Research Article

Antioxidative and anti-inflammatory effects of Thai traditional topical herbal recipe for osteoarthritis of knee

Jintana Junlatat[1*], Suphannachat Nusawat[1], Warinee Sangprapai[1], Saran Chaweerak[1]

[1] Faculty of Thai Traditional and Alternative Medicine, Ubon Ratchathani Rajabhat University, Ubon Ratchathani, 34000

[*] Corresponding author, Email address: jintana.j@ubru.ac.th

Naresuan Phayao J. 2022;15(1):11-22.

Received; 22 April 2021; Revised: 17 July 2021; Accepted: 30 September 2021

Abstract

The purpose of the study was to evaluate the anti-oxidative and anti-inflammatory effects of a Thai traditional topical herbal recipe for osteoarthritis of knee (OA knee). This recipe has been used to treat and relieve pain and swelling around the knee, it was called Pok-Kao recipe (PK) in Thai. The recipe was prepared, extracted by maceration with 50%ethanol and then freeze-dried to obtain the extract (PKE). PKE was tested for anti-oxidation using DPPH, FRAP and ABTS assays. Anti-inflammatory mechanisms of PKE were investigated by measuring nitric oxide (NO) production using Griess reagent. In addition, the inflammatory-related gene expression and prostaglandin E_2 (PEG_2) inhibition level was investigated by reverse transcription-polymerase chain reaction technique (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) in LPS-stimulated macrophages RAW264.7 cells. The results found that PKE extract showed anti-oxidant activity when tested by using DPPH assay (EC_{50} was 674.90±27.00 µg/ml), FRAP assay (14.03±2.45 µg Trolox equiv./mg) and ABTS assay (EC_{50} was 31.01±7.19 µg/ml). Besides, the extracts exhibited the anti-inflammatory effect at doses 50-200 µg/ml which inhibited the NO production and the expression of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the cells. And, the extract strongly inhibited the PGE_2 production (EC_{50} was 18.97 µg/ml). The results support the traditional uses of this recipe for treating OA knee and are particularly useful for product development.

Keywords: Anti-oxidation, Anti-inflammation, Osteoarthritis of knee, Thai traditional recipe

Introduction

Osteoarthritis of the knee (OA knee) is one of the most common degenerative joint diseases and is rapidly becoming a global public health problem. The evidence shows that it affects more than one-third of people older than 65 years [1]. OA knee leads to disability of joint function and the need for continuous treatment [2]. Osteoarthritis is a result of both mechanical and biological events that destabilize the normal coupling of degradation and synthesis of the articular cartilage chondrocytes, extracellular matrix, and subchondral bone. The clinical features of OA are characterized by joint pain, tenderness, limitation of movement, crepitus, the occasional effusion, and variable degrees of inflammatory effects [3]. A growing body of evidence supports the notion of an important role of both local and systemic inflammation in

promoting structural damage in OA joints, as well as pain and reduced physical function. Numerous cytokines, chemokines, and other proinflammatory factors have been identified in OA synovial fluid [4]. During inflammatory process, the inflammatory biomarkers are highly produced such as reactive oxygen species (ROS), reactive nitrogen species (RNS), tumornecrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, cyclooxygenase (COX)-2. COX-2 is an inducible isoform of prostaglandin synthase found in activated phagocytes that are responsible for inflammation. In addition to TNF-α, IL-1 and IL-6 also play roles as endogenous pyrogens by stimulating the release of prostaglandins catalyzed from COX [5]. In the case of inflammation in the response to pathogen invasion, there are several free radical generations for pathogen elimination, the RNS such as nitric oxide (NO) is produced from inducible nitric oxide synthase (iNOS), and ROS such as superoxide (O2-) is also generated [6]. In this study, we have focused on the effect of this recipe on antioxidation and inflammatory-related factors inhibition. Antioxidants are found in many natural plant and various natural phytochemicals which are extremely influential agents in health promotion and disease prevention. They catch oxidant effectively or terminate the free radical chain-reaction and reduce the oxidative stress from external and intrinsic resources in human physical condition [7]. The exposure to oxidative stress induces inflammation of healthy organs and tissues [8].

There are many therapeutic modalities for OA knee. Some of these include nonpharmacologic treatment (patient education, weight control, and physical therapy) and pharmacologic treatment (acetaminophen, nonsteroidal anti-inflammatory drugs, glucosamine with chondroitin sulphate, steroids, viscosupplement injections, and surgery). Regarding pharmacologic treatment, several countries use NSAIDs as a first line drug for treatment of OA knee [9]. NSAIDs inhibit cyclooxygenase enzyme (COX) and prostaglandins resulting in many side effects. Some of these side effects manifest as peptic ulcers, liver dysfunction, and renal dysfunction. Therefore, the alternative medicines which have the potential for anti-inflammation with less systemic side effects may have a role in the treatment of OA knee.

