

Comparison in Efficacy and Safety of Koklan Mixture Formula 3 and Diclofenac for Pain Relieving in Primary Knee Osteoarthritis

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ABSTRACT

Knee Osteoarthritis is a common disease in aging society. In general, Diclofenac which belongs to Nonsteroidal Anti-inflammatory drugs, has analgesic and Anti-inflammation effects. Although Diclofenac is often prescribed, it conveys high incidence of side effects especially in stomach ulcer. Therefore, the researcher realizes the importance of using the Koklan Mixture Formula 3 which is National Drug Information to replace the using of foreign medicine. The objective of this research was 1) to study comparison of efficacy of the Koklan Mixture Formula 3 and Diclofenac 2) to study safety of the Koklan Mixture Formula 3 and Diclofenac in reducing pain in primary osteoarthritis 3) to study the effectiveness of the Koklan Mixture Formula 3. Research methodology was a Randomize-double blinded, controlled trial at Suan Sunandha Medical Clinic, with criteria for selection of volunteers aged 50-70 years of knee pain no more than moderate using randomize-double blinded, controlled trial. According to the Oxford Knee Score evaluation, 68 subjects using random sampling method, blocked Randomization divided into 2 groups, consisting of 34 patients received the Koklan Mixture Formula 3 dose 30 cc before 3 meals and 34 received Diclofenac 25 mg after 3 meals for 14 days. In this study showed that the Koklan Mixture Formula 3 and Diclofenac were efficient and safe for the treatment of primary knee osteoarthritis and there were no differences statistically significant. Beside Koklan formular 3 also has effect on muscle relaxation, reduce edema and the blood nourishment.

Keywords: Koklan Mixture Formula 3, WOMAC, Primary Knee Osteoarthritis

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INTRODUCTION

Knee Osteoarthritis (OA) is a joint arthritis disease with damagingly changed to knee joint articular cartilage causing chronic pain, bone spurs growth, stiff joint with crackling noise in the knee. As the disease progresses, it produces more harmful metalloproteinase enzymes further deteriorates the cartilage and surrounding soft tissues, more bone spur growth and deformed causing misalignment and severe chronic pain. As such, the Knee OA joint disease is one major cause of disability to old people worldwide. Knee OA is one of major diseases that significantly impact health, quality of life, work productivity and economics costs the in Thailand [1]. The Knee OA chronic pain is typically treated by a prescription Diclofenac (Nonsteroidal anti-inflammatory drug) which has affected analgesic and inflammatory, but its side effects include internal bleeding, ulcer and heart related issue [2]. The Koklan Mixture Formula 3 was a National Drug Information from Thai traditional medicine and has sufficient scientific data of pharmacology properties supporting the pain relieving, anti-inflammatory results and clinical study also showed support for the safety of using this medicine instead of NSAIDs. Koklan (Scientific Name: *Mallotus Repandus* (Willd.) Mull.Arg. Family: Euphorbiaceae) has major substance of Bergenin and there was medical properties of anti-inflammatory and analgesic. It is 100 grams included 20 grams each of koklan Vine, En-onh Vine (*Cryptolepis Buchanani*), Khaen-phang (*Caesalpinia sappan* L.), Sakhan (*Piper interruptum* Opiz), and also 10 grams each of White crane flower (*Rhinacanthus nasutus* (L.) Kurz) and Prickly-leaved elephant's foot (*Elephantopus scaber* L.). The Koklan Mixture Formula 3 of National Drug Information for relieving pain and inflammatory muscle system in Traditional Thai Medicine and no side effects have been reported [3]. Therefore, the researchers realize the importance of Knee Osteoarthritis (OA) is a joint arthritis

disease with damagingly changed to knee joint articular cartilage causing chronic pain, disability and loss of function and The Koklan Mixture Formula 3 has effect on muscle relaxation, reduce edema and the blood nourishment. Beside Koklan formular 3 also properties supporting the pain relieving, anti-inflammatory muscle system in Thai Traditional Medicine and replace the using foreign NSAIDs drugs which will be benefit economic of the nation.

METHOD

This research was a clinical experimental controlled trial using comparative study of parallel design with a control group. The trial was the randomize-double-blinded study. By keeping doctor and patient without knowledge of who was receiving which treatment, the participant patients were randomly placed into two groups and agreement to participated in the research.

Population and Sampling

The volunteer patients were recruited and diagnosed for Primary knee osteoarthritis by modern medicine doctor and Thai Tradition doctor based on patient' history and examination at SuanSunandha Medical Clinic, Suan Sunandha Rajabhat University in Bangkok, Thailand from March 01st, 2020 to May 30th, 2020. The study protocol was approved by the Ethics Committee on Human Research of Suan Sunandha Rajabhat University Certificate Number: COA.1-055/2019 and completed a research article of Thai Clinical Trials Registry Number TCTR20201020010. Informed consent was obtained from each participating subject. The eligible subject was an adult with primary knee osteoarthritis by the American Rheumatism Association American College of Rheumatology [4], according to the criteria proposed by Oxford Knee Score with scores of pain level 1-2, and consent to participate in the determining research of the sample size was compared in two groups: the

experimental group were received Koklan Mixture Formula 3 and the control group received Diclofenac. Sample Size (n) determined to the sample size of each group A and group B which received the experiment pain treatments. Procedure formula was being used for calculation the sample size (n) refers to Cochran, [5-6] as following equation:

$$\text{Sample Size Formula (One Group): } n = \frac{2(Z_{\frac{\alpha}{2}} + Z\beta)^2 \sigma^2}{(\mu_1 - \mu_2)^2}$$

$Z_{\frac{\alpha}{2}}$ = value of distribution curve intersected at 1.960 for Confidence Interval of 95%

$Z\beta$ = power equal to 80%, the point of intersection under the normal curve or equal to 0.84

$\sigma = 1.2$ = Standard Deviation value from previous related research project

$\mu_1 - \mu_2 = 0.85$ (an average value before and after treatment from previous similar research refer to Vilai Kuptniratsaikul *et al.* [7]).

$$\text{Calculation: } n = \frac{2(1.96 + 0.84)^2 1.2^2}{(0.85)^2} = 31.2514$$

Independent by group sampling method, Block Randomization divided the subjects into two groups, adjust Sample Size, the sample size calculation had derived n=31 patients for each group. For a possibility of some future patient issue, a 10% (3) buffer was added and the final size for each group was 34. The two-group total population was therefore 68 patients.

