

Effect of Oral Supplementation of *Channa striatus* Extract on Total Antioxidant Status during Wound Healing in Post Lower Segment Caesarean Section Women

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ABSTRACT

Objective: *C.striatus* has been suggested to have antioxidant and anti-inflammatory properties for wound healing. This study was conducted to determine the level of Total Antioxidant Status (TAS) and the effectiveness of oral supplementation of *C.striatus* extract in post Lower Segment Caesarean Section (LSCS) patients during wound healing.

Design: This was a randomized; double blinded, placebo-controlled study among post LSCS women conducted in Department of Obstetric and Gynaecology, Hospital Universiti Sains Malaysia from March 2010 to December 2012.

Material and Methods: A total of 39 patients from *C.striatus* and 34 patients from placebo group were included in this study. After randomization, the treatment group consumed 500 mg of freeze dried *C.striatus* extract daily while the placebo group consumed 500 mg of placebo (maltodextrin) daily for 6 weeks. For each subject, venous bloods for TAS were taken postoperatively at day 1, day 3, week 2, week 4 and week 6. Repeated measure for analysis of variance (ANOVA) was used to determine the changes of TAS between and within both groups throughout the 6 weeks intervention.

Results: The result suggested an increment of TAS level within *C.striatus* group from second week postoperative onwards which might involve in the enhancement of wound healing.

Conclusion: The administration of *C.striatus* extract has beneficial effect during wound healing by enhancing the process in post-operative LSCS women.

KEY WORDS

Channa striatus, wound healing, total antioxidant status, lower segment caesarean section, reactive oxygen species

INTRODUCTION

Channa striatus (*C.striatus*) or Haruan is a fresh water, air breathing fish from family Channidae. It is widely distributed within Malaysia¹⁾. It is extensively consumed to promote wound healing and proven to have high antioxidant activities²⁾. The content of *C.striatus* which high in amino acids and fatty acids contribute for better wound healing^{1,3)} and to the antioxidative property of *C.striatus*⁴⁾.

Wound healing comprises of three overlapping phases which is inflammation, new tissue formation and remodeling of the tissue. Reactive oxygen species (ROS) involved in all stages of the wound healing process. During inflammatory phase, there is production of large amounts of ROS. ROS is very important and essential to protect against the unwanted microorganisms and be part of the innate immune

system of the organism⁵⁾. Process of wound healing depends on these oxidative stress and low levels of ROS. Suppression or overexposure to oxidative stress can lead to impaired and delayed wound healing⁶⁾. Antioxidant play an important role in wound healing by reducing the ROS and enhance the healing process⁷⁾.

C.striatus extract has been studied and suggested to have antioxidant and anti-inflammatory content for better healing of the wound^{1,8)}. This study was to assess the possible role of *C.striatus* in wound healing in post Lower Segment Caesarean Section (LSCS) women by analyzing the Total Antioxidant Status (TAS). The aim of this study was to determine the level of TAS during wound healing in post LSCS women supplemented with *C.striatus* extract and placebo (maltodextrin).

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Table 1. Age and baseline TAS level of the subjects.

Demographic characteristics	<i>C.striatus</i> (N = 39)	Placebo (N = 34)	<i>P</i> -value
	Mean (SD)	Mean (SD)	
Age (years)	28.03 (5.05)	28.47 (5.22)	0.713
Baseline TAS (mmol/L)	1.27 (0.20)	1.24 (0.21)	0.460

Independent sample t-test

Table 3. Comparison of TAS level (mmol/l) within *C.striatus* and placebo group based on time (ITT analysis).

Comparisons	<i>C.striatus</i> group (N = 39)		Placebo group (N = 34)	
	Mean diff (95% CI)	<i>P</i> -value	Mean diff (95% CI)	<i>P</i> -value
Day 1-Day 3	-0.07 (-0.14, 0.01)	0.081	-0.04 (-0.12, 0.03)	1.000
Day 1-Week 2	-0.13 (-0.22, -0.03)	0.005	-0.04 (-0.17, 0.09)	1.000
Day 1-Week 4	-0.11 (-0.20, -0.02)	0.007	-0.06 (-0.15, 0.03)	0.416
Day 1-Week 6	-0.16 (-0.25, -0.07)	< 0.001	-0.12 (-0.25, 0.01)	0.101

Repeated measures ANOVA with Bonferroni correction.

Table 2. Comparison of TAS level (mmol/l) at different time between *C.striatus* group and placebo group.