Thai traditional medicine (TTM) is the use of alternative treatments for OA knee which include Thai traditional massage, herbal hot compression, topical herbal medicines, and oral herbal medicines. The Thai traditional topical herbal recipe that was chosen for this study has been used to treat and relieve pain and swelling around the knee for a long time. This recipe was included in the Thai massage book (Volume I) of the Public Health Foundation and Development on the topic of Thai medicine for massage, page 374. This recipe contains 5 medicinal plants including *Zingiber cassumunar* roxb. rhizome, *Tamarindus indica* L. leaves, *Acacia concinna* (Willd.) DC. leaves, *Aloe vera* (L.) Burm. F. resin, and *Cinnamomum camphora* (L.) J. Presl in equal proportions. The traditional way of using this recipe was to mix it with a small amount of alcohol and place it on the knee for 10-15 minutes. This drug has been used in clinics for a long time, but no biological activity has been reported to support the use of it. The data from this study will be supported using this recipe in a hospital and be based for continuous study in clinical trials. From these reasons, the recipe might be an alternative choice for OA knee treatment.

Materials and Methods

Preparation of the recipe

The OA knee treatment was prepared according to the traditional herbal recipe. The components of this recipe consist of *Zingiber cassumunar* roxb. bark, *Tamarindus indica* L. leaves, *Acacia concinna* (Willd.) DC. leaves, *Aloe vera* (L.) Burm. F. resin, and *Cinnamomum camphora* (L.) J. Presl crystal (camphor) in equal proportions. These components were purchased from herbal shops in Ubon Ratchathani Province, identified and authenticated by Nusawat, S (taxonomist, lecturer on Thai pharmacy from Faculty of Thai Traditional and Alternative Medicine). The voucher specimens were kept at Thai Medicine Research Center Building, faculty of Thai Traditional and Alternative Medicine, Ubon Ratchathani Rajabhat University. The dried and grounded samples were prepared in accordance with the traditional process by maceration with 50%ethanol for 5 days, then filtered through thin cloth and centrifuged at 3000 g, 25°C for 10 min using laboratory centrifuge (Kubota, Japan). The clear supernatant was concentrated using a rotary evaporator (EYELA, Japan) at 45-50°C, then freeze-dried (Christ, Germany). The ethanolic extract of this recipe was also obtained (PKE).

Determination of antioxidant activity

DPPH assay Anti- oxidant activity was determined by using DPPH radical scavenging [10]. Briefly, the extract was diluted to various concentrations by using methanol and placed in a 96-well plate. Solution of 1 mM DPPH (2, 2 – diphenyl – picryl hydrazine) in methanol was freshly prepared and then 40 µl of DPPH solution was added. After incubation at room temperature for 30 min, absorbance of bleaching solution was measured at 540 nm. Radical scavenging inhibition was calculated and expressed as 50% effective concentration (EC_{50}). Ascorbic acid and α-tocopherol were used as positive compounds for the comparison. The experiment was performed in triplicate.

FRAP assay Anti- oxidative activity was also determined by using ferric reducing antioxidant power (FRAP) assay [11]. Briefly, FRAP reagent was freshly prepared by mixing of 300 mM acetate buffer pH 3. 6, 10 mM 2,4,6- tripyridyl- s- triazine (TPTZ) solution and 20 mM $FeCl_3$ solution. Trolox was used as a standard and diluted to various concentrations in acetate buffer. The extracts were diluted to various concentrations in ethanol. The diluted trolox or extract solution (6 µl) was mixed with acetate buffer (18 µl) and FRAP reagent (180 µl). After incubation at room temperature for 4 min, the solution was measured at an absorbance of 600 nm. The sample absorbance was calculated by relative antioxidant activity in comparison with a standard curve. The experiment was performed in triplicate.

ABTS assay The 2,2'-azino-bis (3-Ethylbenzthiazoline-6-sulphonic acid) or ABTS was transformed into free radicals (ABTS +•) by oxidation with potassium persulfate ($K_2S_2O_8$) by dissolving ABTS and $K_2S_2O_8$ in 1 ml of water and leaving it in the dark for 12-16 hours at room temperature. After that, the ABTS +• solution was diluted with ethanol to obtain an absorbance of 734 nm to 0.7 + 0.02 (ABTS +• stock solution). ABTS +• stock solution and extracts were mixed together and left in the dark for 15 minutes, then absorbance was measured at a wavelength of 734 nm [7]. Trolox was used as positive compounds for the comparison. The experiment was performed in triplicate.