Inclusion Criteria

1. Age between 50-70 years old, no sex limitation.
2. Has diagnosed with Primary knee OA by modern medicine doctor and Thai Tradition medicine doctor and Oxford Knee Score with pain level 1-2 (mild to moderate pain level).
3. No personal severe chronic disease: ulcer, heart, liver, lung, or kidney diseases.
4. Did not take NSAID pain reliever in the last 1 week before participates in the project.

Exclusion criteria:

1. History of medical allergy used during investigation.
2. Severe knee osteoarthritis and currently using NSAIDs.

Research Tool

1. Experimental Materials, 4 types as following:

- 1.1 Koklan Mixture Formula 3 was a medicine solution 30 ml 3 times a day for 14 days.
- 1.2 Diclofenac dose 25 mg 1 capsule 3 times a day for 14 days.
- 1.3 Placebo Solution made of tapioca flour resemble a solution similar to Koklan Mixture Recipe 3.
- 1.4 Placebo Capsule made of corn flour resemble the Diclofenac for 14 days.

2. Data Collection Materials

2.1 Medical History Form: Medical History Form for primary-Knee OA patient, information included sex, age, education, profession, body mass index, pain level, pain duration and any illness. There was a patient personal medical data and its confidential was protected by privacy law. Therefore, it will be kept separate from the research report.

2.2 Pain Evaluation Form: Knee OA Pain Level Evaluation Form using Oxford Knee Score as an evaluation criterion to

summarize and screen patient total score based on pain severity from primary-Knee OA at 3 levels: mild 30-39, moderate 20-29, severe 10-19 [4].

2.3 ROM (Range of Motion): Testing Goniometer measured the degree of knee movement and evaluated flexion and extension abilities [8].

2.4 Total Modified WOMAC Index Form: Evaluation Questionnaire Form using modified WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index.). The Questionnaire had 3 parts: Pain Level, Stiffness Level, and Physical Function level [9]. These data were an indication of efficacy of Koklan Mixture Formula 3 and Diclofenac.

2.5 Safety Form: Examination of blood test results from operation laboratory for CBC (complete blood count), ESR (Erythrocyte sediment rate), BUN (Blood Urea Nitrogen), Creatinin, SGOT (Serum Glutamic Oxalocetic Transaminase), SGPT (Serum Glutamate Pyruvate Transaminase) and Alkaline Phosphatase before and after taking the medicines. These data were an indication as safety of the clinical experiment trial reserch.

2.6 Safety Form: Side effects and adverse drugs reactions of Koklan Mixture Recipe 3 and Diclofenac

Preparation of Medication

1. Koklan Mixture Formula 3, 100-gram solution, the ingredients were 20 grams each of Koklan vine, En-onh Vine, Khaen-phang, Sakhan Vine, and also 10 grams each of White Crane Flower and Prickly-leaved elephant's foot. The mixture solution was manufactured at Applied Thai Tradition Medicine's Medical Factory, College of Allied Health Sciences, Suan Sunandha Rajabhat University follow GMP (Good Manufacturing Practice). The researcher had a conducted laboratory study to find the pharmacological activity of Koklan Mixture Formula 3 as follows:

1.1 Anti-inflammatory activity in macrophage cell cultures (RAW 264.7).

1.2 DPPH antioxidant activity of liquid formulation drugs, Cattle formulation 3, showed antioxidant activity.

1.3 Determination of Bergenin in Koklan Mixture Formula 3 by High Performance Liquid Chromatography (HPLC).

2. Placebo Solution, the solution was made to appear similar to the KMF 3 solution using natural brown color.

3. Diclofenac 25 mg ordered by New Life Pharma Co., Ltd, a pharmacy company which had passed the Department of Medical Sciences' quality inspection for safety and contamination criteria. It's a white opaque capsule.

4. Placebo Capsule, made from corn flour to likely the same as Diclofenac capsule.

Clinical trial procedure

Researcher and a modern medicine doctor had assessed the recruited volunteers to screen for the required criteria to participate in the project. Research data were collected in primary knee OA by the American Rheumatism Association for Knee OA pain level using Oxford Knee Score, Range of movement (ROM), Modified WOMAC Index, Blood test chemistry at Sunandha Medical Clinic, Suan Sunandha Rajabhat University. The efficacy of treatment was monitored at 1, 3, 7, 14 and 21 day. The clinical experiment research duration was 3 weeks. The volunteer patient received the drugs as follow:

1. For 14 days, each day volunteer patient Group A received KMF 3, solution 30 ml, before meal and then take 1 capsule of placebo, 25 mg that look like Diclofenac capsule after meals 3 time a day.

2. Similarly, for 14 days, each day volunteer patient Group B received Placebo solution that likely KMF 3 solution before meals 3 time a day. And then took a Diclofenac capsule after meals 3 time a day. Therefore, both patients and doctor would not know what medicine they took – it's

double-blinded. The experimental had method of Randomize-double blinded, controlled trial (RCT) as below (Figure 1).

