Study on the antioxidant properties of pollen extracts

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The study on the antioxidant and hyolipidemic effect of pollen extracts (Cernitins) was conducted in male mongrel rabbits and Wistar rats. The animals were fed a high-fat diet (HFD) composed of cholesterol, coconut oil and cholic acid, and received pollen extracts (Cernitins) orally (the rabbits over a period of 12 weeks and the rats over a period of 2 weeks). The levels of malondialdehyde (MDA) as an indication of the degree of peroxidation and lipids (cholesterol, triglyceride, separation of lipoproteins into fractions) were measured.

The study demonstrated the reduction of MDA concentrations under the influence of Cernitins, suggesting their antioxidant properties. Total cholesterol and triglyceride content was also decreased.

During the last ten years there have been reports on the toxicity of oxygen and oxygenated free radicals. The enzyme prostacyclin synthese is very sensitive to inhibition by lipid peroxides[10], which also stimulate arachidonic acid release from phospholipids[6] and thereby possibly enhance platelet thromboxane A_2 (TXA_2) formation. Lipid peroxides are present in many tissues, especially in atherosclerotic plaques[5] and possibly in hyperlipidemia. Moreover, it has been established that excessive lipid peroxidation occurs during the aging proceess[12].

The present study included an examination of lipid peroxidation in hyperlipidemic animals under the influence of pollen extracts. Malondialdehyde (MDA), a product of reduction during the oxidative process, was measured as an indicator of the degree of peroxidation.

The pollen extracts (Cernitins), supplied by AB Cernelle, Vegeholm (Sweden) contained mainly water soluble substances (Cernitin T60) and fat soluble components (Cernitin GBX). It has earlier been demonstrated that Cernitins have a remarkable lipid lowering effect, both in animals[1] and in humans[4]. In addition to this, it was established that they have a beneficial effect against the development of atherosclerosis[15].

Materials and Methods

The study was carried out on 30 male mongrel rabbits, with an initial body weight of 3.0-3.8 kg, and 30 male Wistar rats, with an initial body weight of 220-260 g. The animals were fed a standard basic diet, and were divided into three equal random groups: group 1 – control; group 2 – fed HFD; group 3 – fed HFD + pollen extracts (Cernitin T60 50 mg per 24 hrs + Cernitin GBX 10 mg per kg per 24 hrs) orally.

The HFD consisted of the following doses in grams per kg per 24 hrs: cholesterol – rabbits 0.5, rats – 4.0; hydrogenated coconut oil – rabbits 1.0, rats 10.0; cholic acid – rabbits 0.1, rats 0.2.

The experiment was conducted over a period of 12 weeks for rabbits and 2 weeks for rats. On the last day of the experiment the animals were fasted for 18 hrs, and blood samples were taken for biochemical analysis.

MDA (standard: 1-1-3-3-tetramethoxy-propane, supplied by Fluka AG) was measured using the technique described by Stuart and others[13]. The total cholesterol was assayed using a method based on the LIBERMANN-BURCHARDT reaction[1], and triglyceride level was determined by the technique described by Eggstein and Kreutz[3]. Lipoproteins were separated into fractions by agarose electrophoresis[4].

The results were analyzed statistically using Duncan's test.

Results

The MDA concentration in the plasma of the rabbits in group 2 (fed HFD) was markedly higher (Table 1), showing an increase from 2.89 nmol/ml (control group) to 8.04 nmol/ml (i.e. by 372%). The addition of Cernitins to the diet produced a significant drop in the MDA concentration compared with that in the plasma of rabbits in group 2.

In the blood serum of rabbits fed with the HFD, the total cholesterol level was increased by 579%, while the level of triglyceride remained practically unchanged (Table 2). Only two fractions were separated by lipoproteins electrophoresis: practically pre-β and β-fractions remained unseparable. The percentage content of α-lipoproteins in the rabbits in group 2 was considerably reduced. In group 3, the increase in serum cholesterol was markedly and significantly suppressed, while the α-lipoprotein content was increased.

The MDA concentration was distinctly higher in rats in group 2 fed HFD as compared with those in group 1 (Table 3). The MDA concentration in rats in group 3 was significantly lower than that in rats in group 2.

An equally significant increase in the cholesterol level (428%) and in the triglyceride level (116%) was noted in the serum of rats in group 2 fed HFD (Table 4). Electrophoretic separation of lipoproteins revealed a suppression of the percentage content of the z-fraction. The addition of Cernitins to the HFD resulted in a significant reduction in the levels of cholesterol and triglycerides in the serum, and a marked increase in the percentage content of z-lipoproteins.

