

**BIOCHEMICAL INDICATORS OF HEPATOTOXICITY  
IN BLOOD SERUM OF RATS UNDER THE EFFECT OF NOVEL  
4-THIAZOLIDINONE DERIVATIVES AND DOXORUBICIN AND THEIR  
COMPLEXES WITH POLYETHYLENEGLYCOL-CONTAINING  
NANOSCALE POLYMERIC CARRIER**

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*The aim of this study was to compare the effect of new synthetic 4-thiazolidinone derivatives (compounds 3882, 3288 and 3833) and doxorubicin (positive control) in free form and in their complexes with synthetic polyethyleneglycol-containing nanoscale polymeric carrier on the biochemical indicators of hepatotoxicity in blood serum of rats. The activity of enzymes considered as the markers of hepatotoxicity, as well as the concentration of total protein, urea and creatinine were measured in blood serum of rats. It was found that after injection of investigated compounds the activities of alanine aminotransferase, alkaline phosphatase and  $\alpha$ -amylase increased in comparison to control. Doxorubicin injection was accompanied by 4-fold increase in the activity of  $\gamma$ -glutamyltransferase, and injection of compound 3833 led to 2.5-fold elevation of the activity of this enzyme. Complexation of these antineoplastic derivatives with a synthetic nanocarrier lowered the activity of the investigated enzymes substantially if compared to the effect of these compounds in free form. The most evident decrease was measured for  $\alpha$ -amylase,  $\gamma$ -glutamyltransferase and lactate dehydrogenase activities. The normalization of concentrations of total protein, urea and creatinine in blood serum of rats treated with complexes of the studied compounds with a polymeric carrier comparing with their introduction in free form was also detected. Thus, the immobilization by novel polymeric carrier of anticancer drugs possessing high general toxicity in the treated organism mitigates their toxic effect, which is evident as normalization of specific biochemical indicators of the hepatodestructive effects of the anticancer drugs.*

*Key words: hepatotoxicity, 4-thiazolidinone derivatives, doxorubicin, polymeric nanocarrier, enzyme activity, blood serum, rats.*

Chemotherapy is one of the most efficient methods for treatment of patients with oncological pathologies [1-3]. Nevertheless, its application is hindered by a number of issues, the main of which are insufficient specificity of anticancer drugs and their high generalized toxicity, especially their hepatotoxicity, cardiotoxicity, nephrotoxicity, and neurotoxicity [1-7]. Drug-induced toxic lesions are the most widespread cause for withdrawal of medicinal preparations that had been approved for use in clinical practice [6, 7]. Hepatotoxic action of anticancer compounds is frequently associated with numerous clinical and morphological manifestations of pathology of liver, which is a central organ of metabolism [2, 3, 5-7]. The general interrelated

mechanisms that lie in the basis of cytotoxicity of xenobiotics include imbalance of energy metabolism, changes in intracellular calcium homeostasis, activation of free-radical peroxidation in cell, inhibition of protein synthesis and cell division, damage to cell membranes [3, 5-7].

The liver carries out a number of metabolic, hematopoietic, defensive and excretive processes, plays a central role in intermediate metabolism, and is a primary site for detoxification reactions [5-7]. It is a major participant of processing various xenobiotics, including pharmaceuticals [3, 7]. Hepatocytes contain enzymes that modify and conjugate compounds of exogenous and endogenous origin. According to clinical studies, liver lesions by pharmacological

substances are responsible for about 10% of the side effects associated with anticancer treatment [2, 6, 7]. The liver is the focus for toxic effects of most of the cytostatics, as it is the primary site of their metabolism [6-8]. Hepatocytes operate at high concentrations of pharmaceuticals that could already be toxic in their native state, and may gain more toxicity after being modified via metabolic processing [2, 3, 7]. Biotransformation leads to biochemical and physicochemical modifications to pharmaceutical molecules, which may produce polar water-soluble compounds under these conditions, losing their pharmacological activity in the process, becoming less toxic and more easily excreted from the organism [6, 7]. Xenobiotics are purged from an organism in two stages. The first is a metabolic one, directed to introduction of polar groups in the molecule via cytochrome P450 hydroxylase system. The second is a conjugation stage, when the foreign compounds are bound to certain water-soluble ligands [3, 6, 7]. Moreover, the anticancer drugs may exert a cumulative toxic influence on liver functioning [2, 5, 7]. The biological effect of medical substances is dependent on their dosage, concentration of the active substance, manner of introduction, and duration of therapeutic influence. Nevertheless, the hepatocytes are mostly damaged not by pharmaceuticals on their own, but by their toxic metabolites [3, 6, 7].

As a result of a screening program conducted in the National Cancer Institute (USA), we proposed a group of synthetic heterocyclic 4-thiazolidinone derivatives as potential antitumor agents [9, 10]. The compounds of this class are known for their anticancer activity as well as for their other biological effects, such as antibacterial, fungicidal, antiviral, anti-inflammatory, and antidiabetic actions [10]. The results of our previous studies on antitumor efficiency of 4-thiazolidinone derivatives, including those of pyrazoline substitution [9, 10] allowed drawing a conclusion that the pyrazoline-thiazolidinone-indoline conjugates demonstrate the most promising results, and that the most active ones are the compounds 3288, 3833 and 3882 [11, 12]. The molar masses of these synthetic substances are 559.44 g/mol (3288), 530.61 g/mol (3882), and 609.51 g/mol (3833).

