Pakistan Journal of Veterinary and Animal Research

www.pjvar.com; ISSN: 3106-3055 (ONLINE)

RESEARCH ARTICLE

Chitosan gel with Varanus Griseus Body Oil for General Wounds Healing

Muhammad Irfan Haider Jafri[1]*, Tahira Ruby[2], Seemab Fatima[1], Sana Shoukat[3], Muhammad Fahad[2], Muhammad Irfan[2], Kamla Zafar[1]

[1]Department of Zoology, The Islamia University of Bahawalpur, Pakistan; [2]Institute of Zoology, Bahaudin Zakariya University Multan, Pakistan; [3]Department of Animal Nutrition, The Islamia University of Bahawalpur, Pakistan.

*Corresponding author: haider.irfan115@gmail.com

ARTICLE HISTORY
(PJVAR-26-09)

Received: March 15, 2026
Accepted: May 16, 2026
Online: May 30, 2026

Keywords:
Chitosan gel
Wounds healing
Varanus Griseus
Ethno zoological

ABSTRACT

The current study documented the folk knowledge related to the practice of lizard (*Varanus griseus*) products and ethno zoological based drugs used as medicines by the residents of the Cholistan desert of Bahawalpur (Pakistan). In this study, wound healing assay was used to evaluate the healing time in response to five treatments viz chitosan gel, *V. griseus* body oil, chitosan gel combined with *V. griseus* body oil, saline solution (negative control) and FUDIC –H cream (positive control. Wound healing observations were made on day 0, day 3rd, day 7th, day 14th and day 21st. On day 3, best wound healing activity was shown by positive control. On day 7, best wound healing activity was shown by mixture of chitosan gel and oil (group III) and antibiotics (group V). On day 14, best wound healing activity was shown by mixture of chitosan gel and oil (group III) and antibiotics (group V). On day 21, wound was fully recovered in group III and group V rats. Differences among the groups in the total healing time and in the proportional daily reduction of the incision consistently showed that the *Varanus griseus* oil and chitosan-based treatment resulted in a faster healing process compared to the commercial ointment tested. The literature suggests that the efficacy of animal fat on healing may be primarily related to the presence of fatty acids, which have been widely demonstrated to be important for biochemical reactions involved in healing processes. Our findings explored Rohi (Cholistani) communities using *Varanus griseus* oil in folk medicine do not have easy access to pharmacies and hospitals. Based on this work we could recommend that this type of abandoned knowledge should be considered for the management and sustainable use of faunistic resources.

To Cite This Article: Jafri MIH, Ruby T, Fatima S, Shoukat S, Fahad M, Irfan M and Zafar K, 2026. Chitosan gel with Varanus Griseus Body Oil for General Wounds Healing. Pak J Vet and Anim Res, 2(1): 50-55.

INTRODUCTION

The skin is a protective barrier against physical damage, water loss and invasion of toxic agents. The skin has a multilayer structure, and any component has specific properties. The epidermis or outer layer of skin, mainly consisting of keratinocytes, it represents the main barrier against external environmental factors such as ultraviolet radiation (UV), pathogenic microbial agents, and mechanical disturbances. The immediately adjacent layer is dermis or middle layer, some connective tissue rich in collagen; it is a thicker layer, which consists of extracellular matrix (ECM), living cells, nerve endings and blood vessels, providing energy and nutrition to the epidermis. Fibroblasts represent the main cell population of the dermis. They release collagen and elastin which are responsible for mechanical strength and elasticity of the skin. Dermis and epidermis are in constant communication to maintain and eventually restore the hemostasis. The deepest layer is hypodermis or subcutaneous layer. It is constituted by fat tissue and it is responsible for thermal isolation and mechanical protection of the body (Gurtner *et al*., 2008; Kamoun *et al*., 2017).

