International Journal of Pharmacy Teaching & Practices 2011, Vol.2, Issue 4, 165-170.

A Safety Study of Extract Combination of Legundi (*Vitex trifolia L.) Leaves and Temulawak (Curcuma xanthorrhiza R.)* Rhizome as Anti-allergy in Healthy Volunteers.

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Research Article

Please cite this paper as: Hanif Nasiatul Baroroh [(a)], Zullies Ikawati [(b)], Kartono Sudarman [(c)]. A Safety Study of Extract Combination of Legundi (*Vitex trifolia L.) Leaves and Temulawak (Curcuma xanthorrhiza R.)* Rhizome as Anti-allergy in Healthy Volunteers. IJPTP, 2011, 2(4),165-170.

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Abstract

Objective: The effect of extract combination of legundi (*Vitex trifolia* L) leaves and temulawak (*Curcuma xanthorrhiza* Roxb) rhizomes was investigated preclinically as anti allergy.

Methods: This formula have been proven relatively non-toxic. Based on these preliminary studies, this formula was then studied in phase I clinical trials to determine the safety and tolerability of the extract in healthy volunteers.The trial was designed as randomized and double blind study with 30 healthy volunteers divided into 3 groups. Each participants in group 1, 2, and 3 received extract combination of legundi leaves and temulawak rhizomes with the dose of 1500 mg/day, 4500 mg/day, and 9000 mg/day, respectively. Drugs will be administered orally three times daily for 14 days. The primary outcome measured were the absence of tolerability and side effects. The secondary outcome measured were value changes in renal, liver function and physical examination before and after drug administration (*pre and post control group design*).

Results: The results showed that the extract combination at the dose of 1500 mg/day and 4500 mg/day were well tolerated and safe, as seen from the absence of the drug-induced changes in the renal and liver function. However, there were significant changes in the value of AST and serum creatinine after administration of the extract combination with the dose of 9000 mg/day ($p < 0.05$). There were no significant side effects in the Group 1 and 2, while the thrush and decrease of blood pressure were reported with administration of the doses of 9000 mg/day. Thus, extract combination of legundi leaves and temulawak rhizomes at the dose of 1500 mg/day and 4500 m /day were found to be well tolerated and safe.

Keywords: safety, anti allergy, phase I clinical trial, *Vitex trifolia*, *Curcuma xanthorrhiza*

Introduction

Allergic reactions are IgE-mediated immune response against foreign substances that enter the body (Arshad, *et al*, 2001). There were various plants which can be used as antiallergy. The use of herbal medicine has increased in recent years. Legundi (*Vitex trifolia* L.) and temulawak (*Curcuma xanthorrhiza* R.) are medicinal plants which used in traditional herbal medicine for asthma.

Several studies have examined the effect of *Vitex trifolia* and *Curcuma xanthorrhiza* on allergic disease. In the in vitro study, Legundi leaves extract has been reported to inhibit histamine release from RBL-2H3 cell cultures induced by DNP-BSA as antigen (Ikawati *et al*, 2001). Isolated compound from legundi identified as viteosin–A vitexicarpin and vitetrifolin-E have been reported to have tracheospasmolytic activity (Alam *et al*, 2002). Several studies have clearly indicated that 20 mg/kg body weight of curcumin significantly inhibited ovalbumin (OVA)-induced airway constriction and airway hyperactivity (Ram *et al*, 2003). In one of in vitro study, curcumin also show anti-inflammatory activity via inhibition of immunological point extracellular signal-regulated kinase (ERK), which activated the enzyme protein kinase C (PKC) (Baek *et al*, 2003) and inhibited Syk kinase activity (Lee *et al*, 2008).

In the in vivo study, the effect of extract combination of *Vitex trifolia* leaves and *Curcuma xanthorrhiza* rhizome was examined on an active cutaneus anaphylaxis reaction and showed anti allergic activities through mechanism

related to inhibition mast cells degranulation (Ikawati *et al*, 2008) and tracheospasmolytic activity (Melati, et al, 2008).

