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Enzyme therapy is a highly effective and environmentally safe method of treating lacerated wounds in dogs

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The results of the wound-healing effect of Iruxol ® cream in lacerated and crushed wounds of the distal extremities of dogs studies are presented. The injuries were caused by animals falling into arc traps. Such wounds are severe, with maximum tissue damage in the zone of influence of the traumatic factor. It was found that lacerated and crushed wounds are characterized by uneven, swollen, and necrotic edges, crushed tissues of muscles and tendon structures, scalping, and in some cases, bone fractures. The general clinical condition was assessed as intoxication-septic, which is accompanied by traumatic shock, sepsis, and a multi-organ inflammatory reaction. Hypochromic anemia, leukocytosis, accelerated erythrocyte sedimentation rate, left shift of the nucleus indicates intoxication syndrome, and a systemic inflammatory reaction. Lymphocytosis with monocytosis indicates the body's immune system's stimulation to fight destroyed tissues and sepsis, which develops as a complication of a lacerated and crushed wound. Thrombocytopenia is one of the diagnostic criteria for developing disseminated blood clotting syndrome. Together, these complications constitute the pathogenetic axis of shock. The development of vital organs' insufficiency is represented by hepatic and renal insufficiency as the results of intoxication-septic and shock conditions. This clinical status requires infusion and antibiotic therapy. After mechanical cleaning of the wound, the use of Iruxol ® cream based on peptidases for 21 days contributes to the formation of full-fledged structural components of scar tissue in the shortest possible time. The drug's main components that provide its effect are the enzymes collagenase, clostridiopeptidase A, and other peptidases. Enzymes break down the destroyed elements of injured tissues by their proteolysis. The wound is cleaned, and the best conditions are created to form granulation tissue and wound healing. Released under enzymes' action, oxyproline from collagen helps restore the protein components of damaged tissues. These substances are completely bioavailable. Their use is not only therapeutically effective but also environmentally safe. The need for complex treatment of lacerated and crushed wounds, which contributes to the restoration of the general clinical condition and adequate healing of a severe wound, is shown.
Keywords: lacerated and crushed wounds, Iruxol® cream, collagenase, clostridiopeptidase A, shock, sepsis, intoxication, insufficiency of organs, enzymes.

Introduction

In the conditions of the modern world, with an abundance of mechanisms, transport, and other technological acquisitions of humankind, injuries among people and animals have increased. Technical means can cause serious injuries. Some of these injuries are wounds (Frame & Meier, 2007; Payne-James, 2016).

The problem of wound healing and treatment remains one of the most urgent in modern veterinary medicine. There is a need for the use of pharmacological drugs, which, in addition to accelerating the healing of wounds, must be environmentally safe (Demidova-Rice et al., 2012; Guo & DiPietro, 2010; Rosenberg et al., 2014; Stevens et al., 2014; Velnar et al., 2009).

Since ancient times, it has been known about the use of agents that contain proteolytic enzymes to improve wound healing (Stanley et al., 2014; Zhao et al., 2012). The use of enzyme therapy topically leads to better cleansing and faster healing of wounds, especially infected ones (Patry & Blanchette, 2017; Pham et al., 2012).

Extremely popular preparations for local external use in complex crushed, thermal, chemical, and other types of wounds, are ointments based on enzymes. Such preparations are environmentally safe since they are biological and entirely bioavailable for the animal body (Eckhard et al., 2009, 2013; Giladi & Haase, 2018; Guidice et al., 2017).

Our attention was drawn to the topical preparation of Iruxol ® in the form of an ointment for external use. It contains enzymes that are produced by anaerobic bacteria *Clostridium histoliticum* Weinberg and Séguin 1916, Bergey, Harrison, Breed, Hammer and Huntoon 1923: collagenase (EC 3.4.4.19), clostridiopeptidase A (EC 3.4.24.3) and other peptidases (EC 3.4) (Arora et al., 2015; Eckhard et al., 2013).