In previous studies, we have demonstrated that combining of thiazolidinone, pyrazoline and 2-oxoindoline moieties in a more 'rigid' tricyclic system – 3-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-4-oxo-4H-thiazol-5-ylidene]-1,3-dihydroindol-2-one –

allows us to achieve high cytostatic activity in 60 strains of human cancer cells without a pronounced selective effect [9, 12]. All the three compounds – 3288, 3833 and 3882 – are structurally similar and belong to a group of pyrazoline-thiazolidinone-isatins which were patented by us and have a noticeable antineoplastic activity in vitro that led us to study their effect on laboratory animals [12, 13].

It is well known that clinical application of doxorubicin is associated with a number of side effects [4, 14]. For instance, alternative approaches are in the active development with the aim of achieving targeted delivery of effective doses of the drug packaged in liposomes, polymeric micelles, dendrimers, ceramic nanoparticles, iron oxides, various proteins etc. [15, 16]. Targeted drug delivery with nanocarriers is more efficient in terms of bioavailability, adverse side effects minimization, mitigation of total toxicity to healthy tissues and organs, and reduction in total cost of treatment. The advantage of nanomaterial-based pharmaceuticals over traditional ones lies in the prolonged internal circulation, ability to sustain high efficiency for extended time periods, and ability to be present in the required concentration in the target site [15]. In example, doxorubicin inclusion in liposomes extends its circulation period 300-fold and improves its pharmacokinetics if compared to these properties of free doxorubicin [15, 17, 18]. The application of specific carriers for drug delivery allows us to improve substantially the efficacy of their therapeutic performance as well as to mitigate their adverse side effects [16, 17].

The studies aimed at creation of polymeric nanocarriers capable of transporting a toxic anticancer agent are particularly worth mentioning. There have been described applications of water-soluble comb-like polymer containing primary chain based on copolymer of unsaturated peroxide 2-tert-butylperoxy-2-methyl-5-hexene-3-yne (VEP) and glycidyl methacrylate (GMA) with polyethylene glycol (PEG) side chains as a carrier for anticancer drug doxorubicin [17-19].

It is known that toxic effects of antitumor drugs is associated with their decreased absorption by hepatocytes, changes in activity of certain enzymes, and inhibition of their interaction with blood serum proteins [6, 7]. Marker enzymes and metabolites, the levels of which are taken to reflect the metabolic status of liver, are commonly used to evaluate hepatocellular damage by synthetic compounds [5-7]. Pathophysiologic manifestations of drug-induced

hepatotoxicity are characterized by both intracellular and extracellular changes in hepatocytes [6, 7]. Some symptoms are traditionally taken as pertaining to liver lesion, such as bile ducts damage, hepatocytes lysis, transport protein degradation, T-cell cytolytic activity, apoptosis of hepatocytes, and disintegration of mitochondria [6, 7]. Nevertheless, the particular mechanisms of hepatotoxicity of pharmaceuticals, including anticancer drugs, remain largely unknown. Elevated serum levels of alanine aminotransferase (AlAT), alkaline phosphatase (AIP) and  $\gamma$ -glutamyltransferase (GGT) are observed at liver pathologies. This is explained by their increased biosynthesis in bile ducts cells, solubilization and release in bloodstream [5-7]. These phenomena are observed during intrahepatic as well as extrahepatic obstruction, when activity of the mentioned enzymes may exceed normal levels 5 to 10 times [4-6]. The profound increase in enzymatic activity with no consecutive decrease indicates the veritable toxicity of the pharmaceutical, which indicates the need for immediate termination of treatment.

The aim of the present study was to investigate a possibility of alleviation of hepatotoxic effects associated with novel synthetic derivatives of 4-thiazolidinones (compounds 3882, 3288, 3833) under conditions of their injection to laboratory rats in complex with polyethylene glycol nanocarrier. The effect was compared to that of the action of compounds in free-form. The effects of a well-known anticancer drug doxorubicin with and without nanocarrier particles were used for a positive control. The hepatotoxicity was evaluated by measuring marker enzymes' activity, total serum protein content and serum concentrations of a number of metabolites.

### Materials and Methods

All the manipulations with laboratory animals were conducted in accordance with the bioethics standards [20-22, 25], with the Decree No 944 by the Ministry of Health of Ukraine issued on 14.12.2009 "Establishing the order of conduct for preclinical studies of pharmaceutical substances and expert evaluation of materials from preclinical studies of pharmaceutical substances", and with current methodological recommendations [21].

The study was conducted on mature wild-type white laboratory rats with body mass of 200 to 220 g that were kept on standard fodder in animal facility compound with adequate lighting and temperature conditions.

