**A Study Protocol on the Effect of Haruan Fish (*Channa striatus*) Extract on Wound Healing and Quality of Life in Patients with Sternotomy and Leg Wounds after Coronary Artery Bypass Grafting Surgery: A Prospective, Randomized Controlled Trial**

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Executive Summary

PROBLEM STATEMENT: Coronary artery bypass grafting (CABG) surgery is still the standard procedure in the treatment of advanced coronary artery disease especially in multi-vessel disease. This surgery requires a sternotomy and the harvesting of venous conduits of the legs and occasionally the radial artery. Considering the extensive surgery and long incisions, especially in harvesting the saphenous veins, wound healing remains as one of the main challenges post-operatively. It is also known that many factors contribute to wound healing including clinical, psychosocial, organizational and educational. HYPOTHESIS: *Haruan* or *Channa striatus,* a freshwater snakehead fis*h,* is widely believed to promote wound healing; hence, it would be an interesting and challenging theory to be proven scientifically by introducing evidence-based practice. RESEARCH METHODOLOGY: This study is designed in a way that the wound will be assessed post-operatively with the optimum and standard patient care in two groups of randomized patients. One group would receive placebo and the study group would receive *Haruan* capsules. The wound assessment is done using ASEPSIS scoring method that has been proven to be both objective and repeatable. This method would grade the wound as uninfected, disturbed healing, minor infection, moderate infection and severe sepsis. Visual Analog Scale will be used to assess the severity and quality of pain experienced by patients. The same scale is also used to assess the patients’ mobilization. Finally an important assessment would be on the health related quality of life of patients. This assessment will be done using the Nottingham Health Profile. EXPECTED OUTPUT/OUTCOMES/OMPLICATIONS: It is postulated there will be improvement I wound healing on the study arm and a reduction in post-operative wound morbidity. There will also be improvements in patients’ quality of life. SIGNIFICANCE OF OUTPUT FROM THE RESEARCH PROJECT: The faster recovery and mobility with a reduction in in percentage of would healing would subsequently result in the reduction of total hospital costs and confer an advantage in term of human resources to the overall economic benefits. The possibility of marketing *haruan* as well, which can be reared domestically in fishponds, and commercialization of *haruan* protein capsules for biomedicine use, is highly potential and very attractive.

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| **Introduction**:  **Aims**:  **Hypothesis**:  **Study Design: Place and Duration of Study**:  **Materials and Methods**: ***Randomization***:  ***Measurement***:  ***Sample size calculation***  **Expected Outcomes**: |

***Keywords****: wound healing, channa striatus, quality of life, coronary artery bypass grafting, randomized controlled trial*

1. INTRODUCTION

Despite the advancement in percutaneous trans-coronary angioplasty (PTCA) in managing patients with coronary artery disease (CAD), coronary artery bypass grafting (CABG) surgery remains a dominant operation in managing multi vessel disease. In CABG surgery, the internal mammary artery and greater saphenous vein are the usual vessels chosen as conduits for revascularization1 though radial artery is occasionally utilized as a conduit as well despite two recent randomized studies showing no evidence of its superiority.2.3

Delayed sternal wound healing and sternal wound infections are a major cause of morbidity and mortality in patients after cardiac surgery. The frequency of this complication is generally low, between 0.7%4 and 3.3%5. However, incidences up to 9.7%6 have been reported with prolonged post-discharge surveillance. Potential sequelae of infection of this site include wound dehiscence, mediastinitis, pericarditis, osteomyelitis and endocarditis7 with associated mortality between 14% and 47%8-12.

