Wound Healing Potential of *Aloe vera* in Climbing Perch (*Anabas testudineus)*

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Abstract

Fresh gel portion of *Aloe vera* leaves was evaluated for the wound healing potential in climbing perch (*Anabas testudineus)*. Fifteen adult climbing perch were divided into three groups. Group 1 received *Aloe vera* gel, group 2 received Betadine® antiseptic ointment (positive control) and group 3 received no treatment (negative control). Wound was created at the flank area using a punch biopsy (8 mm in diameter). One topical application was applied on the wound. Assessment of healing progress was carried out on day 10. Gross observation and histological studies were carried out. There was no significant difference in wound reduction among the groups. However, group 2 showed highest rate of healing followed by group 1 and group 3, and groups 1 and 3 had similar rate of healing. Histological examination on cellular activity of healing process showed increased thickness of epithelium layer, infiltration of inflammatory cells, presence of fibroblast cell and rearrangement of the cell.

Keywords: *Aloe vera*, climbing perch (*Anabas testudineus),* wound healing

Introduction

Aloe vera leaves contain biologically active compounds such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones and anthraquinones and various lectins (Wikipedia, 2009). *Aloe vera* (true aloe) has been recognized by many users as having the most effective healing power (Rasik et al., 1983). The Aloe's broad spectrum healing power lies in its purported ability to relieve pain; reduce swelling; penetrate deeply; stimulate cell growth (repair); kill bacteria, fungus, and viruses; stimulate circulation; balance nutrition and reduce inflammation. It has virtually no known toxicity (Coats and Aloha*,* 1980).

Climbing perch (*Anabas testudineus*), also known by its common name, *ikan puyu,* is a fresh water fish that can normally be found in paddy fields. This fish is air breathing type, that is, they can survive longer than other fish without water. It is carnivorous, a voracious eater, likes to eat insect, has hard scales and spiny fins. The superficial wound healing in fish differs greatly from those of mammals and other higher vertebrates. Contrary to mammals, epithelization in fish is a rapid process with movement of the epidermis, starting from the wound edge, within 1 to 4 hours of infliction of the wound (Banerjee and Mittal, 1999). This is achieved perhaps to prevent the heavy influx of water through the damaged skin.

There are not many studies on wound healing potential of *Aloe vera* in fish. Therefore this study was carried to establish the healing potential of *Aloe vera* in fish which included the gross observation, measurement of wound area and histological study.

Materials and Methods

The mature *Aloe vera* leaves were selected and skinned. The inner pulp and gel was collected and applied directly onto the wound. Betadine® antiseptic ointment was used as positive control and petroleum jelly (Vaselin®) was applied to protect the wound when fish was in the water.

Animals

Fifteen four-month-old climbing perch (*Anabas testudineus)* with an average weight of 150 g, were obtained from a commercial farm. They were divided into three groups. The fishes were acclimatized individually in plastic tanks (30 cm x 30 cm x 25 cm) for 3 days. They were fed with commercial pellets once a day.

Treatment schedules

Group 1 received *Aloe vera* gel, group 2 Betadine® antiseptic ointment and group 3 did not receive any treatment and acted as a negative control. All treatments were applied topically immediately after wound was inflicted at the right flank between the abdomen and the tail. Twelve scales were plucked manually with the help of a pair of forceps and the wound was made using punch biopsy of 8 mm (Figure1). The flesh was cut at the base and lifted with a pair of forceps. All surgical procedures were carried out under general anaesthesia using clove oil at the dosage of 0.5 mL/L. The fishes were allowed to recover from anaesthesia in the recovery tank with oxygen supply and removed to the individual tank after full recovery. Only one treatment was given. Vaselin® was then applied to protect the wound.