Experiment trial flow chart

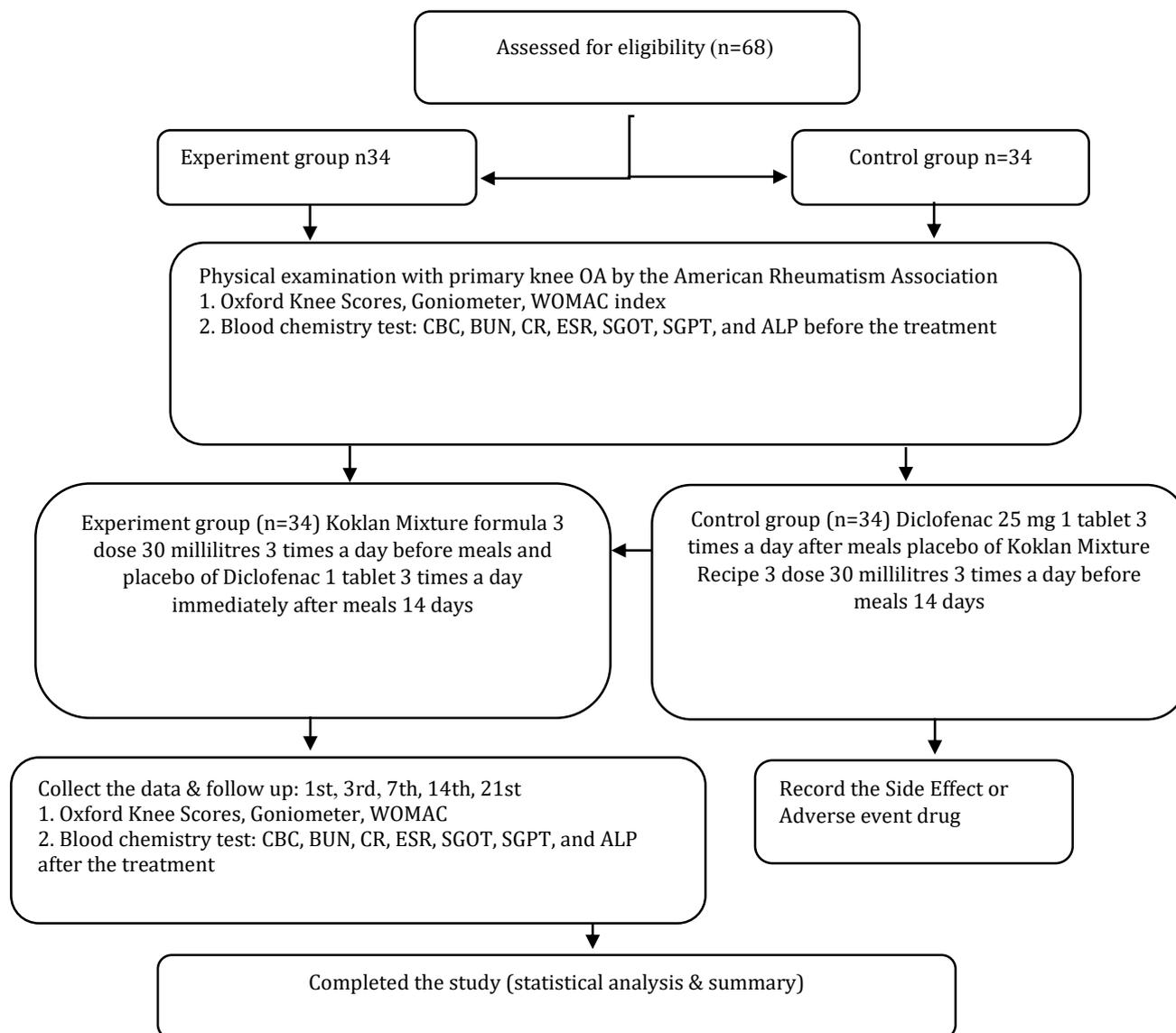


Figure 1. Experimental trial flow chart (Randomize-double blinded, controlled trial).

1. Data analysis of general information data such as sex, age, wellness, education, profession, pain duration, body mass index, use Descriptive Statistic to find Frequency, Percentage and Average. It was patient medical record, and its confidentiality was protected by privacy law. The record would be kept separate form public domain.
2. Data analysis of efficacy of treatment result data from pain severity level evaluation by Oxford Knee Score, ROM, WOMAC Index, use Means and Standard Deviation (SD), Unpaired t-test, Paired t-test. Raw data were collected from both groups (A, B) before and after the experimental treatments for 14 days. There was a final follow up data collected 7 days thereafter (day 21) to be used for further check the treatment effectiveness of both groups.
3. Data analysis of safety data from the experiment group and the control group from lab blood test at operation

room CBC, ESR, BUN, Creatinine, SGPT, SGOT, Alkaline Phosphatase used statistical Paired t-test compared before and after the trial.

4. Data analysis of the Effectiveness of Koklan Mixture Formula 3 from pain severity level evaluation by Oxford Knee Score, WOMAC Index and ROM used statistical Paired t-test compared before and after the trial.

RESULTS

1 Efficacy measurement comparing between Koklan Mixture Formula 3 and Diclofenac as follow:

1.1 Oxford Knee Score were evaluated pain severity level of primary Knee OA by using statistical Unpaired t-test to check the Means difference between Group A and B on Day 1, 3, 7, 14, and 21 found that there was only 1 difference. It was the day 21 data which shown statistically difference

$\alpha=0.05$. As for pain level in experiment group, checking Mean value using statistical Paired t-test to compare before and after administered medicines found that day 3, 7, 14 and 21 had shown improvements to be statistically differed $p < 0.05$. Also, control group Mean value had shown the similar improvement results. As for the pain level before taking medicines, all patients were at

moderate level, and the pain level had improved to mild level after taking the medicines. During day 1 to 14, both groups (A, B) took appropriate medicines and discontinued after day 14. For the day 21 evaluation, it was for a follow up purpose after no treatment for 7 days. All results showed in the Table 1 and Figure 2 below.

Table 1. Evaluation of Pain Level for Primary KOA using Oxford Knee Score.

| DAY | Koklan Mixture Formula 3 Group A (n=34) Pain Level | | | Diclofenac Group B (n=34) Pain Level | | | p-value ab |
|-----|---|------------------|-----------|---|------------------|-----------|------------|
| | before | after | p-value a | before | after | p-value b | |
| | Mean \pm SD | Mean \pm SD | | Mean \pm SD | Mean \pm SD | | |
| 1 | 26.65 \pm 3.04 | 26.65 \pm 3.04 | -- | 25.53 \pm 2.63 | 25.53 \pm 2.63 | -- | 0.110 |
| 3 | 26.65 \pm 3.04 | 29.47 \pm 2.84 | 0.0017* | 25.53 \pm 2.63 | 29.09 \pm 2.27 | 0.0073* | 0.542 |
| 7 | 26.65 \pm 3.04 | 33.24 \pm 1.99 | 0.0011* | 25.53 \pm 2.63 | 32.71 \pm 1.70 | 0.0091* | 0.242 |
| 14 | 26.65 \pm 3.04 | 36.76 \pm 1.69 | 0.0018* | 25.53 \pm 2.63 | 36.12 \pm 1.53 | 0.0019* | 0.103 |
| 21 | 26.65 \pm 3.04 | 36.21 \pm 2.25 | 0.0016* | 25.53 \pm 2.63 | 34.94 \pm 1.94 | 0.0089* | 0.016* |