Determination of phenolic content The total phenolics content was determined by the Folin-Ciocalteu method [12]. A sample (5 mg) was dissolved with methanol and up to 1 mL, and then the sample solution was mixed with 0.25 mL of the 1N Folin-Ciocalteau reagent and 1.25 mL of 20% sodium carbonate. After mixing and standing for 40 minutes at the room temperature, the optical density was measured at 725 nm. The total phenolic contents were expressed as mg gallic acid equivalent (GAE)/g dry basis.

Determination of anti-inflammatory activity

Cell culture The murine macrophage cell line, RAW264.7 cells, was purchased from PromoCell, Germany. The cells were cultured in DMEM media supplemented with 10% heat-inactivated calf serum (HyClone, USA) and 1%penicillin (100U/ml)-streptomycin (100μg/ml) and incubated at 37ºC in a humidified atmosphere with 5%CO_2.

Cytotoxicity test Macrophage RAW264.7 cells were treated with various concentrations of extract and then incubated at 37ºC in the humidified atmosphere with 5%CO_2 for 24h. Cell viability was analyzed by using MTT assay [13] and the absorbance measured at 570 nm. The results were calculated for % inhibition and expressed as 50% inhibitory concentration.

Nitric oxide inhibitory test The nitric oxide (NO) assay was performed as described previously with slight modification [14]. After pre-incubation of RAW 264.7 cells (1.5×10^5 cells/mL) with LPS (1 μg/mL) for 24 h, the quantity of nitrite in the culture medium was measured as an indicator of NO production. Amounts of nitrite, a stable metabolite of NO, were measured using Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid). Briefly, 100 μL of cell culture medium was mixed with 100 μL of Griess reagent. Subsequently, the mixture was incubated at room temperature for 10 min and the absorbance at 540 nm was measured in a microplate reader. Fresh culture medium was used as a blank in every experiment and the NO inhibition was calculated as percentage. And, aminoguanidine was used as a positive control.

Determination of inflammatory-related gene expression The cells were cultured overnight in a 12-well plate and treated with various concentrations of extract and positive control. After incubation at 37ºC in the humidified atmosphere with 5%CO_2 for 22 hr, the LPS was added then further incubated for 2 hr. Total RNA was extracted from the treated cells by using a GE Healthcare extraction kit. The first-strand cDNA was synthesized from total RNA (40ng) with Omniscript reverse transcriptase kit. The primers were used for amplifying the respective fragments. Polymerase chain reaction (PCR) was performed by incubation of each cDNA sample with the primers, Taq polymerase and deoxynucleotide mix. Specific oligonucleotides were based on the published sequences [15, 16]. cDNA was synthesized from 40 ng of total RNA with a two-step RT-PCR kit using a thermal cycler (Biometra, Germany) and the PCR profiles were 3 0 cycles. The PCR products were then analyzed on 1.5% agarose gel, visualized by Novel Juice staining and RT-PCR product densities measured by Gel Documentation and System Analysis machine. The inflammatory-related gene expressions were calculated for the relative mRNA expression level and compared with β-actin.

Determination of PGE_2 The PGE_2 levels in macrophage RAW264.7 cells were measured using an immunoassay (R&D systems a biotechne brand, USA) according to the manufacturer's specifications. The cells were cultured overnight in a 12-well plate and treated with various concentrations of extract and positive control. After incubation at 37ºC in the humidified atmosphere with 5%CO_2 for 22 hr, the LPS was added then further incubated for 2 hr. Cell culture supernates were removed by centrifugation. 150 µl of supernatant samples were put in the wells and then 50 µl of the primary antibody solution to each well. After that, 50 µl of PGE_2 conjugate was added to each well and incubated for 2 hours at room temperature on the shaker. Wash buffer was added and removed for 4 times, then remaining wash buffer was removed by aspirating, inverting the plate and blotting it against clean paper towels. Then, 200 µl of substrate solution was added and incubated for 30 minutes at room temperature and the optical density was measured at 450 nm using a microplate reader (Shimadzu, Japan). The sample absorbance was calculated by comparison with PGE_2 standard curve. The experiment was performed in triplicate.

