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INFLUENCE OF COMPLEX OF PREPARATIONS ON THE STATE OF THE ORAL CAVITY TISSUES IN RATS UNDER THE CONDITIONS OF EXPERIMENTAL HYPOTHYROIDISM

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The research is dedicated to studying the state of the dentoalveolar system and tissues of the oral cavity of rats under the influence of the complex under the action of experimental hypothyroidism. Experimental hypothyroidism was simulated in 8 1-month-old rats (group 2). The intact group (group 1) consisted of 8 rats. 7 rats received a complex under conditions of experimental hypothyroidism (group 3). The duration of the study was 30 days. The complex of preparations in conditions of insufficiency showed a significant caries-preventive and periodontal-protective effect. Under the action of the complex, an increase in the activity of alkaline phosphatase in the periodontal bone tissue and dental pulp was revealed, as well as a decrease in the activity of acid phosphatase in the bone of the alveolar process.

Key words: plant polyphenols, rats, biochemical markers, hypothyroidism modeling, stinging nettle preparation.

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ВПЛИВ КОМПЛЕКСУ ПРЕПАРАТІВ НА СТАН ТКАНИН РОТОВОЇ ПОРОЖНИНИ ЩУРІВ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОГО ГІПОТИРЕОЗУ

Дослідження присвячено вивченню стану зубощелепної системи та тканин ротової порожнини щурів під впливом комплексу при дії експериментального гіпотиреозу. У 8 щурів 1-місного віку проводили моделювання експериментального гіпотиреозу (2-а група). Інтактну групу (1 група) склали 8 щурів. У 3-й групі 7 щурів отримували комплекс на тлі експериментального гіпотиреозу. Тривалість дослідження становила 30 днів. Комплекс препаратів в умовах недостатності виявив значну карієс-профілактичну та пародонтопротекторну дію. Під дією комплексу виявлено збільшення активності лужної фосфатази в кістковій тканині пародонту та пульпі зубів, а також зниження активності кислої фосфатази в кістці альвеолярного відростка.

Ключові слова: рослинні поліфенолі, щури, біохімічні маркери, моделювання гіпотиреозу, препарат кропиви дводомної.

The work is a fragment of the research project “Influence of hypoxia on the processes of collagen formation and mineralization on models of dental pathology and correction of the obtained disorders”, state registration No. 0118U006963.

Hypothyroidism is a syndrome, a condition of the body associated with a reaction to a low level of concentration of thyroid hormones (TH). Hypothyroidism is associated with functional insufficiency of TH. Iodine is a trace element involved in the construction of TH. Between 60 % and 80 % of the total thyroid hormone produced by TH enters the blood in the form of thyroxine [1, 3]. Thyroxine affects all body tissues; there are no specific target cells for it. This hormone is able to cross the membrane and bind to

receptors in every cell of the body. The main function of thyroxine is activating metabolic processes, which is carried out through stimulation of the synthesis of RNA and the corresponding proteins. Thyroxine affects the metabolism and controls the growth and development of the body. Enhances oxidative processes in the cells of the whole body.

It was found that iodine deficiency adversely affects the intensity of caries in permanent teeth at the age of 12–15 years [7, 11, 12]. In connection with the above, the use of the iodine dietary supplement is relevant.

In addition to potassium iodide, this dietary supplement contains selenium oxide. It is known that selenium is a microelement that is involved in many vital processes – the formation of the hormone TH triiodothyronine, in the metabolism of fats, proteins, carbohydrates, in redox processes. Selenium is a synergist of iodine. A deficiency of iodine and selenium leads to more severe hypothyroidism than a deficiency of iodine alone. Selenium exhibits powerful antioxidant properties, prevents the formation of free radicals, and helps protect cell membranes.

Many plants have medicinal properties, as they contain a variety of biologically active substances. The aerial part of the nettle contains polyphenols (PP) – flavonoids, phenolic acids, tannins, carotenoids, ascorbic acid, vitamins of group B, K, etc.

In our experiments, anti-inflammatory properties, increased regeneration and epithelialization rate of gum tissue in rats under the influence of plant origin PP were established [5]. In the laboratory, we have developed a preparation of plant polyphenols from the aerial part of stinging nettle with the working name PPS.

The purpose of the study was to research the state of the dento-jaw systems and tissues of the oral cavity of rats under the influence of the iodine complex of a dietary supplement and the PPS drug under the condition of experimental hypothyroidism.

