Further Studies on Cernitins: Screening of the Hypolipidemic Activity in Rats

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Our previous studies showed that microbiologically fermented pollen extracts in the form of Cernitin reduced the disturbances in lipid metabolism caused by a high-fat diet [6]. The purpose of this study was to check the metabolic activity of two Cernitin products – T60 and GBX administered in various doses separately and in combination, both orally and intraperitoneally.

Materials and Methods

Two hundred and sixteen male Wistar rats between 150 and 200 g on standard laboratory diet were divided into eighteen equal groups [Table 1]. Group 1 included control animals. The remaining rats received a high-fat diet [HFD] and simultaneously were given two Cernitin substances – T60 and GBX [AB Cernelle, Sweden] orally [through a stomach tube] and intraperitoneally, separately or in combination.

The HFD consisted of hydrogenated coconut oil 10.0 g/kg/day, cholesterol 4.0 g/kg/day and cholic acid 0.2 g/kg/day. The experiment lasted 14 days. After this time the thorax of the animals was opened under a mild ether anesthesia, blood was drawn from the ascending aorta and liver was removed and weighed. The animals were fasted for 16 hrs prior to autopsy.

In the blood serum was determined the level of following lipid fractions: total lipids by the method of Postma and Stroes [4], triglycerides according to Eggstein and Kreutz [2], total cholesterol after Blaszcyszyn [1], beta-lipoproteins by the method of Kellen and Belaj [3]. The electrophoretic separation of lipoproteins was carried out on agarose. Glucose concentration in the blood serum was tested with the orthotoluidine method.

Results were analyzed statistically by Student's t-test.

Results

The investigations of lipids in the blood serum showed an increase of the level of total lipids by 94% [Table 2], triglycerides by 134% [Table 3], total cholesterol by 102% [Table 4] and beta-lipoproteins by 395% [Table 5] in animals of group 2, i.e. fed on a high-fat diet. The concentration of glucose increased by 61% [Table 6] and the liver weight was augmented by 47% [Table 7]. The named differences were statistically significant.

In rats receiving HFD alpha-lipoproteins level was decreased with simultaneous marked elevation of pre-beta-lipoproteins [Table 8].

Oral application of Cernitin T60 lowered the lipid fractions in the blood serum [Tables 2-5]. On comparing groups 2-6 with group 2, total lipids and cholesterol level showed statistically significant decrease in animals treated with the substance in a dose 100 mg/kg, while triglyceride concentration was significantly diminished after Cernitin T60 had been given in doses 10-200 kg.

The glucose blood level was significantly decreased in rats receiving Cernitin T60 in doses 100-200 mg/kg [Table 6]. Separation of lipoproteins into fractions did not show an increase of alpha-lipoproteins content under the influence of Cernitin T60 administered orally [Table 8].

Intraperitoneal injection of Cernitin T60 in a dose 50 mg/kg resulted in a considerable reduction of all the examined lipid fractions as well as glucose level, significantly higher than oral application of a dose 50 mg/kg and distinctly higher, as compared with the remaining groups treated with Cernitin T60 orally.

In animals receiving GBX through intubation, depression of the concentration of lipid fractions was lesser pronounced, and the glucose level was practically unchanged [Tables 2-6]. However, electrophoretic separation of lipoproteins to particular fractions has shown en elevation of alpha-lipoproteins and a decrease in the fraction of pre-beta-lipoproteins after oral administration of Cernitin GBX in a dose 200 mg/kg. There were no significant differences revealed, regarding the detailed lipid fractions, between group 9 and group 12, that were given Cernitin GBX orally and intraperitoneally in an equivalent dose 50 mg/kg.

Application of the two examined Cernitins in combination, into the stomach through intubation [groups 13-15], showed the more expressed decrease of total lipids, triglyceride and beta-lipoproteins concentration in the blood serum, than in rats receiving the substances separately. This was confirmed by electrophoretic separation if lipoproteins into fractions.

Simultaneous intraperitoneal introduction of Cernitin T60 and Cernitin GBX did not resulted in a higher reduction of lipid fractions in the blood serum, than in the case if separated application of the evaluated pollen preparations.

Comment

These experiments confirm our previous investigations showing, that both bee-pollen [5] as a raw material and two products obtained from pollen, namely Cernitin T60 as well as Cernitin GBX [6] exhibit lipid lowering properties in animals receiving a high-fat diet.

The results of this study indicate, that Cernitin T60 reveals a higher activity, regarding an improvement of lipid metabolism disturbances, than Cernitin GBX, as it was shown earlier [6]. Moreover, higher effectiveness in that aspect occurs after intraperitoneal administration of Cernitin T60 in comparison with its application through intubation. There was no dose-dependent relationship stated.