The compounds 3882, 3288, 3833 were produced at the Department of Pharmaceutical, Organic and Bioorganic Chemistry of the Danylo Halytsky Lviv National Medical University [10, 23]. Doxorubicin (Arterium, Ukraine) was of standard pharmacy distribution. The polymeric carrier was synthesized at the Department of Organic Chemistry of the Lviv Polytechnic National University [24].

*In vivo* experiments were conducted on rats with daily injections of compounds 3882, 3288, 3833 or their polymeric complexes for 20 days. The study used 10 groups of 20 animals each: 1 – control group (intact animals); 2 – doxorubicin injections group; 3, 4 and 5 – experimental groups, animals received injections of compounds 3288, 3882 and 3833, correspondingly; 6 – experimental group, animals received injections of polymeric carrier and doxorubicin complex; 7, 8, 9 – experimental groups, animals received injections of polymeric complexes with compounds 3288, 3882 and 3833, correspondingly; – 10 polymeric carrier injection group.

The investigated compounds were introduced daily by intraperitoneal injections once a day after fasting. The experiment lasted for 10 days for animals that received doxorubicin and for 20 days for animals that received the synthetic anticancer compounds. Doxorubicin was injected starting with dose of 5.5 mg/kg, compounds 3882 and 3833 – starting with doses of 10.7 mg/kg, and compound 3288 – starting with dose of 24.3 mg/kg. The dosages were gradually elevated 1.5 times for 4 days to achieve cumulative effect. The starting dosage was 10% of maximum injected dosage in experiments for  $LC_{50}$  determination [8, 13, 14, 22].

The rats were euthanized on the 10<sup>th</sup> or 20<sup>th</sup> day by decapitation under thiopental anesthesia. The blood was used to obtain serum in which activities of alkaline phosphatase (AIP; 3.1.3.1),  $\alpha$ -amylase (3.2.1.1),  $\gamma$ -glutamyltransferase (GGT; 2.3.2.2), lactate dehydrogenase (LDH; 1.1.1.27), alanine aminotransferase (AlAT; 2.6.1.2) were measured, and concentrations of total protein, urea, creatinine, calcium ions and iron ions were determined. These parameters were measured with standard kits for automated biochemistry analyzer (Humalyzer 3000, Germany) [26].

The statistical processing of the data was done by conventional methods for analysis of variance using MS Excel software for Student's *t*-test. The difference was considered to be significant at  $P < 0.05$ .

## Results and Discussion

The detailed *in vitro* screening of compounds 3288, 3882, 3833 was done using standard methodology of the National Cancer Institute (NCI, USA). The results indicate high antineoplastic activity of those 4-thiazolidinone derivatives against most of the 60 human cancer cell lines used in the testing. This was determined by mean values of effective ( $pGI_{50}$ ), cytostatic (pTGI) and cytotoxic concentrations ( $pLC_{50}$ ) (Fig. 1). It is worth mentioning that cytotoxic effect of compound 3833 (as indicated by  $pGI_{50}$  and pTGI) was nearly the same as that of doxorubicin, while compound 3288 demonstrated even higher cytotoxicity ( $pLC_{50}$ ) than that of doxorubicin under the experimental conditions (Fig. 1).

The above data allows one to establish correlation between structural properties and activity of the investigated compounds and to assert that the Br atom at position 5 of indolyne moiety is crucial for the increased cytotoxicity. On the contrary, the substitution of phenyl radical at position 3 of pyrazolyne cycle of compound 3288 with naphthyl moiety of compounds 3833 and 3882 is of lesser importance.

As liver tissue gets damaged, the cytoplasmic and mitochondrial enzymes of hepatocytes are released into bloodstream. Thus, increased ALAT, AIP, GGT, LDH, and  $\alpha$ -amylase activities are perceived as important markers of liver-associated pathological conditions [5-7].

Under 10-day doxorubicin injections (group 2) serum GGT activity was found to increase 4 times (Fig. 3), while AIP and  $\alpha$ -amylase activities were approximately doubled in comparison to control (Fig. 4 and 5). The ALAT activity was elevated by a smaller margin of 41% (Fig. 2). Combined injections of doxorubicin and PEG-based carrier under similar conditions led to 30% decrease in serum GGT activity if compared to control which is 6 times lower than the same index of the group that received doxorubicin alone (Fig. 3). Serum  $\alpha$ -amylase activity in the group 6 after 10 days of combined doxorubicin-nanocarrier injections increased by 50%, which is 42% lower than that in group 2 with carrier-less injections (Fig. 5).

