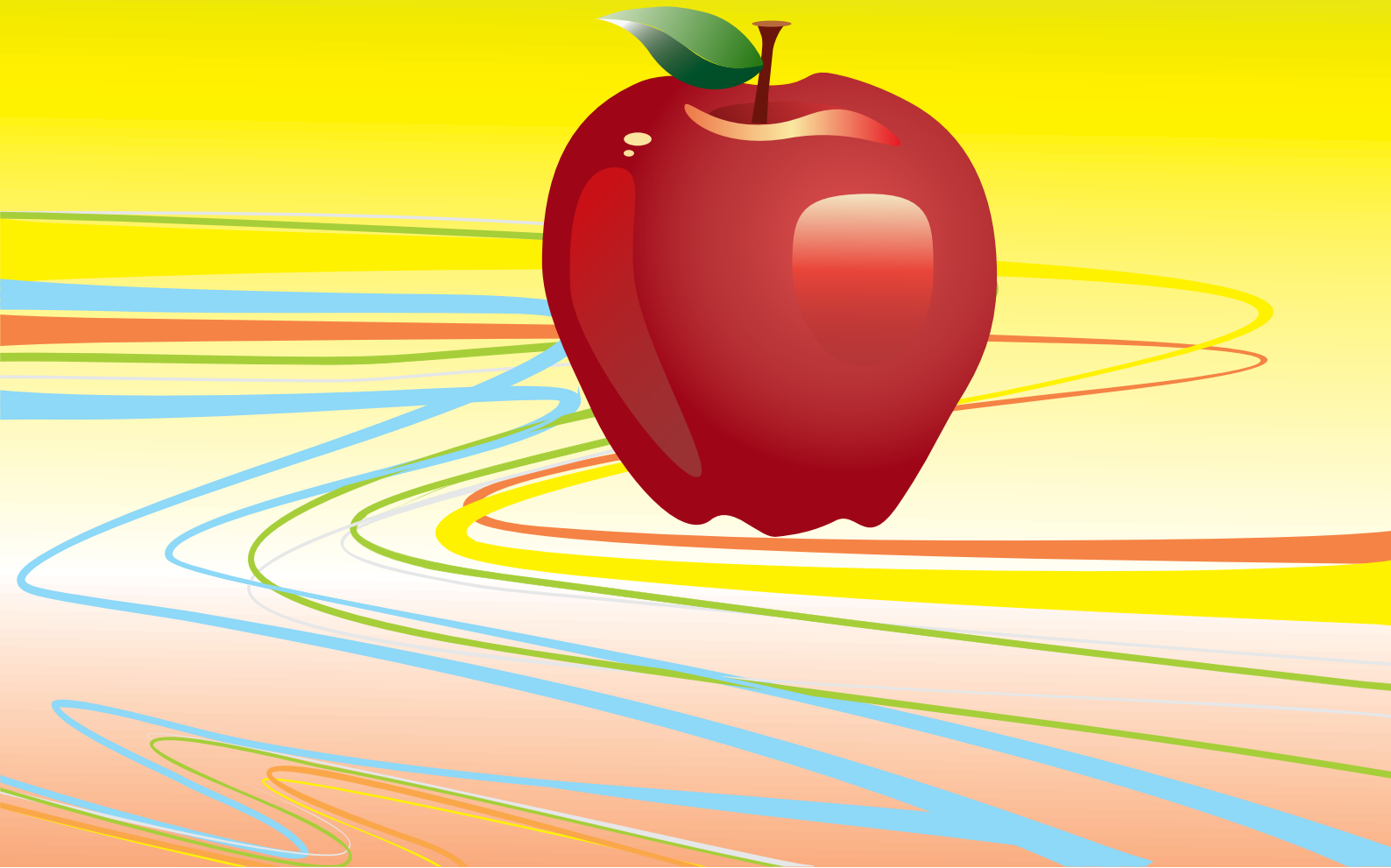
HRANA U ZDRAVLJU I BOLESTI
FOOD IN HEALTH AND DISEASE

ZNANSTVENO-STRUČNI ČASOPIS ZA NUTRICIONIZAM I DIJETETIKU
SCIENTIFIC-PROFESSIONAL JOURNAL OF NUTRITION AND DIETETICS

Specijalno izdanje povodom Međunarodne konferencije "Apiterapija za djecu 2024"
Special edition occasion of the International Conference "Apitherapy for Children 2024"
Prosinac / December 2024

ISSN 2233-1220
ISSN 2233-1239 (online)



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www.hranomdozdravlja.com
ISSN 2233-1220
ISSN: 2233-1239 (Online)
Specijalno izdanje povodom Međunarodne konferencije "Apiterapija za djecu 2024"

Izdavač:
Farmaceutski fakultet Univerziteta u Tuzli, Univerzitetska 7, 75 000 Tuzla, BiH

Suizdavač:
Sveučilište Josipa Jurja Strossmayera u Osijeku, Prehrambeno-tehnološki fakultet Osijek,
Franje Kuhača 18, 31000 Osijek, Hrvatska

Tehnička priprema i dizajn:
Damir Alihodžić (Tuzla, BiH), Ivana Lauš (Osijek, Hrvatska)

Časopis HRANA U ZDRAVLJU I BOLESTI izlazi dva puta godišnje. Ovaj broj tiskan je u 150 primjeraka.

Cijena godišnje pretplate (BiH) 30 €; Cijena godišnje pretplate (Inostranstvo) 50 €
Broj bankovnog računa:
NLB BANKA
Transakcijski račun: 1321000256000080
Budžetska organizacija: 2404019
Poziv na broj: 7013000000

Časopis HRANA U ZDRAVLJU I BOLESTI indeksiran je u/na:
CAB abstracts bazi podataka; FSTA (Food Science and Technology Abstract) bazi podataka;
EBSCO Publishing, Inc. bazi podataka; portalu HRČAK (Portal znanstvenih časopisa Republike Hrvatske);
platformi COBISS (Kooperativni online bibliografski sistem i servisi)

Štampa: ELAK d.o.o Gradačac

FOOD IN HEALTH AND DISEASE
SCIENTIFIC-PROFESSIONAL JOURNAL OF NUTRITION AND DIETETICS
www.hranomdozdravlja.com
ISSN 2233-1220
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Publisher:
Faculty of Pharmacy, University of Tuzla, Univerzitetska 7, 75 000 Tuzla, B&H

Co-Publisher:
Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek,
Franje Kuhača 18, 31000 Osijek, Croatia

Technical preparation and design:
Damir Alihodžić (Tuzla, B&H), Ivana Lauš (Osijek, Croatia)

FOOD IN HEALTH AND DISEASE journal is published twice a year. This issue is published in 150 copies.

Annual subscription price (B&H) 30 €; Annual subscription price (Foreign countries) 50 €
Bank account number:
NLB BANKA
Transaction account: 1321000256000080
Budget organization: 2404019
Reference number: 7013000000

Journal FOOD IN HEALTH AND DISEASE is indexed in:
CAB Abstracts database; FSTA (Food Science and Technology Abstract) database;
EBSCO Publishing, Inc. database; Portal of Croatian Scientific Journals (HRCAK);
COBISS Platform (Co-operative Online Bibliographic System and Services)

Printed by: ELAK d.o.o Gradačac

FOREWORD

It is with great pride and enthusiasm that I welcome readers to this special edition of our scientific newspaper, released in honor of the International Conference *"Apitherapy for Children 2024"*, organized by the Institute for the development of empathy and creativity Eneja and Croatian apitherapy society in February 2024 (https://apisretis.wixsite.com/website-3). This remarkable event gathered leading experts, researchers, and practitioners from across the globe, united by a shared vision: exploring the immense potential of bee-derived products to enhance children's health and well-being. The Conference was supported by professional authorities: Croatin Commission for Medical Education of Doctors, Chamber of Pharmacists of Tuzla Canton (BiH), Association for Nutrition and Dietetics of BiH, Medical Chamber of Slovenia, and point awards for educatiors.

The field of apitherapy, once rooted primarily in traditional medicine, is now witnessing a dynamic evolution as cutting-edge scientific methods bring to light its profound therapeutic applications. With a particular focus on children, a group whose growth and development present unique challenges and opportunities, the potential of bee products becomes especially significant. This edition compiles a curated selection of groundbreaking studies and insights, offering a rich spectrum of knowledge:

- **Upper Respiratory Tract Infections in Children and Apitherapy** – a comprehensive exploration of how bee products can provide natural, effective interventions for one of childhood's most common health issues.
- **The Effect of Royal Jelly on Hormones During Adolescence** – illuminating the influence of this remarkable substance on the hormonal shifts that shape a young person's development.
- **Bee Products Propolis Oil Extract Application in Modeling of Semisolid Pharmaceutical Forms** – a fascinating dive into the innovation of bee-based pharmaceuticals with the potential to revolutionize treatment modalities.
- **Apipedagogy as an Apitherapeutic Method to Support Children's Development** – introducing a novel educational-apitherapeutic approach that integrates bee products into developmental support frameworks.
- **The Water-Soluble Propolis Shows Anti-Influenza Virus Activity** – a vital discovery in the fight against viral illnesses, showcasing the antiviral power of propolis in a form suitable for pediatric care.

These articles not only advance our scientific understanding but also inspire hope for a future where natural solutions like those offered by apitherapy can play a central role in promoting healthier, happier childhoods.

I extend my heartfelt gratitude to the esteemed authors, reviewers, and editorial team whose dedication and expertise made this publication possible. May this special edition ignite curiosity, spark dialogue, and foster collaborations that will continue to advance apitherapy for children worldwide.

Yours sincerely,

Nina Ilič
Guest Editor, Special Edition
Food in Health and Disease
SCIENTIFIC-PROFESSIONAL JOURNAL OF NUTRITION AND DIETETICS

Hrana u zdravlju i bolesti / *Food in Health and Disease*
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ISSN: 2233-1239 (Online)
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(2024) 1 - 37

SADRŽAJ / *CONTENT*

THE WATER-SOLUBLE PROPOLIS SHOWS ANTI – INFLUENZA VIRUS ACTIVITY

Bratko Filipič[1*], Klemen Rihar[2], Dunja Exel Gregorič[3], Adriana Pereyra[4], Hrvoje Mazija[1]

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original scientific paper

Summary

The Influenza virus affects the respiratory tract in humans, causing a range of distinct manifestations including fever, nasal secretions, cough, headaches, muscle pain and pneumonia, which could become violent and severe. Influenza A viruses remain resistant to Amantadine and Rimantadine with a high level of Oseltamivir. Therefore, there is a need for constant improvement of drugs active against resistant Influenza viruses. Propolis has anti-influenza activity both *in vitro* and *in vivo*. Human leukocyte interferon (HuIFN-αN3) is a multi-subtype protein that displays activity against Influenza A, B, and C viruses.
In this study, authors elucidated the anti - Influenza activity of the mixes of Water extract of Propolis and HuIFN-αN3 at different ratios:1:1,1:2 and 2:1. Water extract of Propolis's polyphenols and HuIFN-αN3 were characterized by RP-HPLC. Influenza A and B viruses were separately added to the LLC-MK2 cells treated with Water extract of Propolis and HuIFN-αN3 alone or in ratios 1:1, 1:2, and 2:1.Plates were incubated and cytopathic effects were determined. The best results of ID_{50} were obtained with the mix of 10% Water extract of Propolis and HuIFN-αN3 1:2, showing ID_{50} at 12 ± 0.2 μg/mL for Influenza A and 19 ± 0.6 μg/mL for Influenza B viruses. When comparing the anti-influenza activity of the Water extract of Propolis /HuIFN-αN3 with that of Ribavirin, it was found that 1:2 was the optimal ratio for Water extract of Propolis /HuIFN-αN3 (0.5 and 0.6 for Influenza A and B). This new formulation of Water extract of Propolis and HuIFN-αN3, showing better anti-influenza activity, will improve its application in children' s flu infections *in vivo*.

Keywords: Ethanol extract of Propolis, Influenza viruses, Antiviral activity, Tissue culture

Introduction

Influenza virus infects the respiratory tract in humans and animals causing a variety of different symptoms, including fever, nasal secretions, cough, headache, muscle pain, and pneumonia which often could become severe (Beilharz et al., 2007). During the influenza season, the antigenic drift in the virus occurs often when the formulation of the year's vaccine has already been made. As a consequence, the vaccine became less protective and outbreaks can occur (Dai et al., 1987). The pandemic avian H5N1, H1N1, and already changed A(H3N2) influenza virus strains have spread worldwide, so the emergence of pathogenic influenza virus strains can be predicted (Merigan et al., 1973). In general, it was found that most Influenza A viruses remained resistant to Amantadine and Rimantadine with high levels of Osletamvir resistance (but Zanamivir sensitivity) in seasonal H1N1 (Kugel et al., 2009). Therefore, the constant development of new anti-influenza virus rugs that are effective against resistant Influenza viruses is needed. Bee Propolis is used as folk medicine since 300 BC as a food supplement to maintain or improve human health (Mishima et al., 2005). It is composed of resins (40-55 %), beeswax and fatty acid (20-35 %), essential oils (10%), pollen (5%), and other components such as minerals, vitamins, and sugar. The chemical composition of propolis is complex and more than 180 compounds were identified in it. Biologically most important are polyphenols (Kumazava et al., 2000). Its chemical composition is qualitatively and quantitatively variable, depending on origin and regional plant ecology. The pharmacological properties of Propolis were reported as anticancerogenic (Armstrong, 1981), anti-inflammatory (Uruhisaki et al., 2011), and antimicrobial (Kai et al., 2011). The antiviral activity against several viruses was demonstrated, e.g. Adenovirus (Kujumgijev et al., 1999), HIV (Li et al., 2005), Herpes simplex virus (Hayakari et al., 2013) and anti-Influenza activity (Filipič et al., 2007) (Shi et al., 2007) as *in vitro* as *in vivo*. It was also characterized by the kaempferol flavonoid-related compound (AF-08) responsible for anti-influenza activity (Schanen et al., 2006). HuIFN-αN3 is a multi-subtype protein showing antiviral, antiproliferative, antitumor, radioprotective and antitoxic activity. There are three major classes of IFNs, designated as Types I, II and III (Pavlovich et al., 1990). Type I-IFNs consist of IFN-α, IFN-β, IFN-δ, IFN-ε, IFN-ζ, IFN-κ,IFN-ν, IFN-τ, and IFN-ω. Type II-IFN is composed of a single cytokine, IFN-γ (Prix et al., 1998). Type III-IFNs are IFN-λ1, IFN-λ2,

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IFN-λ3 and IFN-λ4 Type I-and type III-IFNs with similar signal transduction systems are phylogenetically closer to each other than type II-IFN. It is used for the treatment of a variety of different viral diseases and cancers. Among viruses Influenza A and B are being susceptible to HuIFN-αN3 (Weiss et al., 1989) (Mishima et al., 2005). It is important to analyze the effectiveness of the combination of Propolis' Ethanol and Water extracts with HuIFN-αN3 and through this to improve their possible clinical usefulness.
The purpose of the presented experiments was to elucidate the anti-influenza activity against Influenza Viruses A and B, of the combinations of Ethanol extract of Propolis and/or Water extract of Propolis and HuIFN-αN3 *in vitro*.

Materials and methods

Cells and viruses

The LLC-MK2 cells were cultivated in the Eagle's medium with 10% FCS and Antibiotics. Influenza A and B viruses were obtained from the Virological Department of the Institute for Microbiology and Immunology in Ljubljana (Slovenia).