Statistical analysis

All experiments were performed in triplicate and the results were expressed as mean±S.D. The data were compared between treated groups and control group. One-Way ANOVA and multiple comparisons were used to analyze the significant difference results.

Results

Antioxidant activity

The ethanolic extracts of OA knee treatment herbal recipe (PKE) showed antioxidant activity in DPPH, FRAP and ABTS assays as shown in Table 1. For DPPH assay, the half effective concentration (EC_{50}) was 674.90±27.00 µg/ml, compared to the vitamin C standard of 4.18±0.10 µg/ml, it suggested that this extract had low antioxidant effect by mechanism of free radical scavenging activity. In addition, The FRAP assay which was the ferric reducing antioxidant power mechanism exhibited antioxidant activity compared to the Trolox standard curve (14.03±2.45 µg Trolox equiv./mg). However, for the ABTS antioxidant activity test, it was found that this extract had good antioxidant activity, with an EC_{50} value approximately 5.2 times less than the standard Trolox. Moreover, the phenol content of the extract was measured as it is a group of essential substances involved in antioxidant and anti-inflammatory activities. The antioxidant value of each assay in this study differed depending on the mechanism of each assay and the number and position of the hydroxyl groups of the aromatic ring binding site and the type of substituent of phenol [17]. All three methods used in the antioxidant study showed that PKE had antioxidant activity, possibly due to the heterogeneity of chemical constituents in PKE, which were susceptible to different test methods.

Table 1 Antioxidative activity and total phenolic content of PKE

Sample	DPPH* (EC_{50}, μg/mL)	FRAP* (μg Trolox equiv./mg)	ABTS* (EC_{50}, μg/mL)	Total phenolic* (μg gallic acid equiv./mg)
PKE	674.90±27.00	14.03±2.45	31.01±7.19	105.21±7.74
Vitamin C	4.18±0.10	-	-	-
Tocopherol	8.19±0.34	-	-	-
Trolox	-	-	5.95±0.37	-

Anti-inflammatory activity

Macrophage RAW 264.7 cells were treated with various concentrations of the extracts and then incubated at 37ºC in a humidified atmosphere with 5%CO_2 for 24h. Cell viability was analyzed by using MTT assay and the absorbance measured at 570 nm. As shown in Figure 1, the results were calculated for % cell viability and expressed as 50% inhibitory concentration. The results showed that PKE showed low toxicity on the cells (IC_{50} was 2.61 ± 0.12 mg/mL). Based on these results, the extracts at doses 50-200 μg/mL (Concentration for cell viability >90%) were used to evaluate the further anti-inflammatory effect. Anti-inflammatory effects of PKE were investigated by measuring nitric oxide (NO) production using Griess reagent. The NO inhibitory effect of the extracts was evaluated on LPS-stimulated macrophage RAW264.7 cells. The result found that PKE showed NO inhibition and that was very close to the standard substance, aminoguanidine. At 200 μg/ml concentration, PKE and aminoguanidine exhibited NO inhibition at 48.76% and 55.97%, respectively as shown in Figure 2. NO is a free radical synthesized from L-arginine by NO synthetase (NOS). NOS has three isoforms, neuronal, endothelial and inducible; proinflammatory cytokines such as interleukin-1β (IL-1β) activate inducible NO synthetase (iNOS) during inflammation. iNOS and cyclo-oxygenase-2 have been reported to be found in cartilage and synovial tissue. The increase in NO in the joint fluid also indicates an inflammatory feature in the development of OA. Increased IL-1β in the joint fluid suggests that the increase in the activity of local inducible NO synthetase enzyme is also the cause of the increase in NO [18]. The results of this research were explained by the relation of NO inhibition and phenolic content in the recipe [19].

