RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2024.042

ScienceAsia 50 (2): 2024: ID 2024042: **1–8**

Anti-inflammatory activity of a Thai traditional remedy Lom-Pa-Kang and its potential use for knee osteoarthritis therapy

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Received 21 Sep 2023, Accepted 4 Mar 2024
Available online 3 May 2024

ABSTRACT: Lom-Pa-Kang (LPK) is a Thai traditional remedy consisting of four plants: *Piper nigrum* L., *Zingiber officinale* Roscoe, *Citrus hystrix* DC., and *Cynodon aethiopicus*, used to treat headaches from migraines and joint pain. The objectives of this study were to investigate anti-inflammatory activity via nitric oxide (NO) production using Griess reagent assay and MTT assay. In addition, the preliminary clinical trial was investigated in osteoarthritis (OA) of knee patients using the Western Ontario and MacMaster University Scales (WOMAC) and Numeric Rating Scales (NRS). The result showed that the ethanolic extract of the dried LPK remedy exhibited higher anti-inflammatory effect on NO production in the murine macrophage cell lines than that of a standard drug (Ibuprofen) with IC_{50} values of 13.817 ± 0.999 and 56.595 ± 0.116 µg/ml, respectively. Interestingly, all extracts of LPK exhibited non-cytotoxicity upon assessment of cell metabolic activity. The bioactive compounds with anti-inflammatory effect were analyzed using Gas Chromatography Mass-Spectroscopy (GC-MS). Moreover, a four-week preliminary clinical trial in 30 patients of knee OA, aged between 40 and 55 years, had found a statistically significant decrease in NRS pain scores and improvements in WOMAC index scores including pain, stiffness, and physical function, since day seven when compared with baseline ($^{**}p < 0.001$). No adverse effects were identified in any patients. In conclusion, LPK remedy has significant anti-inflammatory activity and offers a potential anti-inflammatory therapeutic approach via localized drug delivery in knee OA patients.

KEYWORDS: Lom-Pa-Kang, Thai traditional medicine, anti-inflammatory, clinical trial, knee osteoarthritis

INTRODUCTION

Osteoarthritis (OA) is the most common chronic articular disease and health problem in elderly people [1]. This disease can affect all joints, especially the knee joint. This painful disease is most frequently involved in up to 10% of men and 13% of women aged over 60 [2]. One of the signaling molecules that plays a major role in the pathogenesis of inflammatory conditions of the joint is NO. It is considered a pro-inflammatory mediator that induces inflammation while being generated in excess according to abnormal conditions [3, 4]. The treatment of OA should be offered with a core set of non-steroidal anti-inflammatory drugs (NSAIDs), which are first-line pharmacologic treatments and have important toxicities such as gastrointestinal irritation, ulceration, bleeding, and decreased renal blood flow with azotemia. Therefore, the procedure of treatment with non-pharmacological interventions, especially biomedicine or herbs, has become popular [5]. Nowadays, traditional medicine and folk medicine are used worldwide for health promotion and processing to relieve several diseases. However, the actual efficacy and possible toxicity of some traditional medicines lack strong evidence-based scientific reports in clinical trials [6, 7]. A review of biomedicine or traditional remedies found that various herbs can be used to treat several symptoms such as headaches, migraines, and joint pain [7]. In this study, we selected a Thai traditional remedy called "Lom-Pa-Kang" from Chawa Dan scripture in the Phaet Thai Doem textbook, which included *P. nigrum*, *Z. officinale*, *C. hystrix*, and *C. aethiopicus* [8]. The dosage form of LPK remedy is used as a poultice to relieve pain. Some previous reports found that piperine, the main active compound in black pepper, exhibited potent inhibition of NO, tumor necrosis factor-alpha (TNF-α), and prostaglandin E_2 (PGE_2) production [9]. *Z. officinale* [Ginger] has been used for reducing pain and treating inflammatory disorders and cancer [10]. Additionally, the ethanolic extract of the leaves and lupeol from *C. hystrix* [Kaffir lime] exhibited a reduction of the pro-inflammatory cytokines and suppressed the expression of both inflammatory genes and NF-κB proteins [11]. Moreover, *C. aethiopicus* [Cynodon] showed anti-inflammatory activities via TNF-α, IL-1β, and IL-6 pathways [12]. Therefore, the objectives of this study were to investigate the anti-inflammatory via NO production using Griess reagent, cytotoxicity using MTT assay, and the chemical constituents using GC-MS of both extracts (fresh and dried) of LPK remedy. In addition, preliminary clinical investigations of the effectiveness of LPK

