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Protective Hepatotropic Effect of Preparations from Mytilidae and Ginseng in Animals Given Dioctyl Phthalate

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It is shown that in a group of animals given dioctyl phthalate alone antipyrine metabolism is enhanced (the urine content of its metabolites rises), and the histological structure of the liver is altered by the end of the experiment: the plasmalemma membranes, the granular endoplasmic reticulum, and mitochondria are destroyed, and fat deposits are observed in the disrupted zones of the mitochondria. In animals given dioctyl phthalate and adaptogens simultaneously antipyrine metabolism normalizes, and the morphological structure of the liver changes slightly, although it does not normalize.

Key Words: *dioctyl phthalate; toxic hepatitis; cytochrome P-450; adaptogens*

Xenobiotics and drugs are metabolized in the liver by the multienzyme complex of cytochrome P-450. The functions of individual enzymes of this system have now been defined more clearly. It has been shown that reactions of antipyrine metabolism are catalyzed by 3 isoforms of cytochrome P-450 [1] (but predominantly by P-450 IAI) and amidopyrine metabolism by P-450 IIBI [3]. Some low-molecular weight antibiotics (benzene, styrene, chlorinated carbohydrates, vinylchloride, and vinylbromide) are metabolized by the isoenzyme P-450 IIEI [9]. Localization of some forms of the cytochrome in the hepatic lobule has been determined by immune assay with special antibody kits. For instance, cytochrome P-450 IA is predominantly localized in the centrilobular zone and is absent in the periportal areas, while P-450 IIIA is heterogenously distributed over the acinus, and P-450 IIB and P-450 IIC9 are homogenously distributed over the acinus [14]. These findings indicate that local lesions of the liver can be caused by active metabolites of xenobiotics. The activity of cytochrome P-450-dependent enzymes is altered under the influence of xenobiotics, which in turn affects their toxicity resulting from the action

Research Clinical Institute of the Moscow Region; Central Research Institute of Gastroenterology, Moscow; All-Russia Research Institute of Fisheries and Oceanography; Chemico-Pharmaceutical Institute, St. Petersburg. (Presented by N. F. Izmerov, Member of the Russian Academy of Medical Sciences)

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of their metabolites. Phenobarbital and 3,4-benzpyrene are generally recognized inducers of the enzymes, and SKF, 525 A, and carbon disulfide are their inhibitors [17].

Adaptogens of plant and animal origin are now believed to play an important role in the treatment and prophylaxis of many diseases. The preparation Unipan is an extract from ginseng roots, grown on a germanium-enriched medium. The preparation exerts an immunomodulating effect due to the induced production of endogenous interferon [5]. MIGI-K, a meat hydrolysate from Mytilidae, contains an entire complex of essential amino acids and an almost entire set of nonessential amino acids; it is rich in trace elements and polyunsaturated fatty acids. The preparation has an immunostimulating, anti-inflammatory, and regenerative activity, and is used as a food supplement [4].

