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Original Article

Channa striatus capsules induces cytokine conversion in pulmonary tuberculosis patients

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Anti-inflammatory;
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Inflammatory;
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Sputum conversion

Abstract

Objective: This study aimed to investigate whether *Channa striatus* capsule induces sputum and cytokine conversion in pulmonary tuberculosis (TB) patients.

Methods: Randomized, placebo-controlled, double-blind pilot study was conducted to pulmonary TB patients who admitted to Department of Internal Medicine, Faculty of Medicine, University of Sam Ratulangi, Manado, North Sulawesi, Indonesia. A total of 36 pulmonary TB patients were randomly divided into two equal groups (n = 18) including one group received standart antituberculosis drugs plus *Channa striatus* capsule and another group received standart antituberculosis drugs plus placebo. *Channa striatus* capsule was given at a dose of 2 g each time, 3 times per day, for 12 weeks. The levels of tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-10 were analyses using enzyme linked immunosorbent assay (ELISA) method.

Results: The rate of positive sputum smear decline was more pronounced in the *Channa striatus* group but did not reach statistically different value between groups. The levels of TNF-α, IFN-γ, and IL-10 were not significantly different in *Channa striatus* group compared to placebo group at baseline (week 0). But at week 12, the supplementation of *Channa striatus* capsule significantly decreased TNF-α, IFN-γ, and IL-10 levels compared to baseline. In placebo groups, there were no significant differences for IL-10 levels at week 12, but the levels of TNF-α and IFN-γ significantly decreased.

Conclusion: Adjunctive supplementation of *Channa striatus* capsules accelerated the beneficial therapeutic effect of TB chemotherapy by improving cytokine response.

INTRODUCTION

Mycobacterium tuberculosis (MTB) is an intracellular pathogen as the causative agent of pulmonary tuberculosis (TB). This pathogen has infected one third of the world's population and accounts for an estimated 1.8 million worldwide deaths annually. The incidence of TB during 2010 worldwide was 8.8 million cases (128 cases/100000 inhabitants), 59% of these cases were detected in Asia [1]. Today, pulmonary TB remains a leading human infectious disease and a major public health problem in low-income countries, including Indonesia. Despite the availability of the Bacillus Calmette-Guerin (BCG) vaccine for more than 80 years, the best tuberculosis vaccine is still far to be generated and its protection is unclear [2].

Deficiencies of micronutrients can reduce host defenses and immune response to combat MTB [3]. This can potentially affect host response to anti-TB chemotherapy and patient outcome. Regulatory T cells and Th2 type immune response appear to predominate in the early clinical evolution of TB and becomes more pronounced as the disease worsens [4-6]. However, successful chemotherapy causes a return back towards a Th1 state. Thus, improving the micronutrient status of treated pulmonary TB patients may accelerate bacterial clearance and clinical healing through improvement of immune response [7].

Cytokines play an important role in orchestrating the immune response which is activated as a network of pro-inflammatory and down-regulatory cytokines

derived from both T cells and macrophages and determine the disease outcome in TB [8]. Cytokine profiles began to shift more towards a Th1 immune response as indicated by increasing interferon (IFN)-γ and tumor necrosis factor (TNF)-α mRNA levels. The status of those two cytokines correlated with decreasing regulatory T cells activity, *i.e.* lower interleukin (IL)-10 mRNA [9]. The steady state between host and pathogen is crucially dependent on TNF-α. Inevitably, the delicate balance is disturbed by anti-TNF-α therapy, leading to reactivation of MTB, and progression to active disease [10].

Channa striatus or snakehead fish, known locally to the Indonesian as haruan, is a freshwater, air-breather and carnivorous fish indigenous to many tropical and subtropical countries including Indonesia [11]. Traditionally, this fish is believed to promote wound healing and alleviates post-operative pain and discomfort [12]. The putative effects of adjunctive *Channa striatus* supplementation on sputum conversion and the immune response TB patients has not been investigated. The randomized, placebo-controlled, double-blind pilot study was conducted to assess the effectiveness of adjunctive *Channa striatus* supplementation on the immune response, and sputum smear conversion of patients being treated for pulmonary TB. It was hypothesized that supplementation would accelerate sputum smear conversion by improving Th1 immune response leading to macrophage activation and MTB killing.

