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FUNCTIONAL AND STRUCTURAL STATE OF RATS' KIDNEYS AND LIVER UNDER THE INFLUENCE OF NANO-POLYMER BASED ON PSEUDOPOLYAMINO ACIDS

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The article presents results of research of the influence of nano-polymer based on pseudopolyamino acids named GluLa-DPG-PEG600 on structural and functional state of kidneys and liver of Rattus norvegicus var. alba. This article precisely describes the influence of GluLa-DPG-PEG600 on activity of alanine transaminase (ALAT), aspartate transaminase (ASAT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGTP) and content of cholesterol and creatinine in blood, — moreover histological analysis of rats liver and kidneys was prepared.

We found that single injection of GluLa-DPG-PEG600 caused increasing activity of ALAT, ASAT, ALP, GGTP and amount of creatinine in blood of rats on the 7th day of experiment. We observed decreased level of enzymes and content of cholesterol and creatinine on the 14th day of experiment comparing with results obtained on the 7th day of experiment. On the 21st day of experiment activity of all enzymes and content of creatinine and cholesterol returned to the same level of activity as in the control group animals. Histological analysis revealed few inflammation processes in ascending convoluted tubules of nephrons on the 7th and 14th days of experiment that confirms increased enzyme activity and content of creatinine and cholesterol. In deed on the 21st day of experiment histological structure of rats and liver were unchanged.

Our results show temporary little toxic effect of GluLa-DPG-PEG600 on rats on the 7th and 14th days of experiment. Decreased toxic effect on the 14th day and its absence on 21st day of experiment can be explained by adaptation process to repeated injections of GluLa-DPG-PEG600 in rats. In conclusion GluLa-DPG-PEG600 could be used as carrier of active ingredients of drugs for future research.

Keywords: RATS, BLOOD, LIVER, KIDNEYS, NANO-POLYMER, PSEUDOPOLYAMINO ACIDS

ФУНКЦІОНАЛЬНИЙ І СТРУКТУРНИЙ СТАН ПЕЧІНКИ ТА НИРОК ЩУРІВ ЗА ВВЕДЕННЯ НАНОПОЛІМЕРНОЇ СИСТЕМИ НА ОСНОВІ ПСЕВДОПОЛІАМІНОКИСЛОТ

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У статті наведені дані досліджень структурного і функціонального стану печінки та нирок щурів Rattus norvegicus var. alba за дії нанополімера на основі псевдополіамінокислот — GluLa-DPG-PEG600. Показано результати впливу GluLa-DPG-PEG600 на активність аланінамінотрансферази (АлАТ), аспартатамінотрансферази, (АсАТ), лужної фосфатази (ЛФ), гамма-глутамілтранспептидази (ГГТП), вміст креатиніну та холестерину і структуру печінки та нирок.

Виявлено, що внутрішньом'язове введення нанополімеру GluLa-DPG-PEG600 спричиняє підвищення активності індикаторних ензимів АлАТ, АсАТ, ЛФ, ГГТП і вмісту креатиніну на 7-у добу експе-

рименту. Після повторного введення нанополімеру на 14-у добу експерименту спостерігалося зниження рівня активності індикаторних ферментів. За наступного введення GluLa-DPG-PEG600 на 21-у добу експерименту активність індикаторних ферментів, вмісту креатиніну та холестеролу були на рівні з контролем. За допомогою гістологічного аналізу було виявлено незначний запальний процес в ділянці висхідних звивистих канальців нирок на 7 та 14 добу експерименту, який, однак, був відсутній на 21-у добу експерименту. Структура печінки дослідних тварин не зазнала патологічних змін.

Проведені біохімічні дослідження крові (активності АсАТ, АлАТ, ЛФ, ГГТП та вмісту креатиніну і холестеролу), а також мікроскопічний аналіз структури печінки і нирок вказують на короткочасний токсичний ефект досліджуваного нанополімеру на організм дослідних щурів. Водночас зменшення токсичного впливу GluLa-DPG-PEG600 на 14-у добу та його відсутність на 21-у добу експерименту свідчить про адаптацію організму щурів до введення нанополімеру GluLa-DPG-PEG600 та низьку токсичність самого нанополімеру. Отримані дані свідчать про безпечність використання GluLa-DPG-PEG600 як носія діючих речовин лікарських препаратів для проведення подальших досліджень.

