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Comparative Evaluation of Different Treatment for Purulent Wounds in Dogs

Ahmad Massoud Niazi*, Mohammad Monir Tawfeeq, Amanullah Aziz, Jahid Zabuli, Shahpoor Rahmati, Abdul Razaq Irshad

Clinic Department, Faculty of Veterinary Science, Kabul University, Kabul, Afghanistan
Email: *niazi.massoud@ymail.com

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Abstract

To compare the healing of purulent wounds with Shilajit dressing vs. Vishnevsky dressing in dogs with purulent wounds of >2 weeks of duration, eight dogs were chosen from same ages in two groups *i.e.*, Shilajit and Vishnevsky dressing group. Dressing was done on every day basis for more than two weeks of follow up period. Main outcome of healing measure was completed at three weeks. Wound healing status was assessed at three days intervals till end of three weeks. Shilajit treated achieved complete decrease in the wound surface area, effect of healing score in Shilajit dressing group in comparison to the Vishnevsky dressing group at $p > 0.05$ level of significance. Shilajit is highly effective in achieving a characteristic feature of regenerative and granulation healing of purulent wounds as compared to Vishnevsky dressing group in dogs.

Keywords

Shilajit Wound Dressing, Purulent Wound of Dog, Granulation Tissue

1. Introduction

Wounds often supervised openly in treatment procedure of veterinary medicine [1]. When a wound is treated in second stage from the wound, it is anticipating that the wound should have enough granulation tissue, contraction, and growth of smooth layer of epithelialization to get closed [2] [3]. For wound healing, a lot of medical agents are used; generally these agents have a great variation due to wound condition and host factors [4] [5]. Shilajits have many essential anti-inflammatory agents which are used as independently for treatment of open wounds in many centuries [6]. Generally, Shilajit is a blackish brown exudation found in the serene surroundings of Himalayas. It is also found in most of the sedimentary rocks especially in Afghanistan, Bhutan, China,

*Corresponding author.

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Nepal, Pakistan, USSR (Tien-Shan, Ural and Caucasus), and Tibet as well in Norway [3]-[7]. Shilajit is a herbo-mineral drug, which oozes out from a special type of mountain rocks in the peak summer months. It is found at high altitudes ranging from 1000 to 5000 meters. The biologically important classes of compounds of Shilajit include dibenzo-alpha pyrenes phospholipids, triterpenes and phenolic acids of low molecular weight, fulvic acids: "carrier molecules", humins and humic acids and trace elements (Fe, Ca, Cu, Zn, Mg, Mn, Mo, and P) [8] [9]. These natural materials have been established as potent wound healing medicines, possessing anti-inflammatory, antimicrobial, antioxidant, and proliferative properties [10]. The effectiveness of Shilajit pharmaceutical formulations on wound healing surely has been not proven in clinical studies in animals [11]. May confirmations of some findings have come from animal (mainly rats or mice) and *in vitro* studies [12]. Vishnevsky ointment contains essential agents such as birch tar, xeroformium and castor oil which are used for management of wounds, burns, skin ulcer and suppurations [13]. Vishnevsky ointment was first used in Russia by a Russian surgeon by the name of Alexander Alexandrovich Vishnevsky and it was broadly used in the Soviet army during the World War II [14]. Wound dressing with Vishnevsky ointment is commonly performed in veterinary medicine as part of open wound management [15]. Our hypothesis was that the use of a Shilajit based ointment may be more beneficial for second intention wound healing than dressing with Vishnevsky ointment. The aim of the present study was to evaluate the effectiveness of a Shilajit based ointment on second intention wound healing of surgically created wounds in the dog compared with Vishnevsky ointment.

2. Material and Methods

2.1. Preparation of Shilajit and Vishnevsky Ointments

Purified Shilajit tablets are bought from Pharmacy (Pure Mummia Asel, Bishkek City, Kargyzactan). Then, 30 gr of dried Shilajit tablets were collected and crushed thoroughly in a grinding stone. When they were converted into fine powder mixed with 70 gr of pure Vaseline, after that the mixed pasty preparation was kept overnight at room temperature and was allowed to cool. Now, the Shilajit ointment preparation was ready for application. Vishnevsky ointment which was prepared in 30 gr bought from Pharmacy (Mus Pharma, Russia). And, it is used in open wounds of dogs in research.

