Materialu k macro-mikroskopicheskoy anatomii. Kiev, 1965: 96-101.

Резюме

ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНА ДІЯ ГЕЛЯ З НАНОЗОЛОТОМ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ПАРОДОНТИТІ

Левицький А.П., Борисенко А.В., Ткач О.Б.

Моделювання пародонтиту у щурів за допомогою аплікацій гелю з протамін-сульфатом викликає в яснах розвиток дисбіоза, знижує вміст гіалуронової кислоти і підвищує ступінь атрофії альвеолярного відростка. Аплікації геля з сорбентом, який містить наночастки золота (5 нм, 500 мкг/г) знижують ступінь дисбіоза, підвищують вміст гіалуронової кислоти та нормалізують показник атрофії альвеолярного відростка.

Ключові слова*: нанозолото, мукозальні гелі, ясна, пародонтит, дисбіоз, гіалуронова кислота.*

Summary

THE THERAPEUTIC-PREVENTIVE EFFECT OF THE GEL WITH NANOGOLD AT THE EXPERIMENTAL PERIODONTITIS

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The simulation of periodontitis in rats with the applications of gel with protamine sulfate causes the development of dysbiosis in gum, reduces the contents of hyaluronic acid and increases the degree of atrophy of alveolar appendage. The applications of gel with the sorbent, containing nanoparticles of gold (5nm, 500 mg/kg) reduce the degree of dysbiosis, increase the contents of hyaluronic acid and normalize the index of alveolar appendage atrophy.

Key words*: nanogold, mucosal gels, gum, periodontitis, dysbiosis, hyaluronic acid.*

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УДК 57.085.23

THE EFFECTS OF ANTIOXIDANTS ON DIABETIC DAMAGE WITH A TOBACCO SMOKE IN BOVINE LENSES

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Investigated the mechanisms involved in the effects of preventing cataract and cigarette smoking (CS) when exposed to the lens antioxidants. Bovine lenses were removed in organ culture for 12 days, exposed to glucose 450 mg % for antioxidants including-Desferioxamine (DFO) and cysteine N-acetyl-L-(NAC). Treated lenses were 4 days in culture medium saturated with cigarette smoke daily dose at 500 psi. The use of laser-lens optical quality was assessed daily. At the end of the incubation period, lenses were analyzed using an inverted microscopy as epithelial layer was used for histochemical assessment method Einarson nucleic acids -RNA-DNA staining.

Reactive Oxygen Species (ROS) were evaluated C6827, to measure the level of cellular oxidation in the epithelial cells of the lens relative to the control cultures by fluorescence microscopy. High glucose with a smoke causes optical and morphological changes in epithelial cells (hypertrophy, hyperplasia). Antioxidants reduce the damage caused by high glucose and CS, to protect the lens from high glucose, reduce cell damage, prevent the increased activity of ROS. NAC protected the lens from damage high glucose better than DFO. We suggest that NAC can serve as an effective advocate for the lens of the eye against damage from smoking diabetics.

Keywords*: antioxidants, cigarette smoking, cataract diabetes, culture lens, Nucleic acids, ROS.*

The purpose of this study was to investigate the mechanisms involved in the effects of antioxidants in preventing glucose damage and cigarette smoking (CS) exposure to the eye lens. Bovine lenses were incubated in organ culture conditions for 12 days. Lenses were exposed to 450 mg % glucose including the antioxidants: Desferioxamine (DFO) and N-acetyl-L-cysteine (NAC). Control lenses were incubated without glucose or antioxidants and other groups were incubated with glucose, glucose and smoke, glucose, CS with antioxidants. Treated lenses were 4 day in the culturally environment daily sated with a cigarette smoke dose of pressure-500 psi. Using our unique laser system lens optical quality of lens was assessed daily throughout the culture period. At the end of the incubation, lenses were analyzed by inverted microscopy and the lens epithelial layer was used for histochemical analysis of the nucleic acids was estimated using the Einarson -DNA-RNA staining method. Reactive Oxygen Species (ROS) were estimated with 5-(and 6-)chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (C6827) to measure the level of cellular oxidation in the cells of lens epithelium. The levels of ROS were measured by monitoring the fluorescent intensity relative to that of control cultures under fluorescent microscopy. Elevated glucose in the culture medium caused optical and morphological damage to bovine lenses. High glucose with smoke causes changes in epithelial cells shape (hypertrophy, hyperplasia). The antioxidants reduced the damage caused by high glucose and CS levels. High doses of glucose in combination with a smoke in the culture medium caused damage to bovine lenses. Antioxidants protect the lens from high glucose (diabetic) damage. Antioxidants reduced the damage to cells shape and prevent the increased activity of ROS. NAC protected the lenses from high glucose damage better than DFO. We suggested that NAC can serve as an effective protector for the eye lens against diabetes damage in smokers.

Introduction

Lois N, Abdelkader E, Reglitz K, Garden C, Ayres JG. [2008] were searched on environmental tobacco smoke (ETS) exposure and eye disease using various combinations of the following terms: passive smoking, environmental tobacco smoke, sidestream smoke, involuntary smoking, secondhand smoke; with eye, conjunctiva, sclera, episclera, cornea, lens, iris, retina, choroid, uvea, optic nerve, uveitis, iritis, blindness, visual loss, cataract, thyroid eye disease, conjunctivitis, age-related macular degeneration, dry eye, tears. A search was further conducted specifically on eye diseases where active smoking has been proposed to be a risk factor, including age-related macular degeneration, Graves ophthalmology, glaucoma, uveitis, refractive errors, strabismus, tobacco-alcohol amblyopia, non-arteritic ischaemic optic neuropathy, Leber optic neuropathy and diabetic retinopathy. Given the scarce number of studies found through the above search, all articles found on ETS and eye disease were included in this review. Very scarce data exist in the literature on the effect of ETS on diseases of the eye. It seems appropriate that ETS should be included in future studies addressing the effect of smoking on eye disease.

