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LONG-TERM STUDIES OF THE CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF SILT-SULFIDE PELOIDS OF THE KUYALNITSKY ESTUARY

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Abstract

Due to anthropogenic impact and climate change, the Kuyalnitsky estuary (Odessa region, Ukraine) has undergone specific changes, which affected the physicochemical composition of the silt-sulfide peloids of the Kuyalnik resort (southern part of the Kuyalnitsky estuary). As a result of these changes, there were corresponding changes in their biological activity. To study the dynamics of these changes, experimental studies of the effect of peloid applications on the structural and functional state of the kidneys of white mice were carried out for the period from 2008 to 2020. If in 2008, the response from the functional state of the kidneys of contact rats was characterized as typical for the effect of moderately mineralized silt-sulfide peloids - a decrease in the volume of daily diuresis due to an increase in tubular reabsorption and an increase in the excretion of potassium and sodium ions against the background of a decrease in the excretion of chlorine ions, then already in 2016, the nature of the response from the outside changed somewhat but remained similar in direction and strength. In 2018, the nature of the response changed dramatically, which manifested itself in a significant increase in the volume of daily urine output only due to a considerable acceleration of GFR, which was accompanied by a significant increase in the excretion of creatinine, urea, sodium and chlorine ions. In 2020, the functional state of the kidneys was characterized by even more powerful negative changes, accompanied by acidification of urine, a significant increase in urea excretion against the background, of a substantial increase in the content of urea and creatinine in the blood. During the same period of research, with a microscopic study of the kidneys, signs of structural changes in the region of the renal glomeruli were first established. The data obtained indicate that the biological activity of peloids has undergone specific negative changes, which should be taken into account when carrying out balneological procedures.

Keywords: *silt-sulfide peloids, biological activity, structural and functional state of the kidneys*

Introduction

In Ukraine, silt deposits of salt water bodies (silt sulfide mud) are traditionally used for medicinal purposes, the deposits of which are concentrated in the southern region of the country. These are the Black Sea estuaries and the Azov Sea estuaries [1, 2, 3]. Sulfide-silt mud is a soft, velvety, plastic mass of black color with the smell of hydrogen sulfide and ammonia, consisting of a skeleton (or crystal skeleton), colloidal fractions, and mud solution. The crystalline stucture makes up 20-50% of the total mass of peloids and consists of silicate particles, gypsum, phosphates, calcium carbonates, magnesium, and other salts, as well as organic residues. To a large extent, the biological activity of silt-sulfide peloids is determined by a spesific concentration of macro- and microelements and biologically active substances [1, 4].

The following effects manifest various medicinal properties of mulo-sulfide peloids: anti-inflammatory, decongestant, analgesic, reparative-regenerative, immunomodulatory, defibrosing, antioxidant, adsorptive-resorptive, detoxifying, desensitizing, etc. [5]. Because silt-sulfide peloids significantly affect the leading pathophysiological factors in many chronic diseases, they are used in balneotherapy in many countries of the world [6 - 9].

One of the necessary conditions for using peloids for medicinal purposes is a comprehensive assessment of their composition and bioactivity, due to complex geological, geographic, physicochemical, microbiological, and other factors [10, 11]. Such an assessment makes it possible to conclude the prospects of using these peloids in restorative treatment in sanatoriums, for therapeutic and cosmetic purposes, etc. [1, 12].

Considering that peloids are natural medicinal resources, they can be influenced by environmental factors such as climate change, anthropogenic load, etc. One of the examples of the need for such a comprehensive assessment is long-term studies of the peloids of the Kuyalnitsky estuary (Kuyalnik resort), which is located near the city of Odessa (Ukraine), the southwestern coast of the Black Sea. Over the past 20 years, the Kuyalnitsky estuary has undergone significant environmental changes, which causes concern and initiates monitoring studies [13 - 16].

In this aspect, conducting experimental studies using laboratory animals is of particular importance since it allows you to obtain quick and more close to the human body results, which primarily give an idea of the response of organs and functional systems to the effect of the investigated agents to assess their safety (toxicity) and biological activity [17, 18]. The essential function of the body is to maintain homeostasis, which is characterized by the stability of the volume of fluids (isovolemia), their osmotic concentration (isoosmia), ionic composition (isoionium), and the concentration of hydrogen ions (isohydria). The kidneys maintain these main parameters of homeostasis [19, 20, 21]. That is why the assessment of the safety and biological activity of the peloids of the Kuyalnitsky estuary was studied by us based on the structural and functional state of the kidneys of experimental animals.

