

# The Potential of Squid Pen Powder as an Alternative Wound Healing Agent

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## ABSTRACT

Chitin is found widely in nature as a skeletal material of crustaceans. Chitin is the second most abundant natural polysaccharide after cellulose on earth. Chitin and its derivative, chitosan, have attracted more attention recently because of their physiochemical and biological characteristics. The study determined the effectiveness of Squid Pen Powder (SPP) in accelerating wound healing. The experimental study utilized the crossover design to measure the wound reduction rate. The research was conducted in Basak, San Nicolas, Cebu City where forty respondents were selected based on definite inclusion criteria and through simple random sampling. Twenty of the respondents composed the experimental group that received SPP as wound healing agent while the other twenty composed the control group that received only wound

composed the control group that received only wound cleansing, which was also given to the experimental group. All wounds, after baseline assessment, were measured by taking a picture of the wounds beside a ruler and evaluated daily using the Royal Marsden Wound Assessment Chart to monitor wound changes. Wound photo was then used to obtain wound area using the KLONK Image Measurement Software. After data gathering, T-test of two-independent samples was used to determine if there was a significant difference in the mean wound healing reduction rates and the mean wound healing duration between two groups. Calculations were done using the Microsoft Excel 2007 and MiniTab software. Results reveal that SPP significantly accelerated wound healing reduction rate (p-value=0.001, 95% CI: -5.68, -1.58) and wound healing duration (p-value=0.002, 95% CI: 0.535, 2.465).

**Keywords** - Chitin, wound healing agent, Squid Pen Powder, wound reduction rate, wound duration

## INTRODUCTION

Wound, as defined by Merriam-Webster, is an injury to the body as from violence, accident or surgery that typically involves laceration or breaking of a membrane, as the skin, and usually damage to underlying tissues. It is acquired during accident (e.g. motor vehicular accidents, falls, cuts, etc.), immobility, and bites (human and animal). It is a common occurrence in the locality that sometimes it is already considered as “nothing” or just a part of daily living thus, leaving it as it is, open and untreated, which may sometimes lead to a serious complication such as infection.

Wound healing, on the other hand, is a complex and dynamic process of restoring cellular structures and tissue layers. The human adult's wound healing process can be divided into three distinct phases: the inflammatory phase, the proliferative phase, and the remodelling phase. Within these three broad phases is a complex and coordinated series of events that includes chemotaxis, phagocytosis, neocollagenesis, collagen degradation, and collagen remodelling. However, some conditions may interrupt the process of wound healing such as infection and other medical conditions like diabetes mellitus that causes delayed wound healing (Dougherty & Lister, 2004).

However, factors, such as proper wound dressing, good nutrition and medication, can speed up wound healing process. A polysaccharide, under study, that can speed up wound healing is chitin. Chitin is the second ubiquitous natural polysaccharide after cellulose on earth (Dutta, Dutta & Tripathi, 2004). It is the white hard, inelastic, nitrogenous polysaccharide found in the exoskeleton as well as internal

structure of invertebrates such as insects, crabs, shrimps, lobsters, and squids. Various studies show a lot of promise of the effectiveness of chitin in wound healing. Some studies revealed that it has a fungicidal and fungistatic nature due to its organic acid property and some found that it accelerates macrophages migration and fibroblast proliferation and promotes granulation and vascularisation.

Philippines, a country that has a large body of water, is highly accessible by any marine animal such as squid that among its popular seafoods. In contrast to the squid's popularity, less importance is given to its pen, where chitin can be found. On the other hand, based on the accidental observation made by the researchers in some of the locals in Mindanao, squid pen powder has been utilized as wound healing agent and its effectiveness is attested by the locals. Thus, curious of the effectiveness of chitin in wound healing, this study was conducted to determine the effectiveness of to determine scientifically the effectiveness of Squid Pen Powder (SPP) as wound healing agent. Data of this study may contribute to the body of knowledge on alternative medicine.

## **OBJECTIVES OF THE STUDY**

This study determined the effectiveness of Squid Pen Powder in wound healing among selected individuals in San Nicolas, Cebu City. Specifically, the study determine (1) the mean wound reduction rate between the experimental and the control groups, (2) any significant difference in the mean reduction rate between the experimental and control group after the daily application of the Squid Pen Powder, and (3) any significant difference in the mean wound healing duration between the experimental and control groups after the daily application of the Squid Pen Powder.

## **MATERIALS AND METHODS**

This study used the basic crossover experimental design. Data was gathered daily to monitor changes wound measurement. The research was conducted in Basak, San Nicolas, Cebu City where forty respondents were sought based on an inclusion criteria and through simple random sampling. The inclusion criteria were (a) 6-12 year old regardless of sex, (b) with scab or abrasion wound on the lower extremities, (c) no pus (d) not taking antibiotics and supplements, (e) wound should only be 1-2 days old, (f) no treatment received since the day of injury, (g) with no underlying medical conditions, (h) no crustacean allergies, (i) not be at any cause obese any cause obese or underweight, and (j) voluntary consented to be part of the study.

