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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.

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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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LIVER SUPPORT:

GRAMINEX Flower Pollen Extract

The Protective Effect of Pollen Extracts against Allyl Alcohol Damage of the Liver

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In male Wistar rats the hepatoprotective effect of pollen extracts (Cernitins) against pra;;y introduced 1% allyl alcohol (0.4 ml per 100g body weight) was investigated. Cernitins were applied orally at 0.6, 24 and 30 h after allyl alcohol administration. After 48h an autopsy was performed and blood was collected for biochemical tests. Liver damage was evaluated by measurement of aminotransferases (AspAT, AIAT) and alkaline phosphatase activity, total bilirubin level in the blood serum as well as by histological examination of the livers. Cernitins significantly reduced the serum enzymes elevations induced by allyl alcohol. The hepatoprotective properties of Cernitins were confirmed by histopathological studies.

Previously we have demonstrated the protective effect of Cernitins* against carbon tetrachloride, ethionine, and galactosoamine-induced damage of the liver. The aim of the present report is an examination of the effect of Cernitins on the hepatitic injury evoked ny allyl alcohol. It possesses the advantage of creating morphological features of damage, which may be observed in humans.

Numerous components belonging to various classes of chemical substances have been identified in pollen: essential amino acids, carbohydrates, deoxyribosides, enzymes, coenzymes, vitamins, sterols, minerals, and trace elements.

MATERIALS AND METHODS

Eighty male Wistar rats weighing 180-240 g were divided into 10 equal groups:
Group 1- controls
Group 2- received allyl alcohol (AA)
Group 3- rats were given AA and Cernitin T60 2.5 mg/kg/day,
Group 4- animals were administered AA and Cernitin T60 50 mg/kg/day,
Group 5- animals received AA and Cernitin GBX 2.5 mg/kg/day,
Group 6- rats were given AA and Cernitin GBX 50 mg/kg/day,
Group 7- was administered AA and Cernitin GBX 2.5 mg/kg/day + Cernitin T60 50 mg/kg/day,
Group 8- rats received AA and Cernitin GBX 50 mg/kg/day +Cernitin T60 50 mg/kg/day,

* Extracts from the pollens of specially selected plants: Cernitin T60 and Cernitin GBX (AB Cernelle Vegeholm, Sweden) free from antigens and other high molecular weight substances. Cernitin T60 contains water-soluble (6.0-9.2 percent of α-amino acids) while Cernitin GBX comprises mainly fat-soluble (10-16 percent of phytosterols) substances.

Allyl alcohol prepared as 1% solution was administered as a single dose of 0.4 ml per 100 g body weight orally to rats, which were fasted for 18 h. Cernitin substances were applied orally through intubation at 0.6, 24 and 48 h after intoxication with allyl alcohol. After 48 h the autopsy of and rats was performed and blood was collected for biochemical tests: alanine aminotransferase (AIAT) and aspartate

aminotransferase (AspAT) according to Reitman and Frankel, alkaline phosphatase according to the method of Bodansky and total bilirubin by the method of Malley and Evelyn. The results were analyzed by Duncan's test.

Specimens for histopathological studies were always taken from the same place of the liver,. For routine microscopic investigations they were stained with hematoxylin and eosin (HE) and for lipids presence with Sudan black.

RESULTS

Exposure of rats to a single oral dose of allyl alcohol caused a marked statistically significant, increase of serum AIAT from 31.5 in the control group to 762.8. AspAT from 61.5 to 797.8 and alkaline phosphatase from 148.8 to 416.6 IU/1 (Table 1). Simultaneously, total bilirubin concentration was elevated from 4.08 to 12.07 μmol/1, and liver weight was increased from 3.56 to 5.22 g per 100 body weight (Table 2)

Application of Cernitin T60 was associated with a marked drop of AIAT and AspAT activity (Table 1) as well as with a decrease of the bilirubin level and liver weight (Table 2), as compared with group 2. Effectiveness, of Cernitin T60 was found to be closely related to the dose given. The administration of Cernitin GBX was particularly effective on the serum enzymes activity as well as on the serum bilirubin concentration the higher dose gave better results. Two Cernitin fractions: T60 and GBX applied in combination caused a significant decrease of serum enzymes activity in comparison with animals receiving allyl alcohol alone.

Table 1. Serum enzymes activity (I.U/1): alanine aminotransferase (A1AT), aspartate aminotransferase (AspAT), alkaline phosphatase (AP), in rats receiving allyl alcohol (AA), and treated with Cernitin T60 and Cernitin GBX (mean ± SE)

Table 2. Total bilirubin level (μmol/l) and liver weight (g/100g body weight) of rats receiving allyl alcohol (AA) and treated with Cernitin T60 and Cernitin GBX (mean ± SE)

Histopathological studies showed, that the liver of rats treated with allyl alcohol developed a typical picture of the toxic effect ascribed to this alcohol. Fatty and vacuolar degeneration of hepatocytes lacated in the marginal zones of the lobules were demonstrated. The hepatocytes revealed the presence of 3-10 fatty droplets or were tightly fulfilled with the lipids (Fig.1). Single, completely degenerated cells were also visible. The degenerated zones of the adjacent lobules often joined each other and formed wide continued bands, which were somewhere accompanied by the focal necrosis of the whole lobules (Fig. 2). All portal spaces were infiltrated with the mononuclear leukocytes among which the single giant policariocytes were also present. The mononuclear infiltrations often continued in the degenerative marginal zones of the adjacent lobules. The liver of rats' receiving Cernitin T60 2.5 mg per kg (group3) demonstrated the widening of the sinusoids. Many lobules looked unchanged (Fig.3), while the others showed some degenerated hepatocytes in their marginal zones.

Fig. 1. Liver of rat receiving allyl alcohol. The hepatocytes revesl the presence of fat droplets or are tightly fulfilled with the lipids. Stain: Sudan black Magn.: x 130

Fig. 2. Necrosis of the liver cells of rat treated with allyl alcohol is visible. Stain: H-E Magn.: x130

Their cells were vacuolated, but there were no fat droplets in the cytoplasm. The leukocytic infiltrations of the portal spaces were negligible and never clongated to the adjacent lobules.

In the liver of animals treated with Cernitin T60 in a dose 50mg per kg/group/4/ only widening of the sinusoids and marked activation of the Browicz-Kupffer cells were demonstrated (Fig. 4).

These cells often contained the single droplets in the cytoplasm while the hepatocytes were unchanged (Fig. %). In rats receiving Cernitin GBX 2.5 mg per kg (group %) the liver still demonstrated foci of acidophilic necrosis, but they were not so numerous as in group 2. Some hepatocytes located in the marginal zones of the lobules were highly vacuolated; however, complete cell degeneration was scarce. The liver of animals that were given Cernitin GBX 50 per kg (group 6) did not differ substantially from the control. There were no signs of hepatotoxicity except for widening of the sinusoids (Fig. 6).

Fig. 3. Liver of rat receiving allyl alcohol and Cernitin T60 2.5 mg/kg. Many lobules look unchanged. Stain: H-E. Magn.: x130

Fig. 4. Liver of rat treated with allyl alcohol and Cernitin T60 50 mg/kg. Only widening of the sinusoids and activation of Browicz-Kupffer cells can be demonstrated. Stain: H-E. Magn.: x130

Fig. 5. The picture shows the beneficial effect of Cernitin T60 50 mg/kg on allyl alcohol induced hepatic injury, No signs of necrosis are present. Stain: H-E. Magn.: x130

Fig. 6. Liver of rat receiving Cernitin GBX 50 mg/kg. There are no signs of hepatotoxicity except for widening of the sinusoids. Stain: H-E. Magn.: x130

Fig. 7. Protective effect of Cernitin GBX 2.5 mg/kg applied in combination with Cernitin T60 50 mg/kg on the liver cell is clearly visible. Stain H-E. Magn.: x 130

Fig. 8. Liver of rat treated with Cernitin GBX 50 mg/kg and Cernitin T60 50mg/kg. No signs of necrosis are present, nevertheless vacuolar degeneration of hepatocytes can be noticed. Stain: H-E. Magn.: x 130

Protective effect of Cernitin GBX 2.5 mg per kg administered in combination with Cernitin T60 in a dose 50 mg per kg (group 7) against allyl alcohol induced hepatic alterations was evident ad well (Fig. 7). No symptoms of necrosis or fatty degeneration were observed. In some areas widening of sinusoids and activation of Browicz-Kupffer cells occurred. It seems, that the treatment of animals with a higher dose of Cernitin GBX (50 mg per kg) in combination with the same dose of Cernitin T60 9group *) did not improve the beneficial effect ascribed to the single pollen extract. Although the focal necrosis and leukocytic infiltrations were not present, nevertheless the marked vacuolar degeneration of the hepatocytes located in the marginal and intermediate zones of the lobules could be noticed (Fig. 8).