Even leg wound infection, which rarely represents a threat to life, adds importantly to morbidity; the average cost of maintaining a patient in hospital with a wound infection is three times that of patients with an uncomplicated postoperative course10. Some other known complications arising from leg wound infection are wound breakdown, cellulitis, lymphangitis, fat necrosis and delayed healing. Rates of these complications vary among published reports, with an incidence as high as 24%.13-16

Findings of previous studies have demonstrated little variation in dressing effectiveness in relation to infection and healing17-20 in the context of incisional wound. The inability to demonstrate specific dressing effectiveness is related to the nature of the wound itself, where healing is taking place by primary intention20. It is of note that 48% of wound infections were deep infections that are more likely to be associated with preoperative and intraoperative factors rather than specific dressing.21

With that understanding, perhaps a better way of promoting wound healing would be systemic rather than topical; with all other factors remaining constant. Extract of *Channa striatus* or locally known as Haruan (snakehead), a fresh water carnivorous fish has been proven to influence the different phases of the wound healing process.22,23 Animal studies have shown that *Channa striatus* has anti-nociceptive effects that could reduce postoperative pain.24-26 Studies have also shown that *Channa striatus* contains all essential amino acids for wound healing, particularly glycine as well as high content of arachidonic acid and polyunsaturated fatty acids that can promote prostaglandin synthesis.22 It has also been shown that *Channa striatus* promotes remodeling of collagen by the synthesis of inter and intra molecular protein cross-linking and then produces a marked increase in the tensile strength that accentuates wound healing.27 Other than that, the fish also has certain fatty acids that have been reported to produce anti-inflammatory activity such as stearic acid and oleic acid, which might also help to explain its ability to exhibit anti-inflammatory activity.28

Up to this point in time, all the above studies on *Channa striatus* have been on animal models. We find it rather interesting to conduct a prospective randomized control trial (RCT) and assess not only wound healing and wound infection after CABG, but also to look into the health-related quality of life (HRQoL) of such patients, considering the anti-inflammatory and anti-nociceptive effect of *Channa striatus*. The potential value of patenting such natural product and it’s usage on other types of surgical wounds including laparotomy and Caesarean section, compounded with the commercial value associated with it, makes it even more attractive.

**2. OBJECTIVE & HYPOTHESES**

The objective of this study is to prospectively examine the effect of extracts of the local fish Haruan (*Channa striatus*) on surgical wound healing and HRQOL in patients who have undergone CABG using the RCT approach.

The hypotheses are as follows:

(1) The Haruan extract increases the surgical wound healing rate, and

(2) The Haruan extract improves the HRQOL in post-surgical CABG patients with surgical wounds.

3. material and methods

**Study Design**

***Laboratory***

The Haruan fish to be used in this study is locally reared in a special pond for Haruan; the sex of the fish is not determined, and the Haruan capsules are manufactured using strict Good Manufacturing Practice (GMP) Guidelines.

*Channa striatus* will be cleansed and all internal organs will be removed. The fish is then placed in an autoclave machine at 1210C for one hour. Subsequently the fish will be dried in an oven at 600C.

The dried fish is then grinded to 700 micron and filled into capsules with 250mg of *Channa striatus* per capsule.

***Clinical***

A RCT is designed where patients with CAD requiring CABG surgery will be prospectively and randomly divided into two parallel groups. One group will receive *Channa striatus* capsule formulation derived from the fillet of *Channa striatus* while the control group will receive identical placebo (maltodextrine) capsules.

Inclusion Criteria:

1. Male or female

2. More than 18 years of age

3. Elective, urgent or emergency coronary artery revascularization

Exclusion Criteria:

1. Less than 18 years of age

2. Refusal to have surgery

3. Inability to give informed consent

4. Documented allergy to fish or fish product

**Sample Size Estimation**

We use the PS Software for sample size calculation. We are planning a study of a continuous response variable from independent control and experimental subjects with 1 control(s) per experimental subject. Based on a previous study [29] we are planning a study of independent cases and controls with 1 control(s) per case. Prior data indicate that the failure rate among controls is 0.027. If the true relative risk of failure for experimental subjects relative to controls is 6.9, we will need to study 58 experimental subjects and 58 control subjects to be able to reject the null hypothesis that this relative risk equals 1 with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. We will use an uncorrected chi-squared statistic to evaluate this null hypothesis. Assuming a drop-out rate of 20%, the minimum sample size required is about 70 (58 + 12) subjects per group.