2.2. Animals

Research protocol was approved by Animal Care Committee of veterinary faculty. Eight mixed breed dogs (8 males and females) aged 3 to 4 months were used. In order to ascertain the good health of the dogs, a complete physical examination, complete blood (cell) count, and serum biochemical analyses were done on each dog before wound creation. Research animal adjust to a new environment 10 days prior to research. Animals were brought twice a day for exercise. Healthy foods were prepared and were given twice a day for each dog.

2.3. Presurgical Protocol

Preparation of animals for presurgical procedure (day 0), premedication such as Acepromazine in dose of 0.04 mg/kg bodyweight (BW) was given in every dog. In addition, Morphine was used in dose of 0.2 mg/kg Intramuscular (IM) in each dog. For Induction of anesthesia was used the Propofol in dose of 5mg/kg Intravenous and the maintenance stage of anesthesia was kept with isoflurane in oxygen. The dorsal side of each dog was clipped and cleaned with antiseptic solution for operation.

2.4. Surgical and Postoperative Procedure

First of all 5 cm full-thickness wound was created in the dorsal side of each dog, for clinical observation and treatment. The wounds were treated once daily until total healing with Shilajit based ointment in four dogs and other four dogs the wounds are dressed with Vishnevsky ointment until healing of wounds. Both groups' wounds are flushed with normal saline 0.9%. During the dressing time each wound covered with clean bandage for prevention of flies and other infected materials. Complete wound healing was done in 21 days of research.

2.5. Evaluation of Physiological Parameters, Hematology and Biochemistry

During the wound healing physiological parameters, wound sizes, hematology and biochemistry were evaluated in under research dogs. Physiological parameters were collected and recorded everyday basis in all healing pe-

riod. In mean time, the wound sizes were measured by ruler which had size of 10 cm in both groups in daily basis up to more than three weeks in dogs. Moreover, hematology tests were done in Blood analyzer (ERMA INC. Particle Counter, Model PCE-170) and the biochemistry element tests such as Calcium, Albumin and Glucose were completed by assistance of LiquickCor Laboratory Kit (PZ CORMAY S. A. Wiosenna 22, 05-092 Lomiank, Poland) and also other elements such as Total protein and Iron measured by Bio-TEST Kit (ErbaLachema s. r. o Karasek 1d, 621 33 Brno, CZ). Hence all biochemistry elements were weighted accurately in PD-303 Spectrophotometer (APEL Japan).

3. Results

3.1. Statistical Finding

All parameters were evaluated one by one before moving to the next which allowed for more precise comparison of individual. Data were presented as the mean value ± standard deviation of the mean for wound healing size, hematology and biochemistry. Statistical analysis was performed on one-way ANOVA and T-test. Values of $P < 0.05$ were considered statistically significant.

3.2. Wound Size Observation

There was significant ($P < 0.05$) increase in Size of wounds in Shilajit group in day of 9 and 15 in wound healing compared to Vishnevsky group (**Table 1**).

3.3. Physiological Observation

There was significant ($P < 0.05$) decrease in Pulse rate (per min), in 15 day of research. However, the other parameters remained in normal range in period of wounds healing in Shilajit group compared to Vishnevsky group (**Table 2** and **Table 3**).

3.4. Hematological Observation

There was significant ($P < 0.05$) increase of leukocytes in 3 days of research but it decrease in the on the sixth day after treatment almost doubled in subsequent observation days and downward trend is maintained Shilajit group compared to the Vishnevsky group. In mean time, There was significant ($P < 0.05$) decrease in the number of erythrocytes on the 15 day, significant ($P < 0.05$) reduction in concentration of hemoglobin in red blood cells observed on the 3 day and subsequently maintained in normal level other days. Therefore, it was significant ($P < 0.05$) increase in the number of platelets in the 9 days after the start of treatment by almost 2.5 times, apparently due to the healing process in Shilajit group compared Vishnevsky group (**Table 4** and **Table 5**).

Table 1. Observation on wound size changes during the wound healings process (Shilajit and Vishnevsky group) (mean ± SD) (n = 4), *Significant at 5% level with Shilajit group value ($P < 0.05$).