DISCUSSION

The present report illustrates, that pollen extracts can protect rat liver against acute intoxication induces by allyl alcohol. Thus, in this experiment we were able to find support for our previous investigations, especially those, which showed the beneficial effect of Cernitins on galactosamine-induced hepatic injury in rats. As already was described in the literature, sylimarin also protects against galactosamine induced injury, but contrary to pollen extracts, it is ineffective against that caused by allyl alcohol. The lack of efficacy of a drug in allyl alcohol induced acute liver damage ascertains that this drug cannot be used in acute disenzymia during the development of a liver disease.

Allyl alcohol produces a periportal necrosis which either proceeds of follows the endothelial damage of the capillaries. In the first case, the early biochemical changes are characterized by alkylation of cellular macromolecules, and inhibition of protein synthesis. According to Schon and Steidl the damage is not caused by allyl alcohol itself, but also by it split up products-acrolein and acrylio and acid appearing under the influence of nonspecific alcohol dehydrogenase of the liver. Only together with these substances it does affect intoxication and damage of the organ. The toxicity of allyl alcohol is probably also based on its double bond which binds the SH groups of corresponding enzymes and which blocks them. This principle of action would correspond to the general pathomechanism of toxic substances, which decrease the SH groups in the liver tissue, block them, and in this way finally lead to liver damage and necrosis.

Pollen extracts were applied by us three times at 0.6, 24 and 30 h after allyl alcohol had been administrated. Liver function changes to various extents during 48 h due to the regenerative capacity of the liver cell. According to the above mentioned cyclicity it is proposed that liver-protecting drugs should be given cyclically too, corresponding to the changes noticed in the different enzymatic processes, i.e. at 06, 24 and 30 h after intoxication. It is to be stressed, that the pollen extracts were administrated after poisoning, that means curatively. Only curative testing, using previously damaged liver, appears to be of special importance for therapy, since in human medicine, the liver protecting substances are applied to patients with a diseased liver.

Our studies do not provide adequate information concerning the mechanism by which the protective activity is brought about. Numerous chemical substances contained in pollen extracts favour the polyfactoral basis of the effect of Cernitins on the liver injury caused by

allyl alcohol: supply of carbohydrates (glucose and fructose), vitamins, folic acid, and SH groups from methionine and cysteine.

The positive effect of Cernitins may be also due to the potentiated synthesis of proteins, exhibiting protective properties against the liver cell injury.

Taking into account the present study and our previous investigations on the beneficial effect of Cernitins on different types of experimental hepatic injury, as well as the synergistic effect of both Cernitins on lipid metabolism it could be concluded, that the application of Cernitin T60 and Cernitin GBX, separately or in combination, to patients suffering from liver diseases, should be considered.

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LIVER SUPPORT:

GRAMINEX Flower Pollen Extract

Effect of Cernilton on the Hepatotoxicity of Carbon Tetrachloride [CCl_4] in Rats

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Cernitin correspond to microbiologically fermented pollen extracts [AB Cernelle, Vegeholm, Sweden]. Cernitin T60 contains mainly water soluble, while Cernitin GBX mainly fat soluble substances.

There are many components isolated from pollen and playing an important and fundamental role in the biological processes and cellular metabolism: essential amino acids, vitamins, enzymes, coenzymes, steroids, minerals and trace elements such as calcium, potassium, magnesium, iron, copper, zinc, manganese, titanium, molybdenum, silicon, sulfur, phosphorus, boron [1, 7, 8, 9, 10].

Composition of pollen extracts could raise the possibility, that Cernitins would be useful in preventing and management of liver injury. In this work we preliminary investigate this possibility.

Materials and Methods

Male Wistar rats weighing 170-200 g fed on a standard laboratory chow were administered CCl_4/0.25 ml per 100 g body weight/diluted with an equal volume of liquid paraffin by a stomach tube.

Cernitins were given 30 minutes prior and 4 h after CCl_4 application.

Seventy two animals were divided into six equal groups:

group 1 – controls,
group 2 – received CCl_4,
group 3 – rats were given CCl_4 and Cernitin T60 50 mg/kg by a stomach tube [p.o.],
group 4 – animals were administered CCl_4 and Cernitin T60 50 mg/kg intraperitoneally [i.p.],
group 5 – received CCl_4 and Cernitin GBX 50 mg/kg by a stomach tube [p.o.],
group 6 – rats were given CCl_4 and Cernitin GBX 50 mg/kg i.p

The animals were fasted for 16 h prior to autopsy. After 24 h from CCl_4 administration the blood was collected and the liver was rapidly removed, weighed and homogenized.

The following biochemical parameters were determined: serum glutamic pyruvic transaminase [SGPT] activity according to the method of Reitman and Frankel [12], serum alkaline phosphatase [SAP] activity according to the method of Bodansky [5], bilirubin level in the blood serum by the method of Malloy and Evelyn [5] and serum total protein level according to the procedure described by Gornall et al. [4]. Triglycerides concentration in the liver homogenate was estimated using Eggstein and Kreutz method [3].

Specimens for histopathological studies were always taken from the same place of the liver; for routine microscopic investigations they were stained with hematoxylin and eosin, and for the lipids presence with oil red.

The results were analyzed by Student's t-test.

Results

Treatment with CCl_4 caused a huge increase in SGPT activity and a marked increase in SAP activity [Table 1]. Intraperitoneal Cernitin T60 application was associated with a drop of SGPT activity by 52 per cent [$p<0.01$] and SAP activity by 40 per cent [<0.001] in rats gavaged with CCl_4 [group 4] as compared with group 2. Diminution of tehse enzymes activity was observed also in animals receiving Cernitin T60 orally, but this diminution was less than that noted in group 4. Nevertheless the difference was statistically significant.

Rats given CCl_4 showed a bilirubin level that was increased to 1.93 umol/1 in comparison with the control value 0.03 umol/l [Table 2]. Cernitin T60 reduced bilirubin level, especially when the drug was administered intraperitoneally, from 1.93 umol/1 [group 2] to 0.10 umol/1 [group 4]. The reduction of the bilirubin concentration occurred in rats receiving Cernitin GBX orally, as well.

Total protein level was practically unchanged in all the examined groups [Table 3]. Treatment with CCl_4 caused the expected rise in liver triglycerides by 245 per cent [Table 4]. In animals that were administered Cernitins, there was no decrease in the liver triglycerides concentration observed.

The mean relative liver weight was significantly higher by 77 per cent in rats given CCl_4 in relation to group 1 [Table 4]. Animals of group 4 and group 5 revealed statistically significant decrease in the relative liver weight as compared with group 2.

Histopathological examination showed marked fatty infiltration [Table 5] and remarkable centrilobular necrosis in all the rats of group 2. The characteristic centrilobular changes consisted of degeneration and necrosis of parenchymal cells around central veins, while peripheral part of the lobules contained a lot of cells revealing balloon degeneration.

In animals receiving Cernitin T60 intraperitoneally [group 4] fatty infiltration of the liver cells was to some degree diminished [Table 5], and necrotic changes were less severe in 6 of 10 rats. In 4 of the 6 mentioned rats necrosis of the liver parenchymal cells was even not shown, however balloon degeneration occurred. The necrosis was also less severe in rats given Cernitin T60 orally [group 3]. In 2 rats of this group necrosis disappeared almost completely.

Discussion

Various forms of treatment have been suggested for hepatic lesions: cysteine, glutathione, methionine, choline, vitamins, hormones and organ extracts. Although there are many drugs used in treatment of liver diseases, their effectiveness is very often insufficient and questioned. Search for new drugs and methods of pharmacotherapy of liver damage require therefore further perpetual attention.

Severe injury of liver cells has been evoked by CCl_4 in our study. Remarkable centrilobular necrosis and ballooning, as well as fatty infiltration of liver cells were observed. This was accompanied by marked and significant elevation of serum enzymes activity and bilirubin concentration. Such a model of liver damage [6] can be useful for evaluation of potentially protective and therapeutic agents. Such a strong destruction of liver cell would require a special and strong drug that could be able to remove completely all the alterations appearing in the form of histopathological a biochemical abnormalities. So potent and effective a drug has not existed, in our opinion, until now.

Our results can be assumed as promising. Cernitin T60 administered intraperitoneally and in less degree given orally possesses benefit effect on the liver of animals treated with CCl_4. Serum glutamic pyruvic transaminase, accepted as a sensitive parameter in detecting structural abnormalities [2], and alkaline phosphatase activities were distinctly and significantly decreased in animals receiving Cernitin. Marked lowering of bilirubin level in the blood serum, as well as diminution of liver weight was also stated. These results were confirmed by the histopathological studies of the liver. Although triglycerides concentration per 1 g of liver homogenate was unchanged, nevertheless it was decreased when calculated per total organ.