remedy in patients with knee OA have been conducted.

MATERIALS AND METHODS

Chemicals and reagents

Commercial grade 95% ethanol was purchased from C.M.J. Anchor Co., Thailand. Analytical grade reagents, e.g. methanol, were purchased from Labscan® Ltd., Bangkok, Thailand. The standard drugs (Prednisolone and Ibuprofen) were purchased from the Department of Medical Sciences, Thailand. Fetal bovine serum (FBS), penicillin-streptomycin (P/S), trypan blue, trypsin-EDTA, and Dulbecco's modified eagle medium (DMEM) were purchased from Gibco® BRL Life Technologies (NY, USA). Phosphate buffer saline (PBS) was purchased from Amreso® (OH, USA). Murine macrophage cell line (RAW 264.7) was purchased from American Type Culture Collection (ATCC® TIB-71™, VA, USA). Lipopolysaccharide (LPS, Serotype: *Escherichia coli* O55:B5), sulfanilamide, *N*-(1-naphthly) ethylenediamine dihydrochloride, phosphoric acid, and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma-Aldrich® Inc., (MO, USA).

Sample collection

The sources and parts of plant ingredients used in LPK remedy are shown in Table 1. Plant materials were cleaned, cut into small pieces, dried at 45 °C in an oven for 48 h, and coarsely ground. The ground plant samples were weighed and mixed according to the LPK remedy proportion shown in Table 1.

Sample extraction and preparation

Ethanolic extraction

The polyherbal LPK remedy displays general information in Table 1. The 500 g of fresh LPK remedy and dried LPK remedy were then macerated with 95% ethanol (1,000 ml, ratio 1:2 polyherbal:95% EtOH) for 7 days. Additionally, all extracts were filtered through Whatman filter paper No. 1 and evaporated using a rotary evaporator (Buchi®, Switzerland). Finally, the fresh and dried extracts of the LPK remedy were stored at −20 °C before testing the bioactivities.

Preparation of Thai traditional dosage form: Poultice of LPK remedy

A hundred grams of a fresh LPK remedy consisting of 7.5 grams of *P. nigrum* fruit, 15 g of *Z. officinale* rhizome, 22 g of *C. hystrix* peel, and 55 g of *C. aethiopicus* stem were prepared. All herbs were crushed, and 5 ml of DI water were added and mixed until homogeneous before use. Our research was conducted according to Chawa Dan scripture from the Phaet Thai Doem textbook in Thai Traditional Knowledge [8].

In vitro bioactivities on NO production and cytotoxicity

Anti-inflammatory activity by Griess reagent assay and MTT assay [13]

The murine macrophage cell lines (RAW 264.7) were prepared for Griess reagent assay [14, 15], seeded into 96-well microplates with a density of 1×10^5 cells/well, and incubated at 37 °C in a 5% CO_2 atmosphere at 95% humidity for 24 h. Next, the fresh medium containing 10 ng/ml of lipopolysaccharide (LPS) and various concentrations of test samples (10, 30, 50, and 100 µg/ml) in DMSO (100 µl/well) were added and incubated for 24 h.

After 24-h incubation, NO production was determined with Griess reagent. A draw-up of 100 µl of the supernatant was transferred into a new 96-well microplate and mixed with 100 µl of Griess reagent containing 1% sulfanilamide, 0.1 N-(1-naphthly) ethylenediamine dihydrochloride, and 2.5% phosphoric acid. The absorbance of the supernatants was measured with the microplate reader at 570 nm.

