UNIVERSITI SAINS MALAYSIA
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LAPORAN AKHIR

THE EFFECT OF CHANNA STRATUS (IKAN HARUAN) EXTRACT ON POST LOWER SEGMENT CAESARIAN SECTION (LSCS) WOMEN

PENYELIDIK

PROF. MADYA DR. AZIDAH ABDUL KADIR

PENYELIDIK BERSAMA

NIK HAZLINA NIK HUSSAIN
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SARINGAT BAI
NORHAYATI MOHD NOOR
INTAN IDIANA HASSAN
WAN HASLINDAWANI WAN MAHMOOD
ASRENEE ABD RAZAK

2015

BUKU LAPORAN AKHIR

GERAN RUI

PENYELIDIK UTAMA:

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TAJUK GERAN:

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UNIVERSITI SAINS MALAYSIA

UNIVERSITY RESEARCH GRANT FINAL REPORT
Geran Penyelidikan Universiti Laporan Akhir

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A.	**PARTICULARS OF RESEARCH** / *MAKLUMAT PENYELIDIKAN:*
(i)	**Title of Research:** *Tajuk Penyelidikan:* THE EFFECT OF *CHANNA STRIATUS* (IKAN HARUAN) EXTRACT ON POST LOWER SEGMENT CAESARIAN SECTION (LSCS) WOMEN
(ii)	
B.	**PERSONAL PARTICULARS OF RESEARCHER** / *MAKLUMAT PENYELIDIK:*
(i)	**Name of Research Leader:** *Nama Ketua Penyelidik:* PROF. MADYA DR AZIDAH ABDUL KADIR
	Name of Co-Researcher: *Nama Penyelidik Bersama:* NIK HAZLINA NIK HUSSAIN JULIA OMAR SARINGAT BAI NORHAYATI MOHD NOOR INTAN IDIANA HASSAN WAN HASLINDAWANI WAN MAHMOOD ASRENEE ABD RAZAK
(ii)	**School/Institute/Centre/Unit:** *Pusat Pengajian /Institut/Pusat/Unit:* Pusat Pengajian Sains Perubatan
D.	**Duration of this research:** *Tempoh masa penyelidikan ini:* ***Duration :** 3 tahun *Tempoh :* **From** : 15.03.2011 **To** : 14.09.2014 *Dari:* *Ke :*

E. ABSTRACT OF RESEARCH

(An abstract of between 100 and 200 words must be prepared in **Bahasa Malaysia and in English.** This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Channa striatus or its local name Haruan, is a fresh water fish consumed for decades as a remedy to promote wound healing by women during postpartum period. The objectives were to compare post-operative pain score; wound healing, safety profile, anteroposterior (AP) measurement of the uterus, pulsatility index (PI), resistive index (RI) of uterine artery and superficial skin wound artery between *Channa striatus* and placebo groups following six weeks of Lower Segment Caesarean Section (LSCS) delivery. This is a randomized, double blind, placebo-controlled study, done in post Lower Segment Caesarean Section (LSCS) women. Subjects were randomized into either *Channa striatus* or placebo group with daily dosage of 500mg of *Channa striatus* extract or 500mg maltodextrin respectively for six weeks post-operation. The women were assessed on Post-operative pain score, Wound Evaluation Scale (WES), Visual Analogue Scale (VAS), Patient's Satisfaction Score (PSS), anteroposterior (AP) measurement of the uterus in longitudinal and oblique transverse planes, pulsatility index (PI), resistive index (RI) of uterine artery and superficial skin wound artery at baseline (day 3), two weeks, four weeks and six weeks post-operatively. The safety profile (RFT, LFT and FBC) were assessed at baseline and six weeks post-operatively.Seventy six subjects were successfully randomised into this study with 38 in *Channa striatus* group and 38 in placebo group. There were no significant difference detected in terms of post-operative pain (p=0.814), WES (p=0.160), pulsatility index (PI), resistive index (RI) of uterine artery and superficial skin wound artery between *Channa striatus* and placebo group. However, (VAS) and (PSS), AP measurement of uterus on longitudinal plane and oblique transverse plane in *Channa striatus* group was significantly better compared to placebo group. Safety profile showed no significant difference between both groups. In conclusion, the consumption of 500mg of *Channa striatus* extract daily showed marked differences in cosmetic appearance, patient's satisfaction towards wound healing and reduction of uterine size in post LSCS women. It is also safe for human consumptions.

Abstrak Penyelidikan

(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris**.
Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Channa striatus atau nama tempatannya Haruan, telah digunakan berdekad lamanya untuk mempercepatkan penyembuhan luka oleh wanita sewaktu berpantang selepas bersalin. Objektif kajian adalah untuk membandingkan tahap kesakitan selepas pembedahan, penyembuhan luka, profil keselamatan ekstrak, pengiraan panjang *anterior posterior (AP)* rahim, *index pulsatiliti (PI), index resistif (RI)* arteri rahim dan arteri *superficial* luka kulit selama enam minggu selepas melahirkan anak secara pembedahan *Lower Segment Caesarean Section (LSCS)*. Dengan itu, satu kajian rawak, rabun dua pihak dengan kawalan plasebo telah dijalankan. Semua subjek telah dibahagikan secara rawak dengan nisbah 1:1 sama ada ke dalam kumpulan *Channa striatus (*500mg sehari) atau kumpulan plasebo (500mg maltodextrin sehari). Tahap kesakitan selepas pembedahan, Skala Penilaian Luka (*WES*), Skala Analog Visual (*VAS*) dan Skor Kepuasan Hati Pesakit (*PSS*), pengiraan panjang *anterior posterior (AP)* rahim, *index pulsatiliti (PI), index resistif (RI)* of arteri rahim dan arteri *superficial* luka kulit telah dinilai pada hari ketiga (peringkat permulaan), minggu kedua, minggu keempat dan minggu keenam selepas pembedahan. Manakala profil keselamatan berdasarkan fungsi hati (*LFT*) , ginjal (*RFT*) dan pengiraan darah menyeluruh (*FBC*) pula dinilai pada hari ketiga dan minggu keenam selepas pembedahan. Keputusannya, seramai tujuh puluh enam orang subjek telah berjaya di bahagikan secara rawak; dengan 38 orang dalam kumpulan *Channa striatus* dan 38 orang lagi dalam kumpulan plasebo. Tiada perbezaan yang signifikan daripada segi pengurangan tahap kesakitan selepas pembedahan , *index pulsatiliti (PI), index resistif (RI)* of arteri rahim dan arteri *superficial* luka kulit di antara kumpulan *Channa striatus* dan kumpulan plasebo. Walaubagaimanapun, *VAS* , *PSS* dan pengiraan panjang *anterior posterior (AP)* rahim,dalam kumpulan *Channa striatus* didapati lebih baik dan signifikan berbanding dengan kumpulan plasebo . Sementara itu profil keselamatan tidak menunjukkan sebarang perbezaan yang signifikan di antara kedua-dua kumpulan. Kesimpulannya, pengambilan ekstrak *Channa striatus* sebanyak 500mg sehari didapati berkesan dalam meningkatkan tahap kosmetik dan kepuasan hati pesakit terhadap penyembuhan luka. Ektrak ini juga terbukti selamat untuk dimakan oleh manusia.