Significance of our observations should be proved by using another models of liver cell damage, and in human beings suffering from acute or chronic liver injury and its consequences.

Cernitin could be applied alone or in combination with other substances known as liver protecting agents.

Conclusion

Significance of Cernitin as liver protecting agent should be considered.

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Table 1. Serum glutamic pyruvic transaminase [SGPT] and serum alkaline phosphatase [SAP] activity in rats receiving Cernitin [50 mg/kg] T60 and GBX [mean±SE]

Group	Treatment	SGPT [units]	SAP [units/1]
1		38± 3	100± 4
2	CCl_4	9900± 946	380± 28
3	CCl_4 + T60 p.o.	6251± 429	286± 27
4	CCl_4 + T60 i.p.	4775± 1036	230± 15
5	CCl_4 + GBX p.o.	8214± 615	310± 21
6	CCl_4 + GBX i.p.	10509± 340	252± 15
P	1/2	<0.001	<0.001
	2/3	<0.01	<0.05
	2/4	<0.01	<0.001
	2/5	>0.1	>0.2
	2/6	>0.5	<0.001

Table 2. Effect of Cernitin [50 mg/kg] T60 and GBX on serum bilirubin level [µmol/1] in rats receiving carbon tetrachloride

Group	Treatment		Mean ± SE
1			0.03± 0.001
2	CCl_4		1.93± 0.21
3	CCl_4 + T60 p.o.		1.13± 0.28
4	CCl_4 + T60 i.p.		0.10± 0.02
5	CCl_4 + GBX p.o.		0.38± 0.06
6	CCl_4 + GBX i.p.		1.76± 0.31
P		1/2	<0.001
		2/3	<0.05
		2/4	<0.001
		2/5	<0.001
		2/6	>0.5

Table 3. Total protein level [g/100m³] in the blood of animals treated with Cernitin [50 mg/kg] T60 and GBX

Group	Treatment		Mean ± SE
1			6.7± 0.12
2	CCl_4		6.6± 0.11
3	CCl_4 + T60 p.o.		6.1± 1.67
4	CCl_4 + T60 i.p.		6.1± 0.10
5	CCl_4 + GBX p.o.		6.4± 0.10
6	CCl_4 + GBX i.p.		6.0± 0.2
P		1/2	>0.5
		2/3	>0.5
		2/4	<0.001
		2/5	>0.3
		2/6	<0.01

Table 4. Triglycerides concentration in the liver homogenate [mmol/g] and liver weight calculated in g per 100 g body weight of animals receiving Cernitin [50 mg/kg] T60 and GBX [mean±SE]

Group	Treatment		Triglycerides	Liver Weight
1			0.20±0.02	2.83±0.06
2	CCl_4		0.69±0.07	5.01±0.12
3	CCl_4 + T60 p.o.		0.66±0.08	4.50±0.25
4	CCl_4 + T60 i.p.		0.66±0.08	4.04±0.12
5	CCl_4 + GBX p.o.		0.87±0.05	4.17±0.11
6	CCl_4 + GBX i.p.		0.60±0.09	3.98±0.44
P		1/2	<0.001	<0.001
		2/3	>0.5	>0.5
		2/4	>0.5	<0.001
		2/5	<0.05	<0.001
		2/6	>0.4	<0.01

Table 5. Fatty infiltration of the liver cell examined histologically in rats receiving Cernitin [50 mg/kg] T60 and GBX

Group	Treatment	Rat nr									
		1	2	3	4	5	6	7	8	9	10
1			+	+						+	++
2	CCl_4	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
3	CCl_4 + T60 p.o.	++++	++++	+++	++++	+++	++++	++++	++	++	+++
4	CCl_4 + T60 i.p.	++++		++++	++	++++	++++	++	++++	+++	++
5	CCl_4 + GBX p.o.	++++	++	++++		++++	++++	++++	++++	++++	++++
6	CCl_4 + GBX i.p.	++++	++++	+++	++++	++	++++	++++	++++	++++	++++

Infiltration was graded:
- none
+ mild
++ moderate
+++ marked
++++ Severe

LIVER SUPPORT:

GRAMINEX Flower Pollen Extract

The Protective Effect of Pollen Extracts against Allyl Alcohol Damage of the Liver

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In male Wistar rats the hepatoprotective effect of pollen extracts (Cernitins) against pra;;y introduced 1% allyl alcohol (0.4 ml per 100g body weight) was investigated. Cernitins were applied orally at 0.6, 24 and 30 h after allyl alcohol administration. After 48h an autopsy was performed and blood was collected for biochemical tests. Liver damage was evaluated by measurement of aminotransferases (AspAT, AIAT) and alkaline phosphatase activity, total bilirubin level in the blood serum as well as by histological examination of the livers. Cernitins significantly reduced the serum enzymes elevations induced by allyl alcohol. The hepatoprotective properties of Cernitins were confirmed by histopathological studies.

Previously we have demonstrated the protective effect of Cernitins* against carbon tetrachloride, ethionine, and galactosoamine-induced damage of the liver. The aim of the present report is an examination of the effect of Cernitins on the hepatitic injury evoked ny allyl alcohol. It possesses the advantage of creating morphological features of damage, which may be observed in humans.

Numerous components belonging to various classes of chemical substances have been identified in pollen: essential amino acids, carbohydrates, deoxyribosides, enzymes, coenzymes, vitamins, sterols, minerals, and trace elements.

MATERIALS AND METHODS

Eighty male Wistar rats weighing 180-240 g were divided into 10 equal groups:
Group 1- controls
Group 2- received allyl alcohol (AA)
Group 3- rats were given AA and Cernitin T60 2.5 mg/kg/day,
Group 4- animals were administered AA and Cernitin T60 50 mg/kg/day,
Group 5- animals received AA and Cernitin GBX 2.5 mg/kg/day,
Group 6- rats were given AA and Cernitin GBX 50 mg/kg/day,
Group 7- was administered AA and Cernitin GBX 2.5 mg/kg/day + Cernitin T60 50 mg/kg/day,
Group 8- rats received AA and Cernitin GBX 50 mg/kg/day +Cernitin T60 50 mg/kg/day,

* Extracts from the pollens of specially selected plants: Cernitin T60 and Cernitin GBX (AB Cernelle Vegeholm, Sweden) free from antigens and other high molecular weight substances. Cernitin T60 contains water-soluble (6.0-9.2 percent of α-amino acids) while Cernitin GBX comprises mainly fat-soluble (10-16 percent of phytosterols) substances.

Allyl alcohol prepared as 1% solution was administered as a single dose of 0.4 ml per 100 g body weight orally to rats, which were fasted for 18 h. Cernitin substances were applied orally through intubation at 0.6, 24 and 48 h after intoxication with allyl alcohol. After 48 h the autopsy of and rats was performed and blood was collected for biochemical tests: alanine aminotransferase (AIAT) and aspartate

aminotransferase (AspAT) according to Reitman and Frankel, alkaline phosphatase according to the method of Bodansky and total bilirubin by the method of Malley and Evelyn. The results were analyzed by Duncan's test.

Specimens for histopathological studies were always taken from the same place of the liver,. For routine microscopic investigations they were stained with hematoxylin and eosin (HE) and for lipids presence with Sudan black.

RESULTS

Exposure of rats to a single oral dose of allyl alcohol caused a marked statistically significant, increase of serum AIAT from 31.5 in the control group to 762.8. AspAT from 61.5 to 797.8 and alkaline phosphatase from 148.8 to 416.6 IU/1 (Table 1). Simultaneously, total bilirubin concentration was elevated from 4.08 to 12.07 μmol/1, and liver weight was increased from 3.56 to 5.22 g per 100 body weight (Table 2)

Application of Cernitin T60 was associated with a marked drop of AIAT and AspAT activity (Table 1) as well as with a decrease of the bilirubin level and liver weight (Table 2), as compared with group 2. Effectiveness, of Cernitin T60 was found to be closely related to the dose given. The administration of Cernitin GBX was particularly effective on the serum enzymes activity as well as on the serum bilirubin concentration the higher dose gave better results. Two Cernitin fractions: T60 and GBX applied in combination caused a significant decrease of serum enzymes activity in comparison with animals receiving allyl alcohol alone.

