ANTI-INFLAMMATORY ACTIVITIES OF TEMULAWAK, GINGER, SOYBEAN AND SHRIMP SHELL EXTRACTS IN COMBINATION COMPARED TO DICLOFENAC SODIUM

(Ability in Reducing the Pain and Synovial Fluid Leucocyte Count of Osteoarthritis)

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ABSTRACT

Background: *The prevalence of osteoarthritis (OA) in the community is high. This disease is the second most common cause of physical disability worldwide. Pain in OA is caused by several factors, such as inflammation. Non steroidal anti-inflammatory drugs (NSAIDs) were the most common drugs given worldwide to reduce pain in OA. NSAIDs were also associated with a high incidence of gastrointestinal side effects. An alternative to manage this problem is by using the combination of Curcuma xantorrhyza Roxb. (commonly known as temulawak) extract, ginger (Zingiber officinale) extract, soybean (Glycine max), and shrimp shell. Curcuma xantorrhyza contains curcumin which has anti-inflammatory effect by suppressing cyclo-oxygenase (COX-2) enzyme activity, suppressing lipo-oxygenase enzyme activity, and play a role as a free radical scavenger. Ginger can inhibit COX-2 activity in PGE-2 production. Shrimps shell contains glucosamine and chondroitin which can increase proteoglycan in articular chondrocytes and inhibit COX-2 synthesis. Isoflavone in soybean can inhibit articular cartilage degradation and COX-2 synthesis.*

Study Aims: *The purpose of this study is to compare the effect of the combination to diclofenac sodium in reducing synovial fluid leukocyte count and joint pain in patients with osteoarthritis.*

Sudy Method:*This study was a prospective randomized open end blinded evaluation (PROBE). Twenty one patients with knee osteoarthritis diagnosed by American College of Rheumatology criteria were included in this study. Patients were randomized into two groups to receive either diclofenac sodium 25 mg (control group) or the combination of Curcuma xantorrhyza extract 50 mg, ginger extract 100 mg, shrimp shell 100 mg, and soy bean flour 50 mg (treatment group) three times daily for 14 days. Independent t-tests and Mann-Whitney-Wilcoxon tests were used to evaluate changes between prior and post intervention.*

Results: *There were significantly reduction of synovial fluid leukocyte count in both control group ($p=0.017$) and treatment group ($p=0.008$) respectively. The reduction of synovial fluid leukocyte count was not significantly different between control group and treatment group ($p=0.929$). There were significant Improvement of joint pain (VAS score) in both control group ($p=0.012$) and treatment group ($p<0.001$). The reduction of VAS score was not significantly different between diclofenac group and treatment group ($p=0.607$).* ***Conclusion:*** *These results indicate that the evicacy of this combiation was not significantly different with diclofenac sodium in reducing the synovial fluid leukocyte count and joint pain in patients with osteoarthritis.*

Keywords: *osteoarthritis, Synovial fluid leukocyte count, Pain, VAS, Diclofenac sodium, Combination of curcuma, ginger, shrimp shell and soybean*

INTRODUCTION

Osteoarthritis is a joint disease which occurs because of joint cartilage changes, sclerosis of subchondral bones, and inflammation. Osteoarthritis (OA) is a rheumatic disease with the highest prevalence among all rheumatic diseases. Osteoarthritis is the second leading cause of physical disability in the world after ischemic heart disease. This disease causes a major loss of working hours and has a high cost of treatment[1].

World Health Organization (WHO) estimates that 40% of the population of people above 70 years of age suffers from OA and 80% of OA patients have limitation in movement ranging in various degrees from mild to severe which leads to the deterioration of quality of life. Osteoarthritis prevalence increases with age[2].

