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Full Length Research Paper

Effect of topical application of mixture of cod liver oil and honey on old (chronic) wounds and granulation tissue in donkeys

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A comparative study was conducted from November 2015 to May 2016 with the objective of investigating the beneficial effects of the mixture of cod liver oil and honey (group 1) comparing with routine treatment (group 2), combination of chlorhexidine (0.3%) and cetrimide (3%) in healing of contaminated old wounds in donkeys at Bahir Dar, North western Ethiopia. Out of 18 donkeys, 12 (6 male and 6 female) were treated with mixture of cod liver oil and honey and 6 donkeys (3 male and 3 female) were routinely treated. At the 35th day of treatment in group 1, the areas of the wounds were markedly decreased from 4.2% to 66.7% and in group 2 from 66.7% to 85.7% out of 100% of the initial area. The treatment outcome between group 1 and group 2 were significantly different ($p<0.05$).In group 1, no swelling and hyperemia of perilesional skin appearance, no inflammatory exudate, reduced wound area and short time to clinical healing of wound were recorded after treatment. This study also demonstrated that difference in wound healing process between sex groups, in which wound healing in male was significantly ($p<0.05$) faster than female. Mixture of cod liver oil and honey is beneficial in treatment of old traumatized wounds in the donkeys. This effect was primarily mediated by formation of healthy mature scars, clinical healing in short period of time. The owners, institutions or organization working with donkeys and governments may use this mixture for treating old traumatized wounds.

Key words: Cod liver oil, donkey, healing, honey, wound.

INTRODUCTION

More than 72% of the world's equine population resides in developing countries kept for draft purpose (Swann, 2006). Ethiopia has more than 6 million donkeys, the second largest donkey population in the world next to China, 1.9 million horses and over 350,000 mules (FAOSTAT, 2012). Equines are important animals to the resource-poor communities in rural and urban areas of Ethiopia, providing traction power and transport services at low cost (Dinka et al., 2006).

In Ethiopia, the rugged terrain characteristics, absence of well-developed modern transport networks and the prevailing low economic status of the community necessitate the use of equines for transportation (Alemayehu, 2004). In rural and per-urban area, people

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used equines to transport crops, fuel wood, water, building materials and people can be transported by carts or on their back from farms and market to the home (Mohammed, 1991).

Despite their uses, the husbandry practices of working equines are poor. Some methods of hobbling to restrain equines cause discomfort and inflict (Alujia and Lopez, 1991; Mohammed, 1991). As per Alujia and Lopez (1991) report, loading of donkeys without padding and over loading in long distance causes external injury on donkeys and mules. Poorly designed harnesses or yokes that may be healthy and ragged have an effect on the animal health and safety (Alujia and Lopez, 1991).

Wound is an open mechanical injury of the epidermis, underlying the tissues and organs. It is characterized by pain, and gaping bleeding functional disturbance. The most common cause of wounds in working equine are over loading, accidents, improper position of load predisposing to falling, hyena bites, donkey bites, injuries inflicted by horned Zebu (DACA, 2006). Some hobbling methods, inappropriate harnesses or yokes that may be heavy and ragged, long working hours may cause discomfort and inflict wounds (Mekuria et al., 2013).

Wounds are one of the primary welfare concerns of working equines (Sells et al., 2010). The type of wound in working donkeys includes tissue damage with or without blood or exudates or pus, abscess formation, or any secondary bacterial complication. Bites (lacerated wounds) will be identified by irregular edges with underlying tissues removed as well as hemorrhage (Sevendsen, 2008).

Wounds can be either traumatic or surgical in origin; both types can fail to heal and become chronic although traumatic wounds are more commonly affected by healing difficulties. The incidence and prevalence of traumatic wounds in equine is considered to be high (Singer et al., 2003) and a high percentage become chronic, adding more complexity to wound healing management strategies.