Table 1. Serum enzymes activity (I.U/1): alanine aminotransferase (A1AT), aspartate aminotransferase (AspAT), alkaline phosphatase (AP), in rats receiving allyl alcohol (AA), and treated with Cernitin T60 and Cernitin GBX (mean ± SE)

Table 2. Total bilirubin level (μmol/l) and liver weight (g/100g body weight) of rats receiving allyl alcohol (AA) and treated with Cernitin T60 and Cernitin GBX (mean ± SE)

Histopathological studies showed, that the liver of rats treated with allyl alcohol developed a typical picture of the toxic effect ascribed to this alcohol. Fatty and vacuolar degeneration of hepatocytes lacated in the marginal zones of the lobules were demonstrated. The hepatocytes revealed the presence of 3-10 fatty droplets or were tightly fulfilled with the lipids (Fig.1). Single, completely degenerated cells were also visible. The degenerated zones of the adjacent lobules often joined each other and formed wide continued bands, which were somewhere accompanied by the focal necrosis of the whole lobules (Fig. 2). All portal spaces were infiltrated with the mononuclear leukocytes among which the single giant policariocytes were also present. The mononuclear infiltrations often continued in the degenerative marginal zones of the adjacent lobules. The liver of rats' receiving Cernitin T60 2.5 mg per kg (group3) demonstrated the widening of the sinusoids. Many lobules looked unchanged (Fig.3), while the others showed some degenerated hepatocytes in their marginal zones.

Fig. 1. Liver of rat receiving allyl alcohol. The hepatocytes revesl the presence of fat droplets or are tightly fulfilled with the lipids. Stain: Sudan black Magn.: x 130

Fig. 2. Necrosis of the liver cells of rat treated with allyl alcohol is visible. Stain: H-E Magn.: x130

Their cells were vacuolated, but there were no fat droplets in the cytoplasm. The leukocytic infiltrations of the portal spaces were negligible and never clongated to the adjacent lobules.

In the liver of animals treated with Cernitin T60 in a dose 50mg per kg/group/4/ only widening of the sinusoids and marked activation of the Browicz-Kupffer cells were demonstrated (Fig. 4).

These cells often contained the single droplets in the cytoplasm while the hepatocytes were unchanged (Fig. %). In rats receiving Cernitin GBX 2.5 mg per kg (group %) the liver still demonstrated foci of acidophilic necrosis, but they were not so numerous as in group 2. Some hepatocytes located in the marginal zones of the lobules were highly vacuolated; however, complete cell degeneration was scarce. The liver of animals that were given Cernitin GBX 50 per kg (group 6) did not differ substantially from the control. There were no signs of hepatotoxicity except for widening of the sinusoids (Fig. 6).

Fig. 3. Liver of rat receiving allyl alcohol and Cernitin T60 2.5 mg/kg. Many lobules look unchanged. Stain: H-E. Magn.: x130

Fig. 4. Liver of rat treated with allyl alcohol and Cernitin T60 50 mg/kg. Only widening of the sinusoids and activation of Browicz-Kupffer cells can be demonstrated. Stain: H-E. Magn.: x130

Fig. 5. The picture shows the beneficial effect of Cernitin T60 50 mg/kg on allyl alcohol induced hepatic injury, No signs of necrosis are present. Stain: H-E. Magn.: x130

Fig. 6. Liver of rat receiving Cernitin GBX 50 mg/kg. There are no signs of hepatotoxicity except for widening of the sinusoids. Stain: H-E. Magn.: x130

Fig. 7. Protective effect of Cernitin GBX 2.5 mg/kg applied in combination with Cernitin T60 50 mg/kg on the liver cell is clearly visible. Stain H-E. Magn.: x 130

Fig. 8. Liver of rat treated with Cernitin GBX 50 mg/kg and Cernitin T60 50mg/kg. No signs of necrosis are present, nevertheless vacuolar degeneration of hepatocytes can be noticed. Stain: H-E. Magn.: x 130

Protective effect of Cernitin GBX 2.5 mg per kg administered in combination with Cernitin T60 in a dose 50 mg per kg (group 7) against allyl alcohol induced hepatic alterations was evident ad well (Fig. 7). No symptoms of necrosis or fatty degeneration were observed. In some areas widening of sinusoids and activation of Browicz-Kupffer cells occurred. It seems, that the treatment of animals with a higher dose of Cernitin GBX (50 mg per kg) in combination with the same dose of Cernitin T60 9group *) did not improve the beneficial effect ascribed to the single pollen extract. Although the focal necrosis and leukocytic infiltrations were not present, nevertheless the marked vacuolar degeneration of the hepatocytes located in the marginal and intermediate zones of the lobules could be noticed (Fig. 8).

DISCUSSION

The present report illustrates, that pollen extracts can protect rat liver against acute intoxication induces by allyl alcohol. Thus, in this experiment we were able to find support for our previous investigations, especially those, which showed the beneficial effect of Cernitins on galactosamine-induced hepatic injury in rats. As already was described in the literature, sylimarin also protects against galactosamine induced injury, but contrary to pollen extracts, it is ineffective against that caused by allyl alcohol. The lack of efficacy of a drug in allyl alcohol induced acute liver damage ascertains that this drug cannot be used in acute disenzymia during the development of a liver disease.

Allyl alcohol produces a periportal necrosis which either proceeds of follows the endothelial damage of the capillaries. In the first case, the early biochemical changes are characterized by alkylation of cellular macromolecules, and inhibition of protein synthesis. According to Schon and Steidl the damage is not caused by allyl alcohol itself, but also by it split up products-acrolein and acrylio and acid appearing under the influence of nonspecific alcohol dehydrogenase of the liver. Only together with these substances it does affect intoxication and damage of the organ. The toxicity of allyl alcohol is probably also based on its double bond which binds the SH groups of corresponding enzymes and which blocks them. This principle of action would correspond to the general pathomechanism of toxic substances, which decrease the SH groups in the liver tissue, block them, and in this way finally lead to liver damage and necrosis.

Pollen extracts were applied by us three times at 0.6, 24 and 30 h after allyl alcohol had been administrated. Liver function changes to various extents during 48 h due to the regenerative capacity of the liver cell. According to the above mentioned cyclicity it is proposed that liver-protecting drugs should be given cyclically too, corresponding to the changes noticed in the different enzymatic processes, i.e. at 06, 24 and 30 h after intoxication. It is to be stressed, that the pollen extracts were administrated after poisoning, that means curatively. Only curative testing, using previously damaged liver, appears to be of special importance for therapy, since in human medicine, the liver protecting substances are applied to patients with a diseased liver.

Our studies do not provide adequate information concerning the mechanism by which the protective activity is brought about. Numerous chemical substances contained in pollen extracts favour the polyfactoral basis of the effect of Cernitins on the liver injury caused by

allyl alcohol: supply of carbohydrates (glucose and fructose), vitamins, folic acid, and SH groups from methionine and cysteine.

The positive effect of Cernitins may be also due to the potentiated synthesis of proteins, exhibiting protective properties against the liver cell injury.

Taking into account the present study and our previous investigations on the beneficial effect of Cernitins on different types of experimental hepatic injury, as well as the synergistic effect of both Cernitins on lipid metabolism it could be concluded, that the application of Cernitin T60 and Cernitin GBX, separately or in combination, to patients suffering from liver diseases, should be considered.

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LIVER SUPPORT:

GRAMINEX Flower Pollen Extract

The Effect of the Pollen Extracts Quercitin and Cernitin on the Liver Lungs and Stomach of Rats Intoxicated with Ammonium Fluoride

HUMICZEWSKA M., HERMACH U., PUT A. 1994. The effect of the pollen extracts Quercitin and Cernitin on the liver, lungs, and stomach of rats intoxication with ammonium fluoride. Folia biol. (Krakow) 42: 157-166.

Quercitin and Cernitin are not in themselves toxic to rats. When administered at the time of intoxication of the animals with ammonium fluoride, they reduced the noxious effects of the toxic agent in the liver and lungs. It is suggested that Quercitin and Cernitin might play a protective role during prolonged exposure to ammonium fluoride. Neither ammonium fluoride nor Quercitin or Cernitin seem to exert any effect o the stomach.

Key words: pollen extracts, Quercitin, Cernitin, liver, lungs, stomach, ammonium fluoride.

