

THE PROPHYLACTIC AND THERAPEUTIC EFFECTS OF PROPOLIS AGAINST HYPERLIPAEMIA IN ALBINO RATS

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ABSTRACT

The aim of this investigation was to study the effect of propolis phenolic extract on hyperlipaemia in rats. The propolis phenolic extract was analyzed by high performance liquid chromatography (HPLC) to 74 constituents. The major known identified phenolic compounds of propolis were caffeic acid, salicylic acid, cinnamic acid, ferulic acid and kaempferol. Propolis extract caused significantly decrease in total lipids, total cholesterol, triglycerides and LDL- cholesterol contents in serum as well as in liver. On the contrary, HDL- cholesterol was increased in normal and hypercholesterolemic rats compared with the control groups. Furthermore, gain of body weight, food intake and feed efficiency ratio were carried out. From the data, it could be deduced that the propolis phenolic constituents had a remarkable ameliorative effect against hyperlipaemia.

Keywords: propolis, Liver, Cholesterol, serum constituents.

INTRODUCTION

Propolis is sticky, gummy, resinous substance collected by honeybees from various plant sources. Bees collect propolis to seal holes in the hives, smooth out the internal walls and protect the entrance against intruders (Burdock, 1998). Propolis is in no way a new discovery. The ancient Egyptians used it to emblem their dead. In the Balkan states, propolis is still one of the most frequently used medication nowadays (Marcucci, 1995).

More than 180 propolis constituents have been identified by gas chromatography-mass spectrometry (GC-MS). These compounds can be grouped as follows: free aromatic, flavonoids, benzyl, methylbutenyl, phenylethyl, cinnamyl and other esters of these acids, chalcones and dihydrochalcones, terpenoides and others as sugars, ketones and alcohols (Greenawasy *et al.*, 1990; Bankova *et al.*, 1992 and Marcucci, 1995). Although in small quantities, these compounds are very important to propolis activity (Bankova *et al.*, 1987).

Propolis has versatile biological activities, such as antibacterial (Kujumgiev *et al.*, 1993), antiviral (Serkedjieva and Manolova, 1992), immunstimulating (Dimov *et al.*, 1991), anti-inflammatory (Strehl *et al.*, 1994), among others. Flavonoids can inhibit the various stages through to be involved in the initiation of atherosclerosis, endothelial damage, leucocyte activation, platelet adhesion, aggregation and secretion (Kedzia *et al.*, 1988).

Total levels of flavonoids in raw propolis were ranged between 19 and 25.9% (Kosalec *et al.*, 2004). Vanillin, isoferulic, caffeic acid, cinnamic acid and 3,4 dimethoxy cinnamic acid were identified in propolis phenolic extract (Walker, P. and Crane, E., 1987).

It is though to be an antitumer agent, and at possibly presents a differentiation inducing agent (Guarini *et al.*, 1992), antioxidant properties

(Sudina *et al.*, 1993), as well successful clinical applications, which has brought a greater interest in propolis effect.

In this work, we tried to evaluate the hypolipaemic activity of phenolic compounds from propolis. So, total lipids, total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol as well as gain of body weight, food intake and feed efficiency ratio were performed in normal and hypercholesterolemic rats.

MATERIAL AND METHODS

Propolis samples

Propolis was obtained from the beekeeping section of Faculty of Agricultural, Cairo University, Giza, Egypt.

Hydroalcoholic solutions of propolis

Propolis obtained was stirred and 10% ethanolic extract of propolis (EPP) were prepared (10g of propolis in 100ml of 95% ethyl alcohol). It was protected from bright light and moderately shaken at room temperature (five h /daily/ 5 days). The extract was filtered and the solvent was removed by evaporation under vacuum. The residue was dissolved in 100ml distilled water (Sforcin *et al.*, 1995).

Identification of phenolic compounds of propolis extract by HPLC

Identification of individual phenolic compounds of the propolis was performed on a Hewlett-Packard HPLC (Model 1100), using a hypersil C₁₈ reversed-phase column (250 x 4.6mm) with 5.0µm particle size. Injection by means of a Rheodyne injection valve (Model 7125).