RAW 264.7 cells viability was determined by the MTT assay [15–17]. The separated supernatant from the incubated plate and 5 mg/ml MTT solution (the thiazolyl blue tetrazolium bromide solution) were added to each well, and the microplate was incubated at 37 °C in a 5% CO_2 atmosphere at 95% humidity for 2 h. Next, the old medium was removed from the wells, and 100 µl of isopropanol buffer including 0.04 M HCl was added to each well to dissolve the formazan produced in the incubated cells, which was measured with a microplate reader at 570 nm. The test sample was considered cytotoxic when the optical density of the sample was less than 30% of that of the control. The control was 2% DMSO (the final concentration of DMSO was 1%).

Calculation of NO assay

$$\%\ \text{Inhibition} = \frac{\text{Mean of OD}_{\text{control}} - \text{Mean of OD}_{\text{sample}}}{\text{Mean of OD}_{\text{control}}} \times 100$$

Calculation of MTT assay

$$\%\ \text{Toxicity} = \frac{\text{Mean of OD}_{\text{control}} - \text{Mean of OD}_{\text{sample}}}{\text{Mean of OD}_{\text{control}}} \times 100$$

Chemical constituents of LPK remedy

Gas chromatography-mass spectrometry (GC-MS)

The chemical composition of both extracts of LPK remedy was investigated by GC-MS technique. Scion 436-GC model (Scion instruments, Goes, The Netherlands) coupled with a single quadruple mass spectrophotometer comprising a CP-8410 autosampler with a fused silica capillary column SCION-5MS (5% phenyl/95% dimethyl polysiloxane) 30 m × 0.25 mm, 0.25 µM run on helium gas with a flow rate of 1 ml/min [18].

Table 1 The ingredients of LPK remedy.

Taxonomic name	Thai name	Part used	Place for collection	Proportion (% w/w)
Piper nigrum	Phrik Thai	Fruit	Phitsanulok, Thailand	7.50
Zingiber officinale	Khing	Rhizome	Phitsanulok, Thailand	15.00
Citrus hystrix	Ma krut	Peel	Phitsanulok, Thailand	22.50
Cynodon aethiopicus	Ya Phraek	Stem	Phitsanulok, Thailand	55.00

All the raw materials passed quality control according to Thai Herbal Pharmacopeia, 2019.

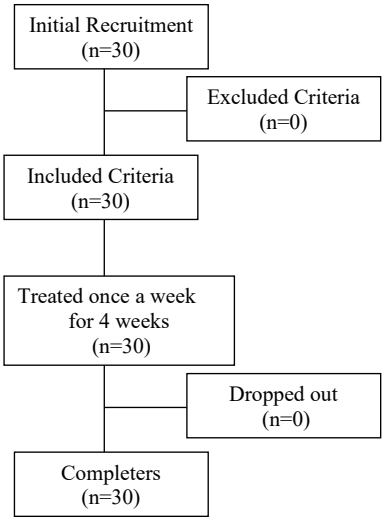

Fig. 1 Flow chart of the study design.

The sample (10 µl) was injected into the capillary column and ran for 60 min throughout the experiment. The initial temperature was maintained at 80 °C, gradually increased up to 250 °C at a rate of 5 °C/min (until 38 min), and finally raised to 280 °C at a rate of 20 °C/min. The component identification was confirmed by comparing the mass spectra with reference data from the National Institute of Standards and Technology (NIST) library.

The preliminary clinical study in OA of knee patients of LPK remedy

Ethical approvals and research design

This four-week trial was aimed at assessing the efficacy of LPK remedy in treating patients with primary knee OA. Patients aged between 40 and 55 years old from the Applied Thai Traditional Medicine Clinic in the Faculty of Public Health, Naresuan University, Thailand, were screened between October 2022 and February 2023. The protocol complied with the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of Naresuan University under the number P3-0002/2565. The inclusion criteria were those who had a knee pain score of $\leqslant 5$ and were diagnosed with primary knee OA based on clinical findings and as defined by the Western Ontario and McMaster University Osteoarthritis Index (WOMAC). Moreover, the participants had to be able to walk and give both verbal and written information regarding the study. The exclusion criteria were patients who had a body mass index of $\geqslant 30$ kg/m^2, injuries in the area affected by OA, pregnancy, lactation, malignant tumors, or severe comorbidities obstructing study participation. The study design of the preliminary clinical study in OA of knee patients is shown in Fig. 1.