Compounds

10% water-soluble propolis prepared from 30% water-soluble propolis that was obtained from BNatural, Corbetta, Italy. 10% Ethanolic extract of Propolis was obtained from Medex d.o.o., Ljubljana, Slovenia. HuIFN-αN3 was from the Institute for Immunology, Zagreb, Croatia.

Cell treatment

The cell treatment experiments were performed in the Interferon research laboratory of the Medical Faculty in Ljubljana, Slovenia, as follows: 100 µl of medium+2% FCS were added from the second to eleventh well on the multiwell plate. In the first well 200 µl of 10% Ethanolic extract, 10% Ethanolic extract+HuIFN-α N3(1:1,1:2 and 2:1), 10% Water extract of propolis, 10% Water extract of propolis + HuIFN- αN3 (1:1,1:2 and 2:1) and 200 µl of HuIFN-αN3 and 200 µl of Ribavirin as a control. All samples were serially diluted and incubated for 8 hours at 37 °C. Influenza A and separately Influenza B viruses were added, and plates were incubated at 37 °C for four days when in the control 100% CPE with small plaques were developed.

Detection of HuIFN-αN3 or Pinocembrin and Galangin by RP-HPLC method

The RP-HPLC analyses were performed in the Medex' HPLC research laboratory which has an accreditation to ISO 17034:2016 to perform the valid analyses. The Vaquish core HPLC sistem with up to 700 bar was used in these experiments. In 10 ml burette, 1.0 mg of Pinocembrin or Galangin are added and diluted to 10.0 ml with Me OH. From this solution, 150 µl of samples were put into the vial and filled with 1350 µl of Me OH. During the analyses 30 different samples were filtered through a 0,45 µm filter and injected 20 µl into the HPLC column. In the experiments the HPLC column Purospher® STAR RP-18; 5 µm 150 x 4,6 mm was used. Conditions of the HPLC system: (a) Temperature of the column: 25 °C, (b) Flow: 0.7 ml/min.; (c) Pressure: 90 – 100 Bar; (d) Atte: 62.5 (e): Absorbance: 290 nm; (f) Injection volume: 20 µl;(g): Gradient: Solvent A = water + 1% Formic acid; Solvent C=Acetonitrile. The Steps of the RP-HPLC run are shown in Table 1 for HuIFN-αN3 samples and in Table 2 for the detection of pinocembrin and galangin.

Table 1. Time course of RP-HPLC chromatography of different HuIFN-αN3 samples

Step: chromatography of different IFN samples in step	Time (min)	Solvent A (%)	Solvent C (%)
0	0	91	9
1	3	80	20
2	6	50	50
3	12	50	50
4	15	91	9
5	20	91	9

Table 2. The steps of the RP-HPLC detection of pinocembrin and galangin

Step: Chromatography of pinocembrin and galangin step	Time (min)	Solvent A (%)	Solvent C (%)
0	0	70	30
1	5	60	40
2	15	60	40
3	20	35	65
4	25	35	65
5	30	70	30
6	35	70	30

The analyses: Plates were washed with PBS, fixed with 5% Glutaraldehyde, washed with PBS and 100 µl of crystal violet was added for 20 minutes. The plates were washed with PBS, and air dried and the OD was measured at 570 nm. The effective concentrations for 50% plaque reduction (ID_{50}) were determined from a curve relating the plaque number to the concentrations of the propolis extracts and huIFN-αN3. The effect of different combinations of Propolis' ethanol or water extract with HuIFN-αN3 in various combinations (1:1, 1:2, and 2:1) on Influenza A and Influenza B virus was also expressed as Eq (1):

$$\text{Ribavirin } ID_{50} \text{ index} = \frac{\text{Propolis' extract and/or HuIFN-αN3(1:1,1:2,2:1) } ID_{50}}{\text{Ribavirin } ID_{50}} \quad (1)$$

Statistical Analysis

The ID_{50} based on the mean plaque number was calculated on the raw data of an in-triplicate assay by regression analysis using Probit (SPSS statistical software package), determining the concentration of drug required to reduce the number of plaques by 50%. Statistical analysis of the experimental data was performed with a two-tailed Student's *t*-test for paired samples with a $p = 0.05$ as the smallest level of significance.

Results

RP-HPLC Analyses of Sendai Virus (Cantell Strain) Induced Interferon (HuIFN-αN3)

HuIFN-αN3 subtypes in different samples (natural or recombinant) are separated according to their relative hydrophobicity using HPLC column Purospher® STAR RP-18 5 µm. The separation of different HuIFN-αN3 subtypes in the samples was achieved by increasing acetonitrile concentration (Kumazava et al., 2000) (Kumazava et al., 2000b). The least hydrophobic interferon subtypes were eluted as early peaks and the most hydrophobic Interferon subtypes eluted as later. As standards, different human recombinant interferons α were used: HuIFN-αA, HuIFN-α2a, and HuIFN-α2b. Their chromatograms and the chromatograms at 280 nm of the Russian HuIFN-αN3 (NDV induced) and HuIFN-αN3 of the Institute of Immunology Zagreb (Croatia) (Sendai virus-induced) were used as standards (Figure 1A). The positions of different HuIFN-αN3 subtypes were determined according to the 214 nm chromatogram in comparison to the protein profile measured at 280 nm. The predominant components of the Sendai virus-induced HuIFN-αN3, are shown in Figure 1B, and are natural IFN subtypes: α1, α2, αA, α2b, and α14. The most important is the relative ratio between α1 and α2 (values of mAU relative units). Various types of HuIFN-αN3 inductors differ in the induction capacity of IFN subtypes: α1, α2, αA, α2b, and α14. The HuIFN-αN3 subtype's antiviral activity in IU/mL was determined by the detection of their' antiviral activity according to the standard procedure: Monolayer received interferon dilution at two-fold increasing levels overnight. The following morning, the medium was removed and 100 µL of challenge virus (Vesicular Stomatitis Virus) in Eagle's medium + 2% FCS were added, and the cell layers were examined under the microscope 24^h later and scored (+4, +3, +2, +1, +0 corresponding to 100% destruction, 75%, 50%, 25%, non-infected, respectively) (Armstrong et al., 1981).

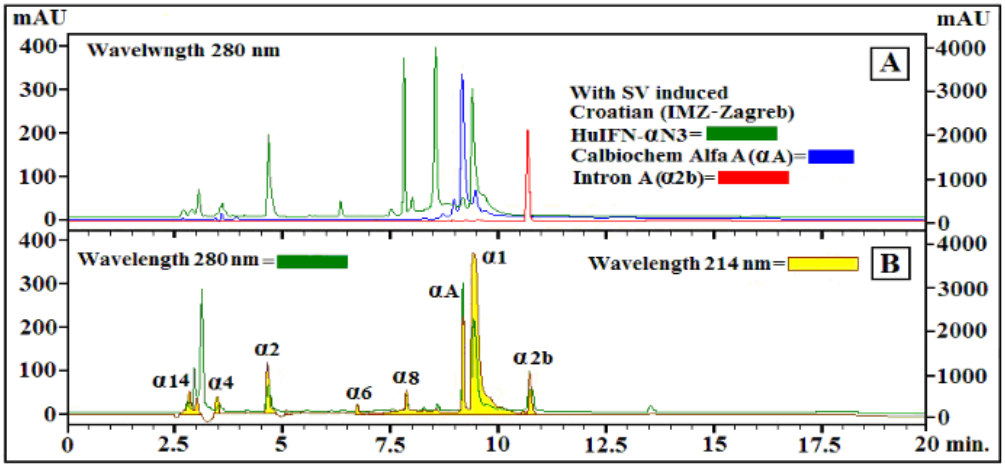

Figure 1. RP-HPLC profiles of the Sendai virus-induced HuIFN-αN3:
(A) SV=Sendai virus (Cantell strain). Protein profiles of the various IFNs at 280 nm
(B) Protein profile at 280 nm ( ) and IFN profile at 214 nm ( ) of
HuIFN-αN3 induced with 100 HA/mL of Sendai virus (Cantell strain)

Quantity of Pinocembrin and Galangin in 10% Propolis' ethanol extract and in 10% Propolis water extract

The 1.0 mg of caffeic acid, chrysin, pinocembrin, and galangin were put and diluted to 10.0 mL with methanol. From this solution, 150 µL of the sample was transferred into a vial and loaded with 1.350 µL of methanol. Samples filtered through a 0.45 µm filter were injected by 20 µL into the HPLC column Purospher® STAR RP-18 5 µm. Their separation was achieved with an acetonitrile gradient in the HPLC column (Figure 2A). The 10% EEP was analyzed under the same conditions in the Purospher® STAR RP-18 5 µm HPLC column. Its separation measured at 290 nm, with an acetonitrile gradient, is presented in Figure 2B (Uruhishaki et al., 2011). The quantity of caffeic acid, chrysin, pinocembrin, and galangin in the experimental sample of 10% EEP was calculated in comparison to standards (Figure 2A). Figure 2C shows the RP-HPLC profile of 10% Water soluble Propolis. Therefore, Table 3 indicates the quantity of caffeic acid, chrysin, pinocembrin, and galangin in the 10% EEP. Table 4 shows a comparison of 10% EEP and 10% Water soluble Propolis in regard to the content of Pinocembrin and Galangin.

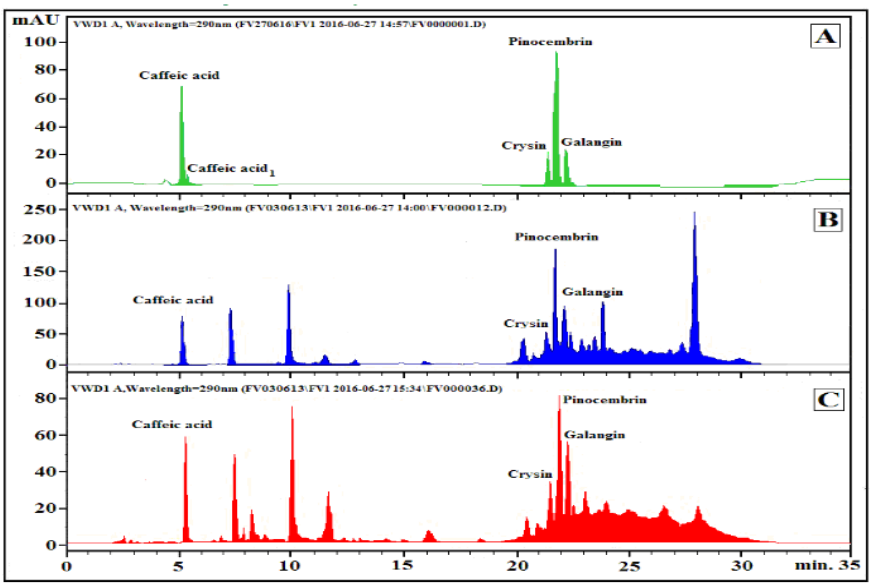

Figure 2. A = The RP-HPLC profile of the Bio-Flavonoid's standards (Caffeic acid, Crysin, Pinocembrin and Galangin); B = The RP-HPLC profile of 10% Ethanol extract of Propolis; C = The RP-HPLC profile of 10% Water soluble Propolis

Table 3. Quantity of caffeic acid, crysin, pinocembrin and galangin in 10% EEP

Extract	Caffeic acid (μg/mL^{-1})	Crysin (μg/mL^{-1})	Pinocembrin (μg/mL^{-1})	Galangin (μg/mL^{-1})
10% EEP	19 ± 0.18	5.4 ± 0.48	0.32 ± 0.08	0.29 ± 0.11

Table 4. Quantity of Pinocembrin and Galangin in 10% Propolis' ethanol extract and 10% Propolis' water extract

Extracts:	Pinocembrin (μg / mL^{-1})	Galangin (μg / mL^{-1})
10% Propolis' ethanol extract	0.32±0.08	0.29±0.11
10% Propolis' water extract	0.05±0.01	0.06±0.02

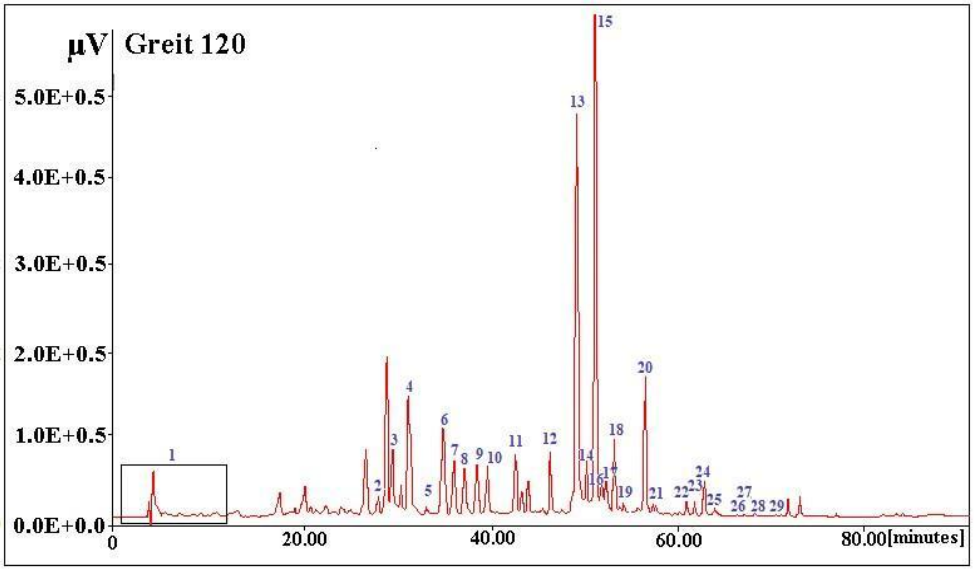

Figure 3. Molecular Composition of Water extract of Propolis Determined by HPLC-UV-ESI-MS 504971
1 = phenolic acids (caffeic, coumaric, ferulic, isoferulic); 2 = quercetin; 3 = pinobanksin 5-methyl ester;
4 = quercetin 3-methyl ester;5 = pinobanksin; 6 = apigenin; 7 = kaempferol; 8 = isorhamnetin; 9 = luteolin 5-methyl ester; 10 = quercetin 5-7-dimethyl ester; 11 = galangin 5-methyl ester; 12 = quercetin 7-methyl ester;
13 = chrysin; 14 = pinocembrin; 15 = galangin; 16 = pinobanksin-3-O-acetate;17 = CAPE; 18 = metoxychrysin; 19 = pinobanksin-3-O-propionate; 20 = caffeic acid cinnamyl ester; 21 = pinobanksin-3-O-butyrate;
22 = pinobanksyn-3-O-pentenoate; 23 = other pinobanksin derivative; 24 = pinobanksin-3-O-hexanoate;
25 = other pinobanksin derivative. (Obtained by kind help of Dr. Nicola Volpi from Department of Life Sciences, University of Modena & Reggio Emilia, Modena 41125, Italy)

Figure 3 shows the HPLC-UV-ESI-MS504971 profile of the Water extract of Propolis. The used ID_{50} 12 ± 2 μg/mL for influenza A and 19 ± 6 μg/mL for influenza B are shown. With 10% EEP and HuIFN-αN3, the best ratio was 1:2, where it was the ID_{50} 22 ± 7 μg/mL for influenza A and 15 ± 4 μg/mL for influenza B.