A constant flow rate of 1.0ml/ min was used with two mobile phases:

- a) 0.5% acetic acid in distilled water at PH 2.65.
- b) 0.5% acetic acid in 99.5% acetonitrile.

The elution gradient was linear starting with (a) and ending with (b) over 35 min using an UV detector set at wavelength 534nm.

Phenolic compounds of sample were identified by comparing their relative retention times with those of the standard mixture chromatogram which are composed of hydroquinone (2.47), gallic acid (8.17) resorcinol (8.75), protocatechoic acid (10.29), hydroxybenzoic acid (14.56), chlorogenic acid (16.20), caffeic acid (16.80), vanillic acid (19.94), P- coumaric acid (20.73), ferulic acid (22.04), salicylic acid (23.27), O-coumaric acid (25.92), coumarin (26.72), apigenin (29.02), kaempferol (31.96) and cinnamic acid (33.29). the concentration of an individual compound was calculated on the basis of peak area measurements.

All chemicals and solvents used were HPLC spectral grade. Standard phenolic compounds were obtained from sigma.

Nutritional experiments

Male albino rats (32) weights (ranged between 100 and 120g) were obtained from the private market, Helwan Experimental Animal Station, Cairo, Egypt. The animals were housed individually in cages with screen bottoms and fed on a basal diet and water was available *ad-Libitum* for one week as

an adaptation period. After adaptation period, the rats were randomly divided into two main groups.

Experiment I

The first main group (16 rats) was divided into two subgroups each of 8 rats. Both of two subgroups were fed on the standard normal diet for whole experiment (30 days). The first subgroup was considered as normal control. The second subgroup of the first main group was continued to be fed on a standard normal diet and administrated by 1.0ml/ 100g b.w. of propolis extract. Food intake was recorded every two days and body weight gain weekly.

Experiment II

The second main group comprised two subgroups each of 8 rats and fed on hyperlipaemic diet rich in fat and cholesterol (Kahlon *et al.*, 1991). The second subgroup was given orally propolis phenolic extract at a dose 1.0ml/ 100g b.w. Food intake and gain of body weight were recorded. The first subgroup was continued to be fed on hyperlipaemia diet and considered as a hyperlipaemic control.

At the end of the experiment (30 days) blood samples were taken, centrifuged at 300 rpm for 20 min to obtain the sera and kept in a deep freezer at 7°C until analysis. The rats were then killed by decapitation and the liver was excised and washed with ice cold isotonic solution (0.15M KCl) and liver samples were stored at -20°C until used for biochemical analysis.

Biochemical analysis

The rat liver was individually homogenized according to the method of Radin (1981). The homogenates were used to determine total lipids and total cholesterol (Kinght *et al.*, 1972). On the other hand, total lipids (Kinght *et al.*, 1972), total cholesterol (Allan *et al.*, 1974), triglyceride (Fossati and Prencipe, 1982), HDL- cholesterol (Lops-Virella *et al.*, 1977) and LDL-cholesterol (Steinberg, 1981) were determined in serum. In addition, gain of body weigh and food intake were recorded. The obtained results were statistically analyzed according to the method described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Flavonoids are a class of aromatic compounds called polyphenols. Flavonoids are natural antioxidants and can inhibit various stages of atherosclerosis. The plasma total cholesterol and atherogenic were reduced by supplementation of 1 – 2% catechins to rats fed cholesterol containing diet (Marcucci, 1995). In this study, an attempt was made to evaluate the hypolipaemic activity of polyphenols from propolis which are listed in indigenous medicine.