Statistical Paired t-test a, b, Unpaired t-test ab * Statistical significance $p < 0.05$.*

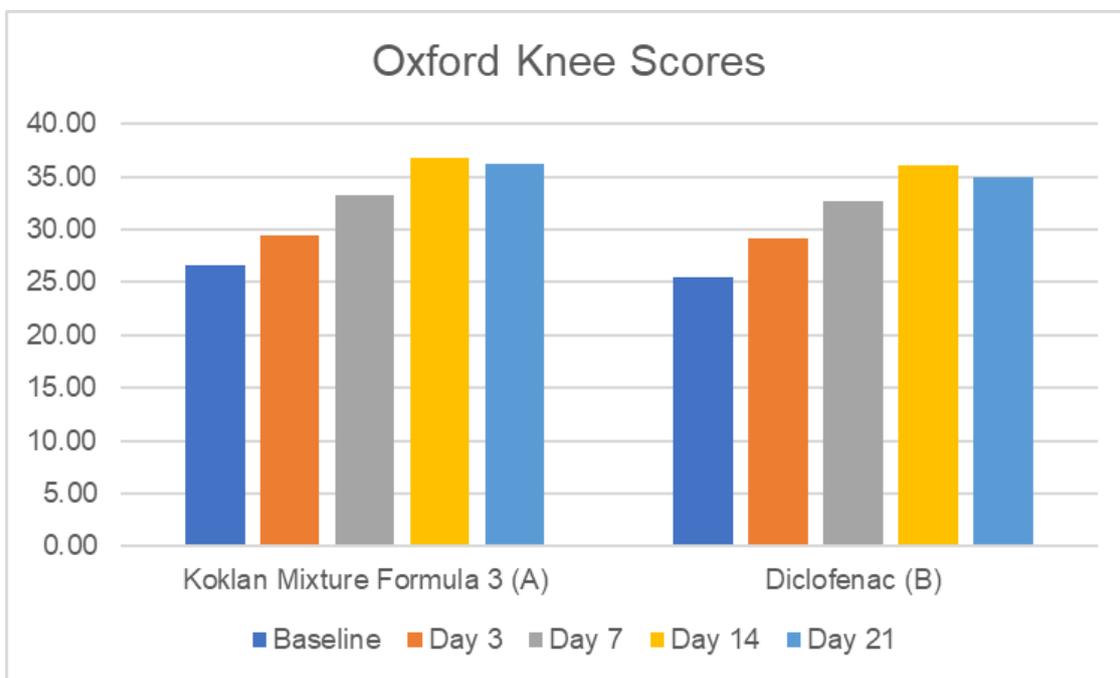


Figure 2. Pain Level Oxford Knee Score.

1.2 ROM (Range of Motion): Using Goniometer to evaluate the ability of flexion and extension for both right and left knees. The student t-test (A/B) of group A and B showed that were not statistically difference ($p > 0.05$) at $P=0.54, 0.55, 0.07, 0.07, 0.58$ and 0.64 accordingly. But the paired t-test of ROM movement in both groups showed significant

statistically differences ($p < 0.05$.) In summary, the knee ROM movement result for both groups is similar in the same respect which were the knee ROM before and after the experimental trial. The comparison showed statistically significant improvement ($p < 0.05$). The analysis results shown in Table 2.

Table 2. The comparison showed statistically significant improvement.

| Range Of Motion (ROM) | | Koklan Mixture Formula 3 Group A (n=34) | | | Diclofenac Group B (n=34) | | | p-value ab |
|-----------------------|-------|--|-----------------|-----------|------------------------------|-----------------|-----------|------------|
| | | before | after | p-value a | before | after | p-value b | |
| | | Mean \pm SD | Mean \pm SD | | Mean \pm SD | Mean \pm SD | | |
| Extension | right | 7.29 \pm 3.89 | 6.86 \pm 3.35 | 0.0010* | 8.79 \pm 2.75 | 8.23 \pm 2.36 | 0.0001* | 0.07 |

| | | | | | | | | |
|---------|-------|--------------|-------------|---------|-------------|-------------|---------|------|
| | left | 6.53±2.82 | 6.26±2.50 | 0.0360* | 7.79±2.82 | 7.05±2.33 | 0.0004* | 0.07 |
| Flexion | right | 123.50±11.11 | 132.78±7.03 | 0.0001* | 125.59±9.41 | 133.65±5.88 | 0.0008* | 0.58 |
| | left | 125.29±10.77 | 131.75±9.85 | 0.0002* | 125.32±6.99 | 132.63±4.96 | 0.0003* | 0.64 |

Range of Motion statistical Paired t-test a, b, Unpaired t-test ab * Statistical significance at $p < 0.05$.

1.3 WOMAC Index: Comparison between KMF 3 and Diclofenac using Modified WOMAC score assessed pain. Validation of a modified Thai version of WOMAC consisted of 22 questions, analyzed in 3 subscales as the average score for 5 questions on pain, 2 questions on stiffness and 15 questions on physical function. The total score was calculated as the mean score for all 22 questions. Test data were analyzed using paired t-test for individual test and

student t-test for group comparison. Outcome data showed as follow:

1.3.1 Individual score of KMF 3 and Diclofenac: paired t-test for all tests before and after administered medication were statistically significant difference ($p < 0.05$). This signified the good efficacy after using either medicines (Table 3).

