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CORRECTION OF METABOLIC CHANGES OF TISSUES OF RATS ORAL CAVITY BY COMPLEX OF PREPARATIONS UNDER CONDITIONS OF INTRAUTERINE HYPOXIA AND CARIOGENIC DIET

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The research is dedicated to the study of the effect of the complex of preparations on the dental status and the state of the tissues of the oral cavity of rats under conditions of intrauterine hypoxia and cariogenic diet. The experiment was carried out on 35 white rats of both sexes: 29 female and 6 male rats. It was revealed caries-prophylactic effect and a decrease in the aggressiveness of the pathological process under the action of the complex in the conditions of intrauterine tissue hypoxia and cariogenic diet. The complex showed an anti-inflammatory effect in the oral mucosa. The levels of metabolic markers of pyruvate hypoxia in the liver and in the oral mucosa returned to normal state. The complex significantly improved the state of collagen and glycosaminoglycans in the parodontal bone tissue.

Key words: hypoxia, caries-prophylactic effect, metabolic markers, plant polyphenols, rats.

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КОРЕКЦІЯ МЕТАБОЛІЧНИХ ЗМІН ТКАНИН РОТОВОЇ ПОРОЖНИНИ ЩУРІВ КОМПЛЕКСОМ ПРЕПАРАТІВ В УМОВАХ ДІЇ ВНУТРІШНЬОУТРОБНОЇ ГІПОКСІЇ ТА КАРІЄСОГЕННОГО РАЦІОНУ

Дослідження присвячено вивченню впливу комплексу препаратів на стоматологічний статус і стан тканин ротової порожнини щурів в умовах дії внутрішньоутробної гіпоксії та карієсогенного раціону. Дослід проведено на 35 білих щурах обох статей: 29 самок і 6 самців. Було виявлено карієс-профілактичну дію і зниження агресивності патологічного процесу при впливу комплексу в умовах дії внутрішньоутробної тканинної гіпоксії і карієсогенного раціону. Комплекс проявив протизапальну дію в слизовій оболонці порожнини рота. Рівні метаболічних маркерів гіпоксії пірувату в печінці і в слизової оболонки порожнини рота нормалізувалися. Комплекс в кісткової тканини пародонту значно поліпшував стан колагену і глікозаміногліканів.

Ключові слова: гіпоксія, карієс-профілактична дія, метаболічні маркери, рослинні поліфеноли, щури.

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.

Hypoxia is a state of oxygen starvation of the body as a whole and of individual organs and tissues, caused by various factors. To assess the patterns of development of metabolic changes in different forms of pathology, endogenous hypoxia is more important, which include tissue hypoxia. This type of hypoxia occurs due to impaired oxygen extraction by tissues from the flowing blood and the inability of cells to utilize oxygen [2].

The triggers for the development of tissue hypoxia are diverse and may be associated with various factors. In three parts of the respiratory chain, respiration is associated with oxidative phosphorylation and

the synthesis of adenositriphosphate. In order for oxidative phosphorylation to be associated with respiration, the mitochondrial membrane must maintain its integrity, be impermeable to H^+ and other ions. The terminal link in the development of a number of hypoxias is the excessive accumulation of hydrogen ions in the tissue, the development of metabolic acidosis, followed by an increase in the permeability of mitochondrial and lysosomal membranes, and adenositriphosphate deficiency. [5]. Activation of lysosomal phospholipases ensures the development of a cascade of reactions for the formation of prostaglandins, leukotrienes, free radicals [14]. Thus, tissue hypoxia secondary formed in hypoxia of various origins.

Recently, dentists-clinicians have been paying considerable attention to the study of the influence of negative factors during the mother's pregnancy. In children who have undergone intrauterine hypoxia, there is a high incidence of colds in the first year of life, an increase in the incidence of caries, a decrease in the mineralizing properties of enamel and dentin, a more frequent occurrence of stomatitis, gingivitis [4]. Children with hereditary and congenital pathology have hypoxic syndrome with impaired redox processes in mitochondrial membranes [2].

General pathogenetic therapy of caries provides the normalization of disturbed metabolic processes in the macroorganism, which contributes to an increase in the nonspecific resistance of the organism, an increase in its resistance to the effects of general adverse factors. One of the manifestations of complex therapy is the oral administration of calcium fluorides, macro- and microelements, vitamins. Thus, in the complex treatment of decompensated caries, it is recommended to take medications to increase the body's resistance, regulate metabolism, and improve the processes of tooth mineralization. Many plants are currently attracting the attention of researchers not only as an object of study. They are promising because contain a variety of biologically active substances and have medicinal properties. Experimental data from biochemical and cytomorphological studies indicate anti-inflammatory properties and a significant role of plant polyphenols in ensuring the resistance of the epithelial tissue of the oral cavity to damaging factors of different nature [11].