All peptidases are known to be proteolytic enzymes. They break off the terminal amino acid residues from the protein or peptide molecules (Zhao et al., 2012).

Collagenase has a highly specific orientation and breaks down collagen. In this case, the free aminoacid oxyproline is formed. Clostridiopeptidase A catalyzes the hydrolytic cleavage of peptides that contain proline, and it also hydrolyzes collagen and gelatin. The enzyme can destroy animal tissues in the wound (Arora et al., 2015; Guidice et al., 2017; Stanley et al., 2014).

In addition to the enzyme components, the drug contains chloramphenicol - a bacteriostatic antibiotic with a broad spectrum of action (Ariyan et al., 2015).

Taking into account its composition, Iruxol ® cream provides enzymatic cleansing of wounds, does not affect the intact epithelium, granulation, muscle, adipose tissue of the body. Given at complex wounds in most cases are infected, the enzymatic breakdown of dead tissue deprives bacteria of the nutrient medium. In general, the ointment has an antimicrobial, wound-cleaning, and wound-healing effect (Arora et al., 2015; Eckhard et al., 2013).

The work aims to conduct a clinical trial of a wound-healing ointment based on enzymes, study the state of the body in the dynamics of wound healing, and demonstrate the wound-healing effect.

Materials and methods

The research was conducted during 2019–2020 in the Clinic of Veterinary Medicine of the Polissya National University, Zhytomyr, Ukraine.

Formation of experimental animal groups

The study involved six dogs injured by arc traps, with varying degrees of tissue damage, which made up the study group. The control group consisted of clinically healthy dogs based on the principle of pairs of analogs in the number of 10 animals (the animals had no clinical signs of other diseases, no pathogens were registered in the blood, and the hematological parameters were within the reference values). All dogs were 2-4 years old, weighing 20-40 kg.

Clinical researching

Clinical studies were conducted using general methods.

Determination of the nature and rate of healing of open wounds was carried out by recording the rate of reduction of the wound surface in time by the planimetric method (Foltynski et al., 2015; Rogers et al., 2010; Sundeep & Eswara, 2014).

The wound contraction was evaluated by the method of "ink marks" (Sundeep & Eswara, 2014).

Hematological and biochemical techniques of blood study

Blood sampling for the study was carried out from the vena subcutanea antebrachii. As an anticoagulant, heparin's solution with an activity of 1000 IU/ml in an amount of 0.01–0.02 ml per sample was used.

The hemoglobin content, the number of shaped elements (red blood cells, white blood cells, platelets), the rate of erythrocyte sedimentation, the leucogram were determined using a hematological analyzer Mindray BC-3600 (Mindray Medical Rus Co. Ltd, Russian Federation).

The content of total protein, albumins, globulins, creatinine, urea in the blood serum of dogs, as well as the activity of enzymes aspartate aminotransferase (AsAT) and alaninaminotransaminasa (AlAT) was tested using a semi-automatic biochemical analyzer Chem 7 (Erba, Czech Republic).

Composition of the treatment

Infusion therapy of sick dogs was carried out for five days according to the following scheme:

Reosorbilact in a dose of 10 ml per 1 kg of body weight,

0.9% Sodium chloride solution at a dose of 10 ml per 1 kg of body weight,

4% Glutargine solution at a dose of 5 ml per 20 kg of body weight,

2% riboxin solution in a dose of 3 ml per 10 kg of body weight,

5% ascorbic acid solution in a dose of 2-4 ml per animal.

Complex antibiotic therapy: Kobactan at a dose of 0.5 ml per 10 kg of body weight and Lincomycin 10% at a dose of 1 ml per 10 kg of body weight intramuscularly once a day for 5 days.

Local treatment of wounds was carried out by scarifying crushed and dead tissues and fragments of tissue elements. Daily performed debridement of wound irrigation solutions Decasan 0.2% and dusting powder Gentasept within 5 days.