Table 3. Efficacy of comparison between Koklan mixture Formula 3 and Diclofenac using modified womac index.

| WOMAC Index | Koklan Mixture Formula 3 Group A (n=34) | | | Diclofenac Group B (n=34) | | | P-value ab |
|------------------------------------|--|------------------|---------------|------------------------------|------------------|---------------|---------------|
| | before | after | P-value a | before | after | P-value b | |
| | Mean ±SD | Mean ±SD | | Mean ±SD | Mean ±SD | | |
| Pain Dimension | | | | | | | |
| 1 Walking | 5.18±0.63 | 3.24±0.50 | 0.000* | 4.74±0.62 | 2.91±0.45 | 0.000* | 0.003* |
| 2 Stair Climbing | 5.35±0.73 | 3.12±0.54 | 0.000* | 5.41±0.56 | 3.21±0.41 | 0.000* | 0.808 |
| 3 Nocturnal | 4.29±0.80 | 2.35±0.60 | 0.000* | 3.65±0.69 | 2.21±0.59 | 0.000* | 0.026* |
| 4 Resting | 2.38±0.78 | 1.09±0.29 | 0.000* | 2.15±0.78 | 1.26±0.45 | 0.000* | 0.874 |
| 5 Weight Bearing | 5.24±0.74 | 3.47±0.56 | 0.000* | 5.24±0.43 | 3.62±0.49 | 0.000* | 0.430 |
| Total Pain Dimension | 4.49±0.45 | 2.65±0.27 | 0.000* | 4.24±0.34 | 2.64±0.24 | 0.000* | 0.154 |
| Stiffness Dimension | | | | | | | |
| 1 Morning Stiffness | 4.03±0.94 | 2.79±0.69 | 0.000* | 3.41±0.56 | 2.35±0.49 | 0.000* | 0.002* |
| 2 Daytime Stiffness | 3.56±0.79 | 2.38±0.60 | 0.000* | 3.15±0.36 | 2.15±0.36 | 0.000* | 0.020* |
| Total Stiffness Dimension | 3.79±0.81 | 2.59±0.58 | 0.000* | 3.28±0.41 | 2.25±0.37 | 0.000* | 0.003* |
| Physical Function Dimension | | | | | | | |
| 1 Going-down Stairs | 5.44±0.66 | 3.56±0.56 | 0.000* | 5.50±0.51 | 3.56±0.50 | 0.000* | 0.889 |
| 2 Going-up stairs | 5.41±0.96 | 3.59±0.56 | 0.000* | 5.56±0.56 | 3.65±0.54 | 0.000* | 0.413 |
| 3 Get Up | 4.50±0.75 | 2.97±0.52 | 0.000* | 4.59±0.56 | 3.21±0.48 | 0.000* | 0.127 |
| 4 Standing | 4.74±0.71 | 2.97±0.63 | 0.000* | 4.47±0.56 | 2.97±0.30 | 0.000* | 0.473 |
| 5 Bending Down | 4.26±0.67 | 2.71±0.58 | 0.000* | 3.79±0.59 | 2.68±0.53 | 0.000* | 0.081 |
| 6 Getting In/Out of Car | 4.44±0.61 | 2.88±0.41 | 0.000* | 4.47±0.51 | 3.09±0.29 | 0.000* | 0.112 |
| 7 Shopping Walking | 4.47±0.56 | 2.94±0.49 | 0.000* | 4.24±0.50 | 2.88±0.41 | 0.000* | 0.121 |
| 8 Putting On Socks | 3.15±0.61 | 2.09±0.51 | 0.000* | 3.47±0.56 | 2.35±0.49 | 0.000* | 0.017* |
| 9 Getting Up from Bed | 3.47±0.66 | 2.21±0.54 | 0.000* | 4.03±0.46 | 2.79±0.41 | 0.000* | 0.000* |
| 10 Remove Socks | 3.15±0.66 | 1.97±0.52 | 0.000* | 3.09±0.38 | 2.06±0.24 | 0.000* | 0.775 |
| 11 Shower/Bath | 3.09±0.51 | 1.88±0.54 | 0.000* | 3.15±0.36 | 2.03±0.39 | 0.000* | 0.189 |
| 12 Sitting Down | 4.38±0.65 | 2.97±0.46 | 0.000* | 4.41±0.50 | 2.97±0.30 | 0.000* | 0.951 |
| 13 Using Toilet | 3.68±0.64 | 2.50±0.56 | 0.000* | 4.09±0.38 | 2.94±0.24 | 0.000* | 0.000* |
| 14 Heavy Housework | 5.32±0.53 | 3.41±0.56 | 0.000* | 4.68±0.53 | 3.24±0.50 | 0.000* | 0.000* |

| WOMAC Index | Koklan Mixture Formula 3 Group A (n=34) | | | Diclofenac Group B (n=34) | | | P-value ab |
|--------------------------------|---|------------------|---------------|---------------------------|------------------|---------------|---------------|
| | before | after | P-value a | before | after | P-value b | |
| | Mean ±SD | Mean ±SD | | Mean ±SD | Mean ±SD | | |
| 15 Light Housework | 3.24±0.43 | 2.12±0.33 | 0.000* | 3.09±0.29 | 2.06±0.34 | 0.000* | 0.123 |
| Total Physical Function | 4.18±0.30 | 2.72±0.25 | 0.000* | 4.17±0.23 | 2.83±0.17 | 0.000* | 0.248 |
| Total Mod WOMAC Index | 4.15±0.40 | 2.65±0.25 | 0.000* | 3.90±0.24 | 2.57±0.16 | 0.000* | 0.020* |

Paired t-test for a, b: The Student’s t-test for ab *Statistical significance $p < 0.05$. All Mean values from Table 3 were plotted into line graph (Fig. 3) below to compare the quality-of-life trend line of both groups (A, B). In

summary, the pain level, stiffness level and physical function of knee joint after using either medicine have improved from moderate level to mild level as following in Figure 3.