This work aims to assess the effect of silt-sulfide peloids on the features of the structural and functional reaction of the kidneys of experimental animals for the period 2008 - 2020.

Methods

Object of research: silt-sulfide peloids of Kuyalnytsya estuary, white rats.

The studies were performed on 56 white female rats, Wistar outbred breeding line, aged 6-8 months, weighing 180 - 210.

Experimental studies were conducted in accordance with the rules established by the Directive of the European Parliament and the Council (2010/63 / EU), by the order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 of March 1, 2012 "On Approval of the Procedure for conducting scientific experiments, experiments on animals by scientific institutions " and methodical recommendations [22, 23, 24].

During the experiment, the animals were in the experimental biological clinic (vivarium) of the State Institution "Ukrainian Research Institute of Medical Rehabilitation and Resort Therapy of the Ministry of Health of Ukraine", Odesa in the conditions of free access to food and water. The animals were kept in standard laboratory conditions: photoperiod - light /

darkness 12:12; air temperature - 22 ± 2 ° C; humidity - 55 ± 10%.

The researchers gently fixed the rat with their hands and shaved an area of skin below the ribs, measuring approximately 20.0 mm × 20.0 mm. On the shaved side of the rat's skin, the experimenters applied a cake with the corresponding sample of peloids at a temperature of 38 ± 2° C. The course consisted of 6 applications every other day. The duration of the application was 20 minutes.

The functional state of the kidneys was assessed by the urinary function (glomerular filtration rate, tubular reabsorption, the volume of daily urine output), excretory function (by the content of creatinine and urea in the blood and their excretion in the urine), and ion-regulating function (daily excretion of sodium, potassium, and chloride ions). Also, the acid-base reaction of daily urine was determined by indicators of the concentration of hydrogen ions. Each rat was placed in a special separate cage for the correct measurement of daily urine. The amount of urine was measured in ml. For unification, it was calculated per unit of body surface ml / dm2, which is equivalent to the filtering surface of all kidney nephron glomeruli. According to generally accepted methods the study of the functional state of the kidneys was carried out [21]. According to the Jaffe reaction (Popper's method) determining of creatinine in blood and urine was carried out. The reduction of urea in urine was carried out by the urease method using Nessler's reagent.

Determination of urea in blood was carried out according to the method, which is based on the interaction of urea with diacetyl monooxine in a strongly acidic medium in the presence of thiosemicarbazide and ferric ions with the formation of colored substances. To determine the excretion of sodium, potassium, calcium, and chloride ions in daily urine, their concentration was determined using an electrolyte analyzer in biological fluids "Kveri-01".

At the end of the course, the animals were removed from the experiment by decapitation under ether anesthesia. During the autopsy, in experimental rats, a macroscopic assessment of the kidneys was performed, then two pieces of a kidney with a volume of 1 cm3 were removed. The first piece was fixed for 24 hours in a 4% paraformaldehyde solution, passed through alcohols of increasing concentration, and poured into celloidin. Histological sections of 7-9 μm thick were made from the obtained blocks, which were stained with hematoxilin-eosin [25].

The obtained preparations were used for microscopic studies of structural changes in the kidneys. The second piece was frozen with dry carbon dioxide (-70° C). On the prepared cryostat sections, histochemical reactions were performed to determine the activity of succinate dehydrogenase (LDH) and lactate dehydrogenase (LDH) according to Lojda Z.'s recipe [25]. The enzyme activity was assessed in conventional units of optical density (c.u.). The methods used are given in the guidelines approved by the Ministry of Health of Ukraine. Experimental studies were carried out following legal documents [26, 27].

For further analysis, the results of studies in the following years were selected: 2008 (2nd group), 2016 (3rd group), 2018 (4th group), and 2020 (5th group). The number of animals in each period was 10. Intact animals were used as a comparison group - 16 animals (1st group).

All data were processed using the statistical package Statistica 10.0 (Statsoft/Dell, Tulsa, OK, USA). The descriptive statistics of the data in tables include mean ± standard error of the mean (SEM) or mean ± standard deviation. Significance was assessed by using the one-way ANOVA followed by t-test. Values were considered statistically significant when P value is less than 0.05.

