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The influence of natural mineral water on the structural and functional changes in the rat kidneys under urolithiasis modelling

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Abstract

In the experiment on the white Wistar rats, the authors evaluated kidneys changes in urolithiasis modelling (Kidney stone disease, KSD) and the possibility of correcting these changes by internal use of the mineral water (MW) with high organic matter content. The results of the studies revealed structural pathological changes in the kidneys in the form of changes of the part of glomeruli capillaries and gross changes in the kidneys tubules and interstitial layers. At the same time, the functional kidneys activity is manifested in the form of increased urination and a significant increase in urination. Urine metabolic characteristics are also change. The use of MW leads to an improvement in the capillaries of the renal corpuscles structure and almost complete restoration of the tubules structure. The functional activity of the kidneys and the quality of urine have significantly improving. The authors believe that the organic compounds of MW, which mainly determine its biological activity, contribute to the improvement of the regulation of metabolic processes and, accordingly, have a positive effect on the structural and functional parameters of the kidneys..

Keywords: *urolithiasis, structural and functional characteristics of the kidneys, mineral water,*

Introduction

The problem of prevention and treatment of urolithiasis (KSD) is one of the most common in nephrology both worldwide and in Ukraine of today (1, 2). In recent years, the prevalence of KSD is seen as a consequence of the impact of negative environmental factors in the regions of residence (3, 4). It should be noted that urolithiasis affects not only the elderly but also the young able-bodied population, which requires long-term treatment, leads to temporary and permanent disability. As the global prevalence of KSD continues to rise, the burden on health systems will continue to increase.

The final uncertainty of the pathogenesis of KSD complicating the solution of this problem. Today in the treatment of KSD are widely used natural remedies and especially mineral water (MW). A special place among MW used in nephrology is occupied by the group of weakly mineralized waters with a high content of organic matter. The authors presented data on the effect of the weakly mineralized waters with high organic matter content in terms of total organic carbon (C org.). It should be noted that the quantity of the MWs of this type are insignificant in the world, due to the peculiarities of their geological formation. The deposits of this MW are concentrated in the Carpathian region. For example, in Romania, sources with this type of MW are located in the Southern Carpathians (6). In Ukraine, MW deposits are concentrated in the Carpathian region. In the Carpathians - the foothills of the eastern Carpathians, stretching in the western region of Ukraine in the Lviv and Ivano-Frankivsk regions, the most famous representative of these waters is Naftusya, belonging to the Truskavetsky field of the MV. In the second half of the 20th century, not far from the Truskavetsky field, the Skhodnitsky deposit of the MW with C org. were designed for use.

The formation of the chemical composition of the MW of these fields is associated with rocks enriched in scattered organic matter of petroleum genesis. MW Naftusya has long been used by people for treatment, the study of its healing properties has received much attention over the past hundred years or more, which has greatly expanded the range of its applications in balneology. Historically, this has given rise to the name MW of other deposits with low mineralization and high content of C org. (from 8 mg / l and above) - MW type Naftusya.

A feature of weakly mineralized MW is their hypotonicity (total mineralization is up to 1 g / l), thanks to which (unlike MW with greater mineralization) their rapid and complete absorption by the intestinal mucosa and is ensure of entry of ions and biologically active substances into the blood. Due to this, metabolic processes in the body improve hydrostatic blood pressure increases, ultrafiltration in the kidneys and diuresis also increases.

High biological activity of MW of this type is associated with organic substances, which include low molecular weight fatty acids, amino-like substances, volatile and non-volatile organic acids, phenols, bitumens and humins. In general, the organic complex of such MW is divided into hydrophilic - low molecular weight (<C8) carboxylic acids, amines, polyphenols) and - hydrophobic hydrocarbons with a chain length (> C8), oils, resins, asphaltene compounds. Experimental studies have shown that the first group of substances has a stimulating effect on the secretory-transport apparatus of the kidneys (7).

