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Wound-Healing Potential of Roots of *Hygrophila Auriculata* Schumach. in Swiss Albino Mice

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Abstract

People staying in the rural areas of Assam, northeast India use roots of *Hygrophila auriculata* to treat small scale wounds. Therefore, a preliminary study was carried out to evaluate the wound-healing potential of the roots of the plant in Swiss albino mice using excision wound model. In the study the animals were divided into three groups having 6 (3 males and 3 females) animals in each group. Animals in group 1 were topically treated with carboxymethyl cellulose (control). Mice in group 2 were treated with reference drug (positive control). Group 3 animals were treated with root extract of *H. auriculata*. Healing was assessed by measuring wound area, histo-morphological observations, estimation of protein and DNA content. The results showed that the wound area of the extract treated group was lesser than the control group. The epithelialization was faster in the treated group when compared with control group. The amount of protein and DNA were also more in treated mice than the control. Extent of healing in the treated animals was quite comparable to that of the positive control group. Thus, the results show that the roots of *H. auriculata* has wound-healing potential.

Keywords: Excision wound model; Epithelialization Mice; *Hygrophila auriculata*; Protein; Wound-healing; DNA

Introduction

The skin serves as a protective barrier for animals. Normally, the epidermis and the dermis layers of the skin exist in a steady state of equilibrium. Disturbances of this equilibrium results into wounds. Of the two types of wounds, acute wound is the type of injury where healing progresses normally to restore the function and anatomy, whereas chronic wounds fail to progress through the normal stages of healing [1,2] Whenever there is a wound, the healing process starts immediately. The process has mainly 3 phases i.e. inflammatory, proliferative and remodeling phase. Since the wound healing is a natural process, treatment is provided either to shorten the healing time period or for reducing the unwanted effects [3]. Treatment of wounds is usually done through administration of synthetic drugs like antibiotics, pain reliever etc [4]. But, most of the drugs which are being used have adverse side effects [5]. Therefore, a healing agent chosen for treating wounds should be able to improve the phases of wound healing and also should not cause any adverse side effects. In this context traditional medicine revealed to serve as the most affordable and accessible source for treatment in rural areas of India. Various plants are being used for treating both acute and chronic wounds, as they are easily available, usually non-toxic and lacks unwanted side effects, so they are largely preferred by the rural people [6-10]. The northeastern region of India is inhabited by a large number of tribal communities of different ethnic groups. These ethnic communities have vast knowledge of curative properties of various traditional medicines. Interaction with rural people of Assam revealed that the people are using different medicinal plants for treating different types of wounds. *Hygrophila auriculata* Schumach. is one such plant, roots of which is used by the rural people as traditional wound healing agent.

The plant is also known for its hepatoprotective, neuroprotective, anti-cancer and anti-oxidant properties, however wound healing potential of roots of the plant is not established [11,12]. The present study was designed to evaluate the wound-healing potential of roots of *H. auriculata* in Swiss albino mice.

Materials and Methods

Collection of Plant Material and Preparation of Extract

The plant was collected from Chirang district of Assam, northeast India and identification was done by the Scientist in Botanical Survey of India (BSI), Shillong. The traditionally usable part i.e. the roots were separated from the plant, washed with water, air dried under shade and grinded into fine powder using a blender. The powder was then soaked in 90% methanol (100 g/L) for 10 days, filtered using Whatman filter paper no.1, and the solvent was separated out using a rotatory evaporator [13].

Experimental Animals

Adult Swiss albino mice (age 8-12 weeks, wt. 25-30 g) were procured from Pasteur Institute, Shillong. The animals were housed under standard house conditions and maintained as per guidelines of the Committee for Purpose of Control and Supervision of Experiments On Animals (CPCSEA, 1988) [14], India. The approval for experimental procedures was obtained from the Institutional Ethics Committee (IEC), North-Eastern Hill University, Shillong, Meghalaya, India.

Creation of Wound (Excision Wound Model)

The animals were divided into three groups having six individuals (3 males + 3 females) in each group. All animals were anaesthetized before and during the creation of the wounds. Excision wounds were made on the mice following the procedures described by Morton and Malone (1972) [15]. Firstly, the dorsal fur of the animals were shaved and the wound area to be created was then outlined. The shaved area was cleaned with alcohol, after that circular piece of skin was cut off from the predetermined area.

