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# EXPERIMENTAL EVALUATION OF A THERAPEUTIC-PROPHYLACTIC COMPLEX ON BIOCHEMICAL MARKERS IN RAT GINGIVAL HOMOGENATES UNDER MODELLED FLUOROSIS AND ORTHODONTIC INTERVENTION

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The study was devoted to evaluate the effect of a therapeutic-prophylactic complex on inflammatory and oxidative-stress biomarkers in gingival homogenates of rats exposed to chronic excessive fluoride intake and orthodontic tooth movement. 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. Summarising the biochemical findings, orthodontic ligature fixation superimposed on fluorosis markedly disrupted the antioxidant-prooxidant balance and intensified inflammatory processes in the gingival tissues. Administration of the therapeutic-prophylactic complex under conditions of experimental fluorosis and orthodontic intervention improved antioxidant status, decreased lipid peroxidation, and attenuated inflammation, thereby confirming the antioxidant and anti-inflammatory properties of the agents incorporated into the developed therapeutic-prophylactic complex.

**Key words:** fluoride, fluorosis, gingiva, rats, experimental study.

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# ЕКСПЕРИМЕНТАЛЬНА ОЦІНКА ВПЛИВУ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ НА БІОХІМІЧНІ МАРКЕРИ У ГОМОГЕНАТАХ ЯСЕН ЩУРІВ НА ТЛІ МОДЕЛЮВАННЯ ФЛЮОРОЗУ ТА ОРТОДОНТИЧНОГО ВТРУЧАННЯ

Дослідження присвячене визначенню впливу лікувально-профілактичного комплексу на біохімічні маркери запалення та оксидативного стресу у гомогенатах ясен щурів за умов хронічного надлишкового надходження фтору та ортодонтичного переміщення зубів. Експериментальні дослідження проведені на 40 щурах-самцях лінії Вістар (стадне розведення) віком 4 місяці, середньою масою тіла 280±14 г. Узагальнюючи результати біохімічних досліджень тканин ясен дослідних щурів можна відмітити, що фіксація ортодонтичної лігатури на тлі флюорозу негативно діє на антиоксидантно-прооксидантну систему та призводить до посилення запальних процесів у яснах тварин. Застосування лікувально-профілактичного комплексу препаратів у яснах щурів при експериментальному флюорозі та ортодонтичному втручанні призводить до покращення стану антиоксидантної системи, зниження рівня перекисного окислення ліпідів та інтенсивності запалення, що підтверджує наявність антиоксидантної та протизапальної дії препаратів що входять до розробленого лікувально-профілактичного комплексу.

**Ключові слова:** фтор, флюороз, ясна, щури, експеримент.

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Gingival inflammation and dental malocclusions are common conditions in adolescents that can significantly compromise oral hygiene and overall periodontal health. Periodontal disease remains a highly prevalent disorder worldwide (with severe periodontitis affecting roughly 10 % of the population) [8]. Orthodontic appliances (fixed braces) exacerbate this problem by creating additional plaque-retentive

niches on the teeth, leading to increased plaque accumulation and higher rates of gingivitis and bleeding [6]. Inadequate mechanical oral hygiene and standard prophylactic measures often fail to control the heightened inflammation associated with orthodontic treatment, underscoring the need for novel preventive approaches.

Fluorosis – chronic fluoride overexposure – is endemic in many parts of the world and poses additional challenges to periodontal health [10]. While low levels of fluoride benefit enamel and bone, chronic fluorosis (arising from excess fluoride in drinking water or other sources) induces systemic biochemical disturbances [7]. Epidemiological studies suggest that individuals in fluorosis-endemic areas exhibit a higher prevalence and severity of gingivitis and periodontitis [7, 8], although other reports find no clear association between fluorosis severity and periodontal status [8]. At the molecular level, fluoride toxicity is known to disrupt the redox balance: exposed patients show elevated total oxidative stress and diminished antioxidant defences in serum, with excessive reactive oxygen species (ROS) generation triggering inflammatory cascades [7]. Thus, fluorosis (alone and in combination with mechanical stress from orthodontic forces) can exacerbate periodontal tissue damage via oxidative and inflammatory pathways.