Sample size

The size of the sample was established based on the outcomes of an investigation by Chantra et al [19]. The lower level of average NRS pain scores from the previous clinical trial was 6.40 (SD 1.85) for Thai herbal paste and 4.93 (SD 1.76). A sample size of 24 participants per group was estimated for an overall type I error (2-sided test) with $^*p < 0.05$ and a power of 84. Finally, a 20% dropout rate of the recruited patients was considered, resulting in the requirement of 30 patients for this study.

Preparation of LPK remedy and treatment

A total of 30 patients with primary knee OA were selected and recruited into the study by Applied Thai Traditional Medicine practitioners. After completing the screening, all patients were directed to apply LPK remedy topically to their affected knee and the surrounding tissues for 30 min, once a week. This dose of LPK remedy was applied from Chawa Dan scripture in the Phaet Thai Doem textbook [8]. The treatment period lasted 4 weeks with assessments for the clinical investigation every week. The primary efficacy outcome measure was the WOMAC index, which includes pain, physical function, and stiffness [20]. Moreover, NRS scores were executed by the patients themselves at every follow-up [21].

Data and statistical analysis

The biological activities were compared using a one-way ANOVA (Dunnett's comparison) to a standard drug with a statistical significance level of $^*p < 0.05$, and a paired-samples t-test and repeat measure ANOVA were applied to compare the weekly outcomes of the pre- and post-treatment groups at a statistical significance level of $^{**}p < 0.001$.

Table 2 The results of inhibitory activity on NO production and cytotoxic activity in murine macrophage cell lines (RAW264.7) ($n = 3$).

Sample	Anti-inflammatory (IC_{50}, µg/ml)	MTT assay (% Survival)
Dried ethanolic extract	$13.81 \pm 0.99^{a,b}$	72.42 ± 1.66
Fresh ethanolic extract	$95.93 \pm 0.54^{a,b}$	83.58 ± 2.52
Prednisolone[a]	56.59 ± 0.11	79.60 ± 5.93
Ibuprofen[b]	26.89 ± 0.25	40.12 ± 4.65

The extract was considered non-toxic when cell survival was > 70%. Data were analyzed by one-way ANOVA and Dunnett's multiple comparison tests. (a) Significant difference is when $^*p < 0.05$ compared with the prednisolone ($n = 3$). (b) Significant difference is when $^*p < 0.05$ compared with the ibuprofen ($n = 3$).

RESULTS

The quality control of plant materials, which are ingredients of LPK remedy, was performed according to Thai Herbal Pharmacopoeia (2019). The indication of LPK remedy (cold taste) in Thai traditional medicine uses this remedy for treating headaches from migraines and joint pain from OA in the form of a topical poultice. Thai folk medicine wisdom described this cold taste and the properties of taste related to pharmacological, i.e. anti-inflammatory and reducing pain [7, 8].

The results showed that the LPK remedy ingredients were tested for inhibitory activity against LPS-induced NO production in the murine macrophage cell lines (RAW 264.7) in comparison with the standard drugs (prednisolone and ibuprofen). The dried and fresh extracts exhibited anti-inflammatory activity on NO production with IC_{50} values of 13.81 ± 0.99 and 95.93 ± 0.54 µg/ml, respectively. In addition, the standard drugs (prednisolone and ibuprofen) exhibited moderate anti-inflammatory activity on NO production with IC_{50} values of 56.59 ± 0.11 and 26.89 ± 0.25 µg/ml, respectively. In the cytotoxic assay, all extracts were tested for cytotoxicity with the MTT assay to substantiate that the inhibitory effect was not due to cell death. The results indicated that none of the extracts were cytotoxic (Table 2).