In a preclinical toxicity study, the *Curcuma xathorrhiza* extract has been proven to be not toxic at a dose of 960 mg/200 g BW mice for 90 days (Artanti, 2009). The extract combination of legundi leaves and temulawak rhizomes (1:1) is also not toxic, with apparent LD50 of 17.1 g / kg in acute toxicity study, as well as in sub acute toxicity study (Ikawati *et al*., 2010). Based on these preliminary studies, this formula was subjected into phase I clinical trials, before developed as phytopharmaceutical for treating allergic disease. This paper describes phase I clinical trial which carried out in adult (18 -40 years old) Indonesian healthy volunteers.

The clinical trials was carried out to determine the safety and tolerability of extract combination of legundi (*Vitex trifolia* L) leaves and temulawak (*Curcuma xanthorrhiza* Roxb) rhizome.

Methodology

The trial was designed as randomized and double blind study. The duration of administration of the extract was 14 days.

Selection Subject

Subject were eligible for enrollment in this study if they met the following inclusion criteria : 18-40 years old, BMI (Body mass index) 18-30, and healthy which is confirmed by laboratory and physical examination. The main exclusion criteria included the followings : pregnant or breast feeding, having serious disease (hepatitis, liver sirrhosis, hypertension, heart failure, kidney failure, rheumatism, cancer, etc.), having history of alcohol or subtance abuse, smoking, taking medication 7 days prior to study and during study. Thirty healthy volunteers gave their informed concent to participate in this study.

Administration

Thirty healthy volunteers were divided into 3 groups. Each participants in group 1, 2, and 3 received extract combination of legundi leaves and temulawak rhizome with the dose of 1500 mg/day, 4500 mg/day, and 9000 mg/day, respectively. Drugs was administered orally three times daily for 14 days with a glass of water after meals.

Laboratory studies

The laboratory test performed were : liver function test including serum levels of aspartate aminotransferase (SGOT/AST), alanine aminotransferase (SGPT/ALT); renal function test including creatinin serum and blood urea nitrogen; and hematological studies consisting of: haemoglobin, eryhtrocyte, haematocrit, leukocyte, mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), and thrombocyte. Urine samples were tested for pH, albumin, glucose, keton, urobilinogen, bilirubin, leucocytes, nitrite, and sediment. All laboratory values were measured by standard clinical biochemical methods. Liver and renal function test were measured before (on day 0) and after treament (on day 15) with legundi and temulawak extracts. Hematological studies and urine samples were measured at prior to treatment to confirm healthy status.

Physical examination

Before and after treatment with extract combination of legundi leaves and temulawak rhizome, full physical examination were obtained and recorded. Then blood pressure of the group 3 was measured on days 0, 5, 10 and 15 . The blood pressure of participants in Group 1 and 2 was measured on days 0 and 15.

Follow up and Monitoring

During the study, the investigator assessed the compliance of the participants to the drug, and the presence of any adverse events. All patients were followed-up for a periode of 14 days. Follow up evaluation were held every two days. Adverse event on the body vital organ systems such as the cardiovascular system, respiratory system, the system central nervous system, gastrointestinal system, musculoskeletal system and skin were observed. All adverse events either reported by subjects or observed by investigator were recorded.

Statistical analysis

Liver and renal function test parameters are presented as mean ± SEM. Measured variabels were compared by paired t tests or wilcoxon signed rank test. Adverse event were evaluated using Fisher exact test. P-values < 0,05 (2-sided test) were considered statisticaly significant.

Results

Participants characteristic

A total of 54 prticipants were screened and 30 participants were selected based on the criterias. The subjects consisted of 16 women (53.3%) and 14 men (46.7%). All participant were aged 18-38 years old, with . And the mean age of participants was 22 years old. All participants were not pregnant or breast feeding, had serious disease, who had a history alcohol or subtance abuse, had taken drug. All participant were healthy based on the results of medical examination and laboratory test.

Safety results

The results of liver function test are showed in table 1 and 2. From the results, the laboratory data including AST values before and after treatment in all groups were still within the normal range (5-34 U /L). There was no significant differences between serum levels of AST before and after treatment with extract combination of legundi and temulawak 1500 mg/day and 4500 mg(sig> 0.05). However, the serum levels of AST in the group 3 showed significant changes after treatment with the extract combination of legundi and temulawak 9000 mg/day (sig<0,05).

