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# SEARCH FOR CHAPERONE-LIKE ANTICATARACT AGENTS, THE ANTIAGGREGANTS OF LENS CRYSTALLINS

## COMMUNICATION 4. STUDY OF THE EFFECT OF A MIXTURE OF DI- AND TETRAPEPTIDES ON A PROLONGED RAT MODEL OF UV-INDUCED CATARACT

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### Abstract

There is a potential of therapeutic action on certain stages of cataractogenesis, in particular on the aggregation of water-soluble proteins of cytoplasmic lens fiber cells, giving rise to insoluble protein complexes. The effect of a combined preparation (N-acetyl carnosine and D-pantethine), acting by the chaperone-like mechanism, was studied in vivo on a prolonged rat model of UV-induced cataract. The use of the combined preparation consisting of a mixture of peptides of N-acetyl carnosine and D-pantethine in a ratio of 1:1 as ocular instillations and intraperitoneal injections could slow down the development of UV-induced cataract in vivo. Pathomorphological studies suggest that the combined preparation has a protective effect on lens tissue when the rat model of UV-induced cataract is employed.

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According to A. Spector free-radical cataractogenesis theory, age-related cataract is primarily caused by free-radical oxidation of eye lens proteins [12]. The validity of the theory is confirmed by various experimental and clinical works. Due to this, most anticataract preparations, widely used in clinical practice, exhibit varying degrees of antioxidant properties [5].

On the other hand, a large number of experimental studies indicate that therapeutic intervention on other stages of cataractogenesis is potentially possible. In particular, the therapeutic intervention on the aggregation of water-soluble proteins of lens fiber cells cytoplasm would result in the formation of insoluble protein complexes [1, 2, 6, 8–10].

In fact, it turned out that the intensification of chaperone-like properties of the  $\alpha$ -crystallin, i.e. its ability to prevent the aggregation of damaged proteins, is the basis for D-pantethine tetrapeptide anticataract mechanism of action. It is known that the compound effectively thwarts the progression of various types of experimental cataract — radiation induced, selenite, galactose, streptozotocin and congenital cataract in Royal College of Surgeons rats [4, 5].

It has been established that N-acetylcarnosine dipeptide possesses anticataract property and can prevent eye lens opacification [3]. In our recent research N-acetylcarnosine displayed chaperone-like activity during

UV-induced  $\beta_L$ -crystallin aggregation in vitro (refer to Article 1 of the series). Follow-up study showed that the mixture of these peptides (N-acetylcarnosine and D-pantethine) in vitro slows down the process of crystallins aggregation under UV exposure more effectively than each peptide taken separately [11], (refer to Article 2 of the series).

It is expected that above mentioned mixture may be used as a basis for the research of new anticataract preparation with chaperone-like mechanism of action. To evaluate the possibility of clinical use of the coformulated preparation (N-acetylcarnosine and D-pantethine) it is necessary to study its anticataract properties in in vivo experiment.

The goal of this research was to study anticataract properties of the coformulated preparation (N-acetylcarnosine and D-pantethine) in experiment in vivo in a prolonged model of the UV-induced cataract in rats.

### Materials and Methods

The experiment was performed on 33 male Wistar rats (66 eyes) aged 20 to 23 days, weighing 39–41 g. The follow-up period was 10 months (43 weeks). The animals were randomly assigned to 5 groups:

1<sup>st</sup> group consisted of 8 animals with UV-induced cataract but did not receive the coformulated preparation;

2<sup>nd</sup> group, the control one, consisted of 7 rats that were not exposed to UV radiation or the preparation administration;

3<sup>rd</sup> group (6 rats, 12 eyes) consisted of irradiated animals who received the coformulated preparation in the form of 5 per cent instillations of each solution;

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4<sup>th</sup> group (6 rats, 12 eyes) included irradiated animals who received the coformulated preparation in the form of intraperitoneal injections of 25 mg/kg D-pyridoxine and 25 mg/kg N-acetylcarnosine;

In the 5<sup>th</sup> group (6 rats, 12 eyes) irradiated animals received the coformulated preparation in the form of intraperitoneal injections of 150 mg/kg of each peptide.

