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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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### **POLLITIN - EXCLUSIVE STEM CELL SUPPLEMENTS**

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.

Moreover, Pollitin is not only a national achievement but a global triumph, available in over 50 countries. Our exclusive patented production process sets us apart as the sole producer of this unique formulation globally, rendering it impossible for anyone else to replicate our success in extracting and utilizing these flower pollen particles. Pollitin - สารอาหารบำบัดเซลล์อ

สารสกัดธรรมชาติคุณภาพสูง สกัดจากเกสรดอกไม้ จาก "ข้าวไรย์" ที่มีสูตรลับเฉพาะของ บริษัท (Graminex L.L.C.) ที่รัฐโอไฮโอ้ ประเทศ สหรัฐอเมริกา ในการปลูก เก็บ และผลิตสกัด ธรรมชาติคุณภาพสูง G60, G63 จากอณูละอองเกสร ดอกไม้ GBX, Graminex® เอกสิทธิ์เฉพาะของบริษัท Graminex เท่านั่นที่ผลิตได้เพียงเจ้าเดียวในโลก อยู่ ภายใต้การควบคุมมาตรฐานการผลิตยา ตามข้อ กำหนดขององค์การอนามัยโลก

จนเราได้รับการรับรองมาตรฐานการผลิตระดับโลก ระดับเดียวกับการผลิตยาเพราะ Pollitin ได้รับรอง การทดสอบค่า ORAC หรือ ค่าระดับความเข้มข้นของ สารต้านอนุมูลอิสระที่สูงมาก และ CAP-e Test หรือ ค่าความสามารถในการดูดซึมเข้าสู่เม็ดเลือดแแดงใน ระดับที่สูงจนได้รับ

การขึ้นทะเบียนเป็น "NUTRACEUTICAL" หรือ "โภชนเภสัช สารอาหารบำบัดระดับเซลล์" ที่สามารถ แก้ไขปัญหาฟื้นฟูได้ลึกถึงระดับเซลล์ มีฤทธิ์ฆ่าเชื้อ แบคทีเรีย และมีผลเสริมสร้างภูมิต้านทานเมื่อเซลล์ ต่างๆ ได้รับสารอาหารที่เหมาะสมตามระบบต่างๆ ใน ร่างกาย ส่งผลให้ร่างกายสามารถต่อสู้กับ เซลล์ที่ผิด ปกติภายในร่างกายได้ถึง 95% และยังได้รับรอง มาตรฐานการผลิตและประสิทธิภาพจากองค์กรต่างๆ มากมายระดับโลก รวมไปถึงยังได้รับรางวัลการันตีอีก มากมายจาก เอกสิทธิ์สูตรลับพิเศษเฉพาะของ Graminex ทำให้สินค้ามีคุณภาพและเกิดผลลัพธ์ที่ดี และน่าเชื่อถือ จนได้รับการยอมรับระดับสากลอีกด้วย

ตลอดระยะเวลากว่า 50 ปี เราได้มีการวิจัยพัฒนา ประสิทธิภาพอย่างต่อเนื่อง มีการวิจัยจากสถาบัน ทางการแพทย์และเภสัชกรรมรับรองมากกว่า 150 การวิจัย เรามีความภูมิใจอย่างมากในการเป็นผู้ผลิต หนึ่งเดียวของโลกที่ได้ครอบครอง ถือลิขสิทธิ์ เอกสิทธิ์กระบวนการผลิตและสูตรเฉพาะ G60 และ G63 จากละอองเกสรดอกไม้ชนิด GBX ที่ไม่มีใคร สามารถทำได้ ส่งผลให้ Pollitin เป็นที่ยอมรับจากคน จำนวนมากใน 6 ทวีป 50 ประเทศทั่วโลก และได้รับผล ตอบรับที่ดีจากผู้บริโภคในการซื้อซ้ำสินค้าอย่างต่อ เนื่องมากกว่า 50 ปี

"Happy MPM: The exclusive importer and distributor of Pollitin in Thailand, Laos, Vietnam, Myanmar, and Malaysia for over two decades. our commitment to unparalleled reliability has touched the lives of over one billion consumers worldwide."