In human diabetes, the deleterious effects of chronic hyperglycemia are the result of excessive nonenzymatic modification of proteins and phospholipids by glucose and its by-products leading to the formation of irreversible oxidized, aromatic, and fluorescent ligands known as advanced glycation end products. This glycation process has been associated with deleterious health effects [Babizhayev MA, Guiotto A, Kasus-Jacobi A, 2009]. Cigarette smoking has been implicated in the pathogenesis of cataract, but the pathogenic mechanism by which cigarette smoke causes cataract is yet to be com-

pletely understood. Avunduk AM, Yardimci S, Avunduk MC, Kurnaz L, Kozkar MC [1997] have suggested that oxidative damage caused by accumulation of Fenton reagents (iron and copper) in the lens can cause lens damage and possibly cataract. To investigate the accuracy of this assumption the study was planned according. A number of twenty-four male Wistar rats were divided randomly into experimental and control groups. The experimental group of rats were exposed to cigarette smoke for two hours in each day over sixty consecutive days and the controls were treated in identical fashion but only exposed to room air. At the end of the study period, both eyes of all the animals were enucleated and one eye prepared for histopathological examination and the other used for the measurement of metal levels. The lenses of experimental animals showed significantly decreased zinc and increased iron, and calcium concentration relative to those of sham exposed controls. However, no significant difference was found in the copper contents of the lenses of both groups. Distinct histopathological changes such as hyperplasia, hypertrophia, and multilayering of epithelial cells and elevations of calcium concentration detected in the lenses of experimental group animals suggested that the lens damage was a result of in vivo exposure to tobacco smoke. We proposed that increased metal contents in the lens can cause lens damage by the mechanism of oxidative stress through formation of oxygen radicals via metal catalysed Fenton reaction. Distinct histopathologic changes in the anterior lens epithelium, such as hyperplasia, hypertrophy, epithelial multilayering, and the presence of epithelial cells over posterior lens capsule, observed in group rats were not present in other groups [Avunduk AM, Yardimci S, Avunduk MC, Kurnaz L, Aydin A, Kozkar MC, Delibaei T, Dayanir V., 1999]. Cataractogenesis after cigarette smoke exposure was associated with an accumulation of iron and calcium in the rat lens, and vitamin E supplementation protected such accumulations and cataractogenesis.

Oxidative stress plays an important role in cataractogenesis. Karppi J, Laukkanen JA, Kurl S. [2011] study was to examine whether plasma concentrations of lutein and zeaxanthin are related to age-related nuclear cataract in the elderly population. Subjects were participants in the Kuopio Ischaemic Heart Disease Risk Factor Study and they were classified into tertiles according to plasma concentrations of lutein and zeaxanthin. The association of plasma lutein and zeaxanthin concentrations with age-related nuclear cataract in 1689 elderly subjects (aged 61-80 years) was investigated in the present cross-sectional study by using the Cox proportional hazards model. A total of 113 cases of incident age-related cataracts were confirmed, of which 108 cases were nuclear cataracts. After adjustment for age, examination year, sex, BMI, smoking, alcohol consumption, serum LDL-cholesterol, serum HDL-cholesterol, years of education, use of oral corticosteroids, history of diabetes and history of hypertension with current use of antihypertensive medication, subjects in the highest tertiles of plasma concentrations of lutein and zeaxanthin had 42 and 41 % lower risks of nuclear cataract, respectively, compared with those in the lowest tertiles (relative risk (RR) = 0·58, 95 % CI 0·35, 0·98; P = 0·041 for lutein and RR = 0·59, 95 % CI 0·35, 0·99; P = 0·046 for zeaxanthin). In conclusion, they suggest that high plasma concentrations of lutein and zeaxanthin were associated with a decreased risk of age-related nuclear cataract in the elderly population.

To investigate whether a daily intake of a moderate dose of Objective: antioxidants modifies the microcirculatory response to smoking, assuming a major influence on redning oxidative damage. The microvascular response to stress on microcirculation. Henriksson P, Diczfalusy

U, Freyschuss A. [2012] users methods: smoking was assessed in individual capillaries by capillaroscopy before and after two weeks of treatment with oral antioxidants, Smoking prolonged time to peak (TtP) capillary blood flow velocity in all subjects. When the subjects were pretreated with ascorbate, TtP was comparable to baseline values of untreated subjects. No significant effect of vitamin E was observed, neither before nor after smoking. Capillary blood flow velocity increased after treatment with ascorbate as well as after vitamin E. However significant reductions in velocity were still observed in response to smokingeven after subjects consumed ascorbate and vitamin E So study focus on individual capillaries and confirms that smoking has a very pronounced, direct and reproducible microvascular effect possible to demonstrate in vivo in human capillaries. Moderate intake of the antioxidant ascorbate clearly mitigated the effects induced by smoking. TtP after smoking in subjects treated with ascorbate was similar to that observed in untreated subjects before smoking a cigarette. Thus oxidative stress could be assumed to play a role in the effects of smoking on microcirculation.

Shalini VK, Luthra M, Srinivas L, Rao SH, Basti S, Reddy M, Balasubramanian D. [1994] is reported that sigarette smoking increase the risk of cataract. Likewise, the use of smoky cooking fuel is implicated in the etiologyd of cataract. In an effort to understand the cellular and molecular basis, the in vitro and in vivo cataractogenetic effects of these smoke condensates have been studied using isolated rat lenses and pigmented rats. Isolated capsulated rat lenses were incubated with cigarette smoke condensate (CSC) and firewood smoke condensate (FSC) for varying periods, with and without antioxidants, in the presence and absence of light. CSC and FSC permeate the lens capsule, imparted colour and opacify the lens in a light-and dose-dependent manner. Antioxidants offered partial inhibition against the above damage. The condensates contain polycyclic aromatics which generate reactive oxygen species such as O_2 photodynamically, and ppb levels of Fenton metal ions which induce oxidative reactions through. OH. Smoke induced damage possibly occurs through systemic absorption and transport of toxic components to several tissues, and specially into the lens, wherein the turnover is slow, leading to chronic accumulation causing oxidative damage to the constituent molecules and to consequent lenticular opacity.

Rao CM, Qin C, Robison WG Jr, Zigler JS Jr. [1995] have undertaken a study to investigate the effect of wood smoke condensate on the physiological integrity and morphology of organ cultured lenses. Lenses in organ culture are metabolically active and have functional defense systems, thus they provide an appropriate model for studying effects of smoke condensate. This present study indicates that metabolites of wood smoke condensate accumulate in the lens. The ability of the lenses to accumulate rubidium-86 (mimic of potassium) and choline from the medium is compromised by exposure to smoke condensate. Rubidium efflux studies suggest that the damage is primarily at the uptake level and does not involve an overall increase in membrane permeability. Protein leakage experiments corroborate this suggestion. Histological data show distinct morphological changes such as hyperplasia, hypertrophy and multilayering of epithelial cells.

