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# Morphological Studies on Rat's Liver After a Ten-Day Treatment with *Chelidonium majus* L. Alkaloid Thiophosphoric Acid Derivative (Ukrain)

Badania morfologiczne wątroby szczurów poddanych 10-dniowemu działaniu tiofosforowo-kwasowej pochodnej alkaloidów *Chelidonium majus* L. (Ukrain)

Descriptions of substances of plant origin and of their semisynthetic derivatives with oncostatic action are found more and more often in professional literature. One of such medicines is an already used preparation called Ukrain discovered by Nowicki in 1978 (10). It is a semisynthetic product, *Chelidonium majus* L. alkaloid thiophosphoric acid derivative, a preparation to which immunostimulating and cytostatic actions are ascribed (8, 11, 13, 18). The action of Ukrain has become the subject of many scientific papers as a result of the growing incidence of neoplastic diseases and spreading interest in their treatment (4—6, 12, 13). As other medicines, Ukrain administered parenterally penetrates into the most important organ taking part in the distribution and detoxication of drugs, that is, to the liver. Due to that and to the spreading use of the drug it seemed interesting to examine the action of this preparation on the animal liver.

## MATERIAL AND METHODS

The studies were conducted on white rats (males) of Wistar strain, aged 3 months. The animals were kept on the standard diet. Twenty rats were taken for the experiment and they were divided randomly into an experimental group (15 rats) and a control group (5 rats). The animals from the control group were administered distilled water in intraperitoneal injections (i.p.) at a dose of 0.5 ml/100 mg of body mass (the same volume as of the medicine) for ten days. The animals from the experimental group were administered *Chelidonium majus* L. alkaloid thiosphoric acid derivative dissolved in distilled water, Ukrain, by Nowicki (10), in intraperitoneal injections (i.p.) in dose 0.5 ml/100 g of body mass (a single dose of about 1.5—2 ml) for ten days, that is, for the period recommended for a single treatment. The experimental group was divided into 3 subgroups of animal according to the size of the $LD_{50}$ dose of the administered drug (Table 1). Ukrain was administered in doses corresponding to: $^1/_{40}$, $^1/_{20}$ and $^1/_{10}$ $LD_{50}$. The $LD_{50}$ dose was based on a study by Kleinrok et al. (6). The dose for the rats of Wistar strain was 280 mg/kg of the body mass. The rats were then decapitated and their livers were taken for examinations.

Sections fixed in 4% formalin and embedded in paraffin were stained with hematoxylin and eosin (H + E), while connective tissue staining was carried out by the Masson's method (2).

Pictures were taken using the Ergaval microscope and with Exacta Varex camera.

Table 1

| Experimental group / Subgroups | Dose of the administered drug |
|---|---|
| $A_{n=5}$ | 7 mg/kg $^1/_{40}$ $LD_{50}$ |
| $B_{n=5}$ | 14 mg/kg $^1/_{20}$ $LD_{50}$ |
| $C_{n=5}$ | 28 mg/kg $^1/_{10}$ $LD_{50}$ |
| Control group $n=5$ | distilled water 0.5 ml/100 mg |

## RESULTS

### The control group

Liver sections stained with hematoxylin and eosin evidenced a regular architectonics of the liver lobules. Hepatocytes cytoplasm showed an affinity to acidic stains. Cell membranes were clear. Browicz-Kupffer cells were observed in the walls of the lacunar vessels. Single erythrocytes were found in the lumens of the lacunar vessels (Fig. 1). Staining by the Masson's method revealed a small amount of connective tissue in the vinicity of the liver triads and its trace amounts in spaces between liver lobules.

### The experimental group

Subgroup A. The structure of the liver lobules was similar to that of the control group. Microgranular hepatocytes cytoplasm showed an affinity to acidic strains. In single hepatocytes it was stained more intensively than in others. Hepatocytes with an increased transparence of the cytoplasm were observed in the perinuclear area. Single liver cells in the division stage were observed (Fig. 2). Such a picture was evident in the middle sphere of the lobules in the livers of 3 animals. In comparison with the control group, a larger number of binuclear hepatocytes was observed. More numerous Browicz-Kupffer cells were found in the walls of the lacunar vessels (Fig. 3). Staining by the Masson's method has shown a picture similar to that of the control group.