Based on the results of serum levels of ALT, there were no significant changes after treatment with the extract combination of legundi and temulawak 1500 mg/day and 9000 mg/day. While, there was statistically significant differences before and after treatment with extract combination of legundi and temulawak 4500 mg/day. However, serum levels of ALT in all groups before and after treatment are still within the normal range (0-55 U/L).

Table 1. Serum AST levels carried out before and after treatment with the extract combination of *Vitex trifolia* and *Curcuma xanthorrhiza* at the dose of 1500 mg/day, 4500 mg/day and 9000 mg/day

Treatment	Mean of AST(Aspartate aminotransferase)(U/L) ±SEM (n = 10)		p value	Reference range
	before treatment	after treatment		
Legundi+temulawak 1500 mg/day	17,45±0,94	17,2±1,05	0,768	5-34 U/L
Legundi+temulawak 4500 mg/day	18,78±1,46	18,31±1,25	0,588	
Legundi+temulawak 9000 mg/day	15,37±0,93	19,02±2,35	0,022*	

Note : *p<0,05 as compared to before treatment values.

Table 2. Serum ALT levels carried out before and after treatment with the extract combination of *Vitex trifolia* and *Curcuma xanthorrhiza* at the dose of 1500 mg/day, 4500 mg/day and 9000 mg/day

Treatment	Mean of ALT (Alanine aminotransferase)(U/L) ±SEM (n = 10)		p value	Reference range
	before treatment	after treatment		
Legundi+temulawak 1500 mg/day	18,68±2,28	16,24±2,56	0,303	0-55 U/L
Legundi+temulawak 4500 mg/day	22,49±4,20	18,77±3,36	0,046*	
Legundi+temulawak 9000 mg/day	15,74±1,89	17,26±4,35	0,76	

Note : *p<0,05 as compared to before treatment values.

The changes of serum levels of urea nitrogen parameters before and after treatments in all groups were not found and still within the normal range (for male: 8-26 mg / dl and for female: 7-20 mg / dl) (Table 3). The same is true for creatinine values in all groups, which were still within the normal range (female: 0.6 to 1.1 mg / dl and for male : 0.7 to 1.3 mg / dl). There were no significant differences before and after treatment with the extract combination with the dose of 1500 mg and 4500 mg per day (p>0.05), while significant changes before and after was found after treatment with the extract with dose of 9000 mg/day. (Table 4). Overall, based on the results of serum levels of AST, ALT, creatinine, and urea nitrogen, no influence on liver and renal function were observed.

Table 3. Blood urea nitrogen carried out before and after treatment with the extract combination of *Vitex trifolia* and *Curcuma xanthorrhiza* at the dose of 1500 mg/day, 4500 mg/day and 9000 mg/day

Treatment	Mean of Blood urea nitrogen (mg/dL) ±SEM (n = 10)		p value	Reference range
	before treatment	after treatment		
Legundi+temulawak 1500 mg/day	9,76±1,23	9,6±1,30	0,61	8-26,0 mg/dL (Male); 7-20,0 mg/dL (Female)
Legundi+temulawak 4500 mg/day	9,31±0,49	9,11±0,43	0,745	
Legundi+temulawak 9000 mg/day	9,07±0,70	10,36±1,27	0,454	

Note : *p<0,05 as compared to before treatment values.

Table 4. Serum Creatinin carried out before and after treatment with the extract combination of *Vitex trifolia* and *Curcuma xanthorrhiza* at the dose of 1500 mg/day, 4500 mg/day and 9000 mg/day

Treatment	Mean of Serum creatinin (mg/dL) ±SEM (n = 10)		p value	Reference range
	before treatment	after treatment		
Legundi+temulawak 1500 mg/day	0,781±0,05	0,715±0,03	0,095	0,7-1,3 mg/dL (Male); 0,6-1,1 mg/dL (Female)
Legundi+temulawak 4500 mg/day	0,788±0,031	0,781±0,037	0,732	
Legundi+temulawak 9000 mg/day	0,77±0,031	0,8±0,038	0,045*	

The adverse events were shown in table 5. Based on monitoring results, there were 6 types of adverse events in 10 subjects in the Group 1 during treatment for 14 days. Two subjects of 10 subjects reported seven adverse events, including: heartburn complaints (1), nausea (1), palpitations (1), frequent urination (1), diarrhea (1) and dizzines (2). However, the adverse event were minimal, happened in short duration (no more than 3 days) and reversible.

