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HEPATOPROTECTIVE EFFECT OF 2,6-DIMETHYLPYRIDINE N-OXIDE (IVIN) IN EXPERIMENTAL MODEL OF CCl_4-INDUCED HEPATITIS OF RATS

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The effect of concomitant pesticides and plant growth regulators on humans is still not well understood. N-oxide-2,6-dimethylpyridine (Ivin) is the plant growth regulator known to reduce the acute toxicity of pesticides, but its protective mechanisms need to be investigated. The aim of the study was to assess the hepatoprotective ability of 2,6-dimethylpyridine N-oxide (Ivin) using a model of acute CCl_4-induced hepatitis. Male Wistar Han rats received two subcutaneous CCl_4 injections (0.8 ml/100 g b.w.). Oral Ivin (13 or 0.13 mg/kg) and hepatoprotector "Silybor-35" (5 mg/kg) as reference substances were administered orally one hour pre- and 2 hours post-CCl_4 injection. The biochemical assay of blood plasma, estimation of lipid peroxidation products in the liver tissue and histological liver analysis were done. The results of functional tests and histomorphological studies of liver tissue demonstrated that Ivin exhibited a pronounced hepatoprotective effect, more pronounced when it was administered at a low 0.13 mg/kg dose. Calculation of the hepatoprotection efficiency index for Ivin showed that it was comparable to that for "Silybor-35".

K e y w o r d s: CCl_4-induced hepatitis of rats, Ivin, hepatoprotective effect.

Pesticides are well-known environmental pollutants, which can cause significant harm to human health and have serious long-term effects, having entered the human body both solely and in combination with other agrochemicals and environmental pollutants [1-4]. Since the liver is the main organ responsible for biotransformation and detoxification of xenobiotics, and it is constantly exposed to pollutants entering the body through air, water, and food, its hepatobiliary system and homeostasis as a whole may be damaged by the pollutants to the extent depending on the exposition dose and duration [2, 5-8].

Modern techniques for protecting plants from diseases and pests are mostly dependent on combined substances and bulk mixtures thereof. The latter include plant growth regulators (PGRs) as well. In many cases, a combined effect of pesticides on the human body is associated with potentiation or summation of toxic effects [9-12]. Unfortunately, the combined effect of pesticides and PGRs in the case of long-term exposure of a living organism has not been sufficiently studied yet.

Some works [13-15] show that the acute toxicity of the above pesticides may be significantly reduced in case of combined one-time exposure of a living organism to some organophosphorus, pyrethroid, triazole, neonicotinoid plant protection substances and PGRs, including 2,6-dimethylpyridine N-oxide (Ivin). In the case of 13-week-long combined exposure to organophosphorus insecticide Chlorpyrifos and Ivin, the latter was found to neutralize the hepatotoxic effect of chlorpyrifos and reduce its anticholinesterase effect on the brain, which contributed to an easier intoxication course. Authors point out that the protective effect may be associated with the Ivin's hepatoprotective and antioxidant effect.

Based on the above, it looks reasonable to study the hepatoprotective effect of Ivin using the classic model of acute hepatitis caused by carbon tetrachloride (CCl_4). The study may clarify the mechanisms of the Ivin protective action and can be used both for the development of preventive measures in case of acute and chronic intoxications, and plant protection techniques.

Aim of the study. Study of the hepatoprotective properties of 2,6-dimethylpyridine N-oxide (Ivin) in the classic model of acute hepatitis caused by carbon tetrachloride.

Materials and Methods

Materials. For study purposes, we used PGR Ivin, 2,6-Dimethylpyridine N-oxide (99.9%, liquid, clear colorless to yellow, miscible with water), produced by NE ISTC "Agrobiotech", Kyiv, Ukraine. The comparison/reference product was a well-known Silymarin-based hepatoprotective medicine Silybor-35 (active substance: dry extract of Silybum marianum (22-27:1, extractant – acetone 95%), equivalent to Silymarin 35 mg, batch UA/5114/01/01, Pharmaceutical company "Zdorovia", LTD, Kharkiv, Ukraine). For simulation of acute hepatitis, we used carbon tetrachloride produced by Scientific-Industrial Enterprise "Alfarus".

Test system. The study was conducted on mature male Wistar Han rats (SPF), which were obtained from the SPF nursery for small laboratory animals belonging to the SE "L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical, Ministry of Health, Ukraine" and transferred to the SPF vivarium with preserved animal status. During the experiment, the animals consumed balanced granulated feed 1324 P manufactured by Altromin (Germany) and received ad libitum deionized, UV- and reverse osmosis-treated filtered potable water from plastic bottles through metal dispensers. Water and bedding material were subject to periodic microbiological control.