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Fluoride compounds are one of the most potent ecotoxins (MARIER 1972; GROTH 1975; MARKIEWICZ 1981). Several publications discuss the toxic action of fluorine and the problem of reducing its emission (GUMIŃSKA 1981; MARKIEWICZ 1981; DOMINICZAK *et al.* 1982; HUMICZEWSKA *et al.* 1989). In the search for cheap, easily available pharmacological means, devoid of side effects, which would reduce the harmful changes occurring with prolonged intoxication with fluorides, attention was paid to pollen extracts. The latter, which have been employed for years in phytotherapy (c.f. OZAROWSKI 1982), were found to be very useful in various diseases of the kidneys and liver, playing a role also in detoxication processes (SCHWARTZ *et al.* 1982; KULAWIAK 1986). New, previously not described, characteristics of pollen were revealed in investigations carried out in the Department of Pharmacology and Toxicology of the Pomeranian Medical Academy in Szczecin (KULAWIAK 1986; CEGLECKA 1991, 1991a; MYŚLIWIEC 1992). However, those studies do not cover all the possibilities of exploiting pollen extracts. Further investigations are therefore needed to establish their pharmacological characteristics and, possibly, other appliances.

The aim of the present study was to investigate the histological and histochemical changes occurring in the lungs, liver, and stomach of rats exposed to ammonium fluoride (NH4F), and to assess the possible beneficial effect on such changes of two pollen extracts, Quercitin and Cernitin, known as detoxicating agents.

Materials and Methods

Animals

The investigations were carried out on 160 inbred, male Wistar rats, weighing approximately 300g each. Throughout the experiment the animals were fed a standard granulated chow and received water *ad libitum.*

Exposition to ammonium fluoride (NH4F)

The animals were placed in a toxicological chamber in which the parameters of humidity and temperature were adapted each time to those prevailing in the animal room. The air flow through the chamber was 10 m^3/h. Ammonium fluoride was introduced as aerosol at a concentration of 2 mg/m^3 of air, and controlled constantly by means of an ionoselective fluoride electrode. The above concentration corresponds to the so-called Highest Permissible

Concentration established for men exposed to fluoride compounds at 0,0016 mg/m^3 of air(Decree of the Polish Council of Ministers, September 30, 1980).

In the present investigation the animals were exposed to ammonium fluoride for 6 h daily, 5 days a week.

Pollen extracts

As pollen extracts Quercitin and Cernitin were applied. Quercitin (synthesized in the Department of Inorganic Chemistry of the Rzeszów Branch of the Kraków Technical University) is a mixture of natrium salts of quercitin 8.5 disulphonic acid i.e. of Na_2QDSA in which NaQSA-5' and NaQSA-8 appear at the ratio 1:1 (unpublished data).

Cernitin (AB Cernelle, Veqeholm, Sweden) appears in two forms, as a fraction soluble in water (Cernitin T 60), and as a fraction soluble in lipids (Cernitin GBX). Cernitin T 60 contains from 60 to 92% of aminoacids, and Cernitin GBX from 10 to 16% of phytosterols (NIELSON *et al.*1987, SEPPÄNEN 1989). In medicine mixtures of the two fractions are used (NIELSON *et al.* 1987).

On days when the rats were exposed to ammonium fluoride the appropriate groups also received Quercitin and Cernitin preparations, previously added to their chow. The doses applied are given below.

Grouping of animals

The animals were divided into two series, each comprising 8 groups of 10 rats.

Those of series I (Groups 2-8) were exposed to ammonium fluoride and/or given pollen-extracts for 3 months, while those of Series II (Groups 10-16) underwent the same experimental procedure but for 6 months.

Group 1 was the control for Series I, and Group 9 for Series II. The two control groups were neither exposed to NH4F, nor given pollen extracts and remained throughout the experiment in the animal room.

Groups 2 and 10 received Quercitin at dose I, i.e. 32mg/kg b.w./day.

Groups 3 and 11 received Quercitin at dose II, i.e. 20mg/kg b.w./day.

Groups 4 and 12 received Cernitin T 60 (100 mg/kg b.w./day and simultaneously, Cernitin GBX (200mg/kg b.w./day).

Groups 5 and 13 were exposed to NH_4F only.

Groups 6 and 14 were exposed to NH_4F and received Quercitin at dose I (as the animals of Groups 2 and 10, respectively).

Groups 7 and 15 were exposed to NH_4F and received Quercitin at dose II (as the animals of Groups 3 and 11, respectively).

Groups 8 and 16 were exposed to NH_4F and received Cernitin (as the animals of Groups 4 and 12, respectively).

After conclusion of the experiments (Series I, i.e. Groups 2-8, and Control Group 1 after 3 months), and Series II, i.e. Groups 10-16, and control Group 9 after 6 months the animals were killed by decapitation.

Histology and histochemistry

After killing the animals, the lungs, liver, and stomach were dissected out. Tissue specimens intended for histological examination were fixed in Bouin's fluid, embedded in paraffin, sectioned at 8mm, and stained with Mayer's heamatoxylin and aqueous eosin.

For histochemical analysis the tissues were immediately frozen, and alter cut on a cryostat into 10µm sections. Histochemical reactions performed on this (unfixed) material included (1) succinic dehydrogenase (SDH) using sodium succinate as substrate, according to Nichlas (PEARSE 1968), (2) acid phosphatase (AcP), and (3) alkaline phosphatase (AIP), using sodium-glicerophosphate as substrate according to Gomori, (PEARSE 1972). Following incubation at 37° C (SDH for 30min AcP and AIP for 60min), the sections were embedded in glycero-gel. In order to confirm the specificity of the particular enzymatic reactions, control reactions without the substrates were simultaneously run.

Results

Histological observations

Hematoxylin and eosin (HE) stained sections of the liver, lung, and stomach of control animals (Groups 1 & 9) showed that the morphological picture of all the investigations organs was normal (Figs 1 & 5).

L i v e r. In the liver of experimental animals exposed to ammonium fluoride for 3 months (Group 5) the liver cells appeared brighter, this being caused by excessive accumulation of glycogen. The blood vessels were extended, and in places fibrosis could be seen (Fig. 2).

After 6 months exposure to NH_4F (Group 13), apart from the changes described above, the laminar structure of lobules was obliterated, particularly at their peripheral parts. Liver cells seemed to be diffused, and no clear borders between them were seen. The connective tissue strands were more extensive (Fig. 3).

In the animals of groups 2, 3, and 4, which for 3 months received pollen-extracts only, no differences in comparison with control Groups 1 and 9 were detected. Similarly, no changes were found in the liver of animals given only pollen extracts for 6 months (Groups 10, 11& 12).

Rats exposed for 3 months to ammonium fluoride, but receiving simultaneously Quercitin at dose I or II (Groups 6 & 7), of Cernitin (Group 8) also did not reveal any differences, compared with controls.

The same was true for animals of Groups 15 and 16 (intoxicated for 6 months, but simultaneously given Quercitin at dose II or Cernitin). However, in the rats of Group 14 (exposed to NH_4F for 6 months, and given Quercitin at dose I), some parts of the liver showed obliteration of the laminar structure as well as extension of the interlobular blood vessels (Fig. 4).

L u n g s. The results of histological observations of the lungs are summarized in Table 1.

No pathomorphological changes were visible either in control Groups 1 and 9, or in rats which received pollen extracts only (i.e. Groups 2, 3, 4, 10, 11 & 12).