The purpose of the study was to research the effect of the combined action of the complex of dietary supplements with preparation of plant polyphenols from the leaves of *Plantago major* on the dental status and the state of the tissues of the oral cavity of rats under conditions of intrauterine hypoxia and cariogenic diet.

Materials and methods. The experiment involved 35 white Wistar rats of herd breeding of both sexes: 29 females and 6 males, which were kept on a standard vivarium diet. In order to reproduce the offspring, 9 mature female rats and 6 male rats was separated in 3 groups of experiment. Then, in these females, presumably from 10 to 19 days of pregnancy, hypoxia (H) of uncoupling of oxidation and phosphorylation processes was reproduced [11] by intraperitoneal administration during pregnancy of "Warfarin Nycomed". (Takeda Pharma, Poland) at a dose of 1.5 mg/kg body weight of rats. After birth, rat pups were separated in 3 groups. The intact group consisted of 6 rats. Seven rats at 1 month of age were put on a cariogenic diet (CgD) [6]. During 30 days, 7 rats on the background of hypoxia and CgD received *per os* a complex of 2 dietary supplements and preparation of polyphenols of leaves of *Plantago major*:

1. Dietary supplements "Macromol" (PE "EURO PLUS", Dnipro, Ukraine); 1 pill (500 mg) contains magnesium (Mg^{2+}) – 20 mg, calcium (Ca^{2+}) – 15 mg, phosphorus (P^{5+}) – 12 mg, iron (Fe^{3+}) – 10 mg, zinc (Zn^{2+}) – 3 mg, manganese (Mn^{2+}) – 1 mg, molybdenum (Mo^{6+}) – 0.1 mg, iodine (I^-) – 50 μg, selenium (Se) – 50 μg, copper (Cu^{2+}) – 1 mg. Excipients: lactose, calcium stearate, potato starch.
2. Phytotherapeutic supplement "Phytolax rowanberry": 1 pill (400 mg) contains licorice root, zhostera fruits, rowan berries, kelp thallus, root.
3. Preparation of polyphenols of leaves of *Plantago major* (ZAT "Liktravy", Zhytomyr, Ukraine). Working name PPP, obtained by original laboratory technology [15], the amount of polyphenols in PPP preparation is 7.83 mg/g of raw material.

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 [7] and glycosaminoglycans (GAG) in parodontal tissues [3]. The level of lipid peroxidation (LPO) products was assessed by the content of malondialdehyde (MDH) in tissues by the thiobarbituric method [1]. The state of the physiological antioxidant system (PAS) was assessed by the activity of glutathione peroxidase (GPO) and catalase [12].

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 number of carious cavities (per 1 rat), as well as the depth of carious lesions of the teeth of rats by caries (in points) were determined on macro-preparations of the isolated rat jaws. The isolated jaws of rats were subjected to morphometric examination [10].

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 Student's 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 of dietary supplements with PPP preparation on the condition of the dentition in rats under conditions of hypoxia and cariogenic diet reproduction in table 1.

Table 1

The effect of the complex of dietary supplements with PPP on the condition of the dentition in rats under conditions of hypoxia and cariogenic diet reproduction, M±m

Indexes / Groups	Indices of bone resorption of parodontal tissue (%)	Number of carious lesions per 1 rat	Depth of dental caries lesions (in points)
Intact	17.2±0.7	1.9±0.4	1.9±0.4
H+CgD	17.2±0.5	3.4±0.3 p=0.03	3.5±0.2 p=0.016
H+CgD+ Complex	17.5±0.9 $p_1>0.1$	2.6±0.2 $p_1=0.06$	2.8±0.2 $p_1=0.05$

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 number of cavities after 30 days of exposure decreased by 24 % (p=0.06). The depth of carious lesions, i.e. the aggressiveness of the pathological process (in points) decreased by 20 % (p=0.05). At the same time, the parameters of parodontal bone resorption did not change significantly.

The complex is significantly (by 3.3 times; $p_1<0.001$ decreased the activity of acid phosphatase in the pulp of rat teeth, which indicates a decrease in the activity of odontoclasts in this study object. At the same time, the activity of this enzyme was 2 times (p=0.04) lower than in the intact group. (Table 2).