Osteosynthesis was performed by repositioning and submerged bone fixation with titanium plates of the fracture sites.

Iruxol ® cream was applied for 21 days by applying to the wound area once a day and applying a gauze bandage on top.

Statistical analysis

Statistical processing of the results was performed using Statistica 13.3 software. Multiple variances were compared using the Fisher distribution (ANOVA). The analysis of variance determines the statistically significant influence on the studied factors. The obtained data's reliability was evaluated using the Fischer F-test at a confidence level of $p<0.05$.

Results

Now-a-days, hunters often use leg-catching traps for hunting different animals. These are pass-through traps, which are considered the most humane and are used mainly for hunting fur-bearing animals and trace an arc traps of the frame and plate types, used for hunting large and more cautious animals. Quite often, dogs fall into the trap, both hunting and domestic pets, on a walk-in hunting areas. Injuries sustained when caught in a trap are quite severe and can cost an animal the amputation of a limb, especially if it is trapped for a long time (Frame & Meier, 2007).

After being caught in arc traps, dogs admitted to the clinic had lacerated wounds located on the metatarsal's dorsal or ventral surface. The age of the wounds was dated to 4-5 days. The diagnosis was established based on the presence of a traumatic factor, significant damage to the outer integument and underlying tissues, and the presence of a gaping hole. The skin was significantly exfoliated over a large area, the edges of the defect were uneven, and extensive areas of tissue necrosis were found. Tendon, the muscle fibers are crushed. Anatomical interruption of blood vessels and nerves is poorly expressed (Cals Jochen & de Bont Eefje, 2012; Jagodzinski et al., 2010; Pallister, 2016; Sever, 2011). In some cases, the bones were broken (Fig. 1).

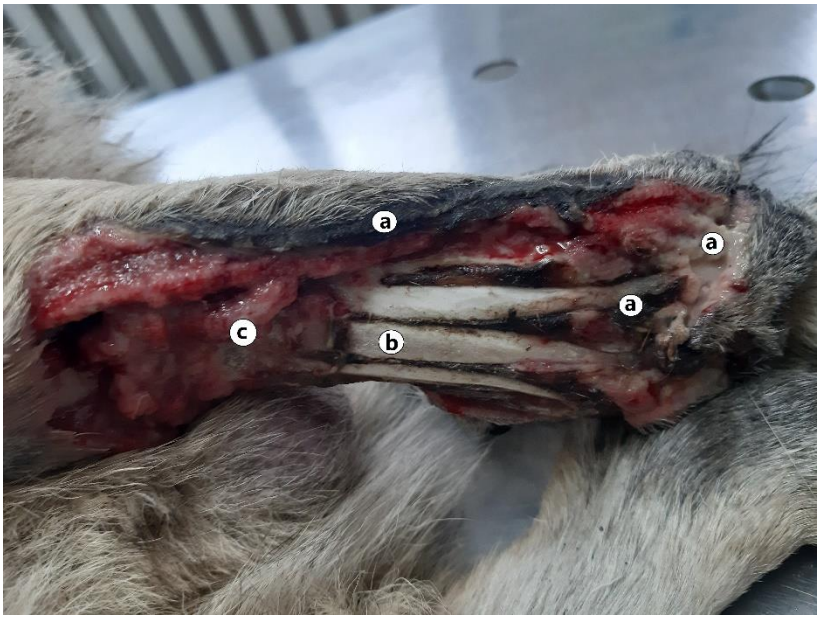

Fig. 1. The lacerated and crushed wound of the dog's metatarsal plantar surface due to falling into an arc trap (a – necrotic wound edges and subcutaneous elements, b – the scalped surface of the metatarsal bones, c – crushed muscle tissue).

The animals' general condition following clinical signs characterized the general condition of the animals: elevated body temperature to 39.8-40.2°C, chills, no appetite, lethargy, sometimes vomiting. Changes in the dogs' general condition were displayed on laboratory blood parameters (table 1, 2).