MATERIALS AND METHODS

This research has been approved by research ethics committee Faculty of Medicine University of Sam Ratulangi, Manado, Indonesia

Subjects

Newly diagnosed pulmonary TB patients attending Department of Internal Medicine, Faculty of Medicine, University of Sam Ratulangi, Manado, North Sulawesi, Indonesia were recruited. Consecutive patients with two times a positive sputum smear, minimal-medium radiologically lesion, 16-23 mg/kg^2 body mass index (BMI), ≥ 2.5-4.5 gr/dl albumin levels, without prior history of TB or treatment, and who were aged 14-50 years were eligible for participation unless they were pregnant, breastfeeding, used corticosteroids, or had HIV, diabetes, or another serious co-morbidity. All subjects gave their written informed consent. The protocol was approved by local ethics committee Faculty of Medicine, University of Sam Ratulangi, Manado, North Sulawesi, Indonesia.

Supplementation

Subjects were randomized to the *Channa striatus* or placebo groups. *Channa striatus* group subjects received four months of supplementation with 2 g/day three times/day of *Channa striatus* in capsule for 12 weeks; placebo group subjects received organoleptically identical, matched placebos. All subjects received short-course, directly observed antibiotic therapy: guidelines: intensive 60-day treatment with isoniazid (300 mg/day), rifampicin (600 mg/day), pyrazinamide (1600 mg/day) and ethambutol (1200 mg/day) followed by a sustained 45-dose therapeutic phase with isoniazid (800 mg/dose) and rifampicin (600 mg/dose).

Follow up

Monitoring of sputum conversion, laboratory indicators, and disease sign and symptoms were done at weeks 0, 2, 4, 6, 8, 10 and 12. Beside that, 10 ml blood samples were collected between 08:00-10:00 am for the immunological analyses at baseline and week 12. The levels of TNF-α, IFN-γ, and IL-10 were analyzed using enzyme linked immunosorbent assay (ELISA) method.

Channa striatus capsules

Channa striatus capsules (VipAlbumin®) were obtained from PT. Royal Medicalink Pharmalab, Makassar, South Sulawesi, Indonesia. Chemical constituents of Channa striatus capsules were analyzed in Indonesian Institute of Science, Bogor, West Java, Indonesia.

Statistical analysis

Data are presented as mean ± SD. Chi-square test was conducted to analyse the difference of sex, sign and symptoms, and sputum conversion between groups. Independent t-test or Mann Whitney test was conducted to detect the different of laboratory parameters and cytokines levels between *Channa striatus* and placebo group. $P < 0.05$ was considered statistically significant.

RESULTS

Subject characteristics, sign and symptoms, and laboratory indicators

Table 1 shows the subject number, sign and symptoms, and laboratory indicators in *Channa striatus* and placebo group. We involved 12 men and 6 women in *Channa striatus* group, 10 men and 8 women in placebo group. The distribution of sex is not significantly different between groups ($P > 0.05$). The frequency of cough, bloody sputum, dyspnea, fever, night sweating, and fatigue is not significantly different between groups ($P > 0.05$). Aspartat aminotransferase (AST) and creatinine level of week 4 was significantly higher in *channa striatus* group than placebo group ($P < 0.05$), but not significantly different in weeks 6 until 12 of treatment in all groups ($P > 0.05$). The levels of albumin of weeks 10 and 12 was significantly higher in *Channa striatus* group compared to placebo group ($P < 0.05$).

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Table 1. Subject characteristics, signs and symptoms, and laboratory indicators.

