THE INFLUENCE OF ORAL AND TOPICAL CHANNA STRIATUS ON TENSILE STRENGTH, EPITHELIAZATION, FIBROBLAST COUNT AND HYDROXYPROLINE ASSAY IN LAPAROTOMY WOUND HEALING OF MALNOURISHED RATS

By

DR REZQA ABDULLAH HUSIN

Dissertation Submitted in Partial Fulfillment
Of The Requirement For The
Degree of Master of Medicine (GENERAL SURGERY)
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DISCLAIMER

I hereby certify that the work in this dissertation is my own except for the quotations and summaries which have been duly acknowledged.

Dated: .....

Dr Rezqa Abdullah Husin

PUM 0201/07

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Introduction: Channa striatus has been used in traditional medicine for centuries to accelerate wound healing. Recently several scientific studies have shown the healing properties of Channa striatus. However study yet to be done on its healing effect on laparotomy wound healing which has greater morbidity and mortality in the event of laparotomy wound failure. There is also no study on its healing effect in malnourished patient whom may benefit the most.

Objective: The aim of the study is to evaluate the effect of Channa striatus on tensile strength, epitheliazation, fibroblast count, and hydroxyprolene level in the healing of laparotomy wound in malnourished rat.

Methods: 40 malnourished wistar rat underwent laparotomy and the wound closed primarily. The rats were divided into two groups by block randomization. Group 1 is the control group. Group 2 received oral and topical Channa striatus daily. The rats were euthanized and full thickness strips of the wound were subjected to tensile strength measurement, histopathological examination for epitheliazation and fibroblast counts. Hydroxyprolene assays was not done due to technical problem.

Results: The results demonstrates that the group treated with oral and topical channa striatus were significantly higher in tensile strength, epithelial and fibroblast cell counts (p value < 0.001).

Conclusion: This study suggests that oral and topical Channa striatus enhances laparotomy wound healing in malnourished rat by increasing the tensile strength, epithelialisation and fibroblast count.

VI ABSTRAK

KESAN CHANNA STRIATUS KE ATAS KEKUATAN TENSIL, KIRAAN SEL EPITHELIAL DAN SEL FIBROBLAST DAN TAHAP HYDROXYPROLENE DALAM PENYEMBUHAN LUKA LAPAROTOMI TIKUS KURANG.

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Latar belakang: Channa striatus telah lama digunakan di dalam perubatan tradisi untuk mempercepatkan penyembuhan luka. Kajian saintifik telah menunjukkan kuasa penyembuhan Channa striatus. Pun begitu masih belum ada kajian yang dilaksanakan ke atas Channa striatus untuk menunjukkan kesannya kepada penyembuhan luka laparotomi yang mana kegagalan penyembuhan luka laparotomi akan menyebabkan kesusahan dan kematian pesakit. Tambahan pula belum ada kajian kesan penyembuhannya ke atas pesakit kurang zat yang mungkin mendapat kesan dan manfaat terbanyak.

Objektif: Sasaran kajian adalah untuk menilai kesan *Channa striatus* ke atas kekuatan tensil, kiraan sel epithelial dan sel fibroblast dan tahap hydroxyprolene dalam penyembuhan luka laparotomi tikus kurang zat.

Metodologi: 40 ekor tikus wistar kurang zat menjalani laparotomi dan luka dijahit semula. Tikus itu kemudiannya dibahagikan kepada dua kumpulan secara blok rawak. Kumpulan satu adalah kontrol manakala kumpulan kedua menerima oral dan topical Channa striatus. Tikus itu dibunuh pada hari ketujuh selepas pembedahan. Luka laparotomi pada abdomen di ambil pada ketebalan penuh dan diuji untuk kekuatan tensil, kiraan sel epithelial dan sel fibroblast. Kajian hydroxyprolene tidak dapat dilakukan kerana masalah teknikal.

Keputusan: Keputusan menunjukkan kumpulan yang dirawat menggunakan *Channa striatus* dengan signifikannya mempunyai kekuatan tensil yang lebih baik, bilangan sel epithelial dan fibroblas yang lebih tinggi berbanding kumpulan yang tidak mendapat rawatan.(nilai $p < 0.001$).

Kesimpulan: Kajian ini mengesyorkan bahawa oral dan topical Channa striatus meningkatkan penyembuhan luka laparotomi tikus kurang zat dengan menaikkan kekuatan tensil, dan menambah bilangan sel epithelial dan sel fibroblast.