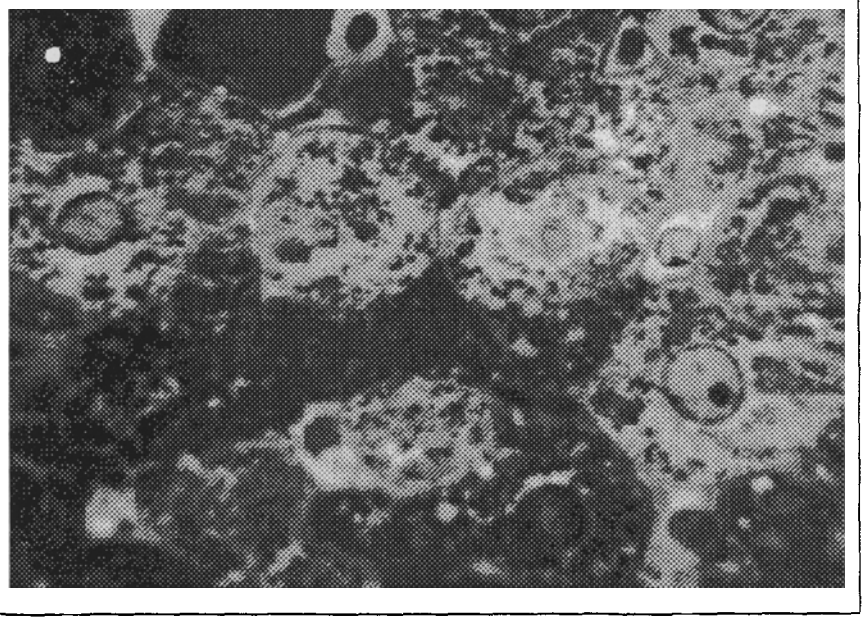

Fig. 1. Dystrophic changes of hepatocytes after injection of DOP (semithin sections). Clumpy cytoplasm; dense areas alternate with less dense areas devoid of structures. (oc. 10, obj. 40 VI).

In our study we investigated the hepatoprotective effect of preparations from Mytilidae and ginseng in animals given dioctyl phthalate (bis-2-ethylhexylphthalate, DOP).

MATERIALS AND METHODS

The experiments were carried out on 91 nonpedigree male rats. An oil solution of DOP (250 and 50 mg/kg) was intragastrically injected daily during one week.

Changes in the liver of rats caused by chronic injections of this ester of phthalic acid in a dose of 50 mg/kg were shown in our previous study [2]. More marked changes in the organ have been observed for doses of 200 and, notably, 1000 mg/kg body weight [13]. MIGI-K was intragastrically injected (0.2 ml per 100 g body weight) to animals for a period of 2 weeks before intoxication with DOP and during 4 weeks of intoxication. The preparation from ginseng, Unipan, was intragastrically injected in a dose of 0.1 ml per 100 g body weight. In the course of the experiment all the animals were divided into 9 groups: 17 intact rats served as the control, two groups, each comprising 12 animals, received the preparations MIGI-K and Unipan alone, two groups received DOP alone in doses of 250 (11 rats) and 50 (7 rats) mg/kg body weight, and 4 groups of animals received DOP (250 mg/kg) + MIGI (13 rats), DOP (250 mg/kg) + Unipan (5 rats), DOP (50 mg/kg) + MIGI (7 rats), and DOP (50 mg/kg) + Unipan (7 rats). In all animals the functional state of the liver was studied using the antipyrine test, which gives an indication of the activity of microsomal oxidation in hepatocytes. Antipyrine was injected in a dose of 50 mg/kg, and urine was collected over 15 h for determination of antipyrine metabolites. Two of the three major metabolites of antipyrine - norantipyrine and 4-hydroxyantipyrine (4-HA) -

TABLE 1. Content of Total and Free Metabolites of Antipyrine in the Urine of Rats Given Therapeutic Preparations, % of Administered Dose

Antipyrine metabolite	Control	MIGI-K	Unipan
Total metabolite:			
norantipyrine	5.9±0.18	4.7±0.37*	5.4±0.37
4-HA	7.9±0.52	6.4±0.60*	6.3±0.54*
Free metabolite:			
norantipyrine	0.52±0.036	0.25±0.039*	0.36±0.045*
4-HA	0.46±0.032	0.34±0.051*	0.37±0.033*

Note. Here and in Table 2 an asterisk denotes reliable differences vs. the control ($p<0.05$).

