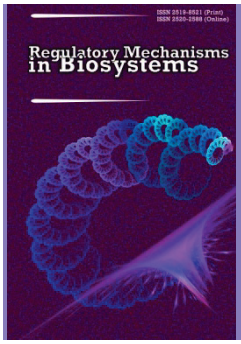

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2023, 14(1), 94–101
doi: 10.15421/022314

Prophylactic efficiency of the administration of vitamin, mineral and sorbent complexes on bone tissue in female rats against the background of chronic alcohol consumption

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Article info

Received 24.01.2023
Received in revised form 16.02.2023
Accepted 18.02.2023

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Makarenko, O. A., Kika, V. V., Khodakov, I. V., & Khromagina, L. M. (2023). Prophylactic efficiency of the administration of vitamin, mineral and sorbent complexes on bone tissue in female rats against the background of chronic alcohol consumption. Regulatory Mechanisms in Biosystems, 14(1), 94–101. doi:10.15421/022314

Among the general effects of alcohol use, it has a negative effect on the bone system, so the development of prevention methods is becoming an increasingly urgent problem. The present study was aimed at evaluating the efficiency of the preventive complexes for the bone system in chronic alcohol intoxication. The study was conducted on 2-month-old female rats for 104 days. Chronic alcoholism in animals was simulated by replacing drinking water with an ethanol solution, the concentration of which was gradually increased from 8–25%. The prophylactic was administered by introducing into the diet a complex of vitamins (P, C, D) and minerals (Cu, Mg, Zn, Se, Mn), the main component of which was crushed oyster shells processed with citric acid. The second prophylactic complex was the clay mineral montmorillonite in combination with vitamins P, C, D. The biochemical markers of resorption (elastase and acid phosphatase activity), osteogenesis (calcium content, alkaline phosphatase activity) and the state of the antioxidant system (catalase, superoxide dismutase, glutathione reductase activity, malondialdehyde content) were determined in bones (jaws and femurs). Chronic alcohol consumption led to an increase in the degree of atrophy of the alveolar process, a decrease in femur bone density due to a decrease in the mineral component in bone tissue, and did not affect the state of the lumbar vertebrae. Chronic alcohol intoxication led to a decrease in the level of calcium in the blood serum of rats, and to a decrease in the alkaline phosphatase activity and the calcium content in the bone tissue of the jaws and femur against the background of increases in the activities of acid phosphatase and elastase. A significant decrease in the activity of the main antioxidant enzymes (superoxide dismutase, catalase and glutathione reductase) in bone tissue of animals against the background of an increase in the malondialdehyde content under conditions of chronic alcoholization has been established. The use of the complex with crushed oyster shells effectively prevented atrophy ofthe alveolar process in the jaws, did not affect the morphometric parameters in the femur and vertebrae, but normalized the activities of serum alkaline phosphatase and catalase, bone elastase, acid phosphatase, superoxide dismutase and glutathione reductase against the background of restoring the levels of calcium and malondialdehyde both in the serum and in the bone tissue of rats subjected to chronic alcoholization. Prophylactic administration of the vitamin complex with montmorillonite had a much weaker effect on the studied indicators. The obtained research results allow us to conclude that the osteoprotective and antioxidant efficiency of the complex with crushed oyster shells is more pronounced than that of montmorillonite under conditions of chronic alcohol intoxication.

Keywords: alcohol intoxication; antioxidant system; alveolar process; femur; lumbar vertebrae.

Introduction

Alcohol is an optional component of the human diet. According to Health at a Glance: Europe 2022, alcohol consumption in 2020 was 9.8 litres of pure alcohol per adult on average across the European Union. On average, more than a third (37%) of European teenagers aged 15–16 drank alcohol sporadically at least once in the last 30 days in 2019 across the European Union.

According to the Global status report on alcohol and health (2018), currently about 2.3 billion people drink alcohol regularly. The death from alcohol consumption is higher than from diseases such as tuberculosis, HIV/AIDS and diabetes. According to forecasts, global alcohol consumption per capita will reach 7–6 litres by 2030 (Manthey et al., 2019).

Ethanol itself, as well as some compounds formed as a result of its metabolism, such as acetaldehyde, ethyl esters of fatty acids, phosphatidylethanol, causes general manifestations associated with toxicity (Birková et al., 2021). Alcohol and its metabolite acetaldehyde can contribute to the development of cancer through the induction of oxidative stress, the development of inflammatory processes, disruption of the carbohydrate metabolism, as well as retinoid metabolism, folic acid absorption (Brygadyrenko et al., 2019; Barron et al., 2020; Rumgay et al., 2021). Ethanol is probably unique among toxins in that it disrupts almost every aspect of lipid metabolism in the liver (You et al., 2018). Of great importance is the effect of ethanol on HIF-1α, which is a component of the transcription factor HIF-1, which regulates hundreds of genes, the changes of which expression can have various consequences in organ damage and in bone tissue as well (Morris et al., 2018).

Alcohol affects the bone system through both direct and indirect mechanisms, and there is consensus that the overall effect of alcohol consumption is a negative effect on quality and homeostasis of bone tissue. Oxidative stress, which is associated with the production of acetaldehyde, which disrupts osteoblastogenesis, plays a significant and possibly initiating role (Gaddini et al., 2016; Eby et al., 2020; Birková et al., 2021; Makarenko et al., 2021). Interestingly, the effect of alcohol on bone loss may

be related to the amount of alcohol consumed. Thus, in a study of the effects of alcohol intoxication on rodents it was shown that in animals which received 10% of total calories from alcohol this did not affect any of the measured bone parameters, while in rodents that received 36% of total calories from alcohol, a marked decrease in bone mineral density and bone strength was observed (Eby et al., 2020).

Chronic alcohol consumption increases the risk of hip fracture due to decreased bone mass. But the overall effect of alcohol consumption on bone health, especially in moderate amounts, has not been sufficiently studied. Animal models of alcohol intoxication attempt to parallel the human state, and many of these models have provided important information about factors mediating medical and psychiatric disorders (Bell et al., 2017). Thus, the effect of alcohol abuse on bone health remains an area for further research.

