Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **S-6 Year 2023** Page **213:218***

The Results of Biochemical and Pathomorphological Studies of The Internal Organs of White Rats on The Model of Experimental Glomerulonephritis

Otajonov Ilkhom Otaboevich[1*], Tajieva Zebo Bakhodirovna[2], Abdullaeva Dilafruz Gayratovna[3], Saidova Gulbakhor Tursunalievna[4], Khayitov Javokhir Bakhodirovich[5], Shamuratova Gulnora Bakhtiyarovna[6], Abdullaeva Diloram Telmanovna[7], Nazarova Malokhat Berdibaevna[8]

[1]Associate professor of the Department of hygiene of children, adolescents and Nutrition, Doctor of Medical Sciences, Tashkent, Uzbekistan. ilhom1802@mail.ru
[2]Head of the Department of Pediatrics and higher khamshiralik affairs of the Urgench branch of the Tashkent Medical Academy, PhD, Urganch, Uzbekistan, zebotajiyeva@gmail.com
[3]Associate professor of the Department of hygiene of children, adolescents and nutrition, Tashkent Medical Academy, Doctor of Medical Sciences, Tashkent, Uzbekistan, abdullaeva.dg1976@gmail.com
[4]Tashkent Medical Academy, assistant of the Department of hygiene of children, adolescents and nutrition, candidate of Medical Sciences Tashkent, Uzbekistan, gulbaxorsaidova595@gmail.com
[5]assistant of the Department of hygiene of children, adolescents and nutrition, candidate of Medical Sciences Tashkent, Uzbekistan, javohirhayitov@mail.ru
[6]Senior Lecturer, Department of Nervous Diseases, psychology and psychotherapy, Urgench, Uzbekistan, Urgench branch of Tashkent Medical Academy shamuratovagulnora8@gmail.com
[7]Associate professor of the Department of children's diseases, Tashkent Medical Academy, Uzbekistan, zvezda-6565@mail.ru
[8]Urgench branch of the Tashkent Medical Academy, Senior Lecturer of the Department of Pediatrics and higher ECHR, PhD, Urganch, Uzbekistan, malohat1975nazarova@gmail.com

**Corresponding author's E-mail: ilhom1802@mail.ru*

Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 22 Nov 2023

Abstract

Objective: to study the effect of bread with the addition of 5 and 10% soy flour on the body of rats and to assess biochemical and pathological changes in animal organs in experimental glomerulonephritis. Material and methods: The glomerulonephritis model was reproduced by injecting 5 ml of isolated adopted lymphocytes intravenously through the tail vein to intact white mongrel rats. Results: the introduction of bread enriched with 5% and 10% soy flour into the diet of animals with experimental glomerulonephritis is safe, does not cause additional pathologies and death of animals and slightly improves the course of the disease. The improvement of the condition is confirmed by the results of biochemical studies of blood serum and histomorphological studies of the internal organs of white rats with experimental glomerulonephritis. Conclusions: the effectiveness of a low-protein diet based on the appointment of bakery products from wheat-soy flour in experimental glomerulonephritis is substantiated.

Keywords: *Experimental Glomerulonephritis, Biochemical Studies, Creatinine, Urea, Total Protein, Urine and Kidney Histomorphology.*

1. Introduction

The increased interest in the study of primary and secondary kidney diseases, especially glomerulonephritis (GL), in the last decade is due to the continued increase in the incidence of terminal renal insufficiency (ESRD), which is acquiring epidemic proportions in some countries. One of the main causes of ESRD is chronic glomerulonephritis (CGN). The clinical manifestations of the latter are very similar, do not depend on the cause and mechanisms of the disease itself [5]. At the same time, the rate of progression of CGN is determined by etiological factors and pathogenetic mechanisms [4].

Significant progress has been made in the study of the pathogenesis of CGN in recent years. So, in addition to clarifying and expanding knowledge in the field of immune phenomena, lifetime morphological changes on the part of the kidneys, reflected in the corresponding WHO classification,

appeared evidence of the presence of nonimmune pathways contributing to the progression of CGN: intraclubular hypertension with hyperfiltration, nephrotoxicity of hyperlipidemia and plasma proteins themselves excreted in urine [8]. The result was a revision of the tactics of treatment of glomerulopathies. New approaches to therapeutic interventions have emerged in various clinical and morphological variants of CGN [4,5].

