



R E V I E W

On Ph.D. Thesis of

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Ph.D. student in self-study form,
Department "Surgery, Radiology, Obstetrics, and Gynecology"
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on the topic:

**"Examination of the regenerative potential of platelet-rich plasma and sodium hyaluronate
with dexpanthenol in experimentally induced corneal ulcers in rabbits"**

for awarding the educational and scientific degree "Doctor"
field of higher education 6. "Agricultural sciences and veterinary medicine"
professional field 6.4. "Veterinary Medicine"
scientific specialty: "Surgery, radiology and physiotherapy of animals"

Scientific consultant: Assoc. Prof. Nadya Zlateva-Panayotova, Ph.D., DVM

Assist. Prof. Mustafa was born on 06.12.1988. He completed secondary education in 2007 at 18th "William Gladstone" secondary school with intensive study of the Chinese language, in Sofia. In 2010, he graduated from the "Yordanka Filaretova" Medical College, Professional Bachelor of Dental Technician at the Medical University - Sofia. In 2019, he graduated with a Master of Veterinary Medicine from the Faculty of Veterinary Medicine at the University of Forestry.

Proficient in English - listening C1, reading C1, writing B1, Turkish - listening C1, reading C1, writing C1, and Chinese - listening A2 reading A2 writing A2.

He is the recipient of a named scholarship for achievements in mastering agricultural sciences, incl. veterinary sciences and forestry in the name of Acad. Doncho Kostov for 2018/2019 from the "Еврика" Foundation.

Assist. Prof. Mustafa has also attached a list of 10 scientific papers. Articles related to the pathology of snakes are of interest.

The thesis is written in 164 standard typewritten pages and has the following sections:

- Content – 2 pages;
- List of abbreviations used – 2 pages;
- Introduction – 2 pages;
- Literature review – 39 pages;
- Own research – 13 pages;
- Results – 41 pages;
- Discussion – 30 pages;
- Findings – 1 page;
- Contributions and recommendations for practice – 3 pages;
- Publications related to the thesis – 1 page;
- Established citations of scientific publications related to the thesis – 1 page;
- Literature – 27 pages;

INTRODUCTION:

In the introduction, Assist. Mustafa briefly presented the application of regenerative medicine for the treatment of various diseases, particularly in ophthalmology. Provides a brief description of platelet-rich plasma, hyaluronic acid, and dexpanthenol and their use in corneal ulcer therapy. The main directions of the recent studies are the standardization of the techniques for the preparation of PRP and the establishment of its most effective form of application in eye diseases, namely the activated gel form, the non-activated liquid form in the form of eye drops or injectable application into different segments of the eye in various pathologies in veterinary ophthalmology.

LITERATURE REVIEW:

The literature review covers 39 standard typescript pages and includes 3 tables and 7 figures. At the beginning of the literature review assist. Mustafa gives a detailed description of the anatomy of the eye and reflects in detail the histological structure of the cornea and the composition and functions of the tear secretion. The review examines the main etiological factors causing corneal damage - mechanical injuries from the impact of foreign bodies or parasites, corneal wounds, and inflammatory processes of the cornea, which may be accompanied by ulceration and difficult-to-restore defects. From the inflammatory processes of the cornea, the mechanical and chemical etiological factors destroying the cornea are described in detail.

Assist. Mustafa describes in detail what is known in the literature regarding the clinical findings in corneal ulcers and diagnostic methods. Much of the literature review covers corneal wound healing at the histological and cytological levels and particularly the role of growth factors and cytokines. Each growth factor is described in detail. In section 1.5 Regenerative therapies in the treatment of corneal ulcers, assist. prof. Mustafa makes a brief retrospective of what has been achieved in the department in this area. In section 1.5.2. Application of Autologous Blood Derivatives in Ophthalmology., the author summarizes what is known so far in the literature about platelet-rich plasma (PRP). The known protocols for extracting PRP and the requirements it must meet are indicated. The literature review would be more comprehensive if experimental and clinical studies on the use of PRP in corneal ulcers in humans and pets were covered in more detail in this section.

Based on an analysis of the literary sources for the purpose of the dissertation, assist. prof. Seven Mustafa believes that the methods for obtaining and applying autologous blood products in animals have not been sufficiently researched. This necessitates additional scientific research in this direction in veterinary medicine.

OWN RESEARCH

1. AIM AND TASKS

The main goal of the Ph.D. thesis is to make a comparative evaluation of the speed and level of healing of experimentally induced corneal ulcers after administration of autologous platelet-rich plasma, sodium hyaluronate with dexpanthenol and surgical method of temporary fixation of the third eyelid in rabbits.

The goal is well-formulated and corresponds to the title of the thesis work. To realize the set goal, assist. Mustafa formulated 5 tasks.