1.0 INTRODUCTION

Traditional medicine is a comprehensive term used to refer to both systems such as traditional Chinese medicine, Indian ayurveda and Arabic Yunani medicine, and to various forms of indigenous medicine. In countries where the dominant healthcare system is based on allopathic medicine, or where traditional medicine has not been incorporated into the national health care system, traditional medicine is often termed "complementary", "alternative" or "non-conventional" medicine (WHO, 2000a).

Traditional medicine is the first point of healthcare for many people in Asia and African countries, where there has been a long and rich tradition of sourcing treatments from natural resources. The uses of natural resources in traditional medicine are mainly based from longstanding observation of the effects on the ailment throughout the generations and personal experience (WHO, 2000a). Extensive accounts of use and experiences from generation to generation provide some evidence of the effectiveness of traditional medicine. The value of traditional medicine has not been fully tested by using modern scientific means. Scientific research is needed to provide evidence of its safety and effectiveness.

Plants and animals have been used as a source of medicines from ancient times, and even in modern times, animal and plant-based systems continue to play an essential role in health care. Wild and domestic animals and their by-products (e.g., hooves, skins, bones, feathers, and tusks) form important ingredients in the preparation of curative, protective and preventive medicine. (Lev, 2003). Additionally, a significant portion of the currently

available non-synthetic and/or semi-synthetic pharmaceuticals in clinical use is comprised of drugs derived from higher plants , followed by microbial, animal and mineral products, in that order (Alves RR, 2006 , Alves RR, . 2006).

The richness in animal biodiversity is still poorly explored as a good source of producing medicine despite some species is well known ingredients for some popular traditional medicine. For instance, frogs, spiders, snails and insects have been reported to produce a therapeutic effects for wound healing and curing fever or controlling blood pressure (Madan Mohan Mahawar, 2008).

In Malaysia, generally traditional medicine can be divided into 5 categories. These include Malay, Indian, and Chinese traditional medicines, homeopathy and complementary medicine. The similarity between these groups of traditional medicine is the used of similar biodiversity that are available in the same area as the source. Example is the widespread use of *Channa striatus* in Malaysia to facilitate wound healing which is the main scope of this study (Mohsin A.K, 1983a).

Channa striatus is a local freshwater snakehead fish, belonging to the *Channidae* family. It is air-breather and carnivorous fish indigenous to many tropical and subtropical countries of South America, Africa and Asia. In Malaysia, the fish is known as haruan. It is widely consumed throughout the nation not only as a food, but also as a remedy for wound healing (Mohsin A.K, 1983a).

Table 1 Some modern drugs derived from traditional medicine (Ghalib, 2007)

Drug	Function	Source	Original Use
Artemisinin	Antimalarial	Chinese Plant *qing hao* or sweet wormwood	Chinese traditional remedy for fevers and colds
Cromolyn	Antiasthmatic	Synthetic compound based on the khellin , the active ingredient of the plant khella	Middle Eastern traditional medicine for asthma , also used in Egypt for kidney stones
Etoposide	Antitumor	Synthesized from podophyllotoxin produced by mandrake	Various folk medicine remedies Chinese, Japanese and East
Hirudin	Anticoagulant	salivary glands of leeches , now produced by genetic engineering	Traditional remedies from around the world, from the *zhi shui* in China to European medicine in the eighteenth and nineteenth centuries.
Lovastatin	It is used to lower cholesterol	girgolas and foods such as red yeast rice . Used to synthesize other compounds such as mevastatin and pravastatin	Fungi are used to treat a broad spectrum of diseases in traditional Chinese medicine , Japanese , Eastern Europe and Russia.
Opiates	Analgesics	Seeds immature poppy	Traditional remedies Arabic, Chinese , Europeans, Indians and North Africans , used to relieve pain and treat a range of conditions such as diarrhea, cough and asthma
Quinine	Antimalarial	Cinchona bark	Traditional remedies for fever and cramps in South America
vinca alkaloids (vincristine , vinblastine)	Antitumor	Rosehip	Various folk remedies around the world , including its use as antidiabetic Jamaica, to treat wasp stings in traditional Indian medicine , eye drops, Cuba and the love potion in medieval Europe .

Many Malaysian believe that *Channa striatus* facilitate wound healing. For this reason, it is widely consumed after an injury or an operation. Women for example, during their postpartum period have *Channa striatus* as their main dish. It is also consumed by post-operative patients with the hope that it can promote and enhance healing and alleviates post-operative pain (Mat Jais, 1994).