Baseline assessment of the wounds was done using the Royal Marsden Wound

Assessment Chart. Before taking all wound photos, wound cleansing was done utilizing Baseline assessment of the wounds of all respondents took place using the Royal Marsden Wound Assessment Chart. Before taking all wound photos, wound cleansing was done utilizing water and clean cotton. Using a digital camera, the researchers took a photo of the wound with a ruler next to it. The photo was taken in a perpendicular angle to maximize wound exposure. It was uploaded in the computer and was generated in the KLONK Image Measurement Software. The photo was calibrated according to the length of the known calibration object, which was the ruler. After the calibration, the outline of the wound was drawn and area was obtained in  $\text{mm}^2$ .

Forty respondents were randomly assigned into two groups: Twenty of them in the experimental group and the other twenty in control group. The experimental group was given Squid Pen Powder (SPP). SPP application was done twice daily, during morning by the respondent's guardian and evening by the researchers. There was no specific amount as to SPP application. Rather, wound area was filled evenly with SPP with no area left uncovered. On the other hand, the control group did not receive SPP and treatment of any kind. All wounds were monitored daily until complete wound closure.

The Royal Marsden Wound Assessment Chart was used for identifying any experienced side effects. There were a total of 134 data collected from the experimental group and 164 data from the control group. T-test of two independent samples was used to determine the statistical difference in the mean wound reduction rates of the experimental and control groups and in the mean wound healing duration values of the two groups.

Wound reduction rate refers to the sum of wound reduction, which is the difference between the two succeeding daily wound measurements of a wound, divided by wound healing duration. It was determined by computing the total wound reduction rates for each respondent and then taking its mean by getting the sum of all the wound reduction rates of all respondents in each group divided by the number of respondents for each group. The mean wound reduction rate for the experimental group was  $8.501 \text{ mm}^2$  while the mean reduction rate for the control group was  $4.895 \text{ mm}^2$ .

Wound healing duration refers to the number of days where the epithelium covers the entire wound surface. The wound healing duration was computed by counting the number of days it took for each wound to heal and getting the mean of the total number of days for the respondents of each group. The mean duration for the control group was approximately by 8 days while the mean duration for the experimental group was approximately 7 days.

In data processing, Minitab version 15.1 English software was utilized.

## RESULTS AND DISCUSSION

Table 1. T-test results for wound reduction rate

### Two-Sample T-Test and CI

Sample	N	Mean	StDev	T-Value	P-Value
control	164	4.89	5.29	-3.51	0.001
experimental	134	8.5	10.9		

The mean reduction rate of the experimental group was 8.502 mm<sup>2</sup>/day while that of the results show that the wounds of those was a larger area (mm<sup>2</sup>) reduced in the wounds (per day) of that those in the experimental group had layer area of their wounds reduced than that of those in the control group. Wounds upon the application of Squid Pen Powder healed approximately 74.67% faster as compared with wounds receiving no treatment of any kind.

As shown in the t-test of two independent samples, there was a significant difference in the mean reduction rates between the experimental (8.5 mm<sup>2</sup>/day) and control (4.89 mm<sup>2</sup>/day) group (95% CI: -5.68, -1.58). As a result, it is safe to reject the null hypothesis and accept the researchers' hypothesis that Squid Pen Powder significantly accelerates wound healing reduction rate.

Table 2. T-test of two independent samples result: Wound healing duration

### Two-Sample T-Test and CI

Sample	N	Mean	StDev	T-Value	P-value
control	164	8.20	4.51	3.06	0.002
experimental	134	6.70	3.95		

As revealed in Table 2, the mean wound healing duration in the experimental group was 7 days while that of the control group was 8 days. The result shows that wounds in the experimental group healed a day faster than that in the control group.

T-test of two independent samples revealed a significant difference between the mean wound healing duration of the experimental (6.70 days) and control (8.20 days) group (p-value=0.002, 95% CI: 0.535, 2.465). The computed p-value of 0.002 suggests that the identified difference between the experimental and control group was unlikely to be a function of chance; thus, it is safe to reject the null hypothesis and accept the researchers' hypothesis that Squid Pen Powder significantly accelerates wound healing duration.

It is evidently shown in the results that the Squid Pen Powder is an alternative wound healing agent. This agent only increases the wound reduction rate but also lessens wound healing duration. The result implies that chitin, the second most abundant polysaccharide in nature, accelerates wounds healing at molecular, cellular, and systemic levels (Jayakumar, et.al, 2011).

Chitin and its derivative chitosan are also considered anti-microbial, biocompatible, biodegradable, hydrating, and nontoxic agents. Chitosan is a hemostat, which helps in natural blood clotting and blocks nerve endings, hence reduces pain. It initiates fibroblast proliferation, helps in ordered collagen deposition, and stimulates increased level of natural hyaluronic acid synthesis at the wound site. Thus, it accelerates wound healing and prevents scarring (Paul & Sharma, 2004). Due to these properties, chitosan shows good biocompatibility and positive effects on wound healing. The antimicrobial properties and high important potential for wound healing are essential for wound care. Healing restores tissue integrity and prevents organisms from deregulation of homeostasis.