During the follow-up period, all instillations and intraperitoneal injections were performed once a day.

UV-induced cataract modeling process and dynamics of cataract development were detailed in the previous article by Avetisov S.E (refer to Part 1 of the series). In brief, the animals were exposed to UV radiation of two quartz lamps URLQ-01 «Solnyshko». 16 minutes exposure sessions were performed every other day. Subjective measurement of cataract development dynamics was done for eye lens digital images by expert evaluation method. Objective evaluation of eye lens opacification dynamics was conducted by means of microdensitometry of biomicroscopic optical sections. Biomicroscopy was performed using slit lamp SL-75 (Opton, Germany) at 45° angle and 0.1 mm diaphragm opening. Examinations were conducted on monthly basis. It is important to note that the total score of left and right eyes was taken as the final estimate of animal eyes damage level. Corresponding data is presented on the images.

Statistical analysis of the results was performed with the use of distribution-free statistics (the Mann–Whitney U test and Spearman's rank correlation coefficient).

After 10 months follow-up, animals were sacrificed using chloroform anesthesia in accordance with the Association for *Research in Vision and Ophthalmology* Statement for the Use of Animals in Ophthalmic and Visual Research [7]. Crystalline lenses were then fixated in order to create semifine sections according to standard practice. Light-optical morphometry of histologic specimens was performed under “Photomicroscope-III” (“Opton”, Germany) with the use of automated morphodensitometry hardware and software complex “Diamorph Objective” (“ZAO Diamorph”). The photorecording was done using “Diamorph” digital camera which was shipped with the complex.

## Results and Discussion

Biomicroscopy of the eye lenses (expert evaluation method) in the course of the experiment enabled to discover the dynamics of opacification development in animals of the studied groups. Thus, the first evaluation performed 1 month after the initial exposure, showed statistically valid differences between 1<sup>st</sup> (exposure) group and 2<sup>nd</sup> (control) group. The valid differences persisted through the whole follow-up period. The exposure group was diagnosed with moderate homogeneous nebulous opacification of the crystalline lens by the end of the experiment, more significant in the nucleus (5 points cataract). At the same time, eye lenses in animals of the con-

trol group were still considerably more transparent (2-3 points cataract).

The development rate of eye lens opacifications was significantly lower in main groups that were exposed to UV radiation and received the coformulated preparation (**Fig. 1**). In terms of the development rate parameter, the differences between animal groups which did and did not receive the preparation, were considered valid as from the 82<sup>nd</sup> day of the experiment ( $p < 0.03$ ). Later on, the validity of the differences increased significantly ( $p = 0.0003$ ). By the end of the experiment (on the 313<sup>th</sup> day), more significant anticataract effects caused by the preparation were observed in the 3<sup>rd</sup> group, thus, instillation of the drug was more effective than injections ( $p = 0.002$ ). On the last examination, the animals of this group had cataract corresponding to 2-3 points estimation, while 4<sup>th</sup> group cataract was estimated as 3 points, and 5<sup>th</sup> group — as 3—4 points by the end of the experiment.

Microdensitometry data allowed to objectify the results of the biomicroscopic study. Optical density index (ODI) of eye lens layers in animals of the 1<sup>st</sup> group (exposure to radiation and no treatment) increased progressively during the follow-up period with highest increase rates in the first 3 months. ODI increase rate curve lowered significantly during 3—10 months. By the end of the follow-up period, the highest ODI increase rates were observed in nucleus (the increase of  $117 \pm 5$  c. u.). ODI dynamics curve of eye lens cortex was similar to the above, except that ODI increase rate was slightly lower and amounted in  $97 \pm 3$  c. u.