Skin lacerations and other traumatic injuries of the integument are frequently seen in equine practices and range from relatively minor cuts to severe, potentially debilitating injuries. The challenges facing the practitioner managing these injuries are numerous, and treatment is dictated by the nature and size of the wound, the area of the body on which the wound occurs, and several aspects of wound healing unique to horses. The age of the wound, integrity of the local blood supply, degree of contamination, location of the injury, skin loss, and local tissue damage must all be considered when deciding on the most appropriate method for managing a particular wound. In addition to biologic factors, the physical size of equine patients and the environment in which they are kept present unique management challenges not encountered in the treatment of soft tissue injuries in other species (Jeremy, 2006).Wounds are of great concern in donkeys as they affect animal productivity and their treatment represents an economic burden to the owners particularly in developing countries (Magda and Khaled, 2011). Granulation tissue is the pebbly or granular appearing tissue which develops in healing wounds anywhere on the horse's body. It is composed of small blood vessels and fibroblasts, but has no nerve supply (Christina, 2002).

Treatment methods that are employed in the management of wounds focus on rapid and efficient evaluation, scrupulous, aseptic surgical techniques, and conscientious and prolonged aftercare (Griffiths et al., 2003).

Many therapeutic agents are used for topical treatment of wounds including yeast cell derivatives (Crowe et al., 1999), cod liver oil (Kietzmann and Braun, 2006), honey (Iftikar et al., 2009), sugar (Cavazana et al., 2009), corticosteroids (Jorissen and Bachert, 2009) and phenytoin (Qunaibi et al., 2009). Honey and cod liver oil are increasingly used as natural products and biological therapies in clinical practice. To accelerate wound healing, modern honey wound dressings have become more widely available and used in wound management (Zumla and Lulat, 1989). This is largely due to the growing clinical problems of antibiotic-resistant bacteria and the combined difficulties for the practitioners in managing chronic wounds such as burns and leg ulcers (Lay-flurrie, 2008). Besides antimicrobial effects of honey (Cooper and Molan, 1999), it has anti-inflammatory and antioxidant properties (Gheldof and Engeseth, 2002), promotes moist wound healing and facilitates debridement (Majtán, 2009; pieper, 2009). Cod liver oil is a nutritional supplement derived from liver of cod fish. It has high levels of omega 3 fatty acid, vitamin A and vitamin D. Terkelsen et al. (2000) reported that cod liver oil was beneficial in wound healing as it enhances epithelization and revascularization.

Management of wound in the study area was practice locally which leads to delay in clinical healing, development of old wounds with or without granulation tissue. Due to this poor practice, the owners lost their money, time, working efficiency, and the donkey itself.

Hence, considering the importance of donkey wound management, the topic was built up to investigate the beneficial effects of the mixture of cod liver oil and honey in the contaminated old wounds without any other topical disinfectants or antimicrobial devices. The general objectives of the study include, investigation of the clinical wound healing process with a mixture of cod liver oil and honey in treatment of old wounds and granulation tissue in donkeys and the specific objectives include evaluation of the time taken for clinical healing of old chronic wound.

MATERIALS AND METHODS

Study area

The study was conducted from November 2015 to May 2016 in and

Figure 1. Natural bee honey (A) and cod liver oil (B).

around Bahir Dar (Amhara Region), North Western part of Ethiopia. It is located 564 km from Addis Ababa, capital of Ethiopia. The study area is covers a total of 197,199 hectares of land which has a summer rainy season with the highest rain fall between June and September (1200-1600 mm) and winter dry season (December to March) with mean annual temperature of 23°C. Located 11'29''N latitude, 37'29''E longitude and with altitude range of 1500-2300 meters above sea level (ANRSAB, 1999).

Topography of the area is characterized with slight slopping covering about 70% of a total land of area, and marked with Lake Tana and Abay River (ANRSAB, 1999). The land is covered by various, low woods, and mainly evergreen plants of various types of, with vegetation cover of land. The main agricultural product is teff, barley, sorghum, wheat, maize and all pulse crops (ANRSAB, 1999). The region has 1.4 million cattle, 1.3 million sheep and goats and 2.8 million equines of which the figure in Bahir Dar and its surroundings are estimated to have about 58 horses, 550 mule and 19517 donkeys (CSA, 2010).