Joint pain can haunt the patients with osteoarthritis every time. Pain in osteoarthritis is still difficult to explain, which occurs because of the combination of many factors, one of them is inflammation[3]. Concept of pain in OA caused by inflammation has some evidences, such as the increase of synovial fluid leukocyte count over 200cells/mm^3 and the presence of many pro-inflammatory mediators in the synovial fluid[4]. Pain in osteoarthritis is chronic, thus need some pain killer or anti-inflammatory drugs. Anti-inflammatory drugs which widely used are non-steroidal anti-inflammatory drugs (NSAIDs) which work by inhibiting cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzyme activities. The suppression of COX-2 enzyme activities can inhibit the forming of E-2 prostaglandin thus hampering the process of joint inflammation. Studies have shown that NSAIDs have serious adverse effects mainly if used in a long period, especially in elderly patients. The adverse effects of NSAIDs can be gastrointestinal bleeding, liver function disorder, kidney disorder, bone marrow disorder, heart attack and stroke [5].

The natural ingredients to treat diseases has been used for thousands years in Indonesia or other countries. Study data in Malang City and District shows that residents using herbal medicine for rheumatic disease treatment are each 476 and 580 per thousand patients in 1995[6].

Curcuma xantorrhyza (temulawak) contains curcumin, essential oil, arabinose, fructose, glucose, starch, tannins and minerals which are magnesium, manganese, iron, copper, calcium, sodium, potassium, lead, zinc, cobalt, aluminum, and bismuth[7]. Curcumin has anti-inflammatory activities[8]. Curcumin is able to block cyclooxygenase, lipooxygenase and has activity as antioxidant[9].

Combination of 15 mg of curcuminoid from *Curcuma domestica* Val. rhizome extract and 100 mg essential oil from *Curcuma xanthorrhiza* Roxb. consumed 2 times daily for 2 weeks is equal to anti-inflammatory drug piroxicam in improving the osteoarthritic pain. Another advantage of the combination of turmeric rhizome extract curcuminoid and temulawak rhizome essential oil combination are the lower cost, higher effectiveness in improving physical conditions, and tends to improve liver, kidney, and gastrointestinal functions[10].

Gingerol, shogaol, diarylheptanoids, and dialdehid diterpenes from ginger are able to inhibit prostaglandin production so they have anti-inflammatory activities[11].

Soybean contains isoflavone proven to suppress COX-2 which is an important pro-inflammatory enzyme which converts arachidonic acid into prostaglandin causing pain and inflammation in osteoarthritis[12].

Shrimp shell contains chondroitin and glucosamine which is a material for the formation of cartilage. Both have anti-inflammation effects and affect the cartilage metabolism by stimulating joint cartilage chondrocyte proteoglycan synthesis[13]. The combination of temulawak extract, ginger extract, soybean, and shrimp shell is hoped to reduce synovial fluid leukocyte count and joint pain in patients with osteoarthritis.

METHOD

Research Equipment

1. Light microscope
2. Leukocyte count chamber
3. Validated visual analogue scale (VAS)

Research Material

1. Capsule which contains curcuma extract 50 mg, ginger extract 100 mg, shrimp shell 100 mg, and soy bean flour 50 mg
2. Diclofenac sodium capsule 25 mg
3. Synovial fluid obtained from knee joints of patients with osteoarthritis as much as 2ml.

Research Subject

The subject of this study were diagnosed with knee osteoarthritis meeting the American College of Rheumatology (ACR) criteria which does not have other arthritis diseases besides osteoarthritis; no liver function, kidney or bone marrow disorder; no history of gastritis, peptic ulceration, or duodenal ulceration; no hypersensitivity to curcuma or diclofenac sodium; and not using anticoagulant or other anti-inflammatory drugs.

Course of Study

This study is a prospective randomized open end blinded evaluation (PROBE) done in the Rheumatology polyclinic, Department of Internal Medicine, Medical Faculty Universitas Gadjah Mada / Dr. Sardjito Hospital – Yogyakarta in September until October 2010.

Before the study began, subjects signed the informed consent. After that, randomization is done using a block 4 randomization so that the subjects are divided into 2 groups which are therapy group and control group.

Patients in treatment group found the combination of those natural drug in capsuls and taken 3 times daily while the control group is given diclofenac sodium 25 mg and taken 3 times daily.

Synovial fluid is obtained from patients before and after 2 weeks of treatment for the leukocyte count.

The visual analogue scale (VAS) was used for evaluated the drug effectiveness in reducing knee pain. Patients were asked to draw a perpendicular line towards the VAS line in which point the pain is felt according to the knee pain of the patient. Assessment is done before treatment and after 2 weeks of treatment.