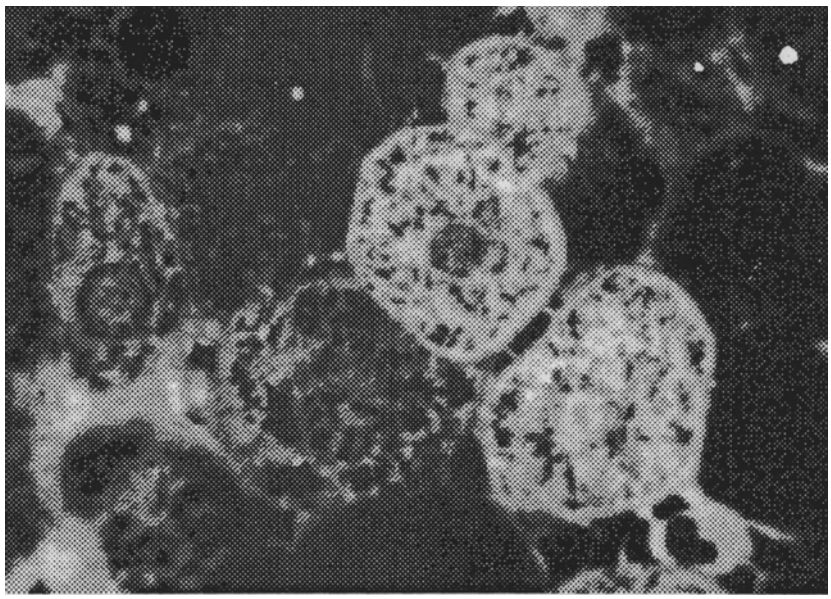

Fig. 2. "Dark" and "light" hepatocytes following administration of DOP + MIGI (semithin sections). (Oc. 10, obj. 40 VI).

were assayed in the urine. In health 39.7% of 4-HA, 14.5% of norantipyrine, and 3.3% of unchanged antipyrine are excreted with the urine in humans [6]. Total and free metabolites were determined by the method of thin-layer chromatography on Silufol plates (Czechoslovakia). One animal from each group was sacrificed for morphological investigation of the liver. Only pronounced changes in the liver for injections of DOP in a dose of 250 mg/kg and for its injection in combination with MIGI-K are described in this paper. For electron-microscopic examination the liver tissue was fixed in 2% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Araldite. The tissue samples and the zones for electron-microscopic study were chosen using one-micron (semithin) sections routinely stained with methylene blue. Ultrathin sections obtained on a UKV-III ultramicrotome were contrasted with uranyl acetate and lead citrate after Reynolds and examined under a JEM-120 EX electron microscope (acceleration voltage 80 kV). The object was photographed at magnifications from 5000 to 50,000.

RESULTS

Table 1 shows that the content of total, as well as free, metabolites in the urine reliably dropped after the injection of the preparation from Mytilidae. After the treatment with Unipan a tendency toward a decrease of the content of metabolites was noted, and for 4-HA and free metabolites the difference was also statistically reliable. These findings suggest that both preparations (but MIGI to a greater degree) have an antioxidant activity, reducing the intensity of metabolism predominantly in its first phase.

The parameters of antipyrine metabolism in animals 4 weeks after injection of DOP are presented in Table 2.

As is seen from Table 2, for the dose of 50 mg/kg a reliable difference from the control was observed only for free 4-HA, whose content in the urine increased. For the dose of 250 mg/kg a reliable increase in the urine content of both total metabolites and an almost 3-fold increase in the content of free 4-HA were noted. An increased urine content of antipyrine metabolites provides evidence that enzymes metabolizing the preparation are activated under the influence of DOP.

Table 3 shows the results of experiments when MIGI-K and Unipan were injected to animals against the background of poisoning with both doses of DOP.

Comparison of the data from Table 3 with the results shown in Tables 1 and 2 invites the conclusion that both preparations administered in combination with DOP, especially in a dose of 250 mg/kg, exert a marked normalizing effect.

