ประสิทธิผลและความปลอดภัยของตำรับยาบำรุงโลหิตกับยาเฟอร์รัสฟูมาเรต ต่อการเพิ่มความเข้มข้นของเลือด
Efficacy and Safety of Thai Traditional Blood Tonic and Ferrous Fumarate on Blood Concentration

นิพนธ์ต้นฉบับ

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วารสารไทยเภสัชศาสตร์และวิทยาการสุขภาพ 2565;17(4):344-351.

Original Article

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Thai Pharmaceutical and Health Science Journal 2022;17(4):344-351.

บทคัดย่อ

วัตถุประสงค์: เพื่อเปรียบเทียบประสิทธิผลและความปลอดภัยของตำรับยาบำรุงโลหิตซึ่งเป็นยาสมุนไพรของแพทย์แผนไทยที่บรรจุอยู่ในบัญชียาหลักแห่งชาติ กับยาเฟอร์รัส ฟูมาเรตต่อการเพิ่มความเข้มข้นของเลือดในหญิงไทย **วิธีการศึกษา:** การศึกษาแบบสุ่มแบบมีกลุ่มควบคุมมีอาสาสมัครเป็นหญิงสุขภาพดีอายุระหว่าง 20 - 45 ปีจำนวน 62 คน แบ่งเป็น 2 กลุ่ม คือ กลุ่มทดลอง (n = 31 คน) ให้กินยาบำรุงโลหิตขนาด 500 มก. ต่อแคปซูล ครั้งละ 2 แคปซูล วันละ 2 ก่อนอาหารเช้าเย็น พร้อมกับยาหลอกของเฟอรรัส ฟูมาเรต ส่วนกลุ่มควบคุม (n = 31) ให้กินยาเฟอร์รัส ฟูมาเรต 200 มก.ต่อแคปซูล ครั้งละ 1 แคปซูลวันละ 1 ครั้งหลังอาหาร พร้อมกับยาหลอกของยาบำรุงโลหิต นาน 4 สัปดาห์ ประเมินประสิทธิผลด้วยค่าค่าฮีโมโกลบิน (Hb) และฮีมาโตคริต (Hct) และความปลอดภัยต่อตับด้วยค่าระดับเอนไซม์ AST และ ALT ในเลือด และต่อไตด้วยระดับยูเรียไนโตรเจน (BUN) และครีเอตินีน (SCr) ในเลือด ที่ก่อนและหลังการทดลอง ทดสอบความแตกต่างของระดับผลลัพธ์ประสิทธิผลและความปลอดภัยหลังการทดลองระหว่างสองกลุ่มด้วยสถิติความแปรปรวนร่วม (ANCOVA) **ผลการศึกษา:** พบว่าทั้งค่า Hb และ Hct หลังการทดลองของสองกลุ่มเพิ่มขึ้นเล็กน้อยและไม่แตกต่างกัน (*P*-value > 0.05) และค่า AST, ALT, BUN และ SCr ก็ไม่แตกต่างกัน (*P*-value > 0.05) พบอาการข้างเคียงน้อยและไม่รุนแรง เช่น อุจจาระอ่อนกว่าปกติ เพราะยาบำรุงโลหิตมีสมุนไพรที่มีฤทธิ์ดังกล่าว **สรุป:** ไม่พบว่ายาบำรุงโลหิตประสิทธิผลและความปลอดภัยที่แตกต่างจากยาเฟอร์รัส ฟูมาเรต ต่อการเพิ่มความเข้มข้นของเลือดในอาสาสมัครไทยเพศหญิงสุขภาพดี แต่ต้องระมัดระวังในการแปลผลเพราะไม่ใช่การทดสอบทางสถิติแบบ non-inferiority test

คำสำคัญ: ตำรับยาบำรุงโลหิต, แพทย์แผนไทย, เฟอร์รัส ฟูมาเรต, ความเข้มข้นของเลือด, ฮีโมโกลบิน, ฮีมาโตคริต

Editorial note
Manuscript received in original form: February 27, 2022;
Revision notified: March 13, 2022;
Revision completed: March 28, 2022;
Accepted in final form: May 15, 2022;
Published online: December 31, 2022.