Table 2

The effect of the complex of dietary supplements with PPP preparation on biochemical parameters in dental pulp and parodontal tissues of rats, M ± m

Groups / Indexes	Animal groups		
	Intact	H+CgD	H+CgD+Complex
	Dental pulp		
AP (nkat/l)	31.0±5.61	51.7±5.00 p=0.03	15.5±1.44 p=0.04 $p_1<0.001$
ALP (mkat/l)	1.98±0.16	1.32±0.29 p=0.08	3.43±0.35 p<0.001 $p_1=0.002$
	Oral mucosa		
AL (nkat/g)	89.8±5.10	265±10.6 p<0.001	115±3.90 p=0.012 $p_1<0.001$
	Alveolar bone		
AL (nkat/g)	61.8±2.00	155±0.025 p<0.001	97.5±1.72 p<0.001 $p_1<0.001$
ALP (mkat/g)	193±2.20	73.5±1.03 p<0.001	102±1.52 p<0.001 $p_1<0.001$
Calcium (mmol/g)	7.30±0.12	2.68±0.090 p<0.001	5.18±0.13 p<0.001 $p_1<0.001$
Phosphorus (mmol/g)	8.07±0.25	4.18±0.080 p<0.001	6.23±0.12 p<0.001 $p_1<0.001$

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.

Under the influence of the complex, the activity of alkaline phosphatase, which is a marker enzyme of odontoblasts, increased by 2.6 times ($p_1<0.001$) compared with the control group and by 1.7 times ($p<0.001$) compared with intact (Table. 2).

The complex improved the mineral metabolism in the parodontal bone tissue. Thus, the activity of alkaline phosphatase increased by 39 % ($p_1<0.001$); the calcium content increased by 1.9 times ($p_1<0.001$); phosphorus – by 1.5 times ($p_1<0.001$).

In the bone of the alveolar bone, the activity of acid phosphatase decreased by 1.6 times ($p_1<0.001$), compared with the group H+CgD, but was significantly higher than in the intact one. In the oral mucosa, a decrease in AP activity was revealed under the influence of the complex and was 2.3 times lower than in the control group ($p_1<0.001$).

The action of the complex did not affect the MDH content and catalase activity in the rat liver. The activity of another antioxidant enzyme, glutathione peroxidase, increased by 1.8 times ($p_1=0.01$). In the mucous membrane of the oral cavity and in the alveolar bone, the complex reduced MDH levels ($p_1=0.07$ and $p_1<0.001$, respectively), which indicates its antioxidant properties. At the same time, the content of MDH in the parodontal tissues was normalized and was practically at the level of intact groups. Catalase activity in the studied objects did not significantly change in comparison with the data of the control groups. In the bone tissue of parodontium, the activity of glutathione peroxidase under the action of the complex increased by 21 % ($p_1=0.02$).

The iunfluence of PPP preparation on content of oxyproline presented in fig. 1.

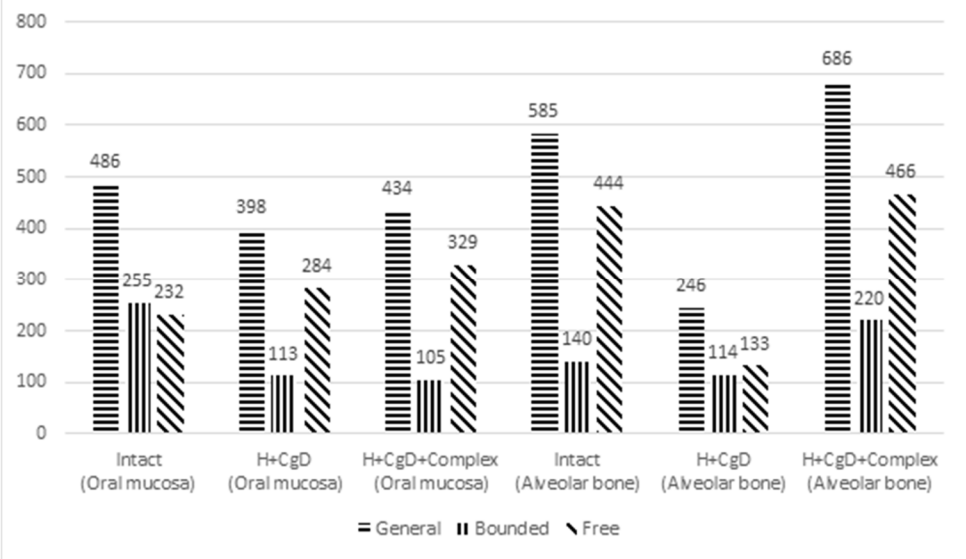

Fig.1. Influence of PPP preparation on content of oxyproline, μmol/g

It should be noted that the content of general and bounded oxyproline in the parodontal bone was significantly higher. On the other hand, free oxyproline practically corresponded to the data of the intact group.