The 10-day long injection course of doxorubicin led to decreased serum urea, total protein, and creatinine concentrations, and to an increased serum calcium concentration (Table 1). Injections of doxorubicin-nanocarrier complex (group 6) led to changes that were less pronounced than those in

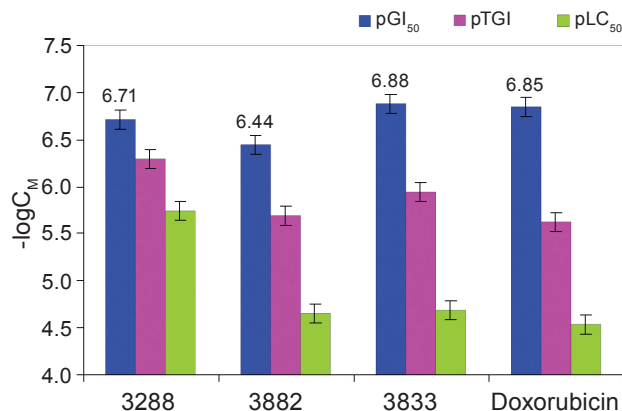


Fig. 1. Results of *in vitro* screening of compounds 3288, 3882 and 3833 in comparison with doxorubicin

the group receiving doxorubicin alone (group 2) (Table 1).

Injections of compound 3288 to animals of group 3 led to an increase in GGT and AIP activities 1.5 times on the 10<sup>th</sup> day of treatment and 2 times on the 20<sup>th</sup> day of treatment (Fig 3, 4). In contrast, ALAT,  $\alpha$ -amylase and LDH activities were not elevated significantly in this group in comparison to control, with the exception of ALAT activity that was found to be 48% increased on the 20<sup>th</sup> day (Fig. 2, 5, 6).

Injection of complexes of compound 3288 and PEG-based carrier (group 7) was associated with diminished activity of all enzymes for 20 days, which indicates hepatotoxicity, in comparison with control group and group 3 (compound 3288 without the carrier) (Fig. 2-6). Compound 3288 injection decreased the concentrations of the metabolites, and returned these parameters to normal values if injected in a complex with the polymeric nanocarrier (group 7) (Table 1, 2).

Compound 3882 injections for 10 days (group 4) led to 2.5 times increased GGT activity, ALAT activity – by 75%, AIP activity – by 52%,  $\alpha$ -amylase activity – by 44%, all of which indicate high hepatotoxicity of this substance (Fig. 2-5). Blood serum activity of the marker enzymes was also elevated after 20 days of compound 3882 injection: 2.3-fold increase in GGT activity, 2-fold in AIP activity and 52% in LDH, while ALAT activity was by 35% lower than that in control group (Fig. 2-4, 6). We found decrease in the activity of these enzymes under the effect of compound 3882 injections in complex with the polymeric carrier for 20 days (group 8): 4-fold for GGT activity and 2-fold for AIP

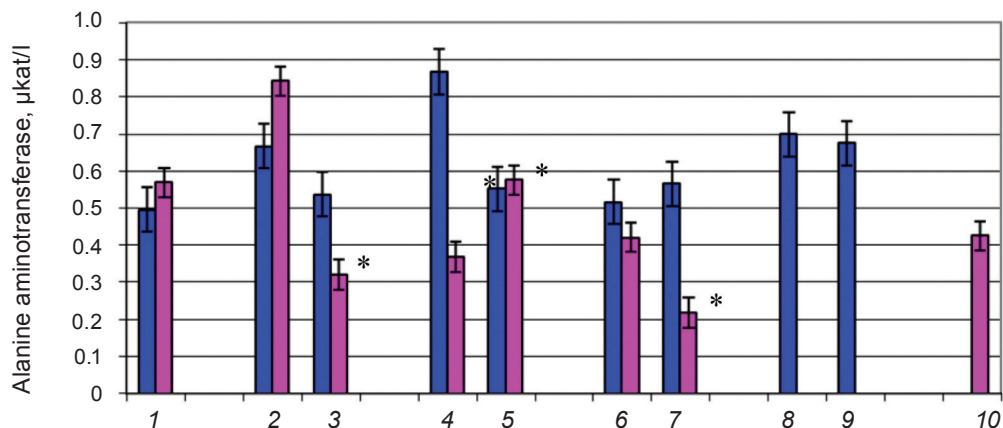


Fig. 2. Serum alanine aminotransferase activity of rats injected with doxorubicin (Dox), compounds 3288, 3882 and 3833, and complexes of these substances with the polymeric carrier (PC) for 10 days (blue) and 20 days (pink). Here and for Fig. 3-6: 1 – control, 2 – 3288, 3 – 3288+PC, 4 – 3882, 5 – 3882+PC, 6 – 3833, 7 – 3833+PC, 8 – Dox, 9 – Dox+PC, 10 – PC. \* $P < 0.05$  in comparison to the corresponding group without PC injections

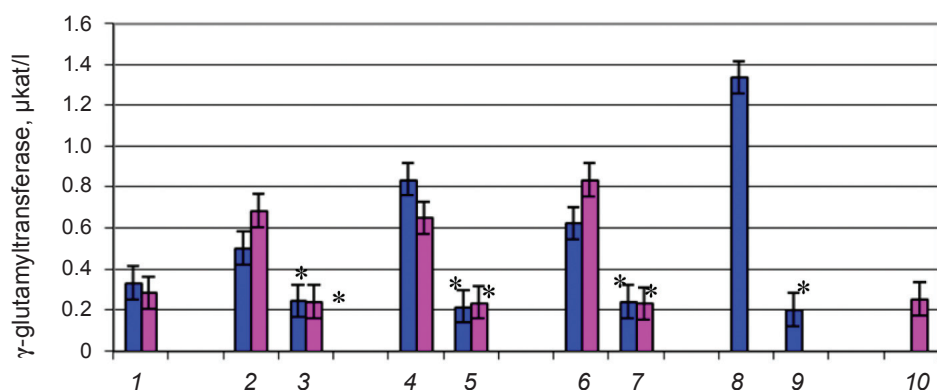


Fig. 3. Serum  $\gamma$ -glutamyltransferase activity of rats injected with doxorubicin, compounds 3288, 3882 and 3833, and complexes of these substances with the polymeric carrier for 10 days (blue) and 20 days (pink)