Ключові слова: ЩУРІ, КРОВ, ПЕЧІНКА, НИРКИ, НАНОПОЛІМЕР, ПСЕВДОПОЛІАМІНОКИСЛОТИ

ФУНКЦИОНАЛЬНОЕ И СТРУКТУРНОЕ СОСТОЯНИЕ ПЕЧЕНИ И ПОЧЕК КРЫС ПРИ ВВЕДЕНИИ НАНОПОЛИМЕРА НА ОСНОВЕ ПСЕВДОПОЛИАМИНОКИСЛОТ

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В статье приведены данные исследований структурного и функционального состояния печени и почек крыс Rattus norvegicus. var. Alba при действии нанополимера на основе псевдополиаминокислот — GluLa-DPG-PEG600. Показаны результаты влияния GluLa-DPG-PEG600 на активность аланинаминотрансферазы (АЛТ), аспартатаминотрансферазы, (АСТ), щелочной фосфатазы (ЛФ), гамма-глутамилтранспептидазы (ГГТП), содержание креатинина и холестерина, а также структуру печени и почек.

Установлено, что внутримышечное введение нанополимера GluLa-DPG-PEG600 провоцирует повышение активности индикаторных ферментов АлАТ, АсАТ, ЩФ, ГГТП и содержания креатинина на 7-е сутки эксперимента. На 14-е и 21-е сутки после повторных введений GluLa-DPG-PEG600 наблюдалось снижение активности индикаторных ферментов, содержание креатинина и холестерина было на уровне с контролем. Гистологический анализ структуры печени и почек крыс показал незначительный воспалительный процесс в области восходящих канальцев почек на 7-е и 14-е сутки эксперимента, который исчезал на 21-й день эксперимента. В структуре печени экспериментальных животных патологических изменений не обнаружено.

Проведенные биохимические исследования крови (активности АсАТ, АлАТ, ЩФ, ГГТП, содержания креатинина и холестерина) и микроскопический анализ структуры печени и почек указывают на кратковременный токсический эффект нанополимера GluLa-DPG-PEG600 на организм крыс. В то же время, уменьшение токсического воздействия GluLa-DPG-PEG600 на 14-е сутки, и его отсутствие на 21-е сутки эксперимента свидетельствует об адаптации организма крыс и низкой токсичности самого нанополимера. Полученные данные свидетельствуют о безопасности использования GluLa-DPG-PEG600 для проведения дальнейших исследований в качестве носителя лекарственных препаратов.

Ключевые слова: КРЫСЫ, КРОВЬ, ПЕЧЕНЬ, ПОЧКИ, НАНОПОЛИМЕР, ПСЕВДОПОЛИАМИНОКИСЛОТЫ

Adjuvants, such as aluminum hydroxide, calcium phosphate and mineral oils, are commonly used in medicine and veterinary. In spite of the fact, that aluminum hydroxide is worldwide spreading adjuvant, its excretion from living organism is problematic [12]. There are several

novel adjuvants, such as lipid micelles forming immunostimulating complexes, interleukin 1, interleukin 2, polyelectrolytes like azoximer bromide and chitosan, components of bacterial cells etc. [10, 11]. In the case of using mineral oils its manufacturing needs emulgators, whose presence in living organism is not completely safe [6, 7]. To minimize risk of adverse reactions, adjuvant selection is made individually for every vaccine.

