O_40

A Clinical Study Phase II of Ginger Extract in Nanostructured Lipid Carrier for Pain Relief in Knee Osteoarthritis Patients

Punyanut Amorndoljai[1*] , Surasak Taneepanichskul[2], Sunyarn Niempoog[3], Ubonthip Nimmannit[4]

Abstract

Introduction: Herbal medicines are used extensively in patients with chronic pain such as osteoarthritis (OA). There are few clinical trials to proof their efficacy and safety. This study was undertaken to examine the efficacy and safety of ginger (*Zingiber officinale* Roscoe) extract in Nanostructured Lipid Carrier (NLC) for relieving pain in knee OA patients. NLC is popular formulation of the topical herbal medicines. **Methods:** Twenty patients (3 males and 17 females aged range of 50-75 years who diagnosed as OA knee. This diagnosis is based on the American College of Rheumatology (ACR) diagnosis criteria. All participants received a treatment with ginger extract in NLC rubbed three times a day for 4 weeks. Efficacy was assessed by the Western Ontario and McMaster Universities Arthritis Index (WOMAC), Knee Injury and Osteoarthritis Outcome Score (KOOS) and patient's global assessment (PGA). The safety of ginger extract in NLC was approved by biochemical tests in serum and hematological parameters. The paired *t*-test was used to compare the average score of efficacy before and after treatment. **Results:** Ginger extract in NLC revealed its statistically significant improvement in patient's global assessment, knee joint pain, physical function, stiffness, symptom, sport & recreation activity and quality of life which measured by PGA, WOMAC and KOOS. Within 4-week treatment with ginger extract in NLC, there were 2 reports of contact dermatitis adverse event. **Conclusion:** A 4-week treatment with ginger extract in NLC could relieve joint pain and improve problematic symptoms and the quality of life in knee osteoarthritis patients.

Keywords: Osteoarthritis, Ginger, *Zingiber officinale* Roscoe, Nanostructured Lipid Carrier, Clinical trial

---

[1] College of Public Health Sciences, Chulalongkorn University, Institute Building 2-3, Soi Chulalongkorn 62, Phyathai Rd, Pathumwan, Bangkok 10330, Thailand

[2] College of Public Health Sciences, Chulalongkorn University, Institute Building 2-3, Soi Chulalongkorn 62, Phyathai Rd, Pathumwan, Bangkok 10330, Thailand

[3] Division of Orthopaedics, Faculty of Medicine, Thammasat University (Rangsit) Klong Nueng, KlongLuang, Pathumthani 12121, Thailand.

[4] National Science and Technology Development Agency,111 Thailand Science Park, Phahonyothin Road, Khlong Nueng, Khlong Luang, Pathum Thani 12120 Thailand.

* **Corresponding author** : Punyanut Amorndoljai, College of Public Health Sciences, Chulalongkorn University, Institute Building 2-3, Soi Chulalongkorn 62, Phyathai Rd, Pathumwan, Bangkok 10330, Thailand

1. Introduction

Osteoarthritis (OA) is a common disease of joint disorder, and the evidences showed that it affects more than one-third of people aged older than 65 years. Patients with OA are at a higher mortality risk 1.54 times of general population (Bliddal, 2009). It is not only a leading cause of chronic disability but also the high cost of treatment. OA has affected on health status of older persons in 3 primary dimensions: physical disability, psychological disability, and pain. Moreover, it also influenced on patient's family, their social support and social activity (Balen and Grazio, 2009; Kapstad, 2007). Unfortunately, in the present, there is no effective therapeutic intervention for OA treatment. Currently, acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) including cyclo-oxygenase II inhibitors are medication used for pain and stiffness relief (Altman, 2010). Although, these pharmaceutical agents could temporarily reduce both pain and improve physical functions but they are no healing effect on the cartilage and sub-chondral damage. Moreover, long term use of NSAIDs is associated with increased risk for renal insufficiency, gastrointestinal bleeding, hypertension and congestive heart failure.