Abstract

Objective: To compare efficacy and safety of the Bumrunglohit formula, a Thai traditional drug for blood tonic listed in the National Essential Drug List, with ferrous fumarate on blood concentration in Thai female volunteers. **Method:** In this randomized, controlled trial, 62 healthy female volunteers aged 20 – 45 years were given the 500 mg Bumrunglohit formula capsule 2 capsules twice daily before meals with dummy capsules of ferrous fumarate (test group, n = 31), or 200 mg ferrous fumarate capsule, 1 capsule once daily after meal with dummy capsules of Bumrunglohit formula (control group, n = 31), for 4 weeks. Efficacy outcomes, i.e., hemoglobin (Hb) and hematocrit (Hct) levels, and safety outcomes on liver functions (AST and ALT levels), and kidney functions (blood BUN and SCr levels) were measured before and after the intervention. Levels of outcomes after the intervention of the two groups were compared using analysis of covariance (ANCOVA). **Results:** After the intervention, Hb and Hct levels of the two groups were both slightly increased and not different (*P*-value > 0.05). For safety, AST, ALT, BUN and SCr levels were also not different between the two groups (*P*-value > 0.05). Adverse events were rare and mild. Loose stool in those taking the Bumrunglohit formula was expected because it contains herb with laxative effect. **Conclusion:** It was not found that the Bumrunglohit formula was different from ferrous fumarate in improving blood concentration in female healthy volunteers. The interpretation should be cautioned since this was not non-inferiority test study.

Keywords: blood tonic formula, Thai traditional medicine, ferrous fumarate, blood concentration, hemoglobin, hematocrit

Journal website: http://ejournals.swu.ac.th/index.php/pharm/index

Introduction

Many individuals have been affected by the blood-related illnesses.[1,2] Anemia, as a blood disorder, has been found along with the disorders that lead the patients to seek medical care. Anemia is also a complication of other diseases or medical treatments. In all age groups combined regardless of gender, anemia is found in about 30% of the population worldwide. In the developing countries, about 35% to 75% of anemia cases of pregnant women were related to iron deficiency.[2,3] Iron deficiency anemia, a common type of anemia, is the reduction of the red cell mass. Anemia could be diagnosed based on the hemoglobin (Hb) level.[4] The World Health Organization (WHO) defines anemia as the Hb of less

than 12 and 13 grams per deciliter (gm/dL) for women and men, respectively.[5] Iron deficiency could be due to a decrease in iron intake, chronic blood loss, or a defective intestinal iron absorption.[6] Severity of anemia depends on duration of iron deficiency[4] and the patient's health status including age and chronic illnesses. Patients with mild anemia may manifest no or mild signs, or only signs of loss of concentration, tiredness with exercise, and more rapid heartbeats.[7] A study of the admission physical examination of the first-year students of Naresuan University in the academic year of 2004 revealed that based on the Hb level, 231 of 2,169 students (or 10.6%) had asymptomatic anemia.[2] For those with more severe anemia, an easy tiredness even with light exercise could be presented. For patients with lung or heart diseases, a slightly low level of Hb could lead to an easy tiredness with regular physical exertion; while healthy individuals could tolerate the anemia when Hb is as low as 6 gm/dL.[8] The most effective treatment of iron deficiency anemia is discontinue the cause of iron deficiency accompanied with iron supplement to achieve a normal Hb level and further restore the iron storage pool. Supplement products with various iron forms are available including ferrous sulfate, ferrous gluconate and ferrous fumarate.[3,9]