Study population

The study was conducted on both sexes of the local breeds of donkeys affected with chronic and old wounds. Donkeys in the study area were mainly used for water, grain, stone, and fire wood transportation. Wounds are mainly due to car accident, heavy loading, loading without padding, improper tying of legs with rope and loading of hot flour. Those donkeys exposed to wound and/or granulation tissue were treated with cod liver oil and honey mixture (n=12) and with routine treatment (n=6) within a given time.

Selection of animals

A total of 18 adult donkeys of both sexes having wounds admitted in the Donkey Sanctuary Veterinary Service Centers for treatment of wounds were used.

Materials used during the study

Cod liver oil, tap water, natural honey, savlon (trade name), cotton gauze, ordinary ruler, shaving blades, 50 ml syringe, cotton and curved and straight scissors (Figure 1).

Treatment of old wounds in donkeys with mixture of cod liver oil and honey

12 donkeys (6 male and 6 female) were presented in the Donkey Sanctuary Veterinary Service Center. After clinical examination, all donkeys were suffering with old and heavily traumatized wound along with the infection on the wound surface. Most wounds were located on the caudal back region of the donkeys. All of these wounds were caused due to heavy loading, loading without padding, improper loading of hot flour, and frequent loading with water for cultivation of chat in the area.

Treatment procedure

Before treatment history was recorded related to age, sex, breed and consent for treatment from the owner of the donkey was obtained. Treatment of wounds was carried out in the following steps: (1) the whole area around the wound up to about 5 cm length from the wound edges was clipped with curved scissor and shaved with shaving blade. (2) The Presence of Inflammatory Exudates (PIE) and Perilesional Skin Appearance (PSA) were assessed. (3) Its area was calculated by multiplying the two largest dimensions to assess the initial size and evaluate the progress in the healing process using ordinary ruler. (4) Grossly contaminated wounds were washed with slight warm water with body temperature water using syringe. (5) Then wound surface was covered with piece of gauze soaked in a mixture of an equal volume of cod liver oil and honey. The amount of the mixture varied according to the wound size. Generally, about 20 ml of the mixture will be used for 100 cm^2 dressing area on the body of the animal. (6) The frequency of changing dressing was decided with how rapidly the mixture is diluted with exudates. The bandage was changed daily up to seven days, every third day for two weeks and then once a week till clinical union of the wound took place. (7) At the end of treatment, the time required for clinical union of the wound, the remaining area, the perilesional skin appearance and the presence of inflammatory exudate were recorded.

Treatment of old wounds in donkeys with routine treatment savlon (chlorhexidine 0.3 + cetrimide 3%)

To investigate the whole effect of the mixture of cod liver oil and honey on the healing of wounds, its effects were compared with the routine treatment. Six donkeys (3 male and 3 female) with old wounds were prepared for this treatment. The wound area was aseptically prepared about a 5 cm from the edge of the wound. Hemorrhage was controlled by pressing of the wound surface for 10 minduring preparation. The wound surface was covered with piece of gauze soaked in savlon. The bandage was changed periodically daily for seven days, and every third day for two weeks and then once a week till the 35th day of treatment. In addition a combination of penicillin (8 mg) and dihydrostreptomycinsulphate (10 mg) per kg

