

## LIVER SUPPORT:

GRAMINEX Flower Pollen Extract

### The Protective Effect of Pollen Extracts Against Allyl Alcohol Damage of the Liver

By

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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, AlAT) 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 hepatic 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  $\alpha$ -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 rats was performed and blood was collected for biochemical tests: alanine aminotransferase (AlAT) 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 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 AlAT 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/l (Table 1). Simultaneously,

total bilirubin concentration was elevated from 4.08 to 12.07  $\mu\text{mol/l}$ , 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 ALAT 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  $\pm$  SE)

*Table 2.* Total bilirubin level ( $\mu\text{mol/l}$ ) and liver weight (g/100g body weight) of rats receiving allyl alcohol (AA) and treated with Cernitin T60 and Cernitin GBX (mean  $\pm$  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 polycariocytes 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 elongated 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 as 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 induced 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 precedes or 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 its split up products- acrolein and acrylo 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 administered. 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 administered 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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