Polyphenolic constituents of propolis

The polyphenolic constituents of propolis were identified by high performance liquid chromatography (HPLC) against standard compounds and the results are shown in Table (1). The identified compounds represented 11.78% of the composition of polyphenols of propolis. The lack of certain standard compounds did not allow the complete identification of the

polyphenols composition. Polyphenols extract was found to contain caffeic acid (2.93%), salicylic acid (2.11), cinnamic acid (1.65%), kaempferol (1.41%) and ferulic acid (1.01%) as the major identified compounds, hydroquinone, resorcinol, hydroxybenzoic acid, vanillic acid, p-coumaric acid, chlorogenic acid, o - coumaric acid and apigenin are present as minor compounds, while gallic acid, protocatechoic acid, phenol and coumarine occurred as trace compounds.

Table (1): Polyphenols content (%) of propolis.

Component	RT *	%
Hydroquinone	2.47	0.73
Gallic acid	8.17	0.06
Resorcinol	8.75	0.13
Protocatechoic acid	10.29	0.03
Catechol	12.95	0.07
Hydroxybenzoic acid	14.56	0.12
Chlorogenic acid	16.20	0.27
Caffeic acid	16.80	2.91
Phenol	17.57	0.08
Vanillic acid	19.74	0.15
P. Coumaric acid	20.73	0.34
Ferulic acid	22.04	1.01
Salicylic acid	23.27	2.11
O - Coumaric acid	25.92	0.51
Coumarin	26.76	0.06
Apigenin	29.02	0.14
Kaempferol	31.96	1.41
Cinnamic acid	33.29	1.65
Identified compounds**		11.78
Unidentified compounds***		88.22

*RT: refers to retention time.

**Number of known compounds = 18 compounds.

***Number of unknown compounds = 56 compounds.

In addition, unknown phenolic compounds were also identified (88.22%) and listed in Table (2). Our results are in accordance with Greenaway *et al.*, (1988) who found the same composition of polyphenolic compounds of propolis especially isoferulate esters.

Gain of body weight, food intake and feed efficiency ratio of normal and hypercholesterolemic rats

The results in Table (3) show that rats fed on basal diet containing propolis phenolic extract did not caused any significant alteration in gain body weight and food intake. Feed efficiency ratio of the normal control was similar to that of propolis phenolic extract treatment.

The gain in body weight, food intake and feed efficiency ratio of hypercholesterolemic rats are shown in Table (3). Propolis phenolic extract did not caused any significant increase in gain body weight, food intake being about the same as in hypercholesterolemic control. In addition, hypercholesterolemic rats fed on propolis phenolic extract had the same feed efficiency ratio (16.94) to that of the control B. In general, the feed efficiency ratio for the normal rats was higher than that found in hypercholesterolemic.

Table (2): The retention time of unidentified phenolic constituents of propolis extract.

Rt	Constituent	Area%	Rt	Constituent	Area%
12.36	Unknown	0.15	32.55	Unknown	0.53
13.36	Unknown	0.03	34.50	Unknown	0.58
13.63	Unknown	0.18	35.47	Unknown	4.27
14.97	Unknown	0.09	36.16	Unknown	1.35
15.61	Unknown	1.17	36.77	Unknown	0.84
16.02	Unknown	0.13	37.41	Unknown	2.14
17.82	Unknown	0.05	38.03	Unknown	1.53
18.02	Unknown	0.09	38.83	Unknown	0.08
18.33	Unknown	0.11	39.72	Unknown	0.04
18.61	Unknown	0.39	40.03	Unknown	1.62
19.37	Unknown	0.12	40.83	Unknown	2.15
20.32	Unknown	2.59	41.83	Unknown	0.23
21.16	Unknown	0.41	42.19	Unknown	2.91
22.88	Unknown	0.34	42.91	Unknown	0.03
23.88	Unknown	0.33	43.19	Unknown	0.45
24.36	Unknown	0.33	43.49	Unknown	1.51
24.70	Unknown	0.14	44.05	Unknown	24.78
24.97	Unknown	0.16	45.61	Unknown	10.36
25.30	Unknown	0.21	46.36	Unknown	3.62
27.23	Unknown	0.04	46.83	Unknown	3.53
27.64	Unknown	0.02	47.19	Unknown	0.73
28.15	Unknown	2.10	47.84	Unknown	0.63
28.54	Unknown	0.29	48.27	Unknown	0.06
29.40	Unknown	0.05	48.54	Unknown	0.37
29.72	Unknown	0.28	49.12	Unknown	1.65
30.26	Unknown	0.05	49.74	Unknown	10.95
30.69	Unknown	0.21	50.42	Unknown	0.29
31.47	Unknown	0.56	50.99	Unknown	0.30

*Rt: refer to retention time.