Table 1. Hematological parameters of dogs with lacerated and crushed wounds before and during systemic treatment, M ± m, n=6.

Parameters	Study time		Healthy animals
	before treatment	Five days after the start of treatment	
Hemoglobin, g / l	80.4 ± 2.8***	107 ± 4.6	119.6 ± 3.8
Erythrocyte sedimentation rate, mm/h	18.2 ± 2.6***	6.0 ± 1.3	3.9 ± 0.8
Red blood cells, T / l	3.8 ± 0.9	5.7 ± 0.4	6.2 ± 0.8
White blood cells, G / l	19.3 ± 2.3*	15.6 ± 2.1	11.3 ± 1.7
Platelets, G / l	111.8 ± 7.6***	200 ± 6.4***	257 ± 13.3
Leukogram:			
- basophils, %	0.02 ± 0.001	0.02 ± 0.001	0.03 ± 0.001
- eosinophils, %	2.6 ± 0.02***	2.4 ± 0.01***	1.1 ± 0.02
- myelocytes, %	-	-	-
- young neutrophils, %	-	-	-
- stick-nuclear neutrophils, %	12.3 ± 1.2***	6.4 ± 0.1***	2.2 ± 0.02
- segment-nuclear neutrophils, %	26.2 ± 3.9***	44 ± 4.8	53.4 ± 1.8
- lymphocytes, %	47.3 ± 4.7	42.4 ± 4.8	40.3 ± 2.6
- monocytes, %	8.3 ± 1.3***	4.6 ± 1.7	2.2 ± 0.06
Core Shift Index	0.47 ± 0.04***	0.15 ± 0.06	0.04 ± 0.001

*p<0.05, ***p<0.001

Thus, the laboratory blood picture determines hypochromic anemia (p<0.001), leukocytosis (p<0.05), thrombocytopenia (p<0.001), acceleration of ESR (p<0.001), left shift of the nucleus (p<0.001), lymphocytosis (p>0.05), monocytosis (p<0.001).

Table 2. Biochemical parameters of dogs with lacerated and crushed wounds before and during systemic treatment, M ± m, n=6.

Parameters	Study time		Healthy animals
	before treatment	Five days after the start of treatment	
Total protein, g / l	48.1 ± 2.6	59.3 ± 5.2	62.4 ± 6.3
Albumins, g / l	20.6 ± 1.9***	28.4 ± 3.6	30.3 ± 1.2
Globulins, g / l	28.3 ± 3.2	30.4 ± 6.2	33.6 ± 1.9
Creatinine, μmol/l	286 ± 12.3***	194.3 ± 7.5***	92.6 ± 4.3
Urea, mmol/l	24.6 ± 2.8***	13.3 ± 2.4**	5.1 ± 0.3
AsAT, IU/I	96.3 ± 4.2***	57.3 ± 9.3***	21.2 ± 3.3
AlAT, IU/l	82.6 ± 6.2***	39.8 ± 8.2*	12.7 ± 0.8

*p<0.05, **p<0.01, ***p<0.001

Biochemical changes in the blood of injured animals were characterized by hypoproteinemia ($p>0.05$), a decrease in the albumin-globulin ratio ($p<0.001$), creatininemia ($p<0.001$), uremia ($p<0.001$), and an increase in transaminase activity ($p<0.001$). Such changes characterize the systemic impact of extensive trauma on the body and determine the development of acute renal failure and the systemic inflammatory response (Pallister, 2016; Dubova et al., 2020; Dubova & Duboviy, 2018; Sever, 2011).

Based on the obtained indicators, infusion therapy was performed according to the same scheme for 5 days. This type of treatment was aimed at detoxifying and nourishing effects.

A course of antibiotic therapy using a combination of drugs kobactan and lincomycin contributed to the healing of inflammatory systemic reactions in the body of injured animals (Ariyan et al., 2015).