1. Reproduction of corneal ulcers by chemical and surgical methods in an experimental animal model - rabbits;

2. Tracking the effect of surgical treatment in experimentally induced chemical and traumatic corneal ulcers by clinical, specific ophthalmological, and imaging-diagnostic methods;

3. Monitoring the effect of the application of sodium hyaluronate in combination with dexpanthenol in experimentally induced chemical and traumatic corneal ulcers by clinical, specific ophthalmological, and imaging-diagnostic methods;

4. Tracking the effect of the application of:

a) platelet-rich plasma in the form of eye drops

b) platelet-rich plasma in the form of subconjunctival injection

in experimentally induced chemical and traumatic corneal ulcers by clinical, specific ophthalmological and imaging-diagnostic methods;

5. Histological assessment of the effect of applied therapies on the healing of experimentally induced chemical and traumatic corneal ulcers.

2. MATERIAL AND METHODS

The experimental studies were carried out in the period 2022-2023 on 28 rabbits of the New Zealand white breed (*Oryctolagus cuniculus*), aged between 12 and 18 months, equally distributed by both sexes, with an average weight of 3.1 ± 0.4 kg.

The experiments were carried out following the Permit for the Use of Experimental Animals No. 337/2022, of the Animal Ethics Committee of the Bulgarian Food Safety Agency, and under the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123, Council of Europe, 2007).

To implement the study, the author formed two experimental stages:

1. Causing a corneal ulcer with a chemical burn by sodium hydroxide (Ch groups);

2. Causing a corneal ulcer with mechanical trauma (Tr groups);

In both stages, 4 groups were formed.

- Group MNF - Ch, Tr – positive control in which a surgical technique for temporary fixation of the third eyelid was applied.

- Group HADP - Ch, Tr – experimental group in which sodium hyaluronate and dexpanthenol were administered by drip.
- Group PRPD - Ch, Tr – experimental group in which autologous platelet-rich plasma was administered by drip.
- Group PRPS - Ch, Tr – experimental group in which autologous platelet-rich plasma was administered through a single subconjunctival injection.

Platelet-rich plasma was obtained according to the method based on Okuda et al. (2003), by double centrifugation in a gradient centrifuge PRP500 Tabletop Low Speed Centrifuge (Cence®, Xiangyi, China).

To induce chemical corneal ulcers, the author used a prepared 1-molar sodium hydroxide solution (1M NaOH), following the method described by Rajamane & Jeyalakshmi (2014).

A traumatic ulcer was induced by superficial keratectomy reaching the stromal layer.

The healing processes of the ulcers in the positive control and experimental groups were followed up by clinical, paraclinical, and specific ophthalmological examinations (slit-lamp ophthalmoscopy, evaluation of the condition of the cornea, measurement of tear production, examination of ulcerative defects with fluorescein dye).

Ulcer healing was monitored by modern optical coherence tomography (Anterior Segment Optical Coherence Tomography, AS-OCT). The method allows for obtaining objective information about the condition of the cornea. Histological examinations of the corneas of the positive control and experimental groups were also conducted.

The obtained primary digital information was processed with statistical analyzes (statistically by one-factor variance analysis ANOVA (Predictive Analytics Software PASW®, SPSS, Version 19), and the least-significant difference (LSD) post hoc test was used for the differences between individual groups compared to the control group). Fisher's coefficient and significance level were calculated, and differences with a significance level of $p < 0.05$ were accepted as statistically significant.

Non-parametric results were processed by non-parametric Friedman's test, and in this test, differences with a significance level of $p < 0.05$ were accepted as statistically significant.

RESULTS:

The results are presented in detail and clearly. The obtained results are reflected in 15 tables and 45 figures. Due to the large number of tables and figures, I will focus on those that best reflect the stated goal of the thesis. Table 5 presents a qualitative analysis of parameters in WB and instillation PRP. The content of formed elements in PRP – WBCs are reduced by 91.8%, and RBCs are reduced by 99.5%. The concentration index of platelets in enriched plasma is equal to 4.75. The author proves that the achieved platelet concentration index in PRP has therapeutic properties. Similar is the information in Table 6 – Qualitative analysis of parameters in WB and PRP for injection.

In these tables, the relevant indicators are presented as average values. Where are the tables with the primary values of these indicators for each rabbit included in the study?

In section 3.2.3 Results of special ophthalmological examinations (from figures 13-20) are shown the results of slit-lamp ophthalmoscopy examinations at the first stage (with chemical burning - figures 13-16) and second stage (with mechanical trauma - figures 17-20) in experimentally induced ulcers of rabbits. In all groups with chemical burns on the first day, corneal opacification was found in the central zone, with complete opacity under both diffuse and focal illumination. Slit-lamp examination shows significant corneal thickening in the central area. The fluorescein test was positive, and the ulcerated defect covered a large central area. On the 10th day, after the release of the third eyelid, a significant opacity was established, resulting in the corneal surface and irregularities in its central part; in the HADP-Ch group on the 10th day, the iris was already visible below the edematous area. The fluorescein test on the 10th day was negative, but edema was still detectable; in the PRPD-Ch group on the 10th day, the iris was already visible below the edematous area. The fluorescein test on the 10th day was negative, but edema was still detectable; in the PRPS-Ch group on the 10th day, single superficial blood vessels were found in isolated cases. The swelling was preserved on the last day as well. The fluorescein test was negative on the 10th day.