Results

First, let us consider the data that demonstrate the dynamics of changes in the physicochemical composition of the peloids of the Kuyalnitsky estuary within the Kuyalnik resort from 2008 to 2020 (Table 1). It should be pointed out that these studies were carried out simultaneously as the experimental studies in rats. During the research period, the content of hydrogen sulfide in the peloids of the Kuyalnik resort ranged from 0.14% to 0.23% with air conditioning requirements of 0.06 - 0.20%; iron sulfide - from 0.14% to 0.32% with conditional requirements of 0.15 - 0.50%. A significant change in the pH values was observed in the peloids. The reaction varied from neutral-weakly alkaline (6.55 pH) in 2008 to slightly acidic (5.80 pH) in 2020, with conditional requirements of 6, 50-7.40 pH. A decrease in pH values negatively affects the balance of forms of hydrogen sulfide in peloids.

Particular attention should be paid to determining the redox potential (Eh index). The redox potential can shift in one direction or another, thereby providing oxidizing or reducing properties and, depending on this, significantly change the conditions for the formation of peloids. An increase in the Eh values from -350 mV in 2008 to -120 mV in 2020, with conditioning requirements of -400 to -150 mV, and a simultaneous decrease in pH values, indicate the attenuation of the peloidogenesis's processes.

The value of the mass fraction of moisture determines the number of physical properties of peloids. Without a liquid phase in peloids, bacterial processes and the migration of many of elements cannot occur. The value of the mass fraction of moisture in the studied samples ranged from 55.79% in 2008 to 45.21% in 2020, which corresponds to the conditioning requirements (35-65%) for peloids. The mass fraction of organic carbon (Corg) tends to decrease its content from 2.34% in 2008 to 1.62% in 2020, which indicates an insufficient supply of fresh organic matter for the processes of peloidogenesis.

When studying the physicochemical composition of silt sulfide peloids, the composition of their mud solution is of great importance, which is a metamorphosed brine of the estuary, which changed its composition under the influence of several biochemical and physicochemical parameters., According to the results of the studies, the mud solution of the estuary peloids is characterized as chloride magnesium-sodium, with mineralization of 143.05 g / l in 2008 and subsequent growth to 324.03 g / l in 2020. Thus, this figure is currently outside the limits established by the corresponding conditioning requirements (90.0-150.0 g / l). There is also an increase in the concentration of the main components of the macro-composition of the mud solution:

- chloride ions from 85.10 g / l to 199.17 g / l;
- sodium and potassium ions from 40.91 g / l to 100.25 g / l;
- magnesium ions from 7.83 g / l to 16.31 g / l.

The content of ammonium ions ranges from 143.05 to 210.99 mg / l.

Data on the effect of course external application of silt-sulfide peloids on the functional state of the kidneys of rats are shown in Table 2. In rats of the 2nd group (2008), the glomerular filtration rate (GFR) does not change. Still, the tubular reabsorption significantly and reliably increases - by 0.4 % (this value varies within 3%), which leads to a decrease in the value of daily urine output by 30% compared with group 1 of intact animals. The content of creatinine and urea in the blood and their excretion do not change. The reaction of daily urine does not undergo significant changes. In this case, the excretion of potassium ions increases by 26%, sodium ions increase by 128%, and the excretion of chlorine ions, on the contrary, decreases by 30%, therefore; the kidneys excrete a small amount of concentrated urine per day.

In rats of group 3 (2016), an increase in GFR by 32% (p <0.01) was found. At the same time, tubular reabsorption significantly increases - by 0.3%, which in combination does not cause an increase in the amount of daily diuresis. The excretion of creatinine increases considerably by 32% and sodium ions by 93%. The latter indicators do not significantly differ from the corresponding indicators of group 1 of intact animals, therefore; there is a moderate stimulation of the functional state of the kidneys.

In rats of group 4 (2018), GFR increases by 36% (p <0.01), which, while maintaining tubular reabsorption at the level of control group 1, leads to an increase in the volume of daily urine output by 41%. There is an increase in creatinine excretion by 36%, urea by 29%, sodium excretion by 600%, and chlorine by 25%. The data obtained indicate (in contrast to the rats of group 2) a more noticeable activation of the kidneys' urinary, excretory, and ion-regulating functions.