Signs and symptoms	*Channa striatus* group (12 m/6 f)	Placebo group (10 m/8 f)	Laboratory indicators	*Channa striatus* group (12 m/6 f)	Placebo group (10 m/8 f)
Cough			**AST**		
Week 0	18	18	**Week 0**	22.72 ± 8.02	21.33 ± 8.65
Week 2	14	10	**Week 2**	28.22 ± 14.04	22.22 ± 9.44
Week 4	8	10	**Week 4**	26.33 ± 11.94*	19.61 ± 4.55
Week 6	7	7	**Week 6**	24.77 ± 9.46	20.94 ± 4.64
Week 8	6	6	**Week 8**	24.11 ± 8.87	25 ± 13.01
Week 10	5	2	**Week 10**	22.66 ± 6.59	21.16 ± 7.1
Week 12	2	2	**Week 12**	21.33 ± 3.80	20.83 ± 6.2
Bloody sputum			**ALT**		
Week 0	11	9	**Week 0**	16.67 ± 7.59	23.50 ± 18.06
Week 2	7	4	**Week 2**	19.11 ± 10.64	18.22 ± 15.75
Week 4	4	2	**Week 4**	20.44 ± 16.11	12.94 ± 7.3
Week 6	4	2	**Week 6**	16.33 ± 8.14	15.00 ± 8.11
Week 8	2	2	**Week 8**	18.17 ± 11.11	18.17 ± 11.11
Week 10	2	2	**Week 10**	16.67 ± 7.41	16.56 ± 9.4
Week 12	1	2	**Week 12**	15.94 ± 4.33	16.78 ± 12.23
Dyspnea			**Creatine**		
Week 0	4	7	**Week 0**	0.78 ± 0.14	0.71 ± 0.15
Week 2	4	5	**Weekth 2**	0.82 ± 0.15	0.70 ± 0.14
Week 4	4	5	**Week 4**	0.77 ± 0.13*****	0.68 ± 0.13
Week 6	2	3	**Week 6**	0.74 ± 0.1	0.68 ± 0.13
Week 8	1	3	**Week 8**	0.74 ± 0.12	0.66 ± 0.16
Week 10	1	2	**Week 10**	0.74 ± 0.09	0.67 ± 0.15
Week 12	1	2	**Week 12**	0.77 ± 0.13	0.69 ± 0.14
Fever			**Uric acid**		
Week 0	18	17	**Week 0**	5.53 ± 1.62	5.30 ± 1.09
Week 2	9	9	**Week 2**	11.40 ± 2.85	11.57 ± 2.51
Week 4	2	2	**Week 4**	10.88 ± 2.43	11.65 ± 2.59
Week 6	1	1	**Week 6**	10.35 ± 2.64	11.28 ± 2.76
Week 8	2	2	**Week 8**	9.37 ± 2.57	10.21 ± 3.59
Week 10	1	2	**Week 10**	7.51 ± 2.66	7.23 ± 2.71
Week 12	0	1	**Week 12**	7.44 ± 2.65	6.76 ± 2.56
Night sweating			**Albumin**		
Week 0	17	17	**Week 0**	3.80 ± 0.36	3.73 ± 0.37
Week 2	8	9	**Week 2**	4.13 ± 0.43	3.95 ± 0.33
Week 4	6	5	**Week 4**	4.17 ± 0.39	4.11 ± 0.28
Week 6	2	3	**Week 6**	4.21 ± 0.4	4.23 ± 0.26
Week 8	1	3	**Week 8**	4.36 ± 0.38	4.17 ± 0.26
Week 10	1	2	**Week 10**	4.42 ± 0.37*****	4.23 ± 0.24
Week 12	1	2	**Week 12**	4.57 ± 0.37*****	4.26 ± 0.25
Fatigue					
Week 0	15	11			
Week 2	15	10			
Week 4	4	6			
Week 6	4	5			
Week 8	3	5			
Week 10	1	5			
Week 12	1	4			

Values are presented as mean ± SD; *****P < 0.05 in comparison with placebo group. (m, male; f, female)

Table 2. Percentage of positive sputum smear in the *Channa striatus* (n = 18) and placebo groups (n = 18) at baseline (week 0) and after supplementation (weeks 2-12).

	Period of supplementation (weeks)						
	0	**2**	**4**	**6**	**8**	**10**	**12**
Channa striatus	100	66	22.2	11.1	5.6	0	0
Placebo	100	100	50	17.7	5.6	5.6	0

Values are presented as percentage of positive sputum smear.