Ribavirin ID_{50} Index

The Ribavirin ID_{50} index was calculated to compare the ID_{50} (antiviral activity) of Water extract of Propolis or 10% EEP in combination with HuIFN-αN3 in ratios 1:1, 1:2, and 2:1 in comparison to Ribavirin. The results are presented in Table 5 and Figures 4 and 5. The lower is, the better it is. The ratio 1:2 was still the best with WSP in combination with HuIFN-αN3 (0.5 for influenza B and 0.6 for influenza A virus). With EEP in combination with HuIFN-αN3, the best was the same ratio of 1:2 (0.7 for influenza B and 1.3 for influenza A virus).

Antiviral activity of combinations of Propolis and HuIFN-αN3

Table 5. The antiviral activity of 10% Ethanolic extract of Propolis, 10% Water extract of Propolis and HuIFN-αN3 in the ratios: 1:1, 1:2 and 2:1 expressed as ID_{50} in µg/mL

Sample:	Bioflavonoids as Caffeic acid (mg mL $^{-1}$)	Influenza A ID_{50}(µg mL^{-1})[1]	Influenza B ID_{50}(µg mL^{-1})[1]
10% Ethanolic extract of Propolis	19±0.18	82±11	62±6
10% Ethanolic extract of Propolis + HuIFN-αN3 1:1	9±0.59	35±7	31±6
10% Ethanolic extract of Propolis + HuIFN-αN3 1:2	6±0.39	22±8	15±6
10% Ethanolic extract of Propolis + HuIFN-αN3 2:1	12±1.78	42±4	31±7
10% Water soluble Propolis	14±1.20	31±9	29±2
10% Water soluble Propolis + HuIFN-αN3 1:1	7±1.10	22±2	31±3
10% Water soluble Propolis + HuIFN-αN3 1:2	4±0.73	12±2	19±7
10% Water soluble Propolis + HuIFN-αN3 2:1	9±0.46	25±6	30±2
Ribavirin		20±2	28±4

[1]ID_{50} = is the concentration of the sample needed to inhibit virus induced CPE (Cytopathogenic effect) on 50%

Ribavirin ID_{50} index

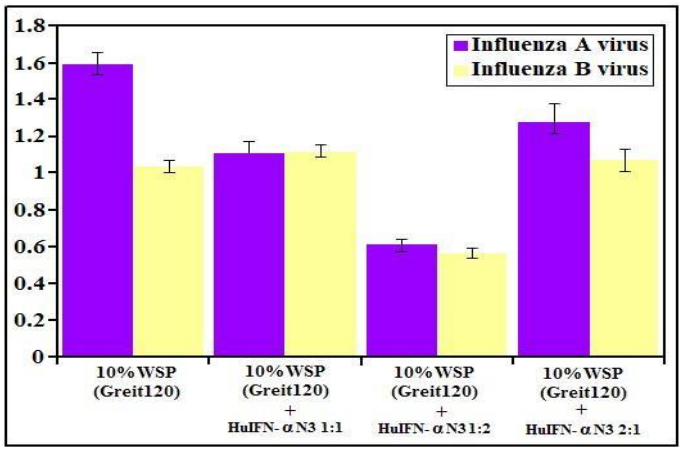

Figure 4. Ribavirin ID_{50} index of Water extract of Propolis and/or combination with HuIFN-αN3 in ratios 1:1, 1:2 and 2:1

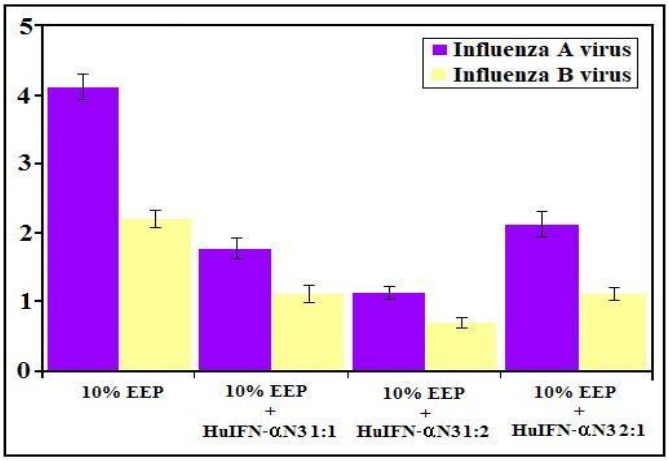

Figure 5. Ribavirin ID_{50} index of EEP and/or combination with HuIFN-αN3 in ratios 1:1, 1:2 and 2:1

Discussion

The samples of very detailed analyzed Water extract of Propolis containing different polyphenols: apigenin (ID_{50} 8.1 ± 4.7 μg/mL), chrysin (ID50 > 100 μg/mL), kaempferol (ID_{50} 24.8 ± 4.3 μg/mL) quercetin (ID_{50} > 100 μg/mL) and caffeic acid (ID_{50} 49.7 ± 5.0 μg/mL) already showed anti-Influenza activity *in vitro* (Kai et al., 2014). The anti-influenca A and B virus activity of complete Water extract of Propolis molecule is: ID_{50} 31 ± 0.9 μg/mL for influenza A virus and ID_{50} 29 ± 0.2 μg/mL for influenza B virus, what is a bit lower, but comparable with ribavirin, having ID_{50} 20 ± 0.2 μg/mL for influenza A and ID_{50} 28 ± 0.4 μg/mL for influenza B. When HuIFN-αN3 is added to Water extract of Propolis in a ratio of 1:1, the ID_{50} 22 ± 0.2 μg/mL for influenza A and 31 ± 0.3 μg/mL for influenza B are found. When this ratio is 1:2, the ID_{50} is 12 ± 0.2 μg/mL for influenza A and 19 ± 0.7 μg/mL for influenza B virus. The ratio 2:1 shows the ID_{50} 25 ± 0.6 μg/mL for influenza A and 30 ± 0.2 μg/mL for influenza B. The highest increase was found when Water extract of Propolis was combined with HuIFN-αN3 in a ratio of 1:2. To elucidate the mechanisms of anti-influenza activity of Water extract of Propolis it was found that caffeic acid from it could restore the viability of cells infected with influenza virus in a dose-dependent manner (Kujumgijev et al., 1999). To find working mechanisms of this anti-influenza activity, it was measured the relative value of influenza virus RNA in cultured cells with and without antiviral compounds. It was found that the relative value of influenza virus RNA/viable cells was not significantly different between groups with different compound concentrations. So it is possible that Water extract of Propolis has no direct influence on an influenza virus or does not interact with influenza virus components, although Li et al. (2005) reported that caffeoylquinic acid from Water extract of Propolis binds to the gp120 of RSV (respiratory syncytial virus) and inhibits virus-cell fusion events in the early stage of the replication cycle. Thus, the anti-influenza activity of Water extract of Propolis is not derived from an inhibition of virus replication, as is true for a neuraminidase inhibitory drug, but may be due to another mechanism, such as an enhancement of cell resistance. As to the effect on antiviral executor genes, Water extract of Propolis enhanced myxovirus resistance 1 (Mx1) expression (Hayakari et al., 2013). Different specificities in antiviral effects of HuIFN-αN3 against influenza A and B viruses were reported as *in vitro* and *in vivo* (Schanen et al., 2006). They share the same specific cell receptor, interferon type I receptor (IFN-αR) composed of two subunits, IFN-αR1 and IFN-αR2, and interact with its different regions (Cook et al., 1996). Antiviral activity of HuIFN-αN3 against influenza A, B, and C viruses is mediated, at least in part, by the induction of intracellular antiviral proteins, such as MxA protein. It is induced by HuIFN-αN3 as a whole and inhibits the replication of various influenza viruses (Pavlovich et al., 1990) (Zurcher et al., 1992). Water extract of Propolis enhances the anti-influenza activity of HuIFN-αN3 in a dose-dependent ratio *via* enhanced resistance of Mx1 expression and MxA induction of influenza virus replication inhibition.

Conclusions

Pinocembrin and Galangin are probably the main antiviral components of Propolis that interact with HuIFN-αN3. The 10% Ethanol extract of Propolis and 10% Water extract of Propolis were analyzed by RP-HPLC. The findings can be seen in Figure 3 and Table 4. The results show a lower amount of Pinocembrin and Galangin in 10% Water extract of Propolis, as in 10% Ethanol extract of Propolis, even here the higher antiviral activity against Influenza A and Influenza B viruses *in vitro* alone and combination with HuIFN-αN3 can be found. The experiments were performed to analyze the anti-influenza activity of 10% Ethanolic extract of Propolis and 10% Water extract of Propolis in combination with HuIFN-αN3 in different ratios (1:1, 1:2 and 2:1). Ribavirin alone was used as acontrol. The results in Table 4 show that the best results (ID_{50})were obtained when the combination of 10% Water extract of Propolis and HuIFN-αN3 in a ratio of 1:2 was used. (ID_{50}12±2 μg/ml for Influenza A and 19±6 μg mL-1 for Influenza B). In the case of 10% Ethanolic extract and HuIFN-αN3, the best ratio was 2:1, where 22±7 $\mu g/mL^{-1}$ for Influenza A and 15±4 $\mu g/mL^{-1}$ for Influenza B. The Ribavirin ID_{50} index was calculated to compare the ID_{50} (AV) activity of Water extract of Propolis or Ethanol extract of Propolis in combination with HuIFN-αN3 in ratios: 1:1, 1:2 and 2:1 in comparison to Ribavirin. The lower is, the better it is. In the case of Ethanol extract of Propolis in combination with HuIFN-αN3, the best was the ratio 1:2 (0.6 for Influenza B and 1.3 for Influenza A). The same ratio was also the best in the case of Water extract of Propolis in combination with HuIFN-αN3 (0.5 for Influenza B and 0.6 for Influenza A). In the future experiments it will be necessary to extend this new formulation to children flu infection.

Acknowledgments

The presented research was supported by Ivan Čermak from the Delta Group International, Zagreb, Croatia, and in the frame of the Croatian Institute for Experimental and Translation Oncology, in the Project "Oncolytic Newcastle disease virus in the veterinary medicine". The presented research is dedicated to the kind memory of Dr. Klemen Rihar.

Conflict of Interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2024) **Specijalno izdanje** 9-14
Food in Health and Disease, scientific-professional journal of nutrition and dietetics (2024) ***Special Edition*** *9-14*

APIPEDAGOGY AS A METHOD TO SUPPORT CHILDREN'S DEVELOPMENT

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original scientific paper

Summary

Apitherapy for children is the targeted use of bee products to strengthen immunity and complementary therapy to official medicine in the treatment process. In the case of Apipedagogical, apitherapy for children takes place as part of regular pedagogical work in a kindergarten or school, which is mainly based on dedicated support for development through nutrition and apitherapeutic services. Bee products that are used in a targeted manner are honey, royal jelly, wax, propolis, and pollen. Apitherapy for children through target-based consumption of bee products relies on three fundamental pillars: sensory integration, nutritional values of bee products, and interoception. The completed study shows that the developmental needs of children correlate with their nutritional needs.
Healthy eating habits of children are of essential importance not only for health but also for development in all areas, including self-esteem, which, among other things, stems from a sense of successful placement in the environment and a sense of the ability to overcome challenges; in bee products, a key supporting role should be recognized in this regard.

Keywords: Apipedagogy, apitherapy, children, nutrition, health

Introduction

Apipedagogy is the name for a specific group of pedagogical programs and didactic approaches. It is mainly characterized by a special pedagogical-apitherapeutic didactic approach, which is strongly focused on healthy life in kindergarten and the direct realization of all the priority tasks of education for sustainable development.
Children learn a lot about bees, but this is a 'side success', the developmental needs of children, safe coexistence with nature, and responsibility towards the environment are in the foreground. The segment of professional apitherapy for children highlights the rights of children through the nutritional value of bee products and apitherapy services and strengthens development in all areas: emotional, psychological, social, motor, and cognitive.
Apitherapy for children is the targeted use of bee products to strengthen immunity and complementary therapy to official medicine in the treatment process. In the case of Apipedagogy, apitherapy for children takes place as part of regular pedagogical work in a kindergarten or school, which is mainly based on dedicated support for development through nutrition. Bee products that are used in a targeted manner are honey, royal jelly, wax, propolis, and pollen.
Apitherapy for children through target-based consumption of bee products in API kindergarten is based on three fundamental pillars:

- sensory integration,
- nutritional and pharmacological values of bee products and
- interoception.

Apipedagogy as a method to support children's development

Senzory integration

This includes apitherapy services, in which the child's body consumes bee products through the skin and/or respiratory system. Certain activities and sensory perceptions trigger certain (predictable) biochemical reactions in the human organism, i.e. activation of endocrine glands to release certain hormones into the blood. These lead to predictable moods and other targeted states.
Thus, for example, playing with experimentation in the child's organism triggers the formation of serotonin, massage with honey or wax triggers the formation of oxytocin, API sensory path with wax triggers the formation of dopamine, and role-playing in api-culinary triggers endorphins. At the same time, bee products have not only educational value but also apitherapeutic and nutritional value - the components of bee products enter the process according to the

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principle of synergy: the pedagogical, pharmacological, nutritional, and therapeutic effects of bee products create a new holistic whole, which represents development support with the help of apitherapy.

Figure 1. Tasting different types of honey with eyes closed

Nutritional values of bee products

As shown in Figure 1, activities are included in which children consume bee products as food. Nutrition affects the organism holistically. Healthy children whose basic needs are satisfied do not have unwanted behavioral deviations, because they feel good, they are in a good mood because they can keep up with the daily routine in kindergarten. Irritability in interaction with peers and mood sensitivity of the child is in most cases related to poor ventilation of the organism (completely or partially blocked airways, asthma, etc.) or other unfavorable health conditions and should not be considered as a character trait of the child in the preschool period, but to improve the condition, take a nutritional approach.

Interoception

Interoception is the ability to perceive inwardly. The five senses, smell, taste, hearing, touch, and sight, are the external perceptual abilities of the body, i.e. the ability to perceive the surroundings. Interoception is the ability to perceive processes or conditions inside the body. Interoception includes all signals from the internal organs, including the cardiovascular system, lungs, intestines, bladder, and kidneys. There is a constant communication dialogue between the brain and internal organs (Robson, 2021). It should be pointed out that interoception is directly related to the feeling of intuition, which is a certain awareness of a need without a tangible explanation for the decision made, resulting from this perception (Kim et al., 2021).
Observations in the implementation of apitherapy for children in kindergartens in the period 2017 - 2023 show that most children in a certain developmental stage show a desire for a certain type of honey, under the needs of physical development and/or current life situation or health condition.

Research of Apipedagogy to support children's development

Methods

Apitherapy for children as a targeted use of bee products through the pedagogical-apitherapeutic approach of the behavior sampling method recognizes the developmental needs of children and prepares pedagogical activities in kindergarten accordingly. In the field of developmental psychology, in addition to the behavior sampling method, the observation method and the time sampling method are also needed.