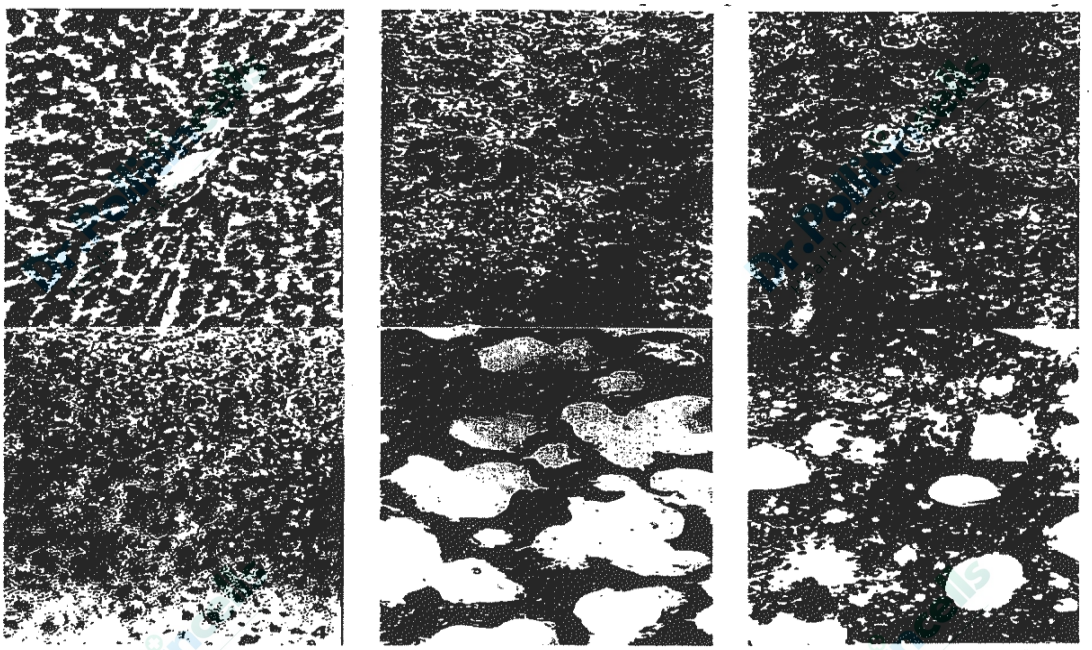

Fig. 1. Liver of a control rat (Group 9); identical pictures were observed in control Group 1. HE × 140. Fig. 2. Liver of a rat exposed for 3 months to ammonium fluoride (Group 5). Note the brightening of liver cells, the extension of blood vessels, and sings of fibrosis HE × 150. Fig. 3. Liver of a rat exposed for 6 months to ammonium fluoride (Group 13). Note the extension of connective tissue strands HE × 150. Fig. 4. Liver of a rat exposed for 6 months to ammonium fluoride, but simultaneously receiving Quercitin at dose I (5 mg/kg/day), (Group 14). Note some extension of capillaries, and, in at places, obliteration of the laminar structure HE × 150. Fig. 5. Lung of control rat (Group 9); identical pictures were observed in control Group 1. HE x 150. Fig. 6. Lung of a rat exposed for 6 months to ammonium fluoride (Group 13). Note numerous extravasations of erythrocytes HE × 150.

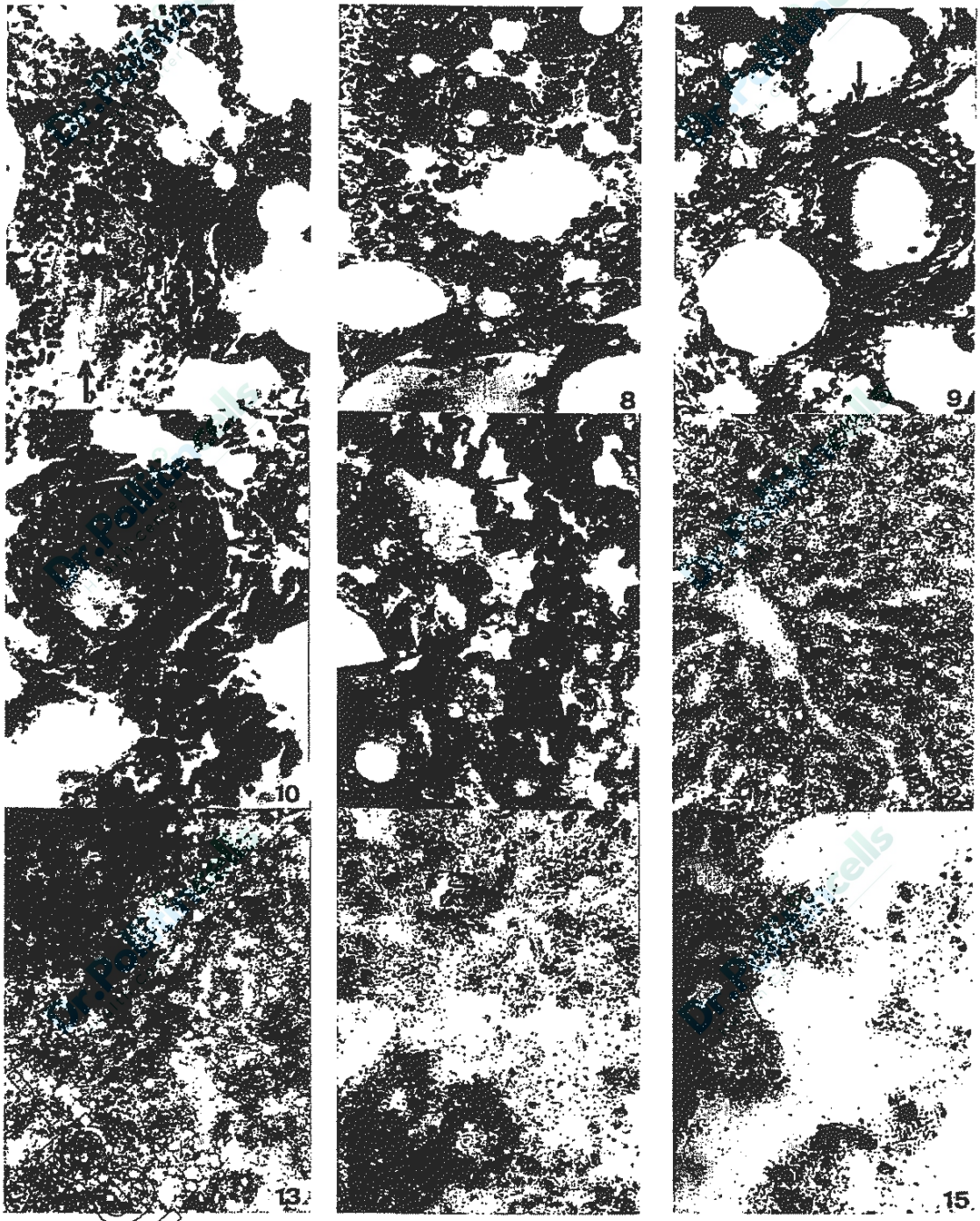

Fig. 7. Lung of a rat exposed for 6 months to ammonium fluoride (Group 13). Note general oedema and, in the alveoli. effusional fluid. erythrocytes, macrophages and desquamated respiratory epithelial cells HE × 150. Fig. 8. Lung of a rat exposed for 6 months to ammonium fluoride (Group 13). Note concretions of haemosiderin in the macrophages HE × 150. Fig. 9. Lung of a rat exposed for 6 months to ammonium fluoride (Group 13). Note numerous accumulations of acidophilic leucocytes HE × 150. Fig. 10. Lung of a rat exposed for 6 months to ammonium fluoride (Group 13). Note numerous accmuulations of limphoidal cells HE × 150. Fig. 11. Lung of a rat exposed for 3 months to ammonium fluoride (Group 5). Note the extension of alveoli filled with effusional fluid HE × 150. Fig. 12. Liver of a control rat (Group 1). The activity of succinic dehydrogenase is moderate × 150. Fig. 13. Liver of a rat exposed for 6 months to ammonium fluoride (Group 13). The activity of succinic dehydrogenase is weak × 150. Fig. 14. Lung of a control rat (Group 9). The activity of succinic dehydrogenase is moderate × 150. Fig. 15. Lung of a rat exposed for 3 months to ammonium fluoride (Group 5). The activity of succinic dehydrogenase is weak × 150.

In the remaining experimental groups, which were all exposed to ammonium fluoride, more or less frequent or pronounced extravasations of erythrocytes, lymphoedema, and hypertrophy of the lymphatics could be observed. These symptoms were strongest in animals intoxicated only with ammonium fluoride for 6 months (Group 13), in which in the alveoli not only effusional fluid but also desquamated respiratory epithelium cells (Fig. 7), erythrocytes, and macrophages were visible. The latter could be seen also in the interalveolar septa, and within the respiratory epithelium (Fig. 6). In the macrophages numerous large concretions of haemosiderin (Fig. 8), originating as the result of phagocytosis of erythrocytes, could be seen. In Group 13 also very distinct hypertrophy of the lymphatics was visible. This was particularly evident around the blood vessels and bronchioli, where often infiltrations of lymphoidal cells (Fig. 10), accompanied by pneumocytes and numerous acidophilic leucocytes (Fig. 9) were observed.

Similar changes, but less intense, were found in Groups 14, 15 & 16 (NH_4F intoxication for 6 months, and simultaneous application of Quercitin or Cernitin), and in Group 5 (rats exposed only to NH_4F for 3 months), (Fig. 11).

In Groups 6, 7 & 8 (intoxication for 3 months plus simultaneous application of Quercitin or Cernitin), the morphological picture of the lungs did not essentially differ from that in controls, the only difference being the accumulation of small quantities of effusion in some of the alveoli.

S t o m a c h. In the stomach only the morphological characteristics of the mucous membrane were analyzed. Neither in the surface and glandular epithelium, nor in the lamina propria and muscularis mucosae could any differences between experimental and control animals be observed.

Histochemical observations

The results of histochemical observations are summarized in Table 2.

Succinic dehydrogenase (SDH)

L i v e r. In control animals (Groups 1 & 9) succinic dehydrogenase appeared as a microgranular reaction, which was usually strongest around the central vein of the lobules. General, SDH activity in both control groups could be classified as moderate (Fig. 12).