Patients who are addicted to alcohol cannot refuse its regular intake, therefore it is important to develop the methods of prevention of negative consequences in the organism, namely in the bone system and jaws, which are caused by regular alcohol consumption. Since alcohol causes a violation of almost all metabolic processes in the body, as well as, based on the data we previously received, a violation of bone metabolism, as a potential remedy of prevention we have proposed for research a complex of vitamins and minerals, the main component of which is calcium from oyster shells. Considering the high toxicity of alcohol and the products of its metabolism, the drug Minerol, which has a high sorption capacity, in combination with vitamins C, D, P and quercetin, was chosen as another means of prevention.

The purpose of our study: to carry out the comparative assessment of two prevention methods against disorders of the states of some bones in female rats treated with alcohol for a long period.

Materials and methods

All procedures with animals and removal of animals from the experiment were carried out in accordance with the principles laid down in the Law of Ukraine "About the Protection of Animals from Cruelty" (No. 1759-VI from 15.12.2009), taking into account the norms of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, March 18, 1986, ETS No. 123) and the World Medical Association statement on animal use in biomedical research. The results of the study were drawn up according to the recommendations of ARRIVE (Kilkenny et al., 2010).

The study has undertaken from January 17 to April 30, 2022 at the Department of Human and Animal Physiology, Faculty of Biology, Odesa I. I. Mechnikov National University. The study used 60-day-old female Wistar rats of colonial breeding, which had an average weight of 160 g. Female rats were used in the experiment due to the fact that we had showed a more pronounced effect of alcohol specifically on bone tissue in females in previous studies (Makarenko et al., 2021). Animals were kept in ordinary cages, two individuals each. All animals had free access to standard rat feed, the daily feed intake was not recorded.

The animals were randomly divided into four groups. The first group was the control group that drank water (n = 8). The second was the group of chronic alcohol intoxication (n = 10), which received an ethanol solution. The third was the group of chronic alcohol intoxication with correction, treated with ethanol solution and the "Calcium from oyster shells" complex (n = 8). The fourth was the group of chronic alcohol intoxication with correction, treated with ethanol solution and the "Minerol" complex (n = 8).

The semi-voluntary alcoholization model was used, in which an alcohol solution was the only liquid available to the animals, animals of the alcoholic groups did not have access to regular drinking water. The oral liquid administration as the alcohol administration technique that most closely resembles human drinking behavior was used in this study. Animals in groups 2–4 were gradually adapted to alcohol intake: the first 7 days they were given an 8% solution, the next 7 days it was increased to 16%, during the next week and for 90 days they were given a 25% ethanol solution (Santos German et al., 2020). The correction complexes were administered to animals of groups 3 and 4 orally at 9 o'clock every day during the 104 days of the experiment. The daily dose of the "Calcium from oyster shells" complex was 500 mg/kg. The daily dose of the "Minerol" complex was 1000 mg/kg.

"Calcium from oyster shells" complex. An optimal diet rich in trace elements such as calcium and vitamin D has long been considered an important component for normal bone physiology (Wilson-Barnes et al., 2022). Calcium is the main element of hydroxyapatite in bone tissue, plays a key role in mineralization of the skeleton and is required for normal growth, development and strength of bones. It has been established that increased calcium intake with food improves the mineral density of bone tissue in a short time. Calcium deficiency is a major risk factor for the development of osteoporosis (Ciosek et al., 2021) due to increased expression of RANKL in bone and an increase in the number of osteoclasts (Xiong et al., 2014). Vitamin D is important for the normal development and homeostasis of the bone system, it is the main link in the hormonal regulation of calcium and phosphorus exchanges due to the induction of the synthesis of the calcium transporter protein from enterocytes of the small intestine into the blood, due to the activation of phosphorus absorption in the intestines and the stimulation of the deposition of calcium salts in the newly formed osteoid matrix. Vitamin D exerts its calcemic and phosphatemic actions by altering the expression of several genes in the small intestine, kidneys, and bone. Activation of vitamin D receptors promotes absorption of calcium and phosphate in the intestine and tubular reabsorption of calcium in the kidneys (Charoenngam et al., 2019). Chronic alcohol consumption is known to affect vitamin D metabolism. These changes caused by alcohol consumption contribute to a decrease in the formation of bone tissue, which leads to osteopenia and increases the risk of fractures (Rosa et al., 2019).

Excessive alcohol consumption increases urinary excretion of calcium, magnesium, and zinc. Deficiency of these minerals, in turn, negatively affects the condition of bone tissue. Thus, magnesium takes part in the mineralization of bones due to the stimulation of the activity of osteoblasts and phosphatases, which mineralize the osteoid matrix. Therefore, magnesium deficiency in the body contributes to the development of osteoporosis (Ciosek et al., 2021). The bones of animals with magnesium deficiency are fragile due to a violation of the mechanical properties of bone (Castiglioni et al., 2013). Studies on the effect of adding magnesium to the diet note an increase in bone mineral density, a reduction in the risk of fractures (Rondanelli et al., 2021). Magnesium deficiency in humans, a known risk factor for bone loss, can lead to hypocalcemia, impaired parathyroid hormone secretion, and low serum concentrations of 1,25-dihydroxyvitamin D, which are commonly seen in individuals who chronically abuse alcohol (Gaddini et al., 2016).

Manganese, zinc, copper and selenium enhance the catalytic activity of antioxidant enzymes (Vona et al., 2021). Zinc is an important element for the development, regeneration and homeostasis of bones. In a study, male Sprague-Dawley rats fed zinc-deficient food lost body weight and developed osteopenia (Eberle et al., 1999). Zinc supplementation may also alleviate liver and intestinal dysfunctions after alcohol exposure. Zinc supplements may prevent hepatocyte death after alcohol exposure (Ballway & Song, 2021). Selenium also participates in normal skeletal development due to its important role in the antioxidant system. To date, many genes associated with the antioxidant properties of selenium have been identified (Hosnedlova et al., 2017). Copper is an important trace element involved in many biological processes. Deficiency of copper can lead to disturbances in the metabolism of the bone system (Qu et al., 2018).

Vitamin C is an important antioxidant and cofactor, as well as a modulator of osteogenic differentiation. Its deficiency leads to the development of bone pathologies, spontaneous fractures, and growth disorders (Aghajanian et al., 2015). Recent studies have shown that vitamin C deficiency affects bone mineral density and leads to the development of osteoporosis (Ratajczak et al., 2020). A correlation between serum vitamin C concentration and the risk of bone fractures was also found (Rondanelli et al., 2021). The use of antioxidants, especially vitamin C, is supported to prevent alcohol-mediated cellular toxicity (Jin et al., 2013).