F. **SUMMARY OF RESEARCH FINDINGS**
Ringkasan dapatan Projek Penyelidikan

In conclusion, the consumption of 500mg of *Channa striatus* extract daily showed marked differences in cosmetic appearance, patient's satisfaction towards wound healing and reduction of uterine size in post LSCS women. It is also safe for human consumptions.

G. **COMPREHENSIVE TECHNICAL REPORT**
Laporan Teknikal Lengkap
Applicants are required to prepare a comprehensive technical report explaining the project.
(This report must be attached separately)
Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.
[Laporan ini mesti dikepilkan]

List the key words that reflect our research:
Senaraikan kata kunci yang mencerminkan penyelidikan anda:

English	Bahasa Malaysia
Post caesarian	Selepas pembedahan bersalin
Post operative pain	Sakit selepas pembedahan
Wound healing	Penyembuhan luka
Uterine measurement	Pengiraan panjang rahim

H. a) **Results/Benefits of this research**
Hasil Penyelidikan

No. *Bil:*	Category/Number: *Kategori/ Bilangan:*	Promised	Achieved
1.	**Research Publications (Specify target journals)** *Penerbitan Penyelidikan (Nyatakan sasaran jurnal)*	2	2 publication (awaiting result) 6 Conference presentation 3 Awards (attachment)
2.	**Human Capital Development**		
	a. Ph. D Students		
	b. Masters Students	**2**	1 MSc 1 M.Med (radiology)
	c. Undergraduates (Final Year Project)		
	d. Research Officers		
	e. Research Assisstants	**1**	**1**
	f. Other: Please specify		
3.	**Patents** *Paten*		
4.	**Specific / Potential Applications** *Spesifik/Potensi aplikasin*		
5.	**Networking & Linkages** *Jaringan & Jalinan*		
6.	**Possible External Research Grants to be Acquired** *Jangkaan Geran Penyelidikan Luar Diperoleh*		

- Kindly provide copies/evidence for Category 1 to 6.

b) Equipment used for this research.
Peralatan yang telah digunakan dalam penyelidikan ini.

Items *Perkara*	Approved Equipment	Approved Requested Equipment	Location
Specialized Equipment Peralatan khusus			
Facility Kemudahan			
Infrastructure Infrastruktur			

- Please attach appendix if necessary.

H.	**COMMENTS OF PTJ'S RESEARCH COMMITTEE** *KOMEN JAWATANKUASA PENYELIDIKAN PERINGKAT PTJ*

General Comments:
Ulasan Umum:

Good achievement and output. 3 gold award.
received with publications.
I reckon the closing of the grant.

UNIVERSITI SAINS MALAYSIA

JABATAN BENDAHARI

PENYATA PERBELANJAAN SEHINGGA 30 NOVEMBER 2014

Projek :

No.Akaun : 1001.PPSP.812090.

Vot	Nama Vot	Peruntukan Projek	Perbelanjaan Terkumpul Sehingga Thn Lalu	Baki Peruntukan Tahun Lalu	Peruntukan Thn Semasa	Jumlah Peruntukan Thn Semasa	Tanggungan Semasa	Bayaran Thn Semasa	Jum Belanja Thn Semasa	Baki Projek
111	GAJI	25,498.33	0.00	25,498.33	0.00	25,498.33	0.00	0.00	0.00	25,498.33
221	PERJALANAN DAN SARA HIDUP	747.94	0.00	747.94	0.00	747.94	0.00	0.00	0.00	747.94
222	PENGANGKUTAN BARANG-BARANG	1,600.00	0.00	1,600.00	0.00	1,600.00	0.00	0.00	0.00	1,600.00
223	PERHUBUNGAN DAN UTILITI	470.68	0.00	470.68	0.00	470.68	0.00	3.71	3.71	466.97
227	BEKALAN DAN BAHAN LAIN	-2,161.20	0.00	-2,161.20	0.00	-2,161.20	0.00	26,823.30	26,823.30	-28,984.50
229	PERKHIDMATAN KHUSUS & HOSPITALITI	23,915.59	0.00	23,915.59	0.00	23,915.59	0.00	12,246.10	12,246.10	11,669.49
	Jumlah	50,071.34	0.00	50,071.34	0.00	50,071.34	0.00	39,073.11	39,073.11	10,998.23

Penyata ini adalah cetakan komputer tiada tandatangan diperlukan

Penyata ini adalah dianggap tepat jika tiada maklumbalas dalam tempoh masa 14 hari dari tarikh penyata

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INTRODUCTION

1.1 Background of the study

Channa striatus, a fresh water snakehead fish consumed in many parts of the Southeast Asian region; for decades is known among local for protein and traditional remedy (Wee, 1982). "Haruan" is the local name for *Channa striatus* in Malaysia. In Philipines, this fish also called as "Haruan". It has been considered as a very good source of health food amongst Asians because it contains high levels of amino acids and fatty acids (Mohd Shafri & Abdul Manan, 2012). *Channa striatus* also normally consumed by women during postpartum to promote wound healing (Mat Jais *et al.*, 1997) as well as to reduce post-operative pain (Mat Jais *et al.*,1994; Mat Jais *et al.*, 1997).