The difficulties encountered in extrapolating biological activity from cigarette smoke composition provide generally applicable lessons as they are representative of the problems encountered with other complex mixtures [Rodgman A, Smith CJ, Perfetti TA., 2000]. Researchers attempting to assess risk are faced with attempting to interpret data from a number of areas including: tobacco science; smoke/aerosol chemistry spe-

cific to tobacco; sophisticated analytical chemistry applications and techniques for trapping, collecting, separating, and quantifying very specific compounds at nanogram to picogram levels; numerous biological testing methodologies; and animal models of tumors and carcinogenesis. Numerous hypotheses have been developed over the past five decades and tested with the technology of the day in attempts to interpret the biological activity of cigarette smoke in relation to the chemistry of this complex mixture. These hypotheses fall into several categories discussed in this review: mechanisms of pyrogenesis of polycyclic aromatic hydrocarbons (PAHs) in tobacco smoke; levels of PAHs in cigarette mainstream smoke (MS) and its tumorigenicity in mouse skin-painting experiments; control of PAH levels in MS; chemical indicators of cigarette smoke condensate (CSC) tumorigenicity; control of levels of MS components partitioned between the vapor phase and particulate phase of MS; tumorigenic threshold limits of CSC and many of its components; tumorigenic aza-arenes in tobacco smoke; MS components reported to be ciliastatic to smokers' respiratory tract cilia; anticarcinogenic tobacco-smoke components. Of 52 hypotheses reviewed in this paper, 15 have excellent data supporting the hypothesis based on today's technology. The remaining 37 hypotheses, although originally plausible, have since become insupportable in light of new and contradictory data generated over the years. Such data were generated sometimes by the original authors of the hypotheses and sometimes by other investigators. The hypotheses presented today are less likely to be supplanted because they are well conceived and have a strong mechanistic basis. The challenge for the future is the generation and interpretation of data relating the chemistry and biological activity associated with the dynamic and complex mixture of tobacco smoke.

Materials and Methodology

Lenses and Organ Culture System

Lenses were excised from eyes obtained from 1-year-old male calves from an abattoir, 2-4 h after enucleation. Each excised lens was placed in a culture chamber consisting of two compartments connected by a round hole. Lenses were completely immersed in culture medium. Culture medium consisted of M199 with Earl's balanced salt solution, 3 % fetal calf serum, and antibiotics (penicillin 100 U/ml and streptomycin 0.1 mg/ml) and was changed daily. The lenses were incubated at 35 ˚C. Experimental treatments started after pre-incubation of 24 h (Dovrat A, Weinreb O.,1995,1999). Oxidative stress represents a mechanism which could lead to diabetic cataract. We exposed bovine lenses in culture conditions for two weeks to high glucose concentration (450 mg %). Bovine lenses were incubated in organ culture conditions for 12 days. Treated lenses were exposed to CS every day for 6 days at dose for amount of smoke of pressure-500 mmHg (equivalent to 5 cigarettes) in a special device. To two of the experimental exposed groups we have added NAC(1mM) and DFO (2.5 mkg/ml) as antioxidants. Exposure to CS was performed by a system consisting of a chamber attached to a vacuum pump and a negative pressure gauge (up to 600 mm Hg) at one end and a cigarette at the other end (Fig. 1). Medium were placed inside the chamber. Then, the vacuum pump was activated, valve B was closed and valve A was opened until a desired level of negative pressure was created inside the chamber. By using the vacuum pump the pressure inside the chamber was reduced relatively to the atmospheric pressure outside. Subsequently, a TIME commercial cigarette containing 14 mg of tar and 0.9 mg of nicotine and filter (Dubek Ltd., Tel Aviv, Israel) was lit, valve A was closed and valve B between the burning cigarette and chamber was opened for 10 seconds allowing CS to

enter the chamber. Creating reduced pressure inside the chamber allowed the drawing of CS from the burning cigarette into the chamber. Thus, the dose of CS entering the chamber equated the level of negative pressure created inside the chamber. Smoke passing through the cigarette filter was considered as vapor phase CS. After exposure to CS the chamber with the medium was sealed and transferred for different incubation times at 35°C. (Rom O. et al., 2013).

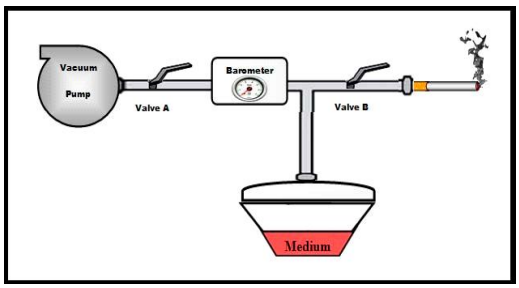

Fig. 1. CS exposure apparatus.

Optical Monitoring System

The optical quality of the lenses was analyzed each day of the culture using a low power laser scanner. The scanner consists of a 2 mW 670 nm helium-neon diode laser mounted on a computer-controlled X-Y table, and a television camera with a video frame digitizer [Sivak et al 1990]. The laser beam was parallel to the axis of the lens and was directed towards the cultured lens along one meridian in 0.5 mm increments. After passing through the lens, the laser beam is refracted and the system determines the back vertex focal length for every beam position. Each scan consists of measurements of the same beam from 22 different points across the lens. A lens of good optical quality is able to focus the laser beam from the various locations. When the lens is damaged its ability to focus the laser beam at the various locations is altered.

Lens Epithelium Microscopy and Histochemistry

At the end of the culture period, lenses were analyzed by inverted microscopy. Lenses were photographed using an inverted microscope in order to assess damage at different depths of the lens. For the subsequent studies we have used epithelium preparations of a monolayer of bull lenses from all experiments. Faddeev's method (1962) was used. For this purpose the used capsule was opened were cleaned and transparent fibers of a lens. On an object plate here was only a capsule and monolayer cellular epithelium.This provided an evaluated account of the different grade of its capsule, i.e. topographical features of central intermediate and equatorial zone epithelium cells (Bormusova E., 1979). Treated lenses were analyzed morphologically and by assessment of the nucleic acid staining of the lens epithelium (Pearse A.G., 1972), the method use gallocyanin-chromalum by Einarsone with enzyme for specificity. The procedure includes fixation of the samples with methanol for 30 min, incubation with the gallocyanin-chromalum solution for 48 hours. The chromalum ($K_2SO_4 \cdot Cr_2(SO_4)_3 \cdot 24H_2O$) with gallocyanin at pH = 1.64 binds to nucleic acids and give it dark blue color.

Reactive Oxygen Species (ROS) in epithelial cells lens were detected with 5-(and 6)chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H2DCFDA, C6827) measuring the level of cellular oxidation in the cells of lens epithelium. When oxidising agent is added to the cells this reagent is converted to fluorescent isomer. The fluorescent signal was detected with a fluorescence microscope, using sources of excitement and filters, corresponding to fluorescein. Levels of ROS were measured by measuring fluorescent intensity of the cells in culture. Nuclei were labled by Propidium Iodide.

The Quantitative intensities of the histochemistry reactions in central and equatorial zones was be done using the program Image-Pro Plus, measuring Integrated Optical Density in each cell us-

ing 30-50 cells of each slide. A change is defined as significant if the difference between control and treated groups reaching value of $P < 0.05$.