To monitor the contrast between a group of children receiving a pedagogical-apitherapeutic approach and a group of children not receiving apitherapy for children, the inductive approach of the comparative-analytical method in combination with the methods of developmental psychology is valid, with careful observation remaining the leading element (Paulus and Stein, 2010).
The study took place over a longer period, 2017 – 2023, and covered all age groups of kindergarten preschool children, i.e. from the age of 1 – 7 years. Two variables were paralleled, namely the children's developmental needs on the one hand and their interoception as an influencing factor in the choice of honey variety on the other hand. The children had five different types of honey at their disposal: acacia, flower, forest, linden, and chestnut. Each type of honey is characterized by a specific ratio of ingredients, which results in differences in the effect on the human body. These deviations are purposefully used in apitherapy. Educators and parents of children participated in the study with the head of the API Kindergarten program.
The study presented in this paper differs from similar studies in the field of interoceptive abilities in preschool children primarily in terms of methodology. While the study from abroad - The new Jumping Jack Paradigm, 2019, which was considered for contrast, was based on the method of talking to children and measuring their heart rate (Luca et al., 2019), the present study, conducted in API kindergartens in Slovenia, is based on a methodology which comes from pedagogy and developmental psychology. In addition, The new Jumping Jack Paradigm focuses on the conscious interoceptive perceptions of preschool children, and the pedagogical-apitherapeutic approach mainly follows the results regardless of whether the interoceptive perceptions of children are conscious or subconscious.

Results and discussion

During 1-3 years, children develop extremely fast in all areas. The areas of development in this age period are inextricably linked: the absence of the possibility of free natural movement, in addition to reduced motor development, results in reduced development in all other areas as well, including a lag in the cognitive area of development. During 1-3 years, children are in the so-called sensory-motor phase, which consequently means that they need a lot of energy for a lot of movement. Since the physical development of the digestive organs is not yet at full strength during this period (significant progress on the motor, cognitive, and digestive levels), it is recommended that during this period they obtain energy from nutrients that provide them with support in this sense.
This is also evidenced by the children's interoceptive perceptions, when in the period 1-3 most children choose honey that has a distinctly sweet taste, in the vast majority it is acacia honey, and it is not just about the pleasure of the sweet taste. Acacia honey, with its high sugar content, is an excellent source of immediate energy. The higher content of fructose in acacia honey is practical support at the digestive level because when consumed, fructose is absorbed more slowly and gradually and is converted into glucose and released into the blood per the needs of the body (Ilič, 2023). In this way, it simplifies the balance in the body, as it does not burden digestion and represents a quick source of energy that is fully absorbed without residues. Based on the results of monitoring children, it can be concluded that the latter is a decisive factor in children's decisions since flower honey also has a distinctly sweet taste - acacia honey contains more fructose, while flower honey contains more glucose.
During 3-4 years, children are getting to know the world more intensively, and they are no longer near their parents all the time. During this period, they increasingly come into contact with a variety of germs, bacteria and other challenges, for which they primarily need a good blood count, which represents the first line of defense in maintaining the body's immunity, since during this period, as a rule, they no longer get antibodies through mother's milk (Ilič, 2023).
This is also evidenced by the interoceptive perceptions of children when in 3-4 years they overwhelmingly prefer darker honey, mostly forest honey. Forest honey is characterized by a higher content of mineral substances, which provide support precisely in the area of good blood count, support for the supply of tissues with oxygen, and balanced digestion.
During 5-7 years, children are mainly full of energy and have a strong need to live it out physically much more independently. During this period, as a rule, they have already formed their taste in terms of food; […] They are physically quite skilled, but while exploring their limitations they also experience injuries more often (Ilič, 2023). This means that during this period of life, they benefit most from foods that have a high nutritional value, support in combating pathogenic organisms that invade the wounds, and are not burdensome to the digestive system (mainly because children are generally in motion a lot during this period).
As can be seen from Figures 2 and 3, this is also evidenced by the interoceptive perceptions of children, who in the period of 5-7 years mostly prefer darker honey, especially chestnut honey. Chestnut honey is a honey with a high nutritional value due to its high pollen content. Among its more important characteristics is the fact that it has the greatest disinfecting and antibacterial power among kinds of honey. Interestingly, children are generally not bothered by the bitterness of chestnut honey during this period, from which we can once again conclude that the choice of the

preferred variety of honey in the preschool period is not solely subject to the sweetness of the honey, but is most likely also a matter of a clear intuitive feeling of what they need. In addition, just like acacia honey, chestnut honey is characterized by a ratio between glucose and fructose in favor of the latter, which means that chestnut honey is a food with high energy and nutritional value, which, like acacia honey, is gentle on digestion.

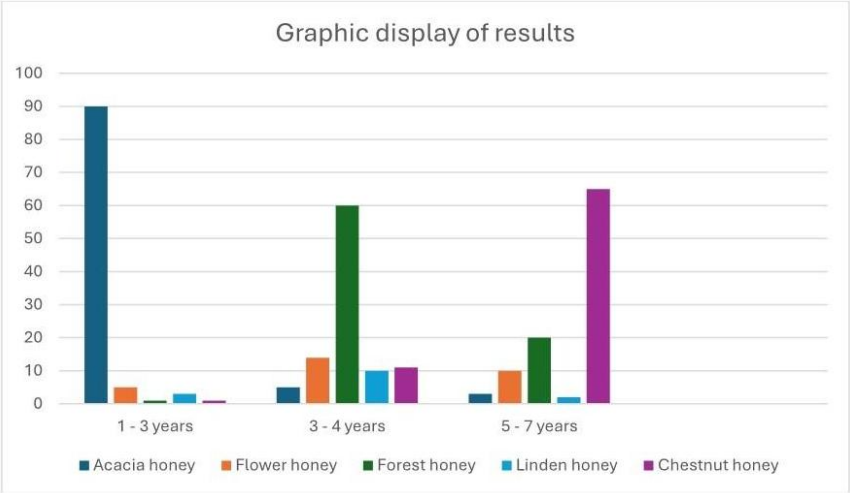

Figure 2. Graphical display of results

New experimental approaches and methods of such research revealed a lot about the processing of afferent signals from the internal organs of the body and enabled more precise descriptions of these processes and a more precise determination of their functional significance in human experience and behavior (Dieter, 1996). In this, all organ systems, neuromuscular, respiratory, gastrointestinal, cardiovascular, and others, play an equally important role, especially because these are factors that can influence perception judgment, which is essential for sensory integration and the child's development resulting from it (Simmons and DeVille, 2017).

This research is important because of the opportunity for further research and finding solutions also in the field of challenges faced by children with special needs, since the pedagogical-apitherapeutic methodology for monitoring needs and results does not condition the level of cognitive or motor skills of the child. In addition, it does not focus only on the question of the level of perception of internal events, but directs attention constructively: what are the nutritional deficits / individual nutritional needs of the child and with which bee products and with what methodology can they be provided in the context of apitherapy for children within Apipedagogy.

Figure 3. Children define the choice of honey

The results of observations in the implementation of apitherapy in Slovenian API kindergartens in the period 2017-2023 can be used constructively in the practice of apitherapy for children. An apitherapist and/or a nutritionist are not competent to make a diagnosis about a child's health condition, but they can identify a child's nutritional needs also based on good observation and a conversation with educators and parents.
There are several ways to obtain concrete information from a child and, taking into account the level of the child's psychological and cognitive phase in the preschool period, communicating the child verbally through conversation is certainly not the main one. Based on the honey that is 'intuitively' chosen by the child, who has not yet mastered verbal communication to the extent that he would participate in an authentic and constructive conversation about his health (health is not the same as well-being), the apitherapist recognizes the general state of the child's organism and constructively acts following these observations.
For optimal development, the child needs those nutrients,

- which build cells, bone and periosteal tissues, muscles, skin, and connective tissues (proteins),
- to reduce the density of food and regulate the passage of food through the digestive tract and slow down the absorption of glucose into the blood (fiber),
- which are a source of energy, for the formation of glycogen in the muscles and food for the nervous system (carbohydrates),
- which represents a reserve source of energy, for building cells, protecting internal organs from extreme temperatures, and enabling the absorption of fat-soluble substances (fats),
- which enables the metabolism of the listed nutrients (vitamins and minerals) and
- which enable resistance to infections (all of the above).

In short, the healthy eating habits of children and the inclusion of apitherapy are of essential importance not only for health but also for development in all areas, including self-esteem, which, among other things, originates from a sense of successful placement in the environment and a sense of the ability to overcome challenges; in bee products, a key supporting role should be recognized in this regard (Critchley and Garfinkel, 2017).
Bee products do not enter the diet of children in the same way as general foods intended for human consumption but as occasional supplements. Honey is energy- and nutritionally rich and at the same time easily digestible food with pronounced pharmacological and nutritional effects. Propolis is stronger in pharmacological than in nutritional effects, which means that it mainly represents direct support in the field of preservation and maintenance of the immune system and in overcoming pathogenic conditions. Pollen, like honey, is energy- and nutritionally rich and at the same time easily digestible food with pronounced pharmacological and nutritional effects. In this research, wax comes into use primarily through the consumption of honey in its original packaging, i.e. in the honeycomb, and through sensory use, and royal jelly in the considered context mainly represents positive pharmacological effects.
The substances available to the child's organism and obtained through nutrition are among the most important elements that build and guide human development from the prenatal period onwards. Neural connections in the brain, which are formed based on experiences in the preschool period, build and shape the foundations for a lifetime. Through apitherapy for children and the apipedagogical methodology, which focuses mainly on prevention in kindergarten, and outside the kindergarten and also on support in the healing process, bee products play an important role in establishing nerve connections that serve as a basis for emotional resistance, academic success and the ability to live healthy enforcement in society. A child who approaches challenges with the attitude that he can do it, because he is getting the substances and experiences he needs for his development, has a better self-esteem, is more successful in both social and cognitive competencies and, as a result, also has a stronger immune system.

Conclusions

According to the results of monitoring children in kindergartens, there is a correlation between their choice of preferred honey and their body condition. Children who faced stress during that period (parental separation, moving, etc.) in 50% of cases more often reached for darker honey or a type of honey that they would not have preferred under normal circumstances than they would have otherwise typical choice. From the above, it can be concluded that there is a possibility that interoception in healthy children takes place with fewer deviations, i.e. children who otherwise feel well can more easily feel what they need on an unconscious level, while in children who face challenges, this internal voice may not be as successful, or perhaps the changed circumstances affect the biochemical sphere in the body to such an extent that the needs regarding the ratio of nutritional substances also temporarily change.

It should be noted that the children monitored were free of developmental problems and congenital disorders. For a more accurate and broader picture, it is necessary to include children with special needs in the observation as well as other bee products, especially propolis and pollen. Due to its effect on endocrine glands in the preschool period, royal jelly is not recommended for regular consumption, except in specific exceptional cases.

In addition, for more credible results in the data analysis itself, it is also necessary to take into account the fact that not all children develop according to the same principle. In the preschool period, two children of approximately the same age can both be healthy, but one develops faster in the motor area and the other in the cognitive area. Optimal nutritional support in physiological terms would be similar for them, but not the same. The latter is not only true because of differences in development, but genetics also has a certain say.

Interoception in apitherapy for children is a perspective element that should be paid attention to. Internal body signals influence children's behavior and emotions, which can be recognized as an opportunity and responsibility for apitherapists, educators, and parents. These are predictable signals that can be used constructively. Food is your medicine and your medicine should be food.

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Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2024) **Specijalno izdanje** 15-18
Food in Health and Disease, scientific-professional journal of nutrition and dietetics (2024) ***Special Edition*** *15-18*

APPLICATION IN MODELING OF PROPOLIS OIL EXTRACT IN SEMISOLID PHARMACEUTICAL FORMS

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original scientific paper

Summary

Bee products are widely used in the production of pharmaceutical and cosmetic products. The purpose of this study is to extract propolis by oil and apply the oil extract to compound the semisolid preparations from natural components. The selected solvent and extraction conditions ensure the isolation of phenolic compounds from the raw material, the total amount of phenolic compounds in the oil extract was determined to be 35.82 ± 1.12 mg/ml. Propolis oil extract is a suitable component in the production of ointments due to its lipophilic properties. The modeled formulations had a homogeneous, semisolid consistency. The viscosity of the produced ointments depends on the amount of wax. As the amount of wax increases, its viscosity increases. All modeled in research formulations were temperature-sensitive. The pH value of the formulations was in the range of 4.17 - 5.77. As the amount of wax increases, less active compounds are released from the formulations.

Keywords: propolis, honey, beeswax, phenolic compounds, ointments

Introduction

Bee products, propolis, and honey are traditionally widely used components in the production of pharmaceutical and natural cosmetic products. Propolis is a complex of biologically active substances with mutually synergistic effects. Propolis can be used in various forms: thick extract, liquid ethanolic extract, and aqueous extract (De Clermont-Gallerande, 2022). When creating propolis products for external use, the optimal form of propolis must be selected for introduction into dermatological semisolid pharmaceutical and cosmetic preparations. Therefore, it is relevant to produce an oil extract from Lithuanian propolis raw material and to evaluate its quality. It is important to choose the right extraction technology so that the produced oil extract is dominated by the same biologically active substances as in the raw material. In the production of propolis oil extract, olive oil was chosen as a solvent, considering its positive properties - it mixes well with solid substances, animal fats, waxes, and paraffins (Gottschlack and McEwen, 2010). Propolis is compatible with olive oil and has even been shown to protect olive oil from autoxidation and lipolysis (Jankowski et al., 2017). Olive oil is used in the production of pharmaceutical and cosmetic products as an auxiliary component and as an active ingredient, as the therapeutic effect of olive oil, when used internally and externally, is proven. Components of olive oil: fatty acids, vitamins (E and carotene), and water-soluble substances. The main fatty acids are oleic (55-58 percent of all olive oil), linoleic acids (9 percent), and linolenic (linoleic) acid (up to 1.5 percent). Olive oil also contains phenolic compounds, etc. of reactive substances (Gottschlack and McEwen, 2010; Jankowski et al., 2017). Olive oil used externally has skin moisturizing properties; due to its antioxidant action, it reduces the skin-damaging effect of free radicals; brightens and evens skin color and the appearance of wrinkles; pleasant to use and non-irritating, suitable for sensitive skin; works against skin aging. Olive oil can be used to treat dermatitis, atopic dermatitis, xerosis, eczema, rosacea, seborrhea, psoriasis, burns, and various skin inflammations (Jankowski et al., 2017; Nilforoushzadech et al., 2018).

Honey is listed in the International Nomenclature of Cosmetic Ingredients (INCI) as a moisturizing, humectant, and emollient product (Öğütcü and Yılmaz, 2014). Honey is useful in skin care products, and its regular use contributes to the youthfulness of the skin and the reduction of wrinkles. Honey is used in different proportions depending on the type of dermatological preparations. Honey is commonly used at 1 - 10 % in products such as lip balms, moisturizers, and gels (Servili, 2013). Cosmetic products are currently using synthetic spermaceti to contribute to animal safety. Spermaceti is a hard wax rich in high molecular weight esters. It is most often found in the composition of ointments, as one of the components of the base, characterized by a moisturizing effect (Silici and Baysa, 2020; Šuran, 2021). Cocoa butter is used in the production of cosmetic products due to its protective and moisturizing properties.

All selected components of the semisolid base must be compatible with each other and ensure the stability and release of the biologically active substances from the modeled semisolid matrix. Therefore, biopharmaceutical release tests *in vitro* are currently performed to assess the quality of semisolid pharmaceutical forms, during which it is determined

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how the modeled semisolid base is appropriate to release active substances, and how the active substances penetrate through biological membranes. This work aims to produce an oil extract of propolis and apply it in the modeling of ointments, using natural components for the formation of a semisolid matrix and to evaluate their influence on formulation quality.