The participation of autochthonous microflora (microbiota) in both the creation and assimilation of organic components in MW is also indicated, which also affects the biological activity of MW, as the metabolic products of microbial cenosis of the MW account for about 1% the total quantity of organic matter of the MW (8). These microorganisms carry out complex biochemical processes and can actively change the pH, Eh, gas and salt composition of MW. Microbial metabolites (sugars, amino sugars, amino acids, etc.) form part of the organic background of underground MW. As a biological environment, MW have a certain composition of its own indigenous microorganisms of different ecological and physiological groups, which adapt to specific living conditions - chemical composition and temperature.

Specific microorganisms that makeup the majority of the autochthonous microbiota of the Truskavets and Skhidnytsia deposits are denitrifying, sulfate-reducing, thionic acid and bacteria that destroy phenols, bitumens and humins. That is, the microbiota plays an important role in the chemical transformations of organic matter in waters such as Naftusya and the formation of their physicochemical properties (8, 9). Therapeutic properties of this type of MW have been proved and successfully used by experimental and clinical works (9, 10, 11).

Despite numerous studies and the progress made in recent years in this direction, the search for new effective and safe drug and non-drug treatments continues (12, 13, 14). One of the main prerequisites for an effective solution to this problem is the use of adequate experimental models of KSD, which on the one hand allow preclinical trials of drugs in conditions as close as possible to those in the human body with nephrolithiasis, as the use of people for such studies is not ethical. (15, 16). Studies on the effect of MW of this type in experimental nephrolithiasis in the available literature are very few and unsystematized. Based on the above, the purpose of the study: to determine the structural and functional state of the rats kidneys on the model of KSD under the influence of MW with high organic matter content.

Materials and methods. The work was performed on 50 white female Wistar rats. Keeping and research on animals were carried out under existing guidelines and legal documents (17, 18). The KSD model was reproduced using a 1% solution of ethylene glycol, which rats were obtained from drinkers in the mode of free access for 25 days (19).

Next, we investigated the presence and degree of corrective action of natural mineral water, which in chemical composition belongs to the type of weakly mineralized with a high content of organic matter hydro carbonate calcium sodium MW. The well is located near the Skhidnytsky MW field (Drohobych district, Lviv region, Ukraine). The total mineralization of MW is 0.85 g / l and the content of organic matter in terms of organic carbon (Sorghum) is 13.0 mg / l.

Animals were ranked into 3 groups. The first group - 10 intact animals, the results of the study were considered control. Group 2 - 20 rats, were animals with a model of KSD.

Group 3 - 20 rats, were animals with a model of KSD, who received as a course of treatment MW from 25 to 35 days during the experiment. MW rats were injected daily into the esophagus through the soft probe with olive, once a day, at a dose of 2.0% of the animal's body weight.

A comprehensive examination began with a urine test. First of all, the microscopic examination of urine determined the composition of sediments.

Morphological studies determined changes in the structural and functional organization of the kidneys and histochemical reactions to determine the activity of succinate dehydrogenase (SDG) and lactate dehydrogenase (LDH), the activity of enzymes was evaluated in conventional units of optical density (units). The content of urea, creatinine, uric acid and alkaline phosphatase activity was determined by biochemical methods in blood serum. Renal functional activity was assessed by the state of urinary function and excretion - glomerular filtration rate, tubulars reabsorption, daily diuresis volume and the excretion of creatinine, urea and chloride ions. The acid-base reaction of daily urine was also determined by the concentration of hydrogen ions. To obtain urine, rats were placed in special individual cages for 1 day.

Methods and techniques used in the research are published in the methodical recommendations (20). For all means of processing statistical material, reliable shifts were considered to be those that were within the limits of probability according to Student's tables $p < 0{,}05$. Statistical processing of data obtained in a series of experiments was performed using programs for biomedical research Statistics and Excel.