Pseudopolyamino acids (PPA) are a class of polymeric compounds which structure doesn't include peptide bonds. Class of nano-polymers described in this article consists of pseudopolyamino acids which are derived from natural α-dicarboxylic acids and polyols. That has advantages of the polymer that are known by the brand name "Pluronic". The resulting nano-polymer has the ability to self-stable aqueous dispersions like "Pluronic" molecules. Nano-polymer consists of polyols of polyoxyethylene and polyoxypropylene with much lower molecular weight that "Pluronic". This nano-polymer refers to pseudopolyamino acids; it means that its components include natural, inherent human body amino acids which don't contain peptide bounds. For our investigation we created nanopolymer GluLa-DPG-PEG600 that consist of glutamic and lauryl acids (GluLa), dipropylene glycol (DPF), polyethylene glycol (PEG600) which covalently attached to mail macromolecule chain (*fig. 1*) [1]. The main purpose of our research was determination the influence of GluLa-DPG-PEG600 on structure and functional state of liver and kidneys of rats.

Materials and methods

Accordingly to the main our purpose, three experimental groups of male aged 6 months *Rattus norvegicus* var. alba were formed. Each group consisted of 9 rats. The weight of 1 rat was approximately 250–300 gram obtained in normal laboratory conditions.

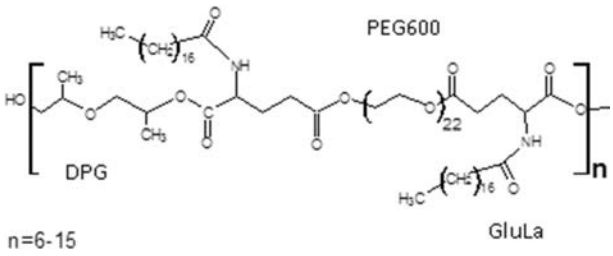

Fig. 1. Structure of nanopolymer system GluLa-DPG-PEG600

On the 1st, 7th and 14th days of experiment the experimental animals were intramuscularly injected by saline, 1 % GluLa-DPG-PEG600 and 1 % bovine serum albumin (BSA) accordingly to scheme (*table 1*). Rats from every experimental group were anesthetized with chloroform and decapitated. Biochemical analysis includes determination of the activity of alanine transaminase (ALAT, EC 2.6.1.2), aspartate transaminase (ASAT, EC 2.6.1.1), alkaline phosphatase (ALP, EC 3.1.3.1), gamma-glutamyl transferase (GGTP, EC 2.3.2.2) and content of cholesterol and creatinine in rats' blood [3]. Liver and kidneys structure were investigated using histological analysis [4, 5]. All experiments were performed in accordance with 1st National Congress on Bioethics (Kyiv, 2001) and European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1985).

Results and their discussions

We chose to determine the activity of ALAT and GGTP as major clinical markers of liver diseases, ALP — marker of liver, bone, kidneys diseases, ASAT and creatinine level as clinical markers of kidneys and heart diseases and

Table 1

Scheme of injections

Groups of animals	Injection
Control	0.3 ml saline
1st	0.3 ml of 1 % GluLa-DPG-PEG600
2nd	0.3 ml 1 % GluLa-DPG-PEG600 with 0,3 ml 1 % serum albumin (BSA)
3rd	0.3 ml 1 % BSA

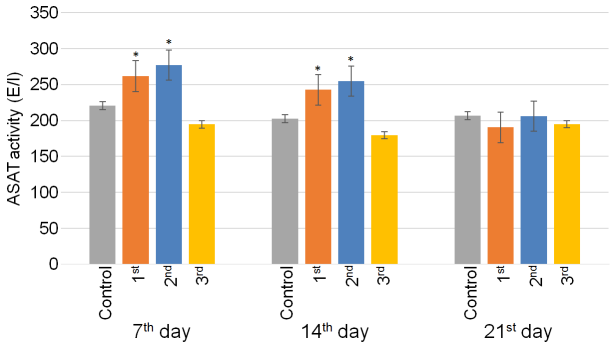

Fig. 2. ASAT activity on the 7th, the 14th and the 21st day of experiment in control, 1st, 2nd and 3rd experimental groups of animals; n=3, M±m, * — P<0.05