Table 1

Morphological changes in the lungs of rats exposed to ammonium fluoride (NH_4F) and/or to the pollen extracts Quercitin (Qu.I, dose 5 mg/kg. b.w./ day; Qu. II, dose 20 mg/kg b.w./day) or Cernitin (C., dose 200 mg/kg b.w./day) during 3 and 6 months

	Groups	Treatment	Extravasations of erythrocytes	Oedemateous changes	Hypertrophy of lymphatics	Acidophilic leucocytes
SERIES I (3 months)	1	Control				
	2	Qu. I				
	3	Qu. II				
	4	C.				
	5	NH_4F	numerous	moderate		numerous
	6	NH_4F+Qu. I	single	weak		single
	7	NH_4F+Qu. II	single	weak		
	8	NH_4F+ C.	single	weak		
SERIES II (6 months)	9	Control				
	10	Qu. I				
	11	Qu. II			moderate	
	12	C.				
	13	NH_4F	very numerous	intense	strong	very numerous
	14	NH_4F+Qu. I	numerous		moderate	numerous
	15	NH_4F+Qu. II	numerous	moderate	moderate	numerous
	16	NH_4F+ C.	numerous	moderate	moderate	numerous

Activity of succinic dehydrogenase (SDH), acid phosphatase (AcP), and alkaline phosphatase (AlP) in the liver, lungs, and stomach of rats exposed to ammonium fluoride (NH_4F), and/or to the pollen extracts Quercitin (Qu. I, dose 5 mg/kg b.w./day; Qu. II, dose 20 mg/kg b.w./day), or Cernitin (C., dose 200 mg/kg b.w./day) during 3 and 6 months

	Enzymes	SDH			AcP			AlP		
	Groups	Treatment	Liver	Lungs	Stomach	Liver	Lungs	Stomach	Liver	Lungs	Stomach
SERIES I (3 months)	1	Control	+++	+++	+++++	++++	+	++++	+	++	+++
	2	Qu. I	+++	+	+++++	++++	+	++++	+	+	+++
	3	Qu. II	+++	++++	+++++	++++	+	++++	+	+	+++
	4	C.	+++	+++	+++++	++++	+	++++	+	+++	+++
	5	NH_4F	+	+	+++++	+	+++	++++	+++	+++++	+++
	6	NH_4F+Qu. I	+++	+++	+++++	++++	+	++++	+	+++	+++
	7	NH_4F+Qu. II	+++	+++	+++++	++++	+++++	++++	+	+++	+++
	8	NH_4F+ C.	+++	+++	+++++	++++	++++	++++	+	+++	+++
SERIES II (6 months)	9	Control	+++	+++	+++++	++++	+++	++++	+	+++	+++
	10	Qu. I	+++	+	+++++	++++	+++	++++	+	+	+++
	11	Qu. II	+++	++++	+++++	++++	+	++++	+	+	+++
	12	C.	+++	++++	+++++	++++	+	++++	+	+++	+++
	13	NH_4F	+	+	+++++	+	+++++	++++	+++	++++	+++
	14	NH_4F+Qu. I	+++	+++	+++++	+++	+++++	++++	+	+++	+++
	15	NH_4F+Qu. II	+++	+++	+++++	++++	++++	++++	+	+++	+++
	16	NH_4F+ C.	+++	+++	+++++	+++	++++	++++	+	+++	+++

Activity: + very weak; ++ weak; +++ moderate; ++++ strong; +++++ very.

In rats exposed only to ammonium fluoride for 3 or 6 months (Groups 5 & 13) SDH activity decreased (Fig. 13).

In the remaining groups, i.e. in both those exposed to NH_4F and receiving pollen extracts and those given pollen extracts only, the activity of SDH did not differ from that observed in the controls.

L u n g s. In untreated control rats moderate SDH activity was observed in all cells of the interalveolar septa and in the walls of bronchioles. In the ciliated epithelium of the latter and in the blood vessels it was higher and could be classified a strong (Fig. 14).

In Groups 5 and 13 (exposed to NH_4F for 3 and 6 months, respectively), and in Groups 2 and 10 (animals intoxicated with ammonium fluoride, and given Quercitin at dose I during 3 and 6 months, respectively), the reaction was less intense (Fig. 15), the only exception being alveolar phagocytes in which SDH activity was in all the mentioned groups fairly strong.

In Groups 3 and 11 (Quercitin at dose II for 3 and 6 months, respectively), and in Group 12 (Cernitin for 6 months) the activity of succinic dehydrogenase was higher than that in the controls, particularly within the endothelium, ciliated epithelium, and in the phagocytes present in the lumen of alveoli and bronchioles (Fig. 16). In all the remaining groups, the reactions for SDH were comparable to those described in animals of the untreated control groups.

S t o m a c h. In the stomach of the control animals (groups 1 & 9) very strong SDH activity was observed in the glandular epithelium, in the lamina propria it was moderate, while in the surface epithelium and muscularis mucosae it remained rather weak.

In none the experimental groups, i.e. intoxicated and/or treated with pollen extracts, did the activity of SDH differ from that found in the controls.

Acid phosphatase (AcP)

L i v e r. In both control groups (Group 1 and 9), the reaction for AcP was in all cells of the liver, including Kupffer cells (Fig. 17), fairly strong.

Intoxication with NH_4F for 3 or 6 months (Groups 5 and 13) reduced AcP-activity in liver cells to weak, and in Kupffer cells (Fig. 18) to moderate. In all the other groups, exposed to NH_4F and/or treated with pollen extracts, the activity of AcP in the liver did not differ essentially from that in controls.

L u n g s. In control animals (Groups 1 & 9), and in those receiving pollen-extracts only (Groups 2, 3, 4, 10, 11 & 12) the cells of the interalveolar walls, as well as the epithelial cells of alveoli, bronchi, and bronchioli, revealed moderate AcP activity, the reaction being stronger only in the granular pneumocytes (Fig. 19). Intoxication for 6 months with NH_4F, with or without simultaneous treatment with pollen extracts (Group 13, 14, 15 & 16), brought about a distinct increase in the activity of AcP, which was particularly evident in the granular pneumocytes and in other cells of the interalveolar walls (Fig. 20).

S t o m a c h. In control Groups 1 and 9 the reaction for AcP was in the glandular epithelium strong, in the surface epithelium and the muscularis mucosae moderate, and in the lamina propria weak. Intoxication with NH_4F and/or treatment with pollen extracts did not cause in any of the experimental groups changes in the above-described situation.

Alkaline phosphatase (AIP)

L i v e r. The reaction for AIP in the liver of control animals (Group 1 & 9) was very weak (Fig. 21). In groups exposed to ammonium fluoride only (Groups 5 & 13) AIP activity increased to moderate (Fig. 22), while in all the other ones it did not differ from that in the controls.

L u n g s. AIP activity in the lungs of control Groups 1 and 9 was fairly evenly distributed in the interalveolar walls, and generally moderate, a slightly more intense reaction being observed in the endothelium of bronchioli and in alveolar pneumocytes (Fig. 23). In animals intoxicated with ammonium fluoride only (Groups 5 & 13), the activity of AIP was very strong, while in those receiving Quercitin , both at dose I and II, and not exposed to NH_4F (Groups 2, 3, 10 & 11), it was moderate (Fig. 24). In all the other groups (Groups 6, 7, 8, 14, 15 & 16) the activity of AIP was comparable to that in controls.

S t o m a c h. AIP activity was found mainly in the surface and glandular epithelium. It was similar in all the experimental and control groups, and could be classified as moderate.

Discussion

Earlier studies (DOMINICZAK & SAMACHOWIEC 1982; HUMICZEWSKA *et al.* 1989), as well as the present one, showed that ammonium fluoride causes various pathological changes in the liver and lungs of rats. It is possible, however, that these changes are not only local responses of the investigated organs but also reflect more general reactions of the whole organism. In the case of the liver it should be borne in mind that it normally accumulates substantial amounts of toxic substances and therefore that, any damage to it may have further, far-reaching consequences.

Prolonged intoxication with ammonium fluoride brings about obliteration of the laminar structure of liver lobules, and more or less extensive fibrosis. These observations are similar to those described in rats with cirrhosis, which developed following intoxication with carbon tetrachloride (GEORGIJEW & KALCZAK 1967; KUNA 1980) sodium fluoride (DOMINICZAK *et al.* 1982), hydrogen fluoride (HUMICZEWSKA *et al.* 1989), and the herbicide Simazin (HUMICZEWSKA *et al.* 1990a).

Although, the changes described in the present investigation were slightly less severe than those described in the papers quoted above, it was interesting to note that when intoxication with ammonium fluoride was accompanied by the simultaneous application of the pollen extracts Quercitin or Cernitin, damage to the liver practically did not occur.