In Thai traditional medicine, weakness, tiredness, and anemia are signs and symptoms of abnormalities of the circulatory system. Lohitang, or blood in Thai traditional medicine, is considered one of the water elements. In Thai traditional medicine, anemia is treated with Bumrunglohit formula (or blood tonic formula). This formula is approved in the National List of Essential Medicines.[10] The formula consists of 10 grams of the wood of *Caesalpinia sappan* and the flower of Anatto tree (*Bixa orellana* L.), 4 grams of lac (*Laccifer lacca*), 2 grams of dried ginger rhizome (*Zingiber officinale*), the flower of *Piper retrofractum* Vahl, roots of *Plumbago indica*, vines of Parsley Panax (Piper ribesioides Wall), roots of Wildbetal leafbush (*Piper sarmentosum* Roxb.), vines of *Arcangelisia flava* L., vines of *Urceola minutiflora* (Pierre) D.J.Middleton, the wood of *Anaxagorea luzonensis* A. Gray, the flower of *Mammea siamensis*, the flower of *Mimusops elengi*, the flower of Iron wood or Indian rose chestnut. (*Mesua ferrea*), and pollens of lotus (*Nelumbo nucifera*), and 1 gram of the flower of Kalamet (*Mansonia gagei* J.R.Drumm. ex Prain.), the fruit of Kalamet, the fruit of Siam cardamom (*Amomum krervanh* Pierre), the flower of clove (*Syzygium aromaticum* L. Merr. & L.M. Perry), Black cumin seeds (*Nigella sativa*), Garden cress seeds (*Lepidium sativum*), Cumin seeds (*Cuminum cyminum*), seeds of *Foeniculum vulgare* Mill. var. dulce Alef., seeds of *Anethum graveolens* L., *Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav., Atractylodes or Atractylis (*Atractylodes lancea* (Thunb.) DC). *Ligusticum striatum* DC., *Angelica sinensis* (Oliv.) Diels, *Artemisia annua* L., dried fruit of *Terminalia chebula* Retz., dried fruit of Yellow myrobalan (*Terminalia citrina* (Gaertn) Roxb. ex Fleming), dried fruit of *Terminalia bellirica* (Gaertn. Roxb., bark of *Alyxia reinwardtii* Blume var. lucida Markgr., bark of *Cinnamomum* spp., bark of *Dracaena loureiroi* Gagnep., the wood of *Senna garretiana* (Craib) Irwin & Barneby, the wood of *Avicennia marina* Forssk, and the wood of *Aquilaria crassna* Pierre ex Lecomte.[11]

Based on modern medicine, certain individual components were found to possess pharmacological effects as follows. The wood of *Caesalpinia sappan* L., with its stringent and salty taste, is used as female blood tonic, and for the treatment of irregular menstruation and menstrual haemagogue. *Caesalpinia sappan* L. contains brazilin which exerts anti-platelet aggregation, cardiotonic effect, and antioxidant activity.[12] In rats stimulated with AYPGKF-NH2 , an agonist of protease-activated receptor 4 (PAR4) , anti-platelet aggregation of *Caesalpinia sappan* L. was tested.[13] It was found that sappanchalcone and brazilin extracted from *Caesalpinia sappan* L. exert anti-platelet aggregation effect with EC_{50} of 114.8 µmol/L and 100.8 µmol/L, respectively.[13] In a study of anti-oxidation activity on 95% ethanol extract of *Caesalpinia sappan* heartwood (ECS), it was found that protosappanin A, protosappanin B and brazilein could inhibit malondialdehyde (MDA) and eliminate superoxide anions, hydrogen peroxide and hydroxyl radicals.[14]

The flower of Anatto tree (*Bixa orellana* L.) with its sweet taste exerts the tonic effects on blood, lymph, heart and brain, and is used for the treatment of anemia, skin itching, kidney defect and menstrual haemagogue. Bixin (Z-bixin) in the flower of Anatto tree exerts inhibition on the skin cancer cells, which could be augmented when tested with dacarbazine, a cytotoxic drug.[15] Lac is also a prominent component in Thai traditional medicine. It is used to as blood tonic and expectorant, and to treat shigellosis and diarrhea. Lac of the tree of *Samanea saman* (Jacq.) Merr. with its astringent taste is used to treat cough and joint bone fracture. The extract of lac was found to have anti-oxidation activity by the DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS+ (2,2'-azino-bis (3-

ethylbenzothiazoline-6-sulphonic acid) when compared with vitamin C as control, but not the O2-assay.[16]