Scientific research carried out in Malaysia have unraveled the biomedical potential of the fish (Mat Jais *et al.*, 1998). The works carried out indicated that *Channa striatus* has a good anti-nociceptive (anti pain) property which is comparable to morphine (Zakaria *et al.*, 2007). The anti-nociceptive property makes it suitable for reduction of post-operative pain (Mat Jais *et al.*, 1997) It also has anti-inflammatory, anti-microbial and anti-cancer property (Michelle *et al.*, 2004; Somchit *et al.*, 2004; Mat Jais *et al.*, 2008).

On top of all those properties, *Channa striatus* also has been proven to influence the different phases of wound healing process by enhancing dermal wound healing based on animal studies (Mat Jais *et al.*, 1997; Mat Jais *et al.*, 1994; Baie & Sheikh 2000a,2000b). Biochemical components of *Channna striatus* such as amino acids and fatty acids are important for the synthesis of collagen fibers during wound healing (Baie & Sheikh 2000a,2000b). *Channa striatus* contains all the essential amino acids for wound healing particularly glycine. High contents of arachidonic acid and polyunsaturated fatty acids that can promote prostaglandin synthesis (Somchit *et al.*, 2004; Zakaria *et al.*, 2005a); also plays a vital role in healing the wounds (Mat Jais *et al.*, 1998). The bioactive compounds in *Channa striatus* also acts synergistically on muscarinic, gama-aminobutyric acid (GABA) and serotonergic receptor systems (Zakaria *et al.*, 2005b). Thus it is postulated that *Channa striatus* might have the same effect in human through this pathways.

1.2 Problem Statement

Channa striatus is consumed by many Malaysians to promote healing in surgical wound especially women in the postpartum period. Although there are extensive studies on *Channa striatus* biomedical properties, the studies were done on animal only and there is no clinical trial in human yet (Mohd Shafri & Abdul Manan, 2012).

Therefore we undertook this study to assess the effect of *Channa striatus* extract that may have a possible role or potential in wound healing and reducing the post-operative pain in human. To our knowledge, there is no published report on the effect of *Channa striatus* extract on uterus involution using conventional or Doppler ultrasound in post lower segment caesarean section women (LSCS). The used of ultrasound in this study would allow objective assessment of the uterus involution during the puerperium. Ultrasound has been suggested to be useful in assessing uterine scar following LSCS [11]. The measurement of resistance flow indices, PI and RI from the flow velocity waveforms of the uterine artery postpartum would provide us new information on the effect of *Channa striatus* extract on the hemodynamic event during the postpartum involution process. Post LSCS women were chosen for the study subjects because they are the major group of *Channa striatus* user in our population. This study was conducted as a randomised, double-blinded, placebo

controlled trial in order to evaluate the effectiveness of *Channa striatus* (Haruan) extract among the women.

1.3 Research objective(s):

1.3.1 General objective:

To evaluate the effectiveness of *Channa striatus* (Haruan) extract as compared to placebo in terms of pain, wound healing and safety profile among post Lower Segment Caesarean Section (LSCS) women.

1.3.2 Specific objectives:

To compare the difference between *Channa striatus* and placebo in terms of:

i. Post-operative pain score

ii. Wound healing based on:

   a. Wound Evaluation Scale (WES)

   b. Visual Analogue Scale (VAS)

   c. Patient's satisfaction score (PSS)

iii. To compare mean anteroposterior measurement of the uterus in longitudinal and oblique transverse planes between patient taking *Channa striatus* and placebo.

iv. To compare mean resistive index (RI) and pulsatility index (PI) of the uterine artery between patient taking *Channa striatus* and placebo

v. To compare mean resistive index (RI) and pulsatility index (PI) and of the artery of the superficial skin wound between patient taking *Channa striatus* and placebo.

vi. Safety profile based on Renal Function Test (RFT), Liver Function Test (LFT) and Full Blood Count (FBC).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Channa striatus is a carnivorous freshwater fish which inhabits almost every river, pond, swamp and paddy field (Wee, 1982). It is also known by the English name as snakehead or stripped murrel (Muhammad Ghalib, 2011). Known by Malays as "Haruan", *Channa striatus* has been reputed as aggressive carnivorous fish (Mohd Shafri & Abdul Manan, 2012).

The name "snakehead" comes from its head that looks like a snake with dark brown or black chevron stripes along its body. It has elongated, widened jaw that is deeply-gaping with fully-toothed mouth that suit its predatory behavior (Mat Jais, 2007

2.2 Traditional Uses of *Channa striatus*

2.2.1 Food fish

Channa striatus is mainly used as food fish (Mohd Shari & Abdul Manan, 2012). For centuries, Malay people cooked the fish in various dishes like soup, roasted, steamed, curried and fried (Mohd Shari & Abdul Manan, 2012). The consumption of *Channa striatus* and other freshwater fishes provides about 70% of total protein requirement (Osman *et al.*,2001). Besides Malays, Malaysian and Singaporean Chinese also take *Channa striatus* in the form of soup or porridge and called it as "sang yue" (The Food Cannon, 2011).

This fish also is being prepared for *Yee Sang* during Chinese New Year (Mat Jais, 2007a). Its firm white boneless flesh (Mat Jais, 2007a), and heavy dark skin is good for soup and makes them suitable to be served in high class hotels and restaurants (The Food Cannon, 2011). *Channa striatus* is also taken as food fish by "Orang Asli" (indigenous people) in Peninsular Malaysia and tribal communities in East Malaysia (Haemalar *et al.*, 2010; Kodoh *et al.*, 2009).