Also affected by fluoride are the lungs. Apart from their known role in various physiological and pathological processes, including the metabolism of many biologically active substances, they also participate in the detoxication of the organism (WATTENBERG & LEONG 1965; HEINEMANN & PISMANN 1969; DOLOFF 1971).

In the lungs of rats intoxicated with ammonium fluoride, numerous acidophilic leucocytes appear in the interstitial tissue, and an all-over

increase in the number of lymphoidal cells takes place. While these reactions seem to be non-specific and reflect the activation of the general defense mechanisms of the organism, the observed simultaneous increase in the number of pneumocytes and the appearance of macrophages ore probably the result of defensive processes of the lung itself, aimed directly at the toxic agent.

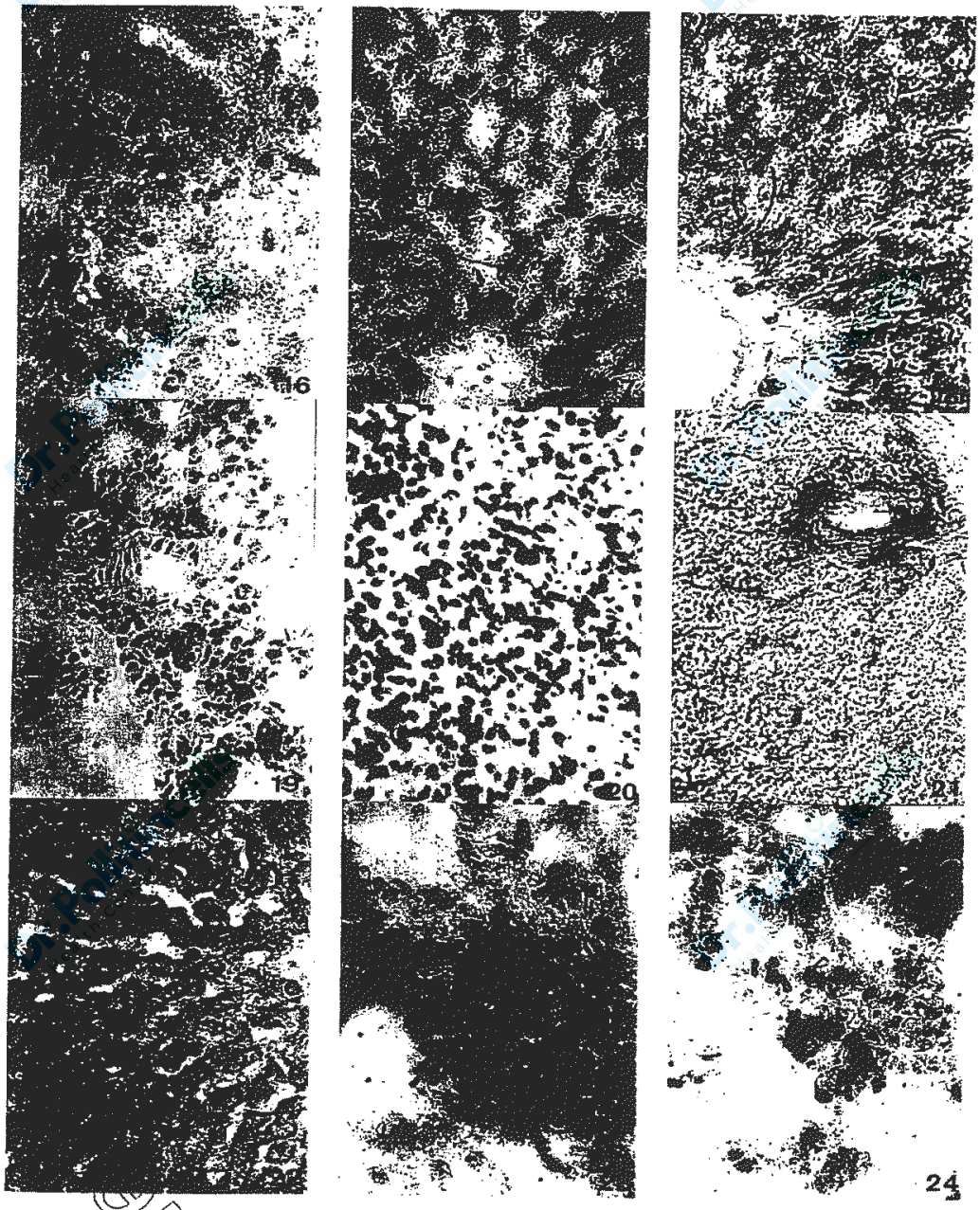

Fig. 16. Lung of a rat for 6 months receiving only Quercitin at dose II (20 mg/kg b.w./day). (Group 11). The activity of succinic dehydrogenase is strong × 150. Fig. 17. Liver of a control rat (Group 9). The activity of acid phosphatase is strong × 150. Fig. 18. Liver of a rat exposed for 3 months to ammonium fluoride (Group 5). The activity of acid phosphatase is weak × 150. Fig. 19. Lung of a control rat (Group 1). The activity of acid phosphatase is weak × 150. Fig. 20. Lung of rat exposed for 6 months to ammonium fluoride (Group 13). The activity of acid phosphatase is very strong × 150. Fig. 21. Liver of a control rat (Group 9). The activity of alkaline phosphatase is very weak x 150. Fig. 22. Liver of a rat exposed for 6 months to ammonium fluoride (Group 13). The activity of alkaline phosphatase is moderate × 150. Fig. 23. Lung of a control rat (Group 9). The activity of alkaline phosphatase is moderate x 150. Fig. 24. Lung of a rat exposed for 6 months to ammonium fluoride (Group 13). The activity of alkaline phosphatase is strong × 150.

A similar increase in the number of acidophilic leucocytes, lymphocytes, pneumocytes and macrophages was found in many other pathological states of the lungs (PARAFINIUK *et al.* 1975; HUMICZEWSKA *et al.* 1990). Often it is also accompanied by more or less extensive extravasations of blood cells, indicating damage to the capillary walls. Injuries to the endothelium of capillaries, increasing their permeability and in consequence causing extravasations, were also described following intoxication with other substances (such as, e.g., benzene and phosphorus), during infectious diseases, and in diseases of the haemopoietic tissue (cf. DOLOFF 1971).

Blood cells extravasated into the surrounding tissues are recognized there as foreign bodies, and induce inflammatory reactions, during which they are imbibed by the accumulating phagocytes. As a result, macrophages often contain haemosiderin concretions.

The application of Quercitin or Cernitin to rats which were at the same time intoxicated with ammonium fluoride substantially reduced the pathological processes described above. The frequency of extravasations was much lower, which suggests that the pollen-extracts, which the animals received had a positive effect also on the capillaries, reducing their fragility.

The results of histochemical studies revealed that ammonium fluoride also affects the metabolic processes in the liver and lungs, but apparently not in the stomach.

The decrease in succinic dehydrogenase activity in the liver and lungs suggests that in their cells the citric acid (Krebs) cycle was blocked, which could be the result of a negative effect of F^- ions on these processes (MACHOY 1981, 1987).

Acid and alkaline phosphatases are similarly considered to be sensitive indicators of disturbances occurring in the course of metabolic processes. The increased activity of AIP in the liver and lungs might in this case reflect the pathological changes described in these organs following NH_4F intoxication. As suggested by SAWICKA (1980), an increase in the level of alkaline phosphatase is often connected with abnormalities in transmembrane transport.

The behavior of acid phosphatase was different, intoxication with ammonium fluoride reducing its activity in the liver, but increasing it in the lungs. On the basis of *in vitro* experiments, GALKA and OGOŃSKI (personal communication) reached the conclusion that F^- ions block the activity of acid phosphatase by binding the Mg^{+2} ions which are necessary for AcP activation. However, it would be difficult to explain in these terms the increase in AcP activity in the lungs, unless one assumes that either in the organism there are mechanisms which counteract the binding of Mg^{+2} and F^- ions or that the quantities of F^- ions reaching the particular organs are too small to block acid phosphatase. In other histochemical and biochemical studies AcP activity was not affected or was even slightly increased following the introduction of fluoride ions (c.f. MESSER & SINGER 1976).

The investigated pollen extracts Quercitin (at dose I or dose II) and Cernitin, when applied evoked practically no negative side effects, but when given to animals simultaneously intoxicated with ammonium fluoride, they substantially reduced its negative action, or even prevented the development of negative changes. This demonstrates that Quercitin and Cernitin should be considered as protective agents in cases when prolonged exposition to fluorides is expected. Unfortunately, so far nothing is known about the mode of action of Quercitin and Cernitin, hence further investigations are needed.

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