Statistical Analysis

After data were obtained, it is analyzed using student's t-test and Mann-Whitney-Wilcoxon test. To analyze the difference in synovial fluid leukocyte count before and after treatment the Wilcoxon signed ranks is used. Analysis of the effectiveness in reducing synovial fluid leukocyte

count in both groups uses Mann-Whitney U test. To analyze the difference in VAS score (pain degree) before and after treatment, paired t-test is used. Analysis of the effectiveness in reducing joint pain in both groups uses unpaired student's t-test. Significance limit is acceptable if $p < 0.05$ with Confidence Interval 95%.

RESULTS AND DISCUSSION

The prospective randomized open end blinded evaluation design used in this study is to avoid biases in the assessment of the response towards the drugs given in this study. The subjects are randomized using the block 4 randomization so that each group represents the subjects. It is expected that results with high validity can be obtained using this method. Before treatment there were 2 patients in which adequate synovial fluid can not be obtained for leukocyte count from treatment group, and after treatment there were 3 patients in whom adequate synovial fluid can not be obtained for leukocyte count from control group, thus results from these patients can not be analyzed.

Table 1. Baseline Data of Subjects Before Treatment

Variable	Number (%)		Mean ± CI		CI 95% MD		P
	control (n=10)	treatment (n=11)	control (n=10)	treatment (n=11)	Lower	Upper	
Gender							0.361 #
- Male	4 (40%)	2 (18.2%)					
- Female	6 (60%)	9 (81.8%)					
Age (years old)			64.00 ± 9.02	62.09 ± 6.64	-5.28	9.09	0.585 *
Education							0.699 ♣
Elementary		2 (18.2%)					
Middle		1 (9.1%)					
High	6 (60%)	7 (63.6%)					
Tertiery	4 (40%)	1 (9.1%)					
Duration of OA (month)			12.75 ± 6.75	24.36 ± 22.90			0.289 ☼
Location of OA							1.000 ♣
- Right	2 (20%)	3 (27.3%)					
- Left	6 (60%)	5 (45.4%)					
- Bilateral	2 (20%)	3 (27.3%)					
BMI			28.85 ± 4.40	25.74 ± 3.19	-0.37	6.60	0.077 *
Leukocyte count			5386.36 ± 12149.49	1000 ± 638.36			0.594☼
VAS score (mm)			56.6 ± 23.23	50.86 ± 12.09	- 10.94	22.41	0.480 *
Comorbidities							
- Hypertension	2 (20%)	4 (36.4%)					0.367 #
- DM	2 (20%)	-					0.214 #
- Heart failure	-	1 (9.1%)					0.524 #
- Dyslipidemia	5 (50%)	6 (54.5%)					0.590 #

# Chi-square / Fisher's Exact test; * Independent t-test, ♣ Kolmogorov Smirnov test, ☼ Mann Whitney U test
N = sample number mm= milimeter
CI = confidence interval
MD CI 95% = Confidence Interval 95% Mean Difference

Baseline data of subjects can be seen in table 1. The baseline data between control and treatment group is not significantly different. Most of the subjects are women. This is consistent with epidemiologic data which shows that osteoarthritis is more prevalent in women compared to men[14]. The mean age of the subjects are 63.00 ± 3.51 years old. This data shows that osteoarthritis is more common among the elderly. This is consistent with epidemiologic data which shows that osteoarthritis is a degenerative disease accompanied by inflammation[15].

Reviewed from education level, the most frequent education level in subjects is high school, thus in the filling of questionnaire there is no large bias[16]. The mean duration of subjects suffering from osteoarthritis is 18.71 ± 3.90 months. This shows that osteoarthritis is a chronic disease. Based on the baseline data of the subjects, there is no significant difference between control group and teatment group.

Synovial fluid leukocyte count mean in the control group declines with p=0.017. This shows that the 25 mg diclofenac sodium capsule if given 3 times daily can reduce synovial fluid leukocyte count significantly in knee osteoarthritis patients.