Results

Optical Analysis

Our optical system can measure minute changes in lens transparency as demonstrated by changes in transmittance. A 670 nm diode laser with the beam parallel to the axis of the lens was directed towards the cultured lens along one meridian in 0.5 mm increments. After passing through the lens, the laser beam is refracted and the system determines the back vertex focal length and transmittance for every beam position. Each scan consists of measurements of the same beam from 22 different points across the lens. Each point on the graph represents the average of the lenses of that treatment. Control lenses (red) show 100 % transparency (Figure 2.). On the other hand, lenses exposed to the different treatments show variation in transparency. High glucose levels reduce lens transparency (violet). Lenses show higher transparency as a result of the treatment with the antioxidants NAC and DFO. Other studies also demonstrated changes in lens optical quality in diabetes (Di Benedetto A, Aragona P, Romano G, et al., 1999) measured lens opacity in diabetic patients using a back-light scattering quantification system. Lens opacity was significantly higher in diabetic patients than in the control group, and showed correlation with glycated hemoglobin levels. Freel CD, Gilliland KO, Wesley Lane C, Giblin FJ, Joseph Costello

M. (2002) compared cytoplasmic textures from a variety of human and animal lenses in electron microscope images in order to relate the extent of roughness with the extent of opacification. Lens cytoplasms exhibiting the greatest roughness correlated with the greatest light scattering.

Tkachov et al. (2006) described his-

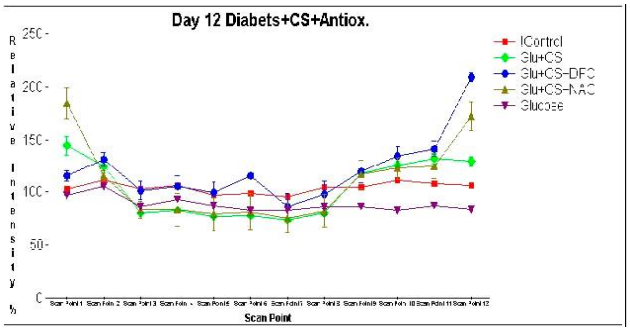

Fig. 2. Lens Optical Quality – Intensity

tomorphological changes in the cataractous lens of diabetic patients using Scheimpflug densitometry and light microscopy. Their study revealed smaller cell density of the lens, larger cell area of lens epithelium, and a lower nucleusplasma ratio in cataractous lenses of diabetics compared to clear non-diabetic lenses.

Inverted Microscopy micrographs

In our study at the end of the culture period, lenses were photographed by inverted microscope. Figure 3A shows a photograph of a control lens with clear sutures and homogeneous epithelial cell layer. Figure 3B demonstrates the severe damage to the lens, when incubated in the presence of high glucose, which is indicated by the blisters under and between the epithelial cells.

Glucose with CS treated lenses show swollen epithelial cells with blisters at the cells borders (Fig. 4A). Each of the two antioxidants used in the study reduces the glucose-induced damage to the lens (Figures 4B, 5).

Results show that high glucose and a smoke of cigarettes does harm to an epithelium of a lens and to fibers, and treatment with antioxidants reduces the damage caused by high levels of glucose in combination with CS.

Histologically, there was a lens of single-layered epithelium in the control group (Fig. 6A). In the experimental group-glucose (Fig. 6B), there was slight pleomorphism in the superficial epithelial cell contours and vacuolization was more

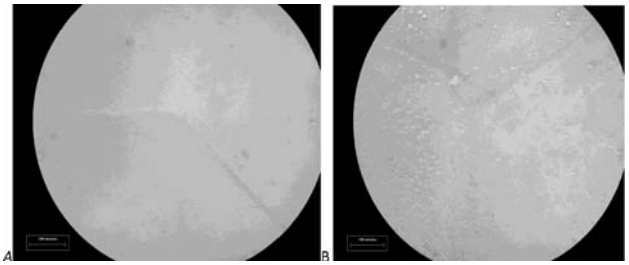

Fig. 3.A. Inverted microscopy micrograph of control lens.
Fig. 3B. Inverted microscopy micrograph of lens incubated with 450 mg % glucose

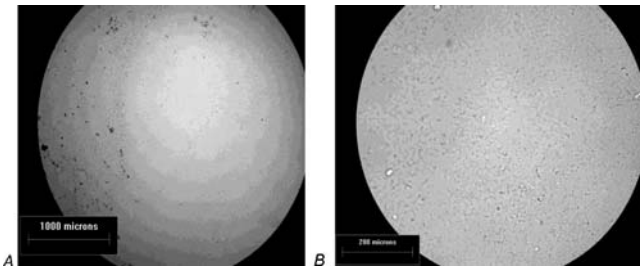

Fig. 4.A. Inverted microscopy micrograph of lens incubated with 450 mg % glucose and CS-500 mmHg.
Fig. 4B. Inverted microscopy micrograph of lens incubated with 450 mg % glucose, CS-500 mm Hg and DFO.

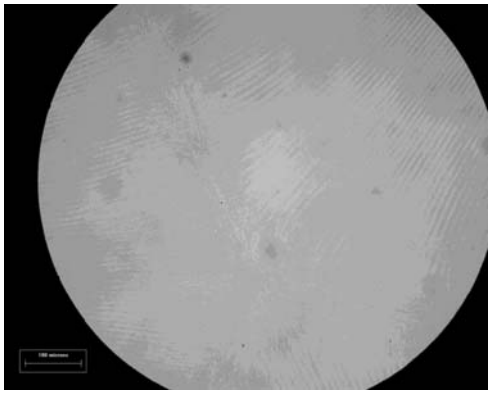

Fig. 5. Inverted microscopy micrograph of lens incubated with 450 mg % glucose, CS-500 mm Hg and NAC

prominent in this group ; the experimental group glucose with CS, had pronounced pleomorphism and pyknosis of the nuclei in the superficial epithelium (Fig. 8A). Some of the epithelial cells had disappeared and were histologically observed as acellular areas. There is larger space between the cells and changes in organization after glucose and CS influence. Cells organization shows integrity is disrupted, such as hyperplasia and hypertrophy of epithelial cells.

These graphics show (Fig. 7) that under the influence of glucose with CS the optical density of nucleic acids in cages of an epithelium varies. As the area of cages changes also (Fig. 10). However, the DFO and NAC correct this shortcoming.

Detection of Oxidative Stress

The antioxidants protective effect of each of the two agents were compared using the 5,6-chloromethyl-2\',7\'-dichlorodihydrofluorescein diacetate (DCF) assay. Formation of ROS in the epithelium was monitored and detected, by fluorescence, in intact bovine epithelial cells layers, from the different treatment groups. Figure 11A, shows a molecule of non (reduced and acetylated) DCF. Note the low fluorescence of the epithelial cells.