The GC-MS chromatogram of the dried and fresh extracts of the LPK remedy demonstrated a total of 13 and 15 peaks, respectively. The phytochemical constituents were recognized by relating the name of the compound to those of the known compounds described by the National Institute of Standards and Technology (NIST) library. The phytoconstitutes in dried extract included 2-methoxy-4-vinylphenol; 1,2,3,4-cyclopentanetetrol; 3,4-altrosan; neophytadiene; lidocaine; 14-methylpentadecanoic acid; 9,12-octadecadienoic acid; 9,12,15-octadecatrienoic acid; phytol; isooxypeucedanin; pabulenol; oleamide; and piperine. The phytoconstitutes in fresh extract included 2,3-dihydropyran-4-one; 2-methyl-6-methyleneoct-7-en-2ylpropionate; dodecamethylcyclohexasiloxane; menthoglycol; 2,6-dimethoxyphenol;3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane; 7,9-di-tert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione; pentadecanoic acid; 9,12-octadecadienoic acid; 9,12,15-octadecatrienoic acid, methyl ester; phytol; 2-methyl-z, z-3,13-octadecadienol; pabulenol; oleamide; and piperine. The bioactivities of chemical constituents from dried and fresh extract LPK remedies are exhibited in Table 3 and Table 4, respectively.

A total of 30 patients completed the study, and their baseline characteristics are shown in Table 5. For the primary outcome of WOMAC index scores, there were statistically significant improvements on all 3 subscales (pain, stiffness, and physical function indexes) and total scores from baseline to after week 4 ($^{**}p < 0.001$), as shown in Table 6. For NRS pain scores, the results demonstrated a statistically significant decrease between before and after each week (Table 7). Moreover, after week 4, NRS dramatically decreased from 4.57 to 2.63 ($^{**}p < 0.001$), which means the severity of the pain was reduced from moderate (4–6) to mild (1–3) [19, 20]. During the research, no patients reported taking acetaminophen as a rescue medication, and no adverse effects were identified in any patient.

DISCUSSION

The dried extract of LPK remedy exhibited stronger anti-inflammatory activity via NO production in *in vitro* conditions than those of the standard drugs (prednisolone (4.3-fold) and ibuprofen (2-fold)) ($^*p < 0.05$). On the other hand, the fresh extract of LPK remedy exhibited weak anti-inflammatory activity via NO production. Also, the result of our study suggests that the dried extract of LPK remedy can be used for product development and *in vivo* studies related to its anti-inflammatory effect. In this study, the dried extract was significantly more active in NO production inhibition than the fresh extract because of the lower humidity of the dried extract, which means it was more concentrated. It was previously reported that high levels of activated articular chondrocytes produce large amounts of NO, and there is increasing evidence suggesting that inflammation plays a key role in the pathogenesis of OA [22]. *P. nigrum* has been used to reduce the production of inflammatory mediators in human chondrocytes with OA, which was stimulated by IL-β. It substantially decreased the IL-1β-stimulated gene expression and synthesis of MMP-3, MMP-13, iNOS, and COX-2. This might indicate that piperine could potentially be utilized as a therapeutic agent for OA [23]. *Z. officinale* can induce an anti-inflammatory response by impeding the migration of leukocytes and activating macrophages and neutrophils. Moreover, 6-

Table 3 Phytochemical constituents identified in the dried plant extract of LPK remedy using GC-MS.