Simulation of acute toxic hepatitis. According to the Methodical recommendations [16], to study Ivin hepatoprotective effect, 30 experimental CCl_4-induced toxic hepatitis male Wistar Han model rats were used.

To reproduce acute toxic liver damage, male rats were subcutaneously administered 50% CCl_4 solution in petroleum jelly oil at a dose of 0.8 ml/100 g of body weight for 2 days.

Curative regimen doses of Ivin and reference substance Silybor were administered orally, 1 h pre-CCl_4 and 2 h post-CCl_4. Ivin was studied at doses of 13 mg/kg and 0.13 mg/kg, which corresponded to 1/100 and 1/1000 of LD_{50}, while Silybor was tested at a dose of 5 mg/kg (therapeutic dose for humans recalculated for rats). Both Ivin and Silybor were administered as water solutions. The control group of animals were administered the same volume of subcutaneous petroleum jelly oil. Each experimental group consisted of 6 animals. Life indicators were taken 24 h after the last CCl_4 administration.

Research on an animal model was conducted in accordance with the guidelines of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the principles of bioethics and the requirements of the Medical and Biological Research Ethics Commission of the "L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical, Ministry of Health, Ukraine" (State Enterprise) (Minutes No. 9/1 dated 09 May 2020).

Study methods. Blood and serum sampling.

For biochemical studies, blood samples were quickly collected from the femoral vein after carbon dioxide sedation with further placement to centrifuge tubes moistened with a heparin/saline solution (1:3). To retrieve blood serum, we used Elmi CM - 6M centrifuge unit (Latvia) at 3000 rpm for 10 min.

Biochemical blood assay. We tested blood serum for the cytolytic activity markers, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), calculated the De Ritis ratio [17]. The cholestasis severity was assessed by the alkaline phosphatase (AP) activity indicators. To assess the functional-and-metabolic activity of the liver, the total protein, cholesterol, total bilirubin, and glucose were studied using standard sets of reagents manufactured by Bio Systems (Spain) and Vitalab Flexor E biochemical analyzer unit (Netherlands). The urea and creatinine levels were determined using sets of reagents for clinical biochemistry produced by company LLC NPP Filisit-Diagnostics (Ukraine) and Thermo Spectronic Helios Alpha 9423 UVA spectrophotometer (UK).

Catalase activity. The state of the antioxidant system was assessed by the activity of one of the key enzymes, namely catalase (KF 1.11.1.6, H_2O_2: H_2O_2-oxidoreductase) in the blood serum. Catalase catalyzes the cleavage of the O-O bond in the H_2O_2 molecule to form molecular oxygen and water. Catalase is localized in peroxisomes, the cytosol of various cells, and mitochondria. Its activity is greatest in the liver and erythrocytes, but lower in the blood serum. In clinical practice and for scientific purposes, the catalase activity in the blood serum is often measured to assess the degree of oxidative stress and endogenous intoxication [18]. The catalase activity was assessed by the ability of hydrogen peroxide to form a stable yellow complex with molybdate salts, the color intensity of which depended on the content

of H_2O_2 in the solution remained intact following catalase activity. A 410 nm wavelength was used for spectrophotometry [19].

Liver sampling for biochemical assay. After blood collection and decapitation of rats, their abdominal cavities were quickly opened, the liver was removed, washed from the blood in cold (+4°C) saline, blotted with filter paper, and placed in a Petri dish on ice. To measure malondialdehyde (MDA), we used 1.0 g of the liver weighed and homogenized in 0.05 M Tris buffer (pH 7.4). To measure other lipid peroxidation (LP) products, we used 0.9% NaCl solution containing 3 mM EDTA.

Measuring lipid peroxidation products. To characterize the state of pro-oxidant system, we studied the MDA content in liver tissues by a pink trimethine complex reaction upon interaction of malondialdehyde with 2-thiobarbituric acid. The supernatant was subject to spectrophotometry at a wavelength (λ) of 532 nm [20]. The levels of diene conjugates (DC), ketodienes (KD), conjugated trienes (CT), and final Schiff base (SB) were measured using the Extractive Spectrophotometric Method [21]. The liver tissues were extracted in the heptane-isopropanol phase. The photospectrum of heptane and isopropanol lipid extract was analyzed at wavelengths of 220, 232, 278 and 400 nm in a cuvette with a 10-mm-thick layer of the corresponding material. The results were presented in oxidation index units (o.i.u.): E_{232}/E_{220} – relative DC content; E_{278}/E_{220} – relative KD and CT content; and E_{400}/E_{220} – relative SB content [21].

