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Chemical composition, oral toxicity and antimicrobial activity of Iranian propolis

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Abstract

Propolis is a resinous natural hive product derived from plant exudates collected by honeybees. Due to biological and pharmacological activities, it has been extensively used in folk medicine. The present study was designed to investigate the chemical composition, subchronic toxicity, antimicrobial activity of Iranian propolis ethanolic extract, which has not been studied previously. One hundred and nine compounds were identified by gas chromatography–mass spectrometry analysis. Forty-five days subchronic toxicity of oral propolis extract was investigated in male rats. During the study no significant behavioral and clinical toxicity has been seen in animals however, hematologic, blood biochemistry and histopathologic data studies exhibited some significant differences between the groups. The ethanolic extract of propolis inhibited the growth of all examined microorganisms including bacteria and fungi with the highest antimicrobial activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis.*

Keywords: Propolis; Chemical compounds; Oral toxicity; Antimicrobial activity

1. Introduction

Propolis or bee glue is a complex resinous mixture of different plant exudates, which is gathered, modified and used by honeybees as a general purpose sealer and draught excluder in their hives. Due to its several biological and pharmacological activities, it has been used in folk medicine. More than 160 constituents have been identified in different propolis samples (Greenaway, May, Scaysbrook, & Whatley, 1991; Marcucci, 1995). It usually consists of waxes, resins, water, inorganic compounds, phenolics and essential oils (Bonvehi, Coll, & Jorda, 1994). In general, propolis composition is directly related to that of bud exudates collected by honey bees from various trees: poplar, birch, beech, horse chestnut, alder and various conifers (Bankova, De Castro, & Marcucci, 2000; Ghisalberti, 1979).

Due to propolis versatile biological and pharmacological effects, it has wide applications in medicine, cosmetics and food industry (Bankova, Christov, & Delgado Tejera, 1998). The ethanolic extract of raw propolis (balsam) has some activities such as antibacterial (Grange & Davey, 1990), antioxidant (Isla, Nieva-Moreno, Sampietro, & Vattuone, 2001), antiviral (Amoros, Simoes, Girre, Sauvager, & Cormier, 1992; Amoros et al., 1994),

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anti-inflammatory (Strehl, Volpert, & Elstner, 1993), anticancer (Frenkel et al., 1993), antibiotic and antifungal (Dobrowolski et al., 1991; Marcucci, 1995) activities. Recently propolis has gained popularity and used extensively in health drink and food to improve health and prevent diseases such as inflammation, heart disease, diabetes, aging and cancer (Banskota et al., 2000). It has been suggested that the presence of a large number of flavonoids, aromatic acids and phenolics compounds are responsible for the most biological and pharmacological activities of propolis (Bankova, Dyulgerov, Popov, & Marekov, 1987; Vennat, Arvouet-Grand, Gross, & Pourrat, 1995).

Contact dermatitis is a well documented allergic reaction to propolis, with approximately 200 cases reported in the literature over the last 70 years (Hausen, Wollenweber, Senff, & Post, 1987a, 1987b), but it has been shown that propolis is not toxic to humans or mammals unless very large quantities are administered (Ghisalberti, 1979; Kaneeda & Nishina, 1994).

Several investigations on propolis have been done in Eastern Europe and South America, but there is no report about chemical composition, oral toxicity and antimicrobial effect of Iranian propolis sample previously. The aim of this study is to identify the chemical composition of 70% ethanolic extract of propolis sample by gas chromatography–mass spectrometry (GC/MS) analysis and to evaluate any changes in biochemical profile of rats after oral propolis ethanolic extract administration by determining some haematologic and seric biochemical factors and histopathological studies. The antibacterial and antifungal activities of propolis ethanolic extract were examined on Gram-positive (*Staphylococcus aureus*; *Staphylococcus epidermidis*; *Bacillus subtilis*) and Gram-negative (*Escherichia coli*; *Pesudomonas aeruginosa*) and fungal strains (*Candida albicans*; *Aspergillus niger*).

2. Materials and methods

2.1. Propolis origin

Propolis sample has been kindly donated by Mr. H. Afrouzan from ''Animal Science Research Institute of Iran (ASRI)'' and was obtained from colonies of honeybees located in Tehran-Khojir (nearly north of Iran) by using plastic nets in fall 2003.