2.2.2 Wound Healer

Channa striatus is synonym with the uses for postnatal diet (Barakbah, 2007; Noor, 1999, 2008) and recuperation from illnesses or surgery (Lee and Ng, 1991). For postpartum mothers, *Channa striatus* is taken to accelerate the wound healing process (Mat Jais, 2007a; Mat Jais *et al.*, 1994) at the episiotomy, Caesarean wound, perineal tear and laceration (Barakbah, 2007; Noor, 1999; Noor, 2008; Mat Jais, Mat Jais, 1994; Mat Jais, 2007a)

Channa striatus is also traditionally used as a medicine to quicken the healing process of newly-circumcised boys (Mustafa *et al.*, 2012). The Malay also take *Channa striatus* as a medicine to heal diabetic foot ulcer and gangrene (Mat Jais *et al,*. 1997; Mat Jais, Matori *et al.*, 1998; Muntaziana *et al,*. 2013)

2.2.3 Pain reliever

It is strongly believed that taking *Channa striatus* not only can accelerate the healing processes but also alleviate the pain sensation of post-surgical operation, circumcisions, Caesarean delivery and all traumatic wounds including injuries due to road accident (Mat Jais *et al.*, 1994; Mat Jais *et al.*, 1998, Mat Jais 2007a).

2.2.4 Curing skin diseases

Channa striatus is also very useful in reducing various skin problems like acne, pimples, allergy, psoriasis, sclerosis and infections (Mat Jais 2007a)

2.2.5 Supplements for elderly

Among the Malays, *Channa striatus* based meals playing the role as functional food which helps the recovery of many illnesses as well as diet supplement for elderly people (Mohd Shafri & Abdul Manan, 2012). It has also been used to reduce muscle and joint pain (Mohd Feroz, 2003).

2.3 Scientific Research: Fatty acids and amino acids composition of *Channa striatus*

2.3.1 Fatty acids composition

Suriah *et al.*, in 1995 did a study to determine fatty acid composition of several Malaysian freshwater fish. They obtained the most common freshwater fish and analyzed their lipid sample. From the findings, they have classiffied *Channa striatus* as a low fat fish compared to other fish with only 3.25% of fat of its whole body weight making the fish a good source of low fat diet.

Wild species of *Channa striatus* contain not more than 3% of fat per body weight, but cultured samples might have higher percentages. Several studies of wild sample reported that it contains low level at 0.99% (Karapanagiotidis *et al.*, 2010); 1.47% (Chedoloh *et al*, 2011) and 2.08% (Mat Jais, 2007a). Whereas other researchers reported a higher level of fat with 11-17% of wet weight (Endinkeau & Kiew 1993), 5.7-11.9% (Ahmad *et al*,. 2005) and up to 35.93% (Zakaria *et al.*, 2007). The major component of the fat is the polyunsaturated fatty acid (PUFA) which is good for health (Kris-Etherthon *et al.*, 2004).

On top of that, *Channa striatus* has been listed as fish of high in arachidonic acid (AA) together with other fish like eel, big head carp, common carp, rohu and freshwater eel.(Suriah *et al.*, 1995). The most abundant fatty acids in *Channa striatus* are Palmitic acid (C16), Oleic acid (C18: 1) and Stearic acid (C18:0). The most interesting figure of its fatty acid and amino acid composition (Zuraini *et. al.*, 2006) can be seen in Table 2.3.1a and Table 2.3.1b respectively.

Table 2.3.1a Fatty acid composition of *Channa striatus*

Fatty acid	Mean±SD
C16:0 (Palmitic acid)	30.39±0.23
C18:0 (Stearic acid)	15.18±0.15
C16:1 (Palmitoleic acid)	2.98±0.07
C18:1 (Oleic acid)	12.04±0.54
C18:2 (Linoleic acid)	8.34±1.01
C18:3 (Linolenic acid)	ND

C20:4 (Arachidonic acid)	19.02±0.78
C20:5 (Eicosapentanoic acid)	ND
C22:6 (Docosahexaenoic acid)	15.18±1.12

Values =mean ±SD , ND= not detected (Source: Zuraini *et al.*, 2006)

Study done by Mat Jais *et al.*, (1994), revealed that the content of arachidonic acid in *Channa striatus* is unusually high, with almost no eicosapentanoic acid (EPA). Then, a study by Zuraini *et al.*, in 2006 has proved this findings by getting the same result.

It also has a high content of docosahexaenoic acid (DHA; C22:6) (Zuraini *et al.*, 2006 and Mat Jais, 2007a). This results contradicted a study done by Dahlan-Daud *et al.*, 2010 when they found that *Channa striatus* traditional extract contains no DHA. The result might be due to wild samples of *Channa striatus* in Zuraini *et al.*, (2006) study, whereas in Dahlan Daud *et al.,* in 2010 study, the samples were collected from cultivated ponds.

However, the study was limited on the *Channa striatus* fillet only, but no other parts of the fish such as roe and mucus (Mat Jais *et al.*, 1998). In 1998, Mat Jais and his colleagues (Mat Jais *et al.*, 1998) studied about fatty acid compositions in mucus and roe of *Channa striatus*. They found that oleic acid was the major component of fatty acid in all parts of *Channa striatus*. The abundance of arachidonic acid in *Channa striatus* fillet and mucus was also detected, with higher percentages in mucus, but low percentage in the roe (Table 2.3.1b).

Non-freeze dried roe contains large amounts of arachidonic acid which was totally absent in the freeze dried sample, leading to the conclusion that arachidonic acid might have been destroyed during the process of freeze drying (Mat Jais 2007a, Hui *et al.*, 2010). Hence, all the extract were suggested to be used as crude extracts without going through freeze drying process (Dahlan-Daud *et al.*, 2010).

2.3.2 Amino acids composition

Gam *et al.*, 2005 found that the most abundant amino acid in *Channa striatus* in decreasing order was glutamic acid, aspartic acid, lysine, arginine, leucine, alanine, valine, threonine and glycine. Mat Jais *et al* (1994) had also conducted a study on amino acid profile on filleted *Channa striatus* extract. The study found the extract rich in glycine. This result has been confirmed by more recent studies (Zuraini *et al.*, 2006, Dahlan-Daud *et al.*, 2010).