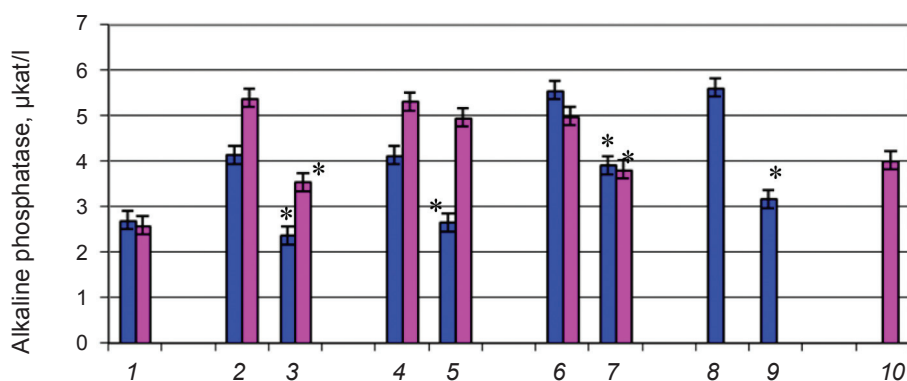


Fig. 4. Serum alkaline phosphatase activity of rats injected with doxorubicin, compounds 3288, 3882 and 3833, and complexes of these substances with the polymeric carrier for 10 days (blue) and 20 days (pink)

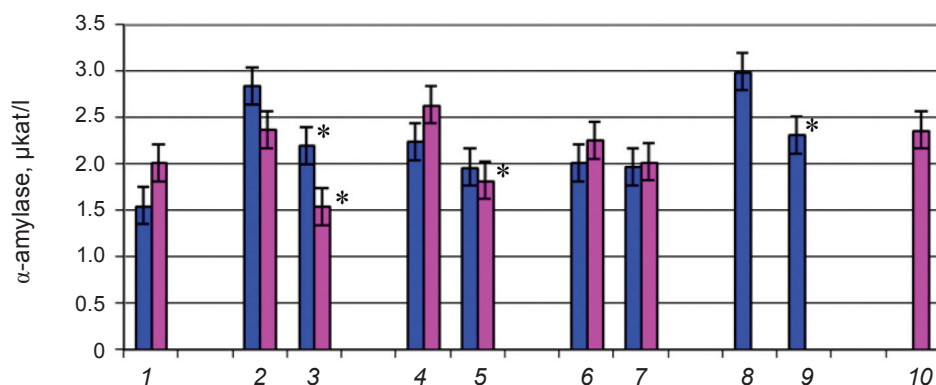


Fig. 5. Serum  $\alpha$ -amylase activity of rats injected with doxorubicin, compounds 3288, 3882 and 3833, and complexes of these substances with the polymeric carrier for 10 days (blue) and 20 days (pink)

Table 1. Blood serum concentrations of metabolites in rats injected for 10 days with investigated compounds or their complexes with the polymeric carrier (PC)

Experimental groups	Total protein, g/l	Urea, mmol/l	Creatinine, $\mu$ mol/l	Ca <sup>2+</sup> , mmol/l	Iron ions, $\mu$ mol/l
Control (group 1)	75.8 $\pm$ 3.2	4.8 $\pm$ 0.4	74.6 $\pm$ 2.6	2.47 $\pm$ 0.45	37.7 $\pm$ 2.2
Doxorubicin (group 2)	55.8 $\pm$ 4.1	1.9 $\pm$ 0.2	56.7 $\pm$ 2.9	3.60 $\pm$ 0.39	31.4 $\pm$ 2.9
Doxorubicin & PC (group 6)	71.8 $\pm$ 4.3*	3.5 $\pm$ 0.4*	77.35 $\pm$ 3.90*	2.49 $\pm$ 0.28*	38.4 $\pm$ 2.8
3288 (group 3)	57.1 $\pm$ 3.4	3.3 $\pm$ 0.3	64.1 $\pm$ 3.2	2.80 $\pm$ 0.31	28.3 $\pm$ 3.1
3288 & PC (group 7)	85.4 $\pm$ 5.1*	5.2 $\pm$ 0.5*	70.7 $\pm$ 3.8	2.29 $\pm$ 0.26	30.9 $\pm$ 3.3
3882 (group 4)	77.0 $\pm$ 4.5	4.7 $\pm$ 0.6	53.4 $\pm$ 2.8	3.00 $\pm$ 0.21	35.0 $\pm$ 3.1
3882 & PC (group 8)	87.8 $\pm$ 4.8	4.7 $\pm$ 0.9	72.9 $\pm$ 3.1*	2.84 $\pm$ 0.18	49.3 $\pm$ 3.8*
3833 (group 5)	50.3 $\pm$ 2.7	3.4 $\pm$ 0.5	67.7 $\pm$ 3.5	3.10 $\pm$ 0.35	15.0 $\pm$ 6.2
3833 & PC (group 9)	81.4 $\pm$ 4.4*	4.3 $\pm$ 0.4	68.5 $\pm$ 3.4	2.37 $\pm$ 0.29	46.2 $\pm$ 3.7*