Table 3. Comparison of cytokine levels in the *Channa striatus* (n = 18) and placebo groups (n = 18) at baseline (week 0) and end of supplementation period (week 12).

	***Channa striatus* group**		**Placebo group**	
	Week 0	**Week 12**	**Week 0**	**Week 12**
Tumor necrosis factor-alpha	4.93 ± 3.04	2.67 ± 0.89*****	6.42 ± 4.43	4.7 ± 5.02*****
Interferon-gamma	2.93 ± 5	0.25 ± 0.24*****	3.22 ± 4.49	0.3 ± 0.25*****
Interleukin-10	0.37 ± 0.26	0.28 ± 0.12*****	0.48 ± 0.26	0.39 ± 0.23

Values are presented as mean ± SD; *****P < 0.01 in comparison with placebo group at same period of supplementation.

Channa striatus capsules and sputum conversion

Table 2 displays the proportion of subjects in the two study groups with a positive sputum smear from baseline through study weeks 12. The proportion of positive smears in both groups decreased over time until by the 12 weeks, none remained positive. The rate of decline was more pronounced in the *Channa striatus* group but did not reach significant difference at any week (P > 0.05).

Channa striatus capsules and cytokine conversion

Table 3 shows the levels of cytokines changes from *Channa striatus* group compared to placebo in 12 weeks period of supplementation. The levels of TNF-α, IFN-γ, and IL-10 were not significantly different in *Channa striatus* group compared to placebo group at baseline (week 0) (P > 0.05). Supplementation of *Channa striatus* capsule significantly decreased TNF-α, IFN-γ, and IL-10 levels at weeks 12 than those in baseline (P < 0.01). In placebo groups, there were no significant differences of IL-10 levels at weeks 12 compared with baseline (P > 0.05), but the levels of TNF-α and IFN-γ decreased significantly (P < 0.01).

Finally, the chemical composition of *Channa striatus* capsules are presented in Table 4.

DISCUSSION

We found that 12 weeks of *Channa striatus* supplementation only significantly induces cytokine levels, but not sputum conversion. The rate of conversion of signs and symptoms was also higher in *Channa striatus* group compared with placebo group, week 4, the levels of AST and creatinine were but did not reach statistical significant difference. At significantly higher in *Channa striatus* group than that in placebo group. At weeks 10 and 12, the levels of albumin were significantly higher in *Channa striatus*

Table 2. Chemical constituents of *Channa striatus* capsules

Chemical constituents (per 100 g)	***Channa striatus***
Protein (%)	85.6
Albumin (%)	30.2
Lipid (%)	5.1
PUFA ω3 (%)	2.03
PUFA ω6 (%)	2.11
PUFA ω9 (%)	0.92
Vitamin A (IU)	1500
Vitamin B1 (IU)	0.9
Vitamin B2 (mg)	1.11
Vitamin B6 (mg)	0.7
Vitamin B12 (mg)	0.76
Vitamin E (mg)	9.11
Vitamin D3 (IU)	51.5
Calcium (mg)	186
Sodium (mg)	126
Magnesium (mg)	39
Zinc (mg)	3
Arachidonic acid (mg)	20.11
Aspartate (g)	1.04
Glutamate (g)	15
Serin (g)	1
Glycine (g)	1.11
Alanine (g)	2.11
Leusin (g)	1.6
Isoleusin (g)	0
Valine (g)	2.11
Triptophane (g)	3
Hydoxyproline (g)	8.1
Proline (g)	1
Phenilalanine (g)	0.81
Histidine (g)	1
Cysteine (g)	1.07
Lysine (g)	1.46
Tyrosine (g)	0.92

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group than that of placebo group. The concentration of albumin (30.2%) in *Channa striatus* capsules maybe the main reason for increasing albumin levels as provided in Table 4.