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#### ATHEROSCLEROSIS SUPPORT:

**GRAMINEX Flower Pollen Extract** 

# 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  $\alpha$ -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 highmolecular weight substances. Cernitin T60 contains water-soluble (6.0-9.2% of  $\alpha$ -amino acids) while Cernitin GBX comprises mainly fatsoluble (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 aminoacids (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 (Cb), HDL CHOLESTEROL (HDL-Ch), TRIGLYCERIDE (TG), PHOSPHOLIPIDS (P),  $\beta$ -LIPOPROTEINS ( $\beta$ -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	$3.74 \pm 0.29$	$2.60 \pm 0.23$	$0.99 \pm 0.10$	$0.98 \pm 0.09$	0.36 + 0.01	$1.13 \pm 0.30$	251.93 + 25.71
2	$25.40 \pm 2.90$	$32.60 \pm 4.48$	$0.69 \pm 0.07$	$1.06 \pm 0.06$	0.79 + 0.09	$12.25 \pm 2.70$	$359.22 \pm 40.92$
3	$10.69 \pm 2.52$	$10.63 \pm 3.79$	$0.82 \pm 0.08$	$0.79 \pm 0.08$	$0.57 \pm 0.11$	8.35+2.44	150.44 + 22.74
4	$21.76 \pm 1.07$	$19.50 \pm 1.60$	$0.91 \pm 0.14$	$1.02\pm0.14$	$0.78 \pm 0.07$	$13.56 \pm 1.14$	$171.01 \pm 26.41$
Р							
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],  $\beta$ -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.



SEPARATION OF LIPOPROTEINS INTO FRACTIONS Values are means ± SE.

Group	Lipoproteins (%)		
	α	pre- $\beta + \beta$	
1	57.33±3.10	42.67 ± 3.10	
2	$7.73 \pm 1.26$	$92.27 \pm 1.26$	
3	$21.73 \pm 6.22$	$78.27 \pm 6.22$	
4	$5.21 \pm 0.76$	$94.79 \pm 0.76$	
р			
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%,  $\beta$ -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

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  $\pm$  SE.

IL.	TG	Cc		
		in homogenate	in microsomes	
87.30 ± 9.76	$16.50 \pm 1.52$	$7.03 \pm 1.33$	0.22+0.02	
$123.30 \pm 10.90$	$31.10 \pm 1.59$	$17.29 \pm 1.27$	$0.39 \pm 0.07$	
$107.03 \pm 19.82$	$15.14 \pm 2.28$	$9.49 \pm 0.99$	0.23 + 0.03	
$62.90 \pm 6.22$	$11.46 \pm 2.28$	$13.67 \pm 1.33$	$0.30 \pm 0.06$	
< 0.05	< 0.001	< 0.001	< 0.05	
> 0.4	< 0.001	< 0.001	< 0.05	
< 0.01	< 0.001	> 0.05	> 0.1	
> 0.1	> 0.1	< 0.05	> 0.1	
	$87.30 \pm 9.76$ $123.30 \pm 10.90$ $107.03 \pm 19.82$ $62.90 \pm 6.22$ $< 0.05$ $> 0.4$ $< 0.01$ $> 0.1$	$87.30 \pm 9.76$ $16.50 \pm 1.52$ $123.30 \pm 10.90$ $31.10 \pm 1.59$ $107.03 \pm 19.82$ $15.14 \pm 2.28$ $62.90 \pm 6.22$ $11.46 \pm 2.28$ $< 0.05$ $< 0.001$ $> 0.4$ $< 0.001$ $< 0.01$ $< 0.01$ $> 0.1$ $> 0.1$	$1.0$ $1.0$ $1.0$ in homogenate         in homogenate $87.30 \pm 9.76$ $16.50 \pm 1.52$ $7.03 \pm 1.33$ $123.30 \pm 10.90$ $31.10 \pm 1.59$ $17.29 \pm 1.27$ $107.03 \pm 19.82$ $15.14 \pm 2.28$ $9.49 \pm 0.99$ $62.90 \pm 6.22$ $11.46 \pm 2.28$ $13.67 \pm 1.33$ < 0.05	

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  $\beta$ -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- $\beta$ - and  $\beta$ -fractions remained inseparable (Table 2). The percentage content of  $\alpha$ -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





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,  $\times 160$ .

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



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,  $\times 160$ .



#### CONTENT OF CYTOCHROM P-450 IN LIVER

Group *	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	$1.34 \pm 0.11$	$6.76 \pm 0.43$	$850.86 \pm 61.92$	
3	$2.06 \pm 0.13$	$13.39 \pm 1.22$	$1601.05 \pm 473.91$	
4	$1.60 \pm 0.09$	$8.17 \pm 0.33$	$1433.71 \pm 161.12$	
Р				
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	
<sup>a</sup> Four animals in e	ach group.			*******

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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	$83.5 \pm 1.55$	$34.07 \pm 1.49$	
3	33.7±10.65	$29.49 \pm 2.01$	
4	58.1± 5.91	33.25±1.76	
Р			
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 7ahydroxylase is involved in the metabolism of cholesterol [21]. Thus, administration of pollen extract to rabbits may be responsible for stimulation of the liver microsomal 7αhydroxylation 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 antiinflammatory 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 oilinduced edema [23]. In the cotton pellet test in rats pollen extract showed an anti-inflammatory activity corresponding to the inflammationinhibiting 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 dietinduced atherosclerosis in rabbits.

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