Clinical Evaluation of Cernilton as Lipid-Lowering Agent

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Cernilton® - a pollen preparation (AB Cernelle, Sweden) is composed of the following constituents (in 1 tablet): Cernitin T60 *(Extr. Pollin. sicc.) 60* mg and Cernitin GBX *(Extr. Pollin. dialys.) 3* mg.

Numerous studies proved the effectiveness of pollen in patients with chronic prostitis [10, 19]. Cernilton removes the oedema of the urethreal mucose surface from the bladder neck to the external sphincter, and in consequence improves urination [27]. Anti-inflammatory properties of Cernitin were shown as well [15]

Dubrisay [11] reported quite interesting results, concerning usefulness of substances prepared from pollens in geriatric patients.

Taking into account the above mentioned clinical data and the results of experimental studies [25, 26] we decided to perform investigations on the significance of Cernilton in subjects with hyperlipidemia. A search for new drugs with greater efficacy and safety continues because now available are not ideal hyperlipidemia drugs.

Materials and Methods

Twenty eight patients (24 males and 4 females) with mean age 44 (range 21-62) entered and completed the study. In all cases hyperlipidemia was diagnosed: 15 patients were classified as having type IV, 3 type IIA and 10 type IIB. No patient suffered from secondary hyperlipoproteinemia due to renal disease, myxedema, diabetes mellitus or liver disease. Serum lipoprotein electrophoresis had been performed on agarose gel to classify hyperlipidemia into Fredrickoson's types.

Patients underwent a complete physical examination including blood pressure, heart rate, x-ray of the chest, and detailed laboratory investigations: ECG, complete blood count, urinaanalysis, as well as bilirubin, urea, creatinine and uric acid concentration in the blood serum and activity of enzymes (SGOT, SGPT, alkaline phosphatase).

Patients were chosen among those, who had not responded to dietary management. They were instructed to continue their diet throughout the study.

Cernilton was given orally 1 tablet three times daily before meals over 1 month. The data reported are based on a comparison between the results of analyses on entry into the trial and after 1 month of management.

The treated patients were divided into two groups. Group 1 included 15 patients with hyperlipidemia that was not controlled pharmacologically previously. In the subjects the following determinations were carried out: in the blood serum - total lipids, triglycerides, total cholesterol, time of fibrinolysis in euglobulins, soluble complexes of fibrine monomers, fibrinogen, platelet aggregation, separation of proteins; and in urine - 17-ketosteroids. Group 2 contained 13 patients with hyperlipidemia resistant to clofibrate. They had been treated previously with clofibrate in a dose *1.5* g daily for 1 month without any effect on the blood lipid level. In the blood serum of these patients total lipids, triglycerides, total cholesterol, phospholipids and free fatty acids were assayed.

Total lipids were determined according to Zöllner and Kirsch [28], triglycerides by the method of Eggstein and Kreutz [12], total cholesterol after Blaszczyszyn [2], free fatty acids according to Duncombe [9] and phospholipids by the method of King and Wootton [16]. Time of fibrinolysis and fibrinogen concentration was measured after Niewiarowski [20], while the index of soluble complexes of fibrine monomers was calculated to according Lipiński et. al. [18].

The platelet aggregation was tested using an Elvi 840 apparatus with the method of Born [3]. 55 μM solution of ADP in the volumes of 3-50 μl was added to the platelet rich plasma (containing 200-400 thousands of platelets in 1 mm^3). Besides, ADP induced aggregation under the influence of Cernitin T60 was determined in *vitro.* 1, 5 and 10% solutions of Cernitin T60 were used.

Urinary 17-ketosteroids were detected according to Callow-Callow, as modified by Kandrac [17). Blood samples were drawn in the morning after 12h of fasting. Significance of the mean differences between the individual values were estimated with Student's t-test.

Results

Effects on serum lipids

The results of one month's treatment with 3 tablets of Cernilton daily are summarized in Tables 1 and 2. Considering all the patients treated (two groups) the positive response to Cernilton was noted in 22 patients among 28 persons receiving the drug. Triglycerides decreased by 49%.

In group 1 (Table 1) normalization of lipid fractions occurred in 5 patients, while improvement was shown in 8 patients. Mean of total lipids level decreased by 21% ($p<0.0l$), while triglycerides concentration was lowered by 32% ($p<0.0l$).

In group 2 (Table 2), i.e. in patients with hyperlipidemia resistant to clofibrate, 1 patient revealed normalization and further 8 patients showed improvement. Mean of triglycerides

level was diminished by 32% *(p<0.05),* as compared with the initial value. Total lipids decreased insignificantly by 14% (p>0.1) and total cholesterol was unchanged.