Therefore, wound healing is a multi-factorial physiological process, but its complexity can result in several irregularities. In these cases, a wound treatment must promote and guide the healing process. A general care procedure includes swabbing for infection, cleaning of the wound bed from tissue debris, even if it needs to the split-thickness skin auto-grafts or allo-grafts, and finally applying the wound dress (Dreifke *et al.*, 2015). Due to the complexity of healing mechanisms, in which several factors are involved and due to the big variety of wound types, the choice of an adequate wound dress is crucial. Successful wound management relies on an accurate assessment of the wound type and the patient, combined with know-how on the features of potential dressing materials. An ideal wound dressing should promote rapid healing, with minimal inconveniences for the patient. In particular, it should be able to remove excessive exudate, improve autolytic debridement, and keep the moisture adequate for healing. Along with these properties, the wound dress should be flexible, adhesive and easy to remove (Suarato *et al*., 2018).

Chitosan allows rapid blood clotting and has gained US approval for use in bandages and other haemostatic agents. The product reduces blood loss compared to gauze and increases patient survival. These products were sold to the United States Army which has already made use of the bandages. Chitosan is hypoallergenic and has natural antibacterial properties. Among the applications, it has also been used in hemodialysis membranes (Becker *et al*., 2000). Many authors have already proposed and studied the application of chitosan in wound healing. Chitosan membranes are not cytotoxic when tested with fibroblasts, the human wounds treated with chitosan membranes heal faster, the chitosan oligosaccharides from their enzymatic degradation have a stimulating effect on macrophages and attract neutrophils to the lesion site. The action of chitosan-based dressings on acute and chronic skin wounds, verified in studies, demonstrates its performance in all stages of the healing. Porous membranes of chitosan provide excellent permeability to oxygen, control water loss through evaporation and promote drainage of exudate from the wounds (Balbino *et al*., 2005; Becker *et al*., 2000; Berger *et al*., 2004).

Chitosan gel has to show potential to be used as a wound healing system. Ideal properties include: the ability to stimulate the proliferation of fibroblasts; to inhibit the growth of inflammatory cells; to act against bacteria and fungi; to stimulate the activation of macrophages and neutrophil migration; to promote re-epithelialization; to provide a lower degree of fibroplasia; to stimulate the immune system; to be oxygen permeable; low-toxicity; promote bio-adhesion, to have hemostatic potential; and to have antitumor action. All these properties contribute to medical and pharmaceutical application (Park *et al*., 2009).

The wound healing process involves the stages of hemostasis, inflammation, migration, proliferation, and cell maturation (Honnegowda *et al*., 2015). The presence of infections and contamination by bacteria can delay this process, therefore it is necessary to use medicines that keep the place moist and that allow gas exchange, and also the use of dressings that are easily removable or absorbable (Wang *et al*., 2012).

Chitosan is able to promote adequate formation of granulation tissue, due to the correct deposition of collagen fibres, leading to correct repair of lesions on the skin. The main biochemical effects of chitin and chitosan the activation of fibroblasts, production of cytokines, accelerates the migration of macrophages and stimulation of type IV collagen synthesis. In the studies of a large number of patients with traumatic wounds were treated with chitosan and glycolato dressings and satisfactory results were observed in all cases. So many studies emphasize the combination of chitosan with other materials to improve its effect on healing. Another studies, combined chitosan with other substances and observed acceleration of wound closure and thus, faster healing (Becker *et al*., 2000). The higher the degree of de-acetylation, the greater the efficiency of chitosan in the modulation of human fibroblasts mitogenesis and the greater the cell adhesion and proliferation, both for keratinocytes and fibroblasts, a fact observed in the study by (Ueno *et al*., 2001; Chatelet *et al*., 2001). This increased cell adhesion and proliferation was associated with the fact that, even at neutral pH, there are still cationic sites in chitosan chains, allowing electrostatic interaction with negative charges on the surface of the cell membranes. The antimicrobial activity is believed to be due to the interaction of the amino-positive groups of the glucosamine units with the negative components of the cell walls of the bacteria, suppressing the biosynthesis. It is also observed that chitosan interrupts the transport of nutrients through the cell wall and causes the leakage of cellular organelles, accelerating the death of the bacteria.

Another mechanism studied is the penetration of chitosan of low molar mass in the cell, which binds to DNA, inhibiting transcription and translation (Sohn *et al.,* 2009; Chatelet *et al*., 2001).