Histological liver analysis. For histological studies, we took liver samples from 6 male rats of each group. The samples were fixed in a 10% neutral formalin solution, dehydrated in an alcohol battery of increasing concentration, and embedded in paraffin blocks. Following that, 5-7 μm sections were made using Microm HM 325. Deparaffinized sections were stained with hematoxylin and eosin (H&E) according to the standard technique adopted for morphological studies [22]. Histological preparations were analyzed using OPTON Axioskop light microscope (West Germany) and photographed using a Canon EOS 1000D digital camera (Japan).

Hepatoprotective index. To measure the hepatoprotective effect of Ivin and the reference medicine Silybor, the efficiency index EI (%) was calculated in relation to a positive control (CCl_4).

EI hepatoprotective action was calculated by the formula:

$$EI=(I_{control} - I_{study})/I_{control}\times 100,$$

where EI (%) – a percentage difference in the severity of liver damage in the control group and groups of animals that received the study medicine;

$I_{control}$ and I_{study} are the average indices obtained in the control and study groups, respectively.

EI was calculated separately using enzymatic markers of liver damage (ALT, AST, AP), functional indicators (total bilirubin, total protein, cholesterol, glucose, urea), the levels of LP products in liver tissues in the heptane phase, and by catalase activity.

A positive EI value (plus-effect) indicates a decrease in the damage magnitude. A negative EI value (minus-effect) indicates an increase in the damage magnitude.

Statistics. The results were shown as the arithmetic mean (M) and standard error of the mean (±m). Data variables between groups were analyzed by parametric one-way ANOVA followed by Fisher's LSD post-hoc test. The level of statistical significance was $P \leq 0.05$.

Results and Discussion

The rat model biochemical indicators following Ivin and Silybor administration with underlying CCl_4 are given in Table 1.

As one can see from the data presented in Table 1, CCl_4 caused statistically significant changes in all the studied indicators, except for the blood serum creatinine. The ALT and AST readings increased by 94.88% and 39.23%, respectively, while De Ritis ratio decreased by 28.66%. We also observed AP elevation by 81.40%. The total serum protein and glucose decreased by 5.19 and 23.27%, respectively. The total bilirubin, cholesterol, and urea readings grew by 31.19, 38.83, and 8.05%, accordingly.

The combined effect of CCl_4 and Ivin at a dose of 13 mg/kg caused milder changes in biochemical blood serum indicators than in case of sole CCl_4. Thus, the ALT and AP readings increased by 43.06 and 22.14%, respectively, in relation to the intact control. De Ritis ratio dropped by 15.93%. The blood serum urea increased by 21.73%, while glucose content decreased by 11.9%. We did not observe statistically significant changes in total protein, total bilirubin, cholesterol and creatinine.

No changes in total protein, total bilirubin, creatinine, urea, cholesterol and glucose were observed under the concomitant CCl_4 and Ivin administration at a dose of 0.13 mg/kg. The activity of cytolysis and

Table 1. Changes in the biochemical indicators of male rats after oral administration of Ivin and Silybor under the conditions of acute hepatitis caused by carbon tetrachloride (M ± m, n^a = 6)

Indicator	Control	CCl_4, 0.8 ml/100g	Ivin, 13 mg/kg	Ivin, 0,13 mg/kg	Silybor, 5 mg/kg
AP, U/l	234.83 ± 10.23	426.00 ± 15.97[b]	286.83 ± 13.58[b,c]	293.67 ± 21.16[b,c]	282.33 ± 17.64[b,c]
ALT, U/l	54.88 ± 3.30	106.95 ± 8.35[b]	78.51 ± 9.24[b,c]	73.25 ± 3.69[b,c]	75.64 ± 4.71[b,c]
AST, U/l	86.20 ± 4.93	120.02 ± 8.43[b]	103.88 ± 4.94	107.39 ± 7.75[b]	98.91 ± 13.15[c]
De Ritis ratio	1.57	1.12	1.32	1.47	1.31
Total proteins, g/l	54.23 ± 0.90	51.08 ± 0.79[b]	54.23 ± 1.39[c]	52.35 ± 1.19	51.50 ± 0.75[b]
Total bilirubin, μmol/l	6.99 ± 0.33	9.17 ± 0.67[b]	7.83 ± 0.17	8.38 ± 0.51	7.40 ± 0.83[c]
Cholesterol, mmol/l	1.03 ± 0.05	1.43 ± 0.10[b]	1.17 ± 0.11[c]	1.09 ± 0.11[c]	1.12 ± 0.08[c]
Glucose, mmol/l	7.82 ± 0.13	6.00 ± 0.34[b]	6.89 ± 0.30[b]	7.22 ± 0.23[c]	7.05 ± 0.22[b,c]
Urea, mmol/l	9.57 ± 0.17	10.34 ± 0.26[b]	11.65 ± 0.22[b,c]	9.67 ± 0.21[c]	9.08 ± 0.24[c]
Creatinine, μmol/l	108.36 ± 8.40	98.71 ± 6.00	102.12 ± 9.60	113.70 ± 6.50	166.79 ± 12.01[b,c]