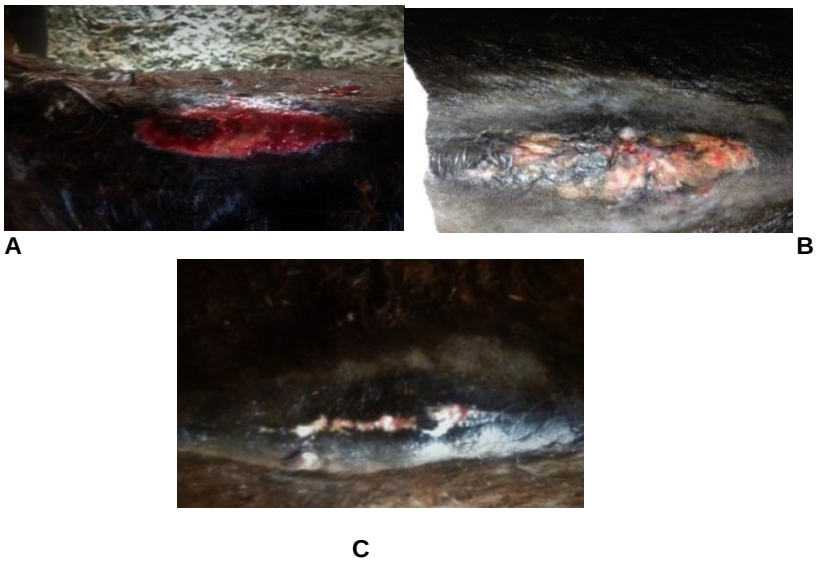

Figure 2. A. After cleaning with tap water. **B.** After one week of treatment with mixture of cod liver oil and honey. **C.** After three weeks of treatment with a mixture.

body weight or 1 ml penicillin and streptomycin per 25 kg bodyweight was given for three days to control the bacterial infection of the wound. The area of the wound, the time required for clinical union, PSA and PIE of the wound were recorded.

Statistical analysis

The data was entered into Mc-soft excel spread sheet and analyzed by STATA version 12.0. Independent t-test for the two groups of treatment and dependent t-test for sex difference within one group of treatment were utilized.

RESULTS

Treatment of old wounds in donkeys with mixture of cod liver oil and honey

The clinical healing of wounds in treated cases with the mixture of cod liver oil and honey took place in a period of time ranged from two to five weeks. It was found that surgical debridement at the beginning of treatment was important in most cases due to presence of granulation tissue. Washing of the wound with tap water was seen to be very successful in removal of debris, necrotic tissue and pus as well as helpful in refreshment of the wounds surface (Figures 2 and 3).

After one week of treatment, the wounds surface appeared bright red in color, moist and not elevated above wound edges. After two to three weeks of treatment, wounds in all cases showed cleanness and healthy surface and observable decrease in the wound surface. Wounds areas were markedly decreased after five weeks of treatment. After four weeks of treatment in eight cases, the remaining wound areas out of 100% of the initial area were from 4.2 to 37.5% and there was no swelling and hyperemia at the perilesional skin appearance (PSA) and no inflammatory exudate (PIE) over the surface of the wound. The rest four cases showed that for 50 to 66.6% area from the initial area, there was mild swelling and hypereamia and thick crust over the wound surface (Table 1).

Wound healing difference between Sex group

Area, perilesional skin appearance (PSA), presence of inflammatory exudate (PIE) and time required to clinical union of wound were significantly different ($p< 0.05$) between both sex groups. In two cases, male donkeys showed remaining wound areas of 30 and 37.5% and in five cases, it was between 4.2 to 17% out of 100% of the initial area. The perilesional skin appearances and presence of inflammatory exudate in male were none except one which showed mild swelling and hyperemia and thick crust over the wound surface while in the female donkey, there was mild swelling and hypereamia and thick crust over the surface of the wound except one case which showed no exudate. The time required for clinical union in male cases took place between a time range of two to five weeks whereas in females four to five weeks.

Treatment of old wounds in donkeys with routine treatment savlon (Chlorhexidine0.3%+cetrimide 3%)