Table 1. Concentration of malondialdehyde (MDA) in the blood plasma of rabbits (mean ± SE)

Group		MDA	
		nmol/ml	nmol/10^9 platelets
1		2.60 ± 0.15	3.92 ± 0.13
2		12.27 ± 0.82	23.93 ± 2.90
3		9.30 ± 0.37	15.26 ± 1.59
P	1/2	< 0.001	< 0.001
	2/3	< 0.01	< 0.05

Table 2. Cholesterol (CH) and triglyceride (TG) levels in blood serum of rabbits, and electrophoretic separation of lipoproteins into fractions (mean ± SE)

Group		CH (nmol/l)	TG (nmol/l)	Lipoproteins (%)	
				α	pre-β + β
1		2.60 + 0.23	0.98 ± 0.09	57.33 + 3.10	42.67 ± 3.10
2		32.60 ± 4.48	1.06 ± 0.06	7.73 ± 1.26	92.27 ± 1.26
3		10.63 ± 3.79	0.79 ± 0.08	21.73 ± 6.22	78.27 ± 6.22
P	1/2	< 0.001	> 0.7	< 0.001	< 0.001
	2/3	< 0.01	> 0.05	< 0.05	< 0.05

Table 3. Concentration of malondialdehyde (MDA) in blood plasma of rats (mean ± SE)

Group		MDA	
		nmol/ml	nmol/10^9 platelets
1		2.89 ± 0.20	5.94 ± 0.32
2		8.04 ± 0.30	17.36 ± 0.38
3		5.29 ± 0.40	11.73 ± 0.69
P	1/2	< 0.001	< 0.001
	2/3	< 0.001	< 0.001

Table 4. Cholesterol (CH) and triglyceride (TG) levels in blood serum of rats, and electrophoretic separation of lipoproteins into fractions (mean ± SE)

Group		CH (nmol/l)	TG (nmol/l)	Lipoproteins (%)	
				α	pre-β + β
1		1.28 ± 0.16	1.25 ± 0.16	51.42 ± 3.92	48.58 ± 3.92
2		6.76 ± 0.62	2.70 ± 0.35	21.68 ± 2.45	78.32 ± 2.45
3		3.73 ± 0.29	0.75 ± 0.10	34.20 ± 3.40	65.80 ± 3.40
P	1/2	< 0.001	< 0.01	< 0.001	< 0.001
	2/3	< 0.001	< 0.001	< 0.01	< 0.01

Discussion

The antioxidant hypothesis assumes that health and recovery involves protection against the free radical injury which may be caused by endogenous oxygen radicals, by exogenous radicals or by secondary radicals propagated as a result of the chain reaction of polyunsaturated fatty acid peroxidation.

The MDA concentrations detected in our experiment show that an increase in lipid peroxidation occurs in animals suffering from hyperlipidemia when compared with controls. The reduction of the MDA concentrations under the influence of pollen extracts suggests that Cernitins are an effective means of reducing lipid peroxidation, i.e., that they have antioxidant properties.

MDA concentrations seem to be produced by the action of the cyclooxygenase. Since MDA is also one of the principal products of the breakdown of the endoperoxides, its measurement offers a simple method of assessing the function of this enzyme. TXA_2 formation occurs in equimolar amounts with that of MDA. TXA_2 is an active vaso-constrictor and platelet aggregating agent. It aggregates platelets via a direct process[9], and causes them to release adenosine diphosphate, which is also a potent aggregating agent[2]. Furthermore, the MDA concentration in plasma is probably relative to the MDA concentration in arterial walls, and lipid peroxidation plays a role in the production of atheromatous plaques and arterial tissue injuries[8].

Although platelet aggregation and lipid peroxidation are not synonymous, the events which lead to the release reaction appear to be accompanied by the generation of free radicals and the peroxidation of lipids. Pollen extracts may block this phenomenon, either by direct enzymatic inhibition of the conversion of arachidonic acid to labile aggregation stimulating substances or intermediary endoperoxides, or by restructuring the fatty acid so that it is rendered impervious to peroxidation.

A number of recent publications have dealt with the possible role of lipid peroxidation in the process of atherosclerosis[8]. Peroxidation involves reduction of molecular oxygen to H_2O with intermediate free radicals, particularly toxic ones. Free radicals may

react with nucleic acids, proteins, polysaccharides and lipids. In lipid peroxidation, these radicals react with unsaturated fatty acid to produce endoperoxides, which are very active substances with cytotoxic properties. Peroxidation can occur as the result of inflammatory or degenerative processes. Atherosclerosis leads to hypoxia of the arterial walls, which is accompanied by inflammation.

The results of this study support our earlier experiments on the significance of pollen extracts in the treatment of lipid metabolism disturbances[11.14.15.] and our clinical studies on the inhibition of platelet aggregation by Cernitins[7]. They explain, and to some extent clarify, the mechanism of beneficial action in the management and prophylaxis of atherosclerosis.

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Received in November 1986