Subgroup B. A different stainability of hepatocytes was observed in the examined preparations of liver stained with H + E. Single hepatocytes with more acid absorbing cytoplasm and clearly seen nuclei were found in the peripheral sphere of the lobules (Fig. 4). The nuclei of some of the parenchymal cells had

a pyknotic appearance. In the central sphere of the lobules hepatocytes cytoplasm was more tranlucent and their nuclei had blurred contours. The increased transparence of cytoplasm in the peripheral area of the cell was observed in many hepatocytes (Fig. 4). Intercellular boundaries were clearly seen. Similarly to the previous group, more numerous binuclear hepatocytes and Browicz-Kupffer cells were observed in comparison with the control group. The amount of the connective tissue was similar to that observed in the sections of the liver taken from the animals from the control group (Fig. 5).

Subgroup C. A regular structure of the lobules was observed in the preparations of livers stained with H+E. Hepatocytes cytoplasm showing an affinity to acidic stains had a macrogranular structure with increased transparence (Fig. 6). The nuclei of hepatocytes had different shapes and sizes. Nuclei contours were indistinct. Hepatocytes sizes were also different. A larger number of Browicz-Kupffer cells was observed. No significant difference in the amount of the connective tissue was found in comparison with the control group.

## DISCUSSION

Taking into account the frequent application of Ukrain as a cytostatic medicine it seemed interesting to examine the extent to which this semisynthetic preparation affects the liver, the organ largely determining the course of changes in the whole organism. Substances absorbed parenterally are transported by blood proteins or its cellular elements to the liver where they undergo transformations to inactive forms (14, 20), Taking into consideration the fact that the transformations of many substances take place in the hepatocytes, it should be assumed that they are exposed to the possible harmful action of the metabolized substances.

In all experimental groups, morphological examinations have shown the occurrence of single hepatocytes with increased transparence in the area of the cytoplasm. These changes were more significant in the group in which Ukrain was administered in the dose 7 mg/kg of body mass and 14 mg/kg of body mass. Such a picture is sometimes called vacuolar degeneration, i.e. changes in the area of smooth endoplasmic reticulum — an organelle connected with drug intoxication; its is one of the adaptative changes of the cell evoked by a pathogenic stimulus. In this case it could have been the administered drug.

In the experimental groups A and B single hepatocytes with acid absorbing cytoplasm were found. Such a condition may be connected with a large concentration of the mitochondria in the cells. It could be supposed that the administered drug caused a change in the energetic state of the liver cells. Such an observation is in agreement with the data in the literature concerning Ukrain. In all the experimental groups changes in the area of the cell nuclei were observed.

Single hepatocytes in the division stages were observed in the group of animals receiving Ukrain in the dose of $^1/_{40}$ $LD_{50}$, the smallest of the administered doses. There have been reports that the most numerous mitotic divisions in the rat's liver take place from the 7th to the 41st day after birth, so that their occurrence in adult animals may be a result of a damage and regeneration of this organ (2, 7, 9, 15). However, a small number of hepatocytes with pyknotic nuclei was observed in group B receiving Ukrain in the dose of $^1/_{20}$ $LD_{50}$. It is considered that in less efficient cells the nuclei are smaller as a result of the condensation of its components (16). It may be supposed that the administered drug caused the impairment of the function of these hepatocytes.

An increase in the dose of Ukrain contributed to the occurrence of cell nuclei of different shapes and sizes, some of them having unclear contours. Hence, it may be supposed that with these hepatocytes an impairment of the functions of the cell nuclei has taken place. The more frequent occurrence of binuclear hepatocytes observed in groups A and B, is regarded as one of the characteristic features of liver regeneration (7).

An increased number of Browicz-Kupffer cells was observed in all the experimental groups. These are macrophages taking part in the phagocytosis processes. The increase in their number is one of the characteristic feaures of their excitation state. There are reports claiming that resting macrophages may exert a nonspecific cytotoxic effect on neoplastic cells, while the stellate cells, additionally activated by immunomodulators (Ukrain is one of them), reveal high cytotoxicity in relation to these cells (3, 17, 19).

The possibility of an activation of Browicz-Kupffer cells is significant from the clinical point of view because liver is a very frequent site of metastases.

Taking into account the amount of the connective tissue in all the experimental groups it may be supposed that Ukrain has no influence on the fibrillation processes in the liver.

