

SUMMARY

The doctoral dissertation named „Curative-prophylactic actualities and perspectives in some surgical affections of herbivores”, tackles a new research direction of treating open traumatic lesions, with the purpose of „optimizing” the tissue regeneration process.

Part I, the Knowledge stage, contains data from the specialty literature regarding „The evolution of the tissue regeneration process”, and it is structured in four chapters which refer to: healing of wounds – physio-pathological aspects and therapeutic inclusion; the tissue regeneration process; tissue regeneration process therapy and normal and pathologic cicatrisation process.

Part II, Personal research, is extended over 133 pages and contains aspects related to the studied material and the used methods, the results of the research, discussions about them and the final conclusions.

It was proven that some individual and some mixed proteases, increase the blood and tissue concentration of antibiotics, of some chemotherapy drugs and some other medicinal concoctions.

Still, in our vision, one of the most important therapeutic actions is that they dissolve any „dead” matter, including cancerous cells, cysts, waste deposited on the cardio-vascular system walls, on the walls of the intestines, wounds etc.

These reasons stood at the basis of using a relatively new group of proteolytic enzymes in our experiment, together with a vegetal component with special therapeutic actions, yet not described in the specialty literature.

Our purpose was that of demonstrating the therapeutic efficiency of a new pharmaceutical formula, respectively of *Euphasia superba* proteolytic enzymes and of the *Inula uliginosa* plant extract ointment.

The proposed objectives were:

- The detection of an enzymatic concentration that has an optimal therapeutic action to remove the „dead”, devitalized tissue.

- The detection of the plant extract which has optimal immune stimulating, germicidal and regenerative therapeutic action.
- Proving the therapeutic superiority of a new local therapy by healing wound with a proteolytic enzymes and plant extract ointment based treatment, vis-à-vis a classic treatment – saline solution 0.9% and Oximanirom.
- To prove this aspect, the following activities have been made:
 - The assessment of the wound load degree with dead, devitalized matter;
 - The determination of the germ contamination degree and the identification of the Flora isolated from the wound;
 - The quantification of the effect on different blood cellular components (leukocytes and leukocyte formula);
 - Establishing the main histological characteristics of the wound biology;
 - Clinical-therapeutic assessment and investigations on the evolution of the main macroscopic aspects of the cicatrization process.

The study was made consecutively on 105 patients, registered in the examination record of the Iepureni private office, who undertook surgical interventions and ambulatory treatment during 2003-2009. The research was made on a number of 66 horses and 39 cows and consisted in the experimental and clinical treatment of some new therapeutic procedures which can facilitate the healing of open traumatic lesions (wounds), potentially patentable, taking as comparison the classic treatment formula.

The inclusion criteria of the studied patients were:

- Adult animal, with an age between 4 and 9 years old;
- Without any symptoms of an associated pathology;
- Without a condition of vital dysfunction (e.g.: cardio-respiratory stability).

The exclusion criteria (applicable to the patients, which initially complied with the inclusion criteria):

- Owners refusal, expressed under any form and at any stage of the study;

- The appearance of any complication during the study (hemorrhage, infection, organ dysfunction, etc.).

The studied batches are representative, them representing 10 % of the statistic total. The male/female/batch ratio is 2:1. The sex differences regarding the evolution of the pre-operator period or the effects of the treatment were not reported in the literature data.

The chematic presentation of the therapeutic protocol

EXPERIMENTAL		CLINICAL	
The determination of the most efficient concentration of <i>Euphasia superba</i> proteolytic enzymes	The determination of the plant extract with the most potent pharmacodynamic, immune stimulating, germicidal and tissue regeneration action	The assessment of the therapeutic efficiency of the proteolytic enzymes 6 UC/ml + the <i>Inula uliginosa</i> 10% plant extract ointment	The assessment of the therapeutic efficiency of the saline solution 0.9% and Oximanirom treatment
WITNESSES		The comparative assessment of the two medicament forms	
Saline solution 0,9 %	Oximanirom		
Tested Concentrations	Tested Plants		
0,6; 1,8; 3,0; 4,2; 6,0 UC/ml	<i>Arnica montana</i> <i>Calendula officinalis</i> <i>Inula uliginosa</i> <i>Sedum album</i>		
The number of treated wounds /the number of treatments applied daily			
n = 10/ n = 2	n =10/ n = 3	n = 47/random, dependent on the gravity of the wound	n = 47/random, dependent on the gravity of the wound

The presence of dead (devitalized) tissue in a wound increases the growth rate of germs, reduces the hosts resistance to infection and delays the formation of granulation tissue and the epidermisation/epitelisation process. The non-traumatic removal of the necrotic tissue from the

lesion, constitutes an essential stage in the therapy of wounds, be it of decubital nature, by sting, slash, etc.