Methods of research

Propolis oil extract technology. Propolis (UAB Medicata Filia (Vilnius, Lithuania) 30.0 g was extracted by maceration for 7 days in a dark place with 70.0 g of olive oil.
Determination of the amount of phenolic compounds in propolis oil. 0.3 g of propolis oil was mixed with 50 ml 96% ethanol. Samples were held at 9 p.m. at -12 ºC. Pour 1 ml of the test solution into a 50 ml volumetric flask containing 15 ml of water and 4 ml of Folin-Ciocalteu reagent. Then 6 ml of 20% sodium carbonate solution was added. Water was added up to the 50 ml mark. The prepared solution was left for 2 hours. Absorbance was measured at a wavelength of 765 nm.
Modeling of ointment formulations with propolis oil extract. Beeswax, spermaceti, and cocoa butter were melted in a water bath, with adding the appropriate amount of olive oil propolis extract and mixing until homogeneous mass. The compositions of the formulations with propolis oil extract are presented in Table 1.

Table1. Composition of ointment formulations with propolis oil extract

Formulation substances	Mass, g		
	F1	F2	F3
yellow beeswax	10.0	5.0	2.svi
honey	2.0	2	2.0
propolis oil extract	50.0	50.0	50.0
spermaceti	0.25	0.25	0.25
cocoa butter	10.0	10.0	10.0
olive oil	till 100.0	till 100.0	till 100.0

Studying rheological properties. The consistency coefficient of the simulated semisolid formulations was determined using a Carri-Med CSL100 rheometer (TA Instruments, Germany).
Quality analysis. The pH value was determined by the potentiometric method (pH-meter 766 Knick SE 104 N, Knick Elektronische Meßgeräte GmbH & Co, Germany). The release of phenolic compounds from the formulations was evaluated *in vitro*. Acceptor phase – 30% ethanol solution. Samples were taken after 1, 2, 3, and 6 hours. The total amount of phenolic compounds was assessed according to p-coumaric acid equivalent by spectrophotometric method, wavelength 765 nm. The organoleptic indicators of the modeled semisolid formulations with propolis oil extract were evaluated visually: color, smell, phase stability, and spreadability/washability.
Student's t-test and $p<0.05$ was used as the research level of significance. All tests were repeated three times.

Result and discussion

Propolis oil extract was produced, and organoleptic properties were determined. Propolis oil extract is characterized by a yellowish-brown color and a specific smell of propolis, it is a viscous oily liquid.
Propolis oil extract is intended for inclusion in semisolid formulations, as well as for external use as a final product. Therefore, it is appropriate to determine the amount of biologically active substances - polyphenolic compounds in propolis oil. The total amount of phenolic compounds in propolis oil extract was determined. The research results showed that the total amount of phenolic compounds according to p-coumaric acid in propolis olive oil extract is 35.82 ± 1.12 mg/ml.
One of the application possibilities of propolis oil is the formulation of a dermatological semisolid preparation. Materials of natural origin were chosen for the ointment modeling: yellow beeswax and olive oil, propolis, honey, spermaceti, and cocoa butter.
The quality evaluation results of the produced propolis oil ointment formulations are presented in Table 2. The research results show that the formulations of the modeled compositions remained stable, and phase separation did not occur. The color and smell corresponded to the characteristics of the ingredients.

Table 2. pH values and organoleptic evaluation of ointment formulations with propolis oil extract

Formulation	pH value	Phase separation	Spreadability / Washability	Colour	Odour
F1	4.17 ± 0.04	unnoticed	spreadable / hard to wash off	yellowish	pleasant, characteristics of propolis
F2	4.69 ± 0.02				
F3	5.77 ± 0.08				

The pH values of the ointment formulations were set within the limits of 4.17-5.77. The research results showed that the lowest pH value was typical for formulation F1, and the highest for F3. The pH value of all modeled formulations with propolis oil extract was weakly acidic, close to the physiological pH value of the skin.
The research results showed that formulation composition and temperature influence the structural properties of modeled ointments (Table 3).

Table 3. Consistency coefficient of semisolid formulations with propolis oil extract in different temperatures

Formulations	*Consistency coefficient* (K) Pa·s^n		
	20 °C	32 °C	37 °C
F1	0.2204 ± 0.041	0.1893 ± 0.024	0.0291 ± 0.032
F2	0.1258 ± 0.033	0.0974 ± 0.028	0.0501 ± 0.028
F3	0.0999 ± 0.027	0.0330 ± 0.031	0.1002 ± 0.024

During the experimental study, it was found that the rheological properties of semisolid formulations - the consistency coefficient depend on the amount of wax in the base of the ointment. A statistically significant difference was found between all the tested formulations. It was also found that the viscosity of semisolid formulations decreases with increasing temperature.
During the *in vitro* release study, the influence of the semisolid formulation substances on the release of phenolic compounds from propolis oil extract was determined (Figure 1).

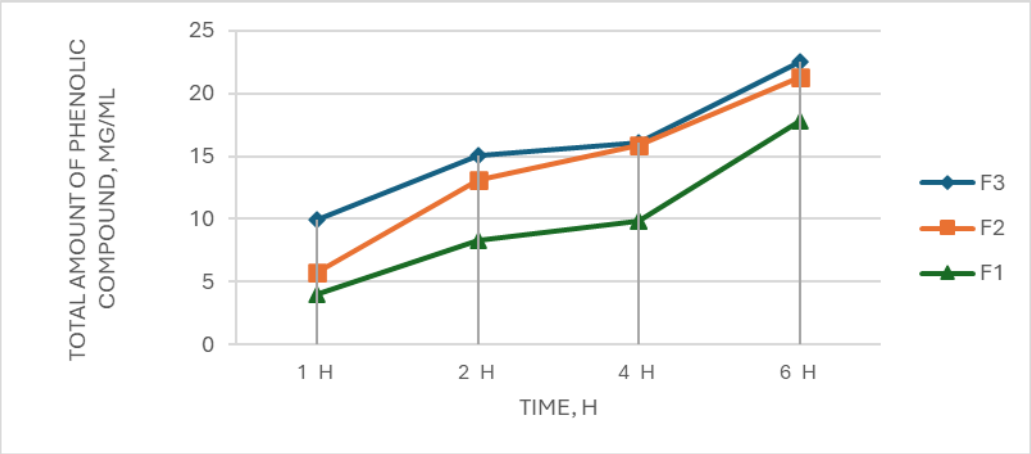

Figure 1. Release of total phenolic compounds from semisolid formulations with propolis oil extract

During the research, it was found that the auxiliary substances forming the basis of the formulations influence the release of bioactive substances from such systems. The compositions of the studied semisolid systems differ in terms of wax content. Therefore, the influence of wax content on the release of phenolic compounds *in vitro* was observed. The systems (F2 and F3) with the least amount of wax release the most phenolic compounds. Statistically significantly less ($p<0.05$) was released by the F1 formulation. This supports the view that due to their lipophilic nature, phenolic acids are difficult to release from the lipophilic semisolid base (Šuran, 2021).

Conclusions

1. The selected propolis extract olive oil and propolis oil technology are suitable for the extraction of biologically active propolis substances from propolis raw material.
2. Propolis oil extract is compatible with the selected base materials and is suitable for inclusion in lipophilic semisolid preparations, for use on the skin due to established organoleptic properties, close to the physiological skin pH value and consistency coefficient at body and skin temperature.
3. Ointment formulations with propolis oil extract ensure the release of propolis bioactive substances from the system.

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Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2024) **Specijalno izdanje** 19-24
Food in Health and Disease, scientific-professional journal of nutrition and dietetics (2024) ***Special Edition*** *19-24*

THE EFFECT OF ROYAL JELLY ON HORMONES DURİNG ADOLESCENCE

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review paper

Summary

Royal jelly is a unique food that is given in the honey bee colony from the egg to the maturity period of the queen bee, and only during the egg period of the other castes. Although both develop from a fertilized egg, the morphological and physiological differences between the worker bee and the queen bee are the result of royal jelly feeding. Royal jelly, which has many beneficial biological activities, has gained importance as a functional food. Scientific research has shown that royal jelly is effective on growth and sex hormones. Its effect on hormonal changes, especially during adolescence, should be taken into consideration, and hormonal changes that will occur in male and female individuals should be investigated.

Keywords: royal jelly, hormone, adolescence, infertility

Introductıon

Today, early puberty is one of the most important problems that concern parents regarding the growth and development of children. Early breast development, especially in girls, worries families. The cause of anxiety is early menstrual bleeding and the thought that growth will stop with bleeding. It covers the periods of adolescence, adolescence, and puberty. Adolescence covers the period between the ages of 10-21. This period is the transition period between childhood and adulthood, where physical, sexual, spiritual, and social changes occur. Puberty is the growth phase that includes physical and sexual maturation that occurs during adolescence. At puberty, secondary sex characteristics are acquired and with the completion of puberty, the person becomes capable of reproduction. The pubertal development process generally occurs between the ages of 8-13 in girls and between the ages of 9-14 in boys. The internal and external reproductive organs (ovaries, uterus, vagina, testicles, and penis) that are inherent in girls and boys constitute the primary sexual characteristics. In addition to the growth of these organs to reach adult sizes, physical changes such as hair growth and breast growth are defined as secondary sex characteristics. Adolescence is the transition period from childhood to adulthood, where secondary sexual characteristics and reproductive capacity are acquired. Puberty is early, starting before the age of 8 in girls and 9 in boys. If puberty does not begin when girls turn 13 and boys turn 14, there is a delay (Delemarre-van de Waal, 2002; Kuiri et al., 2011; Kirivanta et al., 2016; Sultan et al., 2018).

Under normal conditions, the ovaries and testicles, which are the female and male reproductive glands, produce female and male sex hormones (estrogen and testosterone). While the level of sex hormones is low in childhood, estrogen levels in girls and testosterone levels in boys increase dramatically during adolescence, and this increase reveals secondary sex characteristics. The stimulating effect that enables the awakening of the reproductive glands comes from the pituitary gland in the brain. The pituitary hormones, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) stimulate the ovaries and testicles, ensuring the production of both hormones (estrogen, testosterone) and eggs (ovum and sperm) (Angold et. 1999; Mrug et al., 2014). Precocious puberty is the onset of secondary sex characteristics before the age of 8 in girls and 9 in boys. Breast growth or hair growth before the age of 8 in girls, and testicular growth and hair growth before the age of 9 in boys are considered early. Many factors affect the onset and progression of puberty, and genetic factors come first. In addition, racial-ethnic origin, socioeconomic conditions, geographical features, diet, obesity, endocrine disruptors, physical and psychological stress, and chronic diseases affect the onset of puberty. Natural or synthetic substances that have hormonal effects or affect the endocrine system by affecting the production, release, transport, or destruction of hormones are called endocrine disruptors. Natural endocrine disruptors include strawberries, soy products, carrots, apples, coffee, parsley, and legumes. Synthetic endocrine disruptors include DES (diethylstilbesterol), fungicides, herbicides, cleaning agents, polishes, cosmetic products, paints, and plastic materials. Consumption of non-organic agricultural products and living in constant contact with other endocrine disruptors from the development period in the womb may cause early puberty by affecting the brain, pituitary, and reproductive glands (Cassio et al., 1999; Lebrethon et al., 2000; Farello et al., 2019; Ridder et al., 1990; Mrug et al., 2014).

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Effect of royal jelly on hormones

Bee products are used to support the prevention and treatment of many diseases, and this form of treatment is called Apitherapy. Among the bee products, two products that have hormonal effects are royal jelly and apilarnil. Royal jelly is a bee product secreted from the hypopharyngeal (throat) and mandibular glands of young (5-15 days old) worker bees and is the only food that the queen bee feeds on throughout her life. It is a nutritious product with a creamy consistency, whitish, sharp phenolic odor, and a sour and bitter taste, used for the feeding of young larvae and the queen (Šver, 1996; Guo et al., 2021).

During the first four days, all fertilized bee eggs are fed with royal jelly and the queen bee can develop from all of them. However, queen bees are formed only from those fed with royal jelly during the entire larval development period. Due to this nutrition, significant differences are observed between queen bees and worker bees in many respects. While the reproductive organs of the queen bees, who complete their larval stage by being fed royal jelly in their morphological development, are well developed, the organs of the worker bees, such as the pollen basket, mandibles, brood gland, and wax glands, are well developed with the work done in the hive. As a result of this different nutrition for only 6 days, the queen bee gains resistance to diseases, can produce eggs twice its own weight (1500-3000) per day, and can live for 3-5 years. Worker bees, on the other hand, get sick more easily due to their weak immune systems, cannot lay eggs even though they are females, and live only 2-3 months. It is suggested that the main reason for this degree of differentiation between the two individuals is due to their feeding with royal jelly (Collazo et al., 2021).

The composition of royal jelly varies depending on the nutrition of the bees, season, age of the larva, and production method. The main components of royal jelly are 60-70% water, 9-18% proteins, 7-18% sugars, 3-8% lipids, minerals (Fe, Na, Ca, K, Zn, Mg, Mn and Cu), amino acids (Val, Leu, Ile, Thr, Met, Phe, Lys and Trp), vitamins (A, B complex, C and E), enzymes, hormones, polyphenols, nucleotides and small heterocyclic compounds. The pH value of fresh royal jelly is generally between 3.6 and 4.2. Royal jelly is a viscous, creamy, acidic, white-yellow-colored bee product with a pungent odor and sour-sweet taste. Royal jelly is relatively water-soluble and has a density of 1.1 g/mL. Changes in chemical compositions; it is caused by differences in different feeding (without sugars and/or protein supplements), production methods, environmental conditions, flora, bee breed, storage, and processing conditions (Rembold and Dietz, 1996; Xue et al., 2017).

The most important and basic component of royal jelly is 10-hydroxy-2-decanoic acid (10-HDA), which is a fatty acid. This component is the most important value that determines the quality parameter of royal jelly and is only found in royal jelly. Fresh royal jelly produced under appropriate conditions should contain at least 1.40% 10-HDA (Yavuz and Gürel, 2017).

The most important feature of royal jelly is that it is effective in cell renewal, production, and metabolism in the body. It gives the organism strength and vitality, allowing it to renew itself. Studies on these subjects in insects, birds, and mammals have found that it significantly increases lifespan. Royal jelly has cholesterol-lowering, blood pressure-lowering, and vasodilator activity. In addition, it has a hypoglycemic (lowering blood sugar) effect because it contains insulin and similar peptides, and an immunological effect because it is antimicrobial. Since it has a cell-repairing and rejuvenating effect, it is used to heal skin and hair diseases and regulate sexual functions. It is used as a regulator of abnormalities caused by chronic diseases, loss of appetite, and irregular and unbalanced nutrition. It is also recommended to reduce the possible harm of drugs used in the treatment of chronic diseases to the liver and kidneys and to protect these organs (Khazaei et al., 2017).

