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FEATURES OF RAT BLOOD SERUM MARKERS AGAINST THE BACKGROUND OF FLUOROSIS MODELING, ORTHODONTIC INTERVENTION, AND THERAPEUTIC AND PROPHYLACTIC COMPLEX

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The study was devoted to evaluate the influence of a therapeutic-prophylactic complex on blood-serum markers in experimental animals under conditions of modelled fluorosis and orthodontic intervention. Experimental studies were carried out on 40 male Wistar rats (herd breeding), 4 months of age, with an average body mass of 280±14 g. The developed therapeutic-prophylactic complex exhibits pronounced antioxidant and anti-inflammatory (adaptogenic) properties. Its application enhances non-specific resistance under the combined effects of fluoride excess and mechanical stress, as evidenced by normalisation of biochemical markers of oxidative stress and proteolysis. These findings substantiate the feasibility of employing this complex to prevent oxidative damage and inflammatory changes in clinical practice, particularly in orthodontic patients residing in endemic fluorosis areas.

Key words: fluoride, fluorosis, blood serum, rats, experimental study.

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ОСОБЛИВОСТІ МАРКЕРІВ СИРОВАТКИ КРОВІ ЩУРІВ НА ТЛІ МОДЕЛЮВАННЯ ФЛЮОРОЗУ, ОРТОДОНТИЧНОГО ВТРУЧАННЯ ТА ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ

Дослідження присвячене оцінці впливу лікувально-профілактичного комплексу на маркери сироватки крові в експериментальних тварин за умов модельованого флюорозу та ортодонтичного втручання. Експериментальні дослідження проведені на 40 щурах-самцях лінії Вістар (стадне розведення) віком 4 місяці, середньою масою тіла 280±14 г. Розроблений лікувально-профілактичний комплекс виявляє виражені антиоксидантні та протизапальні (адаптогенні) властивості. Його застосування підвищує неспецифічну резистентність організму за комбінованої дії надлишку фтору та механічного стресу, про що свідчить нормалізація біохімічних маркерів оксидативного стресу та протеолізу. Отримані дані обґрунтовують доцільність застосування цього комплексу для профілактики оксидативного пошкодження та запальних змін у клінічній практиці, зокрема в ортодонтичних пацієнтів, які проживають в ендемічних зонах флюорозу.

Ключові слова: фтор, флюороз, сироватка крові, щури, експеримент.

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Fluorosis is a chronic condition that develops as a result of excessive fluoride intake and is characterized by lesions of the teeth, bones, and other organs. Contemporary research pays particular attention to the systemic metabolic shifts associated with fluorosis, notably oxidative stress and the organism's antioxidant status. It has been established that prolonged fluoride overconsumption disrupts the balance between pro- and antioxidant processes: the generation of reactive oxygen species (ROS) and lipid peroxidation products increases, leading to oxidative damage in various tissues (bone, teeth, gastrointestinal mucosa, etc.) [6, 7]. Excessive fluoride exposure exhausts the antioxidant system: the activities of key antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) decrease, while the levels of free-radical oxidation products – particularly malondialdehyde rise [8, 10]. Under these conditions, cells cannot neutralize the excess free radicals, resulting in biomembrane damage, redox-homeostasis imbalance, and, ultimately, apoptosis or other forms of cell death. Chronic fluorosis is also associated with inflammatory reactions and organ dysfunction; notably, oxidative injury to hepatic mitochondria and enterocyte pyroptosis induced by fluoride excess have been described [6]. At present, no specific effective therapies for fluorosis exist, and the disorder remains a pressing medical and social problem in many regions worldwide. Consequently, identifying prophylactic and therapeutic agents capable of mitigating fluoride's deleterious effects is a promising research avenue. In particular, the use of antioxidants is regarded as an effective strategy to minimize oxidative stress in fluorosis [9, 10].

Orthodontic treatment, which involves prolonged mechanical loading of the dentoalveolar apparatus, can likewise provoke oxidative stress in periodontal tissues. Tooth movement under orthodontic forces triggers the release of inflammatory mediators and the generation of ROS in the periodontium, disturbing the equilibrium between free-radical formation and the antioxidant defence system [5]. As a result, cellular membranes, proteins, and matrix components within periodontal tissues may be damaged, influencing bone remodelling and periodontal status in both the short and long term.

A critical element in the pathological alterations associated with chronic inflammation and connective-tissue destruction is the imbalance of the protease-inhibitor system. Under physiological conditions, the activity of tissue proteases (cathepsins, elastase, collagenases, etc.) is strictly regulated by their natural inhibitors, maintaining equilibrium between proteolysis and matrix restoration. In pathological states – especially inflammatory ones, this balance shifts towards excessive proteolytic activity, leading to extracellular matrix damage, collagen degradation, and inflammatory progression [4]. Elevated protease activity coupled with inhibitor deficiency is regarded as a key factor in the development of destructive periodontal lesions under oxidative and other stressors.