The use of quercetin has shown its potential in medicine due to its antioxidant activity *in vivo*. Quercetin may be a useful preventive and therapeutic option for the treatment of bone disorders (Wong et al., 2020). Quercetin promotes the proliferation of bone marrow mesenchymal stem cells, increases the activity of alkaline phosphatase and the expression of

BMP-2 mRNA (bone morphogenetic proteins) (Bian et al., 2021). Quercetin reduces markers of bone resorption. Therefore, quercetin can serve as an important component as a supplement to prevent bone loss (Niu et al., 2020).

The above was the basis for assembling the components of the complex in the following form. The composition of the prophylactic complex "Calcium from oyster shells" (500 mg/kg): calcium (calcium citrate from oyster shells – a laboratory sample of our own technology) – 1500 mg; selenium (active selenium, "Elit-Pharm" LLC, Ukraine) – 500 mg; copper (active copper, "Elit-Pharm" LLC, Ukraine) – 750 mg; manganese (active manganese, "Elit-Pharm" LLC, Ukraine) – 250 mg; zinc (active zinc, "Elit-Pharm" LLC, Ukraine) – 500 mg; magnesium (active magnesium, "Elit-Pharm" LLC, Ukraine) – 500 mg; ascorbic acid (ascorbic acid No. 20, "Gaevskyi's Pharmacy" LLC) – 500 mg; vitamin D (Akvavit-D3, PrJSC "Technolog", Ukraine) IU3 – 300 IU; quercetin (quercetin, tablets of 40 mg, Public Joint-Stock Company "Scientific and Production Center "Borshchagiv Chemical and Pharmaceutical Plant", Ukraine) – 250 mg. Quantitative ratios of components are substantiated depending on their need in pathological conditions.

"Minerol" complex. Since alcohol consumption leads to the accumulation of toxic substances in the body, the drug "Minerol" (NVMP "GOBOR", Ukraine, Bucha, U.S. FDA registration No. 19847105946) was chosen as the main part of this complex. The manufacturer declares this drug as a natural sorbent, the sorption activity of which is up to 380 units, the sorption surface is up to 260 m^2/g, the cation exchange capacity is up to 100 mg-eq. per 100 g of substance. Also, "Minerol" is a source of minerals and contains almost all the macro- and microelements (calcium, silicon, iron, magnesium, sodium, sulfur, manganese, potassium, phosphorus, iodine, lithium, zinc, copper, chromium, selenium) that are necessary for physiological processes. The clay mineral montmorillonite, mined from a depth of 70–80 m, serves as a raw material for "Minerol". There are no vitamins in this drug, so they were added additionally. The composition of the preventive complex "Minerol" (1000 mg/kg): Minerol (NVMP "Hobor", Ukraine) – 900 mg; ascorbic acid (ascorbic acid No. 20, "Gaevskyi's Pharmacy" LLC) – 500 mg; vitamin D (Akvavit-D3, PrJSC "Technolog", Ukraine) IU3 – 300 IU; quercetin (quercetin, tablets of 40 mg, Public Joint-Stock Company "Scientific and Production Center "Borshchagiv Chemical and Pharmaceutical Plant", Ukraine) – 250 mg.

The duration of the experiment was 104 days. Rats were removed from the experiment under thiopental anaesthesia (20 mg/kg) by total heart bleeding. Serum was collected, jaws, femurs, and lumbar vertebrae of female rats were isolated to study the state of the bone system. Morphometric indicators were determined in the selected bones: in jaws – the degree of atrophy of the alveolar process, in femurs and lumbar vertebrae – the bone density and the content of mineral and organic components. The activity of catalase, alkaline phosphatase, the content of calcium and malondialdehyde (MDA) was determined in the serum. The bone tissue resorption indicators (elastase and acid phosphatase activity), the state of the antioxidant-prooxidant system (catalase, superoxide dismutase (SOD) activity, glutathione reductase, MDA concentration), osteogenesis indicators (alkaline phosphatase activity and calcium content) were determined in bone tissue (Makarenko et al., 2022). Data in Tables and text are presented as mean ± standard error (x ± SE). Statistical significance analysis of data was performed in R version 4.2.1 using one-way analysis of variance and Tukey's multiple comparison procedure with significant differences ($P < 0.05$).

Results

The degree of atrophy of the alveolar process in the control group rats is not statistically different from this parameter in the group of animals that received long-term alcohol ($P > 0.05$, Table 1). Nevertheless, a clear tendency to increase in the degree of the alveolar process atrophy is observed, which means the increase in resorption processes in bone tissue of jaws in females of the second group, which chronically consumed alcohol.

The prophylactic administration of the "Calcium from Oyster Shells" complex contributed to a probable decrease in the degree of atrophy by 17.8% compared to the indicator in the chronic alcohol group ($P < 0.05$), which indicates the inhibition of resorption processes in jaw bone tissue against the background of chronic ethanol intake. The daily administration of the "Minerol" complex did not significantly affect the degree of alveolar process atrophy of the 4th group animals with chronic alcoholism, since this indicator completely coincided with the corresponding results in the second group, which received ethanol for a long time. The results in Table 1 demonstrate increased resorption of bone tissue in rat jaws under chronic ethanol exposure and pronounced prophylactic properties of the "Calcium from Oyster Shells" complex.

Table 1
The degree of atrophy of the alveolar process in female rats with chronic alcohol consumption and its prevention (x ± SE, n = 32)

No.	Group	Alveolar process atrophy, %
1	Control	29.74 ± 1.84^{ab}
2	Alcohol intoxication	33.66 ± 0.12^{a}
3	Alcohol intoxication + calcium from oyster shells	27.68 ± 0.61^{b}
4	Alcohol intoxication + Minerol	33.50 ± 1.26^{a}

Notes: values with different superscripts in each lines are significantly different by Tukey's multiple comparison procedure ($P < 0.05$).

It is believed that chronic alcohol intoxication reduces the density and rigidity of tubular bones and vertebrae. Therefore, we also determined the density of femurs and lumbar vertebrae of female rats. In addition, the content of mineral and organic fractions in these bones in female rats was calculated. The results of morphometric studies are presented in Table 2 (femurs) and Table 3 (lumbar vertebrae).