Note: [a]Number of animals in the group; [b]$P \leq 0.05$ in relation to the control; [c]$P \leq 0.05$ in relation to carbon tetrachloride ($P \leq 0.05$ – significantly different between groups by using Fisher's LSD post-hoc test)

cholestasis enzymes was almost at the same level as Ivin action at a dose of 13 mg/kg. Thus, the blood serum ALT, AST, and AP readings increased in relation to the intact control figures by 33.47%, 24.58% and 25.06%, respectively. The De Ritis ratio was slightly different from the control. No statistically significant changes in other indicators were found.

The reference medicine Silybor at a dose of 5 mg/kg caused the lesser harmful effect of CCl_4 on the liver. Thus, compared to the intact control, the ALT and AP readings showed statistically significant elevation by 37.83% and 20.23%, respectively. The De Ritis ratio was 1.31, which was 16.6% less than the one in the intact control animals. Creatinine statistically significantly increased by 53.92%, while glucose content decreased by 9.85%. Observed total serum protein decreased by 5.03% ($P \leq 0.05$). No changes in other studied biochemical indicators were observed.

Histological changes in the liver tissues of intact male rats under CCl_4, Ivin and Silybor are presented in Figure, *A-E*.

Histomorphological studies of liver tissue samples taken from model animals of the intact control group presented no signs of damage (Fig., *A*).

As one can see from Fig. (*B*), CCl_4 at a dose of 0.8 ml/100 g of body weight caused small- and medium-droplet vacuolization of centrilobular hepatocytes, balloon degeneration and necrosis of individual hepatocytes; inflammatory cell infiltrates and isolated cholestasis signs were found at the necrotized sites. At the same time, the hepatocytes in the periportal and intermedial zones of all tested samples presented no pronounced changes. The visual examination of the liver samples revealed the tissue damage area of 30-40%, 40%, and 40-50% in every 2 animals of the total group of 6 subjects.

In the case of Ivin in both studied doses (Fig., *C* and *D*), the reference medicine Silybor (Fig., *E*) with concomitant CCl_4, histological changes in the liver structure were similar to the changes caused by sole CCl_4, were unidirectional in nature, but they differed slightly in severity and area of liver damage. Namely, the visual examination of liver samples revealed a 20-30% area of tissue damage under Ivin at a dose of 13 mg/kg in 3 animals, a 40% damage area in 2 animals, and a 50-60% damage area in one animal. In the case of Ivin at a dose of 0.13 mg/kg, the area of tissue damage was smaller compared to a higher dose and amounted to 20-30%, 30-40%, and 50% in