Based on findings previously mentioned, components in the Bumrunglohit formula have certain pharmacological benefits. According to Thai traditional medicine, the Bumrunglohit formula contains elements with blood tonic benefit (45%), heart tonic benefit of medicines with neutral taste (29.5%) and medicines with heat or hot taste (17%).[17] In Thai traditional medicine, patients with depleted blood volume experience a series of symptoms from tiredness to light pulse and palpitation. Not only blood tonic, but heart tonic is also needed to strengthen the pulse. Patients with depleted blood volume also experience cold hands and feet as the symptoms of cold intolerance and bloating as the symptom of poor digestive system. These individuals need medicines with hot taste to improve the digestive system and to alleviate cold intolerance.[17] The Bumrunglohit formula contains the herbal components that could offer such remedy. With certain individuals being tested for pharmacological benefits as mentioned above, this comprehensive Bumrunglohit formula has been used for a long time with no systematic experiment to prove its efficacy and safety among patients with anemia. However, this Bumrunglohit formula has been widely used for relatively healthy individuals, not clinical anemic patients. Hence, there is the need to prove its benefit on blood concentration on healthy persons. The rationale for using Bumrunglohit formula for anemia was based certain studies on the use of iron supplement in healthy individuals. Iron supplement with a dosage of 60 mg once or twice a week could significantly increase hemoglobin level and reduce the risk of anemia in adolescents in Thailand[18] and other countries.[19] For adults, a study of iron supplement in regular blood donors revealed that 19 mg or 38 mg elemental iron daily with about 2 years follow-up period resulted in an average Hb increase of 0.6 g/dL[20] Another study in regular female blood donors also found that elementary iron of 45 mg daily for 8 weeks resulted in a mean Hb level of 134.6 ± 8.7 g/L compared with 130.0 ± 9.9 g/L in the placebo group (*P*-value < 0.001).[21] It is thus essential to prove whether Bumrunglohit formula could offer such benefit.

Since no systematic research or reports on adverse effects of the Bumrunglohit formula have been published, basic safety measures including short-term detrimental effects on liver and kidney functions should be determined. For liver function, serum glutamic-oxaloacetic transaminase (SGOT) or aspartate aminotransferase (AST) and serum glutamate-pyruvate transaminase (SGPT) or alanine transaminase (ALT) are the basic indicators; for kidney function, blood urea nitrogen (BUN) and serum creatinine (SCr) are the basic ones.[22]

With the promising benefit of the Bumrunglohit formula as an alternative and/or complementary remedy for anemia, it is critical to prove the efficacy and safety in a more systematic fashion. In this present phase I clinical study, we aimed to examine efficacy and safety of the Bumrunglohit formula compared with ferrous fumarate which is the conventional medicine for anemia in healthy volunteers.

Specifically, for the efficacy aspect of the interventions, we aimed to compare the increase in Hb and Hct levels between the Bumrunglohit formula and ferrous fumarate. We also examined adverse events between the two interventions. It was hypothesized that the increase in Hb level in patients receiving the Bumrunglohit formula was not different from those receiving ferrous fumarate. In addition, adverse events in the two groups were not different. In addition to the support for phase II and III clinical trials in patients with anemia, results from this phase I clinical trial in healthy volunteers could be suggestive for the use of the Bumrunglohit formula as blood tonic for women and individuals donating blood, and as appetite stimulant. Economically, the findings could be useful in promoting the acceptance and use of the Thai traditional medicine.

Methods

In this double-blinded, randomized controlled trial (RCT), efficacy and safety of the Bumrunglohit formula and ferrous fumarate were compared. It was conducted from April 1, 2021, to November 30, 2021. Study population was female volunteers aged 20 to 45 years old. To be eligible, they had to be healthy as indicated by physical examinations and laboratory investigations with hematology, liver function and renal function test results within the normal range. They were taking no medications or nutritional supplements which could affect Hb or Hct levels and willing to participate in the study. We excluded women who were pregnancy or breast-feeding, had allergy to the Bumrunglohit formula, ferrous fumarate, or herbal products, had chronic illnesses or took at least one medication regularly for chronic illnesses, had fever (i.e., temperature of 38.5 $^{\circ}$C or higher), had thalassemia, or had

digestion or absorption abnormalities. Those who could not comply with the study protocol, missed the follow-up appointments, or could not be contracted were also excluded. Volunteers were allowed to discontinue the study at their will. Those who did not take study medications at least 2 days in a row were discontinued. They could discontinue if any adverse reactions developed, or side effects were severe enough to ensure medical attention. Those who needed any medical attentions were also allowed to discontinue. Participants taking the study medications less than 80% of scheduled doses were excluded.

For sample size estimation, the equation of $2N = 4 \times (Z_{\alpha/2} + Z_{1-\beta})^2 / \delta^2$ corresponding to independent samples t test was used. Since there are no studies on the Bumrunglohit formula efficacy on Hb level, a conservative small effect size (δ) of 0.3 was assumed.[23] With a type I error of 5% ($Z_{\alpha/2} = 1.96$ for two-sided test), a power of 80% ($Z_{1-\beta} = 0.84$), and an assumed small effect size ($\delta = 0.3$), a sample size of 53 participants for the two groups was required. To compensate for a 15% attrition rate, a sample size of 62 participants was needed.