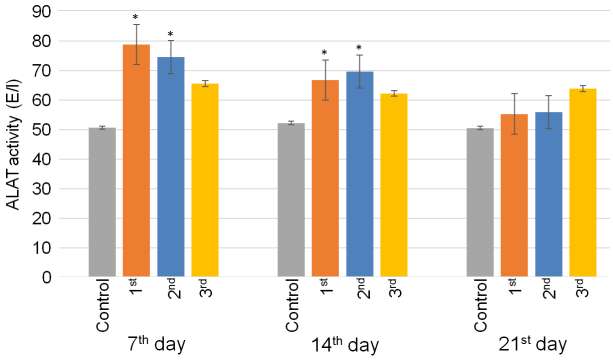

Fig. 3. ALAT activity on the 7th, the 14th and the 21st day of experiment in control, 1st, 2nd and 3rd experimental groups of animals; n=3, M±m, * — P<0.05

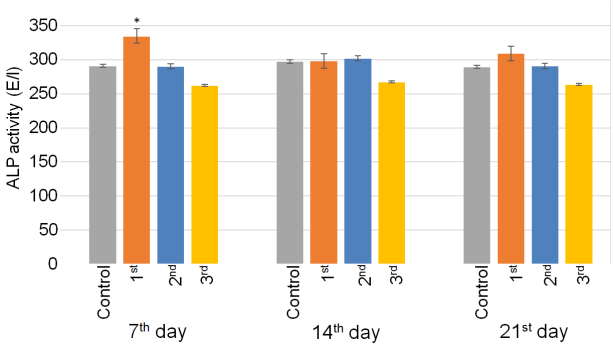

Fig. 4. ALP activity on the 7th, the 14th and the 21st day of experiment in control, 1st, 2nd and 3rd experimental groups of animals; n=3, M±m, * — P<0.05

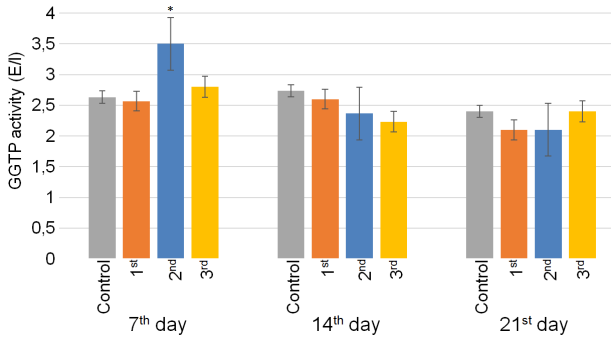

Fig. 5. GGTP activity on the 7th, the 14th and the 21st day of experiment in control, 1st, 2nd and 3rd experimental groups of animals; n=3, M±m, * — P<0.05

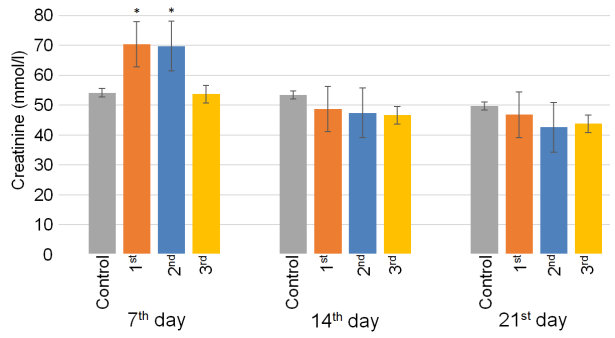

Fig. 6. Creatinine level on the 7th, the 14th and the 21st day of experiment in control, 1st, 2nd and 3rd experimental groups of animals; n=3, M±m, * — P<0.05

determine cholesterol level as clinical marker of cardiovascular system, liver and kidneys diseases.

Biochemical analysis of rats blood revealed that on the 7th day of experiment the animals from the 1st experimental group had increased (P<0,05) ASAT activity on 28 % (*fig. 2*), ALAT — on 55 % (*fig. 3*), ALP — on 13 % (*fig. 4*), creatinine content — on 29 % (*fig. 6*). In fats from the 2nd group the activity of ASAT had increased (P<0.05) on 26 % (*fig. 2*), ALAT — on 47 %, (*fig. 3*), GGTP — on 34 % (*fig. 5*) and creatinine — on 30 % (*fig. 6*). The animals from the 3rd experimental group had unchanged enzymes activity and creatinine level which were the same as in the control group of animals. Level of cholesterol was normal in rats from all experimental groups.