Daily measures, of the traumatic lesions surface treated with different proteolytic enzymes concentration, revealed a dose-dependant pharmaco-clinic effect.

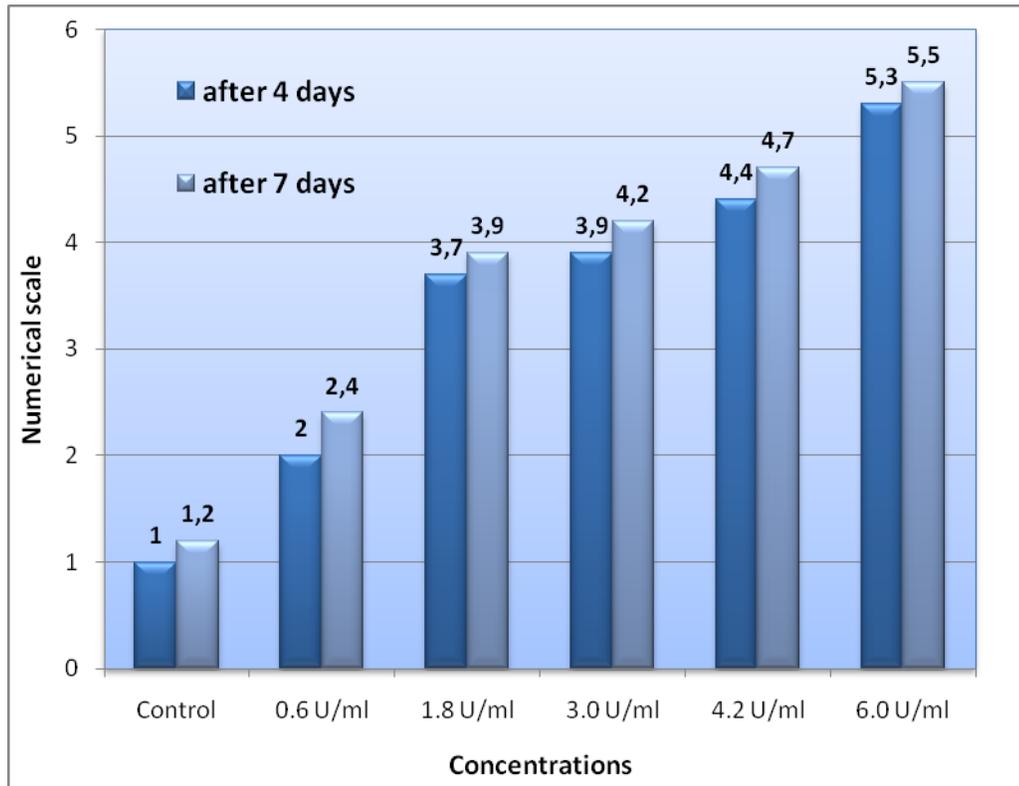


Figure 6.4. Score average (on a numeric scale from 0 to 6) after 4, respectively 7 days of treatment with the de treatment of *Euphasia superba* crustaceous enzymes

After 7 days of treatment, the surface of the wounds covered with necrotic tissue have been cleaned to a rate of 53% for the saline solution and to a rate of 80% for the highest concentration (6,0 U enzyme/ml) of proteolytic enzymes (Figure 6.4.).

After 9 days of observation of the traumatic lesions undergoing treatment, the 4,2 and 6,0 U/ml concentrations lead to a cleaning percentage of 97% of the wound surface. Lower concentrations, respectively 1,8 and 3,0 U/ml registered a wound surface cleaning ratio of over 80%. On average, the wounds treated with 6,0 U/ml *Euphasia superba* enzyme have been cleaned of all necrotic tissue in 9.1 days and healed in 17.4 days. The wounds on which the 4,2 U/ml concentration was used have been cleaned in the 10th day and healed on average after the

17.4 day, while it took for the wounds on which saline solution 0.9% was applied and average of 13.3 days for cleaning and 21.1 days for complete healing from the application of the treatment.

It has been ascertained that the treatment of wounds with proteolytic enzymes hastens the healing rate by attracting mononuclear and multinuclear leukocytes to the wound. Following the proteolytic enzyme's interaction with the extra-cellular protein substratum(enzymatic cleavage) results protein fragments, with a chemo tactic role for monocytes and polymorphonuclear leukocytes.