The combination of fixed orthodontic forces and a background of fluorosis is expected to amplify periodontal inflammation. Orthodontic ligatures themselves are known to impede hygiene and stimulate gingival inflammation due to retained plaque [6]. Inflammation in periodontal tissues involves recruitment of neutrophils and macrophages, which release proteolytic enzymes (e.g. elastase) and reactive oxygen species. Lipid peroxidation products such as malondialdehyde (MDH) rise, and antioxidant enzymes like catalase are consumed. For example, Cherian et al. found significantly higher salivary MDH in periodontitis patients than in healthy controls, confirming MDH as a marker of oxidative tissue damage [5]. Importantly, controlled studies indicate that restoring antioxidant balance can mitigate periodontal oxidative damage. A recent systematic review concluded that oxidative stress is a key factor in periodontitis and that antioxidant supplementation (e.g. vitamins, polyphenols) can effectively reduce oxidative injury in periodontal tissues [4]. Furthermore, clinical trials in orthodontic patients have shown that natural anti-inflammatory/antioxidant agents (aloe vera, propolis, green tea, resveratrol, etc.) significantly reduce braces-associated gingival inflammation and bacterial load [11].

Given the inflammation and oxidative stress burden imposed by both fluorosis and orthodontic treatment, there is a clear rationale for testing adjunctive therapeutic-prophylactic strategies. In the present study, we use a rat model of combined fluoride overload and orthodontic tooth movement to examine whether an orally-administered therapeutic-prophylactic complex can normalize biochemical markers in gingival tissues. Specifically, we focus on indices of neutrophil-driven inflammation (elastase activity), lysosomal enzyme release (acid phosphatase), lipid peroxidation (MDH) and antioxidant defence (catalase activity). These experiments will help evaluate innovative preventive measures aimed at protecting periodontal tissues under the combined challenge of fluorosis and orthodontic treatment [4, 5, 11].

**The purpose** of the study was to evaluate the influence of a therapeutic-prophylactic complex on biochemical markers in gingival homogenates 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 + therapeutic-prophylactic complex (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 necropsy, the gingival mucosae were dissected for biochemical analysis. Gingival homogenates were prepared at a concentration of 20 mg $mL^{-1}$ in Tris-HCl buffer (pH 7.5). The following parameters were assayed: inflammatory biomarkers – elastase activity and acid phosphatase activity; the lipid-peroxidation indicator – malondialdehyde (MDH) content; and the antioxidant defence marker – catalase activity. The antioxidant-prooxidant index (API) was calculated as the ratio of catalase activity to MDH content [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 Student's test. The difference was considered statistically significant at $p<0.01$ [3].

**Results of the study and their discussion.** Elastase – an enzyme of the protease class that cleaves structural proteins – is a potent tissue-destructive proteolytic factor primarily released by polymorphonuclear neutrophils. It also activates procollagenase, converting it to the active collagenase form.

At this stage of our work, we examined inflammatory markers – elastase activity and acid phosphatase activity (pH 4.8) – in gingival mucosal homogenates of rats. The results are presented in Table 1.

Table 1

**Effect of the therapeutic-prophylactic complex on inflammatory markers in rat gingival homogenates under modelled fluorosis and orthodontic intervention, M±m**

| Indicators / Groups | Elastase activity, μkat/kg | Acid phosphatase activity, μkat/kg |
|---|---|---|
| Intact, n=10 | 15.5±1.1 | 22.0±1.7 |
| Fluoride intoxication model, n=10 | 20.1±1.4<br>$p<0.01$ | 34.1±2.2<br>$p<0.001$ |
| Fluoride intoxication + OTM, n=10 | 31.3±2.0<br>$p<0.001$<br>$p_1<0.001$ | 47.2±2.9<br>$p<0.001$<br>$p_1<0.002$ |
| Fluoride intoxication + OTM + TPC, n=10 | 16.2±1.2<br>$p>0.1$<br>$p_1<0.05$<br>$p_2<0.001$ | 23.8±1.4<br>$p<0.02$<br>$p_1<0.02$<br>$p_2<0.001$ |

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.