Materials and methods. The experiment involved 23 white Wistar rats of herd breeding, which were kept on a standard vivarium diet. Modeling of experimental hypothyroidism was carried out in 8 1-month-old rats using the thyreostatic “Mercazolil” (LLC “Zdorovya”, Ukraine) – 0.02 % solution was given to rats for 2 weeks and a 0.01 % solution – for the next 2 weeks with drinking water [9]. The intact group consisted of 8 rats. In the 3rd group, 7 rats received per os a complex of the dietary supplement “Yodo-selen” (LLC PATC “Farmakom”, Ukraine) under the condition of experimental hypothyroidism – 1 pill (250 mg/7 rats) and preparation of herbal PP from stinging nettle leaves (PJSC “Liktravi”, Ukraine) 0.1 ml/100 g body weight of rats. The working name of the PPS preparation was obtained according to the original laboratory technology [6], the amount of PP in the PPS preparation was 7.51 mg/g of the feedstock. The duration of the experiment was 30 days.

The animals were removed from the experiment by total bloodletting from the heart performed under general anesthesia (sodium thiopental 40 mg/kg). Having previously separated the oral mucosa, the jaws were dissected out. The objects of biochemical studies were the liver, the supernatant of the homogenates of the oral mucosa (25 mg/ml), the bones of the alveolar ridge (50 mg/ml), and the pulp of the incisors of rats. The supernatant was obtained by centrifugation in a RS – 6 centrifuge for 15 minutes at 3000 rpm at a temperature of +4°C.

The state of the intercellular matrix (ICM) of the connective tissue (CT) was assessed by the state of the level of collagen (according to the content of bound, free and total hydroxyproline [9] and glycosaminoglycans (GAG) in parodontal tissues [4, 15]. The level of lipid peroxidation (LPO) products was assessed by the content of malondialdehyde (MDH) in tissues by the thiobarbituric method [2]. The state of the physiological antioxidant system (PAS) was assessed by the activity of glutathione peroxidase (GPO) and catalase [10].

To assess the state of rat tissues, biochemical parameters were determined by unified methods using commercial reagent kits: alkaline phosphatase (ALP) activity, acid phosphatase (AP) activity, calcium, phosphorus content, lactate dehydrogenase (LDH) activity, pyruvate content (produced by UV – Abris+).

The results were processed by variational statistical methods of analysis using the Microsoft Office Excel 2016 software. Statistical processing of the experimental study results was carried out by the methods of variation analysis using the Students test. The difference was considered statistically significant at $p < 0.01$.

Results of the study and their discussion. The study of the effect of the complex was carried out under conditions of reproduction of experimental hypothyroidism. The state of the dentoalveolar system of rats when using the complex under conditions of experimental hypothyroidism (EH) is presented in table 1.

Table 1

The effect of the complex of dietary supplements with PPS on the condition of the dentoalveolar system in rats, $M \pm m$

Indices	Indices of resorption of periodontal bone tissue (%)	Number of carious lesions per 1 rat	Depth of dental caries lesions (in points)
Intact	25.4±0.8	2.3±0.2	2.3±0.2
EH	25.3±0.6	3.0±0.3 $p=0.06$	3.0±0.3 $p=0.06$
EH+Complex	19.2±0.7 $p_1<0.001$	1.9±0.2 $p_1=0.006$	1.9±0.2 $p_1=0.006$

Note. p – the index of the reliability of differences relative to the intact group; p_1 – the index of the reliability of differences relative to the control group.

The indices of resorption of the periodontal bone tissue of rats under the action of the complex decreased by 24 % compared to the control group ($p_1<0.001$). The number of carious cavities (per 1 rat), as well as the depth of dental caries lesions (in points) decreased by 63 % ($p_1=0.006$). In the dental pulp of rats, the activity of acid phosphatase (a marker enzyme of odontoclasts) did not differ significantly from the control group. The activity of alkaline phosphatase was 1.6 times ($p=0.001$) higher than in the intact group, which indicated a significant activation of odontoblasts under the action of the complex.

Status of mineral metabolism in the periodontal bone of rats when using the complex under conditions of experimental hypothyroidism (EH) is presented in table 2.