2.2. GC/MS analysis

2.2.1. Sample preparation

One gram propolis sample was chopped in to small pieces and extracted with 50 ml of 70% ethanol with continuous stirring at room temperature (twice after 24 h). After filtration the ethanolic extract was evaporated under vaccum at 50 °C until dryness (Hegazi & Abd El-Hady, 2002). One milligram of dry extract was derivatized with 100 μl bis-(trimethyl) trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) (Fluka, Switzerland) and 50 μl pyridine (Merck, Germany) in a sealed glass tube for 30 min at 100 °C to prepare samples for gas chromatography–mass spectrometry (Greenaway et al., 1991). Sample volume of 1 μl was then injected and analyzed by GC/MS.

2.2.2. Instrument

Gas chromatography–mass spectrometry was carried out on a Hewlett–Packard 6890 GC gas chromatograph coupled to a 5973 mass selective detector under electron impact ionization (EI) mode at 70 eV. The mass scan range was 50–650 atomic mass units (AMU). HP-1 (cross-linked methyl silicone) (30 m × 0.25 mm internal diameter), HP part No. 19091Z-333 purchased from Gulf Bioanalytic, UAE, was employed with helium as carrier gas at a flow rate 1 ml/min and 7.61 psi. Injector temperature was 290 °C. Sample was analyzed with the column held initially at 50 °C for 1 min, increased to 133 °C at 3 °C/min and held for 0.2 min, increased to 164 °C at 2.5 °C/min and held for 0.2 min, increased to 199 °C at 2 °C/min and held for 0.2 min, finally increased to 295 °C at 1.5 °C/min and held at 295 °C for 2 min. The injection was preformed in splitless mode at 200 °C.

2.2.3. Identification of compounds

Peaks were identified by computer searches in commercial reference libraries Wiley 275, PMW TOX2, NIST MS search and user-generated reference library. Good spectral matches for some compounds could be found, but in some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass spectral fragmentation. Reference compounds were co-chromatographed where possible to confirm GC retention time.

2.3. Oral toxicity test

2.3.1. Preparation of propolis hydroalcoholic solution

Propolis sample was stirred at room temperature to prepare 10% ethanolic extract of propolis (10 g of propolis to 100 ml of 95% ethanol). After 24 h, the extract was filtered and used to prepare 30% hydroalcoholic solution for oral rout administration (Sforcin, Novelli, & Funari, 2002).

2.3.2. Acute study

According to our pretest 20 male rats (purchased from Pasteur Institute of Iran and weighing 123 ± 15 g) were acclimated 1 week in animal house prior to experiment. Treatment animals were orally administered 4500, 9000, 13,000 and 20,000 mg/kg body weight of hydroalcoholic solution of propolis extract and control animals received only hydro alcoholic solution to observe ethanol effect as gavage vehicle. The clinical and behavioral signs as well as survival of animals were observed up to 48 h. The results showed that there were no death and toxicological effects in treatment group, so it was suggested that propolis is relatively harmless (Ecobichon, 1997) and the dose of

2000 mg/kg for limit study was performed during subchronic toxicity test.

2.3.3. Subchronic study

Thirty male Wistar rats (purchased from Pasteur Institute of Iran and weighing 116 ± 24 g) were acclimated 1 week in animal house prior to experiment and divided into three groups including treatment (T), received propolis hydroalcoholic solution containing 2000 mg/kg body weight/day of propolis extract; alcohol and water control (C), received 30% hydroalcoholic solution to observe ethanol effect as propolis solvent and blank group (B) received only normal saline. Animals were gavaged 5 days a week up to 9 weeks oral toxicity study (Ecobichon, 1997). Over the entire exposure period the animals were observed for abnormalities and individual body weight. Water and food consumption was measured before each weighing daily.

2.3.4. Haematology and blood biochemistry investigations

After treatment, the animals were deprived of food, but not water, overnight and then euthanized under ether anaesthesia. Cardiac blood samples were collected and centrifuged at 3000 rpm for 20 min. Several hematology and blood biochemistry variables were determined.

2.3.5. Macroscopic and microscopic examination of tissues

Gross observations were made at autopsy and recorded. At terminal sacrifice, the following organs (heart, spleen, liver and kidneys) from each rat were weighted and organ to body weight ratios determined. All organs were fixed in 10% buffered formalin. The tissue specimens were routinely processed into paraffin; 2-μm thick sections were stained with hematoxylin and eosin (H&E). The slides were coded and examined in a single-blind fashion by a veterinary pathologist.

2.3.6. Statistical analysis

The results are reported as mean ± SD. For body weights, hematology, blood biochemistry and organ weight data, the significance of differences was assessed using computer SPSS version 11.5 software. One-way ANOVA and Tukey post hoc multiple comparison tests were used to analyze data. P values less than 0.05 were considered significant.