Randomization

The participants were allocated to either the regimen of Bumrunglohit formula or the regimen of ferrous fumarate. Based on simple randomization, odd numbers were assigned to the regimen of Bumrunglohit formula and even numbers to the regimen of ferrous fumarate.

Study medications

The test intervention was the 500 mg Bumrunglohit formula capsule. The dummy capsule of the Bumrunglohit formula was filled with 500 mg corn starch and had the outside appearance physically identical to the Bumrunglohit formula capsule. The active control intervention was 200 mg ferrous fumarate capsule (elemental iron of 66 mg). Its dummy capsule was filled with 200 mg corn starch with physical appearance identical to the ferrous fumarate capsule.

Dosage regimens

There were two dosage regimens to be tested. For the test group, participants were advised to take 2 Bumrunglohit formula capsules, twice daily, before meals (breakfast and dinner), and 1 dummy capsule of ferrous fumarate, once daily, after meal (breakfast), for 4 weeks. For the active control group, participants were advised to take 1 capsule of ferrous fumarate, once daily, after meal (breakfast), and 2 dummy capsules of Bumrunglohit formula, twice daily, before meals (breakfast and dinner), for 4 weeks.

Participant protection

The study was approved by the Ethics Committee for Human Study of Suan Sunandha Rajabhat University (approval number: COA.1-075/2020; approval date: April 16, 2021). Participation was voluntary with written informed consent. They could withdraw from the study at any time. Data of participant's identity and outcomes were kept anonymous. Results were presented as a summary not individual participants' data.

Study procedure

The recruitment and follow-ups were conducted at Somdet Phraphutthalerdla Hospital, Samutsakorn province. Potential participants were approached and screened for eligibility based on inclusion and exclusion criteria. They were informed about objectives, steps, benefits and risks, anonymity, and voluntary nature of the study. After written informed consent form was obtained, they were recruited to the study. Physical examination by the physician were performed. Laboratory investigations were also performed including hematology tests for blood concentration (i.e., Hb and Hct levels), and tests for safety of the interventions which were liver function tests (i.e., SGOT and SGPT levels) and renal function tests (i.e., BUN and creatinine levels).

For those eligible, they were randomized as mentioned above. Medication regimens for a 2-week supply were given with all necessary instructions. They were instructed to record each of their medication dosing in the diary and adverse events if any. At 2 weeks after starting the medications, participants were followed up at the study center for general physical examinations and adverse events investigation by in-person interview and written records inspections. The adverse events included nausea, vomiting, queasy stomach, loose stool, bloating. Constipation, frequent urination, itching, skin flare, shortness of breath, and others. Medication supply for another 2 weeks was given. At week 4 of the experiment, the participants were scheduled at the study center for laboratory investigations for efficacy and safety outcomes. They were also inquired about any adverse events as mentioned above and menstruation history.

Data analysis

Descriptive statistics including frequency with percentage and mean with standard deviation were used to present demographic and clinical characteristics of the participants. Differences in demographic and clinical characteristics between the two groups were tested using independent t test for continuous variables and chi-square test for categorical variables. Differences in efficacy outcomes (i.e., Hb and Hct) after the 4-weeks intervention between the two groups were tested using analysis of co-variance (ANCOVA) controlling for baseline levels of Hct, Hb, AST, ALT, BUN, and SCr. Differences in safety outcomes (i.e., AST, ALT, BUN, and SCr) after the 4-weeks intervention between the two groups were tested using ANCOVA controlling for baseline levels of AST, ALT, BUN, and SCr. All statistical significance was set at a type I error of 5%. All statistical analyses were performed using the software program SPSS version 23.

Results

Of the total of 73 individuals screened, 62 of them were eligible based on the physical examinations, laboratory investigations and inclusion and exclusion criteria. Participants were randomized to the test group (Bumrunglohit formula regimen) and the active control group (ferrous fumarate regimen) with equal size of 31 individuals. Participants in the test group were slightly older than the control group (35.29 and 32.68 years old, respectively) (Table 1). Majority of participants in the test and control groups had an education of less than the bachelor's degree (51.61% and 54.84%, respectively), and were government employees (45.16% and 41.94%, respectively). The majority of participants in the test group had a monthly income in the range of 5,001 - 10,000 baht per month (35.48%) while those in the control group had the income in the range of 10,001 - 20,000 baht per month (41.94%). None of these characteristics between the two groups were different (Table 1). For participant's clinical status, their temperature, blood pressure, pulse, respiratory rate, weight, height and body mass index were in normal range and not different between the two groups (Table 1).