Table 2

The effect of the complex of dietary supplements with PPS on the condition of mineral metabolism in the periodontal bone in rats, $M \pm m$

Indices	Intact	EH	EH+Complex
Pulp of the teeth			
AP (nkat/g)	0.29±0.020	0.55±0.040 $p=0.001$	0.46±0.070
ALP (nkat/g)	76.9±3.00	–	121±6.68 $p=0.001$
The mucous membrane of the oral cavity			
AP (nkat/g)	84.5±1.92	80.5±3.56	101±1.83 $p=0.001$ $p_1=0.002$
Bone of the alveolar process			
AP (nkat/g)	69.8±2.21	101±1.71 $p<0.001$	84.5±1.92 $p=0.002$ $p_1=0.001$
ALP (nkat/g)	19.3±0.36	8.40±0.20 $p<0.001$	10.4±0.14 $p<0.001$ $p_1<0.001$
Calcium (mmol/g)	6.13±0.0087	6.93±0.035 $p<0.001$	5.19±0.45 $p_1=0.008$
Phosphorus (mmol/g)	4.25±0.076	4.85±0.087 $p=0.002$	3.98±0.44 $p_1=0.06$

Note. p – the index of the reliability of differences relative to the intact group; p_1 – the index of the reliability of differences relative to the control group.

Thus, the activity of alkaline phosphatase increased by 24 % ($p_1<0.001$) compared with the control group. At the same time, the level of calcium and phosphorus did not significantly differ from intact groups data. The complex under conditions of iodine deficiency reduced the activity of AP in the bone of the alveolar process by 16 % ($p_1<0.001$) relative to the control group. In the oral mucosa, AP activity slightly increased.

The influence of the dietary supplement complex with the PPS preparation on LPO processes is presented in fig. 1.

Thus, the content of MDA in the liver of rats did not change significantly. At the same time, the activity of glutathione peroxidase increased in comparison with the control group by 1.5 times ($p_1=0.008$) and 4.3 times ($p<0.001$) relative to the intact group. In the mucous membrane of the oral cavity under the influence of the complex, the content of MDA decreased by 15 % ($p_1=0.03$), which indicates the antioxidant properties of the complex. At the same time, GPO activity increased by 1.5 times ($p=0.04$) compared with the intact group. In the bone of the alveolar process, the state of lipid peroxidation processes

and the activity of antioxidant enzymes did not change significantly. The complex had a positive effect on the state of collagen and glycosaminoglycans of the oral mucosa.

The influence of the dietary supplement complex with the PPS preparation on indicators of the state of the intercellular matrix of periodontium of rats is presented in fig. 2.

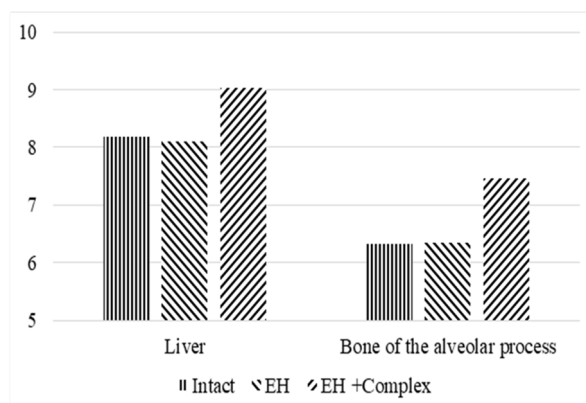


Fig. 1. Influence of the dietary supplement complex with the PPS preparation on the content of MDA in the liver and periodontal tissues of rats, nmol/g.

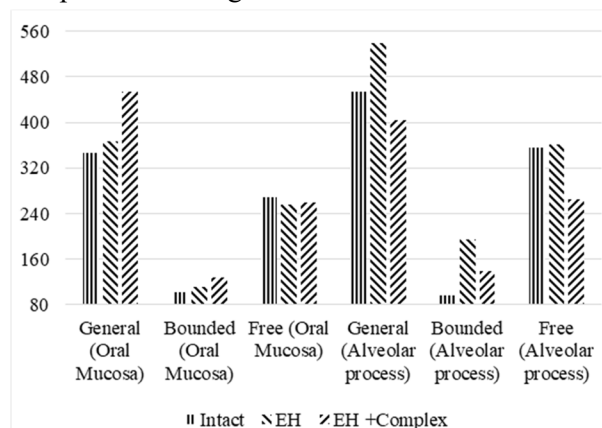


Fig. 2. Influence of the dietary supplement complex with the PPS preparation on indicators of the state of the intercellular matrix of periodontium of rats, μmol/g.

The content of total oxyproline increased by 24 % ($p_1=0.02$); GAG level – by 34 % ($p_1=0.005$) compared with the EH group and 1.4 times ($p=0.002$) relative to the intact group. At the same time, in the bone of the alveolar process, the complex reduced the content of general, bounded and free oxyproline compared with the control group.