Table 2. Results of VAS Score and Synovial Fluid Leukocyte Count Before and After Treatment

Group	control group (mean±CI)		P value	Treatment group (mean±CI)		P value	CI 95% MD	
	Before treatment	After treatment		Before treatment	After treatment		Lower	Upper
Leukocyte count /mm^3	7212.50±14027.26	3033.75±7952.11	0.017#	1000.00±638.35	52.22±131.41	0.008#	9.99	24.91
VAS score (mm)	56.60 ± 23.23	41.20 ± 25.62	0.012*	50.86 ± 12.09	33.41 ± 17.34	<0.001*	4.30	26.50

N= sample number mm= milimeter
CI = confidence interval
CI 95% MD= Confidence Interval 95% Mean Difference
* unpaired T-test
# Wilcoxon signed ranks

From VAS score assessment at the end of therapy in table 2, it can be known that the mean VAS score in control group declined (p=0.012). This shows that the 25mg diclofenac sodium capsule if given 3 times daily can reduce knee pain significantly in osteoarthritis patients.

Pain is the result of an interaction between inflammation and other factors such as radiological disease severity, articular innervations, central and peripheral sensitization, and psychological factors[17].

The decline in synovial fluid leukocyte count and knee pain can be associated with the anti-inflammatory activity of diclofenac sodium. Diclofenac sodium is a preferentially selective COX inhibitor which is a NSAID that suppresses COX-2 activities equal to suppressing COX-1 activities although in reality tends to suppress COX-2 a little stronger. Suppression of COX-2 enzyme activity inhibits prostaglandin E_2 formation thus hampering joint inflammatory process. Diclofenac can also suppress pain stimulated by bradykinin[18,19].

Synovial fluid leukocyte count mean in the treatment group declines with p=0.008. This shows that the therapy capsule can lower synovial fluid leukocyte count significantly in osteoarthritis patients. From VAS score assessment at the end of therapy it can be known that the mean VAS score in therapy group declined (p<0.001). This shows that the therapy capsule which contains curcuma extract 50 mg, ginger extract 100 mg, shrimp shell 100 mg, and soy bean flour 50 mg if given 3 times daily can reduce knee pain significantly in osteoarthritis patients. Lowering of synovial fluid leukocyte count and pain level can occur because each component in the combination has anti-inflammatory effects which lead to the lowering of synovial fluid leukocyte count.

Contained in the rhizome of temulawak are curcuminoids, essential oils, arabinose, fructose, glucose, starch, tannin and minerals such as magnesium, manganese, iron, copper, calcium, sodium, potassium, lead, zinc, cobalt, aluminum and bismuth[7]. The composition of temulawak curcuminoid contains curcumin and curcuminoids demetoxy[20]. Curcumin could

inhibit cyclooxygenase and lipooxygenase enzyme activity and act as antioxidants[9]. Curcumin is proven to be able to inhibit cyclooxygenase and lipooxygenase enzyme activity so that the production of prostaglandin E2 and leukotriene B4 and C4 are inhibited[21].

Ginger contains essential oil which consists of α-pinen, β-felandren, borneol, camphene, limonene, linalool, citral, nolyaldehide, decyladehide, metilheptenon, cineol, bisabolen, 1-α-curcumen, farnesen, humulen, 60% zingiberene and evaporated Zingiber (gingerol pungent substance), namely 60-85% (6)-gingerol, (4)-gingerol, 5-15% (8)-gingerol, 6-21% (10)-gingerol, (12)-gingerol, (6)-methylgingerdiol, shogaol, zingeren, (6)-gingerdiol, diarylheptanoide, β-bisabolene, (E)-α-farnesene. Ginger has anti-inflammatory potential obtained through the action of gingerol, shogaol, diarylheptanoids, and dialdehyd diterpens capable of inhibiting prostaglandin. It is proven *in vitro* that ginger extract has a strong potential for inhibiting production of PGE2, TNF □ and production of COX2 in human synovial by regulating NF - □B activity and degrading from its inhibitor I□B□. In studies conducted in humans, ginger can relieve pain and other symptoms suffered by patients with OA[11, 22, 23].