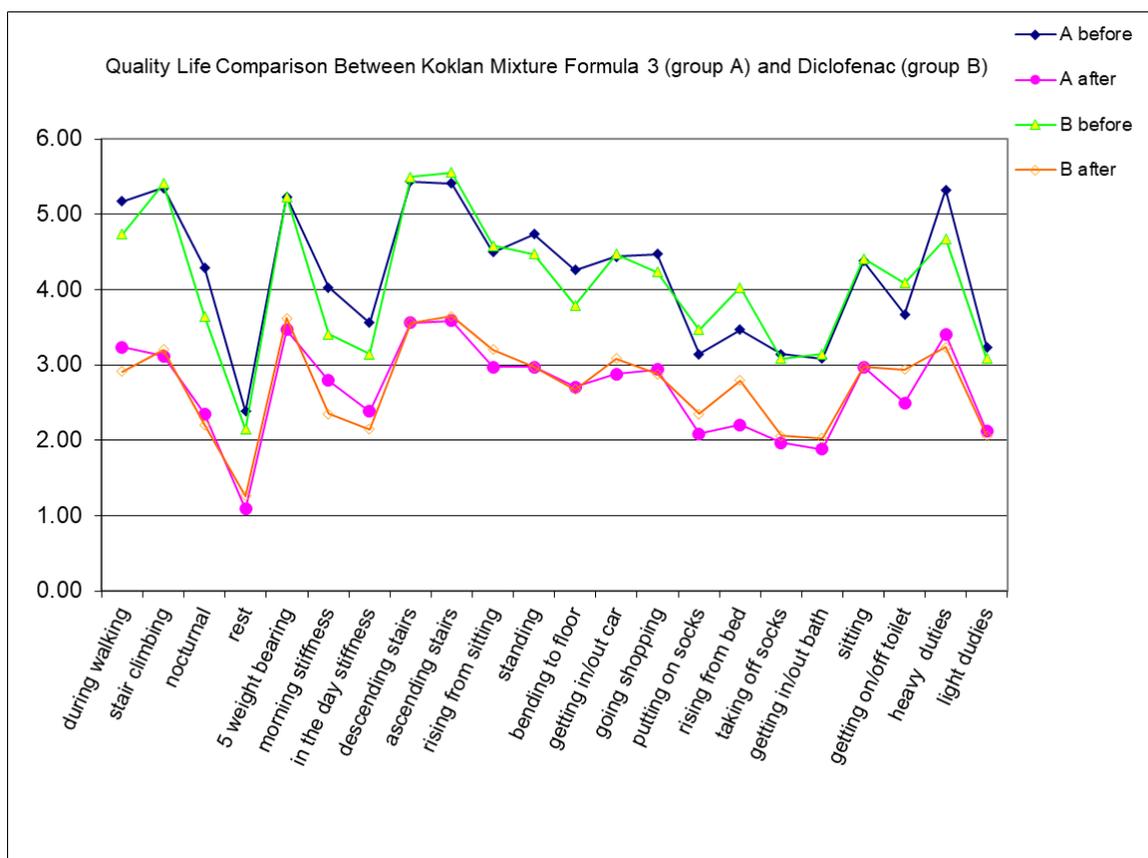


Figure 3. Note: score of WOMAC showed relative activity of compare between 2 groups.

1.3.2 Total Modified WOMAC Index showed that the experiment group (A) before administered pain medicine had Mean value of 4.15±0.40, and after medication Mean value declined to 2.65± 0.25 with p -value =0.000*. Similarly, the control group (B, Diclofenac) showed before and after medication mean values at 3.90±0.24 and 2.57±0.16 respectively, with p -value =0.000*and were statistically significant difference ($p < 0.05$ *). This confirmed the good efficacy of both medicines and comparing group by the student’s t-test (A/B) were 8 distinct items: pain during walking, pain. nocturnal, morning stiffness, in the day stiffness, putting on sock, rising on bed, getting on / out toilet and heavy household were significantly different at the level of $p < 0.05$ *. The remaining items show no differences but summary Total WOMAC were

statistically significant difference p -value ab = 0.02*($p < 0.05$ *) (Table 3).

2. Safety evaluation: Koklan Mixture Formula 3 and Diclofenac were evaluated via laboratory and adverse events drugs as follows:

2.1 Blood Test: Laboratory analysis results of CBC, ESR, BUN, Creatinine, SGOT, SOPT, and Alkaline Phosphate before and after using both medicines used to determine the safety of the medicine in research. The data analysis results in Table 4, showed paired t-test of the experiment group (KMF 3) before and after using the medicine had 4 parameters, ESR, Creatinine, SGOT and Alkaline phosphate, were significantly different $p < 0.05$ *. The Diclofenac group had ESR, Alkaline Phosphate were statistically significant difference $p < 0.05$ *. For group (A,

B) comparison, the student's t-test had 5 variables: ESR, Creatinine, SGOT, SGPT and Alkaline phosphate, were statistically significant difference ($p < 0.05^*$) after using

appropriated medicine for 14 days. But the level of those parameters was still within safety level and there was no effect for difference clinically, see analysis data in Table 4.

Table 4. Blood Laboratory: CBC, ESR, BUN, Creatinine, SGOT, SOPT, Alkaline Phosphatase.

| Blood Chemistry normal range | Koklan Mixture Formula 3 (A) | | | Diclofenac (B) | | | P-value a and b |
|--------------------------------------|------------------------------|-------------------|--------------|-------------------|-------------------|--------------|--------------------|
| | before | after | P value a | before | after | P value b | |
| | Mean \pm SD | Mean \pm SD | | Mean \pm SD | Mean \pm SD | | |
| WBC 4.0-10.0 cell/cu.mm ³ | 8.00 \pm 1.88 | 7.64 \pm 2.07 | 0.085 | 7.26 \pm 1.79 | 7.32 \pm 1.71 | 0.633 | 0.224 |
| RBC 3.5-5.5 M/ul | 4.89 \pm 0.62 | 4.89 \pm 0.62 | 0.948 | 4.63 \pm 0.75 | 4.60 \pm 0.79 | 0.507 | 0.643 |
| Hemoglobin 11.0-15.0 g/dl | 12.49 \pm 1.78 | 12.40 \pm 1.76 | 0.424 | 12.86 \pm 1.79 | 12.71 \pm 1.64 | 0.130 | 0.106 |
| Hematocrit 37.0-48.0 % | 38.94 \pm 4.70 | 38.85 \pm 4.72 | 0.778 | 39.78 \pm 4.87 | 40.36 \pm 5.97 | 0.504 | 0.602 |
| ESR <20 mm/hr | 44.32 \pm 21.54 | 34.18 \pm 16.43 | 0.000* | 43.53 \pm 23.45 | 38.85 \pm 20.09 | 0.018 | 0.000* |
| BUN 6.0-20.0 mg/dl | 12.84 \pm 3.56 | 13.24 \pm 5.64 | 0.565 | 13.34 \pm 4.06 | 13.74 \pm 3.63 | 0.453 | 0.355 |
| Creatinine 0.57-1.11 mg/dl | 0.70 \pm 0.17 | 0.75 \pm 0.24 | 0.018* | 0.74 \pm 0.14 | 0.75 \pm 0.14 | 0.293 | 0.011* |
| SGOT 0.0-32.0 U/L | 26.24 \pm 13.25 | 29.37 \pm 17.41 | 0.046* | 29.33 \pm 16.41 | 30.96 \pm 16.30 | 0.213 | 0.018* |
| SGPT 0.0-33.0 U/L | 28.86 \pm 22.99 | 31.82 \pm 26.21 | 0.068 | 28.16 \pm 14.91 | 31.41 \pm 17.60 | 0.100 | 0.014* |
| Alkaline phosphatase 35.0-104.0 U/L | 64.94 \pm 22.99 | 75.09 \pm 29.35 | 0.000* | 65.97 \pm 22.79 | 74.49 \pm 26.82 | 0.000* | 0.000* |