Statistically significant differences between the density of femur in the control group animals and in the second group rats that chronically consumed alcohol ($P > 0.05$) were not found in our study. The weight, volume, and content of the mineral-organic component of bone in rats with chronic alcoholism did not differ from the corresponding indicators in control animals ($P > 0.05$). And in rats with chronic alcoholism, there was a redistribution of the content of mineral and organic particles in the bone tissue: a decrease in the content of the mineral component ($P < 0.05$) against the background of some increase in the organic component. This indicates a decrease in the degree of mineralization of protein matrix of bone tissue in rats that were treated with ethanol for a long time, and may explain the tendency to decrease in the density of femur in these animals (Table 2).

Daily administration of the "Calcium from oyster shells" complex to rats of the third group did not affect the studied indicators, the level of which remained the same as in the animals of the second group, which were not given prophylaxis. At the same time, the prophylactic use of the "Minerol" complex led to an even greater decrease in the density of femur due to a decrease in the mineral component, which is confirmed by probable changes ($P < 0.05$, Table 2). In our opinion, this may be a consequence of the pronounced sorption properties of "Minerol" and the insufficient amount of the main mineral substances in this complex necessary for the remodelling of bone tissue in the conditions of a violation of its mineralization against the background of ethanol consumption.

Chronic administration of alcohol did not lead to changes in the density, mass, volume, and content of mineral/organic components ($P > 0.05$, Table 3) in the lumbar vertebrae in rats of the second group. The prophylactic administration of both complexes to animals of the third and fourth groups also had no significant effect on the studied morphometric indicators of lumbar vertebrae.

Summarizing the results of the study of the morphometric indicators of various bones of rats (Tables 1–3), it can be concluded that long-term administration of ethanol had a negative effect on the quality of bone tissue (increases resorption processes) primarily in the jaws and to some extent in the femurs of female rats and did not affect lumbar vertebrae. Such a different reaction of bones in response to the effect of alcohol is probably related to their structure, as well as to different functional load on the studied bones. The jaws and vertebrae are represented by spongy bone tissue, which is more metabolically active, and the femurs have a tubular structure. In rodents, jaws and femurs are the most physiologically active with a slight load on lumbar vertebrae. At the next stage of the study, some biochemical parameters of blood serum of rats were determined, the re-

sults are shown in Table 4. In the second group of animals, which were chronically treated with alcohol, enzyme activity increased by 95.5% compared to the control group ($P < 0.05$). Alkaline phosphatase in blood serum has several origins from different tissues: bone tissue, liver, kidneys, the mucous membrane of the small intestine. An increase in the activity of alkaline phosphatase is mainly observed in cholestasis or bone diseases that are associated with the proliferation of osteoblasts. Since the specific bone alkaline phosphatase BALP (bone alkaline phosphatase), which serves as a marker of osteoblast activity, was not determined separately, the increase in the activity of total alkaline phosphatase in the blood serum in rats with chronic alcoholism in our study may primarily be due to the toxic effect of ethanol on liver and the development of cholestasis phenomena (Table 4). The prophylactic administration of the correction complexes to rats of the third and fourth groups led to the decrease in the activity of alkaline phosphatase in blood serum relative to the indicator in the group with chronic alcohol intoxication. It is important to emphasize that after taking the "Calcium from oyster shells" complex, the activity of alkaline phosphatase had normalized and corresponded to the values in the blood serum in control rats ($P > 0.05$), and after the use of the "Minerol" complex, despite the decrease, remained at a significantly high level in relation to the indicator in the control group ($P < 0.05$, Table 4). This indicates the high prophylactic efficiency of the "Calcium from oyster shells" complex in relation to liver and the insufficient properties of the "Minerol" complex in conditions of chronic alcoholism.

Table 2

Morphometric indicators of female rat femurs under conditions of chronic alcohol consumption and their correction ($x \pm SE$, $n = 32$)

No.	Group	Density, μg/mm^3	Weight, mg	Volume, mm^3	Content of mineral-organic complex, % (weight fraction)	Content of mineral component, % (weight fraction)	Content of organic component, % (weight fraction)
1	Control	1633.6 ± 5.3^{a}	617.0 ± 11.0^{a}	377.6 ± 6.1^{a}	70.07 ± 0.41^{a}	47.59 ± 0.26^{a}	22.48 ± 0.34^{a}
2	Alcohol intoxication	1615.9 ± 6.2^{ab}	612.5 ± 11.9^{a}	379.2 ± 8.1^{a}	69.66 ± 0.30^{a}	46.20 ± 0.41^{b}	23.46 ± 0.27^{a}
3	Alcohol intoxication + calcium from oyster shells	1623.6 ± 6.7^{ab}	589.6 ± 20.0^{a}	363.4 ± 13.2^{a}	70.12 ± 0.44^{a}	46.61 ± 0.44^{ab}	23.51 ± 0.47^{a}
4	Alcohol intoxication + Minerol	1605.9 ± 6.7^{b}	591.3 ± 14.7^{a}	368.4 ± 10.1^{a}	68.97 ± 0.38^{a}	45.70 ± 0.42^{b}	23.28 ± 0.26^{a}

Note: see Table 1.

Table 3

Morphometric indicators of lumbar vertebrae in female rats with chronic alcohol consumption and its correction ($x \pm SE$, $n = 32$)

No.	Group	Density, μg/mm^3	Weight, mg	Volume, mm^3	Content of mineral-organic complex, % (weight fraction)	Content of mineral component, % (weight fraction)	Content of organic component, % (weight fraction)
1	Control	1557.1 ± 7.4	159.4 ± 3.6	102.4 ± 2.4	67.03 ± 0.37	42.23 ± 0.53	24.80 ± 0.30
2	Alcohol intoxication	1565.3 ± 5.2	158.2 ± 5.7	101.1 ± 3.7	67.33 ± 0.36	42.90 ± 0.33	24.44 ± 0.28
3	Alcohol intoxication + calcium from oyster shells	1558.0 ± 10.4	151.8 ± 5.6	97.4 ± 3.6	67.14 ± 0.62	42.24 ± 0.68	24.90 ± 0.31
4	Alcohol intoxication + Minerol	1559.8 ± 7.5	163.5 ± 5.1	104.9 ± 3.6	67.41 ± 0.60	42.26 ± 0.42	25.15 ± 0.45

Note: all data in the table are not significantly different by Tukey's multiple comparison procedure ($P > 0.05$).