**Sampling**

***Preoperative***

The two randomized groups [based at the National Heart Institute (IJN)] will be matched according to sex, age, NYHA criteria, ejection fraction, and diabetic status. Operative and perioperative conditions will also be similar for both groups. All subjects will receive an identical prophylactic antibiotic regime consisting of *Cefazolin* 2gm at induction and 1 g 12 hourly for 48 hours. *Gentamicin* 2mg/kg will also be given at induction.

***Intra-operative***

A uniform method of wound closure and disinfection protocol will be followed. Skin will be disinfected with *Betadine* followed by *Povidone Iodine* 10%. The method for wound closure will also be similar for both groups, namely double layer sutures up to the intra-cutaneous skin. Going from deep up to the surface, the pre-sternal fascia will be closed with 1-0 Vicryl suture in a continuous type followed by closure of the subcutaneous tissue. Skin will be closed with Monocryl 3/0 in subcuticular manner. For the leg wound, the subcutaneous tissue will be closed with Vicryl 0, followed by continuous skin suture with Maxon 3/0.

Steel wire sutures will be used to close the sternum in all patients and titanium clips will be used to secure branches of the internal mammary artery on the chest wall and branches ofthe saphenous vein in the leg.

***Post-operative***

Both groups will start taking either 2 capsules (500mg) of *Channa striatus* or 2 capsules of placebo (maltodextrine) daily for a minimum of six weeks, starting from day one post-operatively. If the patient is still ventilated, the capsules will be broken and the content administered via a Ryle’s tube.

**Measurement**

***Wound Infection***

Both sternal and leg wounds will be inspected and assessed daily until subjects are discharged. Information at 6 weeks and 3 months postoperatively will be collected from patients in the clinic or over the telephone. Each wound will be scored using the ASEPSIS system (Appendix A). The important feature of this system is that the final score accrues by a simple and objective assessment of each element on the check-list, each element only being scored once during the 3 month period. Such a scoring system provides a more objective assessment. In general, a clinically significant wound infection will obtain a score of 21 – 30, a deep wound infection usually with a sternal click scoring 31–40 and bone infection scoring >41.

***Wound Healing***

Wound healing will be measured by assessing both wound approximation and skin integrity. Approximation has four categories: total, partial (<2cm of superficial separation), moderate (>2cm of superficial separation), and dehiscence (complete separation of layers). Surrounding skin integrity has three categories: normal (pink, no redness), inflamed (heat, redness, and swelling), or macerated within a 2.5 cm border of the incision. The presence or absence of necrotic tissue will also be noted. Thus, complete wound healing will show total approximation and normal skin integrity whereas incomplete wound healing will encompass other combinations of skin approximation and skin integrity. Measurements will be obtained at 6 weeks and 3 months postoperatively.

***Wound Pain***

A Visual Analogue Scale (VAS) of 0 to 10 cm will be used to assess the degree of wound pain. A score of 0 cm represents “no pain” and a score of 10 represents “severe pain”. Pain assessment will be recorded from day 1 and will continue daily until the subject is discharged. It will be further assessed during the clinic visit at 6 weeks and 3 months postoperatively.

***Mobilization***

Similarly, a VAS of 0 to 10 cm will be used to assess the degree of mobilization. A score of 0 cm represents “inability to walk” and 10 cm represents “excellent mobilization.” Assessment of mobilization will be made on the post-operative days 3 and 4 since there will be restrictions in mobility on days 1 and 2 after surgery. Similarly, it will be assessed again during clinic visit at 6 weeks and 3 months postoperatively.

***Health Related Quality of Life***

The health-related quality of life (HRQOL) analysis will be performed using the Nottingham Health Profile (NHP) questionnaire part 1. NHP has demonstrated its efficiency in clinical studies. NHP focuses not only on patients’ health but also those experiencing discomfort and pain. There is also a lower possibility for patients to ‘medicalize’ their psychological and social stress.

NHP part 1 contains 38 subjective statements divided into six sections: physical mobility, social isolation, emotional reaction, energy, pain and sleep. The scores of each section ranges from 0-100 by adding the item weight to every positive answer. A higher score indicates a higher level of dysfunction and worse QOL.