Glomerulonephritis remains an urgent problem of modern medicine, due to the severity of the course of the disease and the complexity of treatment. Among all kidney diseases, glomerulonephritis has a constant tendency to progress, being the most common cause of the development of chronic renal failure [1,3]. This determines one of the problems of modern nephrology – the problem of disability and rehabilitation, which is associated not so much with the general prevalence of the disease, but with the undoubted predominance among young patients and the fatal development of chronic renal failure [6].

The study of the mechanisms of progression of nephropathies in experimental conditions is dictated by the search for new methods to slow the development of renal failure, which, in turn, is of great medical, social and economic importance. Changing the possibilities of the experiment makes it possible to reproduce specific, predetermined forms of nephritis for a more subtle analysis of the pathogenesis and morphogenesis of the disease, patterns of structural and ultrastructural changes in various departments of the nephron, as well as for preclinical studies of the effectiveness of new drugs or a low-protein diet in this pathology. The positive effect of low-protein diets on the course of glomerulonephritis is associated with their ability to influence glomerular hemodynamics and, accordingly, slow down the formation of glomerulosclerosis. Considering that with a significant decrease in glomerular filtration, the protein content in order to avoid the development of nutritional disorders should not exceed 0.6 g / kg of body weight, the protein component of nutrition should be represented by a protein of high biological value, the source of which is animal products and soy.

The purpose of the study

Study of the effect of bread with the addition of 5 and 10% soy flour on the body of rats and assessment of biochemical and pathological changes in the kidneys of animals with experimental glomerulonephritis.

2. Materials And Methods

To reproduce experimental glomerulonephritis, the method proposed by N.M.Yuldashev, M.K. Nishantaev [7] was used, based on the administration of 5 ml of isolated adopted lymphocytes intravenously through the tail vein to intact white mongrel rats. The experiment was performed on 24 animals weighing 250-300 g. All experimental and control animals were kept in the same conditions of the vivarium Interuniversity Research Laboratory (IRL) TMA on a regular and special diet. Throughout the experiment, the animals were under daily supervision; their general condition, behavior, feed and water consumption, the condition of the hair and mucous membranes were recorded.

Biochemical studies included the determination of creatinine, urea and total protein in blood serum and urine and histomorphological studies of the kidneys of experimental animals with glomerulonephritis who took and did not take complementary foods based on wheat/soy flour bakery products.

Biochemical parameters were determined by standardized methods: total protein – biuretic (Total protein kits from CYPRESS Diagnostics, Belgium), urea-vital urease/glutamate dehydrogenase (Vital development Corp. Russia), creatinine by the Jaffe method (sets of reagents from CYPRESS Diagnostics, Belgium) on the biochemical analyzer VA-88 A (Mindray, P.R. China).

Statistical studies were conducted on the basis of standard clinical recommendations. Quantitative data are presented as the arithmetic mean (M) ± standard deviation (SD) in the case of a normal distribution and as the median (Md) and quartiles (Q) or (SD) for other distributions. The confidence level $p<0.05$ was taken as statistically significant changes. The results of the clinical examination were processed on a Pentium IV personal computer using Microsoft Exell and Microsoft Access office applications with the calculation of the arithmetic mean of the studied indicator (M), its standard error (m), reliability indicators (p) and the student's criterion. At the same time, the methods and existing guidelines for statistical processing of clinical and laboratory research data were taken into account [8].

Before the start of the experiment on modeling glomerulonephritis in all rats weighing 250-300 g under nembutal anesthesia (40 mg/kg weight, intraperitoneal), the anterior abdominal wall was opened, the left kidney was isolated and 250 pathogenic E. coli units were punctured directly into the renal parenchyma with a thin needle in a volume of 0.25 ml on isotonic solution. The wound was tightly sutured. Animals under ether anesthesia were slaughtered on the 7th day of the experiments. After the slaughter of rats in sterile conditions, mesenteric lymph nodes were extracted and placed in a tissue

culture medium No. 199. The nodes were separated from the fatty tissue. Then the organs were crushed in a glass homogenizer and passed through a nylon filter. The cell suspensions were washed twice by centrifugation at 1000 rpm for 10 minutes. In Goryaev's chamber, the number of cells per ml of suspension was counted.

