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Pollitin is a high quality natural extract. extracted from rye pollen under the production and research with technology The same standard as the production of drugs according to the requirements of the World Health Organization. therefore has been registered as “NUTRACEUTICAL” or “nutritional therapeutic nutrition” receiving the ORAC standard or the antioxidant concentration and the CAP-e Test or the ability to be absorbed into red blood cells at a very high level

The body receives almost 100% of the nutrients that are extracted from rye grass pollen. Sold to more than 50 countries on 6 continents around the world for more than 50 years, Swedish researchers have found that research studies. extracted from rye pollen contains Substances that are essential for the creation of new life in the plant family and are fundamental in the food chain. It is a natural anabolic steroid.

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Our premium natural extracts originate from meticulously selected flower pollen found in "Rye." These extracts undergo a unique proprietary production process crafted by Graminex L.L.C. in Ohio, United States. This exclusive process encompasses every stage, from cultivation and harvesting to the creation of high-quality natural extracts, specifically G60 and G63, derived from GBX flower pollen particles. Graminex holds the sole rights to this process and maintains adherence to strict pharmaceutical production standards in alignment with the World Health Organization's requirements.

Our extracts are renowned for their world-class production standards, boasting ORAC certification for exceptionally high antioxidant concentration and CAP-e Test accreditation, which signifies outstanding absorption into red blood cells. Over more than five decades, we have consistently refined and improved our product's efficacy.

Registered as a "NUTRACEUTICAL" or "nutritional therapy," Pollitin addresses issues at the cellular level, offering antibacterial properties and reinforcing immunity. By delivering essential nutrients tailored to various bodily systems, it equips the body to effectively combat abnormal cells. Our dedication to research is exemplified by over 150 certifications from medical and pharmaceutical institutions.

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Pollitin - สารอาหารบำบัดเซลล์อ

สารสกัดธรรมชาติคุณภาพสูง สกัดจากเกสรดอกไม้จาก “ข้าวไรย์” ที่มีสูตรลับเฉพาะของ บริษัท (Graminex L.L.C.) ที่รัฐโอไฮโอ้ ประเทศสหรัฐอเมริกา ในการปลูก เก็บ และผลิตสกัดธรรมชาติคุณภาพสูง G60, G63 จากอณูละอองเกสรดอกไม้ GBX, Graminex® เอกสิทธิ์เฉพาะของบริษัท Graminex เท่านั่นที่ผลิตได้เพียงเจ้าเดียวในโลก อยู่ภายใต้การควบคุมมาตรฐานการผลิตยา ตามข้อกำหนดขององค์การอนามัยโลก

จนเราได้รับการรับรองมาตรฐานการผลิตระดับโลกระดับเดียวกับการผลิตยาเพราะ Pollitin ได้รับรองการทดสอบค่า ORAC หรือ ค่าระดับความเข้มข้นของสารต้านอนุมูลอิสระที่สูงมาก และ CAP-e Test หรือค่าความสามารถในการดูดซึมเข้าสู่เม็ดเลือดแแดงในระดับที่สูงจนได้รับ

การขึ้นทะเบียนเป็น “NUTRACEUTICAL” หรือ “โภชนเภสัช สารอาหารบำบัดระดับเซลล์” ที่สามารถแก้ไขปัญหาฟื้นฟูได้ลึกถึงระดับเซลล์ มีฤทธิ์ฆ่าเชื้อแบคทีเรีย และมีผลเสริมสร้างภูมิต้านทานเมื่อเซลล์ต่างๆ ได้รับสารอาหารที่เหมาะสมตามระบบต่างๆ ในร่างกาย ส่งผลให้ร่างกายสามารถต่อสู้กับ เซลล์ที่ผิดปกติภายในร่างกายได้ถึง 95% และยังได้รับรองมาตรฐานการผลิตและประสิทธิภาพจากองค์กรต่างๆ มากมายระดับโลก รวมไปถึงยังได้รับรางวัลการันตีอีกมากมายจาก เอกสิทธิ์สูตรลับพิเศษเฉพาะของ Graminex ทำให้สินค้ามีคุณภาพและเกิดผลลัพธ์ที่ดีและน่าเชื่อถือ จนได้รับการยอมรับระดับสากลอีกด้วย