Influence of PPP preparation on content of glycosaminoglycans presented in fig. 2.

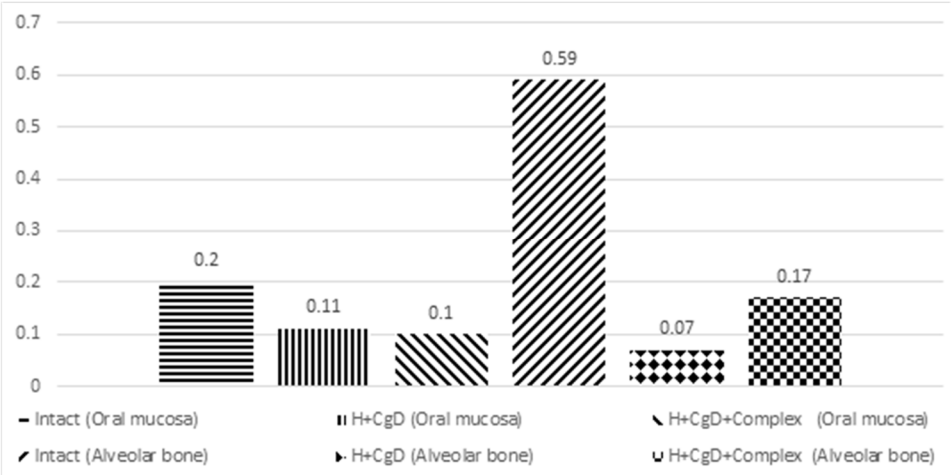

Fig.2. Influence of PPP preparation on content of glycosaminoglycans, mg/g

The content of GAG and oxyproline did not significantly change in the oral mucosa compared with the data of the control groups. In the alveolar bone, the GAG level increased by 2.4 times (p_1<0.001); general oxyproline – by 2.8 times (p_1<0.001); free – by 3.5 times (p_1<0.001); bounded – by 1.9 times (p_1=0.02) regarding control groups data.

Effect of PPP preparation on pyruvate content in rat tissues presented in fig. 3.

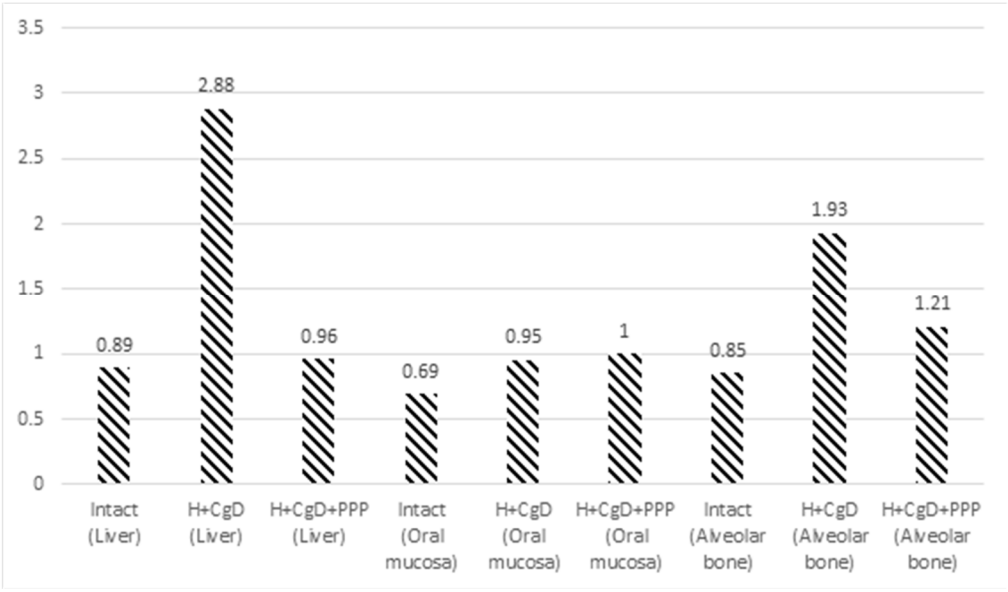

Fig.3. Effect of PPP preparation on pyruvate content in rat tissues, mmol/g

In the oral mucosa, the pyruvate content did not change significantly. At the same time, the content of pyruvate in the alveolar bone also decreased by 1.6 times (p_1<0.001), compared with the control group H+CgD.

