Flower Pollen Extract and its Effect on the Liver

Contents

Hepatoprotective effect of flower pollen lipid extract in paracetamol-induced hepatotoxicity in mice........ 2
The Effect of the Pollen Extracts Quercitin and Cernitin on the Liver, Lungs, and Stomach of Rats Intoxicated with Ammonium Fluoride........................................................................................................ 3
Experimental evaluation of the effect of pollen extract on the course of paracetamol poisoning ........... 7
Effect of pollen extract (Cernitin ™) on the course of poisoning with organic solvents ...................... 8
Effect of Pollen Extracts (Cernitin™ preparation) on Selected Biochemical Parameters of Liver in the Course of Chronic Ammonium Fluoride Poisoning in Rats................................................................. 9
The Protective Effect of Pollen Extracts Against Allyl Alcohol Damage of the Liver............................ 10
The Effect of Pollen on the Changes in the Liver of Laboratory Rats Evoked by Ethionine, Carbon Tetrachloride, Allyl Alcohol and Galactosamine ................................................................. 13
The Effect of Cernitins on Galactosamine-Induced Hepatic Injury in Rat........................................... 14
Hepatoprotective effect of flower pollen lipid extract in paracetamol-induced hepatotoxicity in mice

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Hepatoprotective effects of the lipid flower pollen extract (LEPK) in paracetamol intoxication in mice were shown. Normalisation of A1AT and LDH—biochemical indicators of necrotic changes in the hepatic cells, and high protection ultra structural cell organelle such as mitochondrion substantially testifies to the hepatoprotective effect of investigated lipid flower pollen extract on the hepatic cells.

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


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 on 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 (GUMINSKA 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; MYSLIWIEC 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 humanity and temperature were adapted each time to those prevailing in the animal room. The air-flow through the chamber was 10m3/h. Ammonium fluoride was intruduced as aerosol at a concentration of 2mg/m3 of air, and controlled constantly by means of an iono-selective fluoride electrode. The above concentration corresponds to the so-called Highest Permissible Concentration established for men exposed to fluoride compound at 0.0016mg/m3 of air (Decree of the Polish Council of Minister, 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 Rzeszow Branch of the Krakow Technical University) is a mixture of natrium...
The Effect of the Pollen Extracts Quecertin and Cernitin on the Liver, Lungs, and Stomach of Rats intoxicated with Ammonium Fluoride

salts of quercitin 8.5 disulphonic acid i.e of Na2QDSA 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 T60), and as a fraction soluble in lipids (Cernitin GBX). Cernitin T60 contains from 60 to 92% of aminoacids, and Cernitin GBX from 10 to 16% of phyosterols (NIELSON et al.1987, SEPPANEN 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. 30mg/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 T60 (100 mg/kg b.w./day and simultaneously, Cernitin GBX (200mg/kg b.w./day).

Groups 5 and 13 were exposed to NH4F only.

Groups 6 and 14 were exposed to NH4F and received Quercitin at dose I (as the animals of Groups 2 and 10, respectively).

Groups 7 and 15 were exposed to NH4F and received Quercitin at dose II (as the animals of Groups 3 and 11, respectively).

Groups 8 and 16 were exposed to NH4F 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 8nm, and stained with Mayer's haematoxylin and aqueous eosin.

For histochemical analysis the tissues were immediately frozen, and after 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 glycerol-gel. In order to confirm the specificity of the particular enzymatic 5 reactions, control reactions without the substrates were simultaneously run.

Results

Histological observations

Haematoxylin 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 NH4F (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 cleat 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 *) 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 NH4F for 6 months, and given Quercitin at dose I), some parts of the liver showed obliteration of the interlobular blood vessels (Fig. 4).

Lungs. The results of histological observation 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 signs of fibrosis HE 150. Fig.3. Liver of a rat exposed for 6 months to ammonium fluoride (Group13). 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 (5mg/kg/day), (Group 14). Note some extension of capillaries, and, in at places, obliteration of the laminar structure HE x 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 x 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 x 150.