The questionnaire will be distributed to both groups of patients before CABG and at six weeks and three months during clinic visit postoperatively. The questionnaires are going to be administered using the questionnaire-interview approach.

The research activities and key milestones of the study are shown in **Table 1 and 2**, respectively.

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| **Table 1: Research activities** | | | | | |
| **Research activity** | **Month** | **Year** | **to** | **Month** | **Year** |
| * Presentation for Ethics Committee Approval | Dec | 2013 |  | Jan | 2014 |
| * Preparation of database for patients | Feb | 2014 |  | Mac | 2014 |
| * Preparation and translation of MHIQ for QOL | Mac | 2014 |  | Apr | 2014 |
| * Laboratory preparation of fortified *C striatus* capsules | May | 2014 |  | Jun | 2014 |
| * Randomization and recruitment of patients | Jun | 2014 |  | Jul | 2015 |
| * Follow-up of patients and MHIQ QOL reassessment | Aug | 2014 |  | Sep | 2015 |
| * Mid-cycle data analysis: Clinical and QOL | Dec | 2014 |  | Jan | 2015 |
| * Presentation and publication of preliminary results | Jan | 2015 |  | Feb | 2015 |
| * Complete data analysis: Clinical and QOL | Sep | 2015 |  | Oct | 2015 |
| * Preparation of data for presentation followed by publication and dissemination of knowledge | Nov | 2015 |  | Dec | 2015 |

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| **Table 3: Key milestones** | | |
| **Milestone** | **Month** | **Year** |
| * Ethics Committee approval | Jan | 2014 |
| * Randomization of patients and recruitment into research project | Jun | 2014 |
| * Mid-cycle analysis: Clinical and McMaster Health Index for Quality of Life | Dec | 2014 |
| * Final data analysis: Clinical and McMaster Health Index for Quality of Life | Sep | 2015 |
| * Project completion, presentation and publication of data | Dec | 2015 |

**Statistical Analysis**

Data will be examined for normality using the Kolmogorov-Smirnoff test and stem-and-leaf plot. If normal, we will proceed with the parametric tests; however, if not normal, we will proceed with the non-parametric tests.

The results will be presented as means ± standard deviations for scaled measurements; numbers and percentages for categorical measurements. The unpaired t-test will be used to examine mean differences for wound infection, wound healing, pain, and mobilization across the two groups. Differences in proportions will be examined using the Chi-square test.

To examine the mean QOL score differences between the two groups (pre-operative, six weeks, and three months), the one-way repeated measure ANOVA with post-hoc multiple comparison test (between and within subject) the two groups will be performed.

To determine the factors influencing the change of QOL after CABG, with the dependent variable being binary (improved or worsened), we will perform the simple logistic regression (SLogReg) initially, and examining the statistical significance of each independent variable such as the number of revascularization and duration of surgery on the outcome. Then, we will perform a multiple logistic regression (MLogReg) including variables with a level of significance ≤ 0.20 in the multivariate logistic regression and controlling for the effects of possible confounding variables (sex, age, NYHA Criteria, ejection fraction, and diabetic status). A *p* value of 0.05 will be taken as the level of significance.

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Competing interests

Authors have declared that no competing interests exist.

Authors’ Contributions

AFM designed the study, research protocol and first draft of the manuscript. JD, MEMT, AMY, and RO provided significant input into the protocol of the study. SB provided the processed Haruan and maltodextrine capsules and provided significant input into the research protocol. RBN revised the research protocol and manuscript, managed the literature searches, calculated the sample size and statistical analyses, and applied for research funding. All authors read and approved the final manuscript.

Consent

Written informed consent will be obtained from all subjects prior to commencement of the study. A copy of the written consent form is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

Ethical approval

All authors hereby declare that all experiments will be examined and approved by the appropriate ethics committee namely the National Heart Institute Ethics Committee and will therefore be performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The Clinical Trial will also be registered with the National Medical Research Register (NMRR), Ministry of Health, Malaysia.

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