Therefore, wound healing is a multi-factorial physiological process, but its complexity can result in several irregularities. In these cases, a wound treatment must promote and guide the healing process. A general care procedure includes swabbing for infection, cleaning of the wound bed from tissue debris, even if it needs to the split-thickness skin autografts or allografts, and finally applying the wound dress (Dreifke *et al.,* 2015). Due to the complexity of healing mechanisms, in which several factors are involved and due to the big variety of wound types, the choice of an adequate wound dress is crucial. Successful wound management relies on an accurate assess-ment of the wound type and the patient, combined with know-how on the features of potential dressing materials. An ideal wound dressing should promote rapid healing, with minimal inconveniences for the patient. In particular, it should be able to remove excessive exudate, improve autolytic debridement, and keep the moisture adequate for healing. Along with these properties, the wound dress should be flexible, adhesive and easy to remove (Suarato *et al*., 2018).

Chitosan gel associated with other substances may acquire new properties that optimize and extend its applicability and efficiency in wound healing (Gomes *et al*., 2005). In this work, the substance associated with the chitosan gel was *Varanus griseus* body oil which presents an adequate profile in bioactive and antioxidant compounds and explored wound healing property and can be used as zootherapeutic agent.

MATERIALS AND METHODS

The chitosan gel of medium degreedeacetylation (95%) was obtained from Primex company. *Varanus griseus* body oil was obtained from body fats of the sad animal commonly present in Cholistan desert. Pure chitosan gel (CG) was obtained from the reaction of chitosan powder with 2.0% aqueous acetic acid solution. 3.0 g of chitosan was used for 100 mL of acetic acid solution. The solution was stirred for 30 min. To obtain the chitosan gel with *Varanus griseus* body oil 1.0 mL of the oil was added to the gel and stirred for 10 min.

Ethical approval: This study was carried out according to the guidelines set by the ethics committee for animal handling, Institute of Pure and Applied biology, B.Z.U, Multan.

Study design: The rats of both sexes males and females from 2 to 4 months' age with body mass around 200-250 gms were obtained from the Pharmacy Department, Bahauddin Zakariya University, Multan. The animals were kept under monitored conditions at a temperature equivalent to 26 ± 1 ◦C, with feed and water ad libitum, and a 12 h light/dark cycle. The animals were randomly divided into distinct groups of twelve specimens, according to a specific treatment scheme. The topical treatment with saline and collagenase ointment (negative and positive control groups, respectively), the pure chitosan gel (CG) and mixture of gel with *Varanus griseus* body oil.

After intramuscular anesthesia in rat, composed of xylazine hydrochloride (0.04 mL/100 g and 10 % ketamine hydrochloride 0.08 mL/100 g). Dorsal region trichotomy was performed by circular removal of the tissue with skin and approximately 0.6 cm in diameter with the aid of a puncture to form a skin wound with exposure of the dorsal muscle fascia. Finally, the animals were treated daily with an application of approximately 10 mg ointment, saline solution, saline solution and 10 mg gel, were applied to each animal kept under observation. The non-sutured wounds were not covered with any kind of dressing.

The wound treatment of each group was administered topically in the injured region, with the products assigned to each group. The wounds were cleaned with saline solution every day. The animals were monitored on daily basis to check the recovery rate of wound. The animals treated with the active principles and the control groups under analysis were followed daily by observing the lesion repair, referring to the changes regarding the presence or absence of edema, exudate, crust and wound coloration. Digital photographic recording of the wound of all animals of the experimental groups was daily performed during the treatment, and the lesions size was measured with the aid of an analog caliper on 1st, 3rd, 7th, 14th, and 21st day of treatment.

RESULTS

Wound Healing activity:

Day 0: Rats were grouped into five groups on the basis of treatment. Group I was treated with only chitosan gel. Group II was treated with *Varanus griseus* body oil. Group III was treated with chitosan gel and *Varanus griseus* body oil. Group IV was negative control (saline solution). Group V was positive control (FUDIC-H). On day 0, wound incision was done with the help of punch machine in five groups of rats. On day 0, wound was measured with the help of transparent scale as shown in Table 1 and Fig. 1 (A-E). All the groups were incised a wound of 20 mm (diameter).