Note the high fluoresce in the epithelial cells with glucose(Fig. 11B) and glucose+CS (Fig. 12A) which indicate high oxidation. DFO reduced the oxidation levels in the cells (Fig. 12B), while NAC totally prevents the damage (Fig. 13).

The epithelium from lenses treated with in the presence of NAC looks the

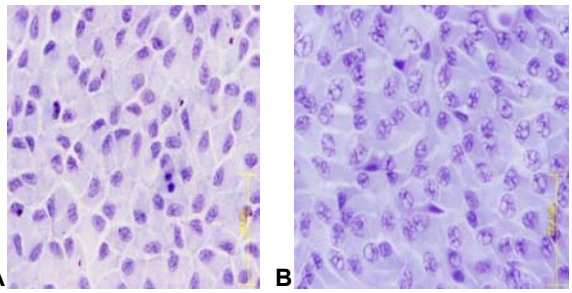

Fig. 6.A. Day 12 Epithelium cells. Control (DNA/RNA Staining).
Fig. 6B. Day 12. Epithelium Cells. Glucose (450 %) (DNA/RNA Staining)

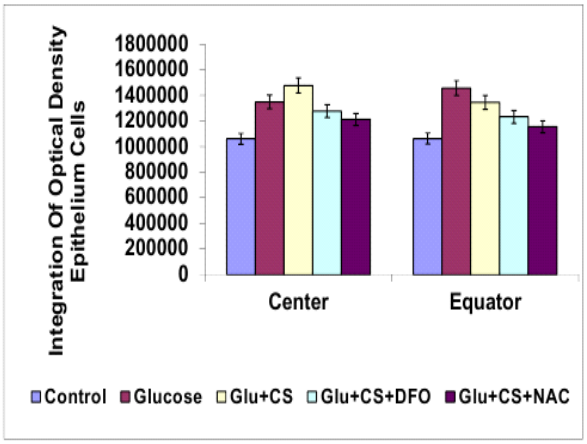

Fig. 7. Day 12. DNA/RNA Diabetes+CS+Antioxidants. Integration of Optical Density Epithelium Cells.

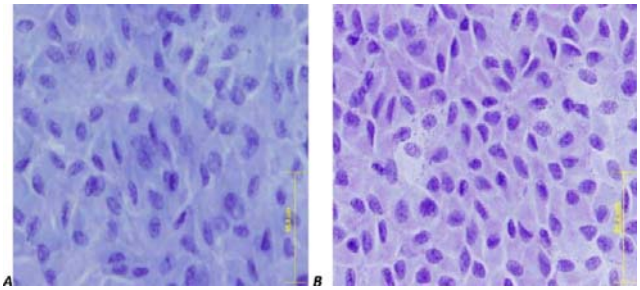

Fig. 8A. Day 12. Epithelium Cells Glucose + CS (DNA/RNA Staining)
Fig. 8B. Day 12. Epithelium Cells Glucose + CS+DFO (DNA/RNA Staining)

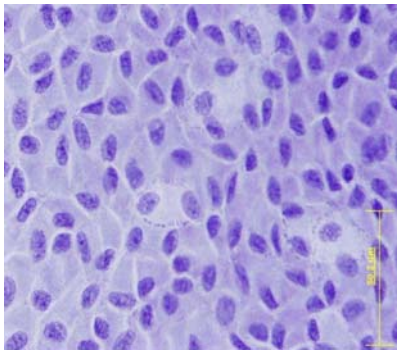

Fig. 9. Day 12. Epithelium Cells Glucose + CS+NAC (DNA/RNA Staining). Antioxidants a reduced the damage caused by the high maintenance of glucose with CS.

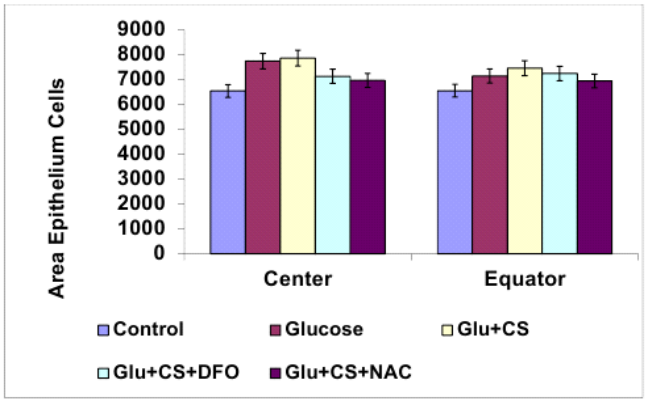

Fig. 10. Day 12. DNA/RNA Diabetes+CS+Antioxidants (Area Cells)

same as the controls.

Hiller R. et al [1997] examine the association between cigarette smoking and the incidence of nuclear and nonnuclear lens opacities in members of the Framingham Eye Study Cohort. During the approximately 12.5 years between eye examinations, lens opacities developed in a total of 381 persons, with nuclear opacities constituting the most frequent type. In logistic regression analyses that controlled for age, sex, education, and diabetes, a significant positive association with increasing duration of smoking and number of cigarettes smoked daily was found for nuclear lens opacities, alone or in combination (test for trend, P < or = 0.002), but not for nonnuclear opacities (test for trend, P = 0.62). Among the heavier smokers (persons who smoked > or = 20 cigarettes per day according to 6 or more biennial Framingham Heart Study examinations), 77 % were still smoking at the time of the first eye examination. Persons who smoked 20 or more cigarettes per day at the time of the first eye examination were at substantially increased risk for the development of nuclear opacities than nonsmokers (odds ratio, 2.84; 95 % confidence interval, 1.46-5.51). There was no apparent excess risk for persons with nonnuclear lens opacities (odds ratio, 1.42; 95 % confidence interval, 0.65-3.07). The City Eye Study is a nine year longitudinal prospective epidemiological study [Flaye DE et al.,1983]. During the first three year phase the study recruited 1029 volunteers, aged between 54 and 65 years, primarily from companies and organisations working in or around the City of London. The analysis of the first cohort data shows a significant association between nuclear lens opacities and moderate to heavy cigarette smoking. The Relative Risk for nuclear lens opacity and cigarette smoking ranges from 1.0 for past light-smokers through 2.6 for past heavy-smokers, to 2.9 for present heavy smokers. There is also a strong association between smoking and a number of common eye diseases, which include Graves' ophthalmopathy, age-related macular degeneration, glaucoma, and cataract [Cheng AC. et al., 2000]. Despite the multifactorial aetiology of these ocular syndromes, smoking is an independent risk factor that has dose-response effects. It causes morphological and functional changes to the lens and retina due to its atherosclerotic and thrombotic effects on the ocular capillaries. Smoking also

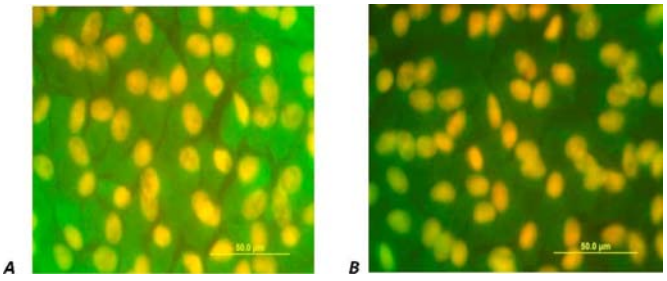

Fig. 11A. ROS. Control lens epithelium after 12 days in organ culture conditions. Fig. 11B. Day 12. Epithelium Cells. Glucose. ROS.