2.4. Antibacterial and antifungal activity

2.4.1. Minimum inhibitory concentration (MIC) determination

The MIC of ethanolic propolis extract was determined by macrodilution method (Andrews, 2001) with respect to different test microorganisms including Gram-positive bacteria (*S. aureus* ATCC 6538p, *S. epidermidis* ATCC 12228, and *B. subtilis* ATCC 6633), Gram-negative bacteria (*E. coli* ATCC 8739 and *P. aeruginosa* ATCC 9027) and fungal strains (*C. albicans* ATCC 10231 and *A. niger* ATCC 16404).

Propolis crude extract was redissolved in ethanol at a concentration of 20 mg/ml which was further diluted with distilled water in a ratio of 1:10 (2 mg/ml). Two-fold dilution of the resulting propolis solution was prepared in tubes containing 1 ml of double strength Muller–Hinton (MH) broth for bacteria and Sabouraud dextrose (SD) broth for fungi to make the concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.91 and 1.95 μg/ml.

The bacteria inocula were prepared by suspending overnight colonies from MH agar media in 0.9% saline. The *C. albicans* and *A. niger* inocula were prepared by suspending colonies from 48 h and 72 h old (SD) agar cultures in 0.9% saline, respectively. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to 0.5 McFarland standards (1.5×10^8 CFU/ml). The suspensions were then diluted in 0.9% saline to give 10^6 CFU/ml and 100 μl aliquots of each prepared microbial suspension were added to the test dilutions. The tubes containing bacteria were incubated at 30–35 °C for 24 h and those containing fungi were incubated at 20–25 °C for 48 h. After incubation the lowest concentration of propolis extract inhibited the visible microbial growth was considered as the minimum inhibitory concentration (MIC).

3. Results and discussion

Propolis composition is directly related to that of bud exudates collected by honey bees from different trees, so analyzing the ethanolic extract of propolis sample will help us to know the botanical sources covering the geographical location which could be used as a source of propolis for the foraging bees. Chemical composition of 70% ethanolic extract of propolis sample from Tehran-Khojir was assessed by GC/MS analysis. The identified compounds have listed in Table 1. The main flavonoid compounds in derivatized ethanolic extract of propolis were flavanones and their derivatives together with flavones. High levels of flavanones like pinobanksin, pinobanksin-3-acetate, pinocembrin, pinostrobin, and flavones like chrysin and galangin indicate that the main plant source of Tehran propolis is *Populus*. Other chemical compounds such as aliphatic acids, aromatic acids and their esters showed the characteristic of poplar propolis. From the aliphatic acids it contained a significant amount of palmitic, oleic, stearic, malic and succinic acids. Benzoic, *trans*-4-coumaric, ferulic, caffeic and 3,4-dimethoxycinnamic acids had the highest amount of aromatic acids. This sample was characterized also by the presence of caffeate, coumarate, ferulate and cinnamate esters. From the above mentioned data it is clear that Tehran sample is a typical poplar propolis (Christov, Bankova, Hegazi, Abd El-Hady, & Popov, 1998; Hegazi & Abd El-Hady, 2001) and the primary source of the plant exudates incorporated into Tehran-Khojir propolis sample is bud exudates of poplar trees. Long chain fatty acids, hydrocarbons, variable amounts of sugars such as glucose and fructose, sugar alcohols like inositol and myoinositol are also present.

Table 1
Composition of Tehran-Khojir propolis assessed by GC/MS of trimethylsilyl (TMS) derivatives