ตลอดระยะเวลากว่า 50 ปี เราได้มีการวิจัยพัฒนาประสิทธิภาพอย่างต่อเนื่อง มีการวิจัยจากสถาบันทางการแพทย์และเภสัชกรรมรับรองมากกว่า 150 การวิจัย เรามีความภูมิใจอย่างมากในการเป็นผู้ผลิตหนึ่งเดียวของโลกที่ได้ครอบครอง ถือลิขสิทธิ์เอกสิทธิ์กระบวนการผลิตและสูตรเฉพาะ G60 และ G63 จากละอองเกสรดอกไม้ชนิด GBX ที่ไม่มีใครสามารถทำได้ ส่งผลให้ Pollitin เป็นที่ยอมรับจากคนจำนวนมากใน 6 ทวีป 50 ประเทศทั่วโลก และได้รับผลตอบรับที่ดีจากผู้บริโภคในการซื้อซ้ำสินค้าอย่างต่อเนื่องมากกว่า 50 ปี

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ATHEROSCLEROSIS SUPPORT:

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Effect of Pollen Extract on the Development of Experimental Atherosclerosis in Rabbits

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Summary

Our previous studies have shown that the pollen extract, Cernitin, reveals lipid-lowering properties in animals and humans. The present study was designed to investigate the influence of Cernitin on the development of experimental atherosclerosis in rabbits over a period of 12 weeks.

Forty male mongrel rabbits were divided into 4 equals groups: (1) controls, (2) animials receiving high-fat diet (HFD) containing cholesterol and coconut oil, (3) HFD + pollen extract, and (4) HFD + clofibrate.

The most pronounced reduction in lipid metabolism and in the severity of plaque formation occurred after the pollen extract had been applied. The total cholesterol content in serum and liver homogenate was depressed by 67% and 45%, respectively, while the serum HDL cholesterol and α-lipoproteins level was increased by 19% and from 7.73% to 21.73% respectively. The cytochrome P-450 content in the liver microsomes was elevated by 98% (nmol/ g liver). Atherosclerotic plaque intensity at 12 weeks, measured planimetrically, averaged 85.5% in HFD-fed animals vs 33.7% in pollen extract-treated rabbits. These findings suggest that Cernitin, in addition to significantly lowering serum lipid levels in rabbits on an experimental diet, may modify lipid disposition in major arteries.

Key words: *Experimental atherosclerosis – High-fat diet fed rabbits – Pollen extract*

Introduction

We previously reported that pollen extract shows a remarkable lipid-lowering effect in animals fed high-fat diet [1,2] and in humans [3,4]. These studies have however, not taken into consideration the possible beneficial effect of the agent on atherosclerosis development.

Pollen extracts – Cernitin T60 and Cernitin GBX (AB Cernelle, Vegeholm, Sweden) are taken from 6 plant species: Rye grass, Maize, Timothy grass, Pine, Alder flower, and Orchard grass. After removing the membrane with a solvent, the content of the pollen grains are flushed out through the hila. The solvent is then removed and the extract is microbiologically digested. During degradation, high-molecular weight material, that may be difficult to absorb, is reduced to low-molecular weight substances that can be easily absorbed in the gastrointestinal tract. Thus, extract from the pollens are free from antigens and other high-molecular weight substances. Cernitin T60 contains water-soluble (6.0-9.2% of α-amino acids) while Cernitin GBX comprises mainly fat-soluble (10-16% of phytosterols) substances.

The chemical composition of pollen has been subjected to several investigations [5-7]. Numerous chemical substances have been identified and isolated from pollen: 21 amino-acids (including 10 essential aminoacids), all known vitamins, enzymes, coenzymes, sterols, minerals and trace elements. As much as 23%

TABLE 1

LEVEL OF TOTAL LIPIDS (TL), TOTAL CHOLESTEROL (Ch), HDL CHOLESTEROL (HDL-Ch), TRIGLYCERIDE (TG), PHOSPHOLIPIDS (P), β-LIPOPROTEINS (β-L) AND FREE FATTY ACIDS (FFA) IN THE BLOOD SERUM OF RABBITS

Values are means ± SE.