Figures 17-20 show the results of the instrumental inspection of rabbits with traumatic corneal ulceration. In all groups with mechanical trauma to the cornea on the 1st day, a significant loss of smoothness of the cornea was detected, as a result of the deepithelialization found in the examination with both diffuse and focal illumination, the slit-lamp confirmed its rough front surface, the fluorescein sample was positive, with the ulcerative defect covering a large central

area of the cornea. On the 10th day, in the MNF-Tr group, the lid margins were swollen, the cornea kept its transparency with the roughness on its anterior surface, no thickening of the layers was detected with the slit-lamp, but the rough surface and change in sphericity were confirmed. No corneal neovascularization was detected, the fluorescein test was positive and retained the dye with high intensity. In the groups HADP-Tr, PRPD-Tr, PRPS-Tr on the 10th day, no neovascularization, edema, opacification, and disruption of the structure of the anterior surface of the cornea was detected.

With the obtained results, the author proves that the therapeutic effect of the applied treatment methods depends on some points of the etiological factor. The results regarding clinical ophthalmological assessment in the positive control and the experimental groups with chemical corneal ulcers and with traumatic corneal ulcers (table 14, 15) correlate with the results of slit-lamp ophthalmoscopy studies and with the results in the dynamics in the area of the ulcer defect in the first and second stages (table 18, 19).

In section 3.3, the results (figures 22-37) of tracking the speed and character of corneal healing with diagnostic imaging studies, are presented with an axial scan at the level of the anterior chamber of the eye with a cornea in a transverse section through its central part, and 3D-reconstruction with a gonioscopic view from the level of the anterior surface of the iris. Through this imaging method, information is obtained from deeper tissues of the anterior segment in a non-contact and non-invasive manner, and corneal edema, stromal structural changes, neovascularization, and inflammatory opacification are identified.

Results of histological examinations of the corneas in the positive control and experimental groups are presented in Figures 38-57. The histological changes occurring after causing the experimental ulcers and the dynamics of the healing processes as a result of the applied means of therapy are reflected.

Unidirectional dynamics are established in all presented results. Each subsequent method builds on the information obtained in the study of experimentally induced corneal ulcers.

DISCUSSION:

In the Discussion section, Dr. Mustafa expertly contrasts his results with those of other authors. At the beginning of the discussion, the candidate can point out, with reason, the choice of the New Zealand white rabbit as the animal species on which he conducted the present study. He skillfully graded the information contained in each of the research methods used. The author

has been able to perfectly interpret and compare the information that each of the results obtained during the research brings.

At the end of the discussion, Assist. Prof. Seven Mustafa concludes that the present study gives him reason to recommend the use of these agents with high regenerative potential on real patients in clinical practice with company animals.

Dr. Mustafa has indicated 7 conclusions, which briefly reflect fully the results obtained by the author. It has included 6 original and 4 confirmatory contributions and 4 practice recommendations.

Three publications are listed in connection with the dissertation work. They have been published in the following journals: Tradition and Modernity in Veterinary Medicine, vol. 6, No 1(10), 55 – 64 (ISSN 2534-9333) (e-ISSN 2534-9341) (2021), Tradition and Modernity in Veterinary Medicine, Online first, 20.12.2022 (e-ISSN 2534-9341) (2022) and Advance Research Journal of Multidisciplinary Discoveries, 80(2), 8 – 14 (e-ISSN-2456-1045) (2023). The three articles are co-authored by Assoc. Prof. Mehandzhiyski and Assoc. Prof. Zlateva.

Despite the negative circumstances that occurred in the department, Dr. Mustafa succeeded in the short time in which he had to conduct experimental research and at the same time lead classes, and today he presented us with a wonderful scientific work. This is also due to the scientific developments in the field of regenerative therapy previously carried out in the department.

The goal has been achieved. The basis for this is the sufficient number of experimental stages included in the study. Modern devices and methods are used. The obtained results are skilfully interpreted in the Discussion section.

LITERARY SOURCES:

Dr. Mustafa has indicated 336 literary sources, of which 9 in Cyrillic and 327 in Latin.

CONCLUSION:

The Ph.D. Thesis is a completed study of the regenerative potential of platelet-rich plasma and sodium hyaluronate with dexpanthenol in experimentally induced corneal ulcers in rabbits. In the process of carrying out the planned tasks, the Ph.D. student significantly increased his knowledge and skills in the application of regenerative medicine in eye pathology.

The proposed Ph.D. Thesis meets the requirements for awarding the educational and scientific degree "DOCTOR" and I propose to the honorable Scientific Jury to award the ESD "DOCTOR" to assistant professor SEVEN RUZHDI MUSTAFA in the scientific specialty: "Surgery, radiology and physiotherapy of animals", field of higher education 6.0 Agricultural sciences and veterinary medicine, professional field 6.4. Veterinary Medicine.

02.08.2023

Review prepared by:

(Prof. Bogdan Yanev Aminkov, PhD, DVMSc)