Compound name[a]	TMS group	%TIC[b]
Aliphatic acids		
2-Hydroxypropanoic acid (lactic acid)	2	0.08
2-Hydroxybutanedioic acid (malic acid)	3	0.31
trans-1,4-Butenedioic acid (fumaric acid)	2	0.01
Butanedioic acid (succinic acid)	2	0.29
Nonanoic acid (pelargonic acid)	1	0.01
Decanoic acid (capric acid)	1	0.02
Dodecanoic acid (lauric acid)	1	0.02
Hexadecanoic acid (palmitic acid)	1	0.54
Oleic acid	1	0.86
Octadecanoic acid (stearic acid)	1	0.28
2-Hydroxy acetic acid	2	0.01
2,3-Dihydroxypropanoic acid (glyceric acid)	3	0.01
Tetradecanoic acid (myristic acid)	1	0.04
Heptadecanoic acid	1	0.06
11-Eicosanoic acid	1	0.11
2,3,4-Trihydroxy butyric acid (tetronic acid)	4	<0.01
Octanoic acid	1	0.01
Palmitelaidic acid	1	0.03
9,12-Octadecadienoic acid (Z,Z)	1	0.16
α-Linolenic acid	1	0.1
Hydroxy malonic acid	3	0.02
Aromatic acids		
Benzoic acid	1	0.19
4-Hydroxybenzoic acid	2	0.03
Benzenepropanoic acid (hydrocinnamic acid)	1	0.02
3-Phenyl, 2-propenoic acid (*trans*-cinnamic acid)	1	0.07
cis-4-Methoxy, cinnamic acid	1	0.01
trans-4-Methoxy, cinnamic acid	1	0.12
cis-3(4-Hydroxyphenyl)-2-propenoic acid (*cis*-4-coumaric acid)	2	0.01
trans-3(4-Hydroxyphenyl)-2-propenoic acid (*trans*-4-coumaric acid)	2	0.18
3(3,4-Dimethoxyphenyl)-2-propenoic acid (3,4-dimethoxy cinnamic acid	1	0.36
3(3-Hydroxy-4-methoxyphenyl)-2-propenoic acid (iso ferulic acid)	2	0.1
3(3-Hydroxy-4-hydroxyphenyl)-2-propenoic acid (ferulic acid)	2	0.87
3(3,4-Dihydroxyphenyl)-2-propenoic acid (caffeic acid)	3	0.44
3-Methoxy,4-hydroxybenzoic acid (isovanillic acid)	2	0.01
3(3-Methoxy, 4-hydroxyphenyl)-2-propenoic acid (isomer 1)	2	0.02
Delta-9-tetra-hydrocannabinol acid	2	0.13
3,4-Dihydroxybenzoic acid (protocatechuic acid)	3	0.01
3(3-Methoxy, 4-hydroxyphenyl)-2-propenoic acid (isomer 2)	2	0.02
Esters		
Hexadecanoic acid, ethyl ester (ethyl palmitate)	–	0.12
1,2-Benzenedicarboxylic acid, diethyl ester (diethyl phthalate)	–	0.03
Benzyl-*trans*-4 coumarate	1	0.12
1-Phenylethyl *trans* caffeate	2	0.19
Cinnamyl caffeate	2	0.11
3-Methyl-3-butenyl-*trans*-ferulate	1	0.21
3-Methyl-2-butenyl-*trans*-ferulate	1	0.35
3-Methyl-3-butenyl-*trans*-iso ferulate	1	0.38
3-Methyl-3-butenyl-*trans*-caffeate	2	2.93
2-Methyl-2-butenyl-*trans*-caffeate	2	1.05
3-Methyl-2-butenyl-*trans*-caffeate	2	3.08
2-Methyl-2-butenyl-*trans*-4-coumarate	1	0.07
3-Methyl-2-butenyl-*trans*-4-coumarate	1	0.14
Phenylethyl *trans*-4-coumarate	1	0.08
Linoleic acid ethyl ether (ethyl linoleate)	–	0.04
2-Propenoic acid, 3-phenyl-, ethyl ester (ethyl cinnamate)	–	<0.01
3(3,4-Dimethoxyphenyl)-2-propenoic acid, methyl ester	–	0.03
3(4-Methoxy, 3-hydroxyphenyl)-2-propenoic acid, methyl ester	1	0.08
3(3-Methoxy, 4-hydroxyphenyl)-2-propenoic acid, methyl ester	1	0.03
3(3,4-Dihydroxyphenyl)-2-propenoic acid, methyl ester	2	0.01
Oleic acid, ethyl ester (ethyl oleate)	–	0.17
Stearic acid, ethyl ester (ethyl stearate)	–	0.04
Flavonoids		
5-Hydroxy-7-methoxy flavanone (pinostrobin)	1	0.51
5,7-Dihydroxy flavanone (pinocembrin)	2	2.62
2′,6′-Dihydroxy-4′-methoxydihydro chalcone	2	0.07
5,7-Dihyroxy-3-acetyloxyflavanone (pinobanksin-3-acetate)(Isomer1)	2	0.17
5,7-Dihyroxy-3-acetyloxyflavanone (pinobanksin-3-acetate)(Isomer2)	2	2.29
5,7-Dihydroxy flavone (chrysin)	1	0.3
3,5,7-Trihydroxy flavanone (piobanksin)	3	0.9
3,5,7-Trihydroxy flavone (galangin)	2	0.06
2′,4′,6′-Trihydroxy chalcone (pinocembrin chalcone)	3	0.13
3,5,7,4′-Tetrahydroxy flavone (kaempferol)	3	0.11
3,5,7,4′-Tetrahydroxy flavone (kaempferol)	4	0.2
5,7-Dihydroxy flavone (chrysin)	2	0.69
5,7-Dihyroxy-3-propanoyloxyflavanone (pinobanksin-3-propanoate)	2	0.17
3,5,7-Trihydroxy flavone (galangin)	3	0.73
5,7-Dihyroxy-3-(iso)butanoyloxyflavanone (pinobanksin-3-isobutanoate)	2	0.05
5,7-Dihyroxy-3-(iso)pentanoyloxyflavanone (pinobankin-3-isopentanoate)	2	0.04
5,7,4′-Trihydroxy flavanone (naringenin)	3	0.07
3,5,7,3′,4′-Pentahydroxy flavone (quercetin)	4	0.13
Quercetin methyl ether	4	0.12
3,5,7,3′,4′-pentahydroxy flavone (quercetin)	5	0.15
Sugars and sugar alcohols		
Inositol	6	0.02
Myo inositol	6	0.02
Sucrose	8	0.06
D-Fructose (isomer 1)	5	0.12
D-Fructose (isomer 2)	5	0.14
Sorbose	5	0.1
D-Glucitol	6	0.01
D-Glucose	5	0.2
α-D-Xylopyranose	4	0.01
L-Gluconic acid	4	0.01
D-Galactose	5	0.01
Aliphatic hydrocarbons		
Heptadecane	–	0.01
2-Nonadecanone	–	0.03
Eicosane	–	0.14
Aldehydes		
4-Hydroxybenzaldehyde	1	0.01
3-Methoxy, 4-hydroxybenzaldehyde (vanillin)	1	0.03

