

## The Influence of Oral and Topical *Channa striatus* on Laparotomy Wound Healing in Malnourished Wistar Rats

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**ABSTRACT:** *Channa striatus* is traditionally used for its salutary and medicinal value as a source of improved wound healing. Many studies have been conducted to scientifically explain this basis. The objective of our study was to evaluate the effects of *Channa striatus* on the tensile strength, epithelialisation, and fibroblastic proliferation in the healing of full thickness laparotomy wound in malnourished rats. 40 malnourished rats underwent laparotomy and the abdomen closed primarily. After randomization, 20 rats were treated with oral and topical *Channa striatus*. The remaining 20 rats received only normal saline orally and topically. After sacrifice on the 7<sup>th</sup> post op day, all laparotomy wounds were excised and analysed for tensile strength, epithelial and fibroblast cell counts. Our results showed significant higher tensile strength in the treated rats (1.579kg/cm<sup>2</sup> vs. 0.428kg/cm<sup>2</sup>; p-value 0.001). Epithelial cell counts in the *Channa* treated group were also significantly raised (100/mm<sup>3</sup> vs. 83/mm<sup>3</sup>; p-value <0.001). The *Channa* treated group also showed significantly raised fibroblast counts (336/mm<sup>3</sup> vs. 142/mm<sup>3</sup>; p-value 0.001). The results show that *Channa striatus* does have an enhancing effect on the healing of laparotomy wounds in malnourished rats.

**Keywords:** *Channa striatus*, laparotomy wound, malnourished rats, wound healing

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### I. INTRODUCTION

*Channa striatus* locally known as “Ikan haruan” or snake-head fish and belonging to the Channidae family is widely distributed in the ASEAN region and Indian subcontinent [1]. Indigenous to Malaysia, this air-breathing fish thrives in freshwater ponds, lakes, agricultural rivers, and paddy fields [2]. It is rich in proteins containing the major amino acids [3]. Besides, it has high content of arachidonic and docohexanoic acids, with low lipid content mainly present in the form of polyunsaturated fats [4]. Micronutrients are also present in the form of manganese, calcium, copper, magnesium, iron and zinc [5]. Traditionally, the fish is consumed for its beneficial effects on health and well being and also for its healing effects especially after abdominal surgery. Its antibacterial, antifungal, and anti-nociceptive properties have been extensively studied [6,7,8,9].

Wounds are a result of separation or discontinuity of skin, mucous membrane or tissue caused by physical, chemical or biological insult. Wounds heal through an interactive process of haemostasis, inflammation, proliferation and re-modelling [10,11,12,13]. Successful healing depends on many factors which includes an adequate supply of blood and nutrients to the site of damage [14].

Although earlier studies had reported on the therapeutic effects of *Channa striatus* in cutaneous wounds, no studies have been done to verify its beneficial effects on the healing of laparotomy wounds as claimed by traditionalists [15,16,17]. Laparotomy incisions fail to heal 11% of the time [18]. Subsequent repairs for incisional hernias fail in 24% to 58% of patients [19]. Each additional operation increases the risk for further intra-abdominal injury [20].

Malnourished patients are specially at risk for delayed wound healing. Loss of protein from protein-calorie malnutrition leads to decrease in tensile strength, decrease in cell mediated immunity, decreased phagocytic activity and decreased antibody and complement levels [21,22]. 50% of all medical and surgical patients attending an urban hospital in 1974, had evidence of malnutrition [23]. Hence we designed an experiment to study the effect of *Channa striatus* on the healing of laparotomy wounds in malnourished Wistar rats.

### II. MATERIALS AND METHODS

The study was conducted at the Laboratory Animal Research Unit (LARU) of Universiti Sains Malaysia, Kubang Kerian, Malaysia, after approval from the Animal Ethical Committee of USM. Forty male three month old Wistar Kyoto rats having mean weight of 290 grams were used. The rats were bred and supplied by LARU.