To date, the combined impact of chronic fluoride intoxication and orthodontic loading on pro/antioxidant balance and the proteolytic system has not been adequately investigated. Children living in endemic fluorosis regions may require orthodontic treatment, and the convergence of these risk factors can exacerbate pathological changes in periodontal tissues. Therefore, devising prophylactic measures for such populations is of great relevance. One potential approach is the use of therapeutic-prophylactic complexes possessing antioxidant and anti-inflammatory properties that enhance the organism's adaptive capacity under multiple adverse influences.

The purpose of the study was to evaluate the influence of a therapeutic-prophylactic complex on blood-serum markers in experimental animals under conditions of modelled fluorosis and orthodontic intervention.

Materials and methods. Experimental studies were carried out on 40 male Wistar rats (herd breeding), 4 months of age, with an average body mass of 280±14 g. The animals were kept in normal vivarium conditions under natural light and with free access to water and food. Throughout the experiment, the microclimatic conditions of the vivarium environment were strictly observed: temperature (19–22°C) and humidity (55–70 %). Experimental studies were conducted at the Laboratory of Biochemistry and Vivarium of the SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (SE "ISMFS NAMS"). All experiments on rats were conducted according to standard operating procedures approved by SE "ISMFS NAMS", developed in accordance with the Guidelines of the Pharmacological Committee of the Ministry of Health of Ukraine and the International Regulations for the Use of Laboratory Animals [2].

The animals were divided into 4 groups as follows:

– 1st group – intact, n=10;

– 2nd group – fluoride intoxication model, n=10;

– 3rd group – fluoride intoxication + orthodontic tooth movement, n=10.

– 4th group – fluoride intoxication + orthodontic tooth movement + TPC, n=10

Animals in the intact group received balanced feed that fully covered their daily requirements for nutrients, vitamins, minerals and trace elements, as well as disinfected and reverse osmosis-filtered water with free access.

Orthodontic tooth movement (OTM) was induced to achieve mesial displacement of the maxillary molars, mimicking natural tooth movement and permitting biochemical assessment of alveolar bone under physiological mechanical loading. A notch was prepared on the incisors to secure a 0.012″ stainless-steel orthodontic ligature (length ≥ 100 mm). The ligature was passed interdentally between the first and second maxillary molars and tightly looped around the first molar; its two free ends were fixed to the incisor notch. Ligature tension was verified every three days throughout a 30-day period. The method followed the model of Horokhivskyi V.N. (2006). Models of experimental fluorosis and OTM were employed separately and in combination to assess the efficacy of the therapeutic-prophylactic complex.

The total duration of the study was 60 days. During the first 30 days, Groups 2–4 received NaF in their drinking water. Subsequently, OTM of the maxillary molars was initiated in Groups 3 and 4 while NaF administration continued. Group 2 received NaF for the entire 60-day period (fluorosis model). In Group 4, the therapeutic-prophylactic complex was administered intragastrically as an aqueous suspension for 30 days against the background of fluorosis and OTM. Group 1 served as the control and received only filtered water.

Animals were withdrawn from the experiment by an overdose of intraperitoneal anaesthesia using sodium thiopental (at a rate of 40 mg/kg) on day 60 of the experiment by total bleeding from the heart. After euthanasia, blood was collected by cardiac puncture to obtain serum for biochemical analysis. In the rat serum we measured the concentration of malondialdehyde (MDH), a marker of lipid peroxidation; the activity of the antioxidant enzyme catalase; and calculated the antioxidant-pro-oxidant index (API) as the ratio of catalase activity to MDH content. In addition, total proteolytic activity (TPA), the level of trypsin inhibitor (TI), and the TI/TPA ratio were determined [1]. 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 $p<0.01$ [3].

Results of the study and their discussion. At this stage of our work we were interested in assessing the state of the antioxidant-pro-oxidant system in experimental rats exposed to the therapeutic-prophylactic complex (TPC) under conditions of modelled fluorosis combined with orthodontic intervention. To this end, catalase activity, malondialdehyde (MDH) concentration and the calculated antioxidant-pro-oxidant index (API) were determined in blood serum (Table 1).