The 2<sup>nd</sup> (control) group showed insignificant steady increase of the ODI in all layers (the increase of  $37 \pm 4$  c.u.) during the whole follow-up period. The steady increase is probably an indicative of natural age-related development of the eye lens in Wistar rats. In comparison to the 1<sup>st</sup> group, the differences in measures were valid at each observation ( $p < 0.001$ ).

The graph of the ODI increase in eye lens cortex and nucleus in the 3<sup>rd</sup> group (UV exposure and preparation instillation) is almost identical to the curve of the 2<sup>nd</sup> group (control); therefore there is a high possibility (more than 95%) that animals of the 2<sup>nd</sup> and 3<sup>rd</sup> groups can belong to the same group (**fig. 2 and 3**). In the 4<sup>th</sup> and 5<sup>th</sup> groups (UV exposure and intraperitoneal injections of 25 and 150 mg/kg of the preparation, respectively) the values of eye lens ODI exceed those of the control group.

The ODI differences between the groups of UV exposed animals and animals which were exposed to UV and received the preparation in instillations or intraperitoneally can be considered valid from the 30<sup>th</sup> day on ( $p < 0.03$ ). Subsequently, validity of the differences grows significantly ( $p = 0.0001$  for anterior cortex,  $p = 0.0007$  for middle nucleus).

Comparing the groups of animals that received the preparation by different routes, it should be noted that in cortex, the instillations were more effective than the injections of 25 mg/kg ( $p = 0.003$ ) from the 104<sup>th</sup> day on