Chitin influences wound healing as several studies had shown. Muzzarelli (2009) presented the unique characteristics of chitin and chitosan based on the latest chemical, biochemical and medical information in terms of animal/human tissue regeneration. Based on the experiment performed, hemostasis is immediately obtained after the application of chitin-based dressing to traumatic and surgical wound because of the platelet activation by chitin. To promote angiogenesis, necessary to support physiologically ordered tissue formation, the production of the vascular endothelial growth factors is strongly up-regulated in wound healing when macrophages are activated by chitin/chitosan.

Though positive results were documented in the study, several methodological limitations were noted that might affect the results such as not considering the baseline wound measurements. The study revealed that the larger the wound area, the longer

the wound healing duration and the smaller the wound area, the shorter the wound healing duration. Environment factors such as wound exposure to microorganisms due to the respondent's activities such as playing and health teaching compliance may as well be included in the limitation of the study.

Environmental factors such as where, for how long, and how the respondents play may affect the wound healing process due to wound exposure to microorganisms. Microorganism may impede deregulation of hemostasis, which helps in natural blood clotting and in blocking the nerve endings (Paul & Sharma, 2004).

Health teaching compliance was considered a limitation due to the possibility that the respondents or the parents/guardians did not comply properly with the health teachings regarding proper wound cleansing. There were instances reported that wound cleansing was done by the respondents alone. However, actions were taken to address this limitation. Demonstrations were performed with simplified instruction appropriate to respondent's age to ensure that even without guidance, proper wound cleansing can be done.

The strength of this study was the control on some external factors that might affect the rate of wound healing. These factors were age, antibiotics, vitamins and supplements usage, nutritional status, type and location of wounds, as and period of time when the wound occurred.

A study found that chitin in crustaceans was less likely to cause any hypersensitivity when applied to individuals with known crustacean allergies. There was also no report of any intolerance or allergy in individual and animals being tested with chitin (Muzzarelli, 2010). But due to ethical considerations, all respondents must be of no known crustacean allergies.

Minimal side effects were noted during the study. In the experimental group, two respondents had redness of wound was on the second day after application, although one admitted that he/she had been scratching the wound due to the 'wound itching'. Two reported itchiness of the wound's surrounding area on the first to second day after application. One reported pain on the second day of application. Not one of the respondents experienced the mentioned side effects and discontinued the treatment.

## CONCLUSION

It is concluded that Squid Pen Powder is an effective alternative agent in promoting twice-a-day wound healing. With daily cleansing of the wound to avoid infections and application of the Squid Pen Powder, the wound heals without of complication. Side effects include inflammation, pain, and itchiness that mostly occur during the first to the second day of application and last for two to three days.

## LITERATURE CITED

Dougherty, L. & S., Lister

2004 The Royal Marsden Hospital Manual of Clinical Nursing Procedures.UK: Blackwell Publishing.

Dutta, P.K., Dutta, J.,& Tripathi, V.

2004 Journal of Scientific and Industrial Research. Department of Chemistry, Motilal Nehru National Institute of Technology, Allahabad 221 004. Date retrieved: March 2, 2012. URL: [http://nopr.niscair.res.in/bitstream/123456789/5397/1/JSIR%2063\(1\)%2020-31.pdf](http://nopr.niscair.res.in/bitstream/123456789/5397/1/JSIR%2063(1)%2020-31.pdf)

Jayakumar, R., Prabakaran, M., Sudheesh Kumar, P.T., Nair, S.V., Furuike, T., & Tamura, H

2011 Novel Chitin and Chitosan Materials in Wound Dressing. . Date retrieved: March 2, 2012. URL: [http://cdn.intechopen.com/pdfs/12794/InTechNovel\\_chitin\\_and\\_chitosan\\_materials\\_in\\_wound\\_dressing.pdf](http://cdn.intechopen.com/pdfs/12794/InTechNovel_chitin_and_chitosan_materials_in_wound_dressing.pdf)

Merriam – Webster (ND). Wound. Date Retrieved: March 2, 2012. URL: <http://www.merriam-webster.com/dictionary/wound>

Muzzarelli, A.A.

2009 Carbohydrate Polymers. Date retrieved: March 2, 2012. URL: [www.elsevier.com /locate/carbpol](http://www.elsevier.com/locate/carbpol)

Muzzarelli, R.A.

2010 Chitins and Chitosans as Immunoadjuvants and Non-allergic Drug Carriers. University of Ancona, Italy. Date retrieved: March 2, 2012, URL: <http://www.ncbi.nlm.nih.gov/pubmed/20390107>



Paul, W. & Sharma, C.P.

2004 Chitin and alginates wound dressings: A short review. Date retrieved: March 2, 2012, URL: <http://www.mendeley.com/research/chitosan-alginate-wound-dressings-short-review-1/#page-1>