Fig.8. Lung of a rat exposed for 6 months to ammonium fluoride (Group 13). Note concretions of haemosiderin in the macrophages HE x 150. Fig.9. Lung of a rat exposed for 6 months to ammonium fluoride (Group 13). Note numerous accumulations of acidophilic leucocytes HE x 150. Fig. 10. Lung of a rat exposed for 6 months to ammonium fluoride (Group 13). Note numerous accumulations of limphoidal cells HE x 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 x 150. Fig.12. Liver of a control rat (Group 1). The activity of succinic dehydrogenase is weak x 150. Fig.14. Lung of a control rat (Group 1). The activity of succinic dehydrogenase is weak x 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 epithelial 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 limphoidal cells (Fig.9) were observed.

Similar changes, but less intense, were found in Groups 14, 15 &16 (NH4F intoxication for 6 months, and simultaneous applications of Quercitin or Cernitin), and in Group 5 (rats exposed only to NH4F 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.

Stomach. In the stomach only the morphological characteristics of the mucous membrane were analysed. 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)

Liver. 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 1Morphological changes in the lungs of rats exposed to ammonium fluoride (NH4F) and/or to the
pollen extracts Quercitin (Qu.I, dose 5mg/kg. B.w/day; Qu.II dose 20mg/kg b.w/day) or Cernitin (C., dose 200mg/kg b.w./day) during 3 and 6 months

Table 2 Activity of succinic dehydrogenase (SDH), acid Phosphatase (AcP), and alkaline phosphatase (AIP) in the liver, lungs, and stomach of rats exposed to ammonium fluoride (NH4F), and/or to the pollen extracts Quercitin (Qu.I, dose 5mg/kg b.w/day; Qu.II, dose 20mg/kg b.w./day), or Cernitin (C., dose 200mg/kg b.w./day) during 3 and 6 months

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 those exposed to NH4F and receiving pollen extracts only, the activity of SDH did not differ from that observed in the 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 NH4F, with or without simultaneous treatment with pollen extracts (Group 12, 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 NH4F 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 lungs 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 & 113), the activity of AIP was very strong, while in those receiving Quercitin , both at dose I and II, and not exposed to NH4F (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 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).

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 NH4F intoxication. As suggested by SAWICKA (1980), an increase in the level of alkaline phosphatase is often connected with the appearance of macrophages and the appearance of macrophages are probably the result of defensive processed of the lung itself, aimed directly at the toxic agent.

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 behavior of acid phosphatase was different, intoxication with ammonium fluoride reducing its activity of AIP in the liver and lungs might in this case reflect the pathological changes described in these organs following NH4F intoxication. As suggested by SAWICKA (1980), an increase in the level of alkaline phosphatase is often connected with abnormalities in transmembrane transport.

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Fig.16. Lung of a rat for 6 months receiving only Quercitin at dose II (20mg/kg b.w./day). (Group 11). The activity of succinic dehydrogenase is strong x 150.

Fig.17. Liver of a control rat (Group 9). The activity of acridophilic leucocytes is strong x 150.

Fig.18. Liver of a rat exposed for 3 months to ammonium fluoride (Group5). The activity of acid phosphatase is weak x 150.

Fig.19. Lung of a control rat (Group1). The activity of acid phosphatase is weak x 150.

Fig.20. Lung of a rat exposed for 6 months to ammonium fluoride (Group13). The activity of acid phosphatase is very strong x 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 x 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 x 150.
activity in the liver, but increasing it in the lungs. On
the basis of in vitro experiments, GALKA and
OGONSKI (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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WATTENBER L. W., LEONG T.L. 1965. Induction of increased
Experimental evaluation of the effect of pollen extract on the course of paracetamol poisoning