Study has shown that *Channa striatus* has high content of essential amino acids such as glycine, aspartic and glutamine, and essential fatty acid such as arachidonic acid which are important in the process of wound healing and, as well as enhancing the antinociceptive activity (Jais *et al.*, 1998, Zuraini A, 2006). Other studies also proved *Channa striatus* enhances cutaneous wound healing (Baie and Sheikh, 2000b).

Wounds heal through several phases of haemostasis, inflammation, proliferation and remodeling (Goldsmith, 1991). Acute wound healing proceeds via unimpaired progression through those four overlapping phases. Each phase is characterized by the infiltration into the wound site of specific cell types, all of which interact and communicate, by chemical signals, to optimize repair. The healing tends to be compromised, for example, by malnutrition, infection, metabolic disturbances, or an underlying disease.

One of the wound that has huge implication on patient morbidity and mortality is the laparotomy wound. The past 50 years has witnessed a dramatic rise in therapeutic abdominal operations with improvements in perioperative critical care, advances in surgical oncology and the success of abdominal organ transplantation. The management of abdominal wall injury has in many ways not kept pace with innovations in abdominal

surgery. Laparotomy incisions fail to heal 11% of the time (Pollock AV, 1989). Subsequent incisional hernia repairs fail to heal 24% to 58% of the time (Flum DR, 2003). This high rate of surgical wound failure results in thousands of reoperations and higher morbidity and mortality. In United State for example, about 4 million laparotomies performed each year, and the true incidence of laparotomy wound failure and incisional hernia formation approaches 400,000 per year. Each additional abdominal operation increases the risk of further intra-abdominal injury (Duepree HJ, 2003).

Burst abdomens, or acute fascial dehiscence with evisceration, are a rarer, but important extreme of acute wound failure. They have for a long time been associated with mortality rates of nearly 50%. It is estimated that 1% to 3% of laparotomy incisions are associated with clinically obvious dehiscence and evisceration (Carlson, 1997). Even at this relatively low rate, with approximately 4 million laparotomy incisions annually in the United States, this predicts 40,000 to 120,000 acute laparotomy wound failures each year in the United States and 20,000 to 60,000 deaths directly attributable to this dreaded wound complication. Other studies have found that more than 50% of the repaired laparotomy dehiscences will go on to form incisional hernias, entering many of these patients into a vicious cycle of surgical repair, reherniation, and acute and chronic wound complications (Hesselink VJ, 1993). Such impaired healing inflicts a huge cost upon society, diminishing the quality of life for millions worldwide.

One of the factors that affect and impair wound healing process is nutritional factor. Patients, who are malnourished or actively catabolic, such as in the systemic inflammatory response syndrome, demonstrate impaired healing. Inadequate nutrition also retards the

immune response limiting opsonization of bacterial and sterilization of wounds (Demling RH, 2000). Loss of protein from protein-calorie malnutrition leads to decreased wound tensile strength, decreased T-cell function, decreased phagocytic activity, and decreased complement and antibody levels, ultimately diminishing the body's ability to defend the wound against infection (Casey J, 1983).

Malnutrition may preexist wounding or may be encountered secondary to the catabolic imbalance of the patient's overall metabolic state during wound healing. A study of orthopedic patients, including post trauma patients and patients undergoing total hip replacement, found that 42% of patients were malnourished (Jensen JE, 1982). A study conducted in 1984 where they evaluated 215 non cancer patients preoperatively found that 12% of the patients showed evidence of malnutrition (Warnold I, 1984). Another study showed that approximately 50% of all medical and surgical patients at an urban hospital in 1974 had evidence of malnutrition (Daley BJ, 1994).

The purpose of this study was therefore to investigate the healing effect of *Channa striatus* on the laparotomy wound. As malnourishment is very much prevalence among the surgical patient, this study was conducted on malnourished subjects.

2.0 REVIEW OF LITERATURE

2.1 *CHANNA STRIATUS*

Channa striatus is a snakehead fish belongs to the Channidae family. It is indigenous to many tropical and subtropical countries including Malaysia and a valuable source of protein throughout the Asia Pacific region (Mohsin A.K, 1983b). *Channa striatus* has been studied for its putative effects on wound healing (Baie and Sheikh, 2000b, Mat Jais, 1994). It is used by patients in the post-operative period to promote wound healing (Mat Jais, 1997) and to reduce pain (Zakaria, 2004).