Increased activity of ASAT and ALAT can be the effect of defection of hepatocytes structure. Increased activity of GGTP can be the result of destruction of the cells that forms intrahepatic bile ducts and ALP — destruction of the cells that forms extrahepatic bile ducts on ultra microscopic level. No changes in liver were detected using micro-histological analysis. High level of creatinine in blood of rats from 1st and 2nd experimental groups can be the result of decreased glomerular filtration rate.

This theory was also confirmed by few sites of inflammation process revealed using histological analysis in ascending convoluted tubules of nephrons.

Accordingly to our data, on the 14th day of experiment the increased activity of ASAT on 20 % in blood of rats from the 1st experimental group and increased activity of ASAT on 25 % in rats from the 2nd group was detected (*fig. 2*).

ALAT activity increased on 27 % and 33 % in animals from the 1st and the 2nd experimental groups, respectively (*fig. 3*). ASAT and ALAT activity in rats from the 3rd experimental group were the same as in the control group. ALP, GGTP activity and level of creatinine and cholesterol of all groups of experimental animals were the same as in the control group. It was also detected few sites of inflammation process in ascending convoluted tubules of nephrons by histological analysis in rats from the 2nd experimental groups that can be the result of decreased glomerular filtration rate, but the mechanism of influence of GluLa-DPG-PEG600 on kidneys are unknown.

On the 21st day of experiment activity of ASAT, ALAT, GGTP, ALP and level of creatinine and cholesterol in blood of rats from all experimental groups of animals were in the normal range and were the same as in the control group. Histological analysis revealed that there were no pathological changes in structure of liver and kidneys of animals from all experimental groups. During all experiment, no changes in liver were detected using micro-histological analysis.

Results of our research show temporary toxic effect of GluLa-DPG-PEG600 on functional state and structure of rats kidneys and liver. It was maximal on the 7th day of experiment and dropped