Juzwiak S

Studies were performed on 670 male mice of Swiss strain. Cernilton (mixture of preparation Cernitin ™ T-60-100 mg/kg and Cernitin ™ GBX--20 mg/kg emulsified by using Imwitor 370) was intraperitoneally administered in a volume of 30 ml/kg of body mass. LD50 of paracetamol was fixed after intraperitoneal administration. Certimilton was given 1 h before or 1 h after paracetamol in a dose LD100 and LD50, thereafter the survival time and the number of deceased animals were determined. The effect of Cernilton preparation on the lesion of the liver induced by paracetamol was studied in 5 groups with 10 mice each: group 1--control; group 2--paracetamol; group 3--Cernilton, after 1 h paracetamol; group 4--paracetamol; after 1 h Cernilton; group 5--Cernilton. Paracetamol was injected in the following manner: a--300 mg/kg in a single dose, estimation after 3 h, b--300 mg/kg in single dose--section after 24 h, c--230 mg/kg/24 h single dose--estimation after 24 h, d--230 mg/kg/24 h four times--section after 24 h, e--230 mg/kg/24 h--seven times--estimation after 24 h. The degree of hepatic lesion was evaluated on the basis of the activity of alanine and asparagine aminotransferase as well as alkaline phosphatase, total bilirubin concentration in serum, the content of reduced glutathione and cytochrome P-450 in the liver as well as histological and histochemical examinations (glycogen, lipids) of the liver. It has been disclosed that Cernilton increases the survival rate of animals and decreases the hepatic lesion in the course of acute paracetamol intoxication, Cernilton is the factor that effectively normalizes the biochemical and morphological indices of hepatic lesions having been caused by repeated use of toxic paracetamol dose. The therapeutic action of pollen extracts is more effective than prophylactic one. The role of glutathione is significant in the mechanism of protective activity of the pollen extract.

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Effect of pollen extract (Cernitin ™) on the course of poisoning with organic solvents

Ceglecka M

The aim of the study has been to experimentally estimate the chronic exposure of selected biochemical parameters of serum and microsomal level fraction in animals to a mixture of organic solvents. An attempt was made to alleviate the eventual changes by applying Cernitin ™ preparation. The experiment was performed on male rats, Wistar strain. The rats were exposed to the organic solvents in a toxicological chamber with controlled parameters. Cernitin ™ preparation was added to standard diet, being given to the animals in the form of balls. The biochemical investigations were carried out after a lapse of 3 and 6 month exposition. The range of the accomplished studies included: activity of enzymes (AspAT, AIAT, AP, ChE) bilirubin level and lipids content in blood serum. Lipids content was determined in liver homogenate. The content of protein, cholesterol, phospholipids and free fatty acids, was studied in liver microsomes. It has been shown that protracted exposure to the mixture of organic solvents elicits an increase in the activity of the studied aminotransferases and alkaline phosphatase, as well as a decrease in the activity of cholesterase. The changes in activity are accompanied by a rise in the content of lipids. Cernitin ™ preparation used prophylactically normalizes impairments affecting the studied enzymatic and lipid parameters.

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Effect of Pollen Extracts (Cernitin™ preparation) on Selected Biochemical Parameters of Liver in the Course of Chronic Ammonium Fluoride Poisoning in Rats

Mysliwiec Z

The aim of the paper has been the experimental evaluation of protracted exposure to ammonium-fluoride vapours exerted on selected biochemical parameters in serum as well as microsomal fraction of the liver in animals. An attempt was made to ameliorate eventual changes by using Cernitin™ preparation. The experiment was performed on male rats of Wistar strain. The rats were exposed to NH4F in a toxicological chamber with controlled parameters. Cernitin™ was added to standard diet and given to animals in the form of balls. The studies were carried out after 3 and 6 month-long exposure. The range of the performed studies covered: activity of enzymes (AspAT, AIAT, AP, ChE) and content of bilirubin as well as lipids were studied in the blood serum. Content of proteins, cholesterol and phospholipids was investigated in the liver homogenate. It has been shown that chronic exposure to NH4F vapours causes a rise in the activity of studied aminotransferases and alkaline phosphatase, and a decreases in activity of cholinesterase. The changes in activity were accompanied by an increase in the content of lipids. Prophylactic application of Cernitin™ preparation normalizes the disorder involving the studied enzymatic and lipid parameters.