Ginger (*Zingiber officinale* Roscoe) is widely used in folk medicine, and it is popular as a food spice. Its therapeutic uses are various such as treatment of flatulence, gingivitis, toothache, asthma, osteoarthritis and rheumatism (Anoy mous, 2003; Levy *et al.,* 2006; Levy and Simon, 2009). Phytochemical studies have shown that the ginger rhizome contains a wide variety of biologically active compounds. Active components have presented in the volatile oil (1-3%) the mono and sesquiterpens (Mascolo, 1989; Chrubasik *et al*., 2005). The non-volatile oil pungent of ginger consists of gingerol, shogaol, paradols and zingerone (4.0-7.5%). The pungent of ginger has demonstrated anti-inflammatory actions in-vitro, inhibiting leukotriene synthesis, the active of cyclooxygenase enzymes (COX-1 and COX-2) (Shibata et al, 1989; Thomson et al., 2002).

However, with its poor absorption, rapid metabolism and elimination of active compound, the bioavailability of polyphenolic compound is not good. With the limitation of insolubility in water, gingerol and shogaol compounds are limited the application in aqueous base systems. An undertaking approach to solve this problem is the nanoparticle of ginger extract. Recently, the beneficial properties of nanoparticles-based delivery systems are increased the biodegradability, biocompatibility as well as non-toxicity and inexpensive (Praditbongkotchp, 2010). However, there were few studies in therapeutic efficacy of the topical ginger extract nanoparticles in patients with knee osteoarthritis. Therefore, the researchers conducted a clinical study phase II to assess the efficacy and safety of ginger extract in Nanostructured Lipid Carrier (NLC), which has amounts of [6]-gingerol for topical pain relief in knee osteoarthritis (OA).

2. Methods

Patient Selection

This study was approved by the ethical committee of Department of Development of Thai

Traditional and Alternative Medicine, Ministry of Public Health (Thai Clinical Trial Registry No.TCTR 20140306001). Informed consent form was signed by all participants at the beginning of the study procedure. Patient recruitment and data collection were done at Tha Chang Hospital, Singburi Province between May and August 2014. Inclusion criteria were (a) both male and female aged 50-75 years, (b) primarily diagnosed of OA by physician, the diagnosis criteria was based on American College of Rheumatology (Altman *et al*., 1986; American college of rheumatology,1986; Kuptniratsaikul and Rattanachaiyanont, 1987) with knee pain classification and radiographic osteoar thritis in grade II-III of Kellgren and Lawrence criteria (Grade 0=Normal; Grade I=Possible osteophytes, doubtful narrowing of joint space; Grade II=definite osteophytes, absent or question able narrowing of joint space; Grade III=moderate osteophytes, marked narrowing of joint space, severe sclerosis, possible deformity; Grade IV=large osteophytes, marked narrowing of joint space, severe sclerosis, definite deformity) (Kellgren and Lawrence,1957). Exclusion criteria were (a) secondary diagnosed of knee OA, (b) history of herb or herbal medicine allergy during investigation, and (c) severe joint instability or severe deformity (grade IV Kellgren and Lawrence).

Study Protocol

Before entry to the study, patients had a 1-week washout for anti-inflammatory and analgesic medication, during that time acetami nophen 500 mg was used three times a day (or every 4-6 hours can be used for pain-relief) but not any other topical analgesics, NSAIDs or COX-2 inhibitors. If subjects took acetaminophen for OA pain during the 7-day wash-out, they had to record its amount. At baseline visit, patients underwent a physical examination, vital signs confirm, laboratory examination included a CBC, renal function test (BUN and Creatinine) and liver function test (SGOT, SGPT and ALP).

The preparation of ginger extract in NLC is produced and proved its quality control by National Nanotechnology Center, National Science and Technology Development Agency, Thailand. Physical stability of ginger extract in NLC is stored in the refrigerator at 4 °C for 48 hours and in hot air oven at 40 °C for 48 hours for one cycle. The overall 3 cycles was carried out. Characterization of preparation is evaluated by physical appearance, particle size, size distribution kinetics (Zeta potential) and particle morphology. Chemical stability of preparation is performed in 3 situations; accelerating condition (40^0C), stored in refrigerated at 4^0C, and ambient for 3 months. Quantitation of active component, [6]-gingerol is determined by HPLC method at 1, 2, and 3 month.

The ginger extract in NLC contained extract of ginger by ratio of about 5% by weight ([6]-gingerol content 11.18 %). The topical of Nanostructured Lipid Carrier which is prepared by weighing the composition of solid lipid, liquid lipid, surfactant, and water mix. The potency of extract preparation were followed and analyzed for the active molecule 6-gingerol with HPCL. In addition, *in vitro* studies of release of preparation

was done by modified Franz diffusion cell method and approximately 92% of 6-gingerol from NLC was released within 24 hours.