In rats of group 5 (2020), daily urine output increases by 20% due to the rise in GFR by 23% and retention of tubular reabsorption at the level of group 1 of intact rats. The excretion of creatinine and urea increases by 23% and 64%. But at the same time, their content in the blood increases - creatinine by 14%, and urea by 34%, which indicates the accumulation of nitrogen metabolism products in the blood and signs of the development of endogenous intoxication. The reaction of daily urine shifts to the acidic side, as evidenced by a decrease in the concentration of hydrogen ions by 14%. The excretion of electrolytes undergoes significant changes - in the case of potassium, it increases by 20%, sodium increases by 771%, and chlorine by 54%. In rats of this group, the same directionality, but more significant than in rats of group 4, was established, the effect on the excretory and ion-regulating functions of the kidneys.

So, in rats of groups 2 and 3, changes in the functional state of the kidneys were established, which coincide in direction but differ quantitatively. First of all, this concerns daily diuresis, the value of which is influenced by a significant increase in tubular reabsorption (which causes the reabsorption of chloride ions) and adequate excretion of potassium and sodium ions. This indicates the regular activity of the processes of water-electrolyte metabolism and activation of the processes of urination - the work of the kidneys is aimed at preserving fluid in the body and maintaining the constancy of the water-electrolyte balance.

In rats of groups 4 and 5, a reaction uncharacteristic for external use of silt-sulfide peloids was established on the part of the functional state of the kidneys, which is mainly evidenced by the absence of significant changes in tubular reabsorption against the background of a high level of GFR, which causes an inadequately high level of diuresis, hypernatriuresis, and chloruresis. The registered phenomena show the presence of pathological changes in the functional state of the kidneys.

In morphological studies, the macroscopic view of the kidneys in rats in all groups (including intact) did not differ. Microscopically, in rats of groups 2 and 3, the structure of the nephron and its components were without visual changes. In rats of group 3, there was edema of the intertubular layers. In group 2, the SDH activity was (7.00 ± 0.30) c.u.; LDH activity - (5.00 ± 0.17) c.u. Thus, in group 3, the SDH activity was - (7.00 ± 0.14) c.u.; LDH activity - (6.00 ± 0.19) c.u. It should be noted that the activity of SDH in the kidney tissues of rats of the 1st group was (7.00 ± 0.29) c. u., and the LDH activity - (6.00 ± 0.12) c. u., that is, no changes in the activity of redox enzymes were observed.

In rats of the 4th group, microscopic examination revealed that the structure of the nephron and its components did not have obvious changes. The lumens of the tubules are well contoured. SDH activity - (7.00 ± 0.30) c.u.; LDH activity - (6.00 ± 0.27) c.u.

In rats of all the aforementioned groups, the redox enzymes activity in the kidney tissues does not change, as evidenced by the absence of significant changes in comparison with the control group ($p > 0.5$). An exception is a significant decrease in LDH activity with (6.00 ± 0.12) c. u. up to (5.00 ± 0.12) c.u. in rats of group 2.

In rats of group 5, microscopic examination of the structure of the nephron is normal. Part of the glomeruli contains a thinned outer membrane. Clusters of lymphocytes are located around them. The tubules are normal. SDH activity - (7.00 ± 0.10) c.u. LDH activity is (7.00 ± 0.12) c.u., against (6.00 ± 0.12) c. u. in control group 1, which indicates an increase in LDH activity. The established changes in the epithelium of the glomeruli can be considered a response to xenobiotics' action.

It should be noted that in rats of this group, the content of urea and creatinine in the blood significantly increases, and their excretion with urine significantly increases, which against the background of morphological changes in the epithelium of the glomeruli indicates a highly negative effect of peloids on the animal organism. In this aspect, attention is drawn to experimental studies, in which the authors suggest that the standard markers of damage to the renal glomerular apparatus are high levels of creatinine and urea in the blood [29, 30, 31].

Conclusions

The functional state of the kidneys of rats that received a course of peloid applications at different periods of observation significantly differed from each other. In rats of group 2 (in 2008) and, to a lesser extent, group 3 (2016), the established changes correspond to a typical reaction on the part of the functional state of the kidneys to the effect of silt-sulfide peloids when applied externally. In rats of groups 4 and 5 (2018 and 2020), adverse changes in the functional state of the kidneys were revealed. A very high level of diuresis allows the body to get rid of the influence of xenobiotics, toxic metabolic products, and extremely excessive intake of electrolytes. Although no gross side effects have been identified, the kidneys are forced to work on

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the verge of depletion. The inability of the kidneys to reduce the content of urea and creatinine in the blood, against the background of the established changes in the structural components of the glomeruli, indicates signs of depletion of the body's adaptive capabilities under the influence of xenobiotics. Thus, the data obtained indicate that from 2008 to 2020, the physicochemical composition of the silt-sulfide peloids of the Kuyalnik resort (southern part of the Kuyalnitsky estuary) changed significantly, which caused a change in their biological activity. The obtained experimental data are recommended to be taken into account when prescribing balneological procedures.