Group	TL (g/l)	Ch (mmol/l)	HDL-Ch (mmol/l)	TG (mmol/l)	P (mmol/l)	β-L (g/l)	FFA (μmol/l)
1	3.74 ± 0.29	2.60 ± 0.23	0.99 ± 0.10	0.98 ± 0.09	0.36 ± 0.01	1.13 ± 0.30	251.93 ± 25.71
2	25.40 ± 2.90	32.60 ± 4.48	0.69 ± 0.07	1.06 ± 0.06	0.79 ± 0.09	12.25 ± 2.70	359.22 ± 40.92
3	10.69 ± 2.52	10.63 ± 3.79	0.82 ± 0.08	0.79 ± 0.08	0.57 ± 0.11	8.35 ± 2.44	150.44 ± 22.74
4	21.76 ± 1.07	19.50 ± 1.60	0.91 ± 0.14	1.02 ± 0.14	0.78 ± 0.07	13.56 ± 1.14	171.01 ± 26.41
P							
1/2	< 0.001	< 0.001	< 0.05	> 0.7	< 0.001	< 0.001	< 0.05
2/3	< 0.01	< 0.01	> 0.2	> 0.05	> 0.1	> 0.3	< 0.001
2/4	> 0.2	> 0.1	> 0.5	> 0.8	> 0.8	> 0.9	< 0.01
3/4	< 0.001	< 0.05	> 0.4	> 0.1	> 0.1	> 0.05	> 0.4

of the fatty acids contained in Cernitin GBX is in the form of linolenic acid.

The objective of the present study was to determine the effect of induced hyperlipidemia and atherosclerotic lesions in rabbits. The rabbit was chosen because of its susceptibility to atherosclerosis and its similarity to man in bile acid metabolism.

Materials and methods

Animals and diets

The study was carried out on 40 male mongrel rabbits with initial body weight 3.0-3.8 kg fed with a standard basic diet, randomly divided into 4 equal groups: group 1 – was control, group 2 – received a high-fat diet (HFD), group 3 was given a HFD and pollen extracts (Cernitin T60 – 50 mg/kg/24 h + Cernitin GBX – 10 mg/kg/24 h) orally, group 4 was administered a HFD and clofibrate (Pharmaceutical Works 'Polfa'/ 25 mg/kg/ 24 h) orally. The HFD consisted of (g/kg/24 h) cholesterol (0.5), hydrogenated coconut oil (1.0), cholic acid (0.1). Pollen extracts and clofibrate were mixed with the diet and given every morning as a pellet to non-fed rabbits.

The experiment lasted 12 weeks. Animals were weighed every 2 weeks. At the end they were deprived of food for 18 h and then killed. Blood samples were taken for biochemical measurements by heart puncture and aorta and liver were excised.

Biomechanical methods

In blood serum the following lipid fractions were assayed: total lipid level [8], triglycerides [9], total cholesterol [10] and cholesterol of HDL fraction [11]. Serum samples were also analyzed for phospholipids [12], β-lipoproteins [13] and free fatty acids [14]. Lipoproteins were separated into fractions by electrophoresis on agarose gel [15]. Total lipids, triglyceride and total cholesterol [8-10] were determined in liver homogenate.

Microsomal cytochrome P-450 concentration was estimated [16] and microsomal total cholesterol content was measured [17]. Protein content of microsomes was also analyzed [18].

Anatomopathogical evaluation

The aorta was opened longitudinally from the aortic valve to the iliac arteries and examined grossly for the extent of atherosclerosis. The percentage of surface of intima covered by atherosclerotic plaques was evaluated planimetrically.

Sections from the aorta were taken, fixed in 10% buffered formalin and embedded in paraffin. They were then stained with hematoxylin and eosin, Sudan black and orcein for microscopic examination.