Treatment of Animals

After the creation of the wound the first group was considered as control and the animals were exposed to carboxymethyl cellulose only. The second group served as positive control and treatment was done with a reference drug (Neosporin). The third was the treated group where methanol extract (500 mg/kg body weight) of roots of *H. auriculata* was used. All the animals received topical dose daily once and the treatment was continued for 14 days.

Calculation of Wound Area and Epithelialization Period

The wound area was measured on different days i.e. day 4, 7, 11 and 14 with the help of graph paper. The epithelialization period was also noted down, which is the time required for complete healing of the wound.

Estimation of Protein and DNA Content

For estimation of protein and DNA content the wound tissues were collected and stored at -20^{0}C after 14 days of treatment. Protein content was determined following the method of Lowry et al. (1951) [16]. For DNA estimation first isolation was done following standard phenol-chloroform precipitation technique [17] and quantification was carried out using spectrophotometer.

Histopathological and Morphometric Evaluation

For histopathological analysis the wound tissues were collected after 14 days of treatment and preserved in 10% formalin solution. The processing of tissues was then carried out for histopathological study. For observing epithelialization, hematoxylin and eosin stains were used. For morphometric analysis of the wounds, photographs were taken on days 4, 7, 11 and 14.

Acute Dermal Toxicity Test

To check the safety parameter of the extract acute dermal toxicity test was also performed following the OECD (2002) [18] guidelines. The animals were first anaesthetized and then the skin was shaved 24 hours before the application of the extract. Then in next day the shaved area was cleaned with alcohol after that a limit dose of 2000 mg extract/kg body weight was applied. The extract was held in contact with skin using a bandage dressing for 24 hours. After 24 hours, the bandage was removed and the treated area was washed. Then the animals were observed for 14 days for any signs of adverse effects.

Statistical Analysis

The results were expressed as mean±SE. Statistical analyses were performed using Students's 't' test. Differences were considered statistically significant at the value P≤0.05.

Results

Wound Area and Epithelialization Period

Wound area (mm^2) of the three different groups (Table 1) were measured on days 4, 7, 11 and 14. It was found that *H. auriculata* (root extract) treated group had lesser wound area than the control group and it was also observed that the wound area of the extract treated groups were quite comparable to the positive control group. Period of epithelialization denotes the complete healing of the wound. In case of control group, the complete healing took 15.71±0.49 days. But, in treated and positive control groups epithelialization was completed in 12.57±0.53 and 11.86±0.69 days, respectively (Figure 1a).

DAYS	CONTROL	POSITIVE CONTROL	TREATED
Day 4	49.77±3.02	35.14±2.97*	44.97±2.49
Day 7	40.34±2.36	24.58±3.35*	35.84±2.65
Day 11	8.84±0.74	2.85±0.26*	4.74±0.49*
Day 14	2.02±0.25	0±0	0±0

Table 1: Wound areas (mm^2) in different groups of experimental mice after 4, 7, 11 and 14 days of treatment. Values are expressed as mean±SE, * P values significant at ≤0.05 as compared to control.

Protein and DNA Content

Quantitative estimation of protein and DNA in the wound tissues were performed after 14 days of treatment. The protein content of control group was found to be 18.93±0.90 µg/mg tissue. For treated and positive control groups the amount were 26.26±1.00 and 31.20±1.28 µg/mg tissue, respectively (Figure 1b). Similarly, the DNA content of control group was 1.86±0.23 µg/mg tissue, whereas for treated and positive control groups the values were 2.73±0.25 and 3.29±0.17 µg/mg tissue, respectively (Figure 1c).