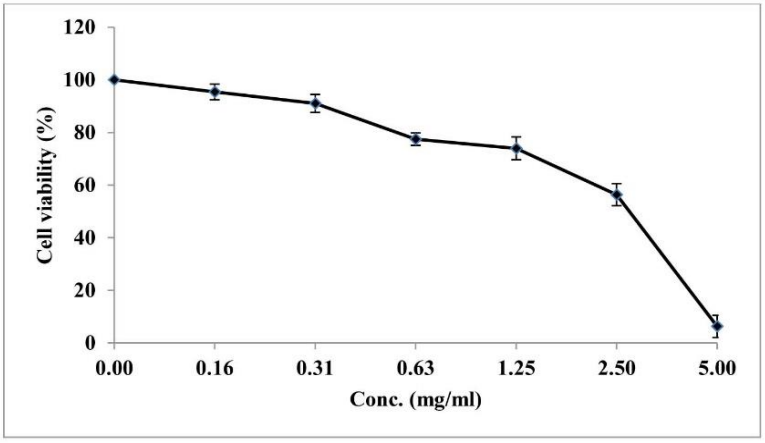

Figure 1 Effect of PKE on the viability of RAW264.7 cells

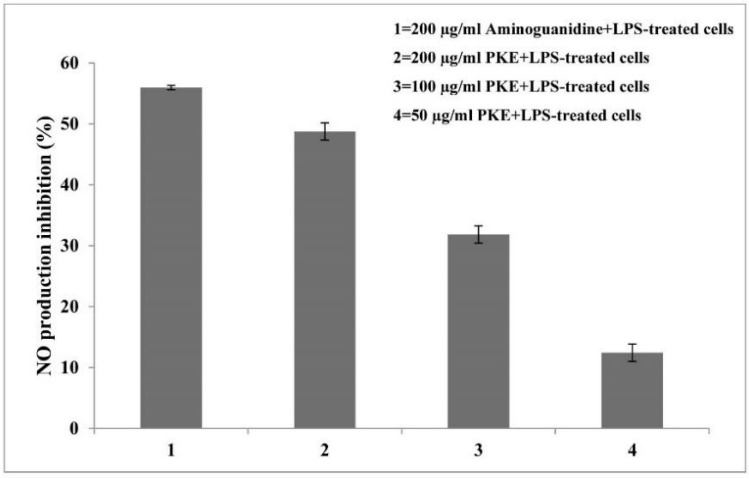

Figure 2 Effect of PKE on nitric oxide (NO) production in LPS-induced RAW264.7 cells. Values were expressed as mean±SD (n=3)

The expression of the pro-inflammatory gene including COX-2, iNOS, IL-1β and TNF-α were not changed when treatment was used with the extracts alone but were up-regulated after treatment with LPS. After incubation for 22 hr with the extracts at 50-200 µg/ml concentration, it was found that the extracts could inhibit the expressions of these genes in the dose-dependent manner. Particularly, at the concentration of 200 µg/ml, PKE exhibited the suppression percentage on IL-1β, TNF-α, COX-2 and iNOS with half inhibition concentration at 35.44%, 72.56%, 97.19% and 296.01%, respectively. In addition, the suppression on these genes was similar to standard drugs, Indomethacin and Aminoguanidine (Figure 3). Moreover, the anti-inflammatory effect of PKE was also found in the protein level, PKE was found to inhibit the PGE_2 production by measuring cell culture supernates from macrophage-stimulated cells (EC_{50} was 18.97 µg/ml) (Figure 4).