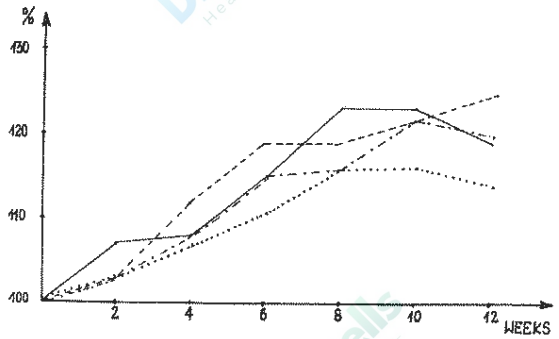

Fig. 1. Body weight of rabbits in the course of experiment expressed in percentage. Initial body weight is taken as 100%. ——— control group; - - - - - HFD; -·-·- HFD + pollen extract; ····· HFD + clofibrate.

TABLE 2

SEPARATION OF LIPOPROTEINS INTO FRACTIONS

Values are means ± SE.

Group	Lipoproteins (%)	
	α	pre-β + β
1	57.33 ± 3.10	42.67 ± 3.10
2	7.73 ± 1.26	92.27 ± 1.26
3	21.73 ± 6.22	78.27 ± 6.22
4	5.21 ± 0.76	94.79 ± 0.76
P		
1/2	< 0.001	< 0.001
2/3	< 0.05	< 0.05
2/4	> 0.1	> 0.05
3/4	< 0.02	< 0.02

Statisitical analysis

The data were analysed by Duncan's test [19].

Results

Body weight of animals

Body weight of rabbits increased and after 12 weeks it was 14-25% higher than the initial values (Fig. 1).

Biochemical studies

In the blood serum of rabbits fed with HFD, the total lipid content was elevated by 579%, total cholesterol by 1154%, phospholipids by 119%, β-lipoproteins by 984% and free fatty acids by 43% (Table 1). HDL cholesterol was decreased significantly by 30%, while the level of triglyceride was practically unchanged. In the group treated with Cernitin, the elevation of serum total lipids, cholesterol and free fatty acids was markedly and significantly suppressed.

HDL cholesterol content in this group of animals was increased in comparison with group 2, while the level of phospholipids and β-lipoproteins was insignificantly diminished. In rabbits on HFD, clofibrate administration did not significantly influence the serum lipid content except for free fatty acids.

Only two fractions were separated by lipoprotein electrophoresis: pre-β- and β-fractions remained inseparable (Table 2). The percentage content of α-lipoproteins in rabbits of group 2 was reduced, from 57.33% in control animals to 7.73% , but in rabbits treated with pollen extract it was significantly elevated to 21.73%, as compared with rabbits on HFD.

In the liver homogenate of animals receiving HFD the level of all investigated lipid fractions significantly increased: total lipids by 41%, triglyceride by 88%, and total cholesterol by146%; total cholesterol in liver microsomes was higher by 77% (Table 3). The content of lipids in liver homogenate of rabbits given HFD and Cernitin or clofibrate was depressed but the change did not reach the control level. Total cholesterol concentration was significantly depressed, both in homogenate and in microsomes under the influence of pollen extract, in comparison with group 2.

The cytochrome P-450 content in the liver microsomes was slightly raised in rabbits receiving the HFD. Animals treated with Cernitin and ingesting simultaneously the HFD, exhibited

TABLE 3

CONTENT OF TL AND TG IN LIVER HOMOGENATE AND Cc IN LIVER HOMOGENATE AND LIVER MICROSOMES (mg/g wet tissue)

Values are means ± SE.

Group	TL	TG	Cc	
			in homogenate	in microsomes
1	87.30 ± 9.76	16.50 ± 1.52	7.03 ± 1.33	0.22 ± 0.02
2	123.30 ± 10.90	31.10 ± 1.59	17.29 ± 1.27	0.39 ± 0.07
3	107.03 ± 19.82	15.14 ± 2.28	9.49 ± 0.99	0.23 ± 0.03
4	62.90 ± 6.22	11.46 ± 2.28	13.67 ± 1.33	0.30 ± 0.06
P				
1/2	< 0.05	< 0.001	< 0.001	< 0.05
2/3	> 0.4	< 0.001	< 0.001	< 0.05
2/4	< 0.01	< 0.001	> 0.05	> 0.1
3/4	> 0.1	> 0.1	< 0.05	> 0.1

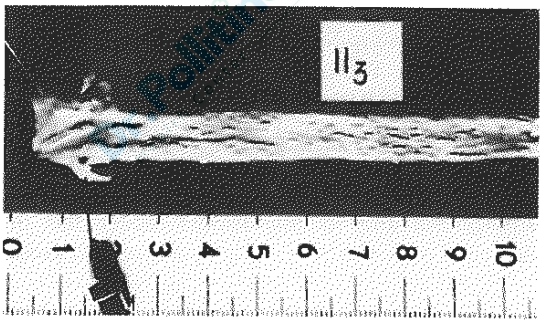

Fig. 2. Macroscopic picture of the aortic internal surface of a rabbit treated with a high-fat diet. The atherosclerotic plaques cover almost the whole surface of intima.