All treated cases with routine treatment, not showing

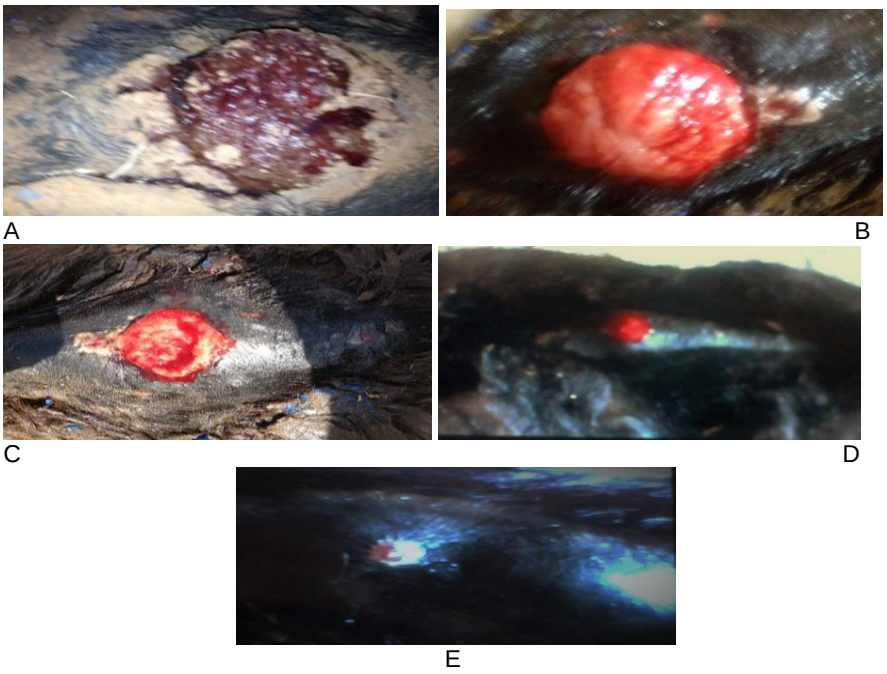

Figure 3. A. Before cleaning. **B.** After cleaning. **C.** After surgical removal of granulation tissue. **D.** After two weeks of treatment with mixture of cod liver oil and honey. **E.** After four weeks of treatment with mixture.

clinical union of wounds within a given period of time (35 days) and the area of wounds were not decreased(Figure 4). After six weeks of treatment only thick exudate was observed (Table 2).

Wound healing difference between two treatment groups

The times taken to clinical union of wound, area, perilesional skin appearance (PSA), and presence of inflammatory exudate (PIE) were significantly different ($p< 0.05$) between the two treatment different groups (Tables 3, 4, 5, 6, 7, 8, 9 and 10).

DISCUSSION

When treating equine wounds, the primary goal is to obtain rapid wound healing with a functional and aesthetically satisfactory outcome. Dressing are used to enhance and support the healing process by decreasing contamination, oedema or exudate, protesting against movement and further trauma and optimizing moisture, temperature, pH and gaseous exchanges at the wound site (Knottenbelt, 2003). Equines are known for their tendency to wound probably due to their inquisitive nature, large size and confinement in areas with potential obstacles such as metal or wire leads in their known difficulties with healing. This study shows the beneficial effect of mixture of cod liver oil and honey in treatment of old traumatized chronic wounds in donkeys. In clinically treated wounds, it was found that surgical debridement washing with slight warm water were valuable steps in removing granulation tissue and debris and in minimizing infection. Formation of granulation tissue in wounds usually occurs as the result of weakness of the initial inflammatory response of wound which leads to chronic inflammation which further inhibits wound contraction and promotes exuberant granulation tissue formation (Wilmink and Weeren, 2005).

Application of mixture of cod liver oil and honey, after debridement, on and around the wounds, brought significance in the requirement of the infection, and observable decrease of wound surface after two weeks to five weeks of treatment. These effects of the mixture appeared to be mediated by the effects of honey, and vitamin A and omega-3 fatty acids in cod liver oil. Gethin et al. (2008) reported that honey dressings were associated with significant reduction in non-healed chronic superficial ulcers. Terkelsen et al. (2000) reported that vitamin A had an important role in accelerating wound healing process. McDaniel et al. (2008) reported that omega-3 fatty acids in cod liver oil can increase in the wound healing through increasing pro-inflammatory cytokines production at wound sites. After application of the mixture, all wounds were covered with protective bandages which were advantageous in controlling

Table 1. Area, PSA, PIE, time taken to clinical union of the wound in group 1.