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Table 1. Physico-chemical composition of peloids of Kuyalnitsky estuary of Kuyalnyk resort area

Year	Cations, g/l			Anions, g/l			Mineralization, g/l	Moisture content, %	Eh, mV	NH_4 %	H_2S, %	FeS, %	C_{org}, %	pH, c.u. pH
	Na^++ K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	HCO_3^-								
Conditioning requirements for the peloids of the Kuyalnitsky estuary	–	–	–	–	–	–	90,0-150,0	35 - 65	-400 - – 150		0,06-0,20	0,15-0,50	–	6,50-7,40
2008	40,91	2,32	7,83	85,10	5,91	0,98	143,05	55,79	-350	143,05	0,23	0,23	2,34	6,56
2016	61,97	1,40	13,13	131,50	6,21	0,45	214,65	42,95	-190	180,61	0,15	0,27	3,52	7,10
2018	81,71	1,60	12,65	160,10	6,44	1,89	264,41	44,74	-125	210,99	0,15	0,32	2,05	6,60
2020	100,25	1,17	16,31	199,17	6,00	1,13	324,03	45,21	-120	158,24	0,14	0,25	1,62	5,80

Table 2. Influence of the peloids of the Kuyalnitsky estuary of the Kuyalnik resort area on the functional state of the kidneys of rats when applied externally

Indexes	1 group	2 group	3 group	4 group	5 group
	$(M_1 \pm m_1)$	$(M_2 \pm m_2)$	$(M_3 \pm m_3)$	$(M_4 \pm m_4)$	$(M_5 \pm m_5)$
Daily diuresis, ml / dm^2 of body surface	1,04 ± 0,07	0,73±0,06*	0,98 ± 0,04	1,47 ± 0,09*	1,25 ± 0,005*
Glomerular filtration rate, ml/(dm^2×min)	0,13 ± 0,009	0,14 ± 0,02	0,17 ±0,006*	0,18 ± 0,004*	0,16 ± 0,001*
Tubular reabsorption, percentage of filtration, %	99,41 ± 0,04	99,82±0,06*	99,70±0,06*	99,39 ± 0,07	99,43 ± 0,004
Excretion of creatinine in the urine, mmol	0,013 ±0,0009	0,014±0,0006	0,017±0,009*	0,018 ± 0,0002*	0,016 ± 0,0001*
Blood creatinine concentration, mkmol/l	48,36 ± 0,65	47,58± 0,21	49,36 ± 0,71	50,55 ± 1,91	55,19 ± 1,03*
Excretion of urea in the urine, mmol	0,78 ± 0,03	0,67± 0,09	0,70± 0,01	1,01± 0,05*	1,28 ± 0,008*
The concentration of urea in the blood, mmol/l	2,80 ± 0,27	2,77 ± 0,69	2,71 ± 0,31	2,89 ± 0,44	3,76 ± 0,43*
Daily urine pH, c.u.	7,17 ± 0,15	7,10 ± 0,48	7,24 ± 0,11	7,20 ± 0,16	6,18 ± 0,07*
Daily excretion of potassium ions, mmol	0,15 ± 0,006	0,19 ± 0,08*	0,15 ± 0,03	0,16 ± 0,008	0,18 ± 0,001*
Daily excretion of sodium ions, mmol	0,14 ± 0,004	0,32 ± 0,005*	0,27 ± 0,002*	0,98± 0,003*	1,22 ± 0,003*
Daily excretion of chloride ions, mmol	0,24 ± 0,02	0,17 ± 0,01*	0,21 ± 0,08	0,30 ± 0,009*	0,37 ± 0,002*

Notes: $(M_1 \pm m_1)$, $(M_2 \pm m_2)$, $(M_3 \pm m_3)$ and $(M_4 \pm m_4)$ - arithmetic means with indicator errors;
* - significant changes ($p < 0.05$) were calculated in comparison between 1 control group and research groups.

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