Table 1 Demographic and clinical characteristics of the participants (N = 62).

Characteristics	N (%)		*P*-value
	Test group (n = 31)	Control group (n = 31)	
Age (years)			
mean ± SD	35.32 ± 7.30	32.65 ± 8.03	0.063*
range	23 - 45	22 - 45	
Education level			0.055†
Lower than bachelor's degree	16 (51.61%)	17 (54.84%)	
Bachelor's degree	15 (48.39%)	13 (41.94%)	
Higher than bachelor's degree	0	1 (3.23%)	
Occupation			0.504†
Agriculture/fishery	3 (9.68%)	2 (6.45%)	
Small-to-medium business	10 (32.26%)	12 (38.71%)	
Government employee	14 (45.16%)	13 (41.94%)	
No occupation/retired	4 (12.90%)	4 (12.90%)	
Monthly income (Baht)			0.540†
Less than 5,000	7 (22.58%)	9 (29.03%)	
5,001 - 10,000	11 (35.48%)	8 (25.81%)	
10,001 - 20,000	10 (32.26%)	13 (41.94%)	
20,001 or higher	3 (9.68%)	1 (3.23%)	
Temperature (°C)	36.46 ± 0.32	36.51 ± 0.32	0.516*
Pulse (beats/min)	72.97 ± 5.74	71.19 ± 5.59	0.217*
Respiratory rate (breaths/min)	17.81 ± 1.74	17.87 ± 1.71	0.745*
Systolic blood pressure (mmHg)	117.87 ± 9.44	116.84 ± 8.45	0.582*
Diastolic blood pressure (mmHg)	76.58 ± 7.31	71.87 ± 8.05	0.025*
Weight (kg)	59.26 ± 13.88	57.89 ± 12.57	0.540*
Height (cm)	157.35 ± 5.50	156.55 ± 5.25	0.495*
Body mass index (kg/m^2)	23.90 ± 5.30	23.74 ± 5.66	0.859*

* Independent t test.
† Chi-square test.

In terms of compliance, all participants completed the study according to the protocol. One participant missed taking the Bumrunglohit formula for two days because of fever associated with covid-19 vaccine. Two participants missed taking ferrous fumarate for 1 day due to the worry over their mild incident of nausea and vomiting. Five participants missed certain doses but not more 20% of the scheduled doses.

For efficacy outcomes, mean Hb level at baseline of participants in the test group (Bumrunglohit formula) (12.23 ± 1.94 g/dL) was slightly higher than that of the control group (11.28 ± 1.82 g/dL). In ANCOVA analysis controlling for baseline Hb level was appropriate. In terms of change, Hb levels in the test group (Bumrunglohit formula) slightly increased from 12.23 ± 1.94 g/dL at baseline to 12.34 ± 1.80 g/dL after the intervention (Table 2). This 0.11 g/dL increase could be viewed as a 0.89% increase of Hb in the test group. Such increase was also found in the control group (11.28 ± 1.82 at baseline and 11.53 ± 1.64 g/dL after the intervention) resulting in a 0.25 g/dL increase or 2.16% increase. The difference of Hb levels between the two groups was not statistically significant (*P*-value 0.961).

For Hct levels, mean Hct level at baseline of participants in the test group (Bumrunglohit formula) (37.59 ± 4.75%) was slightly higher than that of the control group (34.94 ± 4.59%). In accordance with Hb, similar changes of Hct were found in the test group (37.59 ± 4.75% at baseline and 37.82 ± 4.64% after the intervention) and control groups (34.94 ± 4.59% at baseline and 35.65 ± 4.05% after the intervention) with no statistical significance (*P*-value 0.908) (Table 2). The increase of Hct in the test group was 0.23% (or 0.61% increase relatively to baseline value); while that in the control was 0.61% (or 1.99% increase relatively to baseline value).

Table 2 Efficacy outcomes of Bumrunglohit formula compared with ferrous fumarate (N = 62).