Other Clinical Effects

Mean of fibrinolysis (Table 3) was significantly shortened (time from 180 to 129 min), by 29% (p<0.01). We observed depression of the fibrinogen concentration but the difference was insignificant.

Platelet aggregation (Table 4) expressed by aggregation speed in degrees was insignificantly decreased by 13% (p>0.05) after 1 month of Cernilton administration, the remaining parameters describing platelet aggregation were unchanged.

Table 1. Effect of Cernilton on total lipids, triglycerides and total cholesterol in-patients with hyperlipidemia (group 1)

Number of patient	Total lipids (g/l)		Triglycerides (mM/l)		Total cholesterol (mM/l)	
	I	II	I	II	I	II
1	19.37	10.72	2.48	3.70	7.24	8.12
2	10.78	7.40	2.83	2.29	6.67	4.91
3	6.90	7.45	4.79	3.75	5.27	6.39
4	13.00	8.40	3.09	1.23	6.33	4.78
5	8.75	8.88	4.19	2.80	6.46	5.43
6	10.68	10.68	3.42	3.03	5.95	5.82
7	14.20	11.41	2.11	1.73	9.05	6.46
8	14.34	12.00	5.53	3.05	8.27	7.24
9	15.65	15.83	7.65	5.53	5.82	5.69
10	10.00	8.00	3.15	0.82	5.04	5.82
11	15.45	13.21	1.85	1.20	7.55	9.98
12	13.18	11.22	4.42	3.38	4.65	6.34
13	14.20	9.92	2.23	1.64	8.40	8.02
14	11.25	11.00	3.07	2.04	9.57	7.50
15	16.59	9.00	9.62	2.13	5.17	3.88
Mean	12.96	10.34	4.03	2.55	6.76	6.43
± SD	3.26	2.30	2.17	1.24	1.53	1.55
P I/II	< 0.01		< 0.01		> 0.3	

I= Initial value, II= after 1 month of treatment

Table 2. Effect of Cernilton on total lipids, triglycerides, total cholesterol, phospholipids and free fatty acids in patients with hyperlipidemia resistant to clofibrate (group 2).

Number of Patient	Total lipids (g/l)		Triglycerides (mM/l)		Total cholesterol (mM/l)		Phospholipids (mM/l)		Free fatty acids (µM/l)	
	I	II	I	II	I	II	I	II	I	II
16	14.18	15.56	6.61	3.56	8.07	8.66	3.35	4.17	526	598
17	38.89	37.30	26.68	25.65	14.12	15.52	8.98	8.72	706	625
18	11.83	10.06	3.65	3.26	5.69	7.14	3.96	2.94	647	860
19	36.90	19.65	33.40	13.91	6.13	5.90	4.55	3.57	522	510
20	13.43	9.44	6.02	2.30	6.34	5.87	4.32	4.30	625	820
21	28.43	21.27	115.05	8.49	10.86	9.62	5.08	6.67	833	1136
22	20.21	12.05	13.11	2.39	7.40	7.99	5.17	4.37	536	413
23	15.81	13.51	6.48	3.53	7.86	7.47	4.49	3.59	450	756
24	13.63	15.05	6.50	5.30	6.54	7.50	5.38	6.23	370	410
25	28.60	27.40	23.03	23.09	7.40	8.20	5.01	3.75	720	826
26	11.33	14.05	5.15	5.36	6.36	6.21	3.90	3.73	381	352
27	9.09	11.80	3.71	3.71	7.60	8.92	3.87	4.41	560	425
28	10.06	10.63	3.26	3.93	7.14	6.47	2.94	4.31	860	550
Mean	19.41	16.75	11.74	8.03	7.80	8.11	4.69	4.67	595	657
± SD	10.39	8.00	9.96	7.90	2.29	2.51	1.47	1.59	156	220
P I/II	> 0.1		< 0.05		> 0.2		> 0.9		> 0.6	

I= Initial value, II= after 1 month of treatment

Table 3. Time of fibrinolysis, soluble complexes of fibrine monometers, and fibrinogen level

Number of Patient	Time of fibrinolysus (min)		Soluble complexes of fibrine monomers (index)		Fibrinogen (mg/dl)	
	I	II	I	II	I	II
1	160	120	1.7	1.1	450	270
2	240	100	7.0	1.2	400	370
3	240	110	9.0	6.4	370	450
5	135	120	1.6	7.4	340	270
6	180	120	1.0	1.2	270	290
7	180	120	1.0	0.8	400	370
8	120	120	0.6	9.3	340	320
9	150	120	0.9	1.0	370	370
11	120	150	0.9	1.1	320	320
12	150	150	0.7	1.2	450	340
13	300	210	0.8	0.7	450	500
14	180	120	0.6	1.2	470	340
15	180	120	1.2	1.1	240	290
Mean	180	129	2.1	2.6	375	346
± SD	53	28	2.7	3.0	72	68
P I/II	< 0.01		> 0.6		> 0.2	