All the described changes prove the influence of Ukrain on the morphology of hepatocytes in rats.

## Conclusions

1. Intraperitoneal administration of Ukrain in doses $^1/_{40}$, $^1/_{20}$ and $^1/_{10}$ $LD_{50}$ provokes adaptative and regenerative changes in rat's hepatocytes.
2. The administered drug has an influence on the energetic state of liver cells.
3. *Chelidonium majus* L. thiophosphoric alkaloid acid derivative activates Browicz-Kupffer cells.
4. Ukrain has no influence on the fibrillation processes in the liver.

## REFERENCES


1. Bagiński S.: Technika mikroskopowa. PWN, Warszawa 1969.
2. Biedź-Bielewski D., Bilczuk B.: Kliniczna przydatność wskaźników podziałów komórkowych w wątrobie. Pol. Tyg. Lek. **15** (18), 3, 1960.
3. Daemen T. et al.: *In vitro* activation of rat liver macrophages to tumoricidal activity by free or liposome-encapsuled muramyl dipeptide. Cancer Res. **46**, 4330, 1986.
4. Jagiełło-Wójtowicz E., Kleinrok Z.: The effect of thiophosphoric alkaloid derivatives from *Chelidonium majus* on the central dopaminergic and serotonergic system of the rodents. The II Baltic meeting on Pharmacol. Tallin 1990, Abstr. **56**.
5. Kleinrok Z. et al.: Preliminary pharmacological evolution of thiophosphoric akaloid derivatives from *Chelidonium majus* in rodents. The II Baltic meeting on Pharmacol and Clinical Pharmacol. Tallin 1990, Abstr. **65**.
6. Kleinrok Z. et al.: Basic central pharmacological properties of thiophosphoric acid alkaloid derivatives from *Chelidonium majus* L. Pol. Journal of Pharmacol. Pharm. 1991 (in print).
7. Kruś S.: Patomorfologia wątroby. PZWL, Warszawa 1986.
8. Liepins A., Jagiełło-Wójtowicz E.: Modulation of cell mediated lysis of tumor cells by Ukrain. The II Batic meeting of Pharmacol. and Clinical Pharmacol. Tallin—Tartu 1990, Abstr. Suppl.
9. Nowak H. F.: Procesy odrostu, naprawy i adaptacji tkanek. [in:] Podstawy patomorfologii. Red. J. Groniowski, S. Kruś, PZWL, Warszawa 1984.
10. Nowicky J. W.: Patenturkunde Republik Österreich. Wien 1980, nr 354644.
11. Nowicky J. W.: Cancer treatment using anticancer preparation alkaloid derivative Ukrain. Chemotherapia, Suppl. **1196**, 1985.
12. Nowicky J. W. et al.: Clinical and *in vitro* studies with Ukrain. The II Baltic meeting on Pharmacol and Clinical Phamacol. Tallin 1990, Abstr. Suppl.
13. Nowicky J. W. et al.: The application on thiosphoric acid alkaloid derivatives from *Chelidonium majus* (Ukrain) as an immuno-stimulator in patients with various carcinomas. [in:] 9th International Symposium on Future Trends in Chemotherapy. Palexpo—Geneva 1990 (Switzerland), Abstr.
14. Perry M. C.: Toxicity of Chemotherapy. Ed. M. C. Perry, J. W. Jarbro., Grune Stratton Inc. Orlando—San Diego—San Francisco—New York 1984.
15. Sattler C. et al.: Electron microscopic and time lapse studies of mitosis in cultured rat hepatocytes. Hepatology **8** (6), 1540, 1988.
16. Sawicki W.: Jądro komórkowe. [in:] Cytofizjologia. Red. K. Ostrowski, J. Kawiak, PZWL, Warszawa 1990.
17. Skórski T.: Rola makrofagów, monocytów i granulocytów w zwalczaniu nowotworów. [in:] Ultrastruktura i funkcja komórki. Red. J. Kawiak, Z. Osuchowska, M. Jakóbisiak, PWN, Warszawa 1991. T. 5.
18. Staniszewski A. et al.: Immunological profile in patients with small-cell lung cancer (SCLC) treated with alkaloid derivative from *Chelidonium majus* L. [in:] 4-th Congress the European Society of Surgical Oncology. Paris 1988. Abstr.
19. Stukart M. et al.: Induction of tumoricidal activity in isolated rat liver macrophages by liposomes containing recombinant rat γ-interferon supplemented with lipopolysaccharide or muramyldipeptide. Cancer Res. **47**, 3880, 1987.
20. Wiśniewski K. et al.: Farmakodynamika, PZWL, Warszawa 1986.