Number	Sex	Area(size)of wound before treated with oil-honey mixture (cm²)	PSA before treatment	PIE before treatment	Area of after treatment (cm²)	PSA after treatment	PIE after treatment	Time taken to clinical union of wound (in weeks)
W1	M	4*3=12	Swollen and hypermia	Thin	2*1=2(17%)	No swelling and hypereamia	None	2
W2	F	7*4=28	Swollen and hypermia	Thin	5*3=15(53.6%)	Mild swelling and hypermia	Thick crust over the wound surface	4
W3	M	6*3=18	Swollen and hypermia	Thin	1*1=1(5.6%)	No swelling and hypereamia	None	3
W4	M	8*5=40	Swollen and hypermia	Thin	4*3=12(30%)	No swelling and hypereamia	None	3
W5	F	6*4=24	Swollen and hypermia	Thin	4*3=12(50%)	Mild swelling and hypermia	Thick crust over wound surface	5
W6	M	5*3=15	Swollen and hypermia	Thin	1*1=1(6.6%)	No swelling and hypereamia	None	3
W7	M	7*5=35	Swollen and hypermia	Thin	2*2=4(11.4%)	No swelling and hypereamia	None	3
W8	F	6*5=30	Swollen and hypermia	Thin	5*4=20(66.7%)	Mild swelling and hypermia	Thick crust over wound surface	5
W9	F	5*4=20	Swollen and hypermia	Thin	3*3=9(45%)	No swelling and hypereamia	Thick crust over wound surface	4
W10	M	4*3=12	Swollen and hypermia	Thin	1*0.5=0.5(4.2%)	No swelling and hypereamia	None	5
W11	F	6*3=18	Swollen and hypermia	Thin	3*2=6(33.3%)	No swelling and hypereamia	None	4
W12	F	8*4=32	Swollen and hypermia	Thin	6*2=12(37.5%)	Mild swelling and hypermia	Thick crust over wound surface	5

bleeding, reducing the tendency for granulation tissue formation, absorbing exudates, keeping the wounds moist which helps epithelization, protecting the wound from contamination, dust and flies and keeping the topical mixture used better in contact with the wound surface. In wounds treated with routine treatment (group 2) there was very slow clinical healing process and not significant decrease in wound size when compared to those treated with the mixture (group 1). Similar observations reported by Iftikhar et al. (2010) found that honey increased epithelization in wound models in rats. Majtán et al. (2010) observed that honey increased metalloproteinase-9 in cultured human keratinocytes. Metalloproteinase-9 was observed to degrade type IV collagen in the basement membrane and further facilitate migration of keratinocytes (kyriakides et al., 2009). Regarding cod liver oil, it was reported that topical application of cod liver oil ointment to surgically-induced full thickness

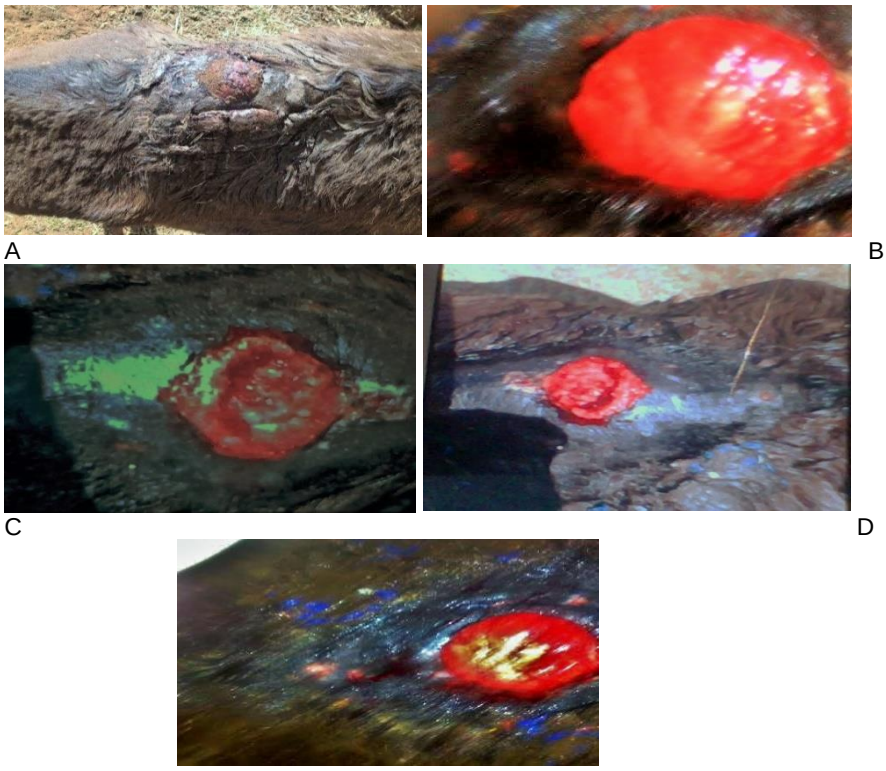

Figure 4. **A.** Before cleaning the wound. **B.** After cleaning. **C.** After surgically removing the granulation tissue. **D.** After three weeks of treatment. **E.** After five weeks of treatment.