**2.1 Acclimatisation** The rats were housed in separate cages devoid of wood or linen which could be chewed. They were fed with standard rat pellets (Gold Coin Feedmills Sendirian Berhad) and water ad libitum. The commercial rat pellets contain crude protein (17-19%), crude fibre (maximum) 15%, crude fat (minimum) 3%,

moisture (maximum) 13% , ash (maximum)10%, calcium 0.8-1.2 % , and phosphorus 0.6-1%. The optimum temperature was regulated by airconditioning .Individual weights were recorded using weighing scale ( ARROW AR 3320 ).The amount of food intake was also measured daily using TANITA kd-200 (ISO/IEC 17025, calibrate 25/BAL-0.03).. Faeces was collected through a tray placed under the metal cages, which were cleaned daily . The rats were assigned randomly to two groups of 20 each , using block randomization . The cages were labelled by indelible marker from 1 to 40. Group 1 will be the control group , and Group 2 will be the test or treatment group. After one week of acclimitisation, the rats were subjected to the study .

## **2.2 Creation of Malnourishment**

Malnourishment in both groups of rats was created by reducing the ad libitum food consumption by 50% of the usual intake. Body weight and food intake was measured daily. Sufficient malnourishment was deemed to have been achieved when the body weight had reduced by at least 10% of the initial body weight ,and reached a plateau for at least 3 consecutive days . After achieving the target weight loss , and plateau, all 40 rats were subjected to laparotomy procedure.

## **2.3 Laporotomy**

The malnourished rats were anaesthetised using intramuscular Ketamine 35mg/kg (100mg/ml),Xylazine 5mg/kg for induction, followed by Isoflurane 2% inhalation for maintainance . The rats were placed and secured in dorsal recumbent position . Abdomen was shaved and skin prepared with Hibiscrub®, alcohol 70%,andPovidone iodine 10%. Operative area draped using sterile towels . Using sterile technique, midline incision starting at the xiphoid process downward to a length of 4 cm made using size 11 Bard Parker blade . Abdomen was opened in layers . Once the peritoneal cavity was entered , the abdomen was closed in two layers,using continuous 4/0 polypropelene suture for the musculo-aponeurotic layer and interrupted 4/0 polypropelene for the skin and subcutaneous tissue. Wounds were cleaned with Povidone iodine 10%, and Isoflurane discontinued . The rats were then monitored for distress, breathing or abnormal movement. The safe and clean environment continued to prevail. Wounds were also observed for complications and progress. All rats tolerated the procedure well.

## **2.4 Subsequent Interventions**

Treatment was started 24 hours after the operation.

Group 1 (Malnourished rats-Control group ) – topical application of 0.9% Normal Saline (NS) over the laparotomy wound and oral administration of NS , once a day until day 7 postop.

Group 2 ( Malnourished rats –Treatment group) – topical application of Channa striatus cream and oral administration of Channa striatus once daily for 7 days . Each Channa tablet contains 80 mg of Channa extract. Tablets were ground and mixed with 10 ml water and given as 100mg/ 100gram weight ( both formulations of tablet and cream were acquired from Major Interest (Malaysia) SendirianBerhad . For the Control group, equivalent volume of NS was used topically and orally respectively.

## **2.5 Termination (Euthanasia ) and Specimen Collection**

On day 7 post laparotomy, both groups of rats were euthanized using overdose of Carbon Dioxide gas ..4.0X4.0 cm. full thickness strips of the anterior abdominal wall containing the laparotomy wound in the centre were harvested . They were further divided into upper and lower caudal segment of 2.0X4.0 cm each. The upper segment was immediately subjected to tensile strength measurement . The caudal strip was used for Histological evaluation

## **2.6 Tensile Strength Measurement**

USSC ( United States Surgical Corporation , Norwalk , Connecticut USA) machine was used. It has a fixed arm to hold one end of the specimen by means of a hook and suture and a movable arm which is used to distract the specimen till the incision gives way. Full thickness cut specimens of 1.0X1.0 cm size are hooked to the machine in such a way that the incision will lie across the opposing forces. The force required to break the laparotomy wound will be the tensile strength of the wound and recorded as kg/cm<sup>2</sup>.