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## EXPLANATION TO FIGURES

Fig. 1. Rat liver from the control group. Staining with H+E. Magn. 200×.
Fig. 2. Rat liver from the experimental group — subgroup A. H+E staining. Magn. 400×.
Fig. 3. Rat liver from the experimental group — subgroup A. H+E staining Magn. 200×.
Fig. 4. Rat liver from the experimental group — subgroup B. Staining with H+E. Magn. 200×.
Fig. 5. Rat liver from the experimental group — subgroup B. Masson's staining. Magn. 200×.
Fig. 6. Rat liver from the experimental group — subgroup C. Staining with H+E. Magn. 200×.

## STRESZCZENIE

Badano wpływ tiofosforowo-kwasowej pochodnej alkaloidów *Chelidonium majus* L. (Ukrain) na morfologię wątroby szczurów. Grupa kontrolna zwierząt otrzymywała dootrzewnowo wodę destylowaną. W obrębie grupy doświadczalnej, której podawano lek dootrzewnowo przez okres 10 dni, wyodrębniono trzy podgrupy ze względu na wielkość dawki $LD_{50}$. Ukrain podawano w dawkach: $^1/_{40}$, $^1/_{20}$ i $^1/_{10}$ $LD_{50}$. U zwierząt grup doświadczalnych zauważono procesy regeneracyjne i adaptacyjne komórek wątrobowych. Obserwowano również wzrost liczby komórek Browicza-Kupffera w miąższu wątrobowym tych zwierząt w porównaniu z grupą kontrolną.