Paired t-test for a, b: The Student's t-test for ab: Statistical significance $p < 0.05^*$.

2.2 Side Effect and Adverse event drugs

There were 6 patients (17.60%) showed side effects and undesirable symptom in the group using Koklan Mixture Formural 3. Two patients showed increased sweat and 1 of each showed mild diarrhea, drowsiness, and felt warm. The Diclofenac group had 10 patients (29.41%) showed side effect or undesirable symptom. Six of them showed dyspepsia, mild diarrhea and drowsiness. The remaining one of each showed itchy rash, warm stomach, urinate more frequent, and sweaty. In summary, a total of 16 patients, 46.98%, showed mild symptom of side effect. None was required any medical treatment or to stop taking the experiment medicines.

3. Effectiveness of Koklan Mixture Formula 3: the primary knee OA in Table 3 and Figure 3 Graph, the paired t-test of Pain, Stiffness, and Physical Function before and after administered KMF3 for 14 days had all tests showed statistically significant difference ($p < 0.05^*$). The detailed summary demonstrated as follow:

3.1 Pain Testing, the total Mean value before taking the KMF3 was 4.49 \pm 0.45, after taking KMR 3 for 14, the total Mean reduced to 2.67 \pm 0.27 with $p = 0.000$. This is a statistically significant difference ($p < 0.05$).

3.2 Stiffness Testing, the total Mean value before taking KMF3 was 3.79 \pm 0.81, after KMR3 for 14 days, the total Mean value had decreased to 2.59 \pm 0.58 with $p = 0.000$. This is also a statistically significant difference ($p < 0.05$).

3.3 Physical Function Testing, the total Mean value before taking KMF3 was 4.18 \pm 0.30, after KMR3 for 14 days, the total Mean value had decreased to 2.72 \pm 0.59 with $p = 0.000$. This is also a statistically significant difference ($p < 0.05$).

In summary, the Koklan Mixture Formula 3 showed the overall effectiveness with the Mean values difference of all tests before and after taking the medicine and resulted patients has reduced pain and suffering in Table 3 and Figure 3.

DISCUSSION

Since there had not been a clinical experiment study of Koklan Mixture Formula 3 previously, therefore the researcher had performed special operation-room studies to test efficacy of anti-inflammatory property of the medicine mixture. The studies' experiment step for this clinical research showed as following:

1. Testing anti-inflammatory in culture macrophage cell line RAW 264.7 from Manose Health and Beauty Research Center found the mixture showed an efficacy of anti-inflammatory inhibited the macrophage cell to produce Nitric Oxide induced by Lipopolysaccharide at the highest level of 33.03 \pm 2.35% at 10 mg/ml concentration. It was similar to Triamcinolone, a Corticosteriod, capable of stop producing Nitric Oxide at 33.65 \pm 3.22% at 1 mg/ml concentration.

2. Testing DHHP free radicals' activity in the Koklan Mixture Recipe 3 solution from Manose Health and Beauty Research Center had showed scavenging activity at SC 50 equal to 9.76 \pm 0.44 mg/ml, 0.0041 ratio of Vitamin C which SC 50 equal to 0.04 \pm 0.03 mg/ml.

3. Using content analysis HPLC (High-performance liquid chromatography) method from Nutroceutical Laboratory Reseach, Faculty of Medicine Thammasat University found Bergenin in the Koklan Mixture Formula 3 at an average 188.46 \pm 3.88 microgram/gm.

In addition, there was a study of Koklan Formula 1 which had the same 3 ingredients: Koklan Vine, Prickly-leaved elephant's foot and White Crane Flower. This study found that had anti-inflammatory effect in laboratory rat experiment [10]. In the Koklan Mixture Formula 3, there are 6 herbal ingredients, which overall showed anti-inflammatory and analgesic pharmacology properties and chemical constituents as detailed as following:

1. Koklan Vine (Scientific Name: *Mallotus Repandus* (Willd.) Mull. Arg. Family: Euphorbiaceae), previous

research studies found its major substance Bergenin and mallorepine had anti-inflammatory properties. The analgesic and anti-inflammatory activities of *Mallotus repandus* were studied using writhing and formalin tests [11].

2. En-onh Vine (*Cryptolepis Buchanani*) was found in several studies exhibited Chondroprotective and Analgesic activity, showed Anti-bacterial activity, and Anti-inflammatory. En-onh Vine is *Cryptolepis Buchanani* is two groups of chemical constituents: (1). Alkaloids b Buchananine and 1, 3, 6-O-trinicotinoyl- α -D-glucopyranose) (2). cardenolides was cryptosin [12] sarmentogenin [13] sarmentocymarin [14] and cardenolide glycosides 15-17.