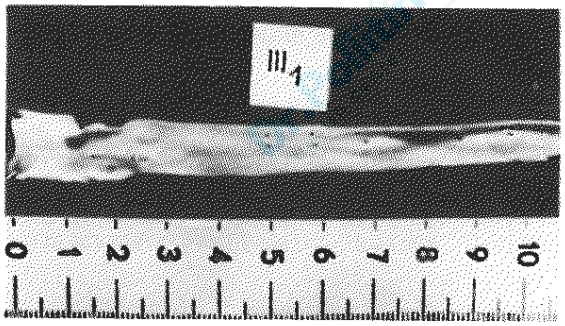

Fig. 3. Macroscopic picture of the aortic intima of a rabbit receiving the pollen extract. Significant inhibition of the formation of atheroma occurs.

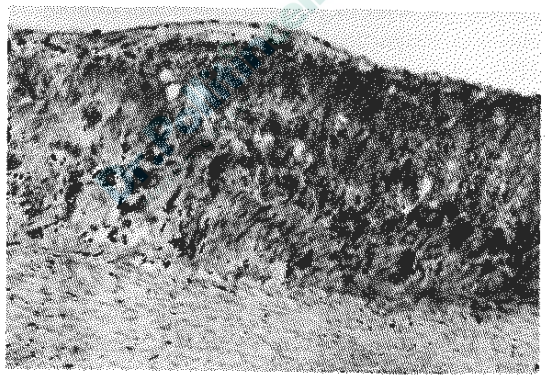

Fig. 4. Micrograph of aorta of a rabbit on a high-fat diet. Well developed atherosclerotic plaque with numerous foam cells and lipid droplet appears. Sudan black + hematoxylin and eosin, ×160.

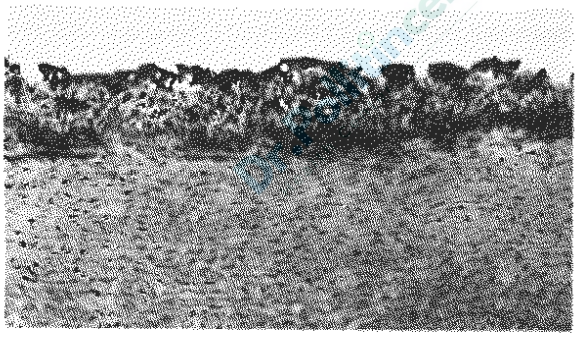

Fig. 5. Histological picture of aorta of a rabbit given pollen extract. Atherosclerotic plaque is thinner and less developed than in group 2. Sudan black + hematoxylin and eosin, ×160.

TABLE 4

CONTENT OF CYTOCHROM P-450 IN LIVER

Group [a]	Mean ± SE		
	nmol/1 mg protein	nmol/1 g liver	nmol/total liver
1	1.13 ± 0.10	5.22 ± 0.97	599.06 ± 116.85
2	1.34 ± 0.11	6.76 ± 0.43	850.86 ± 61.92
3	2.06 ± 0.13	13.39 ± 1.22	1601.05 ± 473.91
4	1.60 ± 0.09	8.17 ± 0.33	1433.71 ± 161.12
P			
1/2	> 0.2	< 0.01	> 0.1
2/3	< 0.01	< 0.01	> 0.1
2/4	> 0.1	< 0.05	< 0.05
3/4	< 0.05	< 0.05	> 0.7

[a] Four animals in each group.

an average cytochrome P-450 content markedly more than that of controls and group 2 as well as rabbits on clofibrate (Table 4).