Peak no.	RT	CAS	Name of the compound	Molecular formula	Molecular weight	Therapeutic used	Ref.
1	11.672	332	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	Anticancer effects (Pancreatic cancer cell lines, Panc-1 and SNU-213)	[29]
2	16.199	552302	1,2,3,4-cyclopentanetetrol	$C_5H_{10}O_4$	134.13	—	—
3	22.108	548229	3,4-altrosan	$C_6H_{10}O_5$	162.14	—	—
4	23.435	10446	neophytadiene	$C_{20}H_{38}$	278.50	Neuropharmacological, anti-inflammatory	[26, 27]
5	24.677	3676	lidocaine	$C_{14}H_{22}N_2O$	234.34	Anti-neoplastic, anti-inflammatory	[30]
6	25.256	36247	14-methylpentadecanoic acid	$C_{16}H_{32}O_2$	256.42	—	—
7	28.439	3931	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	280.40	Antitumor	[31]
8	29.746	860	9,12,15-octadecatrienoic acid	$C_{18}H_{30}O_2$	278.40	—	—
9	28.726	5280435	phytol	$C_{20}H_{40}O$	296.50	Antimicrobial, antioxidant, and anti-inflammatory	[29]
10	35.851	625383	isooxypeucedanin	$C_{16}H_{14}O_5$	286.28	α-Glucosidase inhibitory, antioxidant activity	[32]
11	37.436	3009225	pabulenol	$C_{16}H_{14}O_5$	286.28	Antiproliferative, cytotoxic	[33]
12	40.184	5283387	oleamide	$C_{18}H_{35}NO$	281.50	Anti-inflammatory	[34]
13	41.433	638024	piperine	$C_{17}H_{19}NO_3$	285.34	Antioxidant, anti-inflammatory, immunomodulatory, anti-asthmatic, anti-convulsant, anti-mutagenic, antimycobacterial, anti-amoebic, and anti-cancer	[35]

— means not available; CAS, chemical abstract service; and RT, retention time.

Table 4 Phytochemical constituents identified in the fresh plant extract of LPK remedy using GC-MS.

Peak no.	RT	CAS	Name of the compound	Molecular formula	Molecular weight	Therapeutic used	Ref.
1	7.696	566735	2,3-dihydropyran-4-one	$C_5H_6O_2$	98.10	Antimicrobial	[36]
2	10.610	16206001	2-methyl-6-methyleneoct-7-en-2-yl propionate	$C_{13}H_{22}O_2$	210.31	Anti-inflammatory	[37]
3	10.953	10911	dodecamethylcyclohexasiloxane	$C_{12}H_{36}O_6Si_6$	444.92	–	–
4	12.396	19100	menthoglycol	$C_{10}H_{20}O_2$	172.26	–	–
5	12.550	7041	2,6-dimethoxyphenol	$C_8H_{10}O_3$	154.16	Anticancer (Gastric cancer)	[38]
6	14.991	553025	3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	$C_{18}H_{52}O_7Si_7$	577.20	–	–
7	24.984	545303	7,9-di-tert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276.40	–	–
8	25.256	13828367	pentadecanoic acid	$C_{19}H_{38}O_2$	301.50	Anticancer (Breast cancer)	[31]
9	28.438	3931	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	280.40	Antitumor	[31]
10	28.555	5367462	9,12,15-octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.50	–	–
11	28.723	5280435	phytol	$C_{20}H_{40}O$	296.50	Antimicrobial, antioxidant, and anti-inflammatory	[29]
12	29.628	5364412	2-methyl-z, z-3,13-octadecadienol	$C_{19}H_{36}O$	280.50	–	–
13	37.423	3009225	pabulenol	$C_{16}H_{14}O_5$	286.28	Antiproliferative, cytotoxic	[33]
14	40.188	5283387	oleamide	$C_{18}H_{35}NO$	281.50	Anti-inflammatory	[34]
15	41.435	638024	piperine	$C_{17}H_{19}NO_3$	285.34	Antioxidant, anti-inflammatory, immunomodulatory, anti-asthmatic, anti-convulsant, anti-mutagenic, antimycobacterial, anti-amoebic, and anti-cancer	[35]

— means not available; CAS, chemical abstract service; and RT, Retention time.

Table 5 Baseline characteristics of patients in this study.