I= Initial value, II= after 1 month of treatment

ADP induced platelet aggregation was diminished under the influence of Cernitin T60 solutions added to platelet rich plasma. The reduction of aggregation was observed after 5% Cernitin T60 solution had been used (Fig. 1). In control experiment (without Cernitin) maximal aggregation amounted to 40%. Speed of aggregation was 65º**,** and aggregation after 2 mm amounted to 40%. After Cernitin T60 had been added as 5% solution in the volume of 50 µl, the mentioned parameters were as follows: maximal aggregation –35%, speed of aggregation –60**°** and aggregation after 2 mm—30%.

The platelet aggregation was abolished almost completely after 10% solution of Cernitin had been added, in the volume of 50 µl to the platelet rich plasma (Fig. 2). In control the parameters were as follows: maximal aggregation—55%, speed of aggregation—70% and aggregation after 2 mm—55%.

Urinary 17-ketosteroids (Table 5) increased from 60.6 to 82.8 µM/day i.e. by 37% ($p>0.05$) in patients receiving Cernilton.

Total protein level and separation of proteins into fractions did not alter when comparing initial values with results obtained after 1 month of treatment with Cernilton. There was no effect on blood pressure and heart rate. Another laboratory tests: blood counts, urinaanalysis, bilirubin, urea and uric acid as well as activity of enzymes (SGOT, SGPT, alkaline phosphatase) were unchanged in the course of the trial.

The Cernilton therapy was very well tolerated by all patients without any undesirable side-effects.

Table 4. Platelet aggregation

Number of Patient	Aggregation speed in degrees		Aggregation in 2 min (%)		Aggregation in I phase (%)		Aggregation in II phase (%)		Threshold of aggregation (μM)	
	I	II	I	II	I	II	I	II	I	II
1	80	73	40	42	44	32	97	57	1.5	2.0
2	56	60	25	21	13	14	42	47	0.5	1.0
3	50	50	7	7	12	12	55	55	0.5	0.5
5	70	52	38	35	21	17	53	45	1.5	1.0
6	44	44	16	24	15	17	40	34	0.5	1.0
9	68	75	25	52	20	40	45	80	2.0	1.5
11	76	64	25	43	25	19	47	60	0.3	0.5
12	76	44	38	37	14	20	73	60	0.3	0.5
13	62	58	37	32	27	15	75	40	0.5	0.5
14	71	44	40	10	26	13	50	35	1.5	0.5
15	71	72	37	50	40	33	52	58	1.0	1.0
Mean	66	58	30	32	23	21	55	52	0.9	0.9
± SD	12	12	11	15	11	9	12	12	0.6	.5
P I/II	> 0.05		> 0.6		> 0.4		> 0.6		> 0.9	

I= Initial value, II= after 1 month of treatment

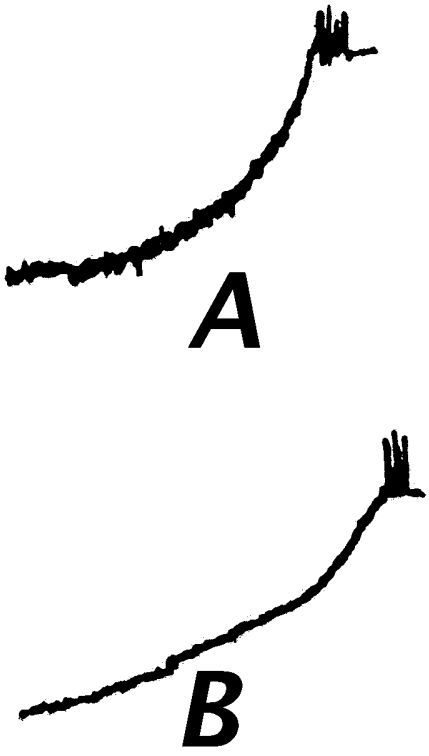

Fig.1. ADP introduced platelet aggregation *in vitro* and influence of 5% solution of Cernitin T60 (B) in comparison with control (A).