Surgical cleansing of the wound with scarification of dead skin, subcutaneous tissue, and muscles were performed locally. To remove purulent exudate, the wounds were treated twice a day with antiseptic drugs Decasan (lotions with an exposure of 10-15 minutes) and Gentasept.

After five days, the animals' condition was much more active; they reacted with interest to events in the external environment. The body temperature was in the range of 38.9-39.2°C.

The changes that developed as a result of the systemic treatment are shown in Tables 1, 2. The data presented shows that the hematopoietic parameters (hemoglobin content, red blood cell count, platelet count) were restored, the number of white blood cells and ESR significantly decreased. In the leucogram, there was a tendency to stabilize the ratio of neutrophils, a part of monocytes returned to the physiological one, and the proportion of lymphocytes remained elevated. The core index is shifted to the left.

Biochemical parameters also moved towards stabilization, although creatininemia ($p<0.001$), uremia ($p<0.01$), and increased transaminase activity (AlAT $p<0.001$, AsAT $p<0.05$) remained significant. After the wound was cleaned of dead parts and purulent-inflammatory elements, osteosynthesis of broken bones was performed and the wound was prepared for wound healing treatment with Iruxol ® cream. (Fig. 2).

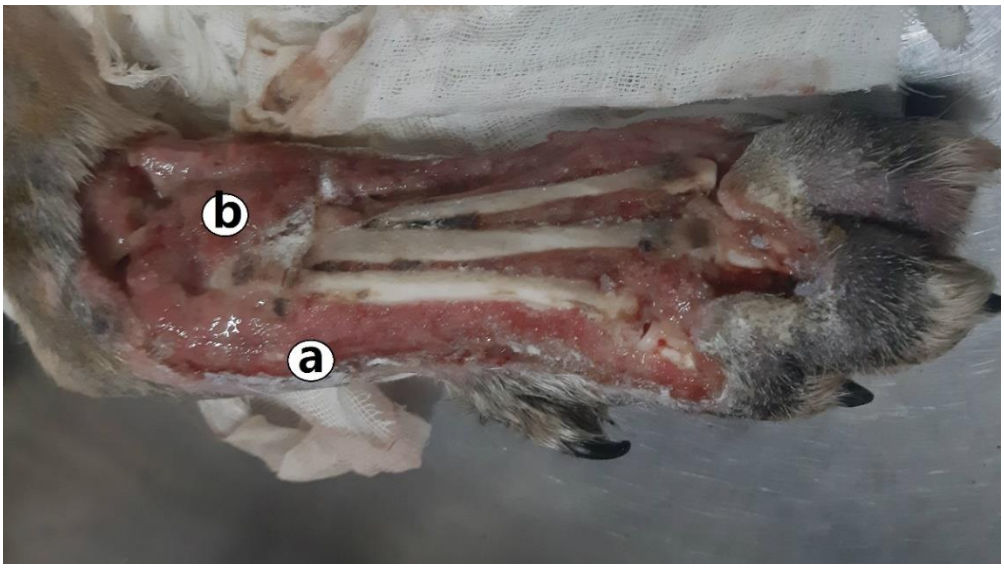

Fig. 2. The lacerated and crushed wound of the metatarsus' dorsal surface after cleaning the wound from purulent-inflammatory and dead elements. Necrotic edges are absent (a), the crushed tissues are covered with early granulation tissue (b).

After five days, progressive granulation of the damaged elements was observed in the wound, the exposed areas of the bones were significantly reduced (Fig. 3).