Through our experimental study on the germicidal, immune modulating and tissue regeneration activity of some plant extracts, it has been proven that *Inula uliginosa* is the most gifted plant with active principles capable of inducing the activities mentioned above..

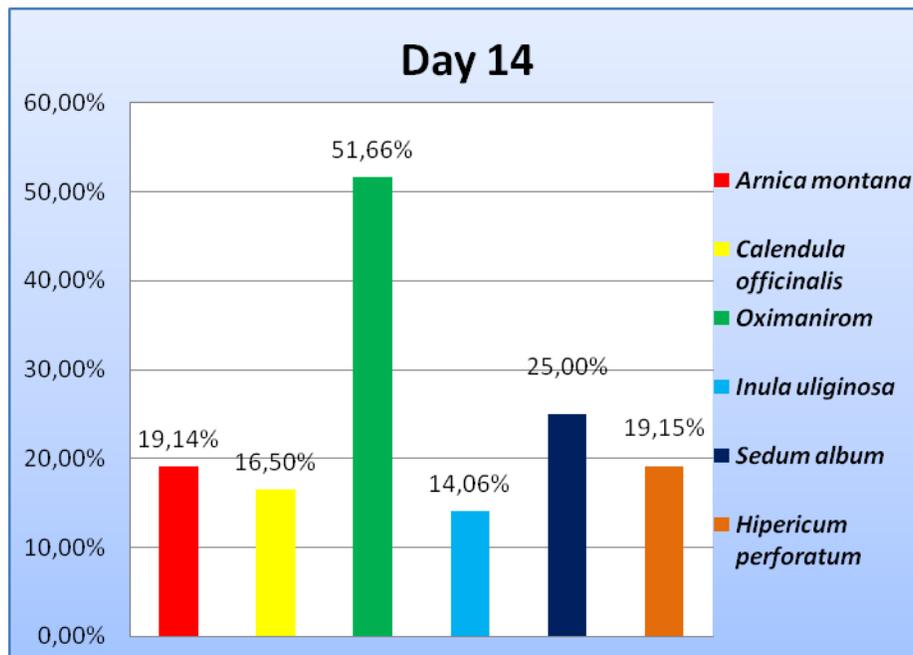


Figure 7. 13. Healing dynamics in the 14th day of treatment

The use of *Inula uliginosa* 10 % plant extract ointment has the following advantages:

- Prevents wound desiccation;
- Prevents the adherence of the bandage to the wound and facilitates it's removal, without traumatizing the granulation tissue(fleshy sprouts);
- The pharmaceuic form remains in contact with the wound for a longer period of time, so the renewal of the bandage will be rare;

- The genesis of the granulation tissue and the epithelialisation starts earlier, ensuring the reduction of both the depth and the surface of the wound.

The comparative assessment of the main clinical aspects of herbivore wound healing, as a follow-up to the application of the two therapeutic formula, the *Euphasia superba* wet bandage with proteolytic enzymes 6 UC/ml plus the *Inula uliginosa* 10 % plant extract ointment and wet bandage with saline solution 0.9% plus Oximanirom, revealed the following:

It has been ascertained that the inflammatory process was much more intense and of a longer period for the O batch wounds (treated with saline solution 0.9% and Oximanirom), compared to the wounds of the E batch (treated with proteolytic enzymes 6 UC/ml and *Inula uliginosa* 10 % plant extract ointment), where it was more discreet and much more reduced.

Through its non-irritating, non-invasive, painless action the wet bandage with proteolytic enzymes, lead to the creation of a biofilm (protective cover) which prevented its adherence to the wound structures. Its removal, even frequently, has not produced tissue damage.

The biggest germ number ($> 10^5$) was registered in the wounds of the O batch animals (treated with saline solution 0.9% and Oximanirom). The lowest germ number ($< 10^5$) was registered in the wounds of the E batch animals, where it seems the enzymatic treatment had a great influence in their reduction, once with the removal of the devitalized tissue.

After the assessment of the chronic wound healing process, treated with the two therapeutic formula, the followed indicators values were superior in the case on the treatment with proteolytic enzymes 6 UC/ml plus *Inula uliginosa* 10 % plant extract ointment.

We recommend the use of the therapeutic formula based on the *Euphasia superba* proteolytic enzymes 6 U/ml plus the *Inula uliginosa* 10 % plant extract ointment in the treatment of chronic wounds, with reduced etiology and healing potential, accompanied by tissue destruction on large areas, relatively deep, with a high contamination factor, even infected and with a high quantity of devitalized tissue (complications with suppurative, necrotic processes) and in all cases when the re-establishment of trophicity and the resolution of some chronic inflammatory processes is necessary.