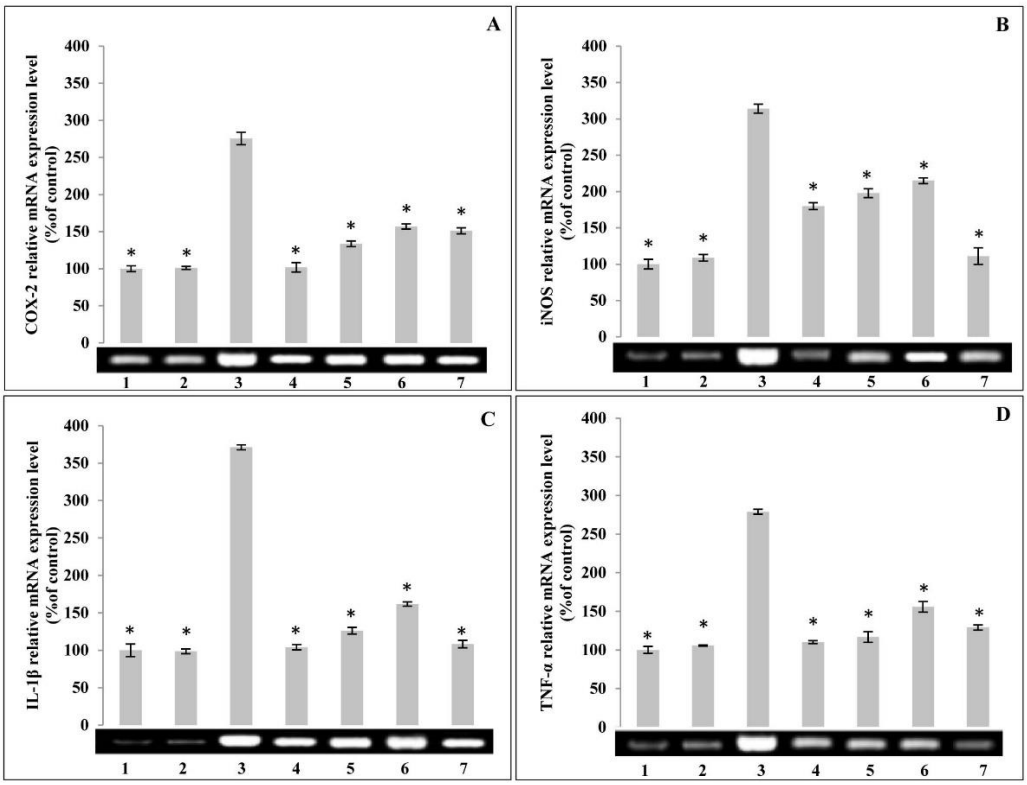

Figure 3 Effects of PKE on mRNA expression of COX-2 (A), iNOS (B), IL-1β (C) and TNF-α (D) (Results were expressed as % of control, **P* < 0.05 vs. LPS-treated cells)

1 = Unstimulated cells 2 = 200 µg/ml PKE-treated cells

3 = LPS-treated cells 4 = 200 µg/ml PKE+LPS-treated cells

5 = 100 µg/ml PKE+LPS-treated cells 6 = 50 µg/ml PKE+LPS-treated cells

7 = 50 µg/ml Positive control+LPS-treated cells (Indomethacin for IL-1β, COX-2 and TNF-α / Aminoguanidine for iNOS)

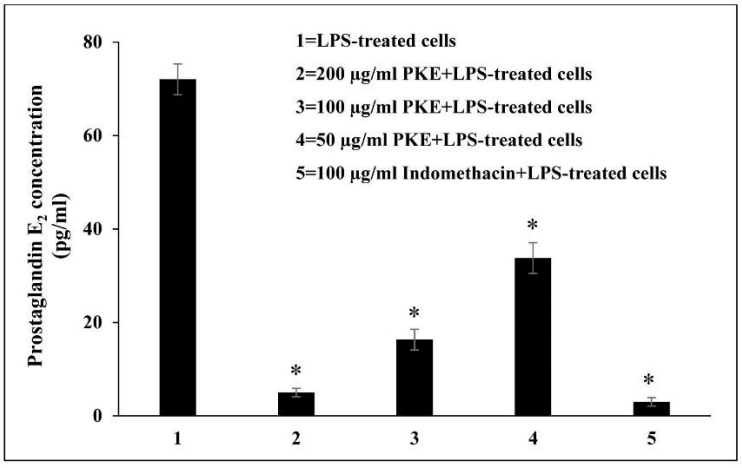

Figure 4 Effect of PKE on prostaglandin E_2 (PGE_2) production in LPS-induced RAW264.7 cells. *represents significant difference from LPS-treated cells (*P* < 0.05)

Discussion

The results of this study support the use of this recipe in the treatment of OA knee. The anti-inflammatory effect of this recipe was evident in suppressing various inflammatory factors, which are directly related to osteoarthritis. Here, especially cytokines are corroborated by data illustrating chondrocytes as a site for pro-inflammatory cytokine production in OA knee [20]. This recipe can inhibit the factors associated with OA knee, possibly due to the herbal composition of the recipe, which contains *Zingiber cassumunar* roxb. rhizome, *Tamarindus indica* L. leaves, *Acacia concinna* (Willd.) DC. leaves, *Aloe vera* (L.) Burm. F. resin, and *Cinnamomum camphora* (L.) J. Presl. *Z. cassumunar* known locally as “Plai” in Thai, has been used for treating bruises, sprains, and musculoskeletal pain. Several pre-clinical and clinical studies demonstrated the anti-inflammatory effect of this plant [21]. *T. indica* or Ma-kam has played roles in traditional medicine as an anti-inflammatory and analgesic drug. The preclinical studies provided strong pharmacological evidence for the anti-inflammatory and analgesic activities of the plant and this may be attributed to the various bioactive compounds in it including alkaloids, flavonoids, tannins, phenols, saponins, and steroids [22]. *A. concinna* or Som-poi, widely used in Southern and Southeast Asia for medicinal purposes, revealed strongly antioxidation [23]. *A. vera* or Wan-hang-ja-ra-kae was described to contain more than 98% water and a large amount of potentially active compounds including water-soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds, organic acids, amino acids, enzymes, sterols and fatty acids. Scientific evidence suggests that *A. vera* has many activities, including anti-oxidant, antidiabetic, and antihyperlipidemic [24]. The last composition of this recipe is *C. camphora* or camphor or Ka-ra-bun in Thai. *C. camphora* has been used for traditional medicine as a therapeutic agent of inflammation-related diseases, including sprains, rheumatic arthritis, abdominal pain, cough and bronchitis, for a long history. Camphor could inhibit the productions of TNF-α, IL-6, and PGE2, and alleviated mRNA and protein levels of iNOS, COX-2, and matrix metallopeptidase-9 [25, 26]. From these data it can be seen that the chemical compounds of the herbs in these formulations all have antioxidant and anti-inflammatory activity profiles, which may be the reason for their antioxidant and anti-inflammatory effects. From the foregoing information, it can be seen that this recipe has been used for a long time and shows the value of Thai traditional medicine wisdom. Thai traditional medicine mostly used herbal recipe rather than single herb because the principle of Thai traditional medicine included major, minor, control herbs, etc., which resulted in the treatment more effective than a single herb. The information obtained from this study provides scientific evidence supporting the use of this recipe for the treatment of OA knee in terms of efficiency, quality and safety. However, for the treatment of Thai traditional medicine is often a holistic treatment that will combine many therapeutic sciences as part of the treatment. OA knee is treated with internal and external drugs, massage, oil tattooing, body curling, acupressure, etc.