Data	LPK ($n = 30$)
Female, number (%)	23 (76.70)
No accident, number (%)	30 (100.00)
Age: yrs, mean (SD)	50.80 (5.69)
BMI: kg/m^2, mean (SD)	23.32 (3.28)
Numeric rating scale (NRS), mean (SD)	4.57 (0.50)
Blood pressure	
Systolic blood pressure (normal ⩽ 140 mm Hg)	124.10 (18.80)
Diastolic blood pressure (normal ⩽ 90 mm Hg)	72.58 (11.08)

Table 6 The average WOMAC index scores of the patients receiving LPK remedy on baseline (Day 0) and after (Day 28) the treatment of OA knee.

WOMAC index score	Before	After	p value**
Pain index	3.81 (4.76)	3.34 (4.83)††	0.001
Stiff index	3.70 (4.82)	3.33 (4.62)†	
Physical function index	60.76 (10.94)	52.96 (9.15)††	
Total score	68.28 (28.36)	59.63 (24.87)††	

Data represent mean (SD), ** Statistical analysis: Repeated one-way ANOVA, †Significant difference from day 0 within group (*p value < 0.05), ††Significant difference from day 0 within group (**p value < 0.001).

gingerol, the main constituent of ginger, can reduce NF-κB activation, thereby inhibiting ROS levels and iNOS expression [24]. *C. hystrix* is prevalent all through Southeast Asian nations. According to some reports, this plant can reduce inflammation by making inflammatory cytokines multiply through NLRP3 adenosine triphosphate [25]. The results of the inhibitory activity on NO production, cytotoxic activity, and previous reports of LPK remedy supported our objectivity of treatment for anti-inflammatory and OA.

The chemical constituents of dried and fresh extracts of LPK remedy exhibited biological and pharmacological effects related to anti-inflammatory, anticancer, antioxidant, and antimicrobial effects [9–12]. Additionally, neophytadiene is a phytochemical from dried extract LPK of remedy that exhibited the significance of inhibiting NO production and inflammatory cytokines (TNF-α, IL-6, and IL-10) both in *in vitro* and *in vivo* conditions [26]. Further, the expression of TNF-α, IL1β, NF-κB, iNOS, PI3k/Akt, and MAPK in the heart tissue was modulated by neophytadiene considerably supporting its anti-inflammatory potential [27].

Table 7 The average NRS pain scores of the patients receiving LPK remedy on baseline (Day 0), during (Day 7 and 14), and after (Day 21) the treatment of OA knee.

Data	Follow-up	Before	After	p value**
Numeric rating scale (NRS)	Week 1	4.57 (0.50)	3.73 (0.64)††	0.001
	Week 2	4.40 (0.49)	3.43 (0.57)††	
	Week 3	3.90 (0.71)††	2.93 (0.74)††	
	Week 4	3.63 (0.62)††	2.63 (0.62)††	

Data represent mean (SD), ** Statistical analysis: Repeated one-way ANOVA, †Significant difference from day 0 within group (*p value < 0.05), ††Significant difference from day 0 within group (**p value < 0.001).

The results of preliminary clinical examinations of the LPK remedy effectiveness in treating OA patients for 4 weeks showed a decrease in pain level and improvement of WOMAC index scores, no rescue drug such as acetaminophen was reported, and no patients experienced any negative side effects. Moreover, other clinical studies of Thai herbal poultice also reported the effectiveness of herbal poultice for knee pain relief in patients with OA of knee [19, 28].

CONCLUSION

The dried extract of LPK remedy exhibited strong anti-inflammatory activity on NO production, which is related to the chronic pain in OA knee. From chemical constituents of dried and fresh extracts, active compounds with anti-inflammatory effects were found. A preclinical trial demonstrated efficacy in reducing pain in knee OA patients. Therefore, the LPK remedy should be further investigated for the development of pharmaceutical products and assessed in animal models and clinical trials to support its use as a therapeutic drug for OA patients.

Acknowledgements: We are grateful to the Faculty of Public Health at Naresuan University for providing the necessary facilities and financial support to carry out this research. We also thank the Division of International Affairs and Language Development for reviewing the manuscript.

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