Groups	Post-wound (3 day)	Post-wound (9 day)	Post-wound (15 day)
Shilajit	5.7 ± 0.2	2.2 ± 0..2*	0.5 ± 0.1*
Vishnevsky	6 ± 0.3	3 ± 0.3	1 ± 0.2

Table 2. Observations on physiological parameters (Shilajit group) (mean ± SD) (n = 4), *Significant at 5% level with pre-wound value ($P < 0.05$).

Parameters with units	Pre-wound	Post-wound (3 day)	Post-wound (9 day)	Post-wound (15 day)
Rectal temperature(˚C)	39.43 ± 0.19	39.6 ± 0.04	39.3 ± 0.3	38.8 ± 0.2
Pulse (per min) ± 1.7	118	119.5 ± 1.6	104 ± 1.3	100 ± 5.5*
Respiration (per min)	19.5 ± 0.7	20 ± 0.7	20 ± 0.6	21.5 ± 0.7
Mucous membrane	pale roseate	Pale roseate	Pale roseate	Pale roseate
Capillary refill time (sec)	<2	<2	<2	<2

Table 3. Observations on physiological parameters (Vishnevsky group) (mean ± SD) (n = 4), *Significant at 5% level with pre-wound value ($P < 0.05$).

Parameters with units	Pre-wound	Post-wound (3 day)	Post-wound (9 day)	Post-wound (15 day)
Rectal temperature (˚C)	39.2 ± 0.2	39.6 ± 0.05	39.6 ± 0.07	39.3 ± 0.08
Pulse (per min)	118 ± 2.3	114 ± 2.1	104 ± 2.2	100 ± 1.3*
Respiration (per min)	22 ± 0.6	20 ± 0.7	20 ± 0.09	20 ± 0.1
Mucous membrane	Pale roseate	Pale roseate	Pale roseate	Pale roseate
Capillary refill time (sec)	<2	<2	<2	<2

Table 4. Observations on hematological parameters (Shilajit group) (mean ± SD) (n = 4), *Significant at 5% level with preoperative value ($P < 0.05$).

Parameters with units	Pre-wound	Post-wound (3 day)	Post-wound (9 day)	Post-wound (15 day)
WBC ($\times 10^3/\mu L$)	129.9 ± 51.6	63.1 ± 4.5*	59 ± 7	86.7 ± 8.8
RBC ($\times 10^6/\mu L$)	8.4 ± 1.4	12.1 ± 0.1	13 ± 0.8	10.7 ± 0.3*
HGB (g/dl)	85.1 ± 12.4	106.5 ± 7.4	114.2 ± 10.3	111.3 ± 1.1
LYM (%)	23.5 ± 16.5	6.3 ± 1.7	3.9 ± 0.6	8.7 ± 1.5
MON (%)	13.2 ± 7.8	3.2 ± 0.6	3.3 ± 0.9	3.9 ± 0.5
MCV (f L)	71.5 ± 1.2	78.2 ± 2.7	77.8 ± 3.7	77.6 ± 1.8
MCH (p g)	29.5 ± 1.8	25.7 ± 0.5	33 ± 5.7	25.8 ± 1.2
MCHC (g/d L)	41.5 ± 3	33.3 ± 1.7	46 ± 6.1	33.5 ± 1.9
PLT ($\times 10^3/\mu L$)	830 ± 162.1	584 ± 74.2	680 ± 171	701 ± 194.8
PDW (f L)	16.73 ± 0.6	17.4 ± 0.1	17.4 ± 0.4	16.4 ± 0.3

WBC (White Blood Cell); MCV (Mean Corpuscular Volume); RBC (Red Blood Cell); MCH (Mean Corpuscular hemoglobin); HGB (Hemoglobin); MCHC (Mean Corpuscular Hemoglobin Concentrations); LYM (Lymphocytes); PLT (Platelets); MON (Monocytes); PDW (Platelet Distribution Width).

Table 5. Observations on hematological parameters (Vishnevsky group) (mean ± SD) (n = 4), * Significant at 5% level with preoperative value ($P < 0.05$).