Variables	<i>C.striatus</i> group (N = 39)	Placebo group (N = 34)
	Mean (SD)	Mean (SD)
Day 1	1.27 (0.20)	1.24 (0.21)
Day 3	1.34 (0.21)	1.28 (0.22)
Week 2	1.40 (0.22)	1.28(0.29)
Week 4	1.39 (0.18)	1.30 (0.25)
Week 6	1.43 (0.17)	1.35 (0.29)

Independent sample t-test

Table 4. Comparison of TAS within *C.striatus* and placebo group based on time (Per Protocol analysis)

Comparisons	<i>C.striatus</i> group (N = 34)		Placebo group (N = 26)	
	Mean diff (95% CI)	<i>P</i> -value	Mean diff (95% CI)	<i>P</i> -value
Day 1-Day 3	-0.08 (-0.16, 0.01)	0.079	-0.06 (-0.16, 0.05)	1.000
Day 1-Week 2	-0.14 (-0.25, -0.03)	0.004	-0.05 (-0.22, 0.12)	1.000
Day 1-Week 4	-0.13 (-0.23, -0.03)	0.006	-0.08 (-0.19, 0.03)	0.409
Day 1-Week 6	-0.18 (-0.29, -0.08)	< 0.001	-0.15 (-0.32, 0.01)	0.093

Repeated measure ANOVA with Bonferroni correction.

MATERIALS AND METHODS

Study design and study population

This was a randomized; double blinded, 2-arm parallel comparative study of *C.striatus* extract versus placebo conducted in Department of Obstetric and Gynaecology, Hospital Universiti Sains Malaysia (HUSM) from March 2010 to December 2012. Postpartum women aged 18 to 40 years who were having elective or emergency LSCS with no active medical, surgical and gynaecological problems were included in the study with informed consent. The patients who had consumed any form of herbal extract in the last three months before and during the study period, took heparin or warfarin and had history of drug or alcohol abuse were excluded from this study. Other exclusion criteria include clinically relevant endocrine, hematological, cardiovascular, connective tissue disease, neurologic, gastrointestinal or other major systemic diseases that can affect the result of this study. Furthermore, the patients on medications that can delay wound healing such as steroid or immunosuppressive therapy were also excluded from this study.

Data collection procedure

Randomization method was used to assign subjects for treatment and placebo group using computer where the subject numbers were allocated based on 1:1 ratio. The subject eligibility was established before treatment randomization and the number was designated strictly and sequentially. In this study, once randomized, the subject and the investigator did not know what study treatment the subjects had received. The oral freeze dried *C.striatus* extract and placebo (maltodextrin) available as a 250 mg capsule each and were prepared by School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang. The treatment group took 500 mg (2 capsules) of *C.striatus* extract while the placebo group took 500 mg (2 capsules) of placebo. Both groups took the *C.striatus* extract/placebo daily for six weeks. The patients who missed three consecutive doses were labeled as non-compliance and excluded from this study. Venous bloods for TAS were taken at day 1, day 3, week 2, week 4 and week 6 from each subject postoperatively. Besides, full blood count, liver and renal function test were also measured at the same time to make sure the safety of *C.striatus* and placebo.

The blood for TAS was determined by colorimetric assay using kit (RANDOX laboratories, United Kingdom) using Vitalab Selectra E analyzer (Vital Scientific N.V). In this assay, 2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate](ABTS[®]) was incubated with peroxidase

(metmyoglobin) to produce the radical cation ABTS[®] which was blue-green in color. This reaction then was measured at 600 nm. Antioxidants in the added sample caused suppression of the color produced which was proportional to the concentration.

Statistical analysis

Sample size for this study was determined using G*Power software (F tests and Repeated measures of ANOVA)⁹. The total number of participants required for this study was 70 after taking the effect size *f* of 0.25 (for medium effect size) to be significant at type I and type II error of 5% and 10% respectively. The total number of participants required after adding 20% anticipated dropout rate was 84 (42 in each group). This calculation was based on 2 groups, 4 times measurements and correlation among repeated measures of -0.2. This study used a (modified) intention-to-treat (ITT) analysis. All subjects who consumed at least one dose of *C.striatus* extract or placebo and had at least one result after baseline are analysed based on their initial randomization. Last Observation Carried Forward (LOCF) principle was carried out for drop-out data (13 subjects). For 60 subjects who completed all the study visits with adequate compliance, Per protocol (PP) analysis was carried out for comparison. Independent t-test was used to analyse the differences in mean or comparison between the treatment and the placebo groups. Repeated measure for analysis of variance (ANOVA) was used to determine the changes of TAS within *C.striatus* and placebo group throughout the 6 weeks intervention. The same analysis was also used to determine between groups differences of TAS with regard to time. *P*-value of < 0.05 was taken as statistically significant. Data analysis was done using software IBM SPSS Version 22.

Ethical consideration

This research was approved by the Human Research Ethic Committee, USM (USM/KK/PPP/JEPeM [227.3.(05)] on July 2010. This study was conducted in concordance to Declaration of Helsinki's principle at all stages.