Table 1

Effect of the therapeutic-prophylactic complex on parameters of the antioxidant-pro-oxidant system in rat serum under conditions of modelled fluorosis and orthodontic intervention, M±m

Indicators / Groups	Catalase activity, mcat/l	Malondialdehyde content, mmol/l	API
Intact, n=10	0.30±0.02	0.40±0.02	7.50±0.30
Fluoride intoxication model, n=10	0.21±0.01 p<0.001	0.76±0.04 p<0.001	2.76±0.12 p<0.001
Fluoride intoxication + OTM, n=10	0.15±0.01 p<0.001 p_1<0.001	1.12±0.08 p<0.001 p_1<0.001	1.33±0.10 p<0.001 p_1<0.001
Fluoride intoxication + OTM + TPC, n=10	0.27±0.02 p>0.1 p_1<0.01 p_2<0.002	0.50±0.03 p<0.01 p_1<0.001 p_2<0.002	5.40±0.21 p<0.001 p_1<0.001 p_2<0.002

Note. p – significance of differences to the intact group; p_1 – significance of differences to the “Fluoride intoxication model” group; p_2 – significance of differences to the “Fluoride intoxication + OTM” group.

Modelling of fluorosis in Group 2 resulted in a marked intensification of lipid peroxidation, as evidenced by a 90 % rise in MDH concentration ($p<0.001$). Simultaneously, catalase activity decreased by 30 % ($p<0.001$), and the API fell 2.7-fold ($p<0.001$).

Considerable disturbances in the antioxidant-pro-oxidant balance were observed in Group 3, where experimental fluorosis was combined with orthodontic ligature fixation. Catalase activity was reduced two-fold, MDH increased 2.8-fold, and the API declined 5.6-fold compared with intact controls ($p<0.001$).

These findings indicate that, under conditions of fluorosis coupled with orthodontic ligature placement, antioxidant defenses are suppressed and lipid peroxidation processes are amplified. Such alterations should be taken into account in clinical orthodontic practice for children living in areas with excessive fluoride exposure.

Administration of the therapeutic-prophylactic complex markedly normalized the antioxidant-pro-oxidant system in Group 4 rats after orthodontic intervention on the background of fluorosis. Relative to Group 3, MDH concentration fell 2.2-fold, catalase activity rose by 80 % ($p_2<0.001$), and the API increased 4.1-fold. These data confirm the high antioxidant efficacy of the applied drug complex in enhancing the non-specific resistance of the organism under the combined impact of experimental fluorosis and orthodontic spring fixation.

It is well recognized that proteolytic system activity plays a pivotal role in regulating numerous physiological processes. Proteolysis-comprising proteolytic enzymes, their inactive precursors, activators and inhibitors maintains homeostasis under normal conditions and during adaptive protective responses. A physiological balance exists between protease activity and their inhibitors; pathological states tip this balance towards protease activation, constituting a key pathogenetic link in destructive and inflammatory disorders and hemostatic disturbances. Proper evaluation of the proteolytic system therefore requires assessment of total proteolytic activity (TPA) and the level of protease inhibitors.

Determination of TPA and trypsin inhibitor (TI) levels in rat serum provides an additional measure of the organism’s non-specific defense. Alterations in the protease-inhibitor system are most clearly reflected by the TI/TPA ratio. The analysis of these indices is summarized in Table 2.

Table 2

Effect of the therapeutic-prophylactic complex on parameters of the protease-inhibitor system in rat serum under conditions of modelled fluorosis and orthodontic intervention, M±m

Groups \ Indicators	Total proteolytic activity, nkat/l	Trypsin inhibitor content, g/l	TI/TPA
Intact, n=10	1.98±0.11	0.50±0.03	0.25±0.02
Fluoride intoxication model, n=10	2.30±0.14 p>0.1	0.48±0.02 p>0.6	0.21±0.02 p>0.2
Fluoride intoxication + OTM, n=10	3.39±0.21 p<0.001 p_1<0.001	0.38±0.01 p<0.002 p_1<0.001	0.11±0.01 p<0.001 p_1<0.001
Fluoride intoxication + OTM + TPC, n=10	2.48±0.16 p<0.002 p>0.4 p_2<0.002	0.63±0.03 p<0.002 p_1<0.002 p_2<0.002	0.25±0.01 p_1>0.8 p_1<0.001 p_2<0.002

Note. p – significance of differences to the intact group; p_1 – significance of differences to the "Fluoride intoxication model" group; p_2 – significance of differences to the "Fluoride intoxication + OTM" group.

Under conditions of experimental fluorosis (Group 2), the serum trypsin-inhibitor level and total proteolytic activity remained comparable to those of intact animals.

Additional orthodontic ligature fixation in Group 3 produced a significant 1.7-fold rise in total proteolytic activity ($p<0.001$), a 24.0 % reduction in trypsin inhibitor ($p<0.001$), and a 2.3-fold fall in the TI/TPA ratio, indicating a marked imbalance in the protease-inhibitor potential. These alterations reflect a pro-inflammatory trend and heightened strain on the non-specific resistance system under combined experimental fluorosis and orthodontic intervention.