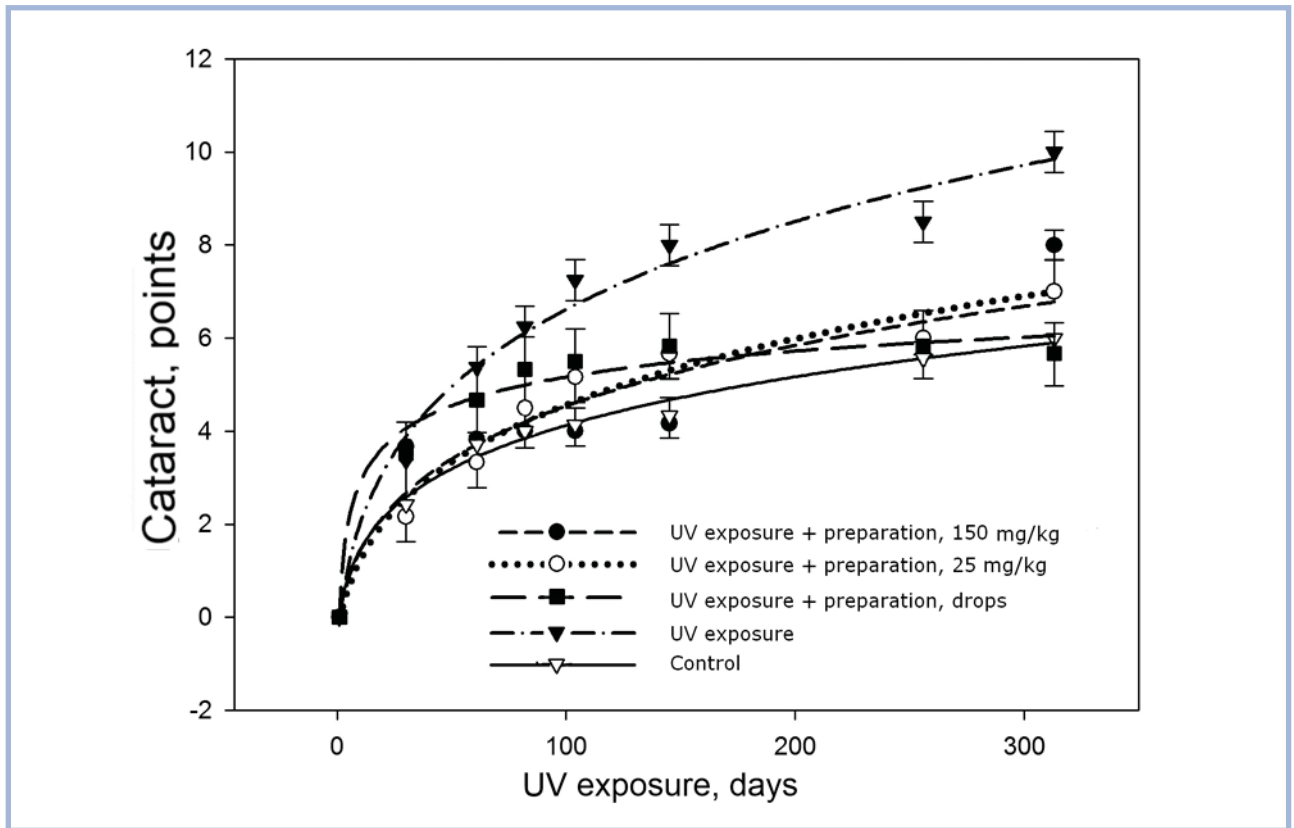


Fig. 1. The effects of the coformulated preparation on the development of UV-induced cataract in rats (expert evaluation method). The data on fig. 2, 3 and 4 is presented in form of mean values per group with an indication of 95 per cent confidence interval.