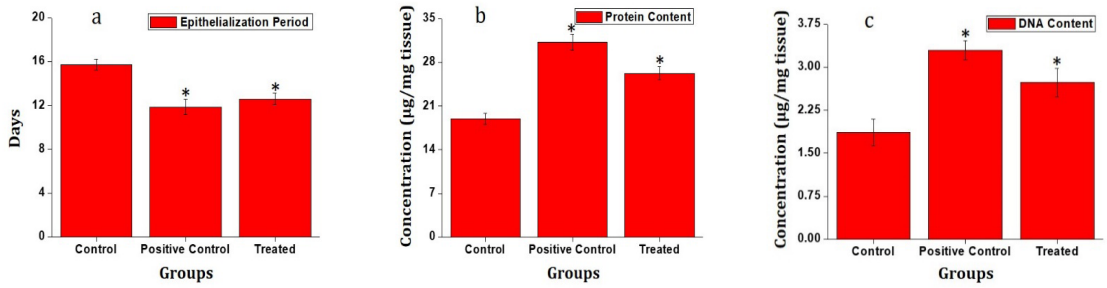


Figure 1: Epithelialization Period (a), Protein Content (b) and DNA Content (c) of three experimental groups after 14 days of treatment. Each bar represents mean ±SE, * P values significant at ≤0.05 as compared to control.

Histopathological and Morphometric Evaluation

Hematoxylin and Eosin stained wound tissues (after 14 days of treatment) showed that the epidermal layer of the control tissue (Figure 2a) was thinner than the extract treated tissue (Figure 2c). Thickness of epidermis in the positive control tissue (Figure 2b) was found to be quite similar to that of the extract treated tissue. A comparative photographs of wounds (Figure 3) in adult mice taken on days 4, 7, 11 and 14 for all the three different groups also showed that the healing was faster in extract treated group when compared to control group and comparable to the positive control group.

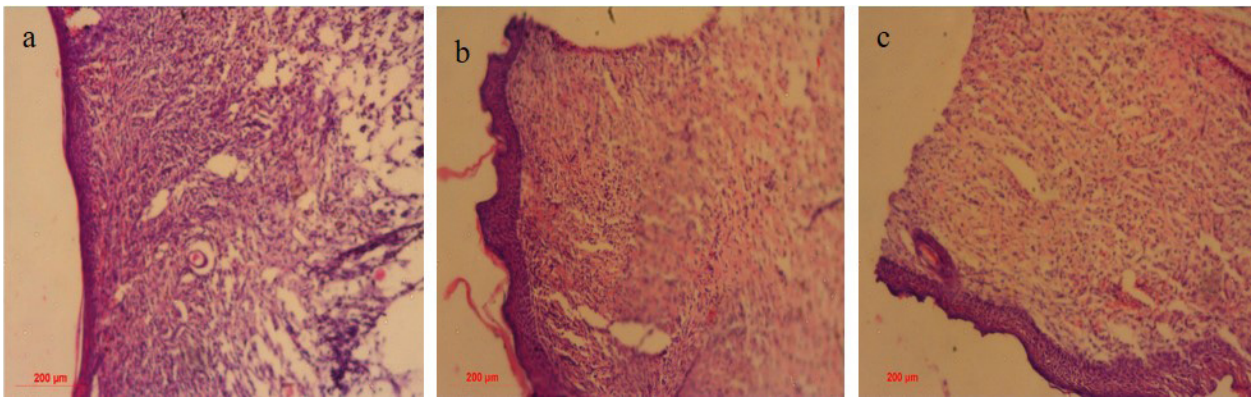
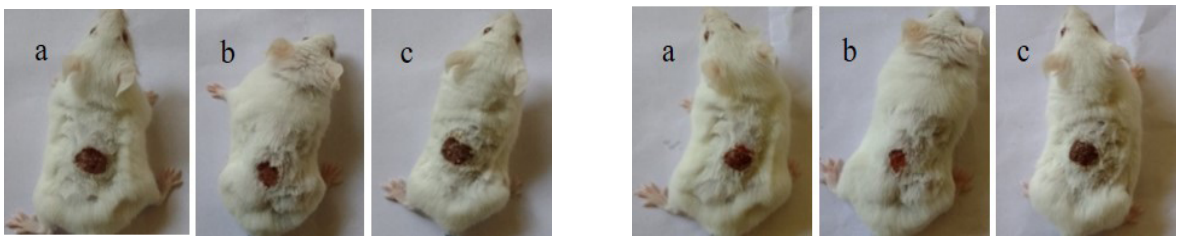
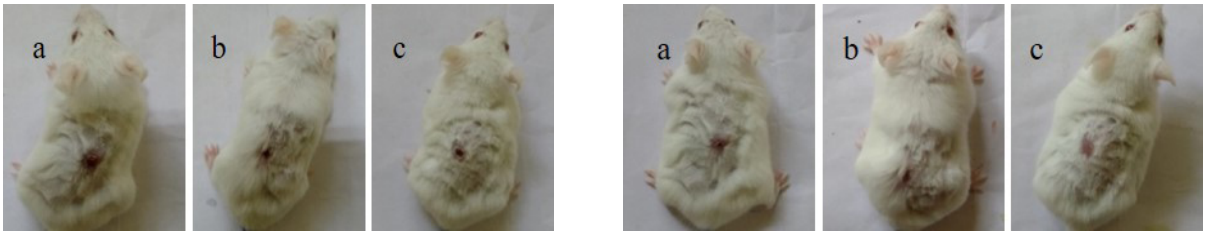