Tabel 2.3.1b Qualitative comparison of the fatty acid composition of freeze-dried roe extract (FD Roe), non freeze dried roe extract (Roe), mucus extract and fillet from *Channa striatus*.

Fatty acid	FD Roe[1]	Roe[1]	Mucus[1]	Fillet[2]
16:0 Palmitic	-	-	+	+

16:1 Palmitoleic	+	_	-	+
17:0 Margaric	+	+	-	-
18:0 Stearic	_	_	+	+
18:1 Oleic	+	+	+	+
18:2 n 6 Linoleic	+	+	+	+
18:3 n 3 Linolenic	+	+	+	+
20:4 n 6: Arachidonic	+	+	+	+
20: 5 Eicosapentanoic	-	-	-	-
22: 6 Docosahexanoic	-	-	-	+

[1] According to Mat Jais *et al.*, (1998); [2] According to Zuraini *et al.*, (2006)

Seventeen amino acids as listed in Table 2.3.2 are the basis element for wound healing (Mat Jais *et al.*, 1994; Mat Jais *et al.*, 1998). Dahlan Daud *et al.*, in 2010 reported the same seventeen amino acids found in *Channa striatus.* Other amino acids that appear to be abundance in *Channa striatus* extracts include glutamic acids (Gam *et al.*, 2006; Ahmad *et al.*, 2005), arginine (Whitte & Barbul 2002), and aspartic acid (Ahmad *et al.*, 2005). Gam *et al* in 2005, found that the fishes with the length between 16cm to 38cm offer relatively similar amino acid value. In the study, when comparing between the different batches of *Channa striatus*, the contents of the amino acids in all three batches of *Channa striatus* were almost similar except for lysine and aspartic acid (Gam *et al.*, 2005).

An analysis of protein content of mucus of *Channa striatus* (Wei *et al.*, 2010) showed that variation exists in different extraction types. Crude extract appears to contain the highest amount of protein, followed by the aqueous and acidic extract. This result could enable an alternative source of amino acid of *Channa striatus* without sacrificing the fish. The functions of all these acids have been described briefly in section 2.4. Furthermore, *Channa striatus* possess a good profile of dietary minerals such as magnesium, copper, calcium, manganese, iron and zinc (Heimann, 1982; Lands, 1986; Mat Jais *et al.*, 1997; Mustafa *et al.*, 2012) and vitamin A (Mat Jais, 2007a).

2.5 Medicinal uses of *Channa striatus* based on scientific research

2.5.1 *Channa striatus* as a wound healer

The effectiveness of *Channa striatus* as a wound healing agent is thought to be influenced by the high level of specific amino acids such as glycine; and fatty acids like arachidonic acid (Baie & Sheikh 200a, 2000b). These two compund were believed to be involved in the promotion of wound healing by the initiation of a series of mechanisms involving remodelling of collagen, re-epithelialization of wound and induction of wound contraction (Baie & Sheikh 200a, 2000b).

Rezqa & Syed Hassan in 2012 had evaluated the effect of oral and topical of *Channa striatus* on tensile strength, epitheliasation, fibroblast count and hydroxyprolene level in the healing of laparotomy wound in malnourished rat.The results demonstrated 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).

Several forms of formulation have been formulated and tested to refine the wound healing effect (Febriyenti *et al.*, 2008). Incorporation of *Channa striatus* extract into palm-oil creams could yield best wound healing result when olein is used as stabilisers (Sheikh *et al.*, 2005). Several types of aerosol formulation of *Channa striatus* extract have also been formulated (Febriyenti *et al.*, 2008). These include aerosol formulations with hydroxylpropyl methylcellulose as polymer and glycerine as plasticisers (Febriyenti *et al.*, 2008), aerosol formulatios incorporated with fusidic acid (Febriyenti *et al.*, 2010a, 2010b) and a water-based extract of *Channa striatus* (Laila *et al.*, 2011).

The film of the spray has been tested for its mechanical stability and water vapour permeability properties (Febriyenti *et al.*, 2010a). Then, the spray formulation has been intervented on created wound on rabbit, mice, and rats (Laila *et al.*, 2011). These aerosol formulations allow an increased rate of wound healing while providing more practical, effective and safe practical application to incision and burn wounds (Febriyenti *et al.*, 2008).

2.5.2 *Channa striatus* as a pain reliever

Channa striatus also has been used to reduce pain for hundred years, especially after road accident or surgery (Mat Jais *et al.*, 1994), but there is no scientific evidence. Mat Jais *et al.*, 1997 done an experiment to find out the pain reliever or antinociception activity of *Channa striatus*. In the experiment, they has tested the antinociceptive effect of whole fillet together with mucus extract in the mice using chemical and thermal pain stimulation.

In the study (Mat Jais *et al.*, 1997), the chemical stimulus is done by administration of the 0.6% acetic acid intraperitoneally and measuring of abdominal constriction. Meanwhile the thermal stimulus is done by application of the hot plate and counting the tail flick of the mice (tail flick test). Results of the experiment showed that mucus extract 12.5% in combination with morphine 0.8mg/kg inhibit 100% the abdominal constriction (chemical stimulus) of the mice whereas fillet extract 10% in combination with morphine 0.8mg/kg showed 98.7% inhibition. Additionally, without combination with morphine, mucus and fillet extract showed 23.4% and 31.6% inhibition respectively. Meanwhile for the thermal stimulus (tail flick test), neither mucus nor fillet extract had any effect of tail flick latency.

These results (Mat Jais *et al.*, 1997) means that both fillet and mucus extract of *Channa striatus* have dose-dependent antinociceptive effects, and both only inhibit chemical pain stimulus, not thermal pain stimulus. In detail, these activities were suggested to be mediated by a peripheral rather than central mechanism, because abdominal constriction test involves peripheral receptors within peritoneal cavity, whereas the tail flick test usually involves a centrally mediated spinal reflex (Mat Jais *et al.*, 1997).