Morphological analysis of semithin sections showed that pronounced changes occur in the liver of animals. In rats given DOP alone an uneven distribution of hepatocytes is characteristic of the

TABLE 2. Content of Total and Free Metabolites of Antipyrine in the Urine of Rats Following Intragastric Injection of DOP, % of Administered Dose

Antipyrine metabolite	Control	MIGI-K	Unipan
Total metabolite:			
norantipyrine	5.9±0.18	6.6±0.42	13.1±1.55*
4−HA	7.9±0.52	9.2±1.65	17.4±2.0*
Free metabolite:			
norantipyrine	0.52±0.036	0.52±0.12	0.64±0.057
4−HA	0.46±0.032	0.74±0.10*	1.22±0.135

liver. The majority of hepatocytes have a clumpy cytoplasm, dense areas alternating with less dense areas devoid of structures (Fig. 1). A normal size, a homogenous structure, and 2-3 nucleoli are typical of hepatocyte nuclei. In individual cells the nucleus is greatly diminished and condensed. The vascular bed of the liver (sinusoids) looks like narrow slits containing solitary erythrocytes. A few Kupffer cells are encountered along the sinusoids.

In rats given MIGI + DOP in a dose of 250 mg/kg changes in the liver are also markedly pronounced. A moderate enlargement of sinusoids, the lumen of which is filled with erythrocytes, and a clear-cut division of hepatocytes into "dark" and "light" cells are worthy of note (Fig. 2). The structure of the "dark" cells is undetectable due to the density of the cytoplasm. In the "light" cells dense mitochondria are clearly seen against the background of the optically translucent cytoplasm. The structure and the size of the nuclei of the majority of hepatocytes are similar irrespective of the density of the cytoplasm.

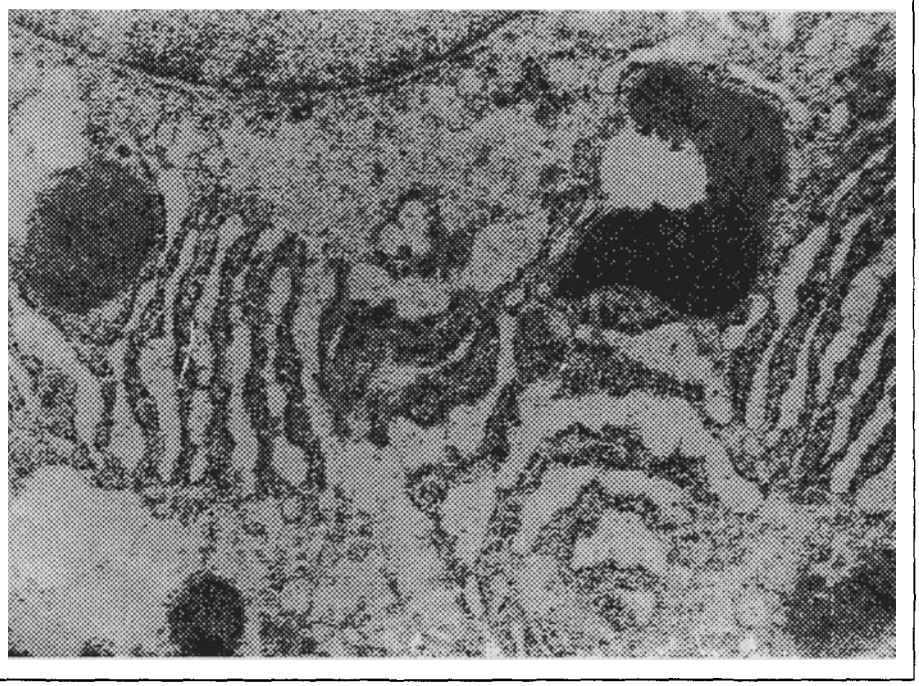

Fig. 3. DOP-induced ultrastructural changes in hepatocytes. Perinuclear zone of hepatocyte. Destruction of membranes of granular endoplasmic reticulum and mitochondria. ×15,000.