Effect of PPP preparation on LDH activity in rat tissues presented in fig. 4.

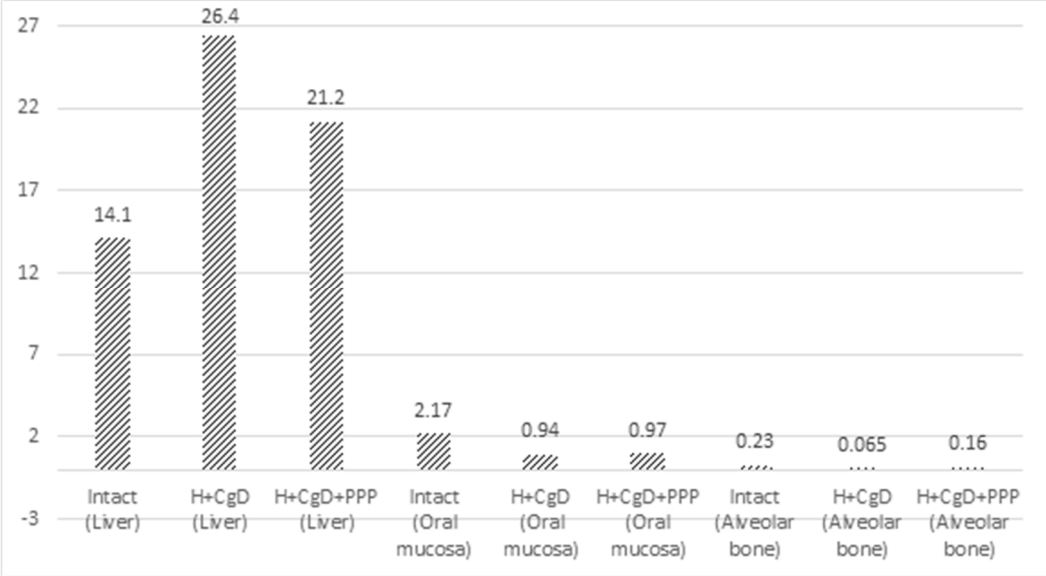

Fig.4. Effect of PPP preparation on LDH activity in rat tissues, μkat/g

LDH activity decreased 2.2 times in the oral mucosa (p=0.002) compared to the intact group, which may indicate an increase in lactate levels. In the alveolar bone, the LDH activity increased by 2.5 times (p_1=0.001), which may indicate a decrease in lactate levels.

The issue of prenatal hypoxia and its long-term consequences has been extremely urgent for many years and attracts the attention of researchers, clinicians of various specialties in terms of explaining the mechanisms of development and possible prevention of various pathological conditions [4, 13, 14]. However, in our opinion, the existing studies devoted to the treatment of disorders in the oral cavity of children who underwent hypoxia in the antenatal period are rather few [8, 9], 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, a caries-preventive effect and a decrease in the aggressiveness of the pathological process were revealed under the combined action of a complex of dietary supplements with a preparation of plant polyphenols from the leaves of *Plantago major* (PPP preparation) under conditions of intrauterine tissue hypoxia and a cariogenic diet. Under the action of the complex, a decrease in the activity of odontoclasts and osteoclasts was revealed in the pulp of rat teeth and bone tissue of the parodontium. The complex showed an anti-inflammatory effect in the oral mucosa. LPO processes normalized in the oral mucosa and parodontal bone tissue. The levels of metabolic markers of pyruvate hypoxia in the liver and in the oral mucosa were normalized, while the LDH activity in all study objects did not correspond to the data of the intact groups. The complex significantly improved the state of collagen and glycosaminoglycans in the parodontal bone tissue, which form the basis of the extracellular matrix of the parodontal connective tissue.

Conclusions

1. It was revealed caries-prophylactic effect and a decrease in the aggressiveness of the pathological process under the action of the complex in the conditions of intrauterine tissue hypoxia and cariogenic diet.

2. The complex showed an anti-inflammatory effect in the oral mucosa.

3. The levels of metabolic markers of pyruvate hypoxia in the liver and in the oral mucosa returned to normal state.

4. The complex significantly improved the state of collagen and glycosaminoglycans in the parodontal bone tissue.

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