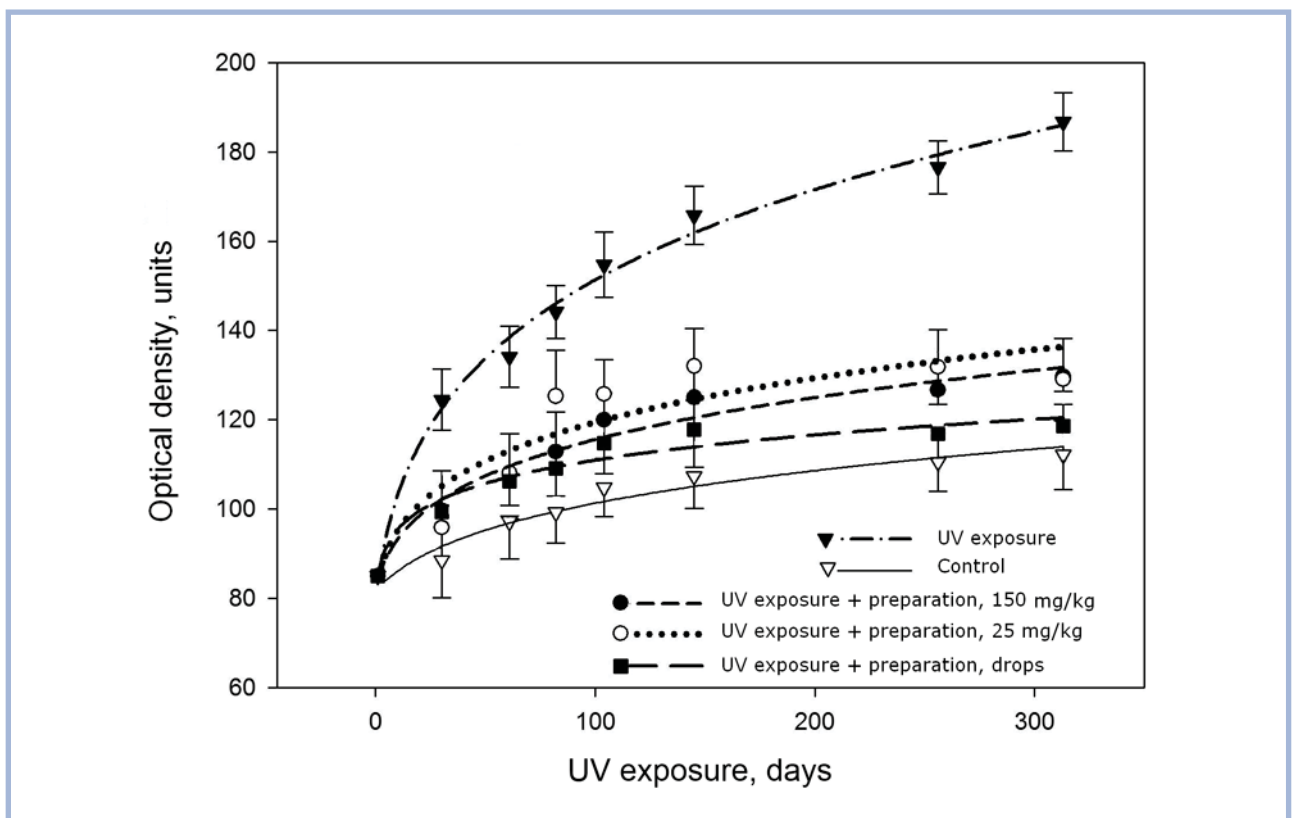


Fig. 2. The effects of the coformulated preparation on the opacification in the anterior cortex of the eye lens (microdensitometry method).

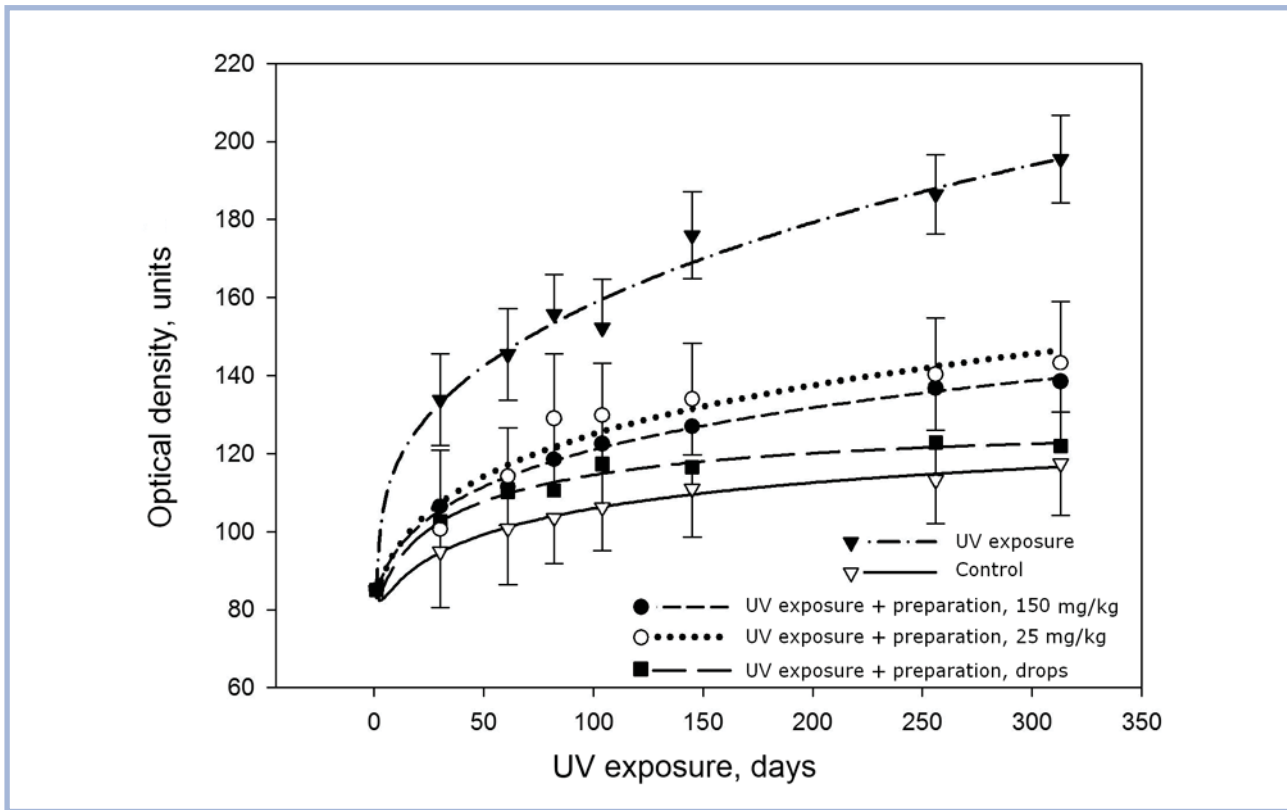


Fig. 3. The effects of the coformulated preparation on the opacification in the middle cortex of the eye lens (microdensitometry method).