RESULTS

Initially, 82 patients were successfully randomized into the study after considering inclusion and exclusion criteria (41 patients for each group). However, 2 patients from *C.striatus* group and 7 patients from placebo group need to be excluded from the study due to no baseline data or no at least one result after baseline. A total of 73 patients remain in this study for ITT analysis; 39 patients were in the *C.striatus* and 34 patients were in the placebo groups. A total of 60 patients completed all the study visits; 34 in *C.striatus* and 26 in the placebo groups (PP analysis). Table 1 shows age and baseline TAS level of the subjects. The result showed no significant difference between the two groups.

Table 2 shows comparison of TAS level between *C.striatus* and placebo group based on time. There was a significant increase of TAS level started from week 2 onwards (higher at the later stage) compared to the placebo group. The level of TAS when compared between both groups showed no significant differences in all the period studied (ITT: $F = 0.65$, $p = 0.629$; PP: $F = 0.58$, $p = 0.676$). Table 3 shows comparison of TAS within *C.striatus* and placebo group based on time for 73 subjects based on (ITT) analysis. There were significant differences in the TAS levels in patients who consumed *C.striatus* when compared between Day 1 with Week 2 till week 6. The level of TAS for placebo group showed no significant differences throughout the study period. Table 4 shows comparison of TAS within *C.striatus* and placebo group based on time (PP analysis) for 60 subjects that completed the entire visit. The results showed similar findings as ITT analysis.

DISCUSSION

The present study has examined the nutritional values of *C.striatus* and its' beneficial effect for wound healing which include anti-microbial, anti-inflammatory, induction of cell proliferation and platelet aggregation, anti-nociceptive and high antioxidant activities^{1,8,10}. The principal finding of this study was the enhancement of the TAS levels from week 2 onwards in *C.striatus* group post-operatively that indicates the beneficial effect of *C.striatus* administration during wound healing of post-operative LSCS women.

C.striatus extract were proven to have high antioxidant activities². The antioxidant property of *C.striatus* is due to its high content in amino acids especially cysteine, methionine, histidine and lysine and to a lesser extent, proline and tyrosine¹⁰. These amino acids work as primary antioxidants and act as metal chelators in linoleic acid system⁹. The antioxidants are most likely to be lipophilic antioxidants which has significant powerful defense tools especially against omega-3 oxidation¹. The antioxidant nature of amino acids are involve against lipid oxidation and also probably due to the action at their side-chain as well¹¹.

The results of this study were similar to the study done by Daud and Dahlan (2011) which showed *C.striatus* has high antioxidant property. In that study, they demonstrated that antioxidant capacity was high in *C.striatus* compared to other extracts. High level of antioxidant play an important role in wound healing by preventing the oxidative damage of cells and enhance the healing process. This study is also consistent with a study done by Ali Khan *et al.* (2014). In that study, the researchers used orally administered freeze dried aqueous extract of *C.striatus* in experimentally induced gastric ulcers in Wistar rats. They found that *C.striatus* possesses anti-secretory and antiulcer activities due to the unsaturated fatty acids and essential amino acids especially aspartic acid that is involved in antioxidant mechanism and was found to be in high amount in *C.striatus*¹².

Besides rich in amino acids, *C.striatus* also contains glutamic acid and dietary mineral such as zinc^{3,8} which might contribute to the enhancement of wound healing. The enhancement of wound healing after supplementation with *C.striatus* has been shown to have shorter time of wound closure that eventually can prevent delayed wound healing. This enhancement also might be due to the antioxidant properties of *C.striatus*. Study done by Blass *et al.* (2012) demonstrated that in patients with delayed wound healing, the wound closure was accelerated and took shorter time to heal after the patients were given glutamine and high dose of oral antioxidants micronutrients such as ascorbic acid and α -tocopherol¹³.

However, this study could not demonstrated significant findings between *C.striatus* and the placebo groups. The possibly could be due to the limited number of samples. It could be a logistic problem to some

patients to come for blood taking at hospital as scheduled after discharge. Apart from that, in the ward, as a standard post-operative pain relief, the patients were given non-steroidal anti-inflammatory drugs (NSAIDs) which were a suppository Diclofenac sodium 100 mg twice a day for three days post operatively and continued with tablet Diclofenac sodium 50 mg, three times a day for the next consecutive four days. NSAIDs could inhibit inflammation which is an integral part of wound healing as a results of inhibition of cyclooxygenases that involved in prostaglandin production, leading to a marked decrease in prostaglandin synthesis¹⁴. The level of TAS perhaps will be higher if NSAIDs were not given to the patients. The dosage of *C.striatus* tablet use in this study could also affect the result. The patients were only given 500 mg of *C.striatus* tablet once daily which might not be sufficient for better wound healing process.

SUMMARY

This study indicates *C.striatus* administration has beneficial effect during wound healing by enhancing the process in post-operative LSCS women. However, further studies are required to confirm and understand the mechanism of action of it.

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