Many studies are showing the effect of royal jelly on infertility. For example, hydroxyurea (HDU), a class of antineoplastic drugs, has many adverse effects, including infertility, especially in men. Therefore, Tohamy et al. (2019) investigated the chemoprotective potential of royal jelly on HDU-induced testicular damage in their study. Experimental animals were given HDU (225 or 450 mg kg) before royal jelly (100 mg/kg) for 60 days. In a dose-dependent manner, sperm count and motility, as well as testosterone, GSH, and catalase concentrations, decreased in the hydroxyurea groups, while MDA, FSH, LH, IL-6, and IFNγ expression levels increased. In hydroxyurea-administered rats, royal jelly intake successfully improved sperm quality, hormonal and antioxidant status, and reproductive organ histochemistry. Researchers concluded that royal jelly can be used as an adjuvant drug to improve hydroxyurea-induced male subfertility, thanks to its antioxidant and anti-inflammatory activities. In another study, Nasir et al. (2017) investigated the effect of royal jelly in protecting rat testicles against carbon tetrachloride-induced testicular damage in male Wistar rats. Royal jelly was given to animals treated with Kabron tetrachloride at doses of 150 and 300 mg/kg. At the end of the experiment (50 days), a significant decrease in sperm concentration, sperm viability, and sperm motility and an increase in sperm abnormalities were observed in the group treated with carbon tetrachloride, while a significant decrease in sperm abnormalities was determined in the rat groups treated with royal

jelly at both doses. In addition, while a significant decrease in sex hormone levels (T, LH, FSH) was observed in the rat group given only CCl_4 in the study, it was suggested that royal jelly could be used in the treatment of infertility problems in men due to the improvement it provided in the examined parameters. In studies conducted on farm animals, royal jelly increased the in vitro fertilization capacity of bull sperm. Consumption of royal jelly increased glycolysis, pentose phosphate pathway, and antioxidant enzyme activity in oocyte and cumulus cells, resulting in higher levels of oocyte maturation, fertilization, and blastocyst formation. It significantly increased sperm motility, luteinizing hormones, and testosterone levels in infertile men. Long-term and regular use of royal jelly stopped the age-related decrease in testicular function of male hamsters and stimulated testosterone levels and spermatogenesis. The consumption of honey and royal jelly together has been beneficial in the treatment of infertility due to asthenozoospermia. In a study examining the effect of tris-yolk extender supplemented with royal jelly on chilled and frozen-thawed ram semen parameters, frozen-thawed sperm total motility, progressive motility, membrane integrity, and viability were found to be significantly higher in the 3% royal jelly-supplemented group. Research shows that royal jelly may be effective in increasing male hormones and sperm count, as well as reducing reproductive toxicity. It has been reported that royal jelly application (gavage 100 mg/kg/day) increases epididymal sperm motility and in vitro fertilization capacity in adult male mice. However, some studies have shown that royal jelly can improve oxidative stress, and male infertility, and inhibit cell proliferation by breaking E2-induced signals. Ahmed et al. (2018) investigated the protective potential of royal jelly against cadmium-induced testicular dysfunction in rats. The royal jelly concentration used in the study was 100 mg/kg and serum and tissue samples were collected and analyzed after the 56^{th} day of the experiment. Results showed decreased serum testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), superoxide dismutase, glutathione reductase, sperm motility and count, and increased levels of malondialdehyde, nitric oxide, tumor necrosis factor-α (TNF-α). Abnormalities with severely damaged seminiferous tubule epithelium with cytoplasmic and nuclear disruptions were observed after cadmium toxicity, including testicular mRNA expression of TNF-α, steroidogenic acute regulatory protein, cytochrome P450 cholesterol side chain cleavage enzyme androgen binding protein, FSH-receptor, LH-receptor. Adverse changes in the androgen receptor, 3β-hydroxysteroid dehydrogenase (HSD), 17β-HSD, and cytochrome P450 17A1 were significantly reduced by royal jelly application. Researchers concluded that royal jelly protects against cadmium-induced testicular toxicity. In another study on infertility, Al-Dujaily et al. (2019) determined the role of royal jelly on some sperm function parameters of obstructive azoospermic men and concluded that royal jelly strongly increased some sperm function parameters of vasectomized male mice. Researchers have suggested the possibility of using royal jelly for male factor infertility, especially in those with obstructive azoospermia.

Spermatogenesis and hormone secretions are very important endocrine and physiological processes to sustain life. In a study on the effects of royal jelly on the testicular development of offspring during neonatal and adolescence consumption, Shi et al. (2019) detected neonatal sexual hormone concentration and histopathological changes in the testicular development of male offspring after orally administering freeze-dried royal jelly to mice for 35 days. Male offspring were given 125, 250, and 500 mg/kg/day royal jelly. Weaned male puppies were given freeze-dried royal jelly for 35 days. It was determined that the diameter of the seminiferous tubule, the height of the seminiferous epithelium, and testicular weight increased significantly in the group given 250 mg/kg/day of royal jelly, while a high dose of royal jelly (500 mg/kg/day) decreased the diameter of the seminiferous tubule, but increased the height of the seminiferous epithelium of male offspring. Moreover, royal jelly administration (250 mg/kg) significantly increased testicular weight and spermatogenesis on the 21st day after birth, while high-dose royal jelly (500 mg/kg) treatment reduced the diameter of the seminiferous tubule and the height of the seminiferous epithelium. On the 35^{th} day after birth, 250 mg/kg royal jelly increased testicular weight, seminiferous tubule diameter, and FSH level. High-dose royal jelly, on the other hand, reduced testicular weight and size (seminiferous tubule diameter and seminiferous epithelium height), apoptotic germ cell rate, and all parameters of missing spermatogenesis. Apart from these, royal jelly (at all doses) reduced sexual hormone secretions (FSH, LH, E2) on the 21^{st} day after birth. The results obtained showed that oral administration of low and medium doses of royal jelly can increase testicular development in the neonatal period until adulthood, but the negative side effects caused by high doses of royal jelly may continue.

In recent years, families often use royal jelly supplements to help their children grow. Pirgon et al. (2019) evaluated the effects of royal jelly supplementation on the growth volume of young rats and hormone levels such as estradiol, growth hormone (GH), and insulin-like growth factor-1 (IGF 1). In the study, 7-day-old rats were given 50 mg/kg royal jelly via gavage once a day for 15 days. Plasma estradiol, growth hormone (GH), and IGF-I levels were then measured. Average weight and tail length changes were found to be significantly higher in the royal jelly group than in the control group at the end of the study. Plasma growth hormone and estradiol levels increased significantly in the royal jelly group, and the total height of the growth plate was found to be significantly higher in the royal jelly

group than in the control group. Moreover, the percentage of estrogen receptor expression in the growth plate was expressed as 81.3% in the proliferative zone of the royal jelly group and 14.3% in the control group. Researchers have shown that royal jelly administration causes longitudinal bone growth as well as estradiol and growth hormone levels, but the findings also provide evidence that royal jelly has some potential estrogenic effects on growth. Bee products have been used to treat various human diseases for decades. It is known that major royal jelly proteins (82-90%), which are the most important bioactive components found in royal jelly, are potential factors in extending honeybee lifespan. Analysis of royal jelly proteins has shown that they consist of some essential free amino acids and complex proteins of the MRJP family, which are necessary for feeding both the queen and the larvae. Some studies show that royal jelly has an estrogenic activity similar to exogenous steroid hormones, including testosterone and 17β-estradiol. These estrogen-like compounds may exert various estrogenic or anti-estrogenic effects in the reproductive systems, commonly by modulating estrogen receptors (ER). Exogenous estrogen or estrogen-like compounds can be found in many foods of plant and animal origin, such as seeds, vegetables, milk, and dairy products. It is also reported that royal jelly prevents the negative effects of exogenous estrogen on the male reproductive system. Studies on reproduction have shown that royal jelly can exert estrogenic effects in vivo and in vitro. It is very important to investigate the reproductive function of offspring when exposed to royal jelly continuously from newborn to maturity.

Sosa-Perez et al. (2017) investigated the effect of intravenous administration of 500 mg royal jelly for seven days before progestogen termination on the synchronization, onset, and duration of estrus, follicular population, and ovulation rate of Pelibuey sheep. Researchers reported that royal jelly could be an alternative for the reproductive management of sheep production units, as it reduces the time to the onset of estrus and increases the number of follicles > 4 mm and the ovulation rate in Pelibuey sheep. Al-Eisa et al. (2018) investigated the protective effect of royal jelly against aluminum chloride ($AlCl_3$) toxicity on testicular histology as well as on pituitary, thyroid, and sex hormones. Animals were given aluminum chloride ($AlCl_3$) (30 mg/kg) intraperitoneally every day for eight weeks, and royal jelly (400 mg/kg) was given once a day in drinking water for eight weeks. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3), percentage of triiodothyronine to thyroxine (T3/T4) and testosterone level were measured in blood serum. Aluminum chloride; It caused a significant decrease in FSH, LH, TSH, T4, T3, T3/T4, and testosterone, while causing the development of oligospermia, hypoplasia, blocked blood vessels, and exfoliated tubules in the testicles. However, royal jelly completely cured these effects.

In a study on male infertility in 2007, royal jelly was given in different doses (25 mg, 50 mg, 100 mg) to 83 infertile men. As a result of the data obtained, it was reported that sperm motility, sluggish sperm, sexual intercourse/week, testosterone level, and LH hormone level increased in infertile men who used royal jelly for three months. The study showed that royal jelly application is safe, has no side effects, and is effective in the treatment of male infertility (Al-Sanafi et al., 2007).

In a study conducted in Egypt, the mid-cycle effectiveness of intravaginal applications of Egyptian bee honey and royal jelly mixture for the treatment of infertility due to asthenozoospermia (low sperm motility) was evaluated on 99 couples. For the research, 3 g of royal jelly and 1 teaspoon of bee bread were added to 100 g of Egyptian honey, and the mixture was given at least 3 days after preparation to ensure sufficient enzymatic interaction between the honey and bee bread. Before use, the mixture was diluted 1:1 in a normal saline solution and then self-administered intravaginally at each coital act starting 1 day after the last menstruation. In this application, repeated for 2 weeks, a plastic piston applicator or a 10 ml syringe was used to introduce the mixture. The application was performed precoital or postcoital, depending on the preference of the couple. 50 couples were studied in this group and 49 couples in the intrauterine insemination group. At the end of the study, a total of 553 cycles were analyzed and it was revealed that there was a significant difference between honey and royal jelly application and vaccination groups. It has been observed that honey and royal jelly application is more effective in the pregnancy cycle. It has been reported that intravaginal use of honey-royal jelly mixture may be a simple and reasonably effective method in the treatment of asthenozoospermia (Abdelhafiz and Muhamad, 2008).

Yang et al. (2012) administered doses of 200, 400, and 800 mg/kg/day to rats for 4 weeks, sperm deformity increased and serum hormone levels were affected in the high-dose group. After the royal jelly administration was stopped, the values returned to normal levels. El-Eisa et al. (2017) determined the effects of royal jelly on pituitary, thyroid, and sex hormones against aluminum chloride toxicity. While aluminum chloride harmed FSH, LH, and TSH hormones when 400 mg/kg of royal jelly was given for eight weeks, royal jelly alleviated these negative effects. Spermatogenesis and hormone secretions are very important endocrine and physiological processes for the maintenance of life. The effects of royal jelly on the testicular development of offspring during neonatal and adolescence have not been adequately studied. Shi et al. (2019) determined neonatal sexual hormone concentration

and histopathological changes in testicular development of male rats after oral consumption of lyophilized RJ (125,250 and 500 mg/kg/day) for 35 days. At the end of the study, they determined that oral M-RJ (250 mg/kg/day) administration significantly increased testicular weight (on the 14th day, the diameter of the seminiferous tubule and the height of the seminiferous epithelium of baby mice. However, high dose RJ (500 mg/kg/day) day) decreased the diameter of the seminiferous tubule but increased the height of the seminiferous epithelium of male offspring. RJ significantly increased testicular weight and spermatogenesis on day 21. On the contrary, oral H-RJ treatment significantly increased the diameter of the seminiferous tubule and the height of the seminiferous epithelium in PNDs on day 21. decreased ($p<0.05$). Oral M-RJ treatment on PND day 35 increased testicular weight, seminiferous tubule diameter, and FSH level. In addition, sexual hormone secretions (FSH, LH, E2) increased significantly after RJ treatment (L-) on day 21, respectively. They concluded that oral administration of low and moderate doses of RJ could enhance testicular development in the neonatal period until puberty, but negative side effects caused by high doses of RJ may remain.

Ghanbari et al. (2018) stated that uterine and ovarian weights and progesterone and estradiol serum levels in immature rats increased in the experimental groups compared to the control group. Additionally, they identified a significant increase in the number of mature follicles and corpora lutea in rats receiving RJ compared to controls. Researchers have reported that Royal Jelly promotes folliculogenesis and increases ovarian hormones. They also stated that it can be considered a natural growth stimulant for immature female animals.

In a study on male infertility in 2007, royal jelly was given in different doses (25 mg, 50 mg, 100 mg) to 83 infertile men. As a result of the data obtained, it was reported that sperm motility, sluggish sperm, sexual intercourse/week, testosterone level and LH hormone level increased in infertile men who used royal jelly for three months. The study showed that royal jelly application is safe, has no side effects, and is effective in the treatment of male infertility (Al-Sanafi et al., 2007).

Conclusıon

Royal jelly is a bee product that contains hormones as well as rich nutritional elements and biomolecules. For this reason, the effect mechanisms of royal jelly on the endocrine system should be considered in many aspects. Its effect on hormonal changes, especially during adolescence, should be taken into consideration, and hormonal changes that will occur in male and female individuals should be investigated. Gynecomastia and early menstruation are just some of these effects.

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Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2024) **Specijalno izdanje** 25-33
Food in Health and Disease, scientific-professional journal of nutrition and dietetics (2024) ***Special Edition*** *25-33*

UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN AND APITHERAPY

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review paper

Summary

Upper respiratory tract infections (URTIs) are an important health problem of childhood. Children struggle with this disease on average 6-8 times a year, each lasting 8-10 days. URI is one of the most common diseases encountered in primary health care institutions and for which antibiotic and analgesic abuse is thought to be common. Reasons such as irrational drug use, inadequate or toxic effects, especially antibiotic resistance, undesirable effects due to wrong drug selection, and unnecessary drug consumption, cannot provide the desired treatment and cause economic losses. Unnecessary use of antibiotics not only causes antibiotic-related side effects and increases the cost of treatment, but also facilitates the emergence of antibiotic-resistant infections. However, it is known that the majority of URTIs are caused by viral factors and are self-limiting, and they usually heal without the need for medication. Acute URTI is the most common reason for outpatient clinic visits and job and school losses. Although URI is often self-limiting, mild, and short-term, when evaluated between quality of life and job losses; The magnitude of its effect is equivalent to chronic diseases. The lack of a definitive treatment, the fast pace of life, and the desire to recover as soon as possible push both patients and physicians to seek different treatments. For this reason, this study draws attention to the role of bee products, which are natural, have no side effects, and are known to have many beneficial effects, in supporting the treatment of URTI in children.

Keywords: URTI, children, propolis, royal jelly, honey

Introductıon

Upper respiratory tract infections (URTIs) encompass local infections above the larynx and are generally conceptualized as such. The most common infectious disease in children is acute upper respiratory tract infections (Wilke et al., 1996). Uncomplicated URTIs typically cause nasal discharge and congestion, sore throat or itchiness, and a tickling sensation leading to cough (Hueston, 1997). The diseases, which can be of viral or bacterial origin, significantly increase during the winter months, posing a threat to public health.

Pharyngitis is an inflammatory disease of the mucosal and submucosal structures of the throat. It can affect the oropharynx, nasopharynx, hypopharynx, tonsils, and adenoids. The normal flora of the upper respiratory tract and oral cavity primarily consists of gram-positive aerobic organisms. Among these, alpha-hemolytic or gamma-hemolytic streptococci, peptostreptococcus, fusobbacterium, and various Bacteroides species are found. In infectious scenarios, these organisms, or mixed infections involving gram-negative and aerobic agents, can occur. Especially in children, Group A beta-hemolytic streptococcus (*Streptococcus pyogenes*) is most commonly implicated in pharyngitis. *Streptococcus pneumoniae*, Group C streptococcus, and other streptococci can also cause pharyngitis. These organisms can also be isolated from healthy individuals. Whether to treat pharyngitis in a symptomatic patient remains controversial. The incubation period for Group A beta-hemolytic streptococci is between 12 hours and 4 days (Incı, 2008).