According to the numerical data obtained, experimental fluorosis in the gingival tissues of rats in Group 2 increased elastase activity by 30.0 % ($p<0.01$) and acid phosphatase (pH 4.8) activity by 55.0 % ($p<0.001$) versus the intact controls. In Group 3, where orthodontic intervention was super-imposed on fluorosis, these markers rose even more sharply – elastase by 101.9 % ($p<0.001$) and acid phosphatase by 114.5 % ($p<0.002$) – indicating intense inflammation in fluorotic gingiva and a marked aggravation of inflammatory processes after ligature fixation.

Oral administration of the therapeutic-prophylactic complex in Group 4 produced a significant 48.2 % reduction in elastase activity ($p_2<0.001$) relative to Group 3. Notably, elastase levels in fluorotic rats subjected to orthodontic force returned to values comparable with intact animals. A similar pattern was

observed for acid phosphatase, whose activity fell by 49.6 % ($p_2<0.001$) compared with Group 3, virtually matching the values of healthy controls.

Thus, the cumulative profile of inflammatory biochemical markers in rat gingival tissue confirms the anti-inflammatory effectiveness of the proposed multi-component therapeutic-prophylactic formulation.

The antioxidant-pro-oxidant system constitutes the primary defence mechanism of organs and tissues, safeguarding their resistance against adverse factors. It regulates and suppresses all stages of free-radical reactions; catalase is one of its key enzymatic components. Catalase, a first-line antioxidant enzyme, inhibits and prevents free-radical formation within cells by decomposing $H_2O_2$ into water and molecular oxygen. Malondialdehyde – the terminal product of lipid peroxidation – reflects the intensity of lipid oxidative processes in the body and serves both as an inflammation marker and an indicator of endogenous intoxication.

Table 2 summarises the biochemical determinations of catalase activity (antioxidant enzyme), MDH content (lipid-peroxidation index), and the calculated antioxidant-pro-oxidant index (API; ratio of catalase activity to MDH content) in rat gingival tissues under experimental fluorosis, orthodontic ligature fixation, and prophylaxis with the therapeutic-prophylactic complex.

Table 2

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

| Indicators / Groups | Catalase activity, mcat/kg | Malondialdehyde content, mmol/kg | API |
|---|---|---|---|
| Intact, n=10 | 11.5±0.5 | 14.2±1.0 | 8.10±0.36 |
| Fluoride intoxication model, n=10 | 9.8±0.3<br>p<0.02 | 21.7±1.3<br>p<0.001 | 4.52±0.21<br>p<0.001 |
| Fluoride intoxication + OTM, n=10 | 10.7±0.7<br>p>0.4<br>$p_1$>0.25 | 31.8±2.0<br>p<0.001<br>$p_1$<0.001 | 3.36±0.18<br>p<0.001<br>$p_1$<0.001 |
| Fluoride intoxication + OTM + TPC, n=10 | 12.5±0.6<br>p>0.5<br>$p_1$<0.001<br>$p_2$>0.2 | 16.4±1.2<br>p>0.2<br>$p_1$<0.001<br>$p_2$<0.001 | 7.38±0.28<br>$p_1$>0.2<br>$p_1$<0.001<br>$p_2$<0.001 |

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.

The presented data indicate a decline in antioxidant defence in the gingival tissues of Group 2 rats with fluorosis: catalase activity decreased significantly by 14.8 % (p<0.02), while the antioxidant-pro-oxidant index dropped 1.8-fold. Consistently, the reduced antioxidant capacity in gingival homogenates correlated with a 52.8 % increase in malondialdehyde content (p<0.001) compared with intact rats.