Isolated adapted lymphocytes were injected intravenously into intact animals through the caudal vein at a dose of 5 ml. Signs characteristic of glomerulonephritis appeared on the 4th day after the introduction of adaptive lymphocytes. On the 5th day after the introduction of adaptive lymphocytes, the rats were divided into 4 groups of 6 in each. Group 1 (healthy rats) and group 2 (animals with glomerulonephritis) were kept on a regular diet. The rats of the 3rd and 4th groups were additionally included in the usual diet bakery products made of wheat / soy flour with a content of 5 and 10% soy flour, respectively.

3. Results and Discussion

The experiment lasted 60 days. During the experiment, the biochemical parameters of blood and urine of animals were evaluated three times: on the 7th, 30th and 60th days. Comparison of biochemical blood parameters of rats of experimental groups and intact group showed that on the 7th day in the blood serum of animals of the 2nd, 3rd and 4th groups, the values of blood parameters increased: urea, creatinine and total protein, which indicates the development of acute inflammatory processes in the kidneys and violation of their basic filtration functions (table).

When bread containing 5 and 10% soy flour is introduced into the diet of experimental animals, there is a statistically insignificant improvement in the condition of animals of the 3rd and 4th groups. In these groups there were no animal deaths, while in the 2nd group, where the animals were kept on a normal diet without the introduction of bread with soy flour, 3 rats out of 6 died 20-26 days after the start of the experiment.

When conducting a qualitative urine analysis with Combina 13 express strips (Human GmbH, Germany), it was found that in groups 2, 3 and 4, protein was detected in the urine throughout the experiment, which normally should not be. The highest values of total protein were recorded in animals of the 2nd group, in rats of the 3rd and 4th groups this indicator was significantly lower.

Creatinine is a substance involved in the systemic energy exchange of muscle fibers. One of the signs of kidney damage is an increase in creatinine. In animals of 3 experimental groups, this indicator was higher than the control (68-104 mmol/l). In animals of the 2nd group, on the 30th and 60th days of the experiments, the creatinine concentration increases, reaching maximum values on the 30th day (158.57 ±9.36, $p<0.01$). On the 60th day, the studied indicator significantly decreased to 125.57±7.55 ($p<0.01$). The creatinine content significantly exceeded the norm and visually corresponded to peak values in group 3 on day 30 at the level of 131.70±24.49, and in group 4 at the level of 138.03±26.30 mmol/l. On the 60th day, the creatinine level in animals of the 3rd and 4th groups decreased to 101.7±18.75 and 108.40±20.40 mmol/l, respectively. Thus, when bread with the addition of 5 and 10% soy flour was introduced into the diet of animals on the 60th day, a significant decrease in creatinine concentration was noted.

Table Biochemical blood parameters of white rats on the 7th, 30th and 60th days of experimental glomerulonephritis, n=6, M±m

Animal groups	Number of days from the beginning of the experiment	Urea, mmol/l	Creatinine mol/l	Total protein, g/l
1 – intact	7 days	15,65±4,07	84,30±16,00	104,26±19,20
	60 days	16,43±5,28	86,47±16,71	103,76±19,05
	60 days	15,08±3,99	79,17±15,11	99,01±18,22
2 – with glomerulonephritis on a regular diet	7 days	74,85±22,22**	120,00±22,47	177,76±33,33
	60 days	87,17±12,40*	158,57±9,36*	185,36±8,56*
	60 days	70,37±10,14*	125,57±7,55*	126,7±11,14*
3 – with glomerulonephritis on a diet with the addition of bread with 5% soy flour	7 days	84,18±22,93**	114,93±21,80	140,17±26,59
	60 days	98,34±27,43**	131,70±24,49	170,11±32,25
	60 days	65,35±18,05**	101,7±18,75	133,14±25,34
	7 days	90,59±23,81**	122,37±22,79	148,69±29,18

4 – with glomerulonephritis on a diet with the addition of bread with 10% soy flour	60 days	98,54±26,24**	138,03±26,30	165,84±31,04
	60 days	60,37±20,54**	108,40±20,40	111,32±20,79

Note. The number of animals in group 2 decreased by the end of the 1st month due to the death of 3 rats; a - $p<0.01$ compared with intact animals; b - $p<0.05$ compared with intact animals

The urea content in the blood of animals of the 2nd, 3rd and 4th groups significantly decreased in dynamics on the 7th, 30th and 60th days, respectively, by 1.06, 1.28 and 1.50 times ($p<0.05$).