Day 3rd: Variable wound healing activity was observed in all the five groups of rats (Fig. 2 (A-E). In Group I animals, wound diameter was calculated as 16 mm. In Group II animals, wound diameter was calculated as 16 mm. In Group III animals, wound diameter was calculated as 17 mm. In Group IV animals, wound diameter was calculated as 18 mm as shown in Table 1. In Group V animals, wound diameter was calculated as 16 mm.

Table 1: Record of wound healing activity under different treatment conditions.

Days/Treatments	Group 1 (G)	Group 2 (O)	Group 3 (G+O)	Group 4 (SS)	Group 5 (P)
Day 0	20 mm	20 mm	20 mm	20 mm	20 mm
Day 03	16 mm	16 mm	17 mm	18 mm	16 mm
Day 07	13 mm	13 mm	10 mm	12 mm	10 mm
Day 14	06 mm	06 mm	03 mm	08 mm	03 mm
Day 21	02 mm	02 mm	Recover	03 mm	Recover

G= gel, O=Oil, G+O= gel and oil, SS= saline solution (negative control), P=positive control

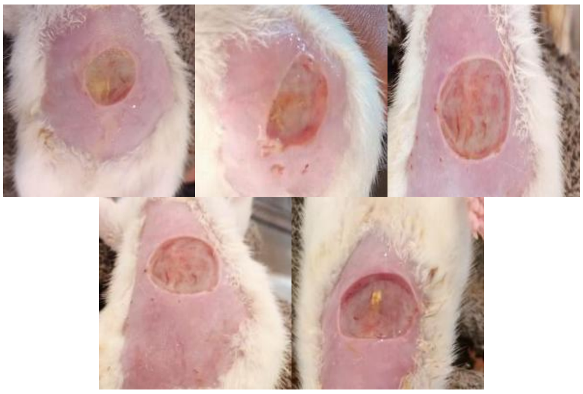

Fig. 1(A-E): Treatment of Rats with gel, oil, gel+oil, saline solution and Fudic-H on day 0.

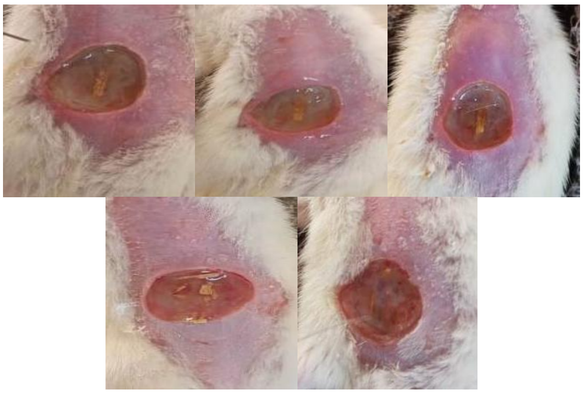

Fig. 2(A-E): Treatment of Rats with gel, oil, gel+oil, saline solution and Fudic-H on day 3rd.

Day 7th: Variable wound healing activity was observed in all the five groups of rats as shown in Fig. 3 (A-E). In Group I animals, wound diameter was calculated as 13 mm. In Group II animals, wound diameter was calculated as 13 mm. In Group III animals, wound diameter was calculated as 10 mm demonstrating the best material for the healing activity. In Group IV animals, wound diameter was calculated as 12 mm. In Group V animals, wound diameter was calculated as 10 mm (Table 1).

Day 21st: Variable wound healing activity was observed in all the five groups of rats for the last day of trail. In Group I animals, wound diameter was calculated as 02 mm. In Group II animals, wound diameter was calculated as 02 mm. In Group III animals had fully recovered. Similar results were observed in case of group V rats. In Group IV animals, wound diameter was calculated as 03 mm. In Group V animals, wound diameter has recovered completely as shown in Fig. 5 (A-E).

At day 3, best wound healing activity was shown by positive control. At day 7, best wound healing activity was shown by mixture of chitosan gel and oil (group III) and antibiotics (group V). At day 14, best wound healing activity was shown by mixture of chitosan gel and oil (group III) and antibiotics (group V). At day 21, wound was fully recovered in group III and group V rats as shown in Fig. 6.