Outcomes	Group	Mean ± SD		*P*-value*
		Before	After	
Hb (g/dL)	Bumrunglohit formula	12.23 ± 1.94	12.34 ± 1.80	0.961
	Ferrous fumarate	11.28 ± 1.82	11.53 ± 1.64	
Hct (%)	Bumrunglohit formula	37.59 ± 4.75	37.82 ± 4.64	0.908
	Ferrous fumarate	34.94 ± 4.59	35.65 ± 4.05	

* Analysis of covariance, adjusted for baseline levels of Hct, Hb, AST, ALT, BUN, and SCr.

In terms of safety, AST, ALT, BUN and SCr levels of participants in the Bumrunglohit formula increased slightly from baseline with no statistical significance (Table 3).

Table 3 Safety outcomes of Bumrunglohit formula compared with ferrous fumarate (N = 62).

Outcomes	Group	Mean ± SD		*P*-value*
		Before	After	
AST (U/L)	Bumrunglohit formula	29.26 ± 11.64	28.48 ± 12.17	0.753
	Ferrous fumarate	28.26 ± 12.69	28.39 ± 12.88	
ALT (U/L)	Bumrunglohit formula	25.23 ± 6.60	25.65 ± 7.25	0.798
	Ferrous fumarate	24.45 ± 9.71	24.81 ± 8.31	
BUN (mg/dL)	Bumrunglohit formula	10.77 ± 2.26	11.25 ± 2.36	0.455
	Ferrous fumarate	12.45 ± 3.62	11.12 ± 2.49	
SCr (mg/dL)	Bumrunglohit formula	0.73 ± 0.13	0.75 ± 0.16	0.594
	Ferrous fumarate	0.70 ± 0.14	0.75 ± 0.17	

* Analysis of covariance, adjusted for baseline levels of AST, ALT, BUN, and SCr.

Mild adverse events were reported in both groups. These events were completely resolved with no intervention discontinuation. In the test group, five participants experienced loose stool with larger stool volume and no cramp. They had bowel movements of 2 – 3 times a day instead of their regular once daily frequency. These bowel irregularities were resolved in 2 days. More appetite was found in eight participants in the test group and resolved within one week. On the other hand, two participants experienced nausea and mild vomiting two days after intervention initiation. The symptom was subsided and completely resolved a few days later.

In addition, since all participants were women, their menstruation was also reported. The participants reported regular menstruation with no lump and no cramp.

Discussions and Conclusion

In this 4-week randomized controlled trial, the Bumrunglohit formula, a Thai traditional medicine was compared with ferrous fumarate for a benefit of improving Hb and Hct level in healthy Thai female volunteers. Of the 62 participants, i.e., 31 in each group, their baseline demographic characteristics and clinical status including temperature, blood pressure, pulse rate, respiratory rate, weight, height, and body mass index were in the normal range and comparable between the two groups which could prevent certain bias.

We found no difference in the level of Hb at the end of the study between the test and control groups (12.34 ± 1.80 and 11.53 ± 1.64 g/dL, respectively). In addition, the increase of 0.11 and 0.25 g/dL in the test and control groups, respectively, were of interest. The increase of 0.25 g/dL of Hb in ferrous fumarate group (elemental iron of 66 mg taken once daily for 4 weeks) is much lower than 0.6 g/dL increase found in the study of Mast and colleagues with 19 to 38 mg elemental iron taken daily for 2 years.[20] The Hb level of 11.53 ± 1.64 g/dL at the end of the 4 weeks period of ferrous fumarate in our study was also lower than 134.6 ± 8.7 g/L of the 8-weeks regimen of elementary iron of 45 mg daily[21] The small increase in Hb level in volunteers taking iron supplement in our study compared with these previous two studies could affect credibility of our study. However, this low level of Hb increase associated with ferrous fumarate regimen in our study could be due to a shorter period of iron supplement. Our finding on a small Hb level increase could be expected.

In addition, Hb levels in our study were relatively lower than those in these two previous studies in healthy volunteers[20,21] In fact, the mean level of Hb of participants in the control group (11.28 g/dL) was slightly lower than the normal range (12 - 16 gm/dl for women adults) while that of the test group was in the normal range. With certain portion of volunteers with Hb and Hct lower than normal limit, a larger increase of the two outcomes could be expected. In patients with iron deficiency anemia, one-month iron supplement could increase Hb as much as 2 g/dL.[24] We also propose that more

studies on the Bumrunglohit formula in anemic patients should be conducted.