The content of calcium in blood serum in female rats as an indicator of the state of bone tissue was also determined. Chronic alcohol intoxication led to a decrease in the level of calcium in the serum of rats by 12.3%, although $P > 0.05$. The level of calcium in the blood is a very stable hormone-dependent indicator of the body's homeostasis, so its decrease even by 12.3% can be interpreted as a certain deficiency of this element important for bone tissue, which developed as a result of long-term alcohol consumption. In blood serum in rats receiving prophylactic complexes, the calcium content was on average 9% higher than the level in serum in animals with chronic alcoholism, which indicates a positive effect of the complexes on the level of calcium in the blood in rats with chronic ethanol intake (Table 4). The activity of catalase, an enzyme of antioxidant protection, decreased in the blood serum of rats of the second group by 35.3% compared to the control group ($P < 0.05$), which indicates the exhaustion of the body's antioxidant capacity under conditions of alcohol intoxication (Table 4). The introduction of the correction complex "Calcium from oyster shells" led to an increase in catalase activity by 27.3% compared to the alcohol group ($P < 0.05$), which indicates the antioxidant properties of this complex in contrast to the "Minerol" complex ($P > 0.05$; Table 4).

The confirmation of the development of general oxidative stress in the body of rats that took ethanol for a long time is an increase in the content of MDA by 18.7% in the blood serum of rats of the second group in comparison with the value in the control group ($P < 0.05$). Since MDA serves as a marker of the intensity of lipid peroxidation, it can be concluded that this process is activated in the body of animals against the background of long-term ethanol consumption. Addition of the correction complexes to the diet of the third and fourth groups effectively prevented an increase in the concentration of MDA in the serum of animals with chronic alcohol intoxication – the level of this indicator corresponded to the normal values in the control group ($P > 0.05$, Table 4). The research results of catalase activity and MDA content in blood serum indicate the development of oxidative stress in rats with chronic alcohol consumption and the antioxidant properties of prophylactic complexes.

Table 4

Serum biochemical indicators in female rats with chronic alcohol consumption and its correction ($x \pm SE$, $n = 32$)

No.	Group	Alkaline phosphatase activity, μkat/L	Calcium content, mmol/L	Catalase activity, μkat/L	Malondialdehyde content, mmol/L
1	Control	0.890 ± 0.123^{a}	2.026 ± 0.089^{a}	0.164 ± 0.010^{a}	24.62 ± 0.93^{a}
2	Alcohol intoxication	1.737 ± 0.175^{b}	1.778 ± 0.050^{a}	0.102 ± 0.004^{b}	29.23 ± 0.83^{b}
3	Alcohol intoxication + calcium from oyster shells	0.929 ± 0.069^{a}	1.937 ± 0.100^{a}	0.143 ± 0.004^{c}	24.33 ± 0.77^{a}
4	Alcohol intoxication + Minerol	1.201 ± 0.050^{c}	1.928 ± 0.085^{a}	0.121 ± 0.004^{b}	25.22 ± 0.73^{a}

Note: see Table 1.

Table 5 shows the results of studies of the biochemical markers of osteogenesis (alkaline phosphatase activity, calcium content) and resorption (elastase and acid phosphatase activity) in the femur of female rats with chronic alcohol intoxication and its correction. The activity of alkaline phosphatase in the femur of female rats of the second group under the influence of the chronic administration of alcohol decreased by 44.6% compared to the indicator in the control group ($P < 0.05$), which indicates a decrease in the functional activity of osteoblasts, and which means inhibition of osteogenesis. The prophylactic complexes did not significantly affect the activity of alkaline phosphatase, which means that they did not

prevent the decrease in the functional activity of osteoblasts in the femur of rats that drank alcohol over a long period (Table 5).

The content of calcium, the main component of hydroxyapatite of bone tissue, decreased in the femur of the second group rats (chronic alcohol) by 21.9% ($P < 0.05$) compared to the level of this indicator in the control group according to the data of our experiment. The regular administration of the "Calcium from oyster shells" complex to the third group rats prevented a decrease in calcium in bone tissue, the level of which was higher by 19.0% ($P < 0.05$) than in bone tissue of the second group animals. The "Minerol" complex contributed to a slight increase in calcium content (by 12.2%) compared to the chronic alcohol group ($P > 0.05$). Therefore, it is the "Calcium from oyster shells" complex that has a more positive preventive effect on the preservation of calcium in the femur in female rats with chronic alcoholization (Table 5).

The activity of elastase, as a marker of femoral bone resorption, that is an enzyme that destroys the protein matrix of bone tissue was determined in the femur of female rats. As shown in Table 5, the activity of bone elastase significantly increased in the group with chronic alcoholism – by 39.7% compared to the control group ($P < 0.05$). The prophylactic administration of both complexes effectively inhibited the activity of bone elastase ($P < 0.05$, Table 5). The level of the second marker of bone resorption of the mineral component – acid phosphatase, which destroys hydroxyapatite, also significantly increased in the second group by 51.1% compared to the control group ($P < 0.05$). The "Calcium from oyster shells" complex contributed to a decrease in the activity of acid phosphatase in the femur in rats of the third group by 31.0% ($P < 0.05$). The effect of the "Minerol" complex on stopping the activity of acid phosphatase in bones in rats of the fourth group was less significant, since the activity of this resorption marker decreased by 19.5% ($P > 0.05$), although its level simultaneously corresponded to the values in the control group ($P > 0.05$, Table 5).

Therefore, long-term alcohol consumption leads to the activation of osteoresorption processes (increase in elastase and acid phosphatase activity) against the background of a decrease in osteogenesis indicators (alkaline phosphatase activity and calcium content). Changes in these biochemical markers in the bone tissue in rats with chronic alcohol intoxication can explain the decrease in the density and content of the mineral component in their femurs (Table 2). The use of preventive complexes did not affect the decrease in the activity of bone alkaline phosphatase, but led to the significant inhibition of the destruction processes in bone tissue due to the decrease in the activity of elastase and acid phosphatase, that is, the functional activity of osteoclasts. An increase in the level of calcium in the bone tissue in rats treated with preventive complexes can be explained by the decrease in the processes of osteoresorption. The "Calcium from oyster shells" complex had a more pronounced osteotropic effect according to the results of Table 5.