and more effective than the injections of 150 mg/kg ( $p = 0.0021$ ) by the 313<sup>th</sup> day. In nucleus, the instillations of the preparation were more effective than the injections of 25 mg/kg ( $p = 0.003$ ) and 150 mg/kg ( $p = 0.006$ ) starting from the 104<sup>th</sup> day.

Figures 2 and 3 show the dynamics of ODI increase in anterior lens cortex and middle nucleus for all groups since the growth of opacifications in both anterior and posterior cortex as well as in nucleus of eye lens developed almost identically.

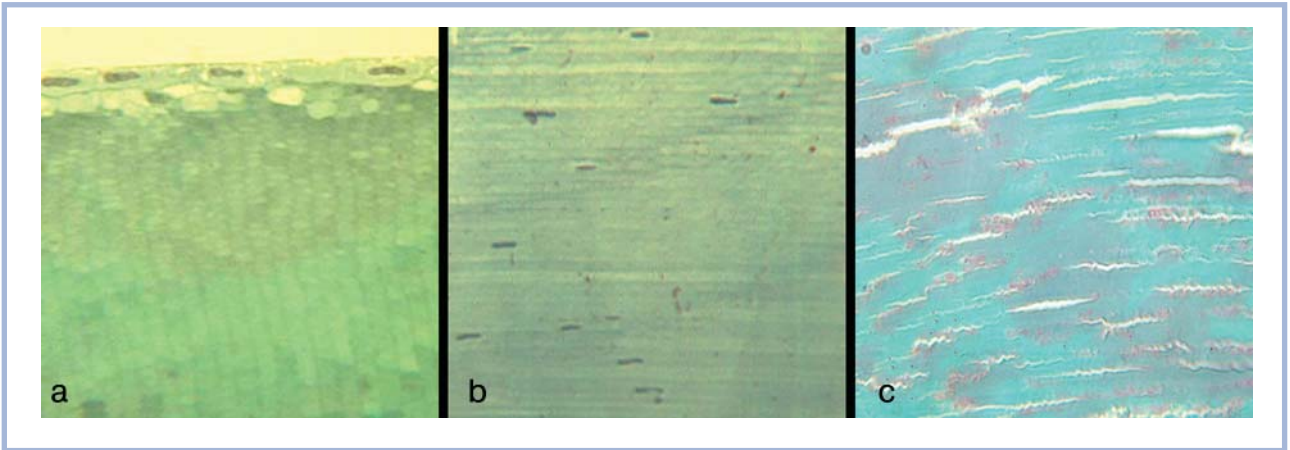
The results of morphologic study of the 1<sup>st</sup> group (UV radiation exposure) and the 2<sup>nd</sup> group (control) animals' eye lenses were extensively described in the previous article (refer to Article 3 of the series). The exposure to UV radiation resulted in apparent structural disturbances in all parts of crystalline lenses of the 1<sup>st</sup> group animals. Insignificant dimensional and structural disturbances were observed in lenticular fibers in nucleus and anterior sub-capsular cortex in the control group.

In animals of the 3<sup>rd</sup> group (UV radiation exposure and instillations of the preparation) anterior capsules of the eye lens were unevenly thinned, and areas of vacuolization, though with intact structure, were seen in crystalline epithelium (fig. 4). Fibers of the exterior cortex could be circumscribed easily, while arcuate nucleus (in the equatorial zone) and intercellular borders were visu-

alized. The medium cortex of eye lens showed localized ecstasies of intercellular areas, which were less significant than in animals of the 1<sup>st</sup> group. Compact placement of the eye lens nucleus fibers and the presence of swellings in them made the nucleus look like a homogeneous tissue.