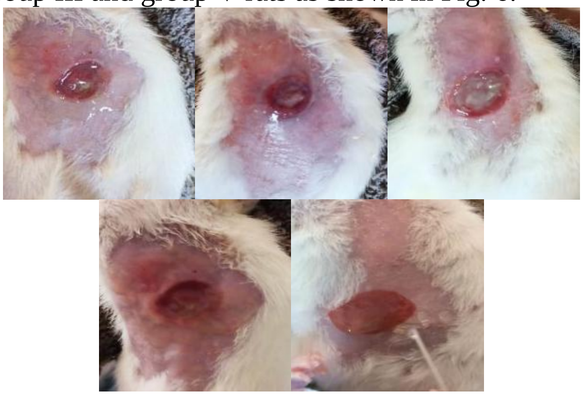

Fig. 3(A-E): Treatment of Rats with gel, oil, gel+oil, saline solution and Fudic-H on day 7th.

Day 14th:

Variable wound healing activity was observed in all the five groups of rats. As shown in Fig. 4 (A-E), In Group I animals, wound diameter was calculated as 06 mm. In Group II animals, wound diameter was calculated as 06 mm. In Group III animals, wound diameter was calculated as 03 mm. In Group IV animals, wound diameter was calculated as 08 mm. In Group V animals, wound diameter was calculated 03 mm. Results of group III and group V were nearly same.

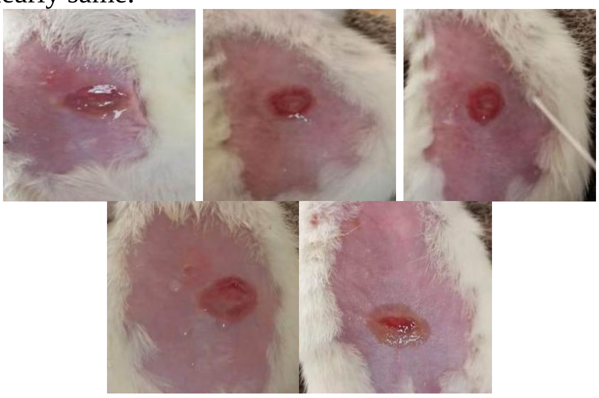

Fig. 4(A-E): Treatment of Rats with gel, oil, gel+oil, saline solution and Fudic-H on day 14th.

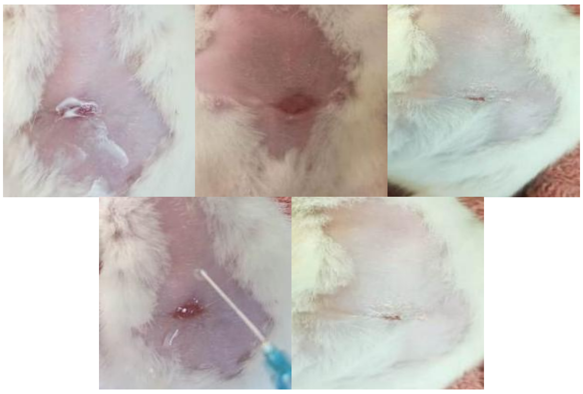

Fig. 5(A-E): Treatment of Rats with gel, oil, gel+oil, saline solution and Fudic-H on day 21st.

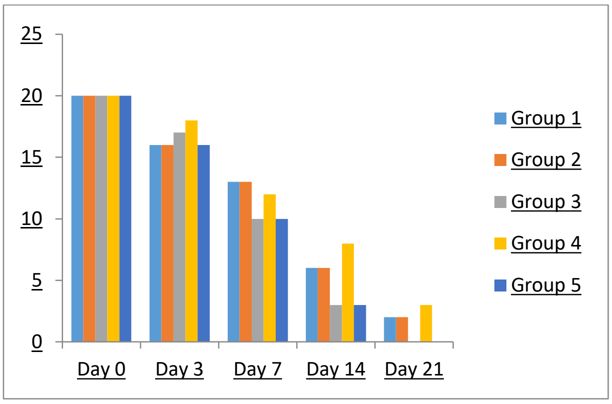

Fig. 6: Record of wound healing activity under different treatment conditions

DISCUSSION

Humans have utilized animals to produce drugs for treating illnesses and injuries since ancient times (Mathias and McCorkle, 2004; Costa and Oliveira, 2000). However, it can be assumed that concern about animal health only originated after the domestication of formerly wild species for use in transportation, agriculture, or as direct food sources. Traditional folk knowledge concerning the use of natural medicines is now present in every known culture.