(*continued on next page*)

Table 1 (*continued*)

Compound name[a]	TMS group	%TIC[b]
3,4-Dihydroxybenzaldehyde	2	0.12
Sesquiterpenes		
trans, *trans*-farnesol	1	0.02
α-Cedrol	1	0.01
4-βH,5α-Eremophil-1 (10)-ene	–	0.04
γ-Cadinene	–	0.01
Diterpenes		
Isopimaric acid	1	0.07
Others		
1,2,3-Propanetriol (glycerol)	3	0.15
Phosphate	3	0.05
1,4-Benzenediol (hydroquinone)	2	0.01
Citric acid	4	0.03
1-(5-ethenyltetrahydro-5-methyl-2-furanyl)-1-methylethanol	1	0.23
trans 1-Phenyl-1-propen-3-ol	1	0.01
2′-Hydroxyacetophenone	1	0.35

[a] The name given does not include the trimethylsilyl (TMS) substituents.
[b] TIC, the ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation.

In order to investigate the oral toxicity of propolis sample extract, 20 male Wistar rats weighing 123 ± 15 g were divided into four groups and 4500, 9000, 13,000 and 20,000 mg/kg body weight of propolis extract were orally administered. Test animals received hydroalcoholic solution of propolis extract and the control animals received only hydroalcoholic solution to observe ethanol effect as propolis solvent. Since there were no death and toxicological changes in clinical and behavioral signs in test animals group up to 48 h, it was suggested that propolis is relatively harmless (Ecobichon, 1997) and the dose of 2000 mg/kg for limit study was performed during subchronic toxicity test.

Through subchronic toxicity testing, thirty male Wistar rats weighing 116 ± 24 g were divided into three groups: Treatment group (T) received propolis hydroalcoholic solution including 2000 mg/kg body weight/day propolis extract, Alcohol and water control group (C) received 30% hydroalcoholic solution to observe ethanol effect as propolis solvent and Blank group (B) received only normal saline as blank animals. Animals were gavaged 5 days a

Table 2
Haematologic and blood biochemistry data in Wistar rats administered 2000 mg/kg Propolis extract