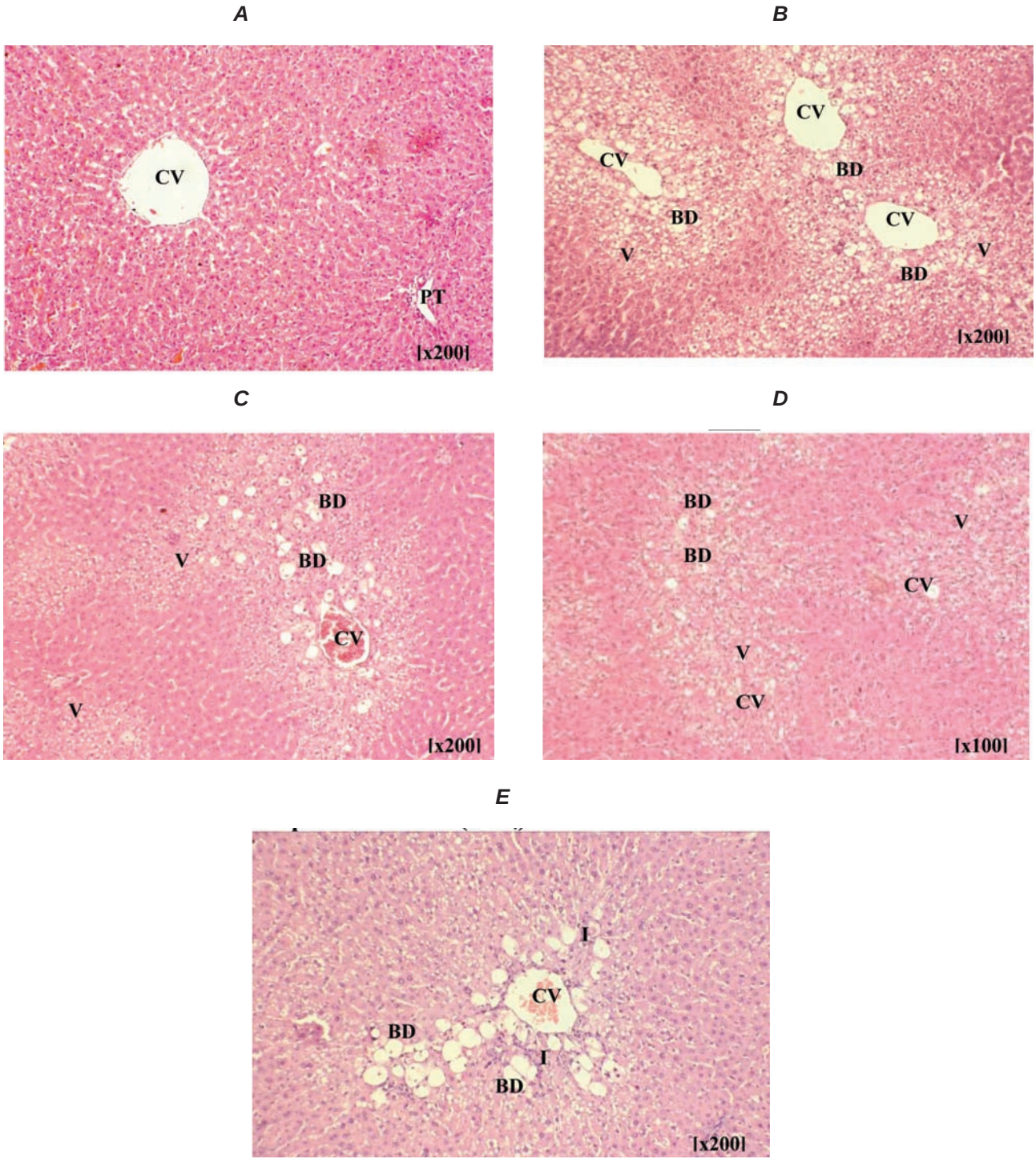

*Fig. Typical histological picture of the liver of control model rats (**A**), rats with CCl_4-induced hepatitis (**B**), rats administered Ivin at doses of 13 mg/kg (**C**) and 0.13 mg/kg (**D**), and rats treated with reference medicine Silybor (**E**) with concomitant CCl4 (H&E). **A** – intact control, no pathological changes, **B** – positive control (CCl_4), pronounced dystrophic changes of hepatocytes around the central veins, **C** – Ivin in a dose of 13 mg/kg with concomitant CCl_4, **D** – Ivin at a dose of 0.13 mg/kg with concomitant CCl_4, **E** – Silybor at a dose of 5 mg/kg with concomitant CCl_4: CV – central vein, PT – portal tract, BD – balloon degeneration of hepatocytes, V – vacuolization of hepatocytes, I – inflammatory cellular infiltrate*

three, two and one model animals, respectively. In the case of Silybor, the area of tissue damage was 10-20%, 20-30%, and 30-40% in one, one, and four animals, respectively.

Ivin and Silybor with concomitant CCl_4 demonstrated much lesser changes in the structure of rat liver than those caused by sole CCl_4.

The obtained data suggest that CCl_4 in the studied dose caused liver damage, characterized by a significant increase in the activity of cytolysis marker enzymes – ALT and AST, a decrease in De Ritis ratio, and an increase in AP activity, resulting in a damage of hepatocyte structure and the development of intrahepatic cholestasis. A drop of total protein, glucose, and an elevation of cholesterol in the blood serum indicate a protein-synthetic dysfunction of the liver, and a disturbance of carbohydrate and lipid metabolism. Elevated content of total bilirubin may indicate a metabolic dysfunction of the liver. The CCl_4-driven moderate acute hepatitis is evidenced by the results of histomorphological studies; it is characterized by the formation of small and medium-sized fatty dystrophy of hepatocytes, balloon degeneration, necrosis of individual hepatocytes, micro-foci of lympho-leukocyte infiltration, and hepatocellular cholestasis. A degree of alteration expressiveness varies from weak to moderately pronounced. The nature, trend, and severity of changes in the functional state and liver structure under acute exposure to CCl_4 are comparable to data from the literature [23].