Table 2. Area, PSA, PIE and time taken to clinical union of the wound.

Number	Sex	Area (size) of wound before treatment (cm²)	PSA before treatment	PIE before treatment	Area (size) of wound after treatment(cm²)	PSA after treatment	PIE after treatment	Time to clinical union of the wound (in weeks)
W1	F	7*2=14	Swollen and hyperemia	Thin	6*2=12(85.7%)	Swollen and mild hypereamia	Thick	No clinical union took pace
W2	F	6*2=12	Swollen and hyperemia	Thin	4*2=8(66.7%)	Mild swelling and hypereamia	Thick	No clinical union took pace
W3	M	5*3=15	Swollen and hyperemia	Thin	5*2-10(66.7%)	Mild swelling and hypereamia	Thick	No clinical union took pace
W4	M	6*4=24	Swollen and hyperemia	Thin	5*4=20(83.3%)	Swollen and hypereamia	Thin	No clinical union took pace
W5	M	4*3=12	Swollen and hyperemia	Thin	3*2=6(50%)	Mild swlling and hypereamia	Thick	No clinical union took pace
W6	F	3*2=6	Swollen and hyperemia	Thin	2*2=4(66.7%)	Swollen and hypereamia	Thin	No clinical union took pace

dermal wounds on the ears of mice resulted in faster epithelization than those coated with vaseline vehicle (Terkelsen et al., 2000). Vitamin A and D in cod liver oil are responsible for such effects. The mixture produced good results in clinically admitted wounds at the end of fourth week. This appeared due to formation of healthy scar that showed higher degree of maturity with an increasing number of fibrocytes and parallel collagen fibers.

This study also demonstrated some sex differences in

Table 3. Area of wound between group 1and 2 after treatment.

S/N	Group 1	Group 2
W1	2*1=2(17%)	6*2=12(85.7%)
W2	5*3=15(53.6%)	4*2=8(66.7%)
W3	1*1=1(5.6%)	5*2-10(66.7%)
W4	4*3=12(30%)	5*4=20(83.3%)
W5	4*3=12(50%)	3*2=6(50%)
W6	1*1=1(6.6%)	2*2=4(66.7%)
W7	2*2=4(11.4%)	
W8	5*4=20(66.7%)	
W9	3*3=9(45%)	
W10	1*0.5=0.5(4.2%)	
W11	3*2=6(33.3%)	
W12	6*2=12(37.5%)	

Table 4. Statistical analysis of area between group 1 and 2, ($p< 0.05$).

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
var1	6	1.5	0.34	0.84	0.62	2.38
var2	6	2.83	0.17	0.41	2.40	3.26
Diff	6	-1.33	0.49	1.21	-2.60	-0.06

Table 5. PIE between group 1 and 2 after treatment.

S/N	Group 1	Group 2
W1	None	Thick
W2	thick crust over the wound surface	Thick
W3	None	Thick
W4	None	Thin
W5	thick crust over the wound surface	Thick
W6	None	Thin
W7	None	
W8	thick crust over the wound surface	
W9	thick crust over the wound surface	
W10	None	
W11	None	
W12	thick crust over the wound surface	

Table 6. Statistical analysis of PIE between group 1 and 2, ($p< 0.05$).

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
var1	6	0.33	0.21	0.52	-0.21	0.88
var2	6	2.33	0.21	0.52	1.79	2.88
Diff	6	-2	0.37	0.89	-2.94	-1.06

clinical healing of wound. Clinical requirement of wound in male donkeys were significantly ($p<0.05$) faster than female donkeys. This may be due to the fact that in male there is larger dermal thickness compared to female, a parameter that has been reported to be under the influence of male hormones (Azzi et al., 2005). A direct

Table 7. PSA between group 1 and 2 of treatment.