Parameters with units	Pre-wound	Post-wound (3 day)	Post-wound (9 day)	Post-wound (15 day)
WBC ($\times 10^3/\mu L$)	81.8 ± 9.8	90.2 ± 3.5	92.3 ± 3.8	89.3 ± 4.3
RBC ($\times 10^6/\mu L$)	11.6 ± 1.04	12.2 ± 1.3	11.5 ± 1.06	13.6 ± 1.7
HGB (g/dl)	3.4 ± 0.3	3.6 ± 0.1	1.9 ± 0.3	4.8 ± 0.4
LYM (%)	6.5 ± 1.9	7.2 ± 2.9	6.9 ± 2.1	5.9 ± 1.3
MON (%)	3.85 ± 0.6	3.9 ± 1.2	4.5 ± 0.8	3.7 ± 0.6
MCV (f L)	80.8 ± 2.5	83.9 ± 2.5	85 ± 2.6	82.4 ± 1.9
MCH (p g)	25.45 ± 1.3	25.8 ± 1.8	27.2 ± 2.1	23.3 ± 1.2
MCHC (g/d L)	32.85 ± 1.5	34.2 ± 1.7	33.4 ± 1.6	30.7 ± 1.2
PLT ($\times 10^3/\mu L$)	809.25 ± 79	812.3 ± 81	808.9 ± 79.1	802 ± 75.3
PDW (f L)	16.5 ± 0.2	18.3 ± 0.5	19.6 ± 0.7	14.7 ± 0.1

WBC (White Blood Cell); MCV (Mean Corpuscular Volume); RBC (Red Blood Cell); MCH (Mean Corpuscular hemoglobin); HGB (Hemoglobin); MCHC (Mean Corpuscular Hemoglobin Concentrations); LYM (Lymphocytes); PLT (Platelets); MON (Monocytes); PDW(Platelet Distribution Width).

3.5. Biochemistry Observation

There was significant ($P < 0.05$) increase total protein content on Shilajit group in the 3 day compared to pre-wound. It should be noted that at 12 days there is increase in the amount of albumin, these changes indicate normalization general condition of the animal due to the wound healing. There was significant ($P < 0.05$) decline on the 9 day in the amount of calcium and iron, but in the following days of study these indicators have tended to increase. The amount of glucose was varied in the opposite direction it mean that, there was significant ($P < 0.05$) increase on the 3, day, and this trend has continued in the following days of observation compared to pre-wound. Changes in the biochemical composition of blood serum in Vishnevsky group during the observation period remained in the normal physiological level (**Table 6** and **Table 7**).

3.6. Clinical Wound Observation (Day 4)

Clinical observation of wounds in Shilajit group was found on day 4 after the start of treatment mentioned cleansing wounds from purulent exudate and dead tissue monitored, and the wound was covered with granulation tissue (**Figure 1**). Subsequently, as a result of investigations at day 4 in Vishnevsky group the surface of the wound contains a certain amount of purulent exudate and the edges of the wound had edema (**Figure 2**). Our study demonstrated that growth of granulation tissues were more prominent in regions treated with particularly Shilajit dressing in comparison with Vishnesvsky dressing.

Figure 1. Clinical observation of Shilajit group at day 4 after operation which was showed growth of granulation tissues with contracted wound edges. Healing of wound passed normal stage of wound healing. In addition, there was no sign of exudate in wound bed and wound size is reduced to 2.5 cm (original image by Nikon Coolpix 16.0 Megapixals).

Figure 2. Clinical observation of Vishnevsky group at day 4 after operation the surface of the wound contains a certain amount of purulent exudate and the edges of the wound had edema the wound size is reduced to 3.5 cm (original image by Nikon Coolpix 16.0 Megapixals).

Table 6. Observations on biochemical parameters (Shilajit group) (mean ± SD) (n = 4), *Significant at 5% level with preoperative value ($P < 0.05$).

Parameters with units	Pre-wound	Post-wound (3 day)	Post-wound (9 day)	Post-wound (15 day)
Total protein (g/L)	43.5 ± 7.4	67.2 ± 8*	63.2 ± 5.2	55 ± 3.6
Albumin (%)	3.5 ± 0.1	3.5 ± 0.09	2.3 ± 0.3	4.5 ± 0.2
Calcium (mmol/L)	2.77 ± 0.4	2.1 ± 0.1	0.6 ± 0.08*	2.3 ± 0.2
Glucose (mmol/L)	3.3 ± 0.21	5.9 ± 0.6	4.9 ± 0.1	6.2 ± 0.3
Iron (mmol/L)	31.3 ± 7.4	24.4 ± 0.9	21.2 ± 2.1*	28.4 ± 3.2

Table 7. Observations on biochemical parameters (Vishnevsky group) (mean ± SD) (n = 4), *Significant at 5% level with preoperative value ($P < 0.05$).