Here and for Table 2 \* $P$  < 0.05 in comparison to corresponding group that did not receive the PC

Table 2. Blood serum concentrations of metabolites in rats injected for 20 days with investigated compounds or their complexes with the polymeric carrier (PC)

Experimental groups	Total protein, g/l	Urea, mmol/l	Creatinine, $\mu$ mol/l	Ca <sup>2+</sup> , mmol/l	Iron ions, $\mu$ mol/l
Control (group 1)	76.5 $\pm$ 3.5	4.3 $\pm$ 0.2	82.5 $\pm$ 4.1	2.50 $\pm$ 0.34	42.4 $\pm$ 2.1
3288 (group 3)	54.2 $\pm$ 2.8	2.8 $\pm$ 0.4	64.3 $\pm$ 3.3	2.60 $\pm$ 0.51	27.7 $\pm$ 2.2
3288 & PC (group 7)	73.2 $\pm$ 3.9*	3.9 $\pm$ 0.6	75.2 $\pm$ 4.2*	2.92 $\pm$ 0.42	36.2 $\pm$ 2.9*
3882 (group 4)	61.8 $\pm$ 3.1	2.4 $\pm$ 0.5	65.1 $\pm$ 3.8	2.60 $\pm$ 0.39	25.7 $\pm$ 1.9
3882 & PC (group 8)	74.2 $\pm$ 4.2	4.8 $\pm$ 0.3*	75.2 $\pm$ 3.9	3.49 $\pm$ 0.32*	53.2 $\pm$ 2.8*
3833 (group 5)	60.2 $\pm$ 2.9	3.9 $\pm$ 0.6	67.1 $\pm$ 2.9	2.60 $\pm$ 0.41	32.6 $\pm$ 3.1
3833 & PC (group 9)	74.3 $\pm$ 4.4*	2.6 $\pm$ 0.4	70.7 $\pm$ 3.4	3.15 $\pm$ 0.33	29.3 $\pm$ 3.2
PC (group 10)	67.5 $\pm$ 3.2	5.1 $\pm$ 0.3	70.7 $\pm$ 2.9	3.22 $\pm$ 0.51	42.5 $\pm$ 3.2

activity, if compared to group 4 (compound 3882 injections without the carrier) (Fig. 3, 4). Concentration of iron ions and creatinine in blood serum was decreased under the effect of compound 3882 10-day injections, and serum concentrations of urea, calcium and iron ions decreased under 20-day injections of this compound (Table 1, 2). All of these parameters were within their normal limits in groups of animals injected with 3882-carrier complex, although total protein level was elevated by 16% (Table 1, 2).

We found that AIP activity activity was doubled in blood serum of rats under effect of 10-day injections of compound 3833 (Fig. 4). In this group, GGT activity was increased by 88%,  $\alpha$ -amylase – by 30%, while AIAT activity remained within normal limits, and LDH activity was by 34% lower than that in control group (Fig. 2, 3 5, 6). The injections of compound 3833 for 20 days led to 3-fold increase in GGT activity and 45% increase in LDH activity in comparison to control group (Fig. 3, 6). The effect of complex of compound 3833 and nanocarrier (group 9) was characterized with by 37% lower GGT activity in comparison with group 5 (the free substance without the carrier), and also by 44% higher AIP activity and by 27% higher  $\alpha$ -amylase activity in comparison to control (Fig. 3-5). AIAT activity was diminished by 58% after 20 days injection of this compound (Fig. 2).

Group 10 of animals who received the polymeric nanocarrier alone, demonstrated 55.4% elevation in the AIP activity on the 20<sup>th</sup> day in comparison with the control one (Fig. 4). The activity of other enzymes was shown to be close to the normal values with minor deviations: GGT activity was decreased by 10%, AIAT – by 18%, LDH – by 33%, while  $\alpha$ -amylase activity was by 17% higher than that of the control group (Fig. 2, 3, 5, 6). Concentrations of blood serum metabolites remained mostly unchanged under effect of 20 days of the polymeric carrier injections (Table 2).

Blood serum AIP activity is generally considered to be an enzymatic marker of the metabolic cholestasis [5-7]. We detected two-fold increase in AIP activity in rats receiving doxorubicin, and 52% increase of AIP activity in animals injected for 10 days with compounds 3288 and 3882. It took 20 days of injections of compounds 3288 and 3882 to achieve the 2-fold increase of the activity of blood serum AIP. Treatments with complexes of the investigated compounds with the polymeric nanocarrier

was found to decrease the AIP activity in comparison with the corresponding experimental groups that did not receive carrier injections (Fig. 4). AIP activity was 4 times lower on the 10 day of treatment in group 4 (doxorubicin in complex with nanocarrier injections) in comparison with that of group 2 (free doxorubicin injections).