## **2.7 Histopathological Examination**

1.0X2.0 cm strips from the centre of the laparotomy wound were immersed in 10% Formalin and despatched to the pathology laboratory of USM on the same day. After embedding in paraffin wax 4.0µm cut sections made using Leica microtome. Slides were stained with Haematoxylin and Eosin (H&E) and analysed by single pathologist. Olympus CX31 microscope with field diameter 0.48mm and low magnifications of 40X and 100X were initially used to scan the slides for maximum concentrations of fibroblasts and epithelial cells. The cells were identified based on their morphology . Individual cells were then counted manually using maximum magnification of 400X , and ocular grid for assistance .

## 2.8 Statistical Analysis

Data was analysed using independent –t test . Results expressed as mean (SD) .Statistical significance was defined as p-value<0.001.

## III. RESULTS

All the animals survived the procedure with no complications till their sacrifice.

### 3.1 Evaluation of Tensile Strength

Mean tensile strength in both the groups is stated in Table 1. At day 7 post laparotomy , the tensile strength for the group of malnourished rats treated with oral and topical *Channa striatus* (Group 2) showed significant increase (1.579 kg/cm<sup>2</sup>) compared to the malnourished group given oral NS and topical NS application (Group 1-control group )(0.428 kg/cm<sup>2</sup>) . The treated group demonstrated better tensile strength with mean difference of 1.151 kg/cm<sup>2</sup> on day 7 post laparotomy (p<0.001).

**Table 1. Tensile Strength Measurement of the Laparotomy Wound (kg/cm<sup>2</sup>)**

Group I Malnourished control group day 7 post -op (n=20) Mean (SD )	Group II Malnourished treated group day 7 post –op (n=20) Mean(SD)	Mean diff. ( 95% CI )	t-statistic (df)	p-value
0.428 (0.14)	1.579 ↑ (0.17)	1.151 (1.27,1.02)	26.448 (38)	<0.001

### 3.2 Epithelial Cell Counts

When the Histopathological examination results of the *Channa* treated group are compared with results from the control , the treated group showed significant higher number of epithelial cells (100 cells/mm<sup>3</sup> vs. 83cells/mm<sup>3</sup>). The mean difference ( 16 cells/mm<sup>3</sup>) on day 7 is also significant (p<0.001) (Table 2 )

**Table 2. Results of Epithelial Cell Counts ( no. of cells /mm<sup>3</sup> )**

Malnourished treated group op (n=20) Mean( SD)	Malnourished (95 % CI ) day 7 post-op Mean (SD)	Mean diff.t-statistic (df)	p value (df)	control group day 7 post- (n=20)
83.80 (17.31 )	100.05 (3.95)	16.25 (24.29,8.20)	20.975 (38)	<0.001

### 3.3 Fibroblast Cell Counts

Table 3 shows fibroblast count in the *Channa* treated group is significantly higher at 336 cells/mm<sup>3</sup> than the control group ( 142 cells/mm<sup>3</sup>). The mean difference between the two groups is also significant at day 7 post laparotomy (68 cells/mm<sup>3</sup>) (p<0.001).

**Table 3. Results of Fibroblast Cell Count (no. of cells/mm<sup>3</sup>)**

Malnourished treated group day 7 post-op (n=20 ) Mean (SD)	Malnourished (95% CI ) day 7 post-op (n=20 ) Mean (SD )	Mean diff.t-statistic (df)	p- value (df)	control group
142.55 (17.79 )	336.9 ( 7.88 )	194.95 (203.16,185.53)	26.18 (38)	<0.001

#### **IV. DISCUSSION**

In this preliminary study in malnourished Wistar rats, laparotomy wound model was chosen as it can be easily extrapolated to the clinical human situation, where morbidity and mortality is high during failed or delayed healing. To our knowledge no previous such studies have been performed. Most surgical patients present with malnutrition either following surgery or trauma, infection or with co morbidities like Diabetes Mellitus (DM). [24]. Inadequate nutrition also retards the immune response by limiting opsonisation of pathogenic bacteria, and sterilisation of wounds. Wound healing can be assessed by clinical methods like observation, testing for mechanical strength, histopathological examination (HPE), or biochemical analysis. In this study we used tensile strength, and HPE to assess the rate of wound healing. Hydroxyproline levels though contemplated earlier, could not be carried out for logistic reasons.