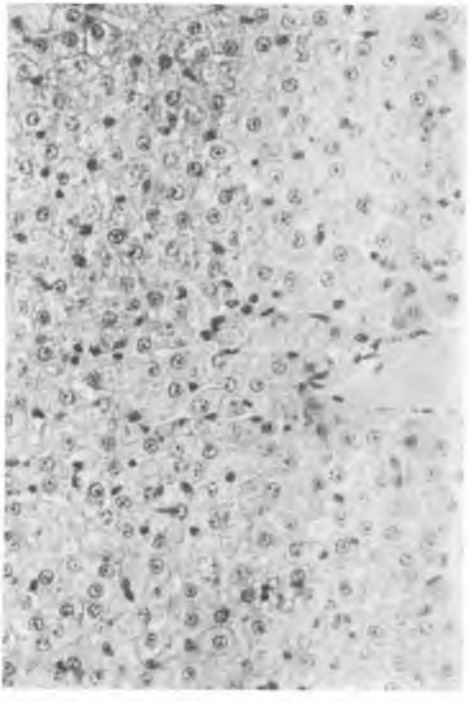

Fig. 1

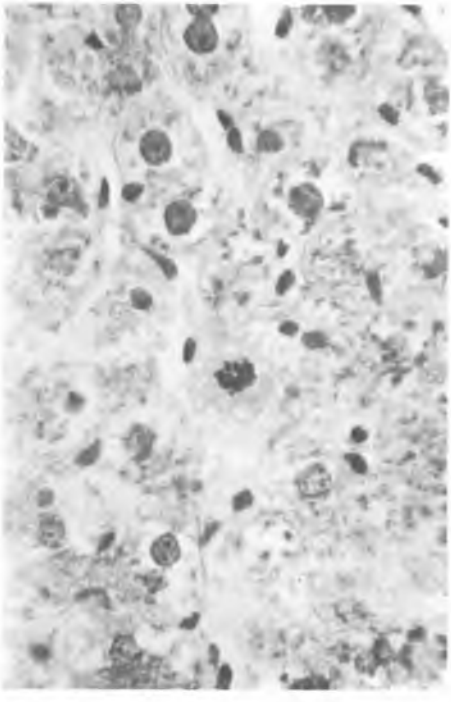

Fig. 2

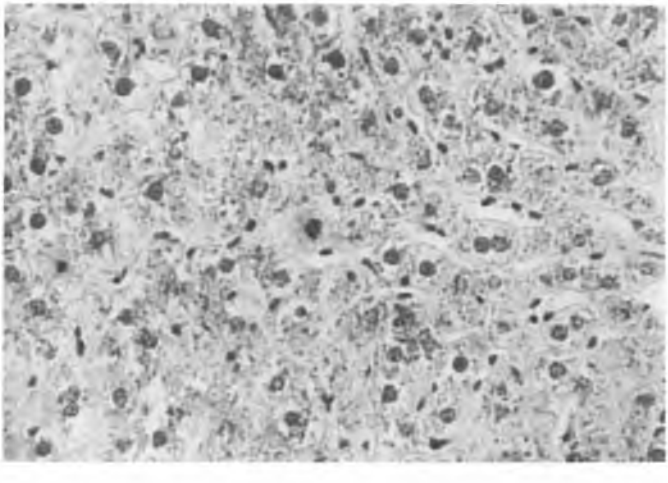

Fig. 3

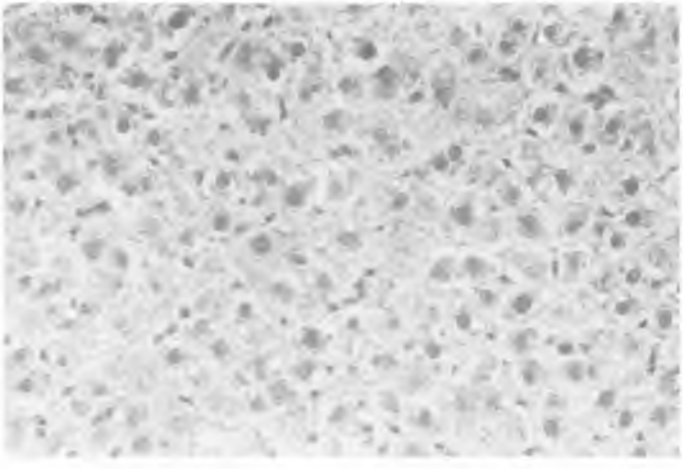

Fig. 4

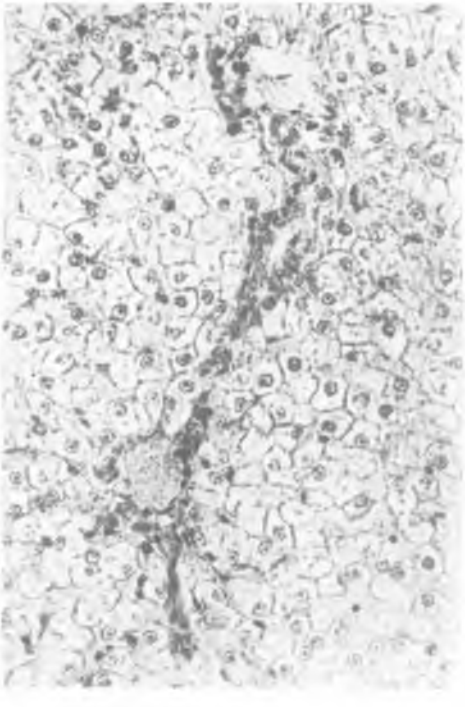

Fig. 5

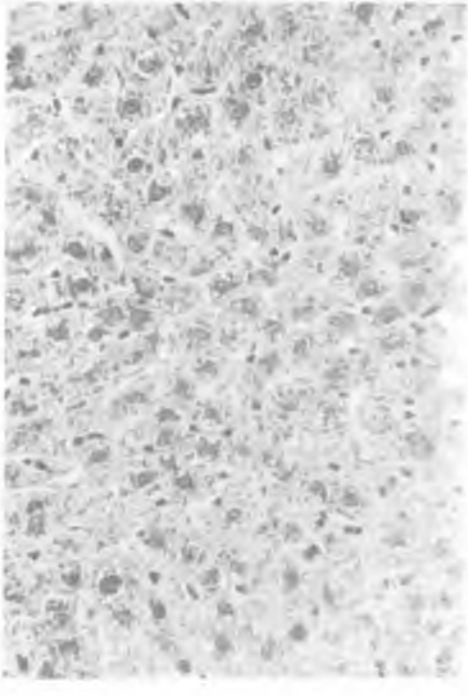

Fig. 6