S/N	Group 1	Group 2
W1	No swelling and hypereamia	Swollen and mild hypereamia
W2	Mild swelling and hyperemia	Mild swelling and hypereamia
W3	No swelling and hypereamia	Mild swelling and hypereamia
W4	No swelling and hypereamia	Swollen and hypereamia
W5	Mild swelling and hyperemia	Mild swelling and hypereamia
W6	No swelling and hypereamia	Swollen and hypereamia
W7	No swelling and hypereamia	
W8	Mild swelling and hyperemia	
W9	No swelling and hypereamia	
W10	No swelling and hypereamia	
W11	No swelling and hypereamia	
W12	Mild swelling and hyperemia	

Table 8. Statistical analysis PSA between group 1 and 2, ($p< 0.05$).

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
var1	6	0.33	0.21	0.52	-0.21	0.88
var2	6	1.5	0.22	0.55	0.93	2.10
Diff	6	-1.17	0.40	0.98	-2.20	-0.13

Table 9. Time taken to clinical union of the wound between group 1 and 2 of treatment.

S/N	Group 1	Group 2
W1	2 weeks	no clinical union took pace
W2	4 weeks	no clinical union took pace
W3	3 weeks	no clinical union took pace
W4	3 weeks	no clinical union took pace
W5	5 weeks	no clinical union took pace
W6	3 weeks	no clinical union took pace
W7	3 weeks	
W8	5 weeks	
W9	4 weeks	
W10	5 weeks	
W11	4 weeks	
W12	5 weeks	

Table 10. Statistical analysis of time taken to clinical union of the wound between group 1 and 2, ($p< 0.05$).

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
var1	6	1.33	0.21	.52	0.79	1.88
var2	6	3	0	0	3	3
Diff	6	-1.67	0.21	0.52	-2.21	-1.12

relationship between dermal thickness and collagen content, and thereby skin strength or mechanical resistance, has been suggested (Shuster et al., 1975). In relation to skin injury, the expression of collagen is markedly increased in fibroblasts in the dermis. This is followed by an extensive remodeling phase, in which the

collagen content is degraded by the coordinated action of several collagenolytic proteases, whose expression and activation have been reported to depend on plasmin (Pins et al., 2000).

CONCLUSION AND RECOMMENDATIONS

This study demonstrated that cod liver oil and honey mixture was beneficial in healing of old traumatized wounds in donkeys before and after treatment, the time required to clinical healing of the wound, perilesional skin appearance and presence of inflammatory exudate. In conclusion, usage of mixture of cod liver oil and honey for old wound help in donkeys early clinical healing, reduce extra expenditure of money and time. Study also demonstrated that there was a significant difference in wound healing between sex groups.

Based on the important major findings and conclusion drawn, the following recommendations are forwarded: The owners, institutions or organization working with donkeys can use this mixture for treating old traumatized wounds as it enhances early wound healing, formation of healthy scar and can reduce risk of antibiotic resistance and also the knowledge of importance and usage of the mixture of cod liver oil and honey in wound healing may be transferred to the community for adoption.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Abbreviations: FAOSTAT, Food and Agricultural Organization Statistical; **DACA,** Drug Administration and Control Authority; **PSA,** perilesional skin appearance; **PIE,** presence of inflammatory exudate; **SIS,** small intestine submucosa; **LYCD,** live yeast cell derivatives; **BCE,** before christian era; **PI,** povidone iodine; **VEGF,** vascular endothelial growth factor; **ATP,** adenosine triphosphate; **DNA,** deoxyribonuclic acid; **MRSA,** methicillin resistant *Staphylococcus aureus*; **HP,** hydrogen peroxide; **ANRSAB,** Amhara National Regional State Agriculture Bureau; **CSA,** Central Statistical Agency; **CM,** centimeter; **ML,** milliliter; **MG,** milligram; **KG,** kilogram.

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