The different types of fat obtained from varieties of animal include crocodile, turtle, lizard, sheep, cobra, shark, python etc. Lack of scientific evidence for the traditional healing practice using animal fats or its oils make researches to scientifically prove the therapeutic activity and this may lead to the new era for the development of a variety of novel formulations. Animal based products are also gained importance along with animal-based medicines. This article focuses on various animal fats, its traditional uses and some of the formulations developed recently.

(Cabral *et al*., 2013) studied the chemical composition of the body fats of *L. macro sternum* and *L. vastus* (amphibian species) and to evaluate their antimicrobial activities as well as the ecological implications of their use in traditional folk medicine. They found both OLM and OLV demonstrated relevant antimicrobial activities against *Pseudomonas aeruginosa* and *Candida krusei.*

The term antioxidant means that it prevents oxidation of other substances that occur in metabolic processes or exogenous factors such as ionizing radiation. In living organisms oxidative stress is mainly responsible for damage and molecular changes (Gomes *et al*., 2005). Macroscopic analysis of mice skin lesions, treatment using saline (negative control), FUDIC-H (positive control), pure gels, and oil-associated gel revealed the evolution of wound tissue repair with hemorrhage in the treated groups with SF, PC, and CG on the third and seventh day of treatment. This is detrimental to healing because it can compromise cell proliferation and promote bacterial growth (Tenci, *et al*., 2016; Tong *et al*., 2016).

Chitosan gel (CG) is an active healing material that aids the complex process of epithelization. Some studies prove that this material accelerates healing by stimulating the intense proliferation of fibro-blasts and angiogenesis, besides preventing the development of infections. The association of this material with bruiti oil may have accelerated the healing action, probably due to the high antioxidant activity and chemical composition of the oil (Du *et al*., 2012).

On day 3, best wound healing activity was shown by positive control. On day 7, best wound healing activity was shown by mixture of chitosan gel and *Varanus griseus* (VG) oil (group III) and antibiotics (group V). On day 14, best wound healing activity was shown by mixture of chitosan gel and oil (group III) and antibiotics (group V). On day 21, wound was fully recovered in group III and group V rats.

In the CG group, there was a decrease in inflammation on the 14th day, with gradually increasing collagen deposition within the incisional scar and the regression of vascular channels. Some macrophages and fibroblasts were observed. The granulation tissue had a little more mature appearance, filling the entire area of the lesion. There was no cellular debris, and it was still possible to observe acanthosis and neoformed vessels

The lesion in the animals of the CG/VG oil group on the 21st day of treatment presented complete reepithelization in the epidermis. In the upper dermis, collagen fibers were observed in horizontal arrangement with some orientation and also hair follicles. Moreover, there is extensive granulation tissue formation with the infiltration of mononuclear cells located in the deepest dermis. It is common knowledge that inflammation is more intense in large tissue defects because a greater volume of necrotic debris, exudate, and fibrin must be removed. Thus, large defects have a greater potential for secondary infection, delaying wound healing.

Differences among the groups in the total healing time and in the proportional daily reduction of the incision consistently showed that the *Varanus griseus* oil and chitosan-based treatment resulted in a faster healing process compared to the commercial ointment tested. The literature suggests that the efficacy of animal fat on healing may be primarily related to the presence of fatty acids, which have been widely demonstrated to be important for biochemical reactions involved in healing processes. Our findings are demonstrated traditional Amazonian communities using anaconda fat in folk medicine do not have easy access to pharmacies and hospitals.

Conflict of Interest: The authors declare no conflict of interest.