Table 5

Biochemical indicators of osteogenesis and resorption in the femur of female rats with chronic alcohol consumption and its correction ($x \pm SE$, $n = 32$)

No.	Group	Alkaline phosphatase activity, μkat/kg	Calcium content, mmol/kg	Catalase activity, μkat/kg	Acid phosphatase activity, μkat/kg
1	Control	28.58 ± 3.38^{a}	6.93 ± 0.34^{a}	12.98 ± 0.90^{a}	1.90 ± 0.20^{a}
2	Alcohol intoxication	15.94 ± 2.71^{b}	5.41 ± 0.33^{b}	18.13 ± 1.29^{b}	2.87 ± 0.31^{b}
3	Alcohol intoxication + calcium from oyster shells	18.40 ± 2.24^{b}	6.44 ± 0.32^{a}	12.87 ± 1.53^{a}	1.98 ± 0.17^{a}
4	Alcohol intoxication + Minerol	18.14 ± 2.23^{b}	6.16 ± 0.24^{ab}	14.19 ± 1.07^{a}	2.31 ± 0.12^{ab}

Note: see Table 1.

The results of research on the activity of antioxidant enzymes and the content of MDA in the femur of animals are presented in Table 6. The activity of the enzyme of antioxidant protection SOD decreased by 32.4% ($P < 0.05$) in the bone tissue in rats of the second group with chronic alcoholism. SOD activity corresponded to the normal level in bone tissue of the third group, and remained at a reliable low level in the bone tissue of the fourth group, which indicates more pronounced antioxidant properties of the "Calcium from oyster shells" complex compared to "Minerol".

The activity of the second antioxidant enzyme catalase under the influence of chronic alcoholization decreased in bone tissue of the second group by 33.0% compared to the control group ($P < 0.05$). The use of complexes contributed to an increase in the activity of catalase in bone tissue in rats of the correction groups relative to this indicator in the chronic alcohol group ($P < 0.05$). At the same time, the activity of bone catalase did not reach a normal level after prophylaxis with both complexes and was probably lower than the corresponding values in bone tissue in the control group ($P < 0.05$, Table 6). The activity of the third enzyme of antioxidant protection – glutathione reductase – also decreased by 29.7% in bone tissue in rats with chronic alcohol consumption compared to the indicator in the control group ($P < 0.05$). The "Calcium from oyster shells" complex contributed to an increase in glutathione reductase activity by 26.7% compared to the chronic alcohol group ($P < 0.07$), almost reaching the normal level in the control group ($P > 0.05$). In the bone tissue of rats of the fourth group that received the "Minerol" complex, glutathione reductase activity did not differ from the values of this indicator in the second group with chronic alcoholism and remained reduced in relation to normal values (Table 6). The concentration of MDA, an indicator of the intensity of lipid peroxidation, significantly increased in the chronic alcohol group by 36.4% ($P < 0.05$) in the femur in female rats. The prophylactic administration of the complexes did not significantly affect the high level of MDA in bone tissue of the third and fourth groups ($P > 0.05$), which remained reliably high in relation to the normal values in the control group of animals ($P < 0.05$, Table 6).

Table 6

Biochemical indicators of the state of the antioxidant-prooxidant system in the femur of female rats with chronic alcohol consumption and its correction ($x \pm SE$, $n = 32$)

No.	Group	Catalase activity, μkat/kg	Glutathione reductase activity, mmol/kg·min	Superoxide dismutase activity, c.u./kg	Malondialdehyde concentration, mmol/kg
1	Control	2.855 ± 0.063^{a}	64.51 ± 5.60^{a}	0.710 ± 0.073^{a}	6.45 ± 0.29^{a}
2	Alcohol intoxication	1.908 ± 0.120^{b}	48.55 ± 4.29^{b}	0.485 ± 0.059^{b}	8.80 ± 0.61^{b}
3	Alcohol intoxication + calcium from oyster shells	2.450 ± 0.114^{c}	61.96 ± 3.88^{ab}	0.584 ± 0.042^{ab}	8.26 ± 0.53^{b}
4	Alcohol intoxication + Minerol	2.327 ± 0.120^{c}	50.51 ± 4.05^{ab}	0.530 ± 0.037^{b}	8.05 ± 0.27^{b}

Note: see Table 1.

Thus, long-term alcohol consumption leads to inhibition of antioxidant protection in bone tissue of femurs in rats, which is evidenced by a significant decrease in the activity of the main antioxidant enzymes. As a result, the intensity of lipid peroxidation, that is the content of MDA, increases, which makes a significant contribution to the destruction of bone tissue under the influence of alcohol. The correction complexes, which were administered to rats against the background of alcohol, contributed to maintaining the activity of bone antioxidant enzymes at a high level, except for the catalase activity, which corresponded to normal values. The "Calcium from oyster shells" complex had a more pronounced antioxidant effect according to the results in Table 6. The prophylactic use of both correction complexes did not affect the reduced of catalase activity and the increased MDA content in bone tissue in rats with the chronic alcoholization.

Table 7 shows the results of determining the biochemical parameters of jaws in female rats with chronic alcohol intoxication and correction. Indicators of osteogenesis – alkaline phosphatase activity and calcium content. Alkaline phosphatase activity in the jaws of the chronic alcohol group decreased by 35.4% compared to the control group ($P < 0.05$), which indicates a decrease in the functional activity of osteoblasts. The correction complexes helped to increase the activity of the osteogenesis marker. The calcium content decreased by 28.5% ($P < 0.05$) in bone tissue of jaws under the influence of long-term alcohol administration. Prophylactic administration of the "Calcium from oyster shells" complex to rats of the third group contributed to a slight increase in calcium content by 7.7% ($P > 0.05$), although at the same time its level did not statistically differ from the indicator in jaws of the control group ($P > 0.05$). The use of the "Minerol" complex did not affect the reduced calcium content in the jaws in rats that consumed ethanol for a long time (Table 7).

Next, the indicators of bone resorption – the activities of elastase and acid phosphatase – in the jaws of rats were determined. Elastase activity significantly (by 53.4%) increased in the jaws in animals of the second group with chronic alcohol intoxication compared to the indicator in the control group ($P < 0.05$). Administration of the preventive complexes equally effectively prevented the increase in bone elastase activity in the jaws of animals that consumed alcohol. Thus, the use of "Calcium from oyster shells" in rats of the 3rd group led to reduction in the activity of bone elastase by 29.6% ($P < 0.05$), and the use of "Minerol" – by 26.9% ($P < 0.05$).