	Treatment (T)	Alcohol and water control (C)	Blank (B)
Haematology			
WBC (ul)	4057 ± 1283	5300 ± 1378	5133 ± 2318
RBC (mil/ul)	7.56 ± 0.27[a,b]	8.57 ± 0.42	8.28 ± 0.43
Haemoglobin (g/dl)	13.21 ± 0.36[a,b]	15.25 ± 0.59	14.83 ± 0.45
Haematocrit (%)	42.34 ± 4.47	44.12 ± 1.88	40.96 ± 1.70
MCV (fl)	54.15 ± 1.72[a,b]	50.4 ± 1.32	49.5 ± 1.82
MCH (pg)	17.5 ± 0.49	17.4 ± 0.27	17.93 ± 0.73
MCHC (%)	32.3 ± 0.43[b]	34.57 ± 0.46[c]	36.20 ± 0.4
Neutrophils (%)	21.28 ± 3.25	25.25 ± 10.14	21.33 ± 5.13
Lymphocytes (%)	75.42 ± 4.07	70.5 ± 9.88	75.33 ± 6.42
Monocyte (%)	1.75 ± 0.95	2.25 ± 0.5	2.33 ± 0.57
Eosinophil (%)	2.66 ± 2.08	2.14 ± 1.06	2.33 ± 0.57
Platelet (×1000/u)	796.42 ± 15.62	892.25 ± 74.98	906.33 ± 3.21
PT (s)	22.33 ± 2.42	17.00 ± 4.83	20.66 ± 3.78
Blood biochemistry			
FBS (mg/dl)	200 ± 38.26	170 ± 76.31	151.66 ± 15.17
Blood urea (mg/dl)	37.14 ± 5.14	40 ± 6.68	45.66 ±14.64
Cholesterol (mg/dl)	22.14 ± 7.35[a,b]	45 ± 5.41	53.6 ± 1.15
Triglyceride (mg/dl)	184.42 ± 66.05[a,b]	83.5 ± 23.15	64 ± 5.56
Creatinine (mg/dl)	0.7 ± 0.08	0.75 ± 0.05	0.66 ± 0.11
Sodium (mEq/L)	142.71 ± 0.95	143.25 ± 0.5	144.6 ± 6.35
Potassium (mEq/L)	4.88 ± 0.47[a,b]	6.67 ± 1.42	6.40 ± 0.43
Total bilirubin (mg/dl)	0.68 ± 0.03[b]	0.65 ± 0.05[c]	0.16 ± 0.05
AST (IU/L)	131.71 ± 15.61	218.75 ± 49.92[a,c]	131 ± 24.24
ALT (IU/L)	49.85 ± 7.77	45.75 ± 2.87	48.33 ± 7.63
LDH (IU/L)	2005.42 ± 284.21[b]	2599 ± 509.36[c]	1074 ± 707.05
CPK (U/L)	761.42 ± 120.12	1268.75 ± 274.10[a,c]	517.66 ± 192.86
Total protein (g/dl)	7.2 ± 0.22	7.05 ± 0.26	7.06 ± 0.15
ALP (IU/L)	848.66 ± 108.33[a,b]	486.66 ± 33.65	455 ± 89.82

WBC, white blood cell count; RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PT, Prothrombin time; PTT, Partial thromboplastin time,FBS, fasting blood sugar; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; ALP, alkalane phophata Results are expressed as mean ± (SD) of 10 animals.
[a] There is significant differences between values of group (T) and (C). ($P < 0.05$).
[b] There is significant differences between values of group (T) and (B). ($P < 0.05$).
[c] There is significant differences between values of group (C) and (B). ($P < 0.05$).

week during 9 weeks oral toxicity study (Ecobichon, 1997). No deaths and clinical signs were observed in any of the groups over the entire period. The animals gained weight in a normal manner in all three groups. There were also no clear differences in food and water consumption between groups. After the treatment, cardiac blood samples were taken and haematologic and seric factors were determined. The results have shown in Table 2. Hematology results showed, slightly, but significant differences in variables including: RBC, hemoglobin, MCV and MCHC between groups. Statistically significant differences in blood biochemistry variables such as cholesterol, potassium, triglyceride, alkaline phosphatase, CPK, AST, total bilirubin and LDH were found in (T) and (C) groups in comparison with group (B). The significant high seric levels of these enzymes are related to specific organ injuries, mainly cardiac and liver damages. Macroscopic and microscopic examinations were carried out for specimens. No macroscopic changes were found in animals at autopsy. Organ: body weight ratios of organs from each rat were determined and have shown in Table 3. Slightly significant increase in organ: body weight ratios of liver and spleen weights have seen in group (T) animals. Histopathological examination revealed some reversible abnormalities in prepared tissue sections of the organs. In brief, hypertrophic and acidophilic cardiocyte; hyperplasia of mesangeal cell and dilated capillary in kidney specimen; dilated sinusoids, microvesicule, mild necrosis in hepatocyte and sub capsular edema in spleen specimen were the observed histological changes in groups (T) and (C) in comparison to group (B), which can be related to 30% hydroalcoholic vehicle used for preparing gavage extract suspension in group (T) and ethanol effect observing in group (C). Antibacterial and antifungal activities of propolis ethanolic extract were examined on Gram-positive (*S. aureus*; *S. epidermidis*; *B. subtilis*) and Gram-negative (*E. coli; P. aeruginosa*) and fungal strains (*C. albicans; A. niger*). Results of minimum inhibitory concentration (MIC) determination have shown in Table 4. The ethanolic extract of propolis exhibited an inhibition in the growth of all examined microorganisms including bacteria and fungi showing the highest antibacterial activity against Gram-positive bacteria such as *S. aureus* and *S. epidermidis* with the MIC of 125 μg/ml. The antibacterial activity probably attributed to the presence of high levels of caffeate esters and flavonoids compounds and high antifungal activity against *C. albicans* may be related to the presence of some aliphatic and aromatic acids, caffeate esters and great amounts of flavanones such as pinostrobin, pinocembrin, pinobanksin and pinobanksin-3-acetate. The results of the antimicrobial activity of Tehran-Khojir propolis sample is in agreement with the findings of Mertzner, Bekemeier, Paintz, and Schneidewind (1979), who found that the antimicrobial of propolis can be attributed to its components as pinocembrin, galangin, pinobanksin, pinobanksin-3-acetate, para-coumaric acid, benzyl ester and caffeic acid esters. Furthermore Kujumgiev et al. (1999) found that all investigated propolis samples were active against fungal and gram-positive bacterial strains.