Authors' contribution: MIHJ executed the experiment. TR write up work. SF write up work. SS analyzed the data. MF revised the manuscript, MI and KZ wrote and revised the manuscript

REFERENCES

Balbino C, Pereira L and Curi RI, 2005. Mecanismos envolvidos na cicatrização: uma revisão. Rev Bras ciê Farm 41(1): 27- 51.

Becker T, Schlaak M and Strasdeit H, 2000. Adsorption of Ni (II), Zn (II) and Cd (II) by new chitosan derivatives. React Funct Polym 44: 289-298.

Berger J, Reist M, Mayer JM, *et al.,* 2004. Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. J Pharm Biopharm 57(1): 35-52.

Cabral RA, Jackson MG, Rose-Koga EF, *et al.,* 2013. Anomalous sulphur isotopes in plume lavas reveal deep mantle storage of Archaean crust. Nature 496(7446): 490-493.

Chatelet C, Damour O and Domard A, 2001. Influence of the degree of acetylation on some biological properties of chitosan films. Biomaterals 22(3): 261-268.

Costa Neto EM and Oliveira MVM, 2000. Cockroach is Good for Asthma: Zootherapeutic Practices in Northeastern Brazil. Human Ecol Rev 7 (2): 41-51.

Dreifke MB, Jayasuriya AA and Jayasuriya AC, 2015. Current wound healing procedures and potential care. Mater Sci Engin C. 48: 651–662. https://doi.org/10.1016/j.msec.2014.12.068.

Du F, Wang H, Zhao W, *et al.,* 2012. Gradient nano-fibrous chitosan/poly ε-caprolactone scaffolds as extracellular microenvironments for vascular tissue engineering. Biomaterials 33(3): 762-770.

Gomes A, Fernandes E and Lima JLC, 2005. Fluorescence probes used for detection of reactive oxygen species. J Biochem Biophys Meth 65: 45-80.

Gurtner GC, Werner S, Barrandon Y *et al.,* 2008. Wound repair and regeneration. Nature 453: 314. https://doi.org/10.1038/nature07039 Retrieved from.

Honnegowda TM, Kumar P, Udupa EGP *et al.,* 2015. Role of angiogenesis and angiogenic factors in acute and chronic wound healing. Plastic and Aesthetic Res 2: 243-249.

Kamoun EA, Kenawy ERS and Chen X, 2017. A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings. J Adv Res 8(3): 217–233. https://doi.org/10.1016/j.jare.2017.01.005.

Mathias E and McCorkle CM, 2004. Traditional livestock healers. Rev Sci Tech Off Int Epiz 23 (1): 277-284.

Park M.J, Gwak KS, I. Yang I, 2009. Effect of citral, eugenol, nerolidol and α-terpineol on the ultrastructural changes of Trichophyton mentagrophytes. Fitoterapia 80: 290-296.

Sohn S and Gido SP, 2009. Wet-spinning of osmotically stressed silk fibroin. Bio Mac Mol 10(8): 2086-2091.

Suarato G, Bertorelli R and Athanassiou A, 2018. Borrowing From Nature: Biopolymers and Biocomposites as Smart Wound Care Materials. Front Bioeng Biotechnol 1-11. https://doi.org/10.3389/fbioe. 2018.00137.

Tenci M, Rossi S, Bonferoni MC, *et al.,* 2016. Particulate systems based on pectin/chitosan association for the delivery of manuka honey components and platelet lysate in chronic skin ulcers. Int J Pharm 509: 59-70.

Tong S, Xu DP, Liu ZM, *et al.,* 2016. Synthesis of and in vitro and in vivo evaluation of a novel TGF-β1-SF-CS three-dimensional scaffold for bone tissue engineering. Int J Mol Med 367-380.

Ueno H, Mori T and Fujinaga T, 2001. Topical formulations and wound healing application of chitosan. Adv Drug Deliv Rev 52(2): 105-115.

Wang T, Zhu X, Xue X, *et al.,* 2012. Hydrogel sheets of chitosan, honey and gelatin as burn wound dressings. Carbohydr Polym 88: 75-83.