Thus, in animals of the 3rd and 4th groups that received a diet with bread enriched with 5 and 10% soy flour, the studied indicators in all periods of experiments are lower than in group 2 rats that did not receive bread with soy flour.

60 days after modeling glomerulonephritis, at the end of a series of experiments, histological studies of organs and tissues of animals of three experimental groups were carried out. Histological diagnostics of kidneys in animals of the 2nd experimental group allowed to establish chronic tubulointerstitial nephritis with focal nephrosclerosis in them (indicated by the arrow). Macroscopically, the kidneys are enlarged in size due to interstitial edema and inflammation, the boundary between the cortical and cerebral layers is not noticeable. Under the capsule and in the cortical layer – petechial hemorrhages. The kidney parenchyma tissue is replaced in places and compressed by focal growths of connective tissue (Fig. 1). Periglomerular and peritubular types of sclerosis prevail (indicated by a double arrow). The glomeruli of the cortical layer outside the interstitial sclerosis zones are enlarged in size (Fig. 2).

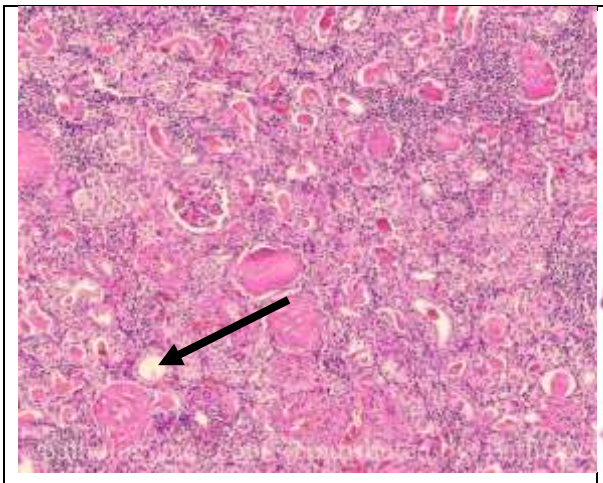

Fig. 1. Kidney of group 2 rats after modeling glomerulonephritis with the help of adopted lymphocytes on the 60th day. Coloring GE. Uv. 10x10.

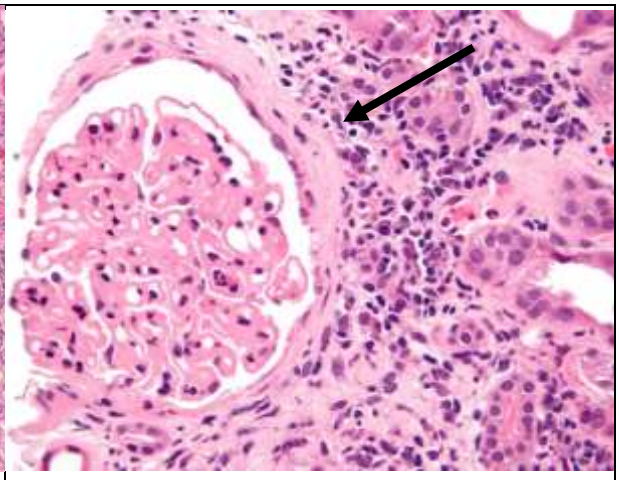

Fig. 2. Kidney of group 2 rats after modeling glomerulonephritis with the help of adopted lymphocytes on day 60. Coloring GE. Uv. 10x20.

Morphological studies of the kidneys of animals of the 3rd experimental group, which were fed with bakery products containing 5% soy flour, revealed less pronounced lymphocytic infiltration in the renal interstitium, interstitial edema was observed only in two micro-preparations. There is a small number of atrophied renal tubules. Unlike animals of the 2nd group, no petechial hemorrhages were observed under the capsule and in the cortical layer. Kidney parenchyma tissue is less replaced by connective tissue growths compared to the first experimental group (Fig. 3). There is a small number of glomerulomerular scleroses (Fig. 4).