Anatomopathlogical findings

Gross alterations

The intime of the aorta of rabbits of group 1 (controls) was unchanged (Table 5). In animals given the HFD, marked development of atherosclerotic plaques had occurred, the plaque coverage averaging 83.5% (Fig. 2), compared to only 33.7% in the pollen extract-treated animals (Fig 3). There was increase in the weight of livers of rabbits of group 2 and group 4, but it was unchanged in animals receiving pollen extract (Table 5).

TABLE 5

PERCENTAGE OF SURFACE OF THE AORTIC INTIMA COVERED BY ATHEROSCLEROTIC PLAQUES AND MEAN LIVER WEIGHT EXPRESSED IN g/1000 g BODY WEIGHT

Values are means ± SE.

Group	Atherosclerotic plaques	Liver weight
1	0	29.94 ± 1.37
2	83.5 ± 1.55	34.07 ± 1.49
3	33.7 ± 10.65	29.49 ± 2.01
4	58.1 ± 5.91	33.25 ± 1.76
P		
1/2	–	< 0.001
2/3	< 0.001	> 0.1
2/4	< 0.001	> 0.6
3/4	> 0.05	> 0.1

Microscopic studies

The aorta of rabbits of group 2 contained numerous atherosclerotic lesions of various intensity (Fig 4). Most large lesions causing the wall to protrude into the lumen on the long segment of the vessel, transgressed the limit of the elastic intima. Numerous foam cells loaded with lipids, fibroblasts and single smooth muscle cells in the lesions appeared. Lipid infiltrations consisting of small droplets, appeared also in myocytes in the media.

We also observed clearly focal proliferation of myocytes present in the media. Atherosclerotic plaques contained myocytes and numerous collagen fibres and less numerous elastic fibers. In rabbits receiving pollen extract less severe histological alterations were observed (Fig. 5). The plaques were thinner and contained less foam cells, than in animals of group 2. No essential inhibition of the formation of atheroma occurred in the aorta of the HFD-fed rabbits given clofibrate.

Discussion

The reports on the serum lipid-lowering effect orally administered pollen extracts to rats [2,20] could be confirmed in the rabbits fed a high-fat diet. Moreover, an apparently less severe atheromata in the aorta of rabbits given pollen extract, than in the high-fat diet animals, alone and in combination with clofibrate, was related to the decreased serum concentration of cholesterol and other lipid fractions.

Our previous studies considered the metabolic activity of Cernitin extracts – T60 and GBX administered separately and in combination [20]. The combination resulted in synergistic and intensified effects on the metabolic processes. We have shown in this experiment the elevated content of cytochrome P-450 in the liver microsomes. Cytochrome P-450 dependent 7α-hydroxylase is involved in the metabolism of cholesterol [21]. Thus, administration of pollen extract to rabbits may be responsible for stimulation of the liver microsomal 7α-hydroxylation of cholesterol to bile acids. Earlier it was demonstrated, that pollen extract is able to diminish platelet aggregation in vitro as well as in vivo [3,4]. The platelets of the arterial blood have been depicted as significant factors in early atherogenesis as well as in late thrombotic complications of advanced atherosclerosis.

Previous studies have revealed that anti-inflammatory agents markedly suppress the development of atherosclerotic plaque formation in cholesterol-fed rabbits [22]. Decreased atherosclerotic plaque formation could result from a reduced rate of cholesterol influx and an increased rate of efflux from rabbit aorta. The definite anti-inflammatory action of Cernitin extracts was revealed in the case of croton oil-induced edema [23]. In the cotton pellet test in

rats pollen extract showed an anti-inflammatory activity corresponding to the inflammation-inhibiting effect of phenylbutazone, but was completely devoid of toxicity.

The mechanism of antiatherosclerotic action of pollen extract may be due to its known constituents, such as polyunsaturated fatty acids and sterols interfering with intestinal absorption of cholesterol [24].

Linoleic acid has antithrombotic effects in animals and man [25]. Moreover, replacement of dietary saturated fatty acids by linoleic acid lowers the risk for myocardial (re)infarction [26] as well as cardiovascular death rate [27]. The cholesterol-lowering effect of linoleic acid is probably due to that fraction which is converted to linolenic acid or some further metabolite [28]. Recently it has been demonstrated that timnodonic acid when administered to rats is able to cause a doubling of the vascular production of prostacyclin-like material [29]. Our conclusions favor the polifactorial basis of the effect of pollen extract on the high-fat diet-induced atherosclerosis in rabbits.