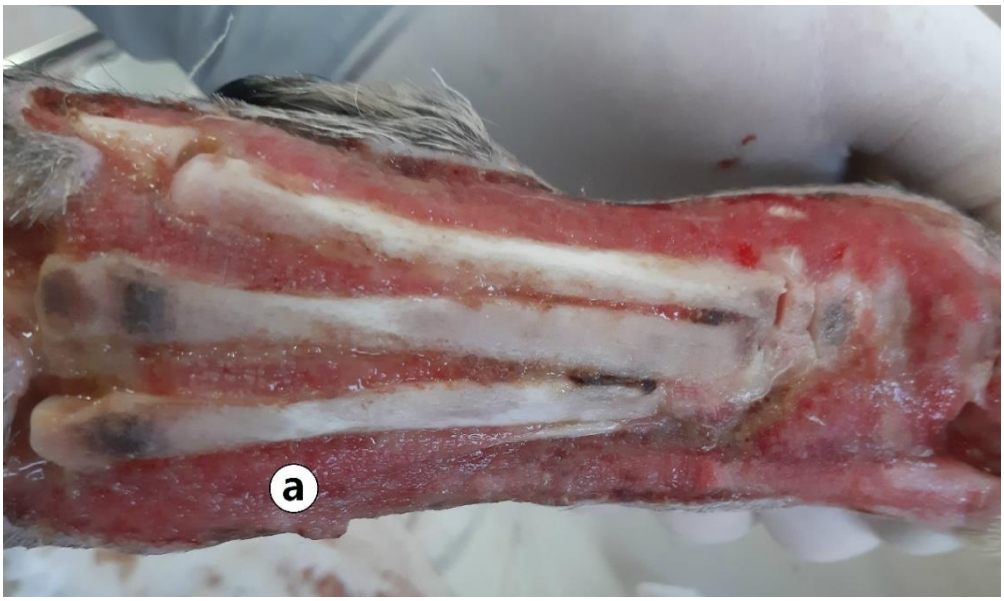

Fig. 3. Progressive granulation of damaged muscle tissue elements (a)

In dynamics, these processes continued progressively, and by the end of the follow-up period (the 21st day from the start of treatment with Iruxol), a significant reduction in the wound defect was observed (Fig. 4).

Fig. 4. Healing of a lacerated and crushed wound 21 days after the application of Iruxol® ointment (a – young granulation tissue).

After the growth of granulation tissue, autoplasty of the skin was performed. The motor ability of the dogs ' limbs was completely restored. The general condition of the animals was assessed as clinically healthy.

Discussion

The problem of injuries in dog breeding is very relevant. As a result of human activity, animals can receive particularly severe injuries (Frame & Meier, 2007; Payne-James, 2016).

Dogs admitted to the clinic ue to falling into traps had lacerated and crushed wounds, which relate to injuries with maximum tissue destruction (Cals Jochen & de Bont Eefje, 2012; Jagodzinski et al., 2010; Pallister, 2016; Sever, 2011).

The clinical condition of the injured dogs was characterized by intoxication, sepsis, and traumatic shock. Laboratory parameters confirmed this. Thus, hypochromic anemia indicates the destruction of red blood cells and their loss of hemoglobin due to the development of traumatic shock and intoxication and blood loss that occurred after the dog received damage (Payne-James, 2016).

Leukocytosis and accelerated erythrocyte sedimentation rate are markers of septic status and systemic inflammatory response. Changes in the leuco confirm this position. Thus, the shift of the nucleus to the left indicates the development of reactive inflammation. Lymphocytosis and monocytosis, determined in sick animals, indicate the stimulation of the body's protective functions, aimed at fighting destroyed tissues and sepsis (Payne-James, 2016).

Thrombocytopenia in injured dogs may indicate the release of these shaped elements into blood clots that form in the microcirculation due to traumatic shock and its obligatory companion-disseminated intravascular coagulation syndrome (DIC) (Dubova et al., 2020). Thus, a decrease in the number of platelets in the bloodstream is defined as"consumption thrombocytopenia".

Biochemical changes in the blood of sick dogs also confirm the development of a systemic inflammatory process. This is evidenced by hypoproteinemia and a decrease in the albumin-globulin ratio, increasing the globulin fraction as a marker of the inflammatory process.

The multi-organ inflammatory response is represented by an increase in the activity of transaminases in the blood and the content of residual nitrogen products (Dubova & Duboviy, 2018).