The activity of acid phosphatase, which destroys hydroxyapatite of bone tissue, increased in the jaws of the second group with chronic alcohol compared to the control group by 23.1% ($P < 0.05$). The correction complexes showed different efficiency. The prophylactic administration to rats of the third group of the "Calcium from oyster shells" complex contributed to a decrease in enzyme activity by 21.9% ($P < 0.05$), and the "Minerol" complex did not affect the activity of bone acid phosphatase in the fourth group, although in both correction groups this indicator did not differ from normal values in the mandibles in rats of the control group ($P > 0.05$, Table 7). The long-term administration of ethanol to rats of the second group led to an increase in the content of MDA in the bone tissue of the jaws by 35.8% compared to the indicator in the control group ($P < 0.05$), which indicates the activation of lipid peroxidation. Both complexes proved to be effective antioxidants ($P < 0.05$).

Table 7

Biochemical indicators in jaws of female rats with chronic alcohol consumption and its correction ($x \pm SE$, $n = 32$)

No.	Group	Alkaline phosphatase activity, μkat/kg	Calcium content, mmol/kg	Elastase activity, μkat/kg	Acid phosphatase activity, μkat/kg	Malondialdehyde, concentration, mmol/kg
1	Control	39.39 ± 1.80^{a}	6.70 ± 0.83^{a}	11.13 ± 0.90^{a}	0.520 ± 0.048^{a}	4.81 ± 0.25^{a}
2	Alcohol intoxication	25.46 ± 2.16^{b}	4.79 ± 0.38^{b}	17.07 ± 0.93^{b}	0.645 ± 0.026^{b}	6.53 ± 0.55^{b}
3	Alcohol intoxication + calcium from oyster shells	43.61 ± 4.37^{a}	5.16 ± 0.44^{ab}	12.02 ± 0.44^{a}	0.495 ± 0.020^{a}	4.67 ± 0.25^{a}
4	Alcohol intoxication + Minerol	33.56 ± 2.84^{ab}	4.85 ± 0.50^{b}	12.47 ± 0.78^{a}	0.561 ± 0.036^{ab}	4.68 ± 0.33^{a}

Note: see Table 1.

Discussion

Our research confirmed the negative impact of chronic ethanol consumption on bone tissue in jaws and femurs. Namely, receiving 12.3 ± 2.3 mL/day of a 20% ethanol solution led to an increase in the degree of the alveolar process atrophy, as well as a decrease in the density of femur bones due to a decrease in the mineral component in bone tissue against the background of some increase in the organic component. The obtained results indicate an increase in resorption processes in bone tissue in the jaws and a decrease in the degree of mineralization of the protein matrix in the bone tissue of the femur in rats that consumed ethanol over a long period. Chronic alcoholization did not affect the state of the lumbar vertebrae of rats, which can be explained by the low functional load on vertebrae of the rodents. The decrease in the length, mass and BMD in tibia bones of animals under chronic alcoholism is recorded in a study on young rats (Rosa et al., 2019). Chronic treatment with alcohol in high doses causes osteopenia due to necroptosis of osteoblasts (Guo et al., 2021). Chronic ethanol consumption did not affect the density of bone tissue, only slightly reduced the mechanical properties of tibia (Clayton et al., 2021).

Morphometric changes in the jaws and femurs in rats which were chronically treated with alcohol are explained by the violation of some biochemical indicators in blood serum and in bone tissue. Thus, the level of calcium decreased in the blood of animals after long-term ethanol consumption, and a decrease in osteogenesis markers (alkaline phosphatase activity and calcium content) in bone tissue of the jaws and femur under the influence of alcohol was established against the background of an increase in resorption markers (acid phosphatase and elastase activity). That is, chronic alcoholism activates the processes of resorption against the background of inhibiting the processes of osteogenesis, as a result of which there is an increase in the alveolar process atrophy in the jaws and a decrease in the density of femur bones. The influence of alcohol on the state of the alveolar bone is often studied in the presence of concomitant factors such as periodontitis or ovariectomy. Chronic alcoholism in combination with periodontitis or ovariectomy led to alveolar bone loss and decreased bone density (de Almeida et al., 2020; Frazão et al., 2020; Nascimento et al., 2020). The research results showed that ovariectomy and ethanol exposure alone can induce alveolar bone loss, and their combination significantly enhances this effect (Nascimento et al., 2020). In the study of the effect of ethanol on bone tissue, although it is noted that the effect of ethanol itself does not lead to an increase in the loss of alveolar bone tissue, the quality of bone tissue changes in all evaluated parameters (trabecular thickness, trabecular number parameter, percent bone density) (Frazão et al., 2020).

In our opinion, oxidative stress could be the starting mechanism of increased resorption processes in bone tissue in animals that have consumed alcohol over a long period. The pro-oxidant effect of ethanol is well known, and bone tissue is no exception to its negative effects. In our study, a significant decrease in the activity of the main antioxidant enzymes (SOD, catalase and glutathione reductase) in bone tissue in animals chronically treated with ethanol was established. We have registered an increase in lipid peroxidation as a result of a decrease in antioxidant protection in bone tissue under conditions of chronic alcoholism, which is evidenced by the high content of MDA in the bone tissue of these animals (Fig. 1). The alcohol-induced increase in the formation of reactive oxygen species (ROS) plays an important role in the regulation of bone homeostasis. Alcohol exposure increases the level of NADP oxidases, which leads to the formation of ROS, which activates the expression of RANKL and stimulates osteoclastogenesis (Eby et al., 2020).

So, it is worth analysing the effect of the correction complexes proposed by us on the state of the bone system in female rats with chronic alcohol intoxication. The "Calcium from oyster shells" complex turned out to be more effective than the "Minerol" complex in many of the studied parameters. Namely, the use of the "Calcium from oyster shells" complex contributed to the inhibition of resorption processes in jaws of rats, which was reflected in a decrease in the degree of alveolar process atrophy by 17.8% compared to the chronic alcohol group ($P < 0.05$), while the introduction of the "Minerol" complex significantly did not affect the degree of the alveolar process atrophy. The complex proved to be less effective in the femur and lumbar vertebrae, without affecting the ethanol-impaired morphometric indicators of the studied bones. Reception of the "Minerol" complex against the background of chronic alcoholism led to a decrease in the density of femur bone, which can be explained by the sorption properties of the complex and the insufficient amount of the main mineral substances in this complex, necessary for the remodeling of bone tissue. The use of the "Minerol" complex did not have a significant

effect on the morphometric parameters in lumbar vertebrae in female rats.