Electron-microscopic investigation of the liver samples revealed changes in the intracellular structures of hepatocytes, which reflected their response to the experimental treatment. The effect of DOP in many hepatocytes manifested itself in destruction of the membranes; as a result, in some cell areas the granular endoplasmic reticulum preserved its shape only due to the ordered distribution of ribosomes. Many canaliculi of the granular endoplasmic reticulum are enlarged and are found near mitochondria. A high density of the matrix and barely distinguishable solitary cristae are typical of the latter. In some mitochondria considerable areas are destroyed and replaced with fat (Fig. 3). The nuclei of such cells do not exhibit any changes. Solitary lysosomes and peroxisomes with a typical structure, well preserved microtubules, and, sometimes, considerable amounts of free ribosomes are present in some hepatocytes.

As in semithin sections, "dark" and "light" hepatocytes are clearly seen in electron microphotographs derived from animals given DOP + MIGI (Fig. 4). They may be located in close proximity, preserving the contacts typical of normal hepatocytes. Light cells exhibit various degree of edema. Sizable areas of their cytoplasm may be

TABLE 3. Content of Total and Free Metabolites of Antipyrine in the Urine of Rats for Administration of DOP in Combination with the Preparations MIGI-K and Unipan

Antipyrine metabolite	MIGI-K		Unipan	
	DOP, 50 mg/kg	DOP, 250 mg/kg	DOP, 50 mg/kg	DOP, 250 mg/kg
Total metabolite:				
norantipyrine	6.85±0.34	6.7±0.66**	6.4±0.69	8.7±1.17**
4-HA	7.14±0.38	9.0±1.1**	7.8±1.04	8.8±1.00**
Free metabolite:				
norantipyrine	0.48±0.072	0.53±0.05	0.41±0.05	0.44±0.05
4-HA	0.74±0.06*	0.70±0.061*,**	0.53±0.09	0.78±0.152*,**

Note. Reliability of differences ($p<0.05$) from the control and from the group of rats poisoned with the corresponding dose of DOP is denoted by one and two asterisks, respectively.

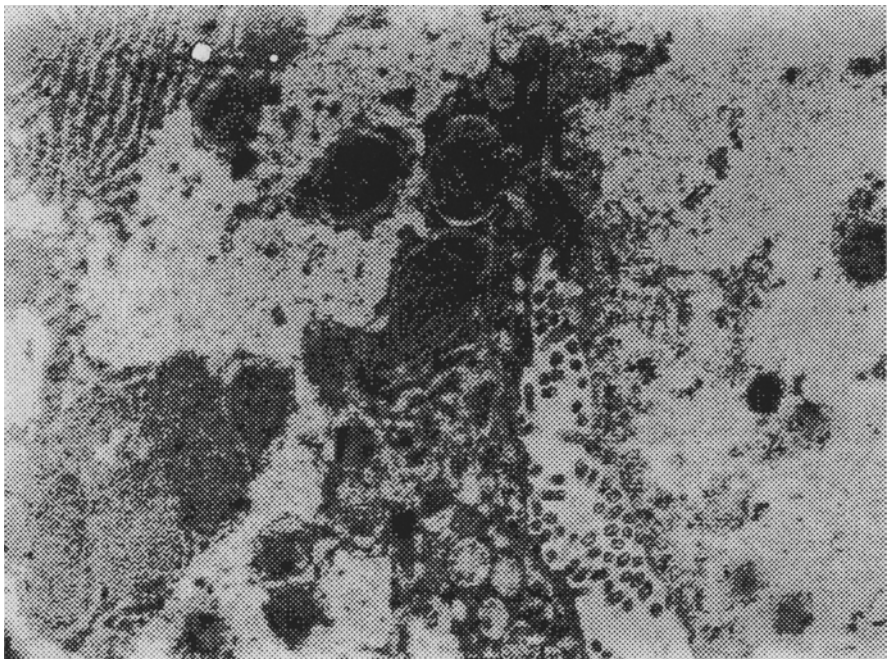

Fig. 4. Ultrastructural changes in hepatocytes under the influence of DOP + MIGI. Boundary of "dark" and "light" hepatocytes. ×8000.

almost entirely devoid of organelles, their place being occupied by just a small amount of floccular detritus.