Shrimp shell contains chondroitin and glucosamine which are formation materials of cartilage. The mechanism of action in the treatment of OA is not fully known. Both seem to have anti-inflammatory effect and affect the metabolism of cartilage proteoglycan by stimulating the synthesis of joint cartilage chondrocytes[13]. Some in vitro experiments showed synthesis stimulation of glycosaminoglycan and proteoglycans stimulate synovial production of hyaluronic acid is estimated as a mechanism in a study. Giving the combination of glucosamine and chondroitin sulfate reduces the intensity of moderate osteoarthritis knee pain compared to placebo[24].

Soybean contains isoflavones which are natural selective estrogen receptor modulators (SERMs), which may indicate partial estrogen agonist or antagonist action in tissue depending on some factors including estrogen receptor prevalence and intrinsic estrogen concentration[25].

Positive effects of isoflavone can be obtained from its direct effect towards cartilage. Articular cartilage is a tissue target of estrogen and for SERM such as isoflavone. Study conducted on animals suggests that intra-articular estrogen injection can increase the frequency and severity of osteoarthritis. Estrogen can also suppress proteoglycan synthesis and cause cartilage degeneration in osteoarthritis. Intra-articular estrogen injection can also disturb lactate dehydrogenase in chondrocytes, which continues with the disruption of collagen matrix. Soybean isoflavone can bind with estrogen receptors and give antagonistic effects towards local estrogen[25].

Soybean isoflavone is proven capable of suppressing pro-inflammation molecules such as COX-2 and NO in LPS-induced chondrocytes, but has no effects towards COX-1[12]. Other than isoflavone, another component of soybean (such as, soybean unsaponifiables) is also proven to hamper pro-inflammation cytokines in chondrocyte in vitro[25].

Table 3. VAS Score and Synovial Fluid Leukocyte Count Difference Before and After Treatment

Variable	Mean±CI		P value	CI 95% MD	
	Control group	Treatment group		Lower	Upper
Δ leukocyte count /mm^3	4178.75 ± 7088.14	853.00 ± 696.22	0.929#		
ΔVAS score (mm)	15.40 ± 15.51	18.45 ± 11.05	0.607*	-15.26	9.16

N= sample number mm= milimeter

CI = confidence interval

CI 95% MD= Confidence Interval 95% Mean Difference

* paired T-test # Mann-Whitney Test

In table 3, it can be seen that both control group and therapy group can reduce synovial fluid leukocyte count significantly. The synovial fluid leukocyte count decline in the therapy group (mean 4178.75) compared to control group (mean 853.00) is not statistically significant (p=0.929). This shows that the effectiveness of the therapy group in reducing synovial fluid leukocyte count in knee osteoarthritis is not significantly different compared to the control group.

In table 3, it can be seen that both control group and therapy group can reduce joint pain symptoms significantly. The decline in joint pain degree in the therapy group (mean 18.45 mm) is more prominent compared to control group (mean 15.4 mm), but statistically the difference is not significant (p=0.607). This shows that the effectiveness of the therapy group in reducing joint pain in knee osteoarthritis is not significantly different compared to the control group.

In this study, therapy is given in 2 weeks time. Addition of the therapy period is expected to increase the activity of the therapy drug in reducing synovial fluid leukocyte count and joint pain in osteoarthritis patients. The addition of sample size is also expected to increase the difference in the reduction of synovial fluid leukocyte count and joint pain in osteoarthritis patients.

An advantage of this study is that the results of this study can be used as a consideration material by health workers to choose which therapy to use in reducing synovial fluid leukocyte count and joint pain in patients with osteoarthritis. Combination of temulawak extract, ginger extract, soybean, and shrimp shell is can be an alternative therapy for osteoarthritis to avoid the adverse effects caused by NSAIDs. Results of this study can also be used as an initial reference to conduct further clinical studies about that combination using a larger sample and a longer time period. This study is also useful in the development of herbal medicine science in Indonesia.

CONCLUSION

1. The combination of temulawak extract, ginger extract, soybean, and shrimp shell given 3 times daily can reduce synovial fluid leukocyte count and joint pain in osteoarthritis patients significantly in 2 weeks treatment.
2. Effectiveness of this combinaion in reducing synovial fluid leukocyte count and joint pain in osteoarthritis is not significantly different compared to diclofenac sodium in 2 weeks treatment.

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