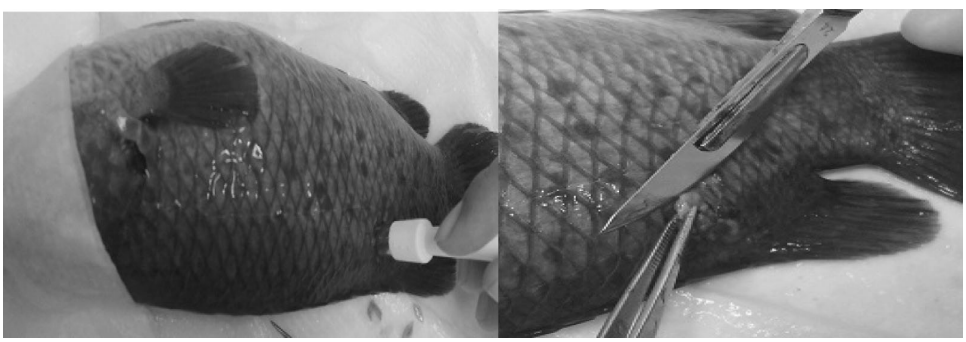


Figure 1. Skin biopsy

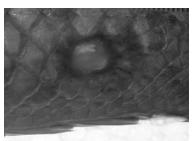
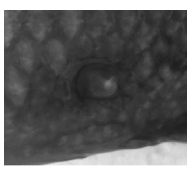
Measurement of wound area

Wound area was measured by drawing wound boundaries around it on a transparent plastic and the area within the boundaries was calculated using a graph paper. The values for each treatment were averaged and presented in mm².

Wound Healing Score

The wound healing scores are summarized as in Table 1.

Table 1. Wound healing scores in climbing perch

Score	Descriptions of lesion	Picture
1. No healing	Large area of wound (8mm) Red in colour	
2. Moderate	Dark shade at periphery of wound Slightly reduced Pale pink colour	
3. Good	Dark shade at the periphery towards centre of wound Whitish colour	
4. Excellent	Normal shade of skin	

Histological studies

Sections were qualitatively assessed under the light microscope for infiltration of inflammatory cells, fibroblastic proliferation and epithelization (Rasik et al., 1999).

Results and Discussion

On day 0, the wound was fresh and red and about 11 mm^2 area. On day 10, the areas surrounding the wound were dark with a small fresh area in the centre. The unhealed wound was pale pink covered by whitish shade and copious slime. There were some new scales growing around the periphery. They were small in size and softer than the normal scales. Reduction of wound area was highest in the positive control group followed by the negative control and *Aloe vera* group. Mean percentage of wound reduction area showed high variability among treatment. However, there was no significant difference in healing between all the groups. This may be due to only a single application of the treatment given, and the early dissolving of the protective gel which hindered further

healing. A more frequent treatment may produce better results. Another reason may be attributed to the fragility of the healing of cells in fish. The emulsion form of aloe vera probably has a better healing potential when made to optimum concentration suitable for healing.

Histology Evaluation

The epidermis appeared thicker especially in the centre and epithelial cells appeared irregularly arranged compared to the normal skin (Figure 2a). The epidermis appeared vacuolated and spongy due to the presence of prominent extracellular space between the epithelial cells which remained connected to each other by prominent intercellular cytoplasmic bridges (Figure 1b). The mucous cells also appeared thickened especially on the surface. The inflammatory cells and fibroblasts appeared prominently at the lesion. The inner layers of the granulation tissue at the level of *stratum compactum* appeared compactly arranged with the presence of blood capillary (Figure 1c).

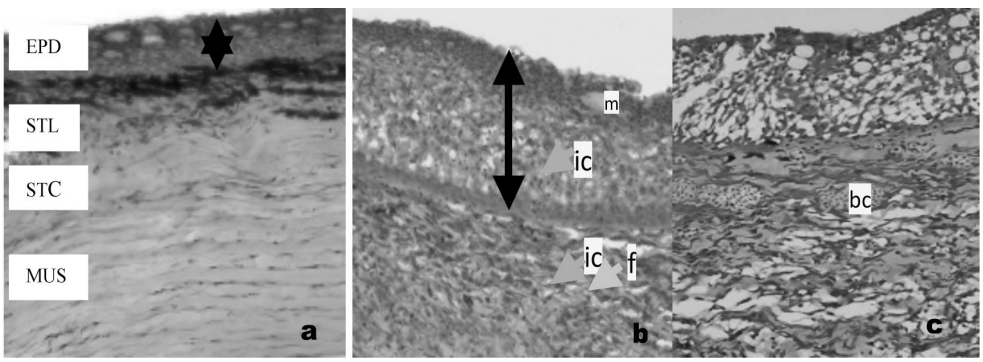


Figure 2. Cross-section of the skin of Climbing Perch

(EPD: epidermis; STL:stratum laxum; STC:stratum compactum; MUS:muscle; bc:blood vessel; f:fibroblast; ic:inflammatory cells)