Parameters with units	Pre-wound	Post-wound (3 day)	Post-wound (9 day)	Post-wound (15 day)
Total protein (g/L)	34.9 ± 6.1	83.2 ± 5.2	79.6 ± 3.1	52.5 ± 4.9
Albumin (%)	3.4 ± 0.3	3.6 ± 0.1	1.9 ± 0.3	4.8 ± 0.4
Calcium (mmol/L)	2.8 ± 0.5	1.7 ± 0.6	0.6 ± 0.4	1.9 ± 0.2
Glucose (mmol/L)	3.7 ± 0.3	5.4 ± 0.4	5.2 ± 0.2	6.8 ± 0.5
Iron (mmol/L)	55.4 ± 8.1	21 ± 3.2	27.2 ± 4.6	34.2 ± 5.3

3.7. Clinical Wound Observation (One and Half Week Post-Operation)

In the clinical wound observation at day 10 in the Shilajit group, after the start of treatment the wound is significantly reduced in size, surface crust in the wound is epithelialization process which result on prevails during healing epithelization planar in the wound bed (**Figure 3**). However, in the Vishnevsky group the wound surface was covered with granulation, and they are fine-grained, contains a certain amount of wound secretion. Swelling, anemic granulation was observed (**Figure 4**).

4. Discussion

Healing of wound is a complicated and running process, however, it has three deferent phases which each phase extending over; the inflammatory, the repair or proliferative, and the remodeling phase [16]. Therapeutic agents has important role in some stage of healing of wound and mainly they have variation in some ways [17]. As mentioned earlier, Shilajit and their derivatives act as potent antimicrobial, anti-inflammatory, and wound healing agents. Their effectiveness in wound healing is attributed not only to their proliferative properties, but also to their anti-inflammatory activity. Therefore, Shilajit based wound healing pharmaceutical preparations modulate both the inflammatory and the proliferative stage of wound healing [18]. In this research, in full-thickness wound not seen any sign of contamination during the healing process. However, the Shilajit dressing had significant healing effect on Shilajit group of wounds. In addition, the swelling of wounds in Shilajit based group was noticeably decreased in days 9 to 12 compared to Vishnevsky dressing group. The most important role of granulation tissue is the shortening of wound, moreover, granulation tissue guard the wound, furnish the border to microbes, and a prepare the smooth layer to epithelialization in wound [6]-[19]. In this research, on day 3 the granulation tissue appeared in Shilajit based wounds and this process completed up to days of 15 of research compared to Vishnevsky based wounds. In the present study, the Shilajit based ointment did significantly improve the rate of wound healing compared to Vishnevsky; the mean wound size as evaluated by planimetry decreased significantly from day 0 to day 15 on Shilajit based wounds. Similar results were obtained from many other therapeutic agents or procedures on open wound healing in dogs; they allowed normal healing or showed minor advantages in some of the proliferative processes on certain days, but they did not actually accelerate the overall wound healing, which usually lasted 3 to 4 week [20]-[24].

5. Conclusion

Based on the observations, the study concludes that the significant changes in the physiological, hematological

Figure 3. Clinical observation of Shilajit group at day 10 after operation, wound is significantly reduced in size up to 0.5 cm and the surface crust in the wound is the epitheliailzation process which in result introduced the planer epithelialiazation (Original image by Nikon Coolpix 16.0 Megapixals).

Figure 4. Clinical observation of Vishnevsky group at day 10 after operation, Although the wound surface is coverd by granulation tissue, the wound contains a certain amount of secration. In additon the wound size is reduced to 1.3 cm (Original image by Nikon Coolpix 16.0 Megapixals).

and biochemical parameters indicate that Vishnevsky is a lavage solution capable of achieving closure of an open wound once it is clean. On the other hand, using of the Shilajit based ointments in clinical canine cases of chronic or contaminated wounds enhances the epithelization and granulation of wounds, which is better demonstrating the effectiveness Shilajit ointment on second intention wound healing in dogs.

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