Daily injections of doxorubicin for 10 days led to 41% increase of serum AIAT activity, and injections of compound 3288 led to 35% increase of this activity (Fig. 2). Compounds 3288, 3882 and 3833 when injected in complexes with the polymeric nanocarrier caused a decrease in AIAT activity by 38%, 10%, and 58%, correspondingly (groups 3, 4 and 5) on the 20<sup>th</sup> day of treatment. We observed insignificant decrease by 18% of AIAT activity under the effect of the polymeric carrier alone (Fig. 2).

Injections of compounds 3288, 3882 and 3833 for 20 days led to increased LDH activity by 37%, 52% and 45%, correspondingly (groups 3, 4 and 5) (Fig. 6), while doxorubicin injections under these conditions led to 100% lethality among animals of the experimental group. The ability of compounds 3288, 3882 and 3833 to increase blood serum LDH activity was largely mitigated if they were injected for 10 days in complex with the polymeric nanocarrier (Fig 6). Blood serum LDH activity after 20 days of such complex injections was diminished more noticeably – by 26%, 47% and 33%, correspondingly (groups 3, 4, and 5) (Fig. 6). We observed similar (33%) decrease in the LDH activity in serum of rats injected with the polymeric carrier alone.

GGT is one of the primary enzymes that mark liver lesion directly, which is corroborated by our study. For instance, under the effect of 10-day doxorubicin injection GGT activity was 4 times higher than that in the control group (Fig. 3). The synthetic 4-thiazolidinones derivatives also increased the activity of this marker of hepatotoxicity, albeit by a much smaller margin than in the control group. All the complexes of the investigated compounds with the polymeric carrier caused significant decrease in GGT activity: 6-fold – for doxorubicin complex with PC (group 6), 2-fold – for 3288 complex with PC (group 7), 4-fold – for 3882 complex with PC, in comparison with corresponding groups that received injections of compounds without the carrier (Fig. 3).

Injections of the anticancer agents were associated with lowered concentrations of iron ions: by 27% – under the effect of doxorubicin, by 35% – under the effect of compound 3288, by 40% – under the

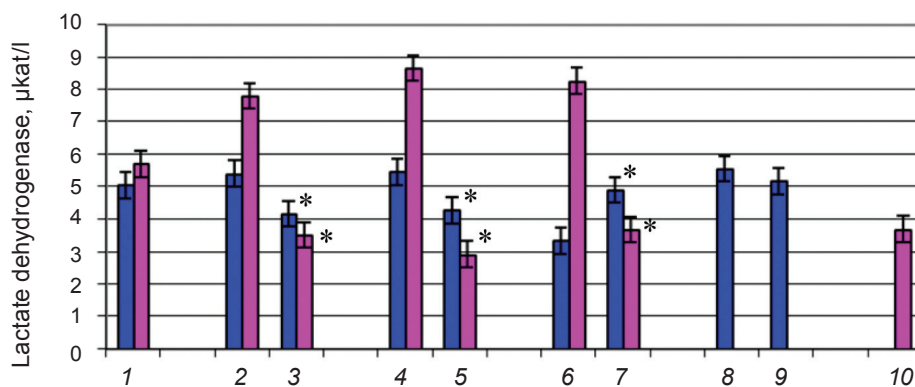


Fig. 6. Serum lactate dehydrogenase activity in rats injected with doxorubicin, compounds 3288, 3882 and 3833, and complexes of these substances with the polymeric carrier for 10 days (blue) and 20 days (pink)

effect of 3882 (Table 1, 2). The combined treatment with the investigated compounds and polymeric nanoscale carrier was accompanied by increased concentration of iron ions in comparison to normal values. Serum iron ions concentration increased by 25% in group 8, which was treated with complex of compound 3882 with the PC. The nanocarrier alone did not influence blood serum concentrations of iron ions (group 10).

AlAT activity was elevated in all experimental groups in comparison to control. AIP and  $\alpha$ -amylase activities were also elevated. We detected 4 times higher GGT activity after doxorubicin injections and 2.5 times higher under the effect of compound 3833.

Thus, we demonstrated that combining anticancer substances with the polymeric nanocarrier serves to decrease blood serum activity levels of most of the measured enzyme markers of hepatotoxicity in comparison with effects of the same compounds applied without the carrier. We also found normalization of concentrations of total protein, urea, creatinine in groups that were treated with complexes of investigated compounds with the polymeric carrier in com-

parison with the corresponding values in groups that received the investigated compounds alone.