Table 3
Final body weight and organ to body weight ratio in Wistar rats administered 2000 mg/kg Propolis extract

Parameters	Treatment (T)	Control (C)	Blank (B)
Final body weight (g)	189.14 ± 12.5	237.25 ± 20.84	271 ± 45.36
Organ to body weight ratio (%)			
Liver	4.92 ± 0.67[a]	3.03 ± 0.35	3.04 ± 0.15
Spleen	0.39 ± 0.08[a]	0.31 ± 0.01	0.24 ± 0.02
Kidney	0.69 ± 0.02	0.66 ± 0.04	0.65 ± 0.04
Heart	0.43 ± 0.05	0.005 ±0.37	0.43 ± 0.05

Results are expressed as mean ± (SD) of 10 animals.
[a] Values significantly different by Tukey multiple comparison test ($P < 0.05$).

Table 4
Minimum inhibitory concentrations (MICs) of ethanolic propolis extract

Microorganism	MIC (μg/ml)
Spore forming G+ bacteria	
Bacillus subtilis	250
G+ bacteria	
Staphylococcus aureus	125
Staphylococcus epidermidis	125
G− bacteria	
Pseudomonas aeruginosa	500
Escherichia coli	500
Fungi	
Candida albicans	250
Aspergillus niger	500

4. Conclusions

Most recent studies have shown that some of the widely used synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisol promote development of cancer cells in rats (Frankel, 1996) and might have some undesirable effects in man too (Nakatani, 1997) then, natural antioxidants have gained popularity day by day. Consumers think that the natural food ingredients are better and safer than synthetic ones. Many of these compounds, such as plant phenolics, often exhibit anticarcinogenic, antiatherogenic and antitumor activity (Kinsella, Frankel, German, & Kanner, 1993), therefore the addition of these compounds into food products may be helpful to consumers' health and also to the stabilization of food products. Due to the presence of some of these effective compounds such as flavonoids (flavones and flavanones), phenolic acids and their esters in propolis and propolis extract, if the positive physiological properties and the non-toxicity of the propolis sample is proven it could be used as a mild antioxidant and antimicrobial preservative which not only may prolong the shelf life of some food products, but also may contribute to the health benefit of consumers.

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References

Amoros, M., Lurton, E., Boustie, J., Girre, L., Sauvager, F., & Cormier, M. (1994). Comparison of the anti-*Herpes simplex* virus activities of propolis and 3-methyl-2-butenyl caffeate. *Journal of Natural Products, 57*, 644–647.

Amoros, M., Simoes, C. M. O., Girre, L., Sauvager, F., & Cormier, M. (1992). Synergistic effect of flavones and flavonols against *Herpes simplex* virus type I in cell culture. Comparison with the antiviral activity of propolis. *Journal of Natural Products, 55*, 1732–1740.

Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*(48), 5–16, Supplement S1.

Bankova, V. S., Christov, R. S., & Delgado Tejera, A. (1998). Lignans and other constituents of propolis from the Canary Islands. *Phytochemistry, 49*, 1411–1415.

Bankova, V. S., De Castro, S. L., & Marcucci, M. C. (2000). Propolis: recent advances in chemistry and plant origin. *Apidologie, 31*, 3–15.

Bankova, V. S., Dyulgerov, A., Popov, S., & Marekov, N. (1987). A GC/MS study of propolis phenolic constituents. *Zeitschrift für Naturforschung, Teil 42C*, 147–151.

Banskota, A. H., Tezuka, Y., Adnyana, I. K., Midorikawa, K., Matsushige, K., Message, D., et al. (2000). Cytotoxic, hepatoprotective and free radical scavenging effects of propolis from Brazil, Peru, the Netherlands and China. *Journal of Ethnopharmacology, 72*, 239–246.