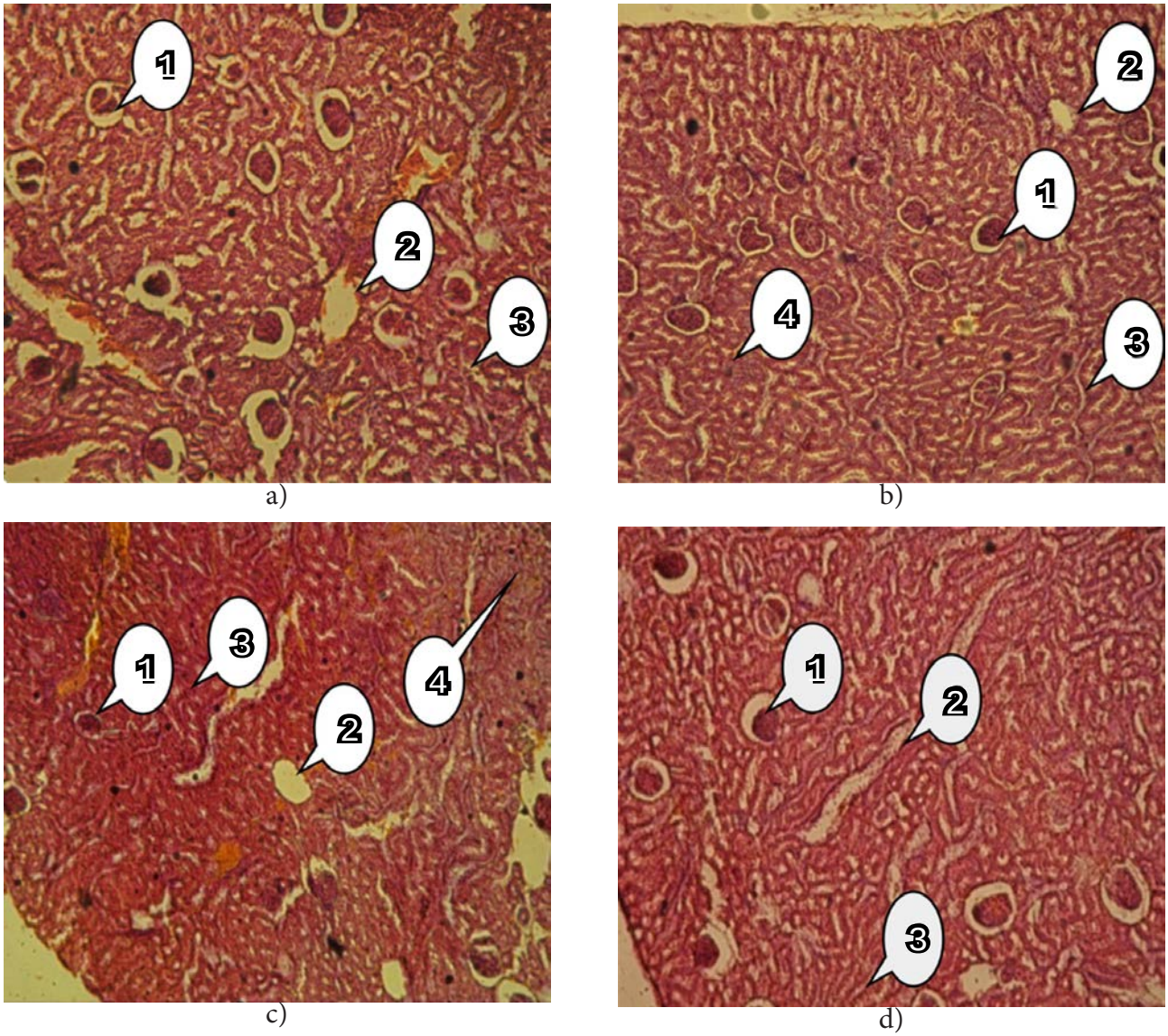

Fig. 7. Microstructure of kidneys on the 7th day of experiment in control, 1st, 2nd and 3rd experimental groups of animals; hematoxylin and eosin staining of tissue (total magnification — 100x): a) — control, b) — the 1st experimental group, c) — the 2nd experimental group, d) — the 3rd experimental group; 1 — renal corpuscles, 2 — vessel wall, 3 — ascending convoluted tubules of nephrons, 4 — site of inflammation process in ascending convoluted tubules of nephrons