Even though baseline Hb level was controlled for in ANCOVA, the effect of Hb discrepancy between the two groups on the change of Hb needs more experiment. For Hct level, changes similar to those of Hb levels were found with no statistical significance. Rationales for the changes were relatively similar to those of Hb level as mentioned above.

Based on our non-significant findings on Hb and Hct, one should not conclude that the Bumrunglohit formula was equivalently beneficial to ferrous fumarate supplement. This study used a traditional test of statistical difference, not the non-inferiority test. Non-significant results of the traditional difference test do not indicate comparable efficacy. Further studies with non-inferiority test should be conducted. However, blood tonic benefit of Bumrunglohit formula was promising as suggested by our findings.

Bumrunglohit formula could increase blood concentration with certain speculated indirect mechanisms including anticoagulation, blood tonic, and anti-oxidation effects of Sappan, Anatto tree, and lac.[11] It was found that extract of lac exerted anti-oxidation effect of 68% by DPPH method and 87% by ABTS method which was higher than that vitamin C.[15] Such anti-oxidation effect of lac is possible with a considerable amount of lac in the Bumrunglohit formula. Based on Thai traditional medicine, this blood concentration effect is also in accordance with the Bumrunglohit formula containing elements with blood tonic benefit (45%), heart tonic benefit of medicines with neutral taste (29.5%) and medicines with heat or hot taste (17%).[17] These effects could directly affect the circulatory system.

In terms of safety of liver and kidney, levels of AST, ALT, BUN and SCr in participants taking the Bumrunglohit formula slightly increased from baseline with no statistical or clinical significance. These levels in the Bumrunglohit formula were not different from those in the ferrous fumarate group. The levels of these measures after the two interventions were also within the normal range. It could be concluded that the 500 mg Bumrunglohit formula taken two capsules twice a day before breakfast and dinner for 4 weeks was safe for liver and kidney.

Bumrunglohit formula was also associated with few mild adverse events. Based on physician's physical examinations and the participant's diary records, no severe nausea or vomiting, diarrhea, shortness of breath or itching were found. The incidents of loose stool with larger volume were experienced in five participants taking the Bumrunglohit formula were not unexpected. The incidents were induced by the laxative effect of myrobalan, yellow myrobalan, and beleric myrobalan in the Bumrunglohit formula. The incidents of 8 participants experiencing an increased appetite could be expected. With the medicines with neutral taste (29.5%) and medicines with heat or hot taste (17%) in the Bumrunglohit formula, indigestion, bloating, cold feet and poor appetite could be improved.[17] Consequently, some participants were worried about weight gain from the increased appetite. For ferrous fumarate, nausea, vomiting, diarrhea and constipation could be expected[17] and were reported by two participants.

This study had certain limitations. With unknown effect size of the Bumrunglohit formula on Hb and Hct, the sample size estimation could be problematic. Further studies could estimate the sample size more precisely. A 4-weeks duration could contribute to the slight increase of Hb and Hct by both interventions. Studies with longer duration should be conducted. Since participants diet habit was not well controlled, outcomes could be confounded. In addition, the covid-19 vaccine made certain participants to have fever, hence the missing of a few doses but with no protocol violation. Future studies should put a stricter diet control and dosing restriction. More frequent follow-ups, either in-person, online, telephone, or all of these means, should be implemented in the future studies. Even though these female participants reported no irregular menstruation with no lump and no cramp, more studies on the effects of Bumrunglohit formula on menstruation should be conducted.

In conclusion, the Bumrunglohit forma, a Thai traditional medicine for blood tonic listed in the Thai National Essential Drug List, with a dosage regimen of 2 of 500 mg capsules, taken twice daily before breakfast and dinner was associated with a slight increase in hemoglobin and hematocrit but no statistical significance was found when compared with ferrous fumarate 200 mg capsule (elemental iron of 66 mg), taken once daily after breakfast, for 4 weeks. The safety was mild and acceptable.

Acknowledgements

The authors would like to thank Dr. Narin Kakathum, Saengsit Kritsadee for invaluable advice, and all faculty members and staff at the Department of Applied Thai Traditional Medicine, Graduate School, Suan Sunandha

Rajabhat University for assistance. We also thank the director and staff at Somdet Phraphutthalerdla Hospital for the courtesy of facilities and personnel in research conduct, and staff at the department of Thai traditional medicine for public relation task in participant recruitment.

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