Table (3): Influence of propolis phenolic extract on gain in body weight, food intake and feed efficiency ratios* of normal and hypercholesterolemic rats.**

Treatment	Normal rats			Hypercholesterolemic rats		
	Gain in body weight (g)	Food intake (g)	Feed efficiency ratio	Gain in body weight (g)	Food intake (g)	Feed efficiency ratio
Control A*	108.90 ± 0.58	587.17 ± 7.42	18.55 ± 0.26	---	---	---
Control B**	---	---	---	92.89 ± 4.11	576.42 ± 2.07	16.10 ± 0.52
Phenolic extract (1.0ml)	117.51 ± 2.49	615.36 ± 14.24	19.10 ± 0.52	101.61 ± 1.96	599.76 ± 3.03	16.94 ± 0.61
L.S.D. (0.05)	6.55	32.04	1.16	9.09	7.32	1.60

*Control A: indicates normal rats fed on standard normal diet during the whole experimental period.

**Control B: represents the hypercholesterolemic rats fed on standard normal diet during experimental period.

*** Feed efficiency ratio was calculated from the equation, Gain in body weight / Food intake x 100.

Table (4): Influence of propolis phenolic extract on total lipids, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol levels in serum of normal and hypercholesterolemic rats.

Treatment	Normal rats						Hypercholesterolemic rats					
	Total lipids	Total cholesterol	Tri-glyceride	HDL - cholesterol	LDL- cholesterol	Riske value**	Total lipids	Total chol-esterol	Triglyceride	HDL-Cholesterol	LDL-cholesterol	Riske value***
Control A*	450.6 ± 2.41	129.76 ± 1.29	155.6 ± 2.74	66.14 ± 0.79	36.81 ± 0.11	1.96	286.53 ± 7.79	560.72 ± 5.36	210.31 ± 1.56	59.16 ± 0.49	92.65 ± 0.78	9.48
Control B**	---	---	---	---	---	---	188.61 ± 3.69	420.32 ± 2.71	157.16 ± 1.06	81.61 ± 0.23	46.82 ± 0.68	5.15
Propolis extract (1.0ml)	380.95 ± 3.76	101.61 ± 0.8	123.71 ± 0.75	75.22 ± 0.45	21.36 ± 0.37	1.35	17.2	11.99	3.76	1.07	2.07	---
L.S.D. (p,0.01)	8.48	3.04	5.67	1.81	0.77	---	---	---	---	---	---	---

The values are means ± S.D. for 8 rats.

* Control A: indicates normal rats fed on standard normal diet during the whole experimental period.

**Control B: represents the hypercholesterolemic rats fed on standard normal diet during the whole experimental period.

*** risk values are means total cholesterol / HDL-cholesterol..

Effect of polyphenols of propolis on levels of lipid fractions in serum and liver rats

Table (4) shows the levels of total lipids, total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol of normal and hypercholesterolemic rats (mg/100ml) fed on hyperlipaemic diet. It was found that the rats received the hyperlipaemic diet showed a significant ($p < 0.5$) increase in serum total lipids, triglycerides, total cholesterol and the risk value (total cholesterol / HDL-cholesterol) compared with the normal control. These results are in accordance with those obtained by Sforcin *et al.* (2002).

The administration of propolis phenolic extract with normal and hyperlipaemic rats induced a significant decrease in serum total lipids, total cholesterol, triglycerides and the risk ratio. also, HDL-cholesterol showed elevated levels, while, the LDL-cholesterol values fraction showed a significant reduction. These results are in accordance with Kedzia *et al.* (1988) who observed blood pressure reduction in rats after propolis ingestion suggesting its beneficial role in preventing atherogenesis. Frankiewicz and Scheller (1984) observed normal concentration of cholesterol and other constituents after treating elderly patients with propolis capsules.