However, the plasma membrane is preserved, and neoplasms directed toward the lumen of the bile capillary are formed at the cell surface near the biliary pole. Solitary lysosomes with different contents, including a microtubule, may occupy this zone of the cell. "Dark" hepatocytes exhibit a nonuniform distribution of organelles: zones with a compact distribution of the elements of endoplasmic reticulum and mitochondria predominate. Rather numerous lysosomes in different stages of formation, as well as peroxisomes with the typical structure, are characteristic of "dark" hepatocytes. As in the "light" (edematous) cells, the plasma membrane and the membranes of many mitochondria are preserved.

Our findings demonstrate that DOP alone, as well as in combination with MIGI, causes dystrophy of the liver in rats. In the group of animals given DOP alone the major toxic effect of the preparation manifests itself in destruction of the cell membranes. This relates not only to the plasmalemma, but also to the membranes of the granular endoplasmic reticulum and mitochondria. Destroyed zones of mitochondria are replaced with fat. The effect of DOP against the background of injections of MIGI manifests itself in a different manner. Membrane destruction is less pronounced, and fat deposits are not observed near destroyed mitochondria. A pronounced edema of some hepatocytes is noted. This may be due, on the one hand, to a moderate enlargement of sinusoids associated with a change in the local hemodynamics and, on the other, to an increased permeability of the hepatocyte plasmalemma. A manifest regeneration was not noted in either series of the experiment.

Thus, our study corroborated the hepatotropic toxic effect of DOP, manifesting itself in an increased activity of cytochrome P-450-dependent enzymes, as well as in dystrophic changes of hepatocytes, notably of their membranes. Fat deposition in the cells, which is characteristic of the effect of typical hepatotropic poisons (carbon tetrachloride, trinitrotoluene, etc.) and which was not observed by us for DOP in a dose of 50 mg/kg [2], was noted.

Injection of the preparation from Mytilidae and ginseng lowered the induced activity of cytochrome P-450-dependent enzymes, evidence of which was suppression of antipyrine metabolism and a decrease in the content of metabolites to the control level. However, the morphological picture of the liver did not normalize; it altered only under the influence of adaptogens: the destruction of membranes diminished, and fat deposition was not observed, but edema of hepatocytes and the phenomenon of so-called "light" and "dark" hepatocytes were noted.

The mechanism of this effect is not entirely clear. Primary metabolites (mono-2-ethylhexylphthalate and 2-ethylhexanone), which are formed in the intestine during hydrolysis of DOP by pancreatic lipase [11], are believed to be responsible for the effect of DOP upon the enzyme system of the liver [16]. Their further metabolism occurs in the liver via ω- and (ω-1)-oxidation to acids, alcohols, and ketones and β-oxidation of the latter [7]. In humans the derivatives of glucuronide monophthalate are the major metabolites [8]. Evidently, MIGI-K and the ginseng extract reduce the oxidative processes in hepatocytes, including the activity of P-450-dependent oxidases, this leading to suppression of antipyrine metabolism. However, the formation

of free radicals and lipid peroxidation is only one mechanism of the hepatotoxic effect of xenobiotics. Some researchers [10] regard a disturbance of glutathione metabolism, leading to modification of the protein thiol residues and destruction of the cell cytoskeleton and intracellular calcium metabolism, as the primary component of toxic damage to the liver [15]. It has been shown that phthalates cause interruption of the energy-dependent processes in the hepatocyte mitochondria and suppression of succinate dehydrogenase activity [12].

Evidently, the adaptogens in question do not exert their effect upon all mechanisms of phthalate-induced toxic damage to the liver, and their protective effect is only partial.

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