Subjects had to apply 2 inches of extruded gel topically around the index knee and rubbed until the gel was dry for three times a day. The returned gel was weighed. Treatment com pliance was measured by assigning the subjects to report a treatment diary. This self-report diary included the reports of other concomitant therapy during the study, pain medication use or nutritional supplements use for OA or related pain. After completion of treatment with NLC of ginger extract at week 4, patients were evaluated the treatment efficacy by using WOMAC parameter, KOOS score (Chaipinyo, 2009; Thai version of KOOS, 2009; Roos and Toksvig-Larsen, 2003) and repeated laboratory tests for safety such as CBC, renal function test (BUN and Creatinine) and liver function test (SGOT, SGPT and ALP). Average parameter of laboratory examination results were compared with normal value and compared with before and after treatment.

Safety and Efficacy Evaluation

Recruitment and retention of patients were reported through descriptive summaries. Treatment protocol adherence was assessed by self-report drug used and returned drug weight. Safety was evaluated by laboratory tests and all adverse events (AE) that occurred during the treatment; in term of its onset, duration and severity of events (mild, moderate, or severe AE), as well as the action taken and outcomes were reported. The relationship between an adverse event and the study medication was assessed by the investigator. Adverse event was classified into 4 levels; unlikely, possible, probable and certain according to Naranjo's alogorithm (Michel and Knodel, 1986) and World Health Organization (WHO) adverse reaction terminology. The adverse events were analyzed by preferred term and by system organ classes.

Efficacy was assessed by using three instruments, (1) the Western Ontario and McMaster Universities (WOMAC) Osteoarthritis Index which assessed pain (classified into pain score, stiffness score and physical function score) WOMAC consists of 22 questions with10-mm visual analog scale, analyzed in 3 subscales as the average score for 5 questions on pain, 2 questions on stiffness, and 15 questions on function. The total score is calculated as the mean score for all 22 questions. (2) Knee Injury and Osteoarthritis Outcome Score (KOOS) which assessed the patient's activities with a 5-point Likert scale; 0=very good 1=good 2=average 3=poor 4=very poor. KOOS is a 42-item, divided into 5 domains; 9 items addressing pain, 7 items addressing other symptoms, 17 items for function in daily living (ADL), 5 items for function in sports and recreation (Sport/Rec), and 4 items for quality of life (QOL) related to knee function. (3) Index of Severity for Osteoarthritis (ISOA) and patient's global assessment (PGA), this self-explanatory questionnaire was assessed by patients and physician with scoring on a 5-point Likert scale (very good=1; good=2; fair=3; poor=4; very poor=5). The responder criteria defined by the Outcome Measures in Rheumatology (OMER-

ACT) committee and The Osteoarthritis Research Society International (OARSI) committee.

End points and Statistics

Patient compliance on treatment medication (NLC ginger extract) was assessed at week 4 by patients self-report of drug use and returned drug weight. Baseline characteristic and demographic data of patients were described by using descriptive statistics; mean, SD, 95%CI, percentage and frequencies. The primary end points of the phase II study were the reduction of the visual analog scale (VAS) score of WOMAC and KOOS from the baseline to the week 4 of medication treatment. The secondary end points were adverse events and OMERACT-OARSI responder criteria [23-24]. The responder criteria covered three domains (pain, function and PGA) that defined as a participant with 1) 50% reduction in pain or 50% improvement function, 2) 20% reduction in pain or 20% improvement function, adding 10% improvement of the PGA from the baseline. The student paired *t*-test was used for comparing the mean difference of WOMAC and KOOS parameter before and after the treatment. All categories of adverse events and complications of the treatment were recorded according to guidelines of WHO (Toxicity Grading Scale for determining the Severity of Adverse Events) and Common Terminology Criteria for Adverse Event (CTCAE) version 4.0.