Results and discussion. Comprehensive assessment of changes in the rats body under the simulation of KSD began with studies of structural and functional changes in the kidneys. Macroscopically, in rats from group 2 with the model of KSD, the kidneys are visually slightly enlarged, their surface is smooth, greyish-brown. Microscopic examination revealed that part of the renal corpuscles of normal appearance. In some bodies, the capillary loops are arranged in a disorderly manner, hemosiderin crystals are scattered between them, and lymphocytes are found. In tubules sharp hypostasis of epitheliocytes is observed, the gleam of tubules is closed. The cytoplasm of epitheliocytes is eosinophilic homogeneous. Interstitial layers are expanded due to roughening of fibrousis fibers and increase in the number of fibroblasts. SDG activity in the epithelium of the tubules - (6.00 ± 0.10) conv. units; LDH activity - (5.00 ± 0.10) conv. units, i.e slightly reduced.

In the study of urinary and excretory functions of the rats kidneys with a model of CSD (group 2), there was an increase in daily diuresis by 45% (p <0,001), due to the increase

of glomerular filtration rate (GFR) by 60% (p <0.001) and a decrease in the value of tubular reabsorption in the tubules of the nephrons (p <0.001) by 0.60% (Table 1). The pH of daily urine is significantly shifted to the acidic side (p <0,01). There was a significant increase in daily products of urinary nitrogen metabolism: urea by 58% (p <0,02), creatinine by 60% (p <0,001), as well as an increase in the excretion of chloride ions - by 36% (p <0,001).

Microscopic studies of the rats urine with the model of KSD determined the presence of unorganized sediments, which are represented by calcium oxalates in a significant amount

(9 points) and a small number of triple phosphates (1 point). That is, the development of a model of rats urolithiasis is accompanied by the phenomenon of polyuria, increased daily excretion of nitrogenous metabolic products and chloride ions, acidification of urine.

Table 1. Indicators of the functional state of the rat kidneys with a model of KSD and the rats that received MW on the background of the development of KSD

Indexes	1 group ($M_1 \pm m_1$)	2 group ($M_2 \pm m_2$)	3 group ($M_3 \pm m_3$)
Glomerular filtration rate, ml/(dm^2×min)	**0,10 ± 0,004**	**0,16 ± 0,0002***	**0,18 ± 0,0002***
Tubular reabsorption, percentage to filter, %	**98,55 ± 0,12**	**97,95 ± 0,05***	**99,28 ± 0,003***
Daily diuresis, ml / dm2 of body surface	**1,52 ± 0,11**	**2,21 ± 0,07***	**1,83 ± 0,012***
pH of daily urine	**8,15 ± 0,17**	**7,25 ± 0,04***	**7,73 ± 0,07***
Excretion of creatinine, mmol	**0,001 ± 0,0009**	**0,016 ± 0,0008***	**0,018 ± 0,0002***
Urea excretion, mmol	**0,81 ± 0,17**	**1,28 ± 0,07***	**1,64 ± 0,009***
Removal of chloride ions, mmol	**0,68 ± 0,08**	**0,93 ± 0,05***	**0,71 ± 0,04***

Note. 1. ($M_1 \pm m_1$), ($M_2 \pm m_2$) and ($M_3 \pm m_3$) — arithmetic means with errors; 2. P_1 — reliability of comparison between M1 and M2; 3. P_2 — reliability of comparison between M1 and M3; 4. Data from the control group of animals were accepted as 100%.

During biochemical studies (Table 2), changes in metabolism were found in the form of the significant increase of uric acid content by 19% (p <0.01) and an increase in alkaline phosphatase activity by 12% (p <0.01), which indicates on to increased process of catabolism and disorders of nucleoprotein metabolism. There was a significant increase in blood creatinine by 24% (p <0.05) and urea by 108% (p <0.01), which indicates the development of pathological changes in the urinary system and also indicates the suppression of detoxification processes.