Nevertheless, it was clearly seen that both extracts have antinociceptive effects on their own and also enhanced the antinociceptive effect of morphine (Mat Jais *et al.*, 1997). Aspartic and glutamic acids were demonstrated to have a role in pain inhibition together with GABA which works together with N-metyhl-D-aspartic acid receptor in brain (Hammer *et al.*, 1993).

Zakaria *et al.*, 2004a, 2004b have shown the involvement of the nitric oxide/cyclic guanosine monophosphate (GMP) pathway of antinociceptive activities in the fillet extract of *Channa striatus*. From the result, in 2005 (Zakaria *et al.*, 2005a, 2005b) they have determined the activity of antinociception by manipulating different antagonists. As a result, they found that the aqueous supernatant extract of *Channa striatus* fillet possessed only a peripheral antinociceptive activity which is concurrent with the result from Mat Jais *et al.*, (1997) study.

According to the study by Zakaria *et al.*, 2005b, the antinociceptive activity was mediated via a non-opiod mechanism and was involved in muscarinic, $GABA_A$-ergic, α-adrenergic and serotonergic receptor systems. Further study was being carried out in 2006 to establish the subtypes of the respective receptors involved and to identify the major compunds present in the extract (Zakaria *et al.*, 2006). The results revealed that there are at least two classes of bioactive compounds responsible for the antinociception with molecular weight less than 5000 dalton and the other one with molecular weight between 10000 to 30000 dalton. This result is also concurrent with the findings from Mat Jais (2007b).

High amounts of arachidonic acid, was believed to play an important functions in the antinociceptive pathways; and this results are confirmed by many studies (Ahmad *et al.*, 2005; Zuraini *et al.*, 2006; Mat Jais *et al.*, 1994; Chedoloh *et al.*, 2011). The compound was detectable in polar lipids but not traceable in neutral lipids. This property was also thought to be played by glycine (Kapoor *et al.*, 2006)

The active ingredient in *Channa striatus* is not clear but is thought to be molecules of peptide origin, possibly a glycoprotein, polypeptide or polysaccharide, whose nature can be either a water-soluble polar compound, or a fat soluble polar compound, or a fat soluble non polar compound, acting alone or in synergy and inter-independent on each other (Zakaria *et al.*, 2004a). It seems that lipase can exert a positive influencing in *Channa striatus* antinociceptive property, and this is leading to the suggestion that lipid is involved either as a carrier to the bioactive molecule or as a component of the bioactive molecule itself (Zakaria *et al.*, 2004b).

To confirm, four major fractions representing four different molecules or types of bioactive compounds have been identified. Ranging from as low as 500 to 30000 dalton in size in the aqueous extract of *Channa striatus* (Zakaria *et al.*, 2006); they are thought to be implicated in affecting at least four types of the non-opioid receptors (muscarinic, GABA, a-adrenergic and the L-arginine/NO/Cgmp pathways (Zakaria *et al.*, 2005b; Raffa & Pergolizzi, 2010). Similar fractions were found with the water-based traditional extract of *Channa striatus* (Dahlan-Daud *et al.*, 2010).

Dambisya *et al.*, 1999 investigated the influence of temperature, pH, and naloxone on the observed antinociceptive effects of *Channa striatus* extract. The purpose is to elucidate the nature and stability of the active component of *Channa striatus* extract. The result was, the bioactive compund in *Channa striatus* extract was stable to pH 6 to 8 and temperature up to

100 ^{o}C. The active components were relatively heat- stable and most likely non-opioid. But the limitation of this study, was that they could not rule out the possibility of polysaccharide characteristic of the extract.

A study by Marimuthu *et al.*, 2011 suggested that cooking *Channa striatus* fillet by grilling was the best method to get the proximate composition as well as mineral composition of *Channa striatus* fillet. Previously in 1997, a study by Mat Jais *et al.*, 1997 suggested that cooking the fish up to 120 minutes does not destroy the analgesic components of the whole fish.

After all, it is convincely concluded that *Channa striatus* extract have superior antinociceptive properties compared to other extracts from other *Channidae* and work in a concentration–dependent manner (Zakaria *et al.*, 2005a, 2005b) in a wide range of temperatures and pH (Dambisya *et al.*, 1999); and enhacing other antinociceptive agents such as morphine (Mat Jais *et al.*, 1997) suggesting a possible interaction with *u*-opioid receptor.

2.5.3 *Channa striatus* as anti-inflammatory and anti-pyretic agent

Channa striatus extract also possessed anti-inflammatory properties (Somchit *et al.*, 2004). The anti-inflammatory effect of *Channa striatus* extract in both acute and chronic inflammation appears to be better than that of other *Channidae* (Somchit *et al.*, 2004). The oleic and stearic acids have been reported to attenuate polymorphonuclear leukocytes activity and undoubtedly influence membrane fluidity, thus supressed inflammatory processes (Dahlan-Daud *et al.*, 2010).

A further test on this property was done by the study from Michelle *et al.*,2004 which found that *Channa striatus* extract had effects in improving osteoarthritis in rabbits. The treated group was given *Channa striatus* extract orally 10 ml/kg body weight three times daily and the control group received normal saline at 10 ml/kg three times daily. The outcomes was evaluated by radiographic and innervations of the synovial membrane after nine weeks of treatment. Results showed there was significant reduction in soft tissue swelling observed in radiograph for treated animals compared with untreated and there was significant improvement in the density of immunoreactive nerve fibers in the synovial membrane of treated animals compared with that of controls.

This study (Michelle *et al.*,2004) suggested that *Channa striatus* treatment could help in remodeling of collagen via the synthesis of inter and intramolecular protein cross-linking. This action would in turn help strengthen the structure of the articular cartilage preventing further degradation (Michelle *et al.*,2004). As a result there would be a marked reduction in the inflammation of the synovium, preventing the loss of immunoreactivity from the synovial membrane (Michelle *et al.*,2004). Thus, *Channa striatus* extract may have role in the treatment of joint diseases with a clearer inflammatory component such as rheumatoid arthritis (Michelle *et al.*,2004).