An increase in the activity of transaminases AsAT and AlAT is most often associated with massive destruction of tissues and damage to hepatocytes as a result of shock factor exposure and intoxication of the body (Dubova & Duboviy, 2018).

As evidenced by creatininemia and uremia, the development of acute renal failure is secondary to severe trauma. The main factor is a violation of intrarenal blood flow, especially at the level of microcirculation. With injuries such as lacerated wounds, the main causes of severe conditions are traumatic shock and acute blood loss, as well as a toxic-allergic factor (Dubova & Duboviy, 2018; Dubova et al., 2020).

Against the background of the obtained data on assessing the general clinical condition of the injured dogs, treatment was carried out with the inclusion of infusion therapy with nutritional and detoxifying solutions. To combat the septic condition established by laboratory tests, the animals were treated with a combination of antibiotics. Also, a whole complex of external treatments aimed at sanitizing the wound made it possible to stop the processes of destruction and the increase in the intoxication-septic symptom complex (Ariyan et al., 2015).

Cleaning the wound from dead tissue and purulent-inflammatory elements became the foundation for wound healing treatment.

Clinical observations have established that the use of Iruxol ® ointment after five days shows a progressive granulation of damaged elements and a significant reduction in the exposed areas of the bones. It is known that granulation tissue is a young connective tissue that replaces the destroyed elements, edema. Initially, it is gently grainy, juicy, rich in thin-walled vessels (Fig. 4), which is why it has a bright red color (Demidova-Rice et al., 2012; Stevens et al., 2014; Velnar et al., 2009).

In the dynamics of the ointment application, the granulation processes continued progressively and by the end of the 21st day of observation, the wound defect significantly decreased.

The indicated wound-healing effects are due to the enzymes collagenase (EC 3.4.4.19), clostridiopeptidase A (EC 3.4.24.3), and other peptidases (EC 3.4). These enzymes break down the injured tissues' destroyed elements, producing their proteolysis, than the wound is cleaned (Arora et al., 2015; Eckhard et al., 2013; Pham et al., 2012; Stanley et al., 2014; Zhao, 2012). The antibiotic chloramphenicol, which is part of the ointment, provides an antimicrobial effect (Ariyan et al., 2015).

Thus, the best conditions are created to form granulation tissue, which means that the wound heals. Moreover, the scar tissue that replaces the granulation tissue, due to enzymes' action, does not contain rigid collagen fibers and makes the scar the smallest possible with these injuries. Oxyproline, which is released by the action of collagenase and clostridiopeptidase A, has proteinogenic properties. It helps restore the protein components of damaged tissues (Pham et al., 2012; Stanley et al., 2014; Zhao, 2012).

We should note that the treatment of animals with lacerated and crushed wounds should be comprehensive. First of all, measures are taken to restore the body's general state and eliminate the systemic inflammatory reaction, traumatic shock, sepsis, and related organ and system lesions. Against this background, the damage must be repaired in order to stop the destructive processes.

The use of the enzymatic ointment Iruksol ® shows a pronounced wound-healing effect, which allows for 21 days to significantly minimize the tissue defect formed as a result of due to a lacerated and crushed wound.

Conclusion

Lacerated and crushed wounds received by dogs resulting from falling into arc traps are severe injuries with maximum tissue damage in the area of the traumatic factor. The clinical condition of injured dogs is defined as intoxication-septic, accompanied by traumatic shock, sepsis, multi-organ inflammatory response.

Infusion nutritional detoxification therapy, antibiotics, and wound rehabilitation for five days allow to remove injured dogs from a severe clinical condition.

Iruksol ® cream application for 21 days can significantly reduce the wound defect with the formation of a full-fledged granulation and then elastic scar tissue.

The wound-healing effect of Iruxol ® is due to the influence of the proteolytic enzymes collagenase and clostridiopeptidase A, as well as the amino acid oxyproline, which contributes to the destruction of damaged tissues and the formation of new structural components of scar tissue.

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