In Group 2 with the dose of 4500 mg/day, there were found 6 types of complaints during treatment. From 10 subjects, there were 5 subjects reported on seven adverse events including: diarrhea (1), mouth itching (1), palpitations (1), flatulence (1), lower abdominal pain (1) and nausea (2). Adverse event were in short duration, not more than one day.

In the Group 3, 7 of 10 subjects reported 13 kind of adverse events, including: thrust (3 subjects), dry mouth (2 subjects), dizziness (2 subjects) and diarrhea (2 subjects). Other adverse events such as heartburn, nausea, palpitations, and abdominal bloating was experienced by 1 subject, respectively. The adverse event of thrust was in short duration, not more than 4 days.

From the vital sign examination, there were decrease of blood pressure in 6 of 10 subjects during treatment with the doses of 9000 mg/day. Two of 10 subjects in Group 2 also reported the decrease of blood pressure. There was no changes of blood pressure parameter in the Group 1.

Discussion and Conclusion:

The main objective of the study was to evaluate the safety in healthy subjects treated with the extract combination of legundi (*Vitex trifolia* L) leaves and temulawak (*Curcuma xanthorrhiza* Roxb) rhizome. Based on preclinical study, both pharmacological and toxicity study, the extract combination is a good candidate to be developed as phytopharmaceutical for antiallergy. Some toxicity studies have examined the safety of *Curcuma xanthorrhiza* and *Vitex trifolia*. It has been reported that administration of ethanol extract of Vitex trifolia leaves with the dose of 11.24 g/kg BW caused degeneration of hepatocyte cell (Mardliyah, 2006). There were not reported any relatif toxicity during treatment *with Curcuma xathorrhiza* at doses of 4.89 g / kg and doses of 960 mg / 200gr BB daily (Farida, 2009; Artanti, 2009). The authors did not record clinical symptoms and weight loss on rats.

Based on the results of clinical and laboratory examinations on 30 healthy subjects, it can be concluded that the extract combination of *Vitex trifolia* leaves and *Curcuma xanthorrhiza* rhizomes with dose of 1500 mg/day and 4500 mg/day did not cause side effects on liver and kidney during treatment for 14 days. While in the dose of 9000mg/days, it caused a change in the value of AST (aspartate aminotransferase) and serum creatinine which affects the liver and kidney function. But this changes were still within normal range. In preclinical study, the ectract combination with the dose 0.79 g kg, 3.15 g/kg and 12.6 g/kg, respectively, did not show any changes in values of AST (Aspartate Aminotransferase) and found not toxic effect on the liver of male and female rats (Ikawati, 2010).

There were increasing of the serum levels of AST after tretament this formula with dose of 9000 mg/ day, but this changes were still within reference range. Aspartate aminotransferase (AST), also refered to as glutamate oxaloacetate transaminase (GOT) is one group of enzymes which cataltyzes the interconversion of amino acids and αketo acids by tranfer amino groups. The greatest concentrations of AST are found in heart, liver, muscle, and kidney tissues.

Table 5. Adverse events during treatment of *Vitex trifolia* and *Curcuma xanthorrhiza* extracts with dose 1500 mg/day, 4500 mg/day and 9000 mg/day

	Legundi+temulawak 1500 mg (n=10)	Legundi+temulawak 4500 mg (n=10)	Legundi+temulawak 9000 mg (n=10)
No of adverse events reported	6	6	8
No (%) of subject reporting events	2 (6,67%)	5 (16,67%)	7 (23,33%)
Adverse events :			
Gastrointestinal disorder			
Hearthbun	1	0	1
Nausea	1	1	1
Diarrhea	1	1	2
Bloating	0	1	0
Dry mouth	0	0	2
Thrust	0	0	2
Lower abdominal pain	0	1	1
Central nervous system disorder			
Dizzines	2	0	1
cardiovasculer disorder			
Palpitations	1	1	1
Decreasing of blood pressure *	0	2	6
Skin disorder			
Itchy and thick mouth	0	1	0
Urinary tract disorder			
frecuent urination	1	0	0

Note : * p<0,05 correlated with treatment

Damage to these tissues can greatly elevate serum AST level. Serum levels in liver may increase to 3 times normal levels normal levels and the increase is roughly proprtional to the degree of the tissue damage. Changes in liver function that occur will affect aspects of pharmacokinetics, especially metabolism (Sacher and McPherson, 2004).