Tensile strength is defined as the force required per unit of cross-sectional area to break the wound. It is a measure of the resistance of tissue undergoing tension. It also indicates the quality and amount of newly developed tissue [25]. It replicates the subdermal arrangement of the collagen fibres in the newly deposited collagen and hence considered significant [26]. Our results show that the tensile strength was significantly increased in malnourished rats treated with oral and topical *Channa striatus*, when compared with the control group treated only with NS orally and topically. ( $p$ -value  $< 0.001$ ). This shows that supplements of oral and topical *Channa striatus* postoperatively in malnourished rats enhances the strength and healing of laparotomy wounds. Similar findings were reported by Baie and Sheikh [27]. However they had used only *Channa striatus* topical cream on cutaneous wounds in well nourished adult Sprague Dawley rats. The increase in tensile strength produced by *Channa striatus* has been attributed to the action of polypeptide formed when amino acids glycine, aspartin, and glutamine combine in the presence of leucine, methionine, alanine and arginine as described by Mat Jais et al [28]. Once formed the collagen molecules are secreted from the cells into the wound site to cross link as fibers. Further re-modelling of the collagen occurs by intra and intermolecular protein cross linkage, enhancing the wound strength [29].

Epithelial proliferation starts at the time of initial wounding, peaking at 48 to 72 hours. Basal keratinocytes from the wound edge and dermal appendages like hair follicles are the main cells responsible for the epithelialisation phase of wound healing. They advance as a sheet across the wound proliferating at its edges and ceasing movement when they reach the center [30]. In this study we found malnourished rats treated with oral and topical *Channa striatus* had significantly higher numbers of epithelial cells compared to the control group ( $p$ -value  $< 0.001$ ). There was no discharge or wound disruption in both groups. The significant difference could be attributed to the high content of hyaluronic acid (HA) in wounds treated with *Channa striatus*. Changes in HA levels influence cellular proliferation and deposition of structural matrix. It is possible that HA provides a more conducive fluid environment facilitating epithelial cellular mobility and faster remodelling. *Channa* treated wounds therefore may result in better wound healing with stable scars [31].

Fibroblasts are the most important component of connective tissue. During the healing process fibroblasts enter the wound site 2 to 5 days after the injury, with the numbers peaking at 1 to 2 weeks later. By the end of the first week, fibroblasts which are the key cells in the wound, begin to secrete collagen necessary for restoring tensile strength to the wound [32,33]. Increase in fibroblast numbers is directly related to increased rate of healing and strength of the wound [34]. In this study we found the fibroblast cell count was significantly higher in the malnourished rats treated with oral and topical *Channa striatus* when compared to the control group. (Mean 336 vs 142,  $p$ -value  $< 0.001$ ). Malnourishment results in delayed fibroblast production and granulation tissue formation. Hence *Channa striatus* given to the malnourished rats provides immediate macro and micronutrients for faster healing by increased proliferation of fibroblasts. In a recent study by Hang, wounds were created in mice and subsequently inoculated with MRSA, to study wound healing in infected mice. Those wounds to which topical *Tilapia piscidin* extract was applied exhibited increased fibroblast cell counts compared to controls. The authors attributed this to the effect of antimicrobial peptides present in *Tilapia* [35]. It is possible *Channa striatus* may have similar properties. This requires further research.

#### **V. CONCLUSION**

The results of this study indicate that *Channa striatus* improves tensile strength, epithelial cell and fibroblast cell counts in the laparotomy wounds of malnourished rats. These results therefore suggest that *Channa striatus* has properties that promote abdominal wound healing when compared with placebo control in malnourished rats. However further studies are needed before adapting its use for clinical surgical practice.

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#### **Conflict of Interests**

The authors have no conflict of interest

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