



LIVER SUPPORT:

GRAMINEX Flower Pollen Extract

Effect of Cernilton on the Hepatotoxicity of Carbon Tetrachloride [CCl₄] 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 CCl₄/0.25 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 CCl₄ application.

Seventy two animals were divided into six equal groups:

group 1 – controls,
group 2 – received CCl₄,

group 3 – rats were given CCl₄ and Cernitin T60 50 mg/kg by a stomach tube [p.o.],
group 4 – animals were administered CCl₄ and Cernitin T60 50 mg/kg intraperitoneally [i.p.],
group 5 – received CCl₄ and Cernitin GBX 50 mg/kg by a stomach tube [p.o.],
group 6 – rats were given CCl₄ and Cernitin GBX 50 mg/kg i.p

The animals were fasted for 16 h prior to autopsy. After 24 h from CCl₄ 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 CCl₄ 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 CCl₄ [group 4] as compared with group 2. Diminution of these 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 CCl₄ showed a bilirubin level that was increased to 1.93 $\mu\text{mol/l}$ in comparison with the control value 0.03 $\mu\text{mol/l}$ [Table 2]. Cernitin T60 reduced bilirubin level, especially when the drug was administered intraperitoneally, from 1.93 $\mu\text{mol/l}$ [group 2] to 0.10 $\mu\text{mol/l}$ [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 CCl₄ 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 CCl₄ 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 CCl₄ 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 and 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 CCl₄. 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 [$\mu\text{mol/l}$] in rats receiving carbon tetrachloride

Group	Treatment	Mean \pm SE
1		0.03 \pm 0.001
2	CCl ₄	1.93 \pm 0.21
3	CCl ₄ + T60 p.o.	1.13 \pm 0.28
4	CCl ₄ + T60 i.p.	0.10 \pm 0.02
5	CCl ₄ + GBX p.o.	0.38 \pm 0.06
6	CCl ₄ + GBX i.p.	1.76 \pm 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 \pm SE
1		6.7 \pm 0.12
2	CCl ₄	6.6 \pm 0.11
3	CCl ₄ + T60 p.o.	6.1 \pm 1.67
4	CCl ₄ + T60 i.p.	6.1 \pm 0.10
5	CCl ₄ + GBX p.o.	6.4 \pm 0.10
6	CCl ₄ + GBX i.p.	6.0 \pm 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