Daily oral administration of the therapeutic-prophylactic complex to rats in Group 4 normalized the protease-inhibitor system: total proteolytic activity decreased by 26.8 % ($p_2<0.002$), trypsin-inhibitor content rose by 65.8 % ($p_2<0.002$), and the TI/TPA ratio returned to values seen in intact controls. These findings are favorable, as a high trypsin-inhibitor level suppresses excessive elastase activity that otherwise contributes to periodontal connective-tissue destruction. The elevated inhibitor level thus attests to the anti-inflammatory action of the therapeutic-prophylactic complex administered throughout the experiment.

Consequently, the applied TPC enhances the non-specific resistance of experimental rats and demonstrates distinct adaptogenic properties.

The present findings confirm that chronic fluoride exposure is a potent inducer of systemic oxidative stress, as evidenced by the sharp rise in malondialdehyde and the concomitant fall in catalase activity observed in Group 2. Similar elevations in lipid-peroxidation products and depression of antioxidant enzymes have been reported in both gastrointestinal [6] and hepatic tissues [9] of fluoride-intoxicated rodents, underscoring that the redox imbalance we documented in serum reflects a generalized metabolic derangement. When orthodontic tooth movement was super-imposed on fluorosis (Group 3), all oxidative indices deteriorated even further, suggesting a synergistic interaction between mechanical loading and fluoride toxicity. This agrees with clinical and experimental data showing that orthodontic forces amplify local reactive-oxygen-species generation and compromise antioxidant defences in periodontal tissues [5]. Restoration of redox homeostasis in the TPC-treated animals (Group 4) highlights the robust antioxidative capacity of the formulation. The 2.2-fold reduction in MDH and 80 % rebound of catalase activity translated into a four-fold improvement of the antioxidant-pro-oxidant index. Such normalisation parallels the protective effects described for exogenous antioxidants that activate Sirt3-dependent mitochondrial pathways and suppress fluoride-induced ROS overproduction [9]. Notably, our data extend these mechanistic insights by demonstrating that systemic antioxidant therapy can remain effective even under the additional mechanical stress of active OTM – a scenario that closely mimics orthodontic management of children residing in endemic fluorosis areas. Equally important is the observed correction of the protease-inhibitor balance. Fluoride alone produced only minor shifts in total proteolytic activity and trypsin-inhibitor levels, but the addition of OTM triggered a 1.7-fold surge in TPA and a 24 % drop in TI, reducing the TI/TPA ratio by more than half. Excessive serine-protease activity is now recognized as a driver of sterile inflammation and connective-tissue breakdown [4]; thus, the dramatic fall in TI/TPA we recorded is pathophysiologically coherent with the heightened oxidative milieu. Oral delivery of the TPC reversed this pattern, raising TI by 66 % and restoring the TI/TPA ratio to baseline values. These shifts imply that the complex not only scavenges ROS but also up-regulates endogenous antiproteases, thereby interrupting the feed-forward loop between oxidative stress and proteolytic tissue injury. From a translational perspective, the combined insults of fluorosis and orthodontic force may accelerate periodontal deterioration through dual pathways – ROS-mediated lipid damage and uncontrolled

proteolysis. The present work demonstrates that a multitarget prophylactic regimen can blunt both processes simultaneously, lending experimental support to recent proposals that orthodontic patients exposed to high environmental fluoride should receive adjunctive antioxidant therapy.

Conclusions

1. Chronic fluoride intoxication combined with orthodontic loading leads to a pronounced imbalance of the antioxidant–pro-oxidant system and proteolytic equilibrium. In rats with experimental fluorosis subjected to orthodontic tooth movement, the activity of the antioxidant enzyme catalase fell by more than half, whereas the malondialdehyde concentration rose 2.8-fold relative to intact animals, resulting in a five-fold decrease in the antioxidant–pro-oxidant index. Concurrently, proteolysis intensified: total proteolytic activity in serum increased 1.7-fold, the trypsin-inhibitor level declined by 24 % compared with the control, and the TI/TPA ratio dropped by more than two-fold. These changes indicate the development of marked oxidative stress and inflammatory-destructive alterations in connective tissue under the combined influence of fluoride excess and mechanical stress.

2. The developed therapeutic-prophylactic complex exhibits pronounced antioxidant and anti-inflammatory (adaptogenic) properties. Its application enhances non-specific resistance under the combined effects of fluoride excess and mechanical stress, as evidenced by normalization of biochemical markers of oxidative stress and proteolysis. These findings substantiate the feasibility of employing this complex to prevent oxidative damage and inflammatory changes in clinical practice, particularly in orthodontic patients residing in endemic fluorosis areas.

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