Conclusions

This study provides scientific information to support the use of this recipe in the treatment of OA knee, showing that the recipe has antioxidant and anti-inflammatory effects, which are associated with OA knee. Ethanol was used in extraction because of its close resemblance to traditional recipe preparations, after which the extract was dried and tested for its antioxidant activity in several models. Overall, the extract was found to have antioxidant activity, and when tested for anti-inflammatory activity, the extract was found to be highly anti-inflammatory in both mRNA and protein levels. All results are consistent with previous reports on the individual herbs found in this recipe. This information is particularly useful for further use of this recipe in the treatment of OA knee and in the development of products for patients with osteoarthritis.

Acknowledgments

This research was financed, and facilities supported by Faculty of Thai Traditional and Alternative Medicine, Ubon Ratchathani Rajabhat University, Thailand.

References

1. Amorndoljai P, Taneepanichskul S, Niempoog S, Nimmannit U. A comparative of ginger extract in nanostructure lipid carrier (NLC) and 1% diclofenac gel for treatment of knee osteoarthritis (OA). J Med Assoc Thail. 2017; 100(4):447–56.
2. Teekachunhatean S, Kunanusorn P, Rojanasthien N, Sananpanich K, Pojchamarnwiputh S, Lhieochaiphunt S, et al. Chinese herbal recipe versus diclofenac in symptomatic treatment of osteoarthritis of the knee: A randomized controlled trial [ISRCTN70292892]. BMC Complement Altern Med. 2004;4:1–8.
3. Pinsornsak P, Kanokkangsadal P, Itharat A. The clinical efficacy and safety of the sahastara remedy versus diclofenac in the treatment of osteoarthritis of the knee: A double-blind, randomized, and controlled trial. Evidence-based Complement Altern Med. 2015;1:1-8.
4. Scanzello CR, Loeser RF. Editorial: Inflammatory activity in symptomatic knee osteoarthritis: Not all inflammation is local. Arthritis Rheumatol. 2015;67(11):2797–800.
5. Huang ZA, Scotland KB, Li Y, Tan J, Kung SHY, Chew BH, et al. Determination of urinary prostaglandin E2 as a potential biomarker of ureteral stent associated inflammation. J Chromatogr B Anal Technol Biomed Life Sci. 2020;1145:122107.
6. Kumar A, Singh KP, Bali P, Anwar S, Kaul A, Singh OP, et al. iNOS polymorphism modulates iNOS/NO expression via impaired antioxidant and ROS content in *P. vivax* and *P. falciparum* infection. Redox Biol. 2018;15:192–206.
7. Li WJ, Lin YC, Wu PF, Wen ZH, Liu PL, Chen CY, et al. Biofunctional constituents from *Liriodendron tulipifera* with antioxidants and anti-melanogenic properties. Int J Mol Sci. 2013;14(1):1698–712.
8. Lee CC, Chen YT, Chiu CC, Liao WT, Liu YC, David Wang HM. *Polygonum cuspidatum* extracts as bioactive antioxidaion, anti-tyrosinase, immune stimulation and anticancer agents. J Biosci Bioeng. 2015;119(4):464–9.
9. Verkleij SPJ, Luijsterburg PAJ, Bohnen AM, Koes BW, Bierma-Zeinstra SMA. NSAIDs vs acetaminophen in knee and hip osteoarthritis: A systematic review regarding heterogeneity influencing the outcomes. Osteoarthr Cartil. 2011;19(8):921–9.

10. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative Properties of Xanthan on the Autoxidation of Soybean Oil in Cyclodextrin Emulsion. J Agric Food Chem. 1992;40(6): 945–8.
11. Famurewa AC, Aja PM, Nwankwo OE, Awoke JN, Maduagwuna EK, Aloke C. *Moringa oleifera* seed oil or virgin coconut oil supplementation abrogates cerebral neurotoxicity induced by antineoplastic agent methotrexate by suppression of oxidative stress and neuro-inflammation in rats. J Food Biochem. 2019;43(3):1–10.
12. Sripanidkulchai B, Fangkrathok N. Antioxidant, antimutagenic and antibacterial activities of extracts from *Phyllanthus emblica* branches. Songklanakarin J Sci Technol. 2014;36(6): 669–74.
13. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1–2):55–63.
14. Adebayo SA, Ondua M, Shai LJ, Lebelo SL. Inhibition of nitric oxide production and free radical scavenging activities of four south african medicinal plants. J Inflamm Res. 2019;12:195–203.
15. Won JH, Im HT, Kim YH, Yun KJ, Park HJ, Choi JW, et al. Anti-inflammatory effect of buddlejasaponin IV through the inhibition of iNOS and COX-2 expression in RAW 264.7 macrophages via the NF-KB inactivation. Br J Pharmacol. 2006;148(2):216–25.
16. Sripanidkulchai B, Junlatat J, Wara-aswapati N, Hormdee D. Anti-inflammatory effect of *Streblus asper* leaf extract in rats and its modulation on inflammation-associated genes expression in RAW 264.7 macrophage cells. J Ethnopharmacol. 2009;124(3):566–70.
17. Ben Ahmed Z, Yousfi M, Viaene J, Dejaegher B, Demeyer K, Mangelings D, et al. Determination of optimal extraction conditions for phenolic compounds from: *Pistacia atlantica* leaves using the response surface methodology. Anal Methods. 2016;8(31): 6107–14.
18. Karan A, Karan MA, Vural P, Erten N, Tascioglu C, Aksoy C, et al. Synovial fluid nitric oxide levels in patients with knee osteoarthritis. Clin Rheumatol. 2003;22(6):397–9.
19. Choi SE, Park KH, Han BH, Jeong MS, Seo SJ, Lee DI, et al. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by phenolic compounds from roots of *Rhododendron mucronulatum*. Phyther Res. 2011;25(9):1301–5.
20. Barker T, Rogers VE, Henriksen VT, Levy M, Schneider ED, Templeton J, et al. Circulating cytokine concentrations are not altered by supplemental vitamin D in knee osteoarthritis: A pilot study. J Nutr Intermed Metab. 2019;18:100103.
21. Chongmelaxme B, Sruamsiri R, Dilokthornsakul P, Dhippayom T, Kongkaew C, Saokaew S, et al. Clinical effects of *Zingiber cassumunar* (Plai): A systematic review. Complement Ther Med. 2017;35:70–7.
22. Komakech R, Kim Y, Matsabisa GM, Kang Y. Anti-inflammatory and analgesic potential of *Tamarindus indica* Linn. (Fabaceae): a narrative review. Integr Med Res. 2019;8(3):181–6.
23. Poomanee W, Chaiyana W, Randall Wickett R, Leelapornpisid P. Stability and solubility improvement of Sompoi (*Acacia concinna* Linn.) pod extract by topical microemulsion. Asian J Pharm Sci. 2017;12(4):386–93.

24. Rahoui W, Merzouk H, El Haci IA, Bettioui R, Azzi R, Benali M. Beneficial effects of Aloe vera gel on lipid profile, lipase activities and oxidant/antioxidant status in obese rats. J Funct Foods. 2018;48:525–32.
25. Li YR, Fu CS, Yang WJ, Wang XL, Feng D, Wang XN, et al. Investigation of constituents from *Cinnamomum camphora* (L.) J. Presl and evaluation of their anti-inflammatory properties in lipopolysaccharide-stimulated RAW 264.7 macrophages. J Ethnopharmacol. 2018;221: 37–47.
26. Wang J, Su B, Jiang H, Cui N, Yu Z, Yang Y, et al. Traditional uses, phytochemistry and pharmacological activities of the genus *Cinnamomum* (Lauraceae): A review. Fitoterapia. 2020;146:104675.