Bonvehi, J. S., Coll, F. V., & Jorda, R. E. (1994). The composition, active components and bacteriostatic activity of propolis in dietetics. *Journal of the American Oil Chemists Society, 71*, 529–532.

Christov, R., Bankova, V. S., Hegazi, A. G., Abd El-Hady, F. K., & Popov, S. (1998). Chemical composition of Egyptian propolis. *Zeitschrift für Naturforschung, 53C*, 197–200.

Dobrowolski, J. W., Vohora, S. B., Sharma, K., Shah, S. A., Naqvi, S. A. H., & Dandiya, P. C. (1991). Antibacterial, antifungal, antiamoebic, antiinflammatory and antipyretic studies on propolis bee products. *Journal of Ethnopharmacology, 35*, 77–82.

Ecobichon, D. J. (1997). *The basis of toxicity testing* (2nd ed.). Boca Raton, FL: CRC Press LLC, p. 49.

Frankel, E. N. (1996). Antioxidants in lipid foods and their impact on food quality. *Food Chemistry, 57*, 51–55.

Frenkel, K., Wei, H., Bhimani, R., Ye, J., Zadunaisky, J. A., Huang, M. T., et al. (1993). Inhibition of tumor promoter mediated processes in mouse skin and bovine lens by caffeic acid phenethyl ester. *Cancer Research, 53*, 1255–1261.

Ghisalberti, E. L. (1979). Propolis: a review. *Bee World, 60*, 59–84.

Grange, J. M., & Davey, R. W. (1990). Antibacterial properties of propolis (bee glue). *Journal of Royal Society of Medicine, 83*, 159–160.

Greenaway, W., May, J., Scaysbrook, T., & Whatley, F. R. (1991). Identification by gas chromatography–mass spectrometry of 150 compounds in propolis. *Zeitschrift für Naturforschung, Teil 46C*, 111–121.

Hausen, B. M., Wollenweber, E., Senff, H., & Post, B. (1987a). Propolis allergy I. Origin properties usage and literature review. *Contact Dermatitis, 17*, 163–170.

Hausen, B. M., Wollenweber, E., Senff, H., & Post, B. (1987b). Propolis allergy II. The sensitizing properties of 1,1-dimethylallyl caffeic acid ester. *Contact Dermatitis, 17*, 171–177.

Hegazi, A. G., & Abd El-Hady, F. K. (2001). Egyptian propolis: I-Antimicrobial activity and chemical composition of Upper Egypt propolis. *Zeitschrift für Naturforschung, 56C*, 82–88.

Hegazi, A. G., & Abd El-Hady, F. K. (2002). Egyptian propolis: II – Chemical composition, antiviral and antimicrobial activities of East Nile Delta propolis. *Zeitschrift für Naturforschung, 57C*, 386–394.

Isla, M. I., Nieva-Moreno, M. I., Sampietro, A. R., & Vattuone, M. A. (2001). Antioxidant activity of Argentina propolis extracts. *Journal of Ethnopharmacology, 76*, 165–170.

Kaneeda, J., & Nishina, T. (1994). Safety of propolis. Acute toxicity. *Honeybee Science, 15*, 29–33.

Kinsella, J. E., Frankel, E., German, B., & Kanner, J. (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technology, 47*, 85–89.

Kujumgiev, A., Tsvetkova, I., Serkedjieva, Y., Bankova, V., Christov, R., & Popov, S. (1999). Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. *Journal of Ethnopharmacology, 64*, 235–240.

Marcucci, M. C. (1995). Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie, 26*, 83–99.

Mertzner, J., Bekemeier, H., Paintz, M., & Schneidewind, E. (1979). Antimicrobial activity of propolis and its constituents. *Pharmazie, 34*, 97–102.

Nakatani, N. (1997). Antioxidants form spices and herbs. In F. Shahidi (Ed.), *Natural antioxidants. Chemistry, health effects, and applications* (pp. 224–244). Champaign, IL: AOCS Press.

Sforcin, J. M., Novelli, E. L. B., & Funari, S. R. C. (2002). Seasonal effect of Brazilian propolis on seric biochemical variables. *Journal of Venomous Animals and Toxins, 8*, 244–254.

Strehl, E., Volpert, R., & Elstner, E. F. (1993). Biochemical activities of propolis extracts III. Inhibition of dihydrofolate reductase. *Zeitschrift für Naturforschung, 49C*, 39–43.

Vennat, B., Arvouet-Grand, A., Gross, D., & Pourrat, A. (1995). Qualitative and quantitative analysis of flavonoids and identification of phenolic acids from a propolis extract. *Journal de Pharmacie de Belgique, 50*, 438–444.