This anti-inflammatoty effect also were studied by a study done by Al Saffar *et al.*, in 2011a and 2011b. However the studies revealed that lower pathology score with markedly improved immunoreactivity were detected in "zerumbone" treated groups compared to *Channa striatus* extract group. Significant different concentrations of PGE_2 but not $PGF_{2\alpha}$ were detected within studied groups. Both remedies ("zerumbone" and *Channa* striatus) significantly improved the immunoreactivity which appeared more prominent in the group

treated with "zerumbone". This finding was significantly confirmed by several studies by Al Saffar *et al.*, 2010, 2011b, 2011c, 2011d.

The anti-inflammatory property may also be the reason behind the observable anti-pyretic activity of the aquaeous extract (Zakaria *et al.*, 2008). This antipyretic activity, however, is absent in the lipid-based extracts which suggest that the anti-pyretic compound may have been a polar based, water soluble substance.

2.5.4 *Channa striatus* as anti-microbial agent (anti-fungal and anti-bacteria)

Results from a study done by Mat Jais *et al.*, 2008, found that ethanol extract of *Channa striatus* have potential properties of narrow spectrum as antifungal agent. The researcher also found that antifungal activities of *Channa striatus* extract have been demonstrated by an ethanolic fillet extract against *Neurospora crassa, Aleurisma keratinophilum* and *Cordyceps militaris.* The same extract also inhibits *Botrytis pyramidalis* and *Paecilomyces fumosa-roseus* on a short term basis. However, further in-depth antimicrobial studies are required before final conclusion could be drawn on the antimicrobial properties of *Channa striatus* extract (Mat Jais *et al.*, 2008).

Apart from fillet of *Channa striatus*, its mucus extract also have antibacterial properties (Wei *et al.*, 2010). Wei *et al.*, 2010 indicated that the acidic mucus extract exhibited a bactericidal activity and inhibited the growth of *Klebsiela pneumonia, Pseudomonas aeroginosa* and *Bacillus subtilis.* The result suggest that the mucus extract of *Channa striatus* may be a potential source of antimicrobial agents.

2.5.5 *Channa striatus* as antioxidant

Among freshwater fishes, *Channa striatus* appears to have a medium level of anti-oxidant activities (Mohd Shafri & Abdul Manan, 2012). This property is possibly contributed by some of the major amino acids and fatty acids in *Channa striatus* extract. Dahlan-Daud *et al.*, 2010 reported that the antioxidants present in *Channa striatus* extract are most likely to be lipophillic antioxidants which represent powerful defence tools particularly against omega-3-oxidation. Rao *et al.* in 2012 found that antioxidant activity also exist in roe of *Channa striatus.* In the study, the researcher compare in vitro antioxidant activity of roe of *Channa striatus* and *Labeo rohita* and found that, antioxidant activity was higher in roe protein hydrosylates of *Channa striatus* compared to *Labeo rohita.*

2.5.6 Channa striatus as anti-depressant and neurophysiology agent

A group of scientist (Saleem *et al.*, 2011) have revealed that *Channa striatus* extract administration showing antidepressant-like effect in mice model of depression. Interestingly, the antidepressant-like effect was not due to psychomotor stimulant activity. But the effect only was limitedly exhibited by mice (Saleem *et al.*, 2011).

Besides anti-deppresant, *Channa striatus* extract also possess neurophysiology functions that could be used as regenerative and restorative agent for treating damage of many types of organs (Mohd Shafri *et al.*, 2011). In the initial study done in 2002, (Karmarkar *et al.*, 2002), the researcher found that the skin extract of *Channa striatus* could initiate apnoea and irreversible blockade of nerve-muscle preparation.

It is also able to exert positive changes in the regenerative potential of neurons involved in traumatic injury as observed by neurite overgrowth and multipolarity of cells which took place in pheochromocytoma PC12 cells treated with *Channa striatus* extract (Mohd Shafri *et al.*, 2011).

2.5.7 *Channa striatus* as a source of albumin

A study from Indonesia (Mustafa *et al.*, 2012) found that *Channa striatus* extract contains abundant of albumin (2.17 ± 0.14g/100mL) which is the largest fraction (64.61%) of protein. This is sufficient to provide albumin for high demand such as hypoalbuminemia and post-surgical patients, and growing children. *Channa striatus* extract is a potential source of albumin as per 100 mL it contains 3.36± 0.29 g protein, 2.17± 0.14 g albumin, 0.77± 0.66 g total fat ; total glucose 0.07 ± 0.02 g, Zinc 3.34 ± 0.8 mg; Cu 2.34 ± 0.98 mg and 0.20 ± 0.09 mg Fe. This result led to the conclusion that *Channa striatus* provides good source of albumin for people who has low albumin serum or injuries, burns or has been in post-operative condition. The researchers also found that the administration of *Channa striatus* extract has been shown to increase the appetite of loss-appetite children. This effect was thought that the presence of Zn in *Channa striatus* extract is likely to be key factor that plays the role in wound healing and children appetite.

2.5.8 *Channa striatus* as gastroprotective agent.

Recently in 2014, Ali Khan *et al.,* did an investigation on the beneficial effects of orally administered freeze dried aqueous extract of *Channa striatus* (AECS) in experimentally induced gastric ulcers in Wistar rats. They found that AECS at concentrations of 40% and 50% w/v significantly decreased the volume of gastric juice and increased the levels of catalase. They also manifested that all the test doses (30%, 40% and 50%) of AECS have markedly decreased ulcer index and malondialdehyde compared to the standard drug whereas AECS 30% w/v did not alter volume of gastric juice, pH, free and total acidities, catalase, and superoxide dismutase.

2.5.9 Other properties of *Channa striatus* (Cardiological and Haematological effect)

In *Channa striatus* extract, the skin extract has been found to contain potent active compound of cardiotoxic factor II (Kamarkar *et al.*, 2002). Apart from cardiotoxic factor II, it also has hypotensive effect and cardiotoxic property that influences the increase in cardiac marker enzyme creatine phosphokinase (CPK) and creatine phosphokinase-MB (CPK-MB) values (Karmakar *et al.*, 2004).