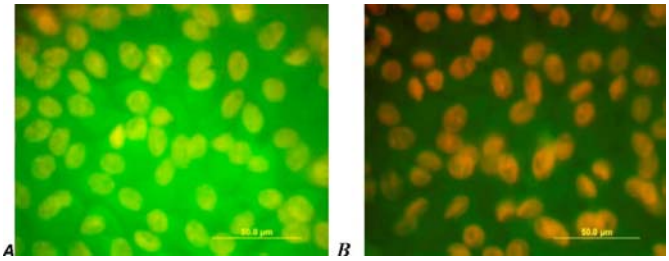

Fig. 12A. Day 12.Epithelium Cells. Glucose+CS. ROS.
Fig. 12B. Day12. Epithelium Cells. Glucose+CS+DFO. ROS.

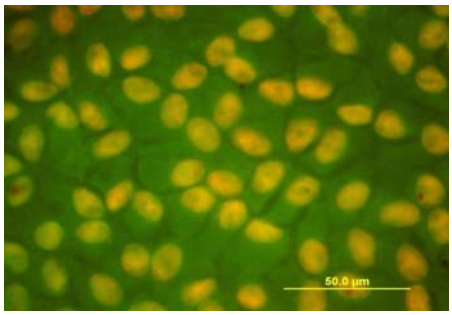

Fig. 13. Day 12. Epithelium Cells.Glucose+CS+NAC. ROS.

enhances the generation of free radicals and decreases the levels of antioxidants in the blood circulation, aqueous humour, and ocular tissue. Thus, the eyes are more at risk of having free-radical and oxidation attacks in smokers. Cataract and age-related macular degeneration (AMD) are the major causes of vision impairment and blindness worldwide [Fletcher AE., 2010]. Both conditions are strongly age related with earlier signs (usually asymptomatic) occurring in middle age and becoming severer and more prevalent with increasing age. The aetiology of these conditions is thought to fit with the 'free radical theory' of ageing which postulates that ageing and age-related diseases result from the accumulation of cellular damage from reactive oxygen species (ROS). Mitochondrial energy production is a major source of endogenous ROS. External sources of ROS include environmental sources especially solar radiation, biomass fuels and tobacco smoking. There is strong evidence from epidemiological studies that smoking is a risk factor for both cataract and AMD. There is moderate evidence for an association with sunlight and cataract but weak evidence for sunlight and AMD. The few studies that have investigated this suggest an adverse effect of biomass fuels on cataract risk. The antioxidant defence system of the lens and retina include antioxidant vitamins C and E and the carotenoids lutein and zinc, and there is mixed evidence on their associations with cataract and AMD from epidemiological studies.

The contribution of chronic tobacco exposure in determining post-myocardial infarction (MI) left ventricular (LV) remodeling and possible therapeutic strategies has not been investigated systematically [Khanna AK, Xu J, Mehra MR., 2012]. Authors small animal investigation, They demonstrate that chronic tobacco smoke exposure leading up to acute MI in rats is associated with greater histological extent of myocardial necrosis and consequent worse LV function. These findings are associated with increased transcriptomic expression of pro-inflammatory cytokines, tissue repair molecules and markers of oxidative stress in the myocardium. The results demonstrate that an N-acetyl cysteine (NAC) treatment significantly reduced tobacco-exposed induced infarct size and percent fractional shortening. A significantly increased LV end-systolic diameter was observed in tobacco-exposed sham compared to tobacco-naпve sham (4.92 ± 0.41 vs 3.45 ± 0.33; $P < 0.05$), and tobacco-exposed MI compared to tobacco-naпve MI (8.24 ± 0.3 vs 6.1 ± 0.49; $P < 0.01$) rats. Decreased intracardiac mRNA expression of the markers of inflammation, tissue repair and oxidative stress and circulating levels of pro-inflammatory cytokines accompanied these positive effects of NAC. The treatment of tobacco-exposed MI rats with NAC

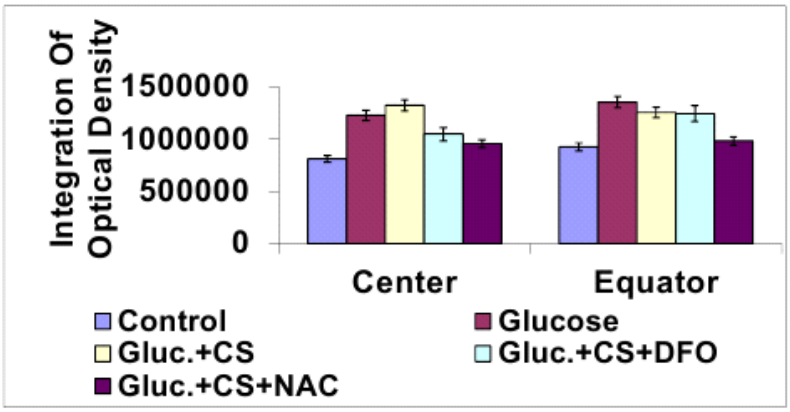

Fig. 14. ROS. Day 12. Diabetes+CS+Antioxidants. Integration of Optical Density.

resulted in significantly increased levels of intracardiac mRNA expression of antioxidants, including superoxide dismutase, thioredoxin and nuclear factor-E2-related factor 2, as well as circulating levels of glutathione (7 ± 0.12 vs 10 ± 0.18; $P < 0.001$), where the levels were almost identical to the tobacco-naпve sham rats. These findings identify a novel post-infarction therapy for amelioration of the adverse effects of tobacco exposure on the infracted myocardium and advocate the use of dietary supplement antioxidants for habitual smokers to prevent and reverse cardiovascular adverse effects in the absence of successful achievement of cessation of smoking.