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Zakładu Toksykologii Instytutu Farmakologii i Toksykologii Pomorskiej Akademii Medycznej
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 orally 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 galactosamine-induced damage of the liver. The aim of the present report is an examination of the effect of Cernitins on the hepatitic injury evoked by 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 analysed 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 hematoxilin 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/l, 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 AA1AT 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 (µU/ml): alanine aminotransferase (AA1AT), 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 located 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 pollicariocytes 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 reveal 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 cytoplasms 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 (group *) 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 acrylco 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 cyclicity it is proposed that liver protecting substances are applied 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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The Effect of Pollen on the Changes in the Liver of Laboratory Rats Evoked by Ethionine, Carbon Tetrachloride, Allyl Alcohol and Galactosamine

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Doses of 50 mg/kg body weight and 200 mg/kg of Cernitin™ T 60 and Cernitin™ GBX may be used over 14 days for effective protection of rat liver cells from toxic action of ethionine. Application of CCl4 caused damage to the liver of rats. Such damage may be mitigated by both Cernitin™ preparations, particularly by Cernitin™ T 60. The damage was further reduced by Cernitin, following administration of allyl alcohol, with increase in transaminase, phosphatase, and bilirubin activities being used as criteria for measurement. The liver-protecting effect of Cernitin™ was confirmed in histopathological investigations. Cernitin™ prevented much of the damage actually caused by galactosamine.

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The Effect of Cernitins on Galactosamine-Induced Hepatic Injury in Rat

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Cernitins correspond to microbiologically fermented pollen extract. Cernitin T60 contains mainly water soluble, while Cernitin GBX mainly fat-soluble substances. The aim of the present work was to examine the effect of Cernitins on the d-galactosamine-induced liver damage in rat. It has been shown, that galactosamine administration to rat resembles viral hepatitis both biochemically and histologically. Our studies proved, that Cernitin T60 given orally or intraperitoneally inhibited or counteracted the elevation of amino transferases activity and the inflammatory process, necrosis and steatosis of the liver cells. The protective effect of Cernitin GBX on the liver parenchyma was only slightly expressed. It is concluded, that application of pollen extracts in patients with liver diseases should be considered.

Previously we have demonstrated the protective effect of linden pollen and Cernitins against fatty infiltration, carbon tetrachloride, and ethionine-induced liver damage. However, hepatic impairment as the result of carbon tetrachloride and ethionine poisoning in laboratory animals does not necessarily represent an adequate experimental model of liver disease in humans. So, we decided to examine the effect of Cernitins in the galactosamine model in rats. Galactosamine administration induces an inflammatory response in the liver that in some aspects resembles the reaction seen clinically in viral hepatitis.

MATERIALS AND METHODS

Ten groups of ten Winstar male rats weighing 190-250 g were used. Animals of group 1 served as the controls. Rats of group 2 received galactosamine. The remaining groups were treated with Cernitins for five days and challenged with galactosamine; four groups were given Cernitin T60: 50mg/kg per day (p.d.), orally (p.o) by intubation (group 3), 200mg/kg p.d., p.o. (group 6). The further four groups received Cernitin GBX: 50mg/kg p.d., p.o. (group 7), 200 mg/kg p.d./ p.o. (group 8), 50 mg/kg p.d., i.p. (group 9) and 200mg/kg p.d., i.p. (group 10). The animals were challenged with d-galactosamine hydrochloride on the third day at the dose of 400 mg/kg i.p. three times within 24 h. They were fasted for 16 h prior to autopsy.

D-galactosamine hydrochloride was purchased from E. Merck (Darmstadt) and Cernitins were kindly delivered by AB Cernelle (Vegeholm, Sweden).

After a mild ether anesthesia, the thorax of the animals was opened and blood was drawn from the ascending aorta. In the blood serum the following biochemical parameters were determined: alanine aminotransferase (A1AT) and aspartate aminotransferase (AspAT) activity according to Reitman and Frankel, alkaline phosphatase activity according to the method of Bodansky and bilirubin level by the method of Malloy and Evelyn. The results were analysed 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), for the lipids presence with Sudan black and for collagen fibres with Van Gieson’s mixture.