Another important finding of this study is, that combination of the two examined Cernitins given orally results in a synergistic effect on the metabolic processes. Therefore, a recommendation of Cernilton, composed of these constituents, seems to be reasonable. Significance of this statement can be supposed by our clinical studies demonstrating, that Cernilton is effective in lowering serum triglyceride level, even in the cases of hyperlipidemia resistant to clofibrate [7].

Conclusions

1. Cernitin T60 reveals higher hypolipidemic activity, than Cernitin GBX.
2. Simultaneous application of the two mentioned Cernitins results in an intensified effect on lipid metabolism disturbances.

References

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Table 1. Groups of animals receiving Cernitins: T60 and GBX [dosage/kg]

Group	Treatment
1	—
2	High — fat diet/HFD
3	HFD + T60 — 10 mg/orally
4	HFD + T60 — 50 mg/orally
5	HFD + T60 — 100 mg/orally
6	HFD + T60 — 200 mg orally
7	HFD + T60 — 50 mg i.p.
8	HFD + GBX — 10 mg orally
9	HFD + GBX — 50 mg orally
10	HFD + GBX — 100 mg orally
11	HFD + GBX — 200 mg orally
12	HFD + GBX — 50 mg i.p.
13	HFD + T60 — 50 mg + GBX — 50 mg orally
14	HFD + T60 — 100 mg + GBX — 100 mg orally
15	HFD + T60 — 200 mg + GBX — 200 mg orally
16	HFD + T60 — 25 mg + GBX — 25 mg i.p.
17	HFD + T60 — 50 mg + GBX — 50 mg i.p.
18	HFD + clofibrate 100 mg orally

Table 2. Effect of Cernitins on serum total lipids level [g/l]

Group	Mean±SE	Statistical significance [p]
1	1.49±0.082	
2	2.89±0.18	1/2<0.001
3	3.13±0.11	2/3>0.2
4	2.72±0.18	2/4>0.2
5	2.27±0.09	2/5<0.01
6	3.17±0.17	2/6>0.2
7	1.92±0.06	2/7<0.001; 4/7 <0.001
8	2.50±0.26	2/8>0.2
9	2.27±0.10	2/9<0.01
10	3.51±0.17	2/10<0.05
11	2.02±0.07	2/11<0.001
12	2.17±0.09	2/12<0.005; 9/12>0.2
13	1.93±0.07	2/13<0.001; 5/13<0.01 10/13<0.001
14	2.85±0.10	2/14>0.5; 6/14>0.1; 11/14<0.001
15	2.23±0.08	2/15<0.005
16	2.08±0.07	2/16<0.001; 7/16>0.1; 12/16>0.2
17	1.78±0.05	2/17<0.001; 13/17>0.1;
18	2.29±0.13	2/18<0.02

Table 3. Serum triglycerides concentration [mmol/l] in rats treated with Cernitins

Group	Mean±SE	Statistical significance [p]
1	0.69±0.02	
2	1.52±0.08	1/2<0.001
3	1.21±0.08	2/3<0.02
4	1.19±0.04	2/4<0.002
5	1.27±0.07	2/5<0.05
6	1.13±0.03	2/6<0.001
7	0.85±0.05	2/7<0.001; 4/7 <0.001
8	1.68±0.13	2/8>0.2
9	0.92±0.05	2/9<0.001
10	1.87±0.10	2/10<0.02
11	1.07±0.05	2/11<0.001
12	0.89±0.03	2/12<0.001; 9/12>0.5
13	0.76±0.06	2/13<0.001; 5/13<0.001 10/13<0.001
14	0.87±0.03	2/14<0.001; 6/14<0.001 11/14<0.005
15	0.82±0.04	2/15<0.001
16	0.99±0.03	2/16<0.001; 7/16<0.05 12/16<0.05
17	0.81±0.04	2/17<0.001; 13/17>0.2
18	0.80±0.04	2/18<0.001

Table 4. Influence of Cernitins on serum cholesterol level [mmol/l]

Group	Mean±SE	Statistical significance [p]
1	1.07±0.04	
2	2.16±0.20	1/2<0.001
3	1.80±0.11	2/3<0.02
4	1.98±0.10	2/4>0.1
5	1.51±0.07	2/5<0.002
6	2.38±0.15	2/6>0.1
7	1.34±0.07	2/7<0.001; 4/7 <0.001
8	1.61±0.13	2/8<0.01
9	1.76±0.09	2/9<0.001
10	2.25±0.16	2/10>0.1
11	1.48±0.05	2/11<0.001
12	1.70±0.07	2/12<0.005; 9/12>0.5
13	1.88±0.06	2/13<0.05; 5/13<0.001 10/13<0.005
14	1.87±0.07	2/14<0.02; 6/14<0.001 11/14<0.001
15	1.47±0.05	2/15<0.001
16	1.57±0.06	2/16<0.002; 7/16<0.001 12/16<0.001
17	1.40±0.06	2/17<0.001; 13/17<0.001
18	1.90±0.02	2/18<0.05