Influenced by Ivin in the studied doses with concomitant CCl_4, changes in the activity of cytolytic and cholestatic markers were expressed to a lesser extent than in the case of sole CCl_4 and slightly differed from the figures of the reference substance. Silybor was associated with a slightly better AST recovery than the one in the case of Ivin. Among the studied parameters characterizing the functional and metabolic liver properties, Ivin restored serum protein synthesis to a greater extent in comparison with Silybor, but total bilirubin was restored to a lesser extent than in the case of Silybor. As for other indicators, the protective effect of Ivin was dose-dependent. Under Ivin, the expressiveness of the effect represented by cholesterol, glucose, and urea readings was the greatest at a dose of 0.13 mg/kg, and was higher than under the one associated with Silybor.

The histomorphological studies showed that Ivin at a dose of 0.13 mg/kg and Silybor with concomitant CCl_4 caused approximately the same changes in the liver structure, manifested to a lesser degree than in the case of sole CCl_4. Influenced by Ivin at a dose of 13 mg/kg, the manifestations of dystrophic changes in hepatocytes were somewhat weaker than those influenced by sole CCl_4 and more moderate than those influenced by Silybor. The combined effect of both Ivin + CCl_4 and Silybor + CCl_4 was characterized by the development of small- and medium-sized fatty dystrophy of hepatocytes combined with balloon degeneration of hepatocytes.

The hepatotoxic effect of CCl_4 is known to be based on boosting free-radical processes and lipid peroxidation [24, 25]. Accumulation of LP products, in particular DC and MDA, which disrupts the structure and function of various body systems, serves as markers of free radical activation. Activation of enzymes of the antioxidant system causes slowing down or interruption of free radical processes in the body, which leads to restoration of the body's condition.

Effects of Ivin and Silybor with concomitant carbon tetrachloride on the state of pro-oxidant and antioxidant systems are presented in Table 2.

As we can see from Table 2, CCl_4 acute influence caused a statistically significant elevation of MDA level in liver tissues by 86.50%. In the heptane phase, an elevation of DC, KD and CT, and SB by 280.30, 142.22, and 370.00%, respectively, was observed. In the isopropanol phase, the accumulation of LP products was somewhat lower. There were statistically significant growth of DC by 25.71%, KD/CT and SB by 130.0 and 180.0%, respectively. The blood serum catalase activity statistically significantly decreased by 23.86%.

Therefore, CCl_4 acute intoxication caused an increase of primary (DC), secondary (MDA, KD and CT) and final (SB) LP products, while the activity of catalase, an enzyme that utilized peroxide radicals (H_2O_2) decreased, which contributed to the development of liver pathology. The discovered effects of CCl_4 acute exposure are consistent with the literature data [26].

No statistically significant changes in MDA, DC, KD and CT, and SB concentration were found under Ivin in doses of 13 mg/kg and 0.13 mg/kg, as well as under Silybor in the heptane phase. In the isopropanol phase, no statistically significant changes in LP product concentration were observed, except for a 90.00% elevation of KD and CT concentration under the influence of Ivin at a dose of

Table 2. Effect of Ivin and Silybor with concomitant carbon tetrachloride on the intensity of LP and the activity of the antioxidant system (M ± m, n^a = 6)

Indicators	Control	CCl_4, 0.8 ml/100 g	Ivin, 13 mg/kg	Ivin, 0.13 mg/kg	Silybor, 5 mg/kg
Catalase, mkat/l	1189.19 ± 67.21	905.41 ± 58.63[b]	1122.97 ± 50.53[c]	1304.05 ± 58.15[c]	1250.45 ± 66.26[c]
MDA, nmol/g of tissue	6.97 ± 0.66	12.99 ± 0.47[b]	7.88 ± 1.04[c]	7.24 ± 0.90[c]	7.76 ± 0.66[c]
Heptane phase					
DC, o.i.u.	0.66 ± 0.07	2.51 ± 0.62[b]	1.12 ± 0.29[c]	0.95 ± 0.16[c]	1.14 ± 0.28[c]
KD and CT, o.i.u.	0.45 ± 0.11	1.09 ± 0.31[b]	0.64 ± 0.13[c]	0.25 ± 0.10[c]	0.75 ± 0.12
SB, o.i.u.	0.040 ± 0.011	0.188 ± 0.057[b]	0.083 ± 0.018[c]	0.056 ± 0.029[c]	0.099 ± 0.011[c]
Isopropanol phase					
DC, o.i.u.	0.35 ± 0.05	0.44 ± 0.03[b]	0.34 ± 0.03[c]	0.39 ± 0.02	0.36 ± 0.02[c]
KD and CT, o.i.u.	0.10 ± 0.04	0.23 ± 0.04[b]	0.15 ± 0.01	0.19 ± 0.00[b]	0.14 ± 0.02[c]
SB, o.i.u.	0.010 ± 0.002	0.028 ± 0.002[b]	0.014 ± 0.001[c]	0.007 ± 0.002[c]	0.016 ± 0.003[b,c]