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LIPID SUPPORT:

GRAMINEX Flower Pollen Extract

Clinical Evaluation of Cernilton as Lipid-Lowering Agent

HERBA POLONICA
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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

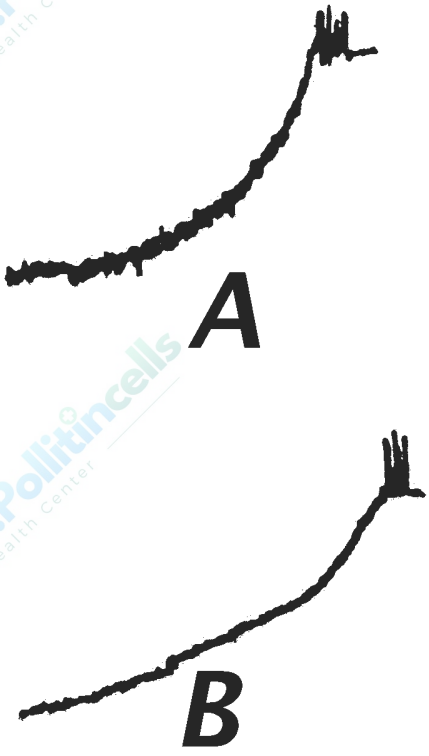

Fig.1. ADP introduced platelet aggregation *in vitro* and influence of 5% solution of Cernitin T60 (B) in comparison with control (A).

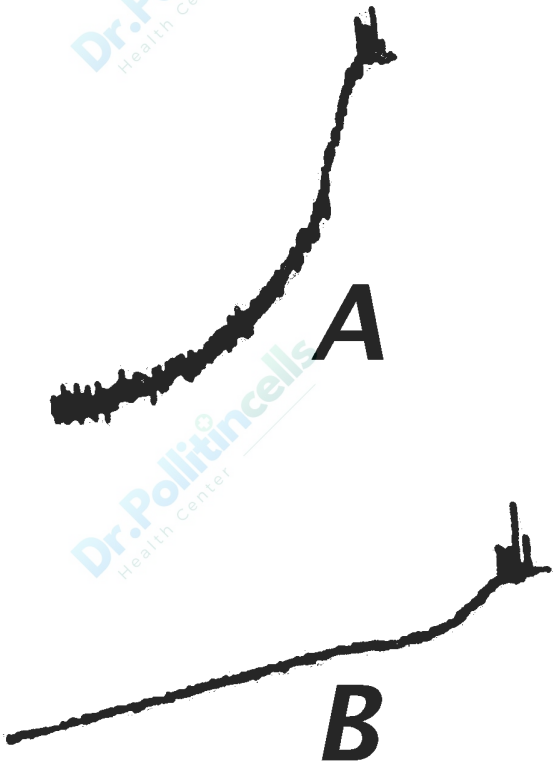

Fig.2. ADP induced platelet aggregation *in vitro* and the influence of 10% solution of Cernitin™ T60™ (B) in comparison with control (A).

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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LIPID SUPPORT:

GRAMINEX Flower Pollen Extract

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.

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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

OTHER SUPPORT:

GRAMINEX Flower Pollen Extract

Effect of Cernilton on Platelet Aggregation In Vivo

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The finding that platelets contain a mitogen for arterial smooth muscle cells [7] has provided a major mechanism for the concept that platelets may be causal agents in atherogenesis. The platelets of arterial blood have been depicted as significant factors in early atherogenesis as well as in late thrombotic complications of advanced atherosclerosis.

Antiplatelet agents are able to suppress the increased platelet aggregation and endothelial cell loss as well as the intimal lesions caused by induced homocystinemia [6].

Our earlier preliminary studies indicated to the possibility of decreased platelet aggregation, especially in vitro, under the influence of Cernitin [8]. The purpose of this investigation is to check the antiplatelet activity of Cernilton in vivo.