Among the pro-inflammatory cytokines, TNF-α in conjunction with IFN-γ plays a key role in the initiation, regulation, and maintenance of the inflammatory response generated by MTB. The effects of pro-inflammatory cytokines are counterbalanced by down-regulatory cytokines such as IL-10 which is produced by the activated macrophages, monocytes, Th2, and Tregs, in response to infection [13].

TNF-α level is thought to be the major cytokine responsible for the formation and maintenance of mycobacterial antigen-induced granulomas through chemokine production [14-16]. TNF-α also plays an important role in preventing reactivation [17]. TNF-α will trigger molecular mechanisms that provide protection against mycobacterial disease [18]. Depletion of TNF using neutralizing antibodies prevented granuloma formation in mice [19]. TNF-α has also been shown to be critical to control long-term and persistent infections, with depletion resulting in failure to maintain granuloma pathology [20]. Previous studies showed that TNF-α will decrease after 4 or 6 months of antituberculosis therapy [21-23]. In this study, we found that TNF-α levels in *Channa striatus* groups was significantly lower than placebo group after 12 weeks of treatment. However, in placebo groups, there were significant decreases of TNF-α levels at weeks 12 compared with baseline as shown in Table 4. This finding indicated that *Channa striatus* accelerates reduction of TNF-α of antituberculosis therapy. Beside that, *Channa striatus* maybe increase granuloma formation or killing acitivity into MTB. These results are consistent with a previous study showing that active TB possessed more monofunctional immune responses primarily characterized by exclusive TNF-α production [24]. Several chemical constituents of *Channa striatus* which may take part as active substances to decrease TNF-α production, including poly-unsaturated fatty acids (PUFA) ω3 and ω6 [25, 26], vitamins A, B, E and D, and several minerals (Ca, Na, Mg, Zn) [9, 26-28] are presented in Table 4.

IFN-γ appears to be essential though not sufficient, for maintenance of latency [9]. IFN-γ activates alveolar macrophages to produce several substances for growth inhibition and killing of MTB. IFN-γ will increase in active status of TB then decreases in ongoing anti-TB therapy [21, 29-31]. Supplementation of *Channa striatus* capsule significantly decreased IFN-γ levels at week 12 than that in baseline. In placebo groups, there were also significant decrease of IFN-γ levels at week 12 compared with baseline as seen in Table 3. This finding indicates that *Channa striatus* accelerated IFN-γ reduction of anti-TB therapy as indicator of alveolar macrophage activation to produce several substances for growth inhibition and killing of MTB. A previous study showed that *Channa striatus* can exhibit anti-inflammatory action [27], in conjuction with its antibacterial effect for MTB [28]. NF-κB and IFN regulatory factor (IR)-3 were involved in IFN-γ production by MTB in dendritic cells [32], so the mechanism of *Channa striatus* to reduce IFN-γ maybe due to inhibiting NF-κB and IR-3. We also found positive sputum conversion in week 2 of supplementation. This finding indicated antibacterial action of *Channa striatus*. An *in vivo* study showed that the ω6 tended to increase survival of MTB in mice, while ω3 tended to increase pathogen killing [33].

IL-10 is an anti-inflammatory cytokine which is produced by response from Th2 cells. IL-10 appears to be important in reducing collateral damage and determination of severity [14]. Overabundance of IL-10 induces failure of regulation from TB infection. In addition, IL-10 also suppresses IFN-γ production by T cells [21]. Several studies showed that anti-TB therapy decrease IL-10 [21, 22, 30]. Supplementation of *Channa striatus* capsules significantly decreased IL-10 levels at week 12 in comparison with baseline. In placebo groups, there were no significant differences of IL-10 as to see in Table 3. This finding indicated that *Channa striatus* prevented overabundance of IL-10 which could induce failure of regulation from TB infection and suppression of IFN-γ.

The results of this study suggest that adjunctive supplementation of *Channa striatus* accelerate the beneficial therapeutic effect of TB chemotherapy by improving cytokine response. Larger clinical studies are required to verify these initial results. If confirmed, adjunctive therapy could be used to shorten the amount of time that TB patients are contagious, thereby reducing the potential for disease spread and allowing them a faster return to work and society.

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