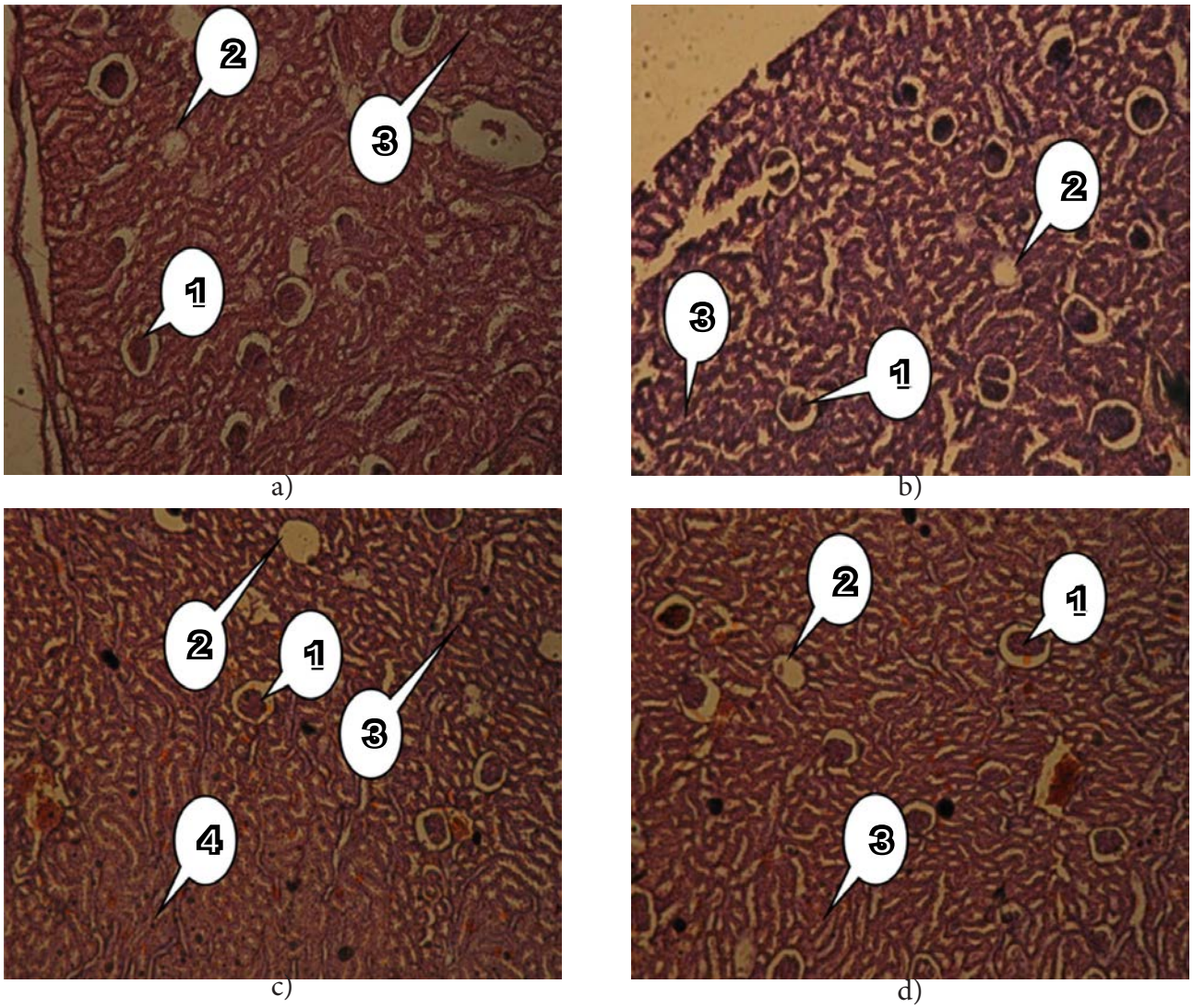

Fig. 8. Microstructure of kidneys on the 14th day of experiment in control, 1st, 2nd and 3rd experimental groups of animals; hematoxylin and eosin staining of tissue (total magnification — 100x): a — control, b — the 1st experimental group, c — the 2nd experimental group, d — the 3rd experimental group; 1 — renal corpuscles, 2 — vessel wall, 3 — ascending convoluted tubules of nephrons, 4 — few inflammation process in ascending convoluted tubules of nephrons

on the 14th day. The absence of toxic effect on 21st day of experiment is a result of possible adaptation process to repeated injections of GluLa-DPG-PEG600 in rats. It can be explained due to the fact that the structure of GluLa-DPG-PEG600 (nano-polymer based on glutamic amino acid) is closely related to nature amino acids.

Conclusions

Our studies show provisionally toxic effect of GluLa-DPG-PEG600 on rats liver and kidneys confirmed by increased activity of ALAT, ASAT, GGTP, ALP, high level of creatinine and local inflammation process in convoluted tubules of nephrons.

Studies show provisionally toxic effect of GluLa-DPG-PEG600 which was maximal on the 7th day of experiment and accompanied by the highest enzymes activity and presence of the sites of inflammation process in ascending convoluted tubules of nephrons. Absence of toxic effect on the 21st day of experiment after three injections of GluLa-DPG-PEG600 was correlated with normal enzymes activity and unchanged structure of rats liver and kidneys. Obtained results showed possible adaptation process in rats to repeated injections of GluLa-DPG-PEG600 confirmed by the fact that the structure of GluLa-DPG-PEG600 (nano-polymer based on glutamic amino acid) is closely related to nature amino acids.

Results show possible safety of usage of GluLa-DPG-PEG600 as a carrier of active ingredients of drugs for future research.

Prospects for future research. In our future research we are planning to study immune response of rats to repeated injections of GluLa-DPG-PEG600. We will also investigate the influence of GluLa-DPG-PEG600 in complex with different vaccines on animals.

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