Materials and Methods

Twenty healthy subjects, ten women and ten men, 39 to 56 [mean 45] years old were studied. Subjects had normal medical histories, physical examinations, screening blood chemistries, blood counts and urinalyses. Each subject was fully informed of the nature of the studies.

Cernilton [AB Cernelle, Vegeholm] was given orally 2 tablets three times daily before meals over 2 weeks.

Platelet aggregation as well as blood serum lipids level was measured in all the examined subjects.

The platelet aggregation was tested using an Elvi 840 apparatus with the method of Born [2].

55 umol solution of ADP was added to the platelet rich plasma.

Total lipids were determined according to Zöllner and Kirsch [10], triglycerides by the method of Eggstein and Kreutz [4] and total cholesterol after Blaszczyszyn [1]. The data reported were based on a comparison between the results obtained on entry into the trial, after 1 week and after 2 weeks of treatment with Cernilton.

Student's t-test was used for comparing differences between means.

Results

Platelet aggregation [Table 1] expressed by means of threshold of aggregation as well as by means of speed of aggregation was diminished

in subjects receiving Cernilton. Threshold after one week was practically unchanged, however after two weeks of treatment with Cernilton it was increased by 82% as compared with initial value, the difference being statistically significant [$p<0.02$]. Speed aggregation was significantly diminished both in the first phase and in the second phase of aggregation.

The effect of Cernilton on serum lipid fractions is summarized in Table 2.

Total lipids level was lowered insignificantly – after 1 week by 11% and after 2 weeks by 18%. Triglycerides concentration in subjects receiving Cernilton was decreased by 18%. Triglycerides concentration in subjects receiving Cernilton was decreased by 18% after 1 week and by 35% after 2 weeks of management. Both differences were statistically significant. Diminution of the total cholesterol level was also observed. It was decreased by 25% after 2 weeks of Cernilton administration in comparison with the initial value.

Discussion

Platelet is reported as playing important roles in cardiovascular diseases. Yamazaki et al. [9] demonstrated hyperaggregable platelets in patients with coronary artery disease, and Frishman et al. [5] proved that platelet aggregation threshold in response to ADP and epinephrine was increased in patients with angina pectoris.

Platelets initiate thrombosis by aggregating at the site of previous vascular injury and it is speculated that altered platelet aggregability may play a significant role in the development and progression of atherosclerotic lesions [3].

The results of this trial show that Cernilton in clinically acceptable doses decreases the platelet aggregation significantly, illustrating the importance of investigating the effect of a drug in vivo.

The present study also clearly indicates that Cernilton is able to affect lipid concentration in the blood serum, even in the cases revealing normal values.

Both factors – platelet aggregation as well as lipid metabolism disturbances are of fundamental importance for development of atherosclerosis and ischemic heart disease. Therefore, therapeutic implications of the obtained results under the influence of Cernilton should be considered and discussed.

Conclusion

Preventive and therapeutic significance of Cernilton for atherosclerosis and ischemic heart disease should be taken into account.

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Table 1. Platelet aggregation [mean ± SE].

Period of examination		Threshold of aggregation [umol]	Aggregation speed [deprees]	
			In I phase	In II phase
Initial value [I]		1.13 ± 0.13	69.5 ± 1.95	41.5 ± 3.16
After 1 week [II]		1.15 ± 0.14	59.3 ± 2.44	33.2 ± 1.60
After 2 weeks [III]		2.06 ± 0.34	53.4 ± 3.25	28.8 ± 1.70
P	I/II	>0,5	<0,005	<0,05
	I/III	<0,02	<0,001	<0,002
	II/III	<0,02	>0,1	>0,05

Table 2. Effect of Cernilton on serum liquid fractions [mean ± SE].

Period of examination		Total lipids [g/l]	Triglycerides [mmol/l]	Total cholesterol [mmol]
Initial value [I]		8.40 ± 0.88	3.39 ± 0.25	7.26 ± 0.23
After 1 week [II]		7.50 ± 0.58	2.80 ± 0.50	6.32 ± 0.42
After 2 weeks [III]		6.86 ± 0.68	2.20 ± 0.21	5.49 ± 0.11
P	I/II	>0,2	>0,2	>0,05
	I/III	>0,1	<0,05	<0,05
	II/III	>0,2	>0,2	>0,5