RESULTS

D-Galactosamine treatment of rats resulted in a marked increase of enzymes activity and bilirubin concentration in the blood serum of animals (Table 1). When compared to the control group, the challenging dose of galactosamine resulted in a 47-fold increase in A1AT activity and 17-fold increase in AspAT activity; alkaline phosphatase activity was elevated by 600% in these animals. Cernitin T60 application was associated with a significant and marked drop of
A1AT, AspAT, and alkaline phosphatase activity as well as the bilirubin concentration in the blood serum as compared with group 2. In rats of group 3, i.e. receiving Cernitin T60 50mg/kg orally, AspAT and alkaline phosphatase activity and bilirubin level remained normal, while A1AT activity appeared to be close to the value established for the control group. Cernitin GBX treatment of rats injected with galactosamine caused distinctly smaller decrease of the examined biochemical parameters than in Cernitin T670 treated animals. Amino-transferrases activity was markedly lower after intraperitoneal administration of Cernitin GBX, as compared with oral dosing. Oral administration of Cernitin GBX 200mg/kg (group 8) did not produce significant changes in A1AT and alkaline phosphatase activity as well as in bilirubin concentration, in comparison with animals receiving galactosamine alone (group 2).

Table 1. Serum alanine aminotransferase (A1AT), aspartate aminotransferase (AspAT), alkaline phosphatase (AP) activity and total bilirubin (B0 level) in rats receiving Cernitin T60, Cernitin GBX and treated with galactosamine (G). Mean ± SE. P.o= orally, i.p.= intraperitoneally

Extensive morphological alterations resembling viral hepatitis were observed in liver sections taken from rats challenged with galactosamine (Fig. 1). Numerous and huge cellular infiltrations comprised mononuclear cells, mainly lymphocytes, and mononuclear phagocytes as well as few granulocytes, accompanied by massive parenchymal cell lysis and vacuolar degeneration, visible in portal spaces and in marginal zones of the lobules (Fig. 2). Cellular infiltrations in sinusoidal vessels surrounded the damaged parenchymal liver cells. Furthermore, fine size droplet fatty degeneration appearing in the peripheral, intermediary and central zones of the lobule was also observed (Fig. 3). A slight increase in fibrous connective tissue existed and there were islands of acute necrosis throughout the section. Protective effect of Cernitin T60 against galactosamine-induced hepatic alterations was evident (Fig. 4). There were no signs of fatty degeneration and necrosis. Cellular infiltrations were almost not visible, slight dilatation of sinusoids as well as moderate hyperemia occurred (fig. 5). Only single mononuclear leukocytes of varying size could be seen in the portal space (Fig. 6). Liver of rats treated with galactosamine did not show quite evident protective action of orally administered Cernitin GBX on the parenchymal liver cells. Extensive leukocyte infiltration was visible (Fig. 7) and fatty degeneration of the liver cell appeared (Fig. 8). However, in rats receiving Cernitin GBX intraperitoneally a slight positive effect could be observed: cellular infiltration necrosis and fatty degeneration of the liver cell was decreased as compared with animals receiving galactosamine alone. Summary of histopathological studies is presented in Table 2.

Fig. 1. Light microscopic appearance of rat liver treated with galactosamine resembling damage evoked by viral hepatitis. Stain: HE. Magn.: x 130

Fig. 2. Liver of a rat treated with galactosamine. Huge leukocyte infiltration of portal space accompanied by massive parenchymal cell lysis and vacuolar degeneration in marginal zones are clearly visible. Stain: HE. Magn.: x 270.

Fig. 3. The multiple fine size droplet degeneration of parenchymal liver cells of a rat treated with galactosamine. Stain: Sudan black. Magn. x 270

Fig. 4. Liver from a rat treated with Cernitin T60, 50 mg/kg orally and challenged with galactosamine. The picture demonstrates the beneficial effect of Cernitin T60 on galactosamine-induced hepatic injury. No signs of leukocyte infiltration, no such a heavy damage of hepatocytes as in the liver of a rat receiving galactosamine alone can be seen. Stain HE. Magn. x 130

Fig. 5. Liver from a rat, which was administrated Cernitin T60 200 mg/kg orally. There are no signs of significant injury of the liver cells. Dilatation of sinusoids and hyperemia occurs. Stain: HE. Magn. x 550

Fig. 6. Grouping of single mononuclear leukocytes of varying size in the portal space of liver from a rat poisoned with galactosamine and protected with Cernitin T60 (50 mg/kg i.p.). The hepatocytes do not demonstrate clear signs of degeneration. Stain: HE. Magn. x 550