Figure 2: Hematoxylin and Eosin stained section of skin tissues (Figure 2a-2c) of different groups of mice after 14 days of treatment (a. Control; b. Positive Control; c. Treated).



After 4 days of treatment

After 7 days of treatmen



After 11 days of treatment | After 14 days of treatment

Figure 3: Photographs showing wounds of the three groups (a. Control, b. Positive Control, c. Treated) of mice after 4, 7, 11 and 14 days of treatment.

Acute Dermal Toxicity Test

Acute dermal toxicity test performed showed no adverse effects on the animals. Animals did not show any drastic changes in the body weight, no change was observed in the skin and fur, nor any behavioral changes.

Discussion

Wound healing is a basic response to tissue injury that leads to the restoration of cellular structures and tissue layers in the damaged tissue. However, successful closure of wounds with the help of curing agents in minimum time period without any adverse effect is a challenge to the scientists. In the present study, it has been observed that roots of *H. auriculata* has wound-healing potential in excision wound model. Here, reduction in physical parameter like wound area (mm^2) and epithelialization period (days) have been accompanied by significant increase in protein and DNA content (µg/mg tissue) in the extract exposed mice was observed. According to Chithra et al. (1998) [19] when the protein and DNA content of treated wounds are greater than the untreated wounds, it indicates that the treatment has stimulated cell proliferation in the wound and thus improved healing process. Similar to our observations, Pather et al. (2011) [20], also showed highest collagen formation at day 7 of treatment leading to significant decrease in the wound area, indicating faster healing. According to Subalakshmi et al. (2014) [21] higher protein and DNA content in the granulated tissue corresponds to rise in synthesis of collagen and also cellular proliferation. Collagen helps in the re-epithelialization of the wound tissue due to the interaction of different binding proteins [22]. Synthesis of collagen is under the regulation of DNA-messenger RNA control system where pro α-chain of type-1 collagen is synthesized by m-RNA. This m-RNA is translated into DNA helping in nucleic acid synthesis [23]. In our present study, an increase in protein and DNA content in the injured tissue treated with root extract of *H. auriculata* further support the above mentioned process. A thicker epidermal layer of the treated tissues as observed in our plant extract treated animals must be due to the proliferation of the keratinocytes, as during wound healing migration, proliferation and differentiation of the keratinocytes takes place to restore the normalcy [24]. When the medicinal plants are used clinically, their safety factor becomes very important. The acute dermal toxicity test using the root extract shows no adverse effects on experimental animals indicating that the topical use of the extract is safe [25].

Conclusion

The results of the present study showed that the healing potential of roots of *H. auriculata* is comparable to that of the reference drug on various physical and biochemical parameters. Hence, our investigation justifies the traditional use of roots of *H. auriculata* in topical management of wounds. But, it is known that growth factors and cytokines play an important role in wound healing [26]. Therefore, further studies involving inflammatory cytokines to compare the healing activity in depth will unveil the mechanism involved in the process of healing.

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Conflict of Interest

The authors hereby declare that there is no conflict of interest.

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