Nagler R et al. [2000] showed that exposure of human plasma in vitro to gas-phase cigarette smoke (CS) causes a marked modification of plasma proteins as measured by protein carbonyl assay. Aldehydes present in CS may cause this elevation of protein carbonyls by reacting with sulfhydryl groups of proteins. Saliva is the first body fluid to confront the inhaled CS. Thus, in vitro exposure of saliva to nine “puffs” of CS also showed a distinct increase in protein carbonyls. Ascorbate and desferrioxamine mesylate had little effect on protein carbonyl formation, while GSH and N-acetylcysteine considerably inhibited the accumulation of protein carbonyls due to CS exposure. Following the exposure to CS, the activities of several salivary enzymes-amylase, lactic dehydrogenase (LDH), and acid phosphatase-were found to be significantly reduced (34, 57, and 77 %, respectively). However, CS had no effect on the activities of aspartate aminotransferase and alkaline phosphatase. Addition of 1 mM of GSH and N-acetylcysteine considerably protected LDH and amylase activities, suggesting that sulfhydryl groups are affected in LDH and amylase. On the other hand, addition of 1 mM ascorbate caused a further loss of LDH and amylase activities, which could be partially prevented by the addition of desferrioxamine mesylate, implicating metal-catalyzed oxidation processes. Finally, loss of acid phosphatase activity was completely unaffected by any of the above antioxidants. It is concluded that the loss of salivary enzyme activities may be due to various agents in the CS that affect the enzyme activities via different mechanisms.

Conclusions

High glucose and CS in the culture medium causes damage to bovine lenses. Antioxidants reduced the damage to cells shape and prevent the increased activity of ROS and Nucleic Acid. We have demonstrated a role for oxidative damage of CS and diabetes formation. It is probable that NAC and DFO can provide favorable result.

NAC protected the lenses from high glucose damage better than DFO. The antioxidant and anti-inflammatory agent N-acetyl-L-cysteine protects the lens from high glucose damage with CS.

Thus, the possible use of antioxidant substances in prevention of a cataract in smokers and treatment is a very attractive possibility that shoved be explored more deeply in the Future.

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References

1. Lois N, Abdelkader E, Reglitz K, Garden C, Ayres JG. Environmental tobacco smoke exposure and eye disease. Br J Ophthalmol. 2008 Oct;92(10):1304-10.
2. Babizhayev MA, Guiotto A, Kasus-Jacobi A. N-Acetylcarnosine and histidyl-hydrazide are potent agents for multitargeted ophthalmic therapy of senile cataracts and diabetic ocular complications. J Drug Target. 2009 Jan;17(1):36-63
3. Avunduk AM, Yardimci S, Avunduk MC, Kurnaz L, Kozkar MC. Determinations of some trace and heavy metals in rat lenses after tobacco smoke exposure and their relationships to lens injury. Exp Eye Res. 1997 Sep;65(3):417-23
4. Avunduk AM, Yardimci S, Avunduk MC, Kurnaz L, Aydin A, Kozkar MC, Delibaei T, Dayanir V. Prevention of lens damage associated with cigarette smoke exposure in rats by alpha-tocopherol (vitamin E) treatment. Invest Ophthalmol Vis Sci. 1999 Feb; 40(2):537-41.
5. Karppi J, Laukkanen JA, Kurl S. Plasma lutein and zeaxanthin and the risk of age-related nuclear cataract among the elderly Finnish population. Br J Nutr. 2011 Oct 18:1-7.
6. Henriksson P, Diczfalusy U, Freyschuss A. Microvascular reactivity in response to smoking and oral antioxidants in humans. Microcirculation. 2012 Jan; 19 (1):86-93.
7. Rao CM, Qin C, Robison WG Jr, Zigler JS Jr. Effect of smoke condensate on the physiological integrity and morphology of organ cultured rat lenses. Curr Eye Res. 1995 Apr;14(4):295-301
8. Rodgman A, Smith CJ, Perfetti TA. The composition of cigarette smoke: a retrospective, with emphasis on polycyclic components. Hum Exp Toxicol. 2000 Oct;19(10):573-95
9. Dovrat A, Weinreb O Recovery of lens optics and epithelial enzymes after ultraviolet A radiation. Invest Ophthalmol Vis Sci. 1995 Nov;36(12):2417-24.
10. Dovrat A, Weinreb O Effects of UV-A radiation on lens epithelial NaK-ATPase in organ culture. Invest Ophthalmol Vis Sci. 1999 Jun;40(7):1616-20.
11. Rom O, Kaisari S, Aizenbud D, Reznick AZ. Cigarette smoke and muscle catabolism in C2 myotubes. Mech Ageing Dev. 2013 Jan-Feb;134(1-2):24-34
12. Sivak JG, Yoshimura M, Weerheim J, Dovrat A. Effect of hydrogen peroxide, DL-propranolol, and prednisone on bovine lens optical function in culture. Invest Ophthalmol Vis Sci 1990; 31: 954-963.
13. Faddeev A.P. Effect of X-ray radiation on an epithelium of lens animals. Abstract. Odessa, 1962, page 8-9.
14. Bormusova E.A. Histochemical characteristics of the lens epithelium of the rabbit eye in ontogenesis (oxyreductase, hidrolase). Ophthalmol. G.,Odessa, 1979, p.494-497.
15. A.G. Everson Pearse. Histochemistry. Theoretical and Applied.3 editon.Ediburgh ahd London, 1972.
16. Di Benedetto A, Aragona P, Romano G, et al. Age and metabolic control influence lens opacity in type I, insulin-dependent diabetic patients, “J Diabetes Complications”, 1999, vol. 13, no. 3, pp. 159-162.
17. Freel CD, Gilliland KO, Wesley Lane C, Giblin FJ, Joseph Costello M. Fourier analysis of cytoplasmic texture in nuclear fiber cells from transparent and cataractous human and animal lenses, “Exp Eye”,2002, vol. 74, no. 6, pp. 689-702.
18. Tkachov SI, Lautenschlager C, Ehrich D, Struck HG. Changes in the lens epithelium with respect to cataractogenesis: light microscopic and Scheimpflug densitometric analysis of the cataractous and the clear lens of diabetics and non-diabetics, “Graefes Arch. Clin. Exp. Ophthalmol.”, 2006, vol. 244, no. 5, pp. 596-602.
19. Hiller R, Sperduto RD, Podgor MJ, Wilson PW, Ferris FL 3rd, Colton T, D’Agostino RB, Roseman MJ, Stockman ME, Milton RC. Cigarette smoking and the risk of development of lens opacities. The Framingham studies. Arch Ophthalmol. 1997, Sep;115(9):1113-8.
20. Flaye DE, Sullivan KN, Cullinan TR, Silver JH, Whitelocke RA. Cataracts and cigarette smoking. The City Eye Study. Eye (Lond).

1989; 3 (Pt 4):379-84.