3. Khaen-phang (*Caesalpinia sappan* L.) which is an extract of the dried heartwood had exhibited attenuation of collagen-induced arthritis in laboratory rat experiment, it was found Anti-inflammatory and Wound Healing Effects of *Caesalpinia sappan* L [10, 18] Chemical constituents were nine compounds which isolated from the heartwood of *Caesalpinia sappan* L. On the basis of spectral data, they were identified as brazilein, brazilin, (:-)-lyoniresinol (3), stearic acid, stigmaterol, (E)-3, 3'-dimethoxy-4,4'-dihydroxystilbene, (-)-syfingaresinol, protosappanin A, and brazilide [19].

4. Sakhan (*Piper interruptum* Opiz): this herbal vine had a property of muting pain in male ICR Mice experiment. Formalin Test method was able to stop pain induced anti-pyretic activity on yeast-induced hyperthermia in rats [20]. Chemical constituents is crotepoxide, eupomatene and pipercolosine [21].

5. White Crane Flower (*Rhinacanthus nasutus* (L.) Kurz): Effects of *Rhinacanthus nasutus* on nitric oxide, prostaglandin E2 and tumor necrosis factor- α releases using RAW264.7 macrophage cells [22] Chemical constituents is Rhinacanthin-c and oxymethylantraquinone. A new naphthoquinone racemate, rhinacastone together with seven known compounds, rhinacanthone, rhinacanthins C, D, N, Q, and E, and heliobupthalmin [23].

6. Prickly-leaved elephant's foot (*Elephantopus scaber* L.): A previous study showed the Sesquiterpene lactones from *Elephantopus mollis* had an effect of anti-inflammatory activities [24] : Chemical constituents is Crepside E, cynaropicrin deacyl; cyanaropicrin-3- β -D-glucopyranoside deacyl; dotriacontan-1-ol; elephantopin, 11-13-dihydro-deoxy; elephantopin, 11-13-dihydro; elephantopin deoxy; elephantopin, iso-deoxy; friedelanol, epi; friedelinol, epi; lupeol; stigmaterol; stigmaterol 3-O-beta-D-glucoside; triacontan-1-ol; zaluzanin C, gluco; scabertopin [25].

According to this research trail experiments and support from previous studies of compounds ingredients and its pharmacology properties, the Koklan Mixture Formural 3 was effective and safety for reduce pain and anti-inflammatory of primary knee osteoarthritis the same as a prescription Diclofenac. In the safety issue, The Koklan Mixture Formula 3 is the National Drug Information for relieving pain and inflammatory muscle system in Thai Traditional Medicine and no side effects have been reported [3]. Thai Traditional Medicine had used for the patient more ten year ago in general hospital and no complication for the treatment. In this paper safety test, blood chemistry of liver and kidney function tests of both groups were statistically significant different, and no severe events were found, but mild adverse events were reported, and the number of volunteers was the sampling

which had appropriate for clinical experimental controlled trial.

CONCLUSION

The researcher summarized the results according to objective as following:

1. Efficacy Comparison: There were no efficacy difference between Koklan Mixture Formula 3 and Diclofenac for relieving pain of primary Knee Osteoarthritis. Both of the medicines showed good efficacy. The medications experiment duration was 14 days used Oxford Knee Score, Range of Motion, and WOMAC Index showed the pain level had decreased statistically significant with $p < 0.05$. The details showed as follow:

1.1 Oxford Knee Score, before administered both medications, the pain level were at moderate level. After 14 days experiment, all patient pain level tests had reduced to mild level. The result was support by statistically data analysis paired t-test P value < 0.05 (Table 1 & Fig. 2).

1.2 Range of Motion (ROM), the range measurement for group A (experiment) and group B (control), before and after the experiment showed a statistically significant difference $p < 0.05^*$. It founded that the extension and flexion of the right knee was significantly difference p -value= 0.001 * and 0.008 *. The extension and flexion of the left knee was significantly different p -value = 0.000 * and 0.000 * (Table 2). This measurement showed that the quality of life improved as well.

1.3 Total Modified WOMAC Index, the Koklan Mixture Recipe 3 group showed Mean value before experiment at 4.15 ± 0.40 and reduced to 2.65 ± 0.25 after 14 days experiment. Diclofenac group showed 3.90 ± 0.24 before and 2.57 ± 0.16 after experiment accordingly. Both groups showed Paired t-test $p < 0.05$ which were the statistically significant difference. Moreover, the two-group using Student's t-test were statistically significant at P-value=0.02* as well. This total modified WOMAC Index, the results showed that the pain level had reduced from moderate to mild level, see support data in Table 3 & Fig. 3. According to this study had improved the patient quality of daily life as well.

2. Safety Comparison Result: There were 2 evaluation categories, blood test and side effect, to determine the safety of using either medications. Blood Test Result: Chemistry Lab analysis in Table 4 showed several organ functions parameters had slightly elevated numbers but those levels were still within safety level and there was no effect of the difference clinically (Table 4). Side Effect and Adverse Events Drug: A total of 16 patients from the 2 groups, 46.98%, showed mild symptom of side effect and some was just undesirable symptom, diarrhea, sweat and drowsiness. None was required any medical treatment or out.

3. Effectiveness of Koklan Mixture Formula 3 (KMF): The paired t-test of before and after using KMF 3 for 14 days had shown the statistically significant difference ($p < 0.05$) improvement for all tests (Table 3 and Fig. 3). It decreased in the mean level of Pain Dimension, stiffness Dimension, and Physical Function with statistically significant differences at the level of $p < 0.05$. The results had shown the effectiveness of Koklan Mixture Formula 3 to reduced pain in primary knee osteoarthritis from moderate to mild level. Finally, according to studies and also supporting information from laboratories of several research of compounding Koklan Mixture formula 3 had

been analgesic & anti-inflammatory effective and safety for pain relieving in primary knee osteoarthritis.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Suan Sunandha Rajabhat University, Thailand, for supporting research activities.

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

FUNDING

No competing financial interests exist.

ETHICAL APPROVAL

The study protocol was approved by the Ethics Committee on Human Research of Suan Sunandha Rajabhat University Certificate Number: COA.1-055/2019 and submitted a research article of Thai Clinical Trials Registry.

AUTHORS CONTRIBUTIONS

All the author has contributed equally.

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