In this study, changes the serum ALT subjects treated with the extracts dose of 4500 mg were still within normal levels. ALT (alanine aminotransferase) also referred to as glutamate pyruvate transaminase (GPT) is

an enzyme found in many tisssues. The highest levels are found in liver and kidney tissues. This enzyme in small amounts found in heart, muscle, and skeletal muscle Tissue destruction leads to the release of the intracellular enzyme into the circulating blood. Markedly elevated serum ALT levels may be found in a variety of diseases which involve the liver cells such as hepatitis and cirrhosis. Serum ALT level is regarded as a reasonably specific indicator of liver disease. Clinical problems occured if there was increasing of serum ALT levels at least 1-3 times the normal levels (Sacher and McPherson, 2004). The chronic use of curcumin can cause liver toxicity. For this reason, this extract should probably be avoided by individuals with liver disease, heavy drinkers and those who take prescription medications that are metabolized by liver (Begum *et al*, 2004).

There were no significant changes of blood urea nitrogen in all groups before and after treatment and was still within normal levels. Low levels are usually not considered abnormal because they reflect the lack of protein in the diet or expansion plasma volume. However, very low levels can indicate severe liver disease. There were significant diferences before and after treatmnet with the extracts at the dose of 9000 mg/ day, but still within reference range.

Based on these laboratory results, it can be concluded that the extract combination of *Vitex trifolia* leaves and *Curcuma xanthorrhiza* rhizomes dose 1500 mg / day and 4500 mg / day was safe for consumption. While the extract with dose of 9000 mg / day still needs a further clinical trials with a longer treatment more than 14 days to ensure safety in liver and kidney function.

Several clinical studies have examined the safety of *Vitex agnus-castus,* other varietas of *Vitex* species. The author reported the adverse events : nausea, gastrointestinal disturbances, fatigue, menstrual disorders, dry mouth, acne, pruritis, and itching (Dugova *et al*, 2008; Padmalatha *et al*. , 2009). Merz *et al* (1996) reported that there were found adverse events: pruritis, eczema with pruritis, indigestion, dizziness, increased activity, and fatigue with treatment *Vitex agnus-castus*(doses 120, 240 and 480 mg). Curcumin have been reported to cause adverse events : diarrhea (0,45 g/day) and nausea (0,9 g/day) (Sharma *et al*, 2004). No apparent side effects have been reported so far. GI upset, chest tightness, skin rashes, swollen skin are said to occur with high dose. A few cases of allergic contact dermatitis from curcumin have been reported.

Curcumin was found to be pharmacologically safe in human clinical trials with doses up to 10 g/day (Begum *et al*, 2008). A phase 1 human trial with 25 subjects using up to 8000 mg of curcumin per day for three months found no toxicity from curcumin (Chainani-Wu, 2004). Based on recent studies that there were similarities with results of this clinical study.

The presence of clinical response difference during treatment with this extracts is probably due to the difference in pharmacokinetic and pharmacodynamic profile of the drug. Pharmacodynamic differences concerning the relationship between test preparation and receptor may be due to differences in receptor sensitivity to the drug. Besides,the unknown of pathophysiological factor, the difference of ages, and tolerability of drug (Shargel *et al*., 2004).

Overall, it can be concluded that the extract combination of *Vitex trifolia* leaves and *Curcuma xanthorrhiza* rhizomes at the dose of 1500 mg / day and 4500 mg / day was safe. Thrush and decrease of blood pressure reported in subjects of Group 3 (dose of 9000 mg/day) need further confirmation with a more varied population, larger number of subjects, and longer duration of treatment.

Acknowledgments

The authors thank to Ministry of Research and Technology, Republic of Indonesia for financial support and PT.Soho Industri Pharmacy for providing the legundi and temulawak extract capsules.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed

CONFLICTS OF INTEREST

The authors declare that they have no competing interests