Table 5. Effect of Cernitins on serum B-lipoproteins concentration [g/l]

Group	Mean±SE	Statistical significance [p]
1	0.22±0.01	
2	1.09±0.09	1/2<0.001
3	0.82±0.06	2/3<0.05
4	0.76±0.06	2/4<0.01
5	0.82±0.07	2/5<0.05
6	0.98±0.07	2/6>0.2
7	0.49±0.02	2/7<0.001; 4/7 <0.001
8	0.95±0.07	2/8>0.2
9	0.58±0.06	2/9<0.001
10	1.01±0.08	2/10>0.5
11	0.71±0.06	2/11<0.005
12	0.67±0.04	2/12<0.001; 9/12>0.2
13	0.51±0.06	2/13<0.001; 5/13>0.5 10/13<0.001
14	0.74±0.04	2/14<0.005; 6/14>0.5 11/14<0.05
15	0.47±0.04	2/15<0.001
16	0.44±0.03	2/16<0.001; 7/16>0.2 12/16<0.001
17	0.49±0.03	2/17<0.001; 13/17<0.01
18	0.59±0.05	2/18<0.001

Table 6. Glucose level [mmol/l] in the blood serum of rats receiving Cernitins

Group	Mean±SE	Statistical significance [p]
1	4.36±0.23	
2	7.05±0.27	1/2<0.001
3	6.66±0.39	2/3>0.2
4	6.27±0.36	2/4>0.1
5	6.05±0.26	2/5<0.02
6	5.89±0.26	2/6<0.01
7	5.38±0.20	2/7<0.001; 4/7 <0.005
8	6.94±0.28	2/8>0.2
9	7.20±0.43	2/9>0.2
10	6.30±0.23	2/10<0.005
11	6.48±0.27	2/11>0.1
12	6.88±0.29	2/12>0.2; 9/12>0.2
13	5.73±0.37	2/13<0.05; 5/13>0.2 10/13>0.05
14	7.26±0.22	2/14>0.2; 6/14<0.005 11/14<0.05
15	6.12±0.47	2/15>0.05
16	7.52±0.25	2/16>0.05; 7/16<0.005 12/16>0.1
17	7.52±0.28	2/17>0.05; 13/17<0.01
18	6.45±0.22	2/18<0.05

Table 7. Liver weight [g per 100 g body weight]

Group	Mean±SE	Statistical significance [p]
1	2.78±0.12	
2	4.10±0.13	1/2<0.001
3	4.31±0.16	2/3>0.2
4	4.64±0.13	2/4<0.01
5	4.59±0.04	2/5<0.005
6	4.62±0.19	2/6<0.05
7	4.52±0.11	2/7<0.05; 4/7 >0.2
8	4.80±0.07	2/8<0.001
9	4.17±0.08	2/9>0.5
10	4.47±0.13	2/10<0.05
11	4.80±0.10	2/11<0.001
12	3.99±0.08	2/12>0.2; 9/12>0.1
13	3.89±0.09	2/13<0.001; 5/13<0.001 10/13<0.001
14	4.07±0.08	2/14>0.5; 6/14<0.02 11/14<0.001
15	3.45±0.10	2/15<0.001
16	3.16±0.04	2/16<0.001; 7/16<0.001 12/16<0.001
17	3.58±0.09	2/17<0.005; 13/17<0.02
18	3.84±0.08	2/18>0.1

Table 8. Separation of lipoproteins into farctions[mean±SE]

Group	Lipoproteins		
	£	pre-β	β
1	76.14±2.92	15.69±2.78	8.17±0.60
2	42.50±2.04	50.76±2.44	6.74±1.15
3	39.50±2.78	43.08±2.40	17.42±1.79
4	29.48±2.21	55.94±3.14	14.58±1.56
5	44.37±1.13	43.07±1.78	12.56±1.55
6	32.62±2.67	58.91±2.01	8.47±0.96
7	42.50±1.41	40.32±1.31	17.18±1.79
8	37.18±2.41	43.32±3.11	19.50±1.82
9	47.48±5.31	34.46±2.93	18.06±3.90
10	46.43±2.45	44.15±2.53	9.42±1.05
11	56.05±2.45	36.02±1.99	7.93±0.81
12	55.80±2.36	32.82±1.81	11.38±1.63
13	61.47±1.74	24.34±2.67	14.19±1.54
14	44.61±2.81	37.33±3.51	18.06±2.69
15	60.29±1.10	29.03±1.30	10.68±0.91
16	65.04±1.88	22.94±1.96	12.02±0.81
17	54.67±2.09	27.33±1.71	18.00±1.61
18	65.48±3.27	24.75±2.35	9.77±1.25