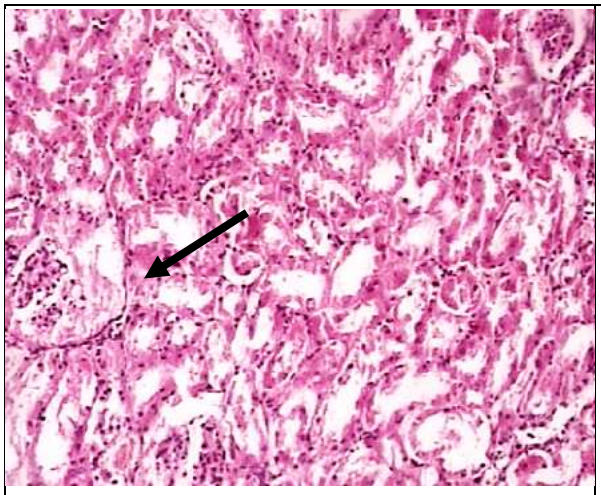

Fig. 3. Kidney of a rat of group 3 who took 5% soy flour for 60 days. Coloring GE. Uv. 10x20.

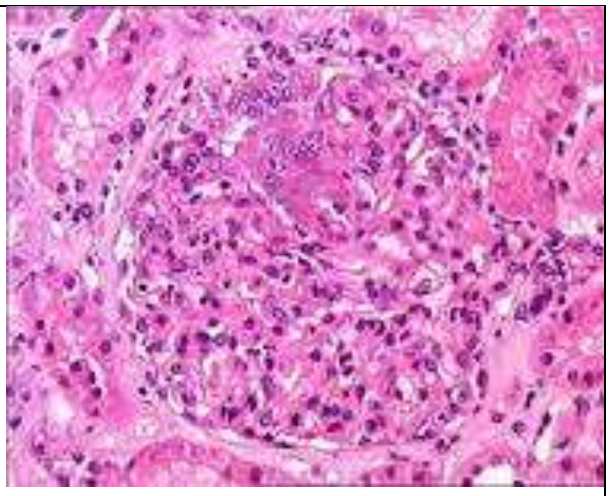

Fig. 4. Kidney of a rat of group 3 who took 5% soy flour for 60 days. Coloring GE. Uv. 10x20.

Morphological studies of the organs of rats of the 4th group, which were fed with bakery products containing 10% soy flour, revealed a suspension of the processes of sclerosis of the kidney parenchyma, which were expressed in animals that were not fed with soy flour. Lymphocytic reactive phenomena decreased in all the studied organs, which manifested itself in improving the functional state of the cell organelles, reducing the swelling of the interstitial tissue.

Macroscopically, the kidneys of animals of this group had a regular bean-shaped shape, a smooth elastic surface, no hemorrhages were detected on the surface and under the capsule, the capsule is easily removed. In the section, the cortical layer is slightly darker than the cerebral one, the boundary between the cortical and cerebral layers is noticeable. The wall of the pelvis is smooth, elastic, not thickened, without hemorrhages, clots and concretions were not found. Histological examination of animal kidney preparations showed the preservation of all structural elements of nephrons, vascular and stromal components of organs (Fig. 5, indicated by an arrow).The tubules are lined with simple cubic epithelium. The renal glomerulus is surrounded by a Bowman capsule (Fig. 6, indicated by a double arrow). These micro-preparations show the urinary space (which appears as a transparent space between the glomerulus and the Bowman capsule). At the same time, signs of membranoproliferative glomerulonephritis remained.

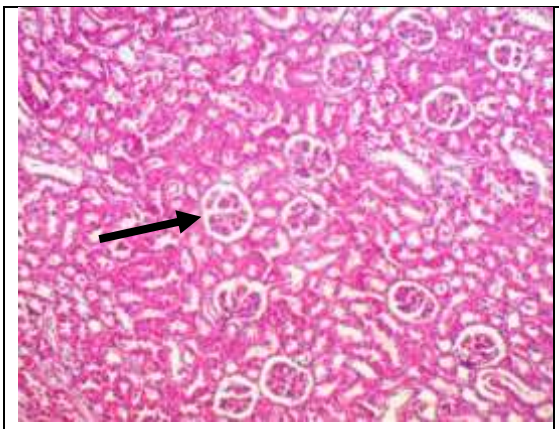

Fig. 5. Kidney of a rat of group 4 after modeling glomerulonephritis with the help of adopted lymphocytes, who took 10% soy flour for 60 days. Coloring GE. Uv. 10x10.