Note: [a]Number of animals in the group; [b]$P \leq 0.05$ in relation to the control; [c]$P \leq 0.05$ in relation to carbon tetrachloride ($P \leq 0.05$ – significantly different between groups by using Fisher's LSD post-hoc test)

0.13 mg/kg and 60.00% increase of SB concentration under influencer of Silybor. The blood serum catalase activity was slightly higher than the one in the control group, but the difference was not statistically significant, however, in relation to the positive control (CCl_4), the activity of the enzyme increased under concomitant Ivin at a dose of 13 mg/kg, Ivin in dose of 0.13 mg/kg, and Silybor by 24.03, 44.03, and 38.11%, respectively.

Since neutral lipids are mainly extracted in heptane, while phospholipids do in isopropanol, the LP products content in the heptane phase indicates the activation or inhibition of LP in neutral lipids, and phospholipids in the isopropanol phase. Based on the study results, we can assume that CCl_4 intensifies LP activity in neutral lipids to a greater extent than in phospholipids of hepatocyte membranes.

The obtained results suggest that Ivin and Silybor prevent the accumulation of LP products in liver tissues and intensify the decomposition of hydrogen peroxide, thus contributing to balancing of pro- and antioxidant systems and reducing the damage of liver tissues caused by CCl_4 acute impact.

The effectiveness of the protective action of Ivin and Silybor in acute liver damage caused by CCl_4 is shown in Table 3.

As we can see from the data in Table 3, the greatest protective effect in the case of CCl_4 liver damage is observed under Ivin administration at a dose of 0.13 mg/kg, as evidenced by EI measured against all studied LP product readings (EI ranging from +44.26 to +77.06) and by catalase activity (–44.03). EI readings associated with the above LP and catalase figures and administration of Ivin at a dose of 13 mg/kg and Silybor are comparable, yet slightly lower than in case of Ivin administration at a lower dose, which is possibly substantiated by lower catalatic activity.

Ivin, in both tested doses, exerts a stabilizing effect on the activity of cytolytic and cholestatic enzymes. The mean EI calculated by the three indicators (ALT, AST and AP) in the case of Ivin administration at doses of 13 mg/kg and 0.13 mg/kg makes +24.10 and +24.36, respectively, and is practically equal to Silybor figures. Compared with Silybor, Ivin in the studied doses contributes to the stability of protein-synthetic liver function, and ensures almost the same level of normalization of cholesterol, glucose and total bilirubin readings. The obtained results suggest that the protective effect of Ivin is not inferior to the one of Silybor.

Based on the above data, it is noteworthy that there is no dose dependence of Ivin for the studied functional indicators and changes in cytolysis and cholestasis enzymes. The effect of Ivin at a lower dose on the state of the pro-oxidative system is manifested to a greater extent than at a high dose. It is known, non-linear effects are characteristic of

T a b l e 3. An efficiency index (EI) range measuring the hepatoprotective effect of Ivin and Silybor, by studied indicators

Indicators	Ivin, 13 mg/kg	Ivin, 0.13 mg/kg	Silybor,5 mg/kg
AP	+32.27	+31.06	+33.73
ALT	+26.59	+31.51	+29.28
AST	+13.45	+10.52	+17.59
Total proteins	–6.17	-2.49	-0.82
Total bilirubin	+14.61	+8.62	+19.30
Cholesterol	+18.18	+23.78	+21.68
Glucose	–14.83	–20.33	–17.50
Urea	–12.67	+6.48	+12.19
MDA	+39.34	+44.26	+40.26
DC	+55.38	+62.15	+54.58
Glucose	–14.83	–20.33	–17.50
Urea	–12.67	+6.48	+12.19
MDA	+39.34	+44.26	+40.26
DC	+55.38	+62.15	+54.58
KD and CT	+41.28	+77.06	+31.19
SB	+55.85	+70.21	+47.34
Catalase	–24.03	–44.03	–38.11