Table 2. The rats blood parameters with the model of KSD and the rats that received MW on the background of the development of KSD

Indexes	1 group ($M_1 \pm m_1$)	2 group ($M_2 \pm m_2$)	3 group ($M_3 \pm m_3$)
Uric acid, μmol / l	**119,12 ± 1,25**	**141,53 ± 5,31***	**126, 78 ± 3,34**
Alkaline phosphatase, U / l	**365,27 ± 12,41**	**408,42 ± 8,23***	**380,65 ± 11,04**
Urea, mmol / l	**2,72 ± 0,31**	**5,67 ± 0,71***	**3,93± 0,38***
Creatinine, μmol / l	**45,24 ± 0,63**	**56,18 ± 1,41***	**50,84 ± 1,92***

Note. 1. (M1 ± m1), (M2 ± m2) and (M3 ± m3) are arithmetic averages with errors of indices; 2. P1 - calculated between ratios (M1 ± m1) and (M2 ± m2) groups of rats; 3. P2 - calculated between ratios (M1 ± m1) and (M3 ± m3) groups of rats; 4. The data of the control group of animals were accepted as 100%.

Macroscopically, in group 3 rats, receiving MW on the background of the development of KSD - kidneys are visually normal size, their surface is smooth, brown. Capsule easily removed.

Microscopically - in the glomeruli of the renal cortex capillary loops are disordered, the cytoplasm of endothelial cells is swollen, but there are no foreign inclusions. The cytoplasm of the tubules epitheliocytes are somewhat swollen, but the lumens are free. The nuclei are light coloured with a clear pattern of chromatin. SDG activity in the part of the tubules - (7.00 ± 0.10), in another part - (4.00 ± 0.20), in the glomeruli - (2.00 ± 0.01) units; LDG activity in the part of the tubules - (6.00 ± 0.10), in the part - (4.00 ± 0.10) units. That is, the activity of redox enzymes is different.

In the rat group with KSD receiving MW, the value of daily diuresis decreases but does not reach the level of the corresponding indicator of group 1. This is largely due to the restoration of tubular reabsorption, which almost reaches the level of the control group. But TFR remains quite high, which causes such a high rate of daily diuresis. The daily excretion of creatinine, urea and chloride ions significantly exceeds the corresponding values of 2 groups of rats with pathology, which indicates an increase in the body's release of nitrogen metabolism products. The pH of the daily urine is somewhat restored, that is confirmed by microscopic urine sediments: in rats of group 3, calcium oxalate crystals are reduced to 5 points, and triple phosphates are not detected.

According to the table. 2, after the use of MW the content of creatinine (p <0.05) and urea (p <0.05) in the blood is significantly reduced compared with group 2 rats but does not reach level 1 of healthy animals. The content of uric acid and the activity of alkaline phosphatase is restored, and do not differ from the indicators of group 1.

Conclusions.

1. In the rats with a model of urolithiasis, structural and functional disorders of the kidneys are determined in the form of disruption of the capillary loops of the renal bodies, deposition of hemosiderin granules, tubular epithelium edema with the closure of the lumen; weakening of the activity of redox enzymes. Impaired urinary and excretory functions of the kidneys are characterized by a significant increase in diuresis, the development of hyperfiltration, increased excretion of urea and creatinine and acidification of the urine. Metabolic disorders are manifested in the activation of catabolic processes and inhibition of detoxification processes.

2. The MW with high levels of organic matter has a corrective effect on changes in the structure and function of the rats kidneys with a model of urolithiasis. The functional state of the kidneys improves: the daily volume of diuresis decreases, the activity of tubular reabsorption and glomerular filtration rate changes against the background of daily urine alkalinization and reduction of crystalluria. Signs of extreme edema of the tubules epithelium, pathological deposits in the renal corpuscles, interstitial disorders disappear. Alkaline phosphatase activity and uric acid content are restored, which against the background of a decrease in the content of creatinine and urea in the blood indicates the restoration of the metabolism of nucleoproteins and proteins

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