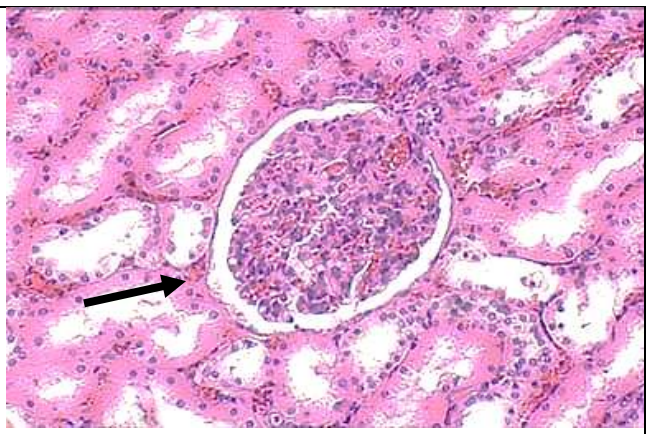

Fig. 6. Kidney of a rat of group 4 after modeling glomerulonephritis with the help of adopted lymphocytes, who took 10% soy flour for 60 days. Coloring GE. Uv. 10x40.

4. Conclusion

The introduction of bakery products enriched with 5% and 10% soy flour into the diet of animals with experimental glomerulonephritis is safe, does not cause additional pathologies and death of animals and slightly improves the course of the disease.

Chronic tubulointerstitial nephritis with focal nephrosclerosis was found in animals of the 2nd experimental group with glomerulonephritis who received bakery products without soy flour. Under

the capsule and in the cortical layer – petechial hemorrhages. The kidney parenchyma tissue is replaced in places and compressed by focal growths of connective tissue. Periglomerular and peritubular types of sclerosis prevail. The glomeruli of the cortical layer outside the interstitial sclerosis zones are enlarged in size.

The histomorphological picture in the kidneys of animals of the 3rd experimental group receiving bakery products with 5% soy flour content was characterized by less pronounced lymphocytic infiltration in the renal interstitium, interstitial edema was observed only in two micro-preparations. There is a small number of atrophied renal tubules. In comparison with animals of the 1st group, there are no petechial hemorrhages under the capsule and in the cortical layer. Kidney parenchyma tissue is less replaced by connective tissue growths compared to the 1st experimental group. There is a small amount of glomerular sclerosis.

Morphological studies of the organs of group 4 rats receiving bakery products with 10% soy flour content indicate a suspension of the processes of sclerosis of the kidney parenchyma. In all the studied organs, dystrophic phenomena decreased, which manifested itself in improving the functional state of the cell organelles, reducing the swelling of the interstitial tissue.The dynamics of changes in the concentrations of urea, creatinine and total protein confirms histomorphological changes in the kidneys.

References:

1. Vorobyeva O.A., Nast S.V., Cohen A.N. Morphological characteristics of the non-amyloid form of deposition of monoclonal immunoglobulins in the kidneys // Arch. pat. – 2006. – No. 6. – pp. 19-22.
2. Zaitsev V.M., Liflyandskiy V.G., Marinkin V.I. Applied medical statistics: Textbook. – St. Petersburg: Folio, 2003. – 432 p.
3. Ignatova M.S. Actual problems of morphology of childhood at the beginning of the XXI century // Pediatrics. - 2007. – No.6. – pp. 6-14.
4. Mukhin N.A., Tareeva I.E., Shilov E.M. Diagnosis and treatment of kidney diseases. – M., 2002. – 381 p.
5. Nephrology: A guide for doctors; Edited by I.E. Tareeva. – M.: Medicine, 2000. – 688 p.
6. Rudov I.V., Kuzmin Yu.F. Complex programs of medical and social rehabilitation of disabled people due to kidney diseases // Gastroenterology of the South of Russia: Annual. scientific and practical publishing house – Rostov n/A, 2008. – pp. 336- 338.
7. Yuldashev N.M., Nishantaev M.K. The functional state of the kidneys when modeling nephritis with the help of adopted lymphocytes // International. journal. prikl. and the foundation. research. - 2016. – No. 6 (part 2). – pp. 318-321.
8. Baud L., Fouqueray B., Belocq A. Cytokines and hormones with antiinflammatory effects: new tools for the therapeutic intervention // Curr. Opin. Nephrol. Hypert. – 2001. – Vol. 10. – P. 49-54.