21. Cheng AC, Pang CP, Leung AT, Chua JK, Fan DS, Lam DS. The association between cigarette smoking and ocular diseases. Hong Kong Med J. Jun; 2000,6(2):195-202.
22. Fletcher AE. Free radicals, antioxidants and eye diseases: evidence from epidemiological studies on cataract and age-related macular degeneration. Ophthalmic Res.44(3):191-8, 2010.
23. Khanna AK, Xu J, Mehra MR. Antioxidant N-acetyl cysteine reverses cigarette smoke-induced myocardial infarction by inhibiting inflammation and oxidative stress in a rat model. Lab Invest. Feb; 2012, 92 (2):224-35.
24. Nagler R, Lischinsky S, Diamond E, Drigues N, Klein I, Reznick AZ. Effect of cigarette smoke on salivary proteins and enzyme activities. Arch Biochem Biophys. 2000, Jul 15; 379(2):229-36.

Резюме

ДЕЙСТВИЕ АНТИОКСИДАНТОВ НА СОЧЕТАНИЕ ДИАБЕТИЧЕСКОГО ПОВРЕЖДЕНИЯ С ДЕЙСТВИЕМ ТАБАЧНОГО ДЫМА НА ХРУСТАЛИК БЫКА

Бормусова Э.А., Резник А.З.

Исследовались механизмы действия антиоксидантов на предотвращение развития катаракты у курящих. Бычьи хрусталики были выдержаны в культуральной среде, содержащей 450 мг глюкозы на 100 г раствора и с антиокислителями — десфероксамином (DFO) и N-ацетил-L-цистеином (NAC) в течение 12 дней. Так же 4 дня хрусталики содержались в культуральной среде, насыщаемой папиросным дымом, ежедневная доза которого составляла 500 psi. Оптическое качество хрусталиков ежедневно оценивалось с помощью лазерной установки. В конце инкубационного периода хрусталики были изучены методом инверсионной микроскопии, а эпителиальный слой использовался для гистохимического метода оценки нуклеиновых кислот окрашиванием ДНК-РНК по методу Эйнарсона. Активные формы кислорода (ROS) были оценены в эпителиальных клетках хрусталика с помощью флуоресцентной микроскопии для измерения уровня клеточного окисления относительно контрольных культур. Высокая доза глюкозы с дымом вызывает оптические и морфологические изменения в эпителиальных клетках (гипертрофия, гиперплазия) и увеличивает флуоресценцию. Антиоксиданты уменьшают это воздействие, снижают количество поврежденных клеток, предотвращают увеличенную деятельность ROS. NAC защитил линзы от повреждения несколько лучше, чем DFO. Мы предполагаем, что данные антиокислители могут служить эффективным средством защиты хрусталика глаза против повреждения у курящих диабетиков. Таким образом, применение антиокислительных веществ в профилактике катаракты у курильщиков и лечение — очень привлекательная возможность, которая может быть исследованной более глубоко в будущем.

Ключевые слова*: антиоксиданты, сигаретный дым, диабетическая катаракта, культура хрусталика, нуклеиновые кислоты, уровень клеточного окисления.*

Резюме

ДІЯ АНТИОКСИДАНТІВ НА ПОЄДНАННЯ ДІАБЕТИЧНОГО ПОШКОДЖЕННЯ З ТЮТЮНОВИМ ДИМОМ НА КРИШТАЛИК БИКА

Бормусова Е.А., Резник А.З.

Досліджувалися механізми дії антиоксидантів на запобігання розвитку катаракти у курящих. Бичачі кришталики були витримані в культуральному середовищі, що містить 450 мг глюкози на 100 г розчину і з антиокислювачами - десфероксаміном (DFO) і N-ацетил-L-цистеїном (NAC) протягом 12 днів. Так само 4 дня лінзи містилися в культуральної середовищі, насичує цигарковим димом, щоденна доза якого становила 500 psi. Оптична якість кришталиків щодня оцінювалося за допомогою лазерної установки. Наприкінці інкуба-

ційного періоду кришталики були вивчені методом інверсійної мікроскопії, а епітеліальний шар використовувався для гистохимического методу оцінки нуклеінових кислот фарбуванням ДНК-РНК по методу Ейнарсона. Активні форми кисню (ROS) були оцінені в епітеліальних клітинах кришталика за допомогою флуоресцентної мікроскопії для виміру рівня клітинного окислення щодо культур контролю. Висока доза глюкози з димом викликає оптичні та морфологічні зміни в епітеліальних клітинах (гіпертрофія, гіперплазія) і збільшує флуоресценцію. Антиоксиданти зменшують цей вплив, знижують кількість пошкоджених клітин, запобігають збільшену діяльність ROS. NAC захистив лінзи від пошкодження дещо краще, ніж DFO. Ми припускаємо, що дані антиокислювачі можуть служити ефективним засобом захисту кришталика ока проти пошкодження у курящих діабетиків. Таким чином, застосування антиокисних речовин в профілактиці катаракти у курців та лікування - дуже привабліва можливість, яка може бути дослідженою більш глибоко в майбутньому.

Ключові слова*: антиоксиданти, сигаретний дим, діабетична катаракта, культура кришталика, нуклеїнові кислоти, рівень клітинного окислення.*

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УДК 636.2.084:577.118

БАЛАНС МЕДИ И ЦИНКА У СУХОСТОЙНЫХ КОРОВ ПРИ ДОПОЛНИТЕЛЬНОМ ВВЕДЕНИИ В РАЦИОН ХЕЛАТНЫХ ФОРМ МИКРОЭЛЕМЕНТОВ

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В настоящее время в животноводстве для восполнения дефицита микроэлементов в кормах все чаще используют хелатные комплексы биометаллов, так как они имеют больше преимуществ по сравнению с их неорганическими источниками. Эффективное использование хелатов позволяет существенно снизить загрязнение окружающей среды за счет повышения биодоступности микроэлементов в организме животных и уменьшения их концентрации в навозе.

Целью исследования было изучение влияния хелатных форм микроэлементов меди, цинка и их сернокислых солей на баланс этих биометаллов у сухостойных коров. Для этого в опытном хозяйстве «Гонтаровка» Института животноводства НААН (Харьковская обл.) был проведен балансовый опыт. По принципу аналогов было подобрано 4 группы сухостойных коров украинской черно-пестрой молочной породы (по 4 головы в каждой), содержание коров – привязное. Основной рацион всех групп был одинаковым и отличался лишь формой и количеством Cu и Zn, скармливаемых дополнительно совместно с концентратами дважды в сутки. Потребность коров в этих микроэлементах была удовлетворена, соответственно, на 100 %, 50 % и 25 % в I, II и III опытных группах за счет компенсации дефицита меди и цинка в основном рационе дополнительным введением хелатов этих биометаллов, а в контрольной группе (IV) – на 100 % за счет их сернокислых солей. Хелатные комплексы Cu и Zn были представлены глицинатами соевого протеина.