Studies by Ghassem *et al.*, in 2011a and 2011b, characterisation of protein hydrolysates from muscle and myofibrillar samples of *Channa striatus* showed different kinetic and proteolytic activities. The result led to isolation of angiotensin converting enzyme (ACE) inhibitory peptides with high ACE-inhibitory activity, further supporting the use of *Channa striatus* as a functional food and preventative medicine in hypertensive patients (Ghassem *et al.*, 2011a, 2011b). The cardiotoxic factor II found in *Channa striatus* skin extract also has blood-modulating properties. This factor could induce a decrease in haemoglobin, total red blood count, white blood count and platelet count (Karmakar *et al.*, 2004).

CHAPTER 3

METHODOLOGY

3.1 Study Design

This is a randomized, double blind, placebo controlled study.

3.2 Duration of the study

The study period was from 15 March 2011 to 14 March January 2014

3.3 Study Population

The study population were women who had elective or emergency Lower Segment Caesarean Section (LSCS) in postnatal wards of Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Kelantan and Hospital Raja Perempuan Zainab II (HRPZ II), Kota Bharu, Kelantan.

3.4 Inclusion and exclusion criteria

These are the inclusion and exclusion criteria for this study.

Inclusion Criteria:

1. Age $\geq$ 18 and $\leq$ 40 years
2. No present active medical, surgical and gynaecological problems
3. First LSCS (elective or emergency)

Exclusion Criteria:

1. Taking any form of herbal extract in the last 3 months before study entry and during the study period
2. History of drug or alcohol abuse.
3. Taking fresh *Channa striatus* during study period
4. Patient taking warfarin or heparin
5. Clinical relevant cardiovascular, gastrointestinal, hepatic, neurologic, endocrine, haematologic, connective tissue disease or other major systemic diseases that would influence the interpretation of results
6. Patients with medical disorder requiring steroid or immunosuppressive therapy
7. Patient with chronic cough or other condition which may cause a rise in intra-abdominal pressure
8. Presence of any congenital anterior abdominal wall defects
9. Mental condition rendering the subject unable to understand the nature, scope and possible consequences of the study.
10. Evidence of uncooperative attitude, including poor compliance including inability to attend follow-up visit.

3.5 Determination of sample size

Sample size were calculated for each objectives. The calculations were done using Power and Sample Size Calculation Software (Dupont & Plummer, 1997) and the one that yielded the biggest sample size was taken as the study sample size.

Sample size for comparing two means between treatment group and placebo group were done. The parameters were as follows:

α = Level of significance = 0.05

Power = 0.9

σ = Standard deviation of the objective measurements [Pain, Wound Evaluation Scale (WES), Visual Analogue Scale (VAS) and Patient's Satisfaction]

δ = Detectable difference in population means (clinically significant difference in objective measurements between *Channa striatus* extract group and placebo (maltodextrin) group based on literature review)

m = Ratio between placebo (maltodextrin) group and *Channa striatus* extract group = 1

Table 3.5 Sample size calculation for each objective

Study objectives	Standard deviation (σ)	Detectable difference (δ)	Sample size	+ 20% drop out
1. Pain	0.6	0.5	31	37
2. WES	0.8	1	14	17
3. VAS	19	15	35	42
4. Patient's Satisfaction	09	1	18	22

The biggest sample size yielded by Objective 3. Based on previous study the detectable difference of VAS between control and treatment group was 15. The VAS measurement has a standard deviation of 19. The minimum required sample size was 35 and after considering the non-response rate of 20%, the sample size calculated for each group was 42. Finally, the estimated total sample size for both the treatment and placebo group was 84.

3.6 Sampling Method

Universal sampling method was applied . All women who had LSCS in HUSM and HRPZ II were approached and asked for eligibility of the study

3.7 Method of assigning and blinding subjects to treatment and placebo groups

The subject eligibility was established before treatment randomization. Subject numbers (randomisation code) were allocated strictly sequentially, as generated using computer (blocks of four) that provided allocation of subject numbers in a ratio of 1:1 to either *Channa striatus* extract or placebo (maltodextrin). Only two co-researcher knew the randomisation code; which were statistician and investigational product preparator. Once a subject has been randomized, the study treatment that they received were not be known by both the

subject and the other investigators that involved with the field work. The codes was broken only after data collection had been completed.

3.8 Investigational product (Study Medication)

The orally administered freeze dried *Channa striatus* extract and placebo was prepared by a certified laboratory, School of Pharmaceutical Sciences Universiti Sains Malaysia. Both the freeze dried *Channa striatus* extract and maltodextrin were available as 250 mg capsule.

3.10 Treatment compliance

Subjects were asked to return all unused medication. The number of capsules issued and minus the number of capsules returned were used to calculate the capsules taken. From this information, compliance was calculated.

$$\text{Compliance} = \frac{\text{Capsules taken during the period}}{\text{Capsules which should have been taken}} \times 100$$

Any subject taking less than 80% or more than 100% was considered non-compliant.

3.11.2 Follow up visit window period

Three days before and three days after planned visit date.

3.13 Informed consent procedure

Above all of the study procedures, informed consent was obtained first. Consent documents (informed consent form and subject information sheet) contain all elements as described in Declaration of Helsinki and the International Conference of Harmonisation (ICH) Guidelines for Good Clinical Practice (GCP) and in accordance with all applicalble laws and regulations.

The informed consent form and subject information sheet describe the planned and permitted uses, tranfers, and disclosures of the subject's personal and personal health information for purposes of conducting the study. The informed consent form and subject information sheet *(Appendix 1)* explain about the nature of the study, its objectives, and potential risks and benefits, and the requirements of the subjects and the facts that she is free to withdraw at any time without giving a reason and without prejudice to her further medical care.

The researcher explained the detailed elements of the informed consent form to the subject. The subjects were given an ample opportunity to inquire about details of the study and decide whether or not to participate in the study. For this study, subjects were also explained in detail about the compulsion of follow up visits.

Subjects who were willing to participate, they were instructed to sign and date using their legal name by black ballpoint ink. Then the researcher also signed and dated the informed consent. Once signed, the original informed consent form was kept in the investigator study