The consumption of both complexes by rats with alcohol intoxication contributed to the improvement of indicators in blood serum of female rats, namely, a decrease in the activity of total alkaline phosphatase ($P < 0.05$), a decrease in the MDA concentration ($P < 0.05$), and an increase in the concentration of calcium ($P > 0.05$). The complexes had different effects on catalase activity in serum: the "Calcium from oyster shells" complex increased catalase activity by 27.3% compared to the chronic alcohol group ($P < 0.05$), the "Minerol" complex did not affect catalase activity. The regular administration of the correction complexes to animals that consumed ethanol effectively prevented the negative effect of alcohol on the biochemical parameters of the femur and jaws in female rats. The "Calcium from oyster shells" complex contributed to the decrease in the activity of markers of bone resorption – elastase and acid phosphatase – in femurs and jaws of rats ($P < 0.05$). The "Minerol" complex contributed to a decrease in the activity of only elastase ($P < 0.05$), while the indicator of acid phosphatase activity did not differ from the level of this parameter in bone tissue of rats with chronic alcoholism. Also, the use of complexes had different effects on the calcium content in femurs and jaws. The use of the "Calcium from oyster shells" complex led to an increase in the concentration of calcium in femurs and jaws of female rats to a normal level ($P > 0.05$). The use of the "Minerol" complex did not affect the calcium content in the examined bones and did not differ from the similar indicator in the second group with chronic alcohol intoxication ($P > 0.05$). Also, the "Minerol" complex had no significant effect on the activity of alkaline phosphatase in the femurs and jaws of rats. The use of the "Calcium from oyster shells" complex led to an increase in the activity of alkaline phosphatase only in the jaws ($P < 0.05$), but did not affect the activity of alkaline phosphatase in the femur. That is, it can be assumed that the use of the "Calcium from oyster shells" complex under conditions of chronic alcoholism effectively inhibits resorption processes in bone tissue, activates osteogenesis in jaws, and does not affect reduced osteogenesis in tissue in the femur.

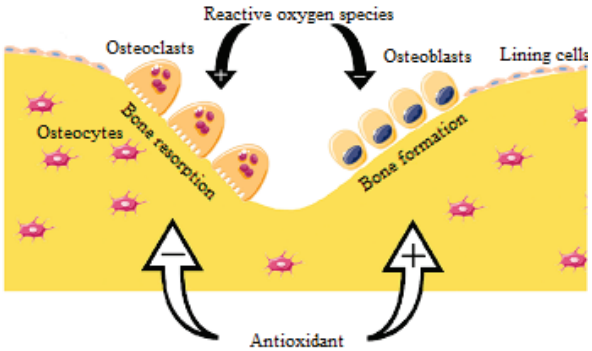

Fig. 1. The effect of antioxidants and reactive oxygen species (ROS) on bone remodeling according to Domazetovic et al. (2017): ROS activate the differentiation of osteoclasts, suppress the activity of osteoblasts, inducing bone tissue resorption; antioxidants activate the differentiation of osteoblasts and inhibit the activity of osteoclasts, inducing bone formation

The prophylactic administration of correction complexes in case of alcohol intoxication showed an antioxidant effect, which was more pronounced after taking "Calcium from oyster shells". Thus, the "Calcium from oyster shells" complex contributed to increase in the activity of bone SOD to a normal level, in contrast to "Minerol", after the use of which SOD activity corresponded to a low level in animals with chronic alcoholism ($P > 0.05$) in bone tissue. Both complexes also contributed to an increase in the catalase activity in the femur of rats ($P < 0.05$) and an increase in the glutathione reductase activity to the level of the activity of these enzymes in the bone tissue in the control group ($P > 0.05$). According to the results of our study more significant antioxidant efficiency was established in bone tissue after the introduction of "Calcium from oyster shells". Figure 1 schematically shows the counteraction of antioxidants to the action of ROS.

The effect of both complexes on the concentration of MDA in the studied bones had a similar effect, namely, a decrease in the concentration of MDA in the jaws of rats ($P < 0.05$) and no effect on this indicator in the femur ($P > 0.05$).

Conclusion

The chronic alcohol consumption led to an increase in the degree of the alveolar process atrophy, a decrease in the density of femur bones due to a decrease in the mineral component in bone tissue, and did not affect the state of lumbar vertebrae. The long-term use of ethanol led to a decrease in the level of calcium in the blood serum of animals, and to a decrease in the activity of alkaline phosphatase and the content of calcium in bone tissue of the jaws and femur against the background of an increase in the activities of acid phosphatase and elastase. A significant decrease in the activity of the main antioxidant enzymes (SOD, catalase and glutathione reductase) against the background of increased lipid peroxidation (MDA content) was found in bone tissue in animals under conditions of chronic alcoholization.

The use of the "Calcium from oyster shells" complex effectively prevented the atrophy of the alveolar process of jaws, did not affect the morphometric parameters of femur and vertebrae, but normalized the activities of serum alkaline phosphatase and catalase, bone elastase, acid phosphatase, SOD and glutathione reductase against the background of restoring the levels of calcium and MDA both in serum and in bone tissue in rats subjected to chronic alcoholization.

The prophylactic administration of the "Minerol" complex showed much lower efficiency on the studied indicators: it had no significant effect on the morphometric indicators of the jaws and vertebrae, but at the same time it further reduced the density of the femur in rats treated with ethanol. Biochemical parameters of serum and bone tissue of these animals improved less after taking "Minerol" than after taking "Calcium from oyster shells", or did not change at all.

The obtained research results allow us to conclude that the osteoprotective and antioxidant efficiency of the "Calcium from oyster shells" complex is more pronounced than that of "Minerol" under conditions of chronic alcohol intoxication.

The research was carried out of the framework of the "Experimental study of changes in oral cavity tissues in rats under the influence of xenobiotics and hypoxia" (state registration number 0120U105477) in State Establishment "The Institute of Stomatology and Maxillo-Facial Surgery National Academy of Medical Science of Ukraine".

All the authors declare that they have no conflicts of interest.

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