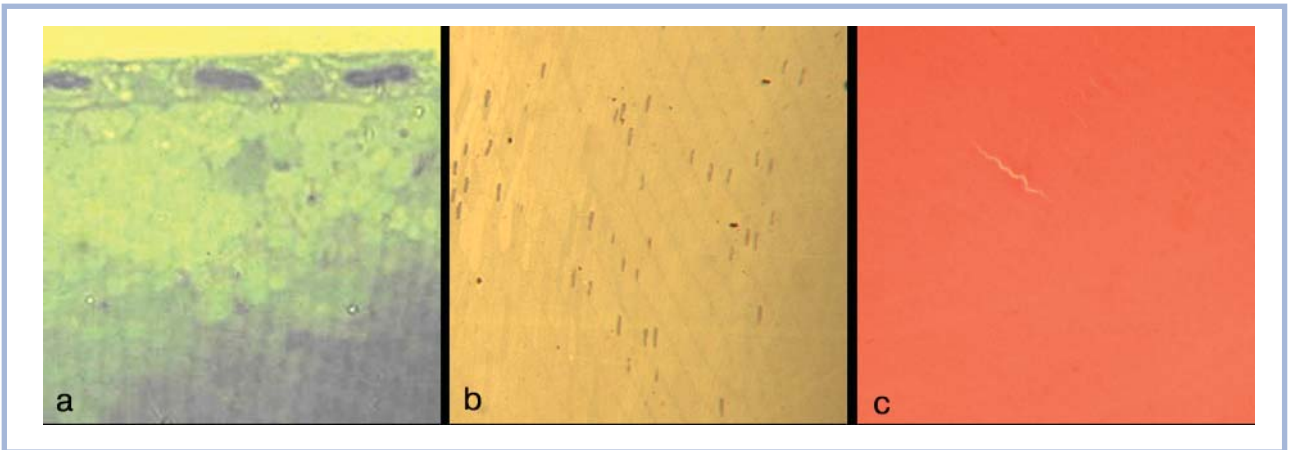
The animals of the 4<sup>th</sup> group (UV radiation exposure and 25 mg/kg intraperitoneal injections of the preparation) showed anterior capsule thinning with functionally intact epithelium (fig. 5). The presence of high number of microvesicles in epithelial cells cytoplasm suggested increase of their transport properties. In contrast to the 1<sup>st</sup> group eyes, the architectonics of the exterior and medium cortex remained unchanged. Eye lens nucleus structure was homogeneous and showed no traces of lysis or structural changes, however, its toluidine blue metachromatic stain (contrary to the cortex), suggested changes of its biochemical composition. Under higher magnification the borders between the fibers were almost indistinguishable, which served as an indicative of a significant induration in this part of the eye lens.

In animals of the 5<sup>th</sup> group (UV radiation exposure and 150 mg/kg intraperitoneal injections of the preparation), eye lens anterior capsules remained nearly unchanged. Histological structure of the anterior capsules was similar to the one of the control group animals, and