Therefore, we established that treatment of experimental animals with novel synthetic 4-thiazolidinone derivatives (compounds 3882, 3288 and 3833) leads to changes in the activity of alkaline phosphatase,  $\alpha$ -amylase,  $\gamma$ -glutamyltransferase, lactate dehydrogenase, alanine aminotransferase in rats' blood serum. The scale of these changes correlates with the antineoplastic activity of the investigated substances. Doxorubicin, serving for a positive control, exhibited the highest antineoplastic activity, however, at the same time it had the most potent hepatotoxic and generalized toxic effect in the animals. Changes in blood serum levels of total protein, urea, creatinine, calcium ions, and iron ions under the effect of the 4-thiazolidinone derivatives were less profound than those of the enzymatic markers of the hepatotoxicity. Combining of compounds 3882, 3288 and 3833 with the nanoscale polymeric PEG-based carrier substantially reduced their hepatotoxicity in comparison with their effects when injected alone.



**БІОХІМІЧНІ ПОКАЗНИКИ  
ГЕПАТОТОКСИЧНОСТІ У  
СИРОВАТЦІ КРОВІ ЩУРІВ  
ЗА ДІЇ НОВИХ ПОХІДНИХ  
4-ТІАЗОЛІДИНОНІВ І  
ДОКСОРУБІЦИНУ ТА  
ЇХ КОМПЛЕКСІВ ІЗ  
ПОЛІЕТИЛЕНГЛІКОЛЬВМІСНИМ  
ПОЛІМЕРНИМ НАНОРОЗМІРНИМ  
НОСІЄМ**

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Мета роботи – порівняти вплив нових похідних 4-тіазолідинонів (сполук 3882, 3288 і 3833) і доксорубіцину (позитивний контроль) та їх комплексів із поліетиленглікольвмісним нанорозмірним носієм на біохімічні показники гепатотоксичності у сироватці крові щурів. Визначали активність ензимів, маркерів гепатотоксичної дії, а також концентрацію загального протеїну, сечовини і креатиніну у сироватці крові щурів. Встановлено, що після введення тваринам досліджуваних сполук зростає активність аланінамінотрансферази, лужної фосфатази та  $\alpha$ -амілази порівняно з контролем. Введення доксорубіцину підвищувало активність  $\gamma$ -глутамілтрансферази у 4 рази, а за дії сполуки 3833 – у 2,5 рази. Полімерні комплекси сполук 3882, 3288 і 3833 за введення їх тваринам істотно знижували активність досліджуваних ензимів порівняно із впливом цих сполук без полімерного носія. Найбільше знижувалась активність  $\alpha$ -амілази,  $\gamma$ -глутамілтрансферази і лактатдегідрогенази. Також встановлено повернення до нормативних показників концентрації загального протеїну, сечовини і креатиніну у сироватці крові щурів, яким вводили полімерні комплекси досліджуваних препаратів, порівняно з цими показниками, які спостерігали за введенням препаратів без полімерного носія.

**Ключові слова:** гепатотоксичність, 4-тіазолідинони, доксорубіцин, полімерний

наноносій, активність ензимів, сироватка крові щурів.

**БИОХИМИЧЕСКИЕ ПОКАЗАТЕЛИ  
ГЕПАТОТОКСИЧНОСТИ  
В СЫВОРОТКЕ КРОВИ КРЫС  
ПОД ДЕЙСТВИЕМ НОВЫХ  
ПРОИЗВОДНЫХ  
4-ТИАЗОЛИДИНОВ  
И ДОКСОРУБИЦИНА  
И ИХ КОМПЛЕКСОВ С  
ПОЛИЭТИЛЕНГЛИКОЛЬ-  
СОДЕРЖАЩИМ ПОЛИМЕРНЫМ  
НАНОРАЗМЕРНЫМ НОСИТЕЛЕМ**

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Цель работы – сравнить влияние новых производных 4-тиазолидинонов (соединений 3882, 3288 и 3833) и доксорубицина (позитивный контроль) и их комплексов с полиетиленглицольсодержащим наноразмерным носителем на биохимические показатели гепатотоксичности в сыворотке крови крыс. Определяли активность энзимов, маркеров гепатотоксичного действия, а также концентрацию общего протеина, мочевины и креатинина в сыворотке крови крыс. Установлено, что после введения животным исследуемых соединений возрастает активность аланинамінотрансферазы, щелочной фосфатазы и  $\alpha$ -амілазы по сравнению с контролем. Введение доксорубіцину повышало активность  $\gamma$ -глутамілтрансферазы в 4 раза, а введение соединения 3833 – в 2,5 раза. Полимерные комплексы соединений 3882, 3288 и 3833 при введении крысам существенно снижали и активность исследуемых энзимов по сравнению с влиянием этих соединений без носителя. Максимально снижалась активность  $\alpha$ -амілазы,  $\gamma$ -глутамілтрансферазы и лактатдегідрогеназы. Также установлено возвращение к нормативным показателям концентрации общего протеина, мочевины и креатинина в сыворотке крови

крыс, которым вводили полимерные комплексы исследуемых препаратов по сравнению с этими показателями при введении препаратов без полимерного носителя.

**Ключевые слова:** гепатотоксичность, 4-тиазолидиноны, доксорубин, полимерный наноноситель, активность энзимов, сыворотка крови крыс.

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