Figure 1 *Channa striatus* species.

(Source: Google Images.net)

2.1.1 Taxonomy, Distribution and Biology

Channa striatus is a tropical, fresh water, carnivorous, air breathing fish species. *Channa striatus* come from the Channidae family, and well known as snakehead fish. There are thirty species of Channidae around the globe, and eight were found in Malaysia. The Channidae are well distributed within China, Taiwan, Indochina, Thailand, Phillippines, Indonesia and India (Mohsin A.K, 1983a).

The natural habitats of *Channa striatus* are remote water but some can be found in close proximity to settlements such as ponds, small lakes, agriculture canals, small rivers and paddy fields. Some can be found in various unexpected places such as a higher ground with water temperature about 20 C. The optimum water PH for *Channa striatus* is between 4.3 to 7.9, temperature is between 20.7 to 26.4 C, water turbidity between 2 to 268 ppm and dissolved oxygen between 1.2 to 6.1 ppm (Jais, 1991). Studies on the genetic variability of *Channa striatus*'s mitochondrial DNA revealed that *Channa striatus* being present in Malaysia for more than 600,000 years thus proving that the fish is truly Malaysian indigenous species (Jais, 2007).

Channa striatus is not a popular in the list of farming fish activity in Malaysia due to its carnivorous behaviour (Mohsin A.K, 1983a). However, some countries in the region, such as Indonesia, Thailand and Indochina are having extensive breeding programs because it is among the popular table dishes in seafood restaurants (Jais, 1991) .

2.1.2 Chemistry of *Channa striatus*

Several studies have been done recently to analyze and identify the main composition of *Channa striatus.* It mainly consist of protein (78.32 +-0.23%). The aqueous extract of *Channa striatus* was found to contain all amino acids, with the major amino acids found being glycine, alanine, lysine, aspartic acid and proline (35.77%, 10.19%, 9.44%, 8.53% and 6.86 % of total protein, respectively) (ZA Zakaria, 2007).

Channa striatus lipid content is quite low at 2.08 %. It also has a high content of arachidonic acid and docosahexaenoic acid (DHA). The most abundant fatty acid present in the aqueous extract of *Channa striatus* is palmitic acid (C16: 0), which accounted for approximately 35.93 % of total fatty acids. The other major fatty acids included oleic acid (C18: 1), stearic acid (C18: 0), linoleic acid (C18: 2) and arachidonic acid (C20: 4), which accounted for the 22.96%, 15.31%, 11.45 % and 7.44 % of total fatty acids, respectively. *Channa striatus* was also found to have ratios of w-3 : w-6 and polyunsaturated fatty acid:saturated fatty acid (PUFA/SFA) lower than 1 (Zuraini A, 2006, ZA Zakaria, 2007).

Channa striatus also has good amount of dietary mineral such as magnesium, calcium, copper, manganese, iron and zinc. Nickel and lead also naturally occurring mineral in *Channa striatus* but they are well below toxic level to human (jais, 1997).

Table 2 Protein composition of Channa striatus (Jais *et al.*, 1998)

Amino acid	Total Protein %
Aspartic acid	8.53
Glutamic acid	4.59
Serine	3.40
Glycine	35.77
Histidine	1.61
Arginine	4.09
Theorine	4.07
Alanine	1.19
Proline	6.86
Tyrosine	1.10
Valine	2.18
Methionine	1.53
Isoleucine	1.28
Leucine	2.91
Phenylalanine	2.48
Lysine	9.44

Table 3 Fatty acid composition of Channa Striatus (Jais *et al.*, 1998)

Fatty acid	% Total fatty acid
Myristic acid	2.15
Palmitic acid	35.93
Stearic acid	15.31
Heptadecanoic acid	2.90
Palmitoleic acid	1.86
Oleic acid	22.96
Linoleic aicd	11.45
Arachidonic acid	0.83

2.1.3 Antimicrobial Properties of Channa striatus

Channa striatus's extract had shown positive although mild results as anti-bacterial and anti-fungal. *Channa striatus's* extract has shown inhibition effects on the growth of 13 filamentous fungus and 3 non-filamentous or yeast species (Mat Jais, 2007).

2.1.4 Antinociceptive Activity of *Channa striatus*

The *Channa striatus* fillet extract produces a dose dependent anti-nociceptive property, which is also essential in healing process (Mat Jais, 1997). Based on studies on rat,