The main viral etiology for upper respiratory tract infections includes rhinoviruses, influenza viruses, adenoviruses, enteroviruses, and parainfluenza viruses. While more than 200 viruses can cause upper respiratory tract infections, rhinoviruses are the most common culprit. Parainfluenza virus, respiratory syncytial virus, and enteroviruses can create a similar clinical picture that is difficult to differentiate. Viruses primarily infect the epithelial cells of the upper respiratory tract, leading to the clinical presentation. Some viruses, like rhinoviruses, enhance the clinical picture by triggering the release of chemical mediators such as histamine and bradykinin from infected mucosa. Influenza virus, on the other hand, causes desquamation of the ciliated epithelium in the respiratory tract, resulting in mucosal damage. Symptoms of viral upper respiratory tract infections typically have a rapid onset. After a short incubation period (24-72 hours), patients experience weakness, widespread body aches, nasal discharge, sneezing, sore throat, and cough. Symptoms continue to worsen during the first 3-5 days and then gradually improve, usually resolving spontaneously within 7-14 days after the recovery phase. Fever is generally normal, although it may mildly elevate (<38 °C) and return to normal within 3 days. While burning and tearing of the eyes are common, true conjunctivitis is mainly seen in adenovirus infections and, to a lesser extent, enterovirus infections. Additionally,

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exudative tonsillitis, which can be mistaken for streptococcal tonsillopharyngitis, may occur in adenovirus infections. In parainfluenza virus infections, hoarseness and cough are prominent, while rhinovirus infections often present with coryza (runny nose), and herpes virus infections may manifest as gingivostomatitis (Akan, 2012; Incı, 2008).
Four main clinical pictures can be mentioned under the heading of URTI: rhinitis, acute tonsillopharyngitis, acute otitis media, and acute rhinosinusitis. While 75% of acute tonsillopharyngitis seen between the ages of 5-15 years is viral, the rest is bacterial and almost all of the bacterial ones are group A beta-hemolytic streptococci (AGBHS). Although acute tonsillopharyngitis can be differentiated as viral or bacterial based on clinical findings and physical examination, it is necessary to make a differentiation based on throat culture, and antibiotic or symptomatic treatment should be given accordingly. A throat culture is necessary for the diagnosis of tonsillopharyngitis; it is a cheap, practical, ubiquitous, and highly sensitive method, and results are obtained after 24 hours. Another laboratory method for the diagnosis of AGBHS tonsillopharyngitis is rapid antigen detection tests performed on throat swabs. Although this test gives results in as short as 20-30 minutes, throat culture is necessary because of high false negativity. Blood tests are also useful in the diagnosis of ABGHS tonsillopharyngitis. While leukocytosis in peripheral blood supports ABGHS, leukopenia and/or lymphomonocytosis are in the direction of viral tonsillopharyngitis. Antistreptolysin-O (ASO) elevation and C-reactive protein (CRP) positivity may also be detected in streptococcal tonsillopharyngitis (Ulusoy, 2008).

Treatment of upper respıratory tract ınfectıon

Symptomatic treatment

Upper respiratory tract infections (URTIs) are among the most common diseases encountered in primary health care organizations and antibiotic and analgesic misuse is considered to be common in their treatment (Steinman et al., 2003; Chlabicz et al., 2004). Irrational drug use, especially antibiotic resistance, inadequate or toxic effects, undesirable effects due to wrong drug selection, and unnecessary drug consumption cause economic losses (WHO, 1993; Mangione-Smith et al., 2003). In studies, it has been reported that the development of resistance to different antibiotics is very high in areas where antibiotic use is high (Spack and Black, 1998; Cizman, 2003), whereas the rate of resistant pneumococcal infections is at the lowest level in areas where antibiotic use is low (Stephenson, 1996; Arason et al., 1996). Unnecessary antibiotic use facilitates the emergence of antibiotic-resistant infections in addition to the occurrence of antibiotic-related side effects and increasing the cost of treatment (Schwartz et al., 1997; Bauman, 2000).
However, it is known that the majority of URTIs are caused by viral agents, are self-limiting and mostly heal without the need for medication (Colgan and Powers, 2001; Bauman, 2000). In a study conducted by Akıcı et al. (2004) in Istanbul, 73.8% of prescriptions diagnosed with URTI included antibiotics, while this rate was 91.8% in a study conducted by Leblebicioglu et al. (2002) in Samsun. As a matter of fact, an article investigating the use of broad-spectrum antibiotics in acute respiratory tract infections in the USA between 1997 and 1999 showed that 63% of patients were prescribed antibiotics, but another study using the 1992 US national health records showed that this rate was 52% (Gonzalez et al., 1995).
The main importance of intervention is to relieve the symptoms of fever, nasal congestion and cough. Various adrenergic agonists, anticholinergics, antihistamine preparations, antitussives, and expectorants are sold for this purpose. Common ingredients of such medications include first-generation antihistamines, antipyretics (paracetamol) or anti-inflammatory agents (ibuprofen), cough suppressants such as dextromethorphan, expectorants (such as guaifenesin), and decongestants such as pseudoephedrine and phenylpropanolamine (MMWR, 2007). While these provide symptom improvement, there is no comparative evidence that they shorten the duration of symptoms (Schroeder et al., 2004; Paul et al., 2004).
The use of antibiotics for URTI in children is controversial because more than 90% of infections have a viral etiology. Current reasons for prescribing antibiotics include diagnostic uncertainty, socio-cultural and economic pressures, malpractice litigation, and parental expectation of an antibiotic (Pschichero et al., 1999). Antibiotics are prescribed at a high rate for the treatment of URTI, which favors antibiotic resistance (Dowel et al., 1998a). However, it is also true that they have a role in defined indications such as severe acute otitis media and severe acute rhinosinusitis lasting more than ten days (Dowel et al., 1998b). Fahey and Stocks (1998) reported in their systematic review of randomized controlled trials comparing antibiotics with placebo for pediatric URTI that antibiotic treatment did not change clinical status and did not reduce the complication rate. However, the researchers emphasized that its efficacy may be higher in a subgroup at a higher baseline risk of developing complications.

Alternative and complementary treatment

As an aid in the treatment of sore throat due to infection, it is recommended to rest and consume plenty of fluids (fruit juice, water, water with lemon, open tea with honey), consume soft foods, gargle frequently with warm salt water, avoid smoking and smoking environment, take aspirin or acetaminophen if there is pain and fever, takes throat lozenges and increase the humidity of the room (Dogan et al., 2012).
In the world, echinacea, vitamin C, zinc, *Pelorgonium sidoides* (Umckaloaba), probiotics, garlic, and saline irrigation have been investigated therapies (Nahas and Balla, 2012; Douglas et al., 2008; Singh and Das, 2013; Timmer, 2008; Lissiman et al., 2014; Kassel et al., 2010). As a result, echinacea, probiotics, and zinc were found to be effective, while vitamin C requires mega doses for protection, and studies on applications such as garlic, saline irrigation, and hot steam are reported to be inadequate and cannot be interpreted.
Apart from these, Biswas et al. (1999) examined the effect of vitamin A supplement in diarrhea and acute respiratory tract infections in 174 children under 6 years of age, and the effect of single oral administration on the incidence of diarrhea and acute respiratory tract could not be determined. The WHO (World Health Organization, 2001) encourages the use of some safe antitussives such as lemon juice and honey in the treatment of cough and reports that such preparations are not expensive. Honey contains more than 200 compounds such as carbohydrates (glucose, fructose, sucrose, maltose, etc.), free amino acids, vitamins, minerals, organic acids, and antioxidants (phenolic compounds, flavonoids, enzymes, carotenoid-like compounds, and other phytochemicals). Studies on the antimicrobial effect of honey have shown that it has a broad spectrum of activity against gram-positive and gram-negative bacteria. It is effective against bacteria such as *Staphyloccoccus aureus, Streptococcus faecalis, Candida albicans, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli,* Salmonella spp., and *Shigella dysenteriae*, which are common agents of upper respiratory tract infections (Adeleye and Opiah, 2003). There are also studies on the antiviral activity of honey (Bogdanov et al., 2008). Indeed, due to these properties, honey has been used in traditional medicine for many years in the treatment of cough (Adeleye and Opiah, 2003). Honey is also used in the treatment of infected wounds and as an ingredient in cough syrups.
Honey reduces inflammation and edema, and stimulates epithelialization, tissue regeneration, granulation, and debridement (Subrahmanyam, 1991; Bogdanov et al., 2008). Honey is recommended for dental hygiene with therapeutic properties in the treatment of gingivitis and periodontal diseases (Bogdanov et al., 2008). WHO (2001) recommends honey as a demulsent agent for cough. Since honey is a sweet product, it stimulates mucus secretion and saliva secretion in the respiratory tract. This effect is combined with the hyperosmolarity of honey and its antioxidant and anti-inflammatory properties.
A Cochrane meta-analysis of 8 pediatric trials of 616 children with viral-induced cough showed that OTC (over-the-counter) medications did not affect cough frequency, severity, number of coughs, and sputum production (Smith et al., 2012). For the treatment of cough in children, dextromethorphan (nonselective serotonin reuptake inhibitor and σ1-receptor agonist) and diphenhydramine (first-generation anticholinergic, antitussive, antiemetic, and antihistamine with sedative properties) are commonly used. The effect of both drugs on cough and sleep quality in children to improve nocturnal syndromes was not higher than placebo (Paul et al., 2004). For years, codeine has also been considered a treatment agent on the grounds that it suppresses cough in the central nervous system. However, there is no evidence that codeine is more effective or safe than placebo (Goldman, 2010).
There is growing interest in the use of complementary and alternative medicines in URTI. Herbal medicines have been studied frequently with conflicting results. The most widely used and studied plants are Echinacea and *Andrographis paniculata*, both of which are believed to be immunostimulants. Similarly, propolis (bee resin) has also been studied and found to increase antibody production. However, the most important problem in the use and study of herbal products is the lack of standardization (Shah et al., 2007). Researchers sought large randomized trials using standardized preparations and measuring well-defined endpoints. There is still insufficient data on safety, especially with long-term use. For example, echinacea is frequently reported to cause rash (Mullins, 1998).

Honey bee products ın the treatment of upper respıratory tract ınfectıons in children

There are studies on the use of honey in the treatment of upper respiratory tract infections and especially coughs. In a partially double-blind randomized study conducted by Paul et al. (2007), they compared buckwheat honey, honey-flavored dextromethorphan (DM), and no drug treatment group given a single night dose (30 minutes before bedtime) in the reduction of difficulty sleeping and night cough in children diagnosed with upper respiratory tract infection. In the two-day study, all groups were instructed not to take any medication or honey on the first night and the application was made on the second night. In the study conducted on 105 children aged 2-18 years with URTI

(duration of illness 7 days or less), the frequency and severity of cough, the quality of cough, and the quality of sleep of families and children were evaluated. While there were differences between the groups, the results of the honey group were better than those of the control group in which no treatment was given, the results of the DM group were not better than those of the control group. The researchers stated that honey can be preferred in the symptomatic treatment of children with sleep difficulties and nocturnal cough due to URTI and that honey can be recommended by physicians as a safe and well-tolerated alternative.

In a similar study, Shadkam et al. (2010) conducted on 139 children aged 24-60 months with cough due to URTI and formed four groups; honey, DM (dextromethorphan), DPH (diphenhydramine), and a control group. Cough frequency and severity were evaluated in the groups, and while there was no statistical difference between the results of DM and DPH groups, the results of the honey group were found to be better than all groups. In conclusion, the researchers reported that 2.5 ml of honey taken before going to sleep had a curative effect on URTI compared to DM and DPH.

The study conducted by Oduwole et al. (2014), which also evaluated the studies of Paul et al. (2007) and Shadkam et al. (2010), focused on the efficacy of honey in the treatment of acute cough in 265 children aged 2-18 years. In the study, DM and DPH, which are the components of commonly recommended cough medicines, and non-medicated groups were compared using a 7-point Likert scale, and although the honey group was better in reducing cough frequency compared to the non-medicated group, it was not found to be different from the DM group. However, irritability, insomnia, and hyperactivity were observed in 7 children in the honey group, moderate adverse reactions in the DM group, and drowsiness in 3 children in the DPH group, but the difference between the groups was not statistically significant. In conclusion, Oduwole et al. (2014) emphasized that honey was better in reducing cough frequency and improving the sleep quality of both families and children compared to the unmedicated group and DPH group, but not better than the DM group.

In a study testing honeys of different botanical origins, Cohen et al. (2012) investigated the effect of three honeys of different botanical origins (eucalyptus, citrus, and honeydew honey) and date syrup extract (brown in color, similar in taste to honey) on night cough and difficulty sleeping in a single overnight dose on 300 children aged 1-5 years with URTI in 2009. The honey group was found to be better in the symptomatic treatment of nocturnal cough and sleep difficulties than the group given date palm extract and was recommended. A review by Eccles (2005) attributed the favorable effects of honey in URTIs to the natural and reflexive salivary secretion of sweet foods, which causes mucus secretion in the respiratory tract, resulting in a demulsant effect on the pharynx and larynx and thus a reduction in coughing. Eccles (2005) reported that the interaction between opioid-responsive perceptual filaments and gustatory nerves can help them produce antitussive effects through the central nervous system mechanism. Raessi et al. (2014) examined the therapeutic effects of prednisolone, coffee, honey, and coffee-honey in the treatment of post-infectious cough (PPC) and compared the effects. Among the groups, the most effective group on cough severity was the group given the coffee-honey combination, which showed success in a short time and was also recommended by the researchers. In a study conducted by Sopo et al. (2014) on 134 children suffering from non-specific acute cough, the effects of a mixture of milk (90 ml) and flower honey (10 ml) were compared with dextromethorphan and levodropropizine (LPD). After three consecutive days of administration, therapeutic success was 80% in the honey+milk group and 87% in the OTC medication groups ($p<0.25$). The researchers showed that milk with honey was at least as effective as DM and LDP groups for non-specific cough. In another study, 30 of 60 patients (group I; mean age 27.4 years) admitted to hospital with cold symptoms in Iran were given the classical treatment regimen (acetaminophen 325 mg/q 6 hours, naproxen 250 mg/q 12 hours + chlorpheniramine 4mg/q 6 hours) while the other 30 patients (group II, mean age 24.4 years) were given 50 g of natural honey daily. Symptoms such as rhinitis, myalgia, fever, throat congestion, cough, and cold were monitored daily. At the end of the study, the symptoms in the group given honey lasted 1-2 days shorter than the control group. This effect was attributed by the researchers to the antioxidant and antimicrobial effect of honey with its phenolic acid, flavonoid and peroxidase content (Pourahmed and Sobhanian, 2009). Naveed et al. (2013) reported that when families with children diagnosed with URTI between the ages of 1-6 years were asked to feed honey, 39 out of 40 families agreed. Raessi et al. (2013) compared the effects of systemic steroids and honey-coffee mixture in the treatment of persistent post-infectious cough (PPC). It was found that the most effective group for PPC was the honey-coffee group. Researchers have shown that a honey and coffee mixture can be used as an alternative in PPC.