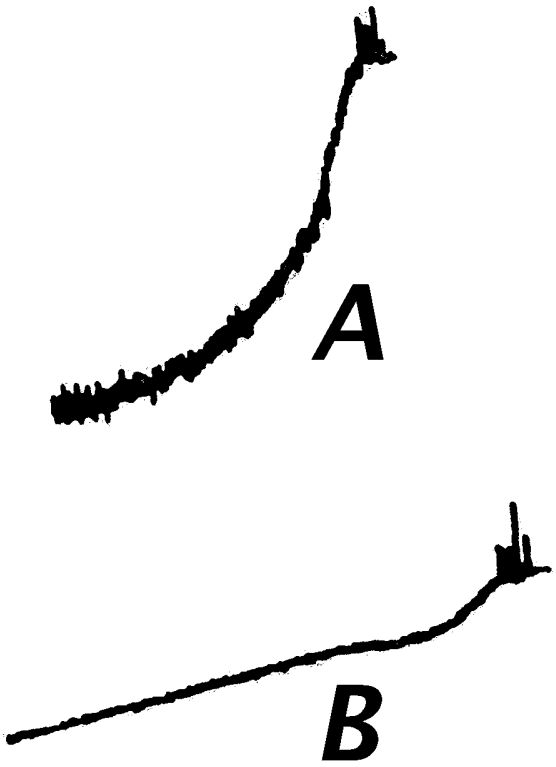

Fig.2. ADP induced platelet aggregation *in vitro* and the influence of 10% solution of Cernitin™ T60™ (B) in comparison with control (A).

Table 5. Elimination of 17 – ketosteroids with urine

Number of patient	Elimination of 17 – ketosteroids (μM/day)	
	I	II
1	111.65	83.65
2	116.55	146.65
3	51.45	116.55
4	17.15	123.55
5	61.60	61.60
6	142.10	109.20
7	16.10	31.15
10	129.85	123.55
11	14.70	66.50
12	11.90	44.80
13	19.25	29.05
14	51.45	109.20
15	43.40	30.80
Mean	60.60	82.80
± SD	48.00	41.20
P I /II	> 0.05	

I= Initial value, II= after 1 month of treatment

Discussion

Our present clinical studies support earlier performed by us experimental investigations on the significance of pollen extract for lipid metabolism disturbances [25, 26]. Data obtained now indicate, that Cernilton is effective in lowering serum triglyceride level, even in the cases of hyperlipidemia resistant to clofibrate. Moreover, in patients receiving Cernilton the activity of the fibrinolytic system is significantly increased. Besides, tendency towards decrease of fibrinogen concentration in the blood, as well as depression of platelet aggregation can be demonstrated.

Enhanced "spontaneous" aggregation has been found in diabetics and in patients, who later had myocardial infarction of thromboembolism [4, 5] Platelets of patients with diabetes, hyperlipoproteinemia and atherosclerosis quite often show an increased sensitivity to aggregating agents [7, 8, 22].

On the other hand, non-steroidal anti-inflammatory drugs are reported to have inhibitory effects on platelet aggregation [141. Taking into account an anti-inflammatory [12], as well as lipid lowering properties of Cernitin, the relationship between Cernitin and platelet aggregation could be assumed.

Inhibition of platelet aggregation by Cernitin T60 has been revealed by us *in vitro.* Considering concentrations of the preparation (5 and 10%) showing such an inhibition, it is to be noticed, that there are many components included: amino acids, vitamins and microelements. When applying for example 5% solution of Cernitin, 0.4% solution of amino acids is being used.

Clinical implication of the obtained results should be taken into account [10]. Importance of those observations is underlined by the reports focused on the relation between atherosclerosis and hyperlipoproteinemia. The association between elevated serum lipid levels and

increased incidence of atherosclerotic disease has been recognized by both epidemiologic and clinical investigations [1, 4]. Recent reports having the association of not type II, but type IV hyperlipoproteinemia (hypertriglyceridemia) with atherosclerotic coronary artery disease [1,4]. Lowering serum lipids may reduce the incidence of atherosclerotic disease. There is also growing evidence, that the changes in the state of the blood such, as increased in hyperlipoproteinemia, fibrinolytic activity [18] and fibrinogen [16] may influence the degree of the consequences of the vascular lesion, and their control may be therapeutically useful.

Cernilton was useful in a clinical trial conducted by the double-blind technique and involving elderly patients [11]. All the patients were suffering from physical and mental asthenia with severe anorexia and loss of weight. Appetite was restored and weight increased. Both physical and mental asthenia disappeared. Biological tests revealed a slight rise in blood protein level and a marked rise in urinary 17-ketosteroid and 17-hydroxysteroid levels which suggest stimulation of adrenocortical secretion.

Significance of our findings can be stressed by the fact, that clofibrate—the basic hypolipidemic agent having been widely used over the last years, can not be recommended as a lipid lowering drug for community-wide primary prevention of ischemic heart disease [21].

There were no complaints, adverse effects or refusal to take Cernilton tablets. Since hypolipidemic drugs may need to be taken for the life of the patients the frequence and type of adverse are important.

In conclusion, Cernilton—preparation obtained from the pollens, should be considered as a drug recommended for prevention and treatment of atherosclerosis.

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