many chemicals of various purposes: antitumor and neurotropic drugs, radioprotectors, neuropeptides, hormones, adaptogens, immunomodulators, antioxidants, dioxins, heavy metals, some PGRs, including pyridine derivatives. Mechanisms of non-linear effects are summarized in [27]. For Ivin, non-linear effects can be explained by the features of the physical state of its molecule. According to [28-31], Ivin exists in a hydrated form in solutions. When in contact with the membrane, Ivin gradually loses bound water. Completed Ivin`s dehydration occurs when it interacts with the membrane at low concentrations ($1 \cdot 10^{-9}$ M). It is noted that the membranotropic activity of hydrated and dehydrated Ivin differs in nature: the first is a membrane destabilizer, and the second is an initiator of crystallization of membrane lipids through the initiation of dispersion interchain interactions.

At high concentrations, Ivin interacts with membranes in a hydrated form, in this case, the membrane modification leads to destabilization and disruption of the lipid matrix structural integrity, resulting in a toxic effect. At low concentrations, Ivin interacts with membrane lipids in a dehydrated state. Dehydrated Ivin causes compression of lipids, which contributes to the stabilization of membranes, and as a result, adaptation to the active factor occurs. Under conditions of equilibrium of the hydrated and dehydrated forms of the Ivin molecule on the membranes, the levelling of the Ivin effect is observed. In this case, there is no membrane modification and inversion occurs (the toxic effect is not observed) [27]. These data indicate that the state of the Ivin molecule and the direction of modification of the lipid matrix of biological membranes can play an important role in the mechanism of non-linear effects.

The obtained Ivin's hepatoprotective effect data will be used in the development of preventive measures for the intoxication of combined pesticides in agricultural applications and further research as a possible hepatoprotective drug.

Conclusion. In the case of subcutaneous BID CCl_4-administration to rats at a dose of 0.8 ml/100 g b.w. under conditions of acute hepatitis, CCl_4 causes moderate liver damage. Ivin in doses of 13 and 0.13 mg/kg exhibits a pronounced hepatoprotective effect, characterized by normalization of cytolytic and cholestatic activity of enzymes, functional indicators, such as total protein, glucose, cholesterol, total bilirubin, and urea; by reducing the LP intensity and activating the antioxidant system. According to cytolytic and cholestatic indicators, results of func-

tional tests and morpho-structural liver changes, the protective effect of Ivin in both studied doses is comparable to the one of the reference substance Silybor. The hepatoprotective effect of Ivin, measured by LP intensity and catalase activity, is manifested to a greater extent at a dose of 0.13 mg/kg than at a dose of 13 mg/kg and is higher than that of Silybor. The absence of a classic "dose-effect" relationship may be related to the properties of the Ivin molecule to be in a hydrated and dehydrated state. Depending on the state of the molecule, Ivin can modify the lipid matrix of biological membranes in different directions.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ГЕПАТОПРОТЕКТОРНА ДІЯ 2,6-ДИМЕТИЛПІРИДИНУ N-ОКСИДУ (ІВІНУ) В ЕКСПЕРИМЕНТАЛЬНІЙ МОДЕЛІ ІНДУКОВАНОГО CCl_4 ГЕПАТИТУ ЩУРІВ

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Сумісна дія пестицидів і регуляторів росту рослин на людину ще недостатньо з'ясована. 2,6-диметилпіридин N-оксид (Івін) є регулятором росту рослин, який, як відомо знижує гострту токсичність пестицидів, але його захисні механізми потребують вивчення. Метою дослідження було оцінити гепатопротекторні властивості 2,6-диметилпіридин N-оксиду (Івіну) за гострого гепатиту, індукованого CCl_4. Самцям щурів Wistar Han під шкіру вводили дві ін'єкції CCl_4 (0,8 мл/100 г маси тіла). Івін (13 або 0,13 мг/кг) та гепатопротектор Силібор-35 (5 мг/кг), як референтну речовину, вводили перорально за 1 годину до та через 2 години після введення CCl_4. Проведено біохімічне дослідження плазми крові, визначено продукти пероксидного окислення ліпідів у тканині печінки та гістологічне дослідження печінки щурів. Результати функціональних проб і гістоморфологічних досліджень тканини печінки щурів показали, що Івін виявляє виражену гепатопротекторну дію, більш виразну при застосуванні в низькій дозі 0,13 мг/кг. Розрахунок індексу гепатопротекторної ефективності для Івіну у разі гострого гепатиту щурів показав, що він співставний з таким для Силібору-35.

Ключові слова: CCl_4 індукований гепатит у щурів, Івін, гепатопротекторна дія.

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