The levels of metabolic markers of hypoxia are presented in fig. 3 and 4.

The content of pyruvate in the oral mucosa under the action of the complex decreased by 26 % ($p_1=0.02$). In periodontal bone tissue, the content of pyruvate increased by 38 % ($p_1=0.007$).

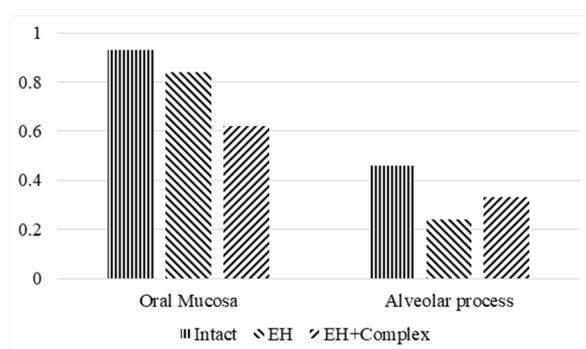


Fig. 3. influence of the dietary supplement complex with the PPS preparation on pyruvate content of rats, mmol/g.

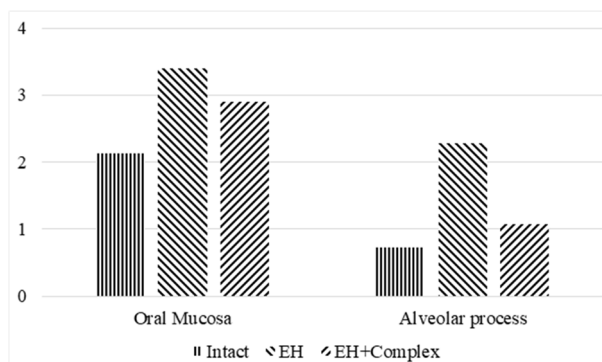


Fig. 4. influence of the dietary supplement complex with the PPS preparation on LDH activity of rats, mkat/g.

LDH activity in the oral mucosa did not differ reliably from the control group, but was 1.4 times higher ($p<0.001$) than in the intact one, which may indicate a decrease in the level of lactate as a substrate of this enzyme. The activity of LDH in the periodontal bone tissue under the influence of the complex decreased by 2.1 times ($p_1<0.001$) relative to the control group, which indicates an increase in the level of lactate. LDH activity was 1.5 times higher than in the intact group.

Recently, researchers have noted an increase in the prevalence and intensity of caries and periodontal diseases in the foci of endemic goiter. With insufficient intake of iodine in the body, thyroid hormones is most affected in children and adolescents [12, 13, 14]. However, in our opinion, the existing studies devoted to the treatment of disorders in the oral cavity of children and adolescents with hypothyroidism are rather few [1, 3], therefore, the obtained results will allow to develop in the future an effective therapeutic and prophylactic complex for the correction of metabolic changes in the oral cavity in such patients. It should be summarized that as a result of the studies carried out, the complex of the dietary supplement “Yodo-selen” with the preparation of plant polyphenols from the stinging nettle leaves in conditions of iodine deficiency showed a significant caries-preventive and periodontal-protective effect. The complex normalized LPO processes and activated glutathione peroxidase in the oral mucosa under conditions of iodine deficiency. Under the action of the complex, the state of collagen improved only in the oral mucosa, as well as GAG in the soft and hard periodontal tissues of rats. The levels of metabolic

markers differed significantly from those of the intact groups. The complex reduced the number and depth of tooth decay by caries, and also significantly reduced the resorption of periodontal bone tissue. Under the action of the complex, activation of osteoblasts and odontoblasts was revealed – an increase in the activity of alkaline phosphatase in the bone of the alveolar process and dental pulp, as well as a decrease in the activity of AP (a marker enzyme of osteoclast metabolism) in the periodontal bone tissue.

Conclusions

1. The developed complex under conditions of iodine deficiency reduced the number and depth of dental caries, and also significantly reduced the resorption of periodontal bone tissue.
2. Activation of osteoblasts and odontoblasts was revealed – an increase in the activity of alkaline phosphatase in the bone of the alveolar process and dental pulp, as well as a decrease in the activity of acid phosphatase in the periodontal bone tissue.
3. The complex normalized the processes of lipid peroxidation and activated glutathione peroxidase in the oral mucosa.
4. The complex improved the state of collagen only in the oral mucosa, as well as glycosaminoglycans in the soft and hard periodontal tissues of rats.

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