Fig. 7. Liver from a rat treated with galactosamine does not show the protective action of Cernitin GBX applied orally 200mg/kg. Massive leukocyte infiltration of marginal zones of the lobules followed by degeneration or lysis of parenchymal cells is visible. Stain: HE. Magn. x 270

Fig. 8. Numerous Sudan-positive granules in the liver of a rat given Cernitin GBX (200 mg/kg orally) and galactosamine. The range of lipid degeneration is almost the same as after administration of galactosamine alone. Stain: Sudan black. Magn. x 270

Table 2. Summary of histopathological abnormalities

Discussion

The liver can be damaged and its functional properties affected in a multiple os ways. The damage may be caused by the direct action of toxic substances or may result from secondary reactions. Direct action is associated with d-galactosamine. It has been shown, that the degree of severity of galactosamine-induced hepatitis is dependent on the species of animals used. Galactosamine administration to rats resembles viral hepatitis both biochemically and histologically.

Exposure of rats to three intraperitoneal doses of galactosamine, three times with in 24h, caused acute hepatitis with increase of AspAT and A1AT activities and intensive histopathological abnormalities of the liver including cellular infiltration, necrosis, fatty degeneration and fibrosis. Amino-transferrases, especially A1AT represent highly specific index of hepatocellular injury and are much more sensitive to minimal or moderate damage of the liver than the other hepatic function tests. The amino-transaminases activity in blood serum is parallel to the degree of liver cell injury. This was confirmed by our investigations, also in respect to prevention of the occurrence of hepatic damage. Elevation of the A1AT activity indicates, that one of the first hepatic responses to galactosamine poisoning comes from parenchymal cells and involves the activity of one or...
more enzymes. Galactosamine toxicity causes the appearance of specific lesions in the liver cells, one characterized by inhibition of nuclear RNA synthesis accompanied by nuclear fragmentation, the other by inhibition of protein synthesis followed by accumulation of aggregates between the stacks of rough endoplasmic reticulum. Since the administration of uridine prevents and reverses in vivo inhibition of both synthesis but not the accumulation of aggregates, it is likely that am acute deficiency of uridine triphosphate due to accumulation of stable uridine diphosphate-galactosamine if the intrinsic mechanism of toxicity.

Our studies proved that Cernitins, corresponding to microbiologically fermented pollen extracts, protected against liver injury caused by d-galactosamine. Cernitin T60 administered both orally and intraperitoneally bought about a rapid, significant reversion to normal or almost normal amino-transferases and alkaline phosphatase activity as well as elevated bilirubin level. Also the damage observed histologically disappeared in animals receiving Cernitin T60, that inhibited or counteracted the inflammatory process, necrosis and steatosis of the liver cell. The protective effect of Cernitin GBX on the liver parenchyma was only slightly expressed. Inhibition of ethionine-induced rat liver injury by pollen extracts was demonstrated by us previously. In rats treated with ethionine and receiving Cernitins we noticed the increased number of nucleoli, attributed probably to the accelerated synthesis of nuclear RNA. Thus, the positive effect of Cernitins may be in consequence due to the potentiated synthesis of proteins exhibiting protective properties against the liver cell injury.

On the other hand, the definite anti-inflammatory action of Cernitin extracts was revealed in the case of croton oil-induced oedema. In the cotton pellet test in rats Cernitin T60 showed an anti-inflammatory activity corresponding to the inflammation-inhibiting effect of phenylbutazone, but was completely devoid of toxicity. It was also possible to confirm the anti-inflammatory action when compared with very active intraperitoneally injected anti-inflammatory agents. It has been proved that the action of pollen extracts is not due to the liberation of corticosteroids.

It is undeniable that Cernitin T60 prevents not only necrosis, but also lipid accumulation. Cernitin T60 has clearly visible lipotropic effect. The mechanism of this action is unknown. It can be attributed to the supply of SH groups which come from methionine and cystine contained in pollen. However, our conclusions favour the polyfactorial basis of the effect of Cernitin T60 on the galactosamine induced liver injury.

Finally, the results of our investigation suggest the possibility of pollen extracts application in patients suffering from acute and chronic liver disease, especially that Cernitins are practically nontoxic and show excellent tolerance properties.

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