The prophylactic effect against hypertriglyceridemia, hypercholesterolemia and risk ratio values were pronounced in the groups of rats received propolis phenolic extract.

The effect of propolis phenolic extract on total lipids and total cholesterol contents in liver of normal and hypercholesterolemic rats is shown in Table (5). The hyperlipaemic diet caused a significant ($P < 0.05$) elevation in total lipids and total cholesterol compared with normal control. These data proved that the hyperlipaemic diet caused a significant effect on liver tissues leading to liver disfunction (Eskander *et al.*, 1995). The administration of propolis extract caused significant decrease in total lipids and total cholesterol in the liver of both normal and hyperlipaemic rats during 30 days experimental period. In general, propolis seems to be the potent agent, whose prophylactic effect against hyperlipaemia was pronounced.

Table (5): influence of propolis phenolic extract on total lipid and total cholesterol content (mg/g) in liver of normal and hypercholesterolemic rats.

Treatment	Normal rats		Hypercholesterolemic rats	
	Total lipids	Total cholesterol	Total lipids	Total cholesterol
Control	230.17 ± 2.79	30.11 ± 0.52	301.76 ± 0.72	48.97 ± 0.60
Phenolic extract (1.0ml)	192.87 ± 0.65	25.67 ± 0.36	210.67 ± 0.19	26.71 ± 0.46
L.S.D. (0.05)	7.95	1.75	2.06	2.08

-The values are means ± S.D. for 8 rats.

These results may be attributed to be remarkable effect of propolis on regulating lipid metabolism of normal and hypertensive rats. On the other hand, caffeic acid is an active component in propolis extract, shows antioxidant properties. The antioxidants were found to reduce serum triglycerides and increases the percentage of HDL-cholesterol in rats (Sudina *et al.*, 1993).

Atherosclerosis results from the accumulation of LDL in the arterial wall and from the cellular response of wall components to injury. Oxidative modification of LDL is a key early event in the pathogenesis of atherosclerosis (Steinberg *et al.*, 1989). Several dietary flavonoids have been shown to lower LDL levels and inhibit the oxidative modification of LDL in vitro (Kwiterovich, 1997 and Catapano, 1997) and hence have the potential to reduce LDL oxidation and atherogenesis in vivo.

On reviewing the results, it can be summarized that the phenolic compounds from propolis offer promising therapeutic value in preventing advancement of atherosclerosis and related cardiovascular anomalies, by inhibiting cholesterol synthesis and alleviating hyperlipaemia.

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التأثيرات العلاجية والوقائية للبروبوليز ضد حالات ارتفاع دهنيات الدم في الفئران البيضاء

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يهدف هذا البحث إلى دراسة تأثير المستخلص الفينولي للبروبوليز على مستوى الدهون في الفئران السليمة والفئران المصابة بارتفاع مستوى الدهون في الدم . وقد لوحظ أن هذا المستخلص سبب انخفاض معنوي في مستوى الدهون الكلية والكوليسترول والتراي جليسريد والليوبروتين المنخفض للكثافة LDL كذلك أدى إلى ارتفاع في مستوى الليوبروتين المرتفع الكثافة HDL في دم الفئران السليمة والمصابة . كما أدى إلى انخفاض معنوي في محتوى الكبد من الدهون الكلية والكوليسترول.

وعند تحليل المستخلص الفينولي بجهاز تحليل الكروماتوجرافي الغازي العالي الكفاءة HPLC وجد أنه يحتوي على أحماض الكافيك والسالسليك والسيناميك والفريوليك والكايامبيغول كموايد فينولية رئيسية والتي قد يرجع إليها هذا التأثير .

لذلك توصي هذه الدراسة بضرورة التفتيش على منتج البروبوليز للوقاية وعلاج حالات زيادة مستوى الدهون في الدم وبالتالي الوقاية من الإصابة بمرض تصلب الشرايين .