In Group 3, the combination of fluorosis and orthodontic intervention caused a 2.2-fold rise in MDH levels (p<0.001) relative to healthy controls. Catalase activity remained comparable to that of Group 2, but the API fell 2.4-fold, underscoring marked disruption of the antioxidant-pro-oxidant system in gingival tissues under orthodontic stress against a background of excess fluoride exposure. These findings highlight the need for antioxidant-based corrective therapy.

Daily administration of the therapeutic-prophylactic complex in Group 4 improved redox balance: catalase activity increased by 16.8 % versus Group 3 ($p_2$>0.2) and by 8.7 % compared with intact animals (p>0.5); the API rose 2.2-fold relative to Group 3, approximating control values, and MDH content returned to normal levels.

The present study demonstrates that chronic fluoride intoxication alone (Group 2) significantly augments neutrophil-derived proteolytic and lysosomal activity in gingival tissues, as reflected by 30 % and 55 % rises in elastase and acid phosphatase, respectively, compared with intact controls (Table 1). These findings corroborate clinical observations that endemic fluorosis is associated with heightened gingival inflammation and periodontal breakdown [4]. When orthodontic tooth movement (OTM) was super-imposed on fluorosis (Group 3), the inflammatory burden more than doubled: elastase activity increased by 102 % and acid phosphatase by 115 % versus healthy animals, emphasising the synergistic pathogenicity of mechanical stress and fluoride toxicity. Lai et al. recently showed that experimental fluorosis amplifies reactive oxygen species (ROS) generation during OTM and accelerates periodontal tissue remodelling [7]; our data extend these observations to protease-mediated matrix degradation in the gingiva. Importantly, daily administration of the therapeutic-prophylactic complex for 30 days (Group 4) reversed these effects. Elastase fell by 48 % and acid phosphatase by 50 % relative to the fluorosis + OTM group, reaching values statistically indistinguishable from the intact group. The ability of TPC to down-

regulate neutrophil elastase is of particular interest, as excessive elastase activity is directly implicated in collagen fibre destruction and extracellular-matrix remodelling within periodontal lesions [13]. The oxidative profile paralleled the inflammatory changes. Fluoride overload reduced catalase activity by 15 % and doubled malondialdehyde levels, lowering the antioxidant-pro-oxidant index (API) by 56 %. Combined fluorosis and OTM further aggravated lipid peroxidation, tripling MDH and driving API down by 64 %. These results are consistent with clinical evidence that MDH is a reliable marker of oxidative periodontal damage and rises linearly with disease severity [5]. After TPC intervention, catalase rose to slightly above physiological values, MDH returned to near-baseline, and API normalised. Such restoration of redox equilibrium aligns with a recent meta-analysis showing that adjunctive antioxidant supplementation significantly improves periodontal outcomes and reduces oxidative biomarkers in both humans and experimental animals [6]. Mechanistically, the protective action of TPC may be attributed to a dual anti-inflammatory and antioxidant mode of action: suppression of neutrophil degranulation (hence reduced elastase release) and reinforcement of endogenous enzymatic defences (catalase), thereby curbing lipid peroxidation. Comparable effects have been reported for multifunctional phytochemical formulations administered during orthodontic therapy, which lowered plaque indices and gingival bleeding while enhancing salivary total antioxidant capacity [12].

## Conclusions

1. In summary, the biochemical analysis of rat gingival tissues showed that orthodontic ligature fixation under fluorotic conditions adversely affected the antioxidant-pro-oxidant balance and intensified inflammatory processes in the gingiva.

2. Administration of the therapeutic-prophylactic complex during experimental fluorosis combined with orthodontic intervention improved the antioxidant status, reduced lipid peroxidation, and attenuated inflammation, thereby confirming the antioxidant and anti-inflammatory properties of the agents included in the developed therapeutic-prophylactic formulation.

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