Grossly, the wound area was not significantly different between all the groups. On day 10, the area surrounding the wound became dark and converging towards the centre. The unhealed wound was pale pink or whitish in color. This is in contrast to dry mammalian wound where the granulation tissue appears first and the epithelization takes place later. The new epithelial cells are produced due to mitotic division of the cells of the stratum germinativum of the epidermis (Fossum et al., 2002). The wound area was covered with copious amount of slime and regeneration of new scales was seen around the periphery of the wound. New scales were smaller in size and softer than normal scales. The mucus protected the body from bacterial or fungal infection and also has a role in the osmoregulatory processes (Hibiya, 1982). Grossly, it was difficult to distinguish between the lesions of the 3 groups because of their similarity. The results could have been different if the duration of study was longer. According to the previous study on *Channa striata* (Banerjee and Mittal, 1999), the normal shade was re-established after

24 hours but in this study the skin was not re-established to the normal shade even at day 10 where the unhealed area could still be observed in the centre of the wound. It can be concluded that the healing process occurs over time and can be shown by the characteristic of wound area reduction, dark shade migrating into centre of the wound and growth of the new scales at the periphery of the wound. In this study the percentage of wound reduction area in positive control (Betadine®) showed better result than negative control and *Aloe vera* treated. However, there was no significant difference in the healing process in all groups. If the frequency of application of the drug is increased, better results may be obtained. Alternatively due to the fragility of the healing cells in fish, *Aloe vera* may be administered as an emulsion in the water. Similarly the healing score should have been done more frequently to obtain more reliable results. The availability of *Aloe vera* after topical application is good as shown by fast healing in some fish in this study. The topical application in fish is a challenge as the drug is easily dissolved when placed in water. To prevent this, petroleum jelly (Vaselin®) was used to cover the wound area. However, there was no advantage of topical application of drug in fishes because of the limited time for the absorption of drug before it dissolved in water.

The cellular activity of the healing process in the climbing perch on day ten showed the middle layer of the epidermis appearing vacuolated and spongy. This characteristic was due to the presence of prominent extracellular space between the epithelial cells which remain connected to each other by prominent intercellular cytoplasmic bridges. This finding is similar to those of Banerjee and Mittal (1999). There was an increase in thickness of the epidermis and mucous cells especially on the surface. The structure of the epithelial layer appeared irregularly arranged. The layer of the epidermis became thicker at the centre of the lesion with high infiltration of inflammatory cells. The layer of the epidermis was thicker to give more protection against the environment. This finding is similar to that of Iwama and Nakanishi (1996) where the epidermal healing is very rapid and within hours a 2- to 3-cell-thick epidermis can cover the wound. In this study, the inner layers of the granulation tissue mostly at the level of *stratum compactum* became compactly arranged with the presence of blood capillaries and elongated fibroblasts. This was also similar to studies by Iwama and Nakanishi (1996) where the elongated fibroblasts appeared within the first week after inflammatory insult and undergo fibroplasis during the second week.

References

Banerjee, T.K. and Mittal A.K. (1999). Histopathological studies on the repair of the excised skin wounds of the air-breathing scalyfish *Channa striata.* (Ed) Narendra Publishing House, Delhi. Pp 1-12.

Coats, B.C. and Aloha, R. (1980). The Silent Healer: A Modern Study of *Aloe vera*. Garland, Texas, BC Coats.

Fossum, T.W., Hedlund, C.S., Hulse, D.A., Johnson, A.L., Seim, H. B., Willard, M.D. and Carrol, G.L. (2002). Surgery of the integument system. In: *Small Animal Surgery*, 2nd ed. Mosby, Inc. St. Louis, Missouri. Pp 134-144.

Hibiya, T. (1982). Skin. In: An Atlas of Fish Histology. Ed. Yokote, M. 1st edi. Kodansha Ltd. Japan. Pp 8-15.

Iwama, G. and Nakanishi, T. (1996) The Non-specific Immune System: Cellular Defences. In. The Fish Immune System. Ed. Secombes CJ. 1st edition. Academic Press, USA. Pp68-70.

Rasik, A.M., Raghubir, R., Gupta, A., Shukla, A., Dubey, M.P., Srivastava, S., Jain, Park, H.K. and Kulshrestha, D.K. (1999). Healing potential of *Calotropis procera* on dermal wounds in guinea pigs. *J Ethnopharmacol* **68**: 261–266.

Rasik, A.M., Raghubir, R., Gupta, A., Shukla, A., Dubey, M.P. and Anderson, B.C. (1983). *Aloe vera* juice: a Veterinary Medicament? *The Compendium in Continuing Education for the Practicing Veterinarian* **5**: S364-S368.