Paul et al. (2007) reported that honey is cheaper and safer than other treatment drugs, and especially processed honey carries little risk of allergy. However, it is noteworthy that honey is not given to children under one year of age due to *Clostridium botulunim* toxicity (Oduwole et al., 2014).

Carr and Nahata (2006) investigated the safety and effects of alternative therapy in the prevention and treatment of URTI in their 2005 review. They evaluated 6 clinical trials examining the use of herbal preparations and 9 studies

involving other CAM (Complementary and Alternative Medicine) therapies. They reported that echinacea reduced the duration and severity of URTIs, and *Andrographis paniculata* (Echinacea) reduced nasal secretions but did not affect URTI symptoms. They reported that echinacea-propolis-ascorbic acid decreased the number of URTI episodes, symptom duration, and number of sick days, while ascorbic acid or homeopathy was not effective. Kumar et al. (2011) reported that traditional and complementary treatment practices are used intensively in children due to the safety phenomenon, especially honey is a traditional medicine used in URTIs. In the study, the opinions of 92 families and 30 health professionals (11 focus families) were taken and 29% of the families reported that they used honey in their children and that honey was traditional, accepted, easily accessible, natural, and safe. They stated that they used it with hot water and lemon, especially in URTIs. Waren et al. (2007) evaluated the effects of honey on nocturnal cough and sleep quality in children and reported that honey improved symptom scores and reduced cough frequency compared to the untreated group, but stated that the study lacked appropriate psychometric data for the survey.

Propolis, another bee product, is a product with high resin content that honey bee colonies collect from the buds of trees, add beeswax and enzymes to it, and use it for various purposes with its protective feature against microorganisms in the hive. The antiviral activity of propolis has also been the subject of numerous studies (Amoros et al., 1992; Harish et al., 1997; Serkedjia et al., 1992). Fouad et al. (2012) investigated the efficacy of propolis by taking 17 throat swab samples from children over 11 years of age with URTI in 2011. Among the patient volunteers, 9 children had positive *S. pyogenes* cultures (52.9% prevalence), followed by *H. influenza* (11.8%) in the cultures of 2 children and *C. albicans* (35.5%) in the cultures of 6 children. All isolates were found to be sensitive to propolis. *S. pyogenes, H. influenza,* and *C. albicans* were inhibited at 200 mg/ml propolis concentration. In addition, the therapeutic effect of propolis in URTI was tested in 41 pediatric patients and complete remission of streptococcal and candidal symptoms was achieved in 2-5 days in all patients. In the study, a propolis+goat milk mixture was reported to be an effective antimicrobial agent in throat infections caused by bacterial and candidal species in children. In addition, Cohen et al. (2004) examined the efficacy of a preparation containing echinacea, propolis and vitamin C in the prevention of URTI in children during a 12-week winter period. The study included 430 children aged 1-5 years. The herbal preparation (Chizukit) group showed a 55% reduction in the number of cases of illness, a 50% reduction in the number of cases per child and a 62% reduction in the number of fevers per child. The duration of individual episodes and the total number of days of illness were significantly reduced in the Chizukit group. In conclusion, the preparation containing echinacea, propolis and vitamin C had a preventive effect on the incidence of URTI. Ophori and Wemabu (2010) identified the bacterial agents of URTI infections and determined the sensitivity of isolates to propolis. Ethanol extracts of 0.25, 0.5, 1, 2, 4, and 10 μg/ml propolis were tested in 250 throat cultures from patients (142 males, 108 females) aged 15-30 years diagnosed with URTI at Benin City Central Hospital in Nigeria. Of the 250 samples, 160 (64%) were *Haemophilus influenzae-positive* cultures with the highest prevalence, followed by *Klebsiella pneumoniae* (19.2%) *Streptococcus pneumoniae* (12%), *Moraxella catarrhalis* (10%), *Streptococcus pyogenes* (2%). While the highest isolate rate was obtained from the 15-18 age group, *M. catarrhalis* and *S. pyogenes* were not isolated from the 23-26 age group. All isolates showed sensitivity to all concentrations of propolis, while *K. pneumonia* and S. *pneumonia* inhibition zones were 32 and 30 mm, respectively. In conclusion, researchers emphasized that propolis is a very effective antimicrobial agent in the management and treatment of URTI caused by bacterial species. In another study, El-Shouny et al. (2012) identified the microbial agents causing URTI in children (17 throat swabs in total) diagnosed with URTI at Al-Thawrah Hospital (Yemen) and determined the sensitivity of the isolates to propolis and antibiotics. In 9 of the 17 samples, *S. pyogenes* was positive and had the highest prevalence (52.9%), followed by *H. influenza* (11.8%) and 6 isolates were identified as *C. albicans* (35.3%). *S. pyogenes* was isolated with the highest rate in the 8-11 age group. All isolates were sensitive to ciprofloxacin, amoxicillin, and cephalexin. Some isolates showed resistance to ampicillin and erythromycin. The growth of *S. pyogenes, H. influenza,* and *C. albicans* was inhibited at 200 mg/ml MIC with inhibition zones of 24, 17, and 19 mm. The efficacy of propolis against URTI infections was also tested in 41 pediatric patients.

Marchisio et al. (2010) studied the effect of propolis and zinc solution in 122 children aged 1-5 years suffering from recurrent otitis media (ROM). During the three-month study period, 31 children (50.8%) received propolis and zinc solution while 43 children constituted the control group. The mean number of OM episodes per child/month was 0.23+0.26 in the propolis group and 0.34+0.29 in the control group. The reduction rate was calculated as 32% ($p=0.03$). It was reported that administration of propolis and zinc in children with a history of OM decreased the risk of OM episodes, and no problems were experienced in terms of trust and tolerability with the satisfaction of the families.

In another study investigating the antimicrobial activity of propolis, Mirzoeva et al. (1997) examined the effect of propolis on the physiology of *B. subtilis, E. coli and R. sphaeroides* microorganisms. In the study, it was determined that ethanolic extract of propolis showed a bactericidal effect. Propolis was found to be effective against gram-

positive and some gram-negative bacteria but this bactericidal effect was species dependent. It is thought that propolis acts on the ion permeability of the bacterial membrane and causes dissipation/disruption of the membrane potential. The electrochemical gradient of protons crossing the membrane is essential for bacterial ATP synthesis, membrane transport, and motility, and thus survival. The effect of propolis on membrane potential and permeability contributes to its overall cytotoxic activity and, together with other antimicrobial components, may reduce bacterial resistance. This is also explained by the synergistic effect of propolis with some antibiotics. Some compounds of propolis such as CAPE and quercetin act as ionophores and cause inhibition of bacterial motility. Considering that bacterial motility and chemotaxis guide the adhesion and invasion of bacteria to the relevant sites, the importance of this effect becomes clear (Finlay and Falkow, 1989; Tamura et al., 1995). In conclusion, the antimotility action of propolis components on bacteria has been shown to play an important role in the inhibition of bacterial pathogenesis and infection development. These compounds are probably flavonoids and caffeic acid esters (Pepeljnajk et al., 1985). Scazzocchio et al. (2006) found that EEP (ethanol extract of propolis) showed a significant antimicrobial effect on all clinical isolates tested. The addition of EEP to the tested antibacterial drugs strongly enhanced the antimicrobial effect of ampicillin, gentamycin, and streptomycin, and moderately enhanced the effect of chloramphenicol, ceftriaxone, and vancomycin, but had no effect on erythromycin. The antimicrobial activity of a propolis sample obtained from 4 different regions of Turkey and Brazil was tested against 9 anaerobic lines. It was determined that the main components of propolis were pinobanksin, quercetin, naringenin, galangin, chrysin, and aromatic acids. In the study, it was suggested that propolis could be used in oral cavity diseases due to its antimicrobial activity (Koru et al., 2007). In addition, Lu et al. (2005) investigated the antibacterial activity of Taiwanese propolis against *Staphylococcus aureus.* They evaluated cell age, incubation temperature, and pH. While bacterial culture age was found to be significant, they observed that propolis was more sensitive during the late exponential phase. Additionally, it was noted that pH values of 35 °C and 37.8 °C were more effective.

In conclusion, the antimotility action of propolis components in bacteria has been shown to play an important role in the inhibition of bacterial pathogenesis and infection development. These compounds are probably flavonoids and caffeic acid esters (Finlay and Falkow, 1989; Tamura et al., 1995). However, it is emphasized that pure propolis components containing cinnamic acid derivatives (caffeic acid and CAPE) and flavonoids (quercetin and naringenin) affect bacterial membrane potential and mobility and that the bacteriostatic and bacteriocidal effects of propolis are probably due to the combined action of such compounds. It is also suggested that the identification and characterization of potent bactericidal components of propolis will be useful for the development of new antibiotic drugs (Pepeljnajk et al., 1995).

Conclusıon

Apitherapy, which involves using bee products, has gained importance in recent years for preventing and treating various diseases. Natural products such as honey, pollen, propolis, and royal jelly are generally beneficial for the human liver and cells, and they possess high antioxidant capacity. These natural products contain various compounds, including carbohydrates, vitamins, coenzymes, polyphenols, aroma compounds, phytosterols, and several terpenes and terpenoids. Therefore, as seen in many studies, the use of bee products such as honey and propolis in upper respiratory tract infections in children can be used safely to prevent and support the treatment of such diseases.

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Original scientific papers report unpublished results of original research. They must contain significant and original observations to be critically evaluated. Experimental data should be presented in a way that enables reproduction and verification of analyses and deductions on which the conclusions are based.

Preliminary communications include short information on the results of scientific research which require immediate publication.

Scientific notes include reports on shorter but completed research or descriptions of original laboratory techniques (methods, apparatus etc.) and should be concise.

Reviews are original, critical and up-to-date surveys of an area in which, preferably, the author himself/herself is active. They should include recent references from international publications.

Professional papers present new possibilities of improvement within the area of food technology. The emphasis is on the application of known methods and facts as well as on broadening the knowledge in the particular area. The acquired knowledge is applied to the object of research.

Procedure

All contributions are evaluated according to criteria of originality and quality of their scientific and professional content. All manuscripts received for consideration will be acknowledged by the Editorial office. All manuscripts are sent to at least two referees. Based on their opinion, the Editor and the Editorial Board bring a decision about the acceptance of the manuscripts. Contributions may be rejected without reviewing if considered inappropriate for the journal.

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Manuscript preparation

The manuscript should consist of max. 15 type written pages including tables, graphs, schemes and photographs. It should be written with 1.5 spacing on one side of the paper and margins 2.5 cm. For the text should be used normal

plain font (Times New Roman, font size 12). The title of the manuscript and the title of the chapter should be short and written in bold. The title of subheading should be written in italic.
Under the title, author/s full name/s and surname/s should be written, with asterisk next to the name of the corresponding author. Footnote at the bottom of the first page should contain information about the corresponding author (address and e-mail). The affiliations for all authors must be given in the following sequence: University/Institution, Faculty/Department, Postal address, City, Country. When authors have different affiliations, should be used superscripted Arabic numbers after last name of the author.
Manuscript has to be written without spelling mistakes, impersonal style. It is the author's responsibility to ensure that papers are written in clear and comprehensible English. Authors whose native language is not English are strongly advised to have their manuscripts checked by an English-speaking colleague prior to submission.
The first word in the paragraph must not be retracted. Paragraphs have to be separated by clicking on enter key. Pages have to be numerated (on the bottom right). For decimal numbers in text and tables dot should be used.

Latin words, phrases and abbreviations, including generic and specific names, should be written in italic.

Manuscripts should be submitted by e-mail, as attached document, to the Editor's office. The manuscripts should be sent to the following address:

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For clearness the paper should be divided into the following sections: **Summary**, *Keywords*, **Introduction**, **Materials and Methods**, **Results and Discussion**, **Conclusions and References**.

Summary

Summary must not exceed 300 words and has to show relevant data, methodology, main results and conclusion. It should not contain abbreviations or references. After summary, authors are asked to list several keywords.

Keywords

Keywords include the main topic of the paper and should not contain more than 5 words or phrases, which should be separated by commas.

Introduction

Introduction should refer to previous research results and explain the purpose of the investigations.

Materials and methods

Experimental part should be written clearly and in sufficient detail to allow the work to be repeated. Materials and Methods should indicate instruments, samples, subjects, chemicals, etc., giving enough details only if new methods and/or procedures and/or principles are used. For the well-known methods and techniques an adequate reference(s) citation will suffice.

Results and discussion

The information given in tables and figures should not be repeated, and only relevant data discussed and explained. Combining the results with discussion can simplify the presentation.
Tables and figures should be completely understandable without reference to the text. For publishing reasons, they have to be delivered in graphical formats (*.xls, *.tif or *.jpg) and at the end of the paper.
All illustrations (graphs, schemes, diagrams, pictures, etc.) should be named as figures. Refer to figures by the abbreviation "Fig.". All figures and tables should be cited in the text and numbered consecutively throughout. Preferred program for preparing figures and tables is Microsoft Office Excel.

Be sure to use lettering, data lines, and symbols sufficiently large and thick to be clearly legible when the figure is reduced to the normal published size. In graphs and charts, curves should be identified by using different symbols for points (•, ◊, ○, □, ■, ▲, etc.) and not by line width or colour. Several figures should be grouped in a plate on one page. Do not place figures into bordered frames. Figure caption and legend should be placed at the bottom of each figure, while table headings should appear above the tables. The text into the figures and tables should be written in the same language as the body text.
Whenever possible formulae and equations are to be written in one line, centred on the page, and consecutively numbered in Arabic numbers between round brackets at the right margin of the paper. Refer to equations by the abbreviation "Eq.".
SI (System International) units should be used. Only symbols (not their subscripts, superscripts or description in brackets) of physical quantities should be written in italic. All physical quantities given in table columns or rows and corresponding table headings with units, or graphical plots and corresponding table headings with units, or graphic plots and corresponding axis labels should conform to the algebraic rules, i.e.

physical quantity / unit = numerical value.

It is preferred to write the measurement units as "kg/m^3".

Conclusions

Conclusions have to briefly explain significance of the research results.

References

References should be selective rather than extensive (with the exception of review articles). Literature citations in the text should be referred by author's last name and year, in brackets, such as (Knowles, 2007), or with the last name of the author as part of the sentence, immediately followed by the year of publication in brackets, such as ("Smith (1963) reported growth on media."). If there are more than two authors, mention the first author and add et al., followed by the year.
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Reference list should be written as shown:

Journals:

Kopjar, M., Piližota, V., Hribar, J., Nedić Tiban, N., Šubarić, D., Babić, J., Požrl, T. (2008): Influence of trehalose addition on instrumental textural properties of strawberry pastes, *Int. J. Food Prop.* 11 (3), 646-655.

Books:

Doyle, M.P., Beuchat, L.R., Montville, T.J. (2001): Food Microbiology: Fundamentals and Frontiers, Washington, USA: ASM Press, pp. 572-573.

Chapter in book:

Varoquaux, P., Wiley, R.C. (1994): Biological and Biochemical Changes in Minimally Processed Refrigerated Fruits and Vegetables. In: Minimally Processed Refrigerated Fruits and Vegetables, Wiley, R.C. (ed.), New York, USA: Chapman, pp. 226-268.

Conference proceedings:

Babić, J., Šubarić, D., Ačkar, Đ., Kopjar, M. (2008): Utjecaj hidrokoloida na reološka svojstva voštanog kukuruznog škroba. In: 43rd Croatian and 3rd International Symposium on Agriculture, Pospišil, M. (ed.), Zagreb, HR, pp. 558-562.