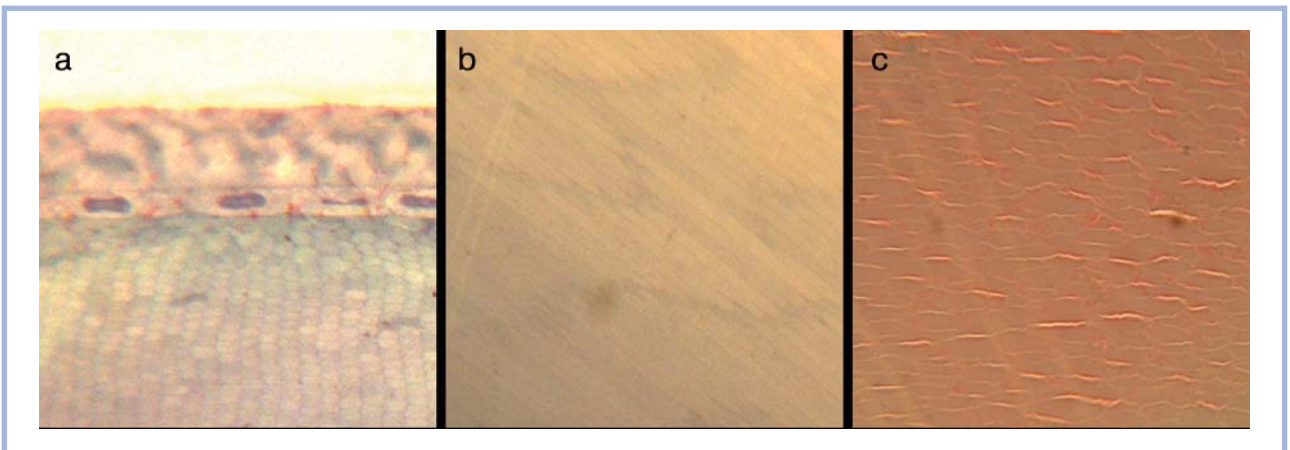
**Fig 4. Histologic pattern of the crystalline lens of a 3<sup>rd</sup> group animal.**

a — thinning of the anterior capsule of the eye lens; b — exterior cortex of the eye lens; c — fibers edema in the eye lens nucleus.  
 In fig. 4, 5 and 6: semifine section, polychromatic coloration. Magnification ×250.



**Fig. 5. Histologic pattern of the crystalline lens of a 4<sup>th</sup> group animal.**

a — thinning of the anterior capsule of the eye lens; b — exterior cortex of the eye lens; c — the eye lens nucleus, induration and homogenization of crystalline fibers.



**Fig. 6. Histologic pattern of the crystalline lens of a 5<sup>th</sup> group animal.**

a — anterior capsule, intact structure; b — exterior cortex of the eye lens; c — the eye lens nucleus, homogenization of crystalline fibers.

no signs of interfiber swellings or vacuolization were seen in the underlying cortex (**fig. 6**). The structure of the deeper cortex and nucleus remained unchanged. Only under higher magnification small concentric fissures were seen in the area of the nucleus reflecting the degree of induration and age-related changes of the lens.

The morphological study data allowed to reveal the effects of the coformulated preparation applied to experimental animals' eye lens in the form of instillations. In this group, as compared to the 1<sup>st</sup> group, the anterior capsule and the deep cortex of eye lens were proved to remain unchanged. Insignificant swelling in the nucleus was not accompanied by fragmentation of the lenticular fibers. However, the best histological results were observed in the 5<sup>th</sup> group, where the architectonics remained intact throughout the whole width of the eye lens substance and was closely similar to the architectonics of the 2<sup>nd</sup> group eyes (control).

## Conclusions

1. Administration of the coformulated preparation made from the mixture of peptides (N-acetylcarnosine and D-pantethine) in the ratio of 1:1 in forms of eye instillations and intraperitoneal injections allow the processes of UV-induced cataract formation *in vivo* to be slowed down.

2. According to biomicroscopic studies data, the coformulated preparation effects are more significant when applied in the form of instillations, as compared to intraperitoneal injections, which may be due to higher drug concentration in the intraocular fluid in that case.

3. The minimum dose of the coformulated preparation (N-acetylcarnosine and D-pantethine) that shows signs of anticataract activity with injection delivery method equals to 25 mg/kg of each peptide. No direct correlation was discovered between the degree of anticataract activity manifestation and increase of the dose size.

4. Pathomorphological studies show that in the context of UV-induced cataract model in rats the coformulated preparation has protective effects on eye lens fibers. Histological study revealed the highest anticataract activity with 150 mg/kg injections of each peptide.

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