3. Results

Baseline demographic and clinical characteristics

During of May to August 2014, thirty patients with OA knee were screened. There were twenty patients who completed the wash out protocol and were eligible for outcome and response assessment. Ten patients were excluded from this study, six patients were in grade I, one patient was in grade IV for radio graphic osteoarthritis of Kellgren and Lawrence criteria, two had gout, and one had rheumatoid and a sciatica nerve pain. No patient had concomitant medication during the study period. The mean of the topical ginger extract in NLC used per patient was 20 g for 30 days. Baseline demographic and clinical characteristics were summarized in Table 1.

Safety

Twenty patients initially enrolled into this study. Two patients dropped out of the study due to an adverse skin reaction; contact dermatitis (Figure1.).

Biochemical evaluation

As a part of the safety evaluation, labo ratory tests were performed for assessment of different biochemical and hematological param eters. The student paired *t*-test was used to compare the parameters at different evaluation points over the 30 days period with the baseline. There was no statistically significant change of these parameters. Although minor changes were observed in some of the biochemical parameters,

they remained within the normal range. Similarly, no significant changes in hematological parame ters when were compared with the baseline.

Adverse Events

The major adverse effect was skin reaction at the applied site and this adverse event led to the discontinuation of 2 participants. One had the total score of Naranjo's algorithm +3 or a possible ADR; that was the adverse event appeared after the suspected drug was given (+1). The adverse reaction was improved when the drug was discontinued (+1). Moreover, the adverse reaction appeared when the drug was re-administered (+2) and there alternative causes that could have affected the reaction (-1). Another also had score +1 as possible ADR. However, all skin reactions were resolved promptly upon withdrawal of treatment.

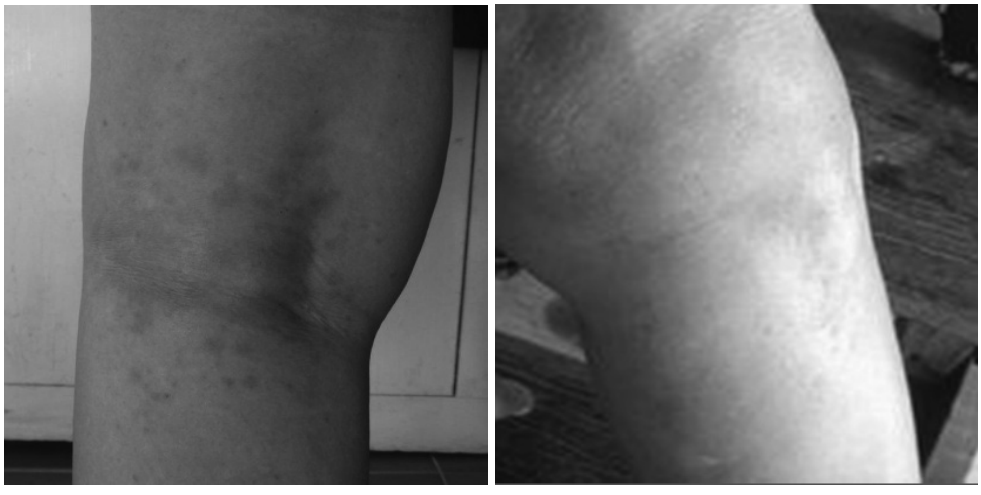

Figure. 1 Contact dermatitis found in the patients who were excluded from the study.

Table1. Demographic characteristics of patients with knee OA (n=20)

Characteristic		**Frequency %**		
Gender; Male		3	15	
Female		17	85	
Radiographic classification of knee OA, Kellgren and Lawrence X-ray				
Grade II		5	25	
Grade III		15	75	
	Mean	**SD**	**Median**	**Range**
Age, years	65	6.3	63	53-75
Duration of disease	2.8	1.5	3	1-7
Body Mass Index, kg/m^2	25	3.4	25	25-30
Heart rate, bpm	72	8	74	62-85
Systolic blood pressure, mmHg	126	14	130	95-150
Diastolic blood pressure, mmHg	70	8	70	52-85
Screening Index of severity for OA (score 0-24)	5.5	2	6.5	4-8
Baseline laboratory values, (normal range)				
Biochemical Parameters				
Alkaline phosphates (30-120 U/L)	83	25	72	52-138
AST (0-50 U/L)	28	18	22	14-93
ALT (0-45 U/L)	33	28	29	10-73
BUN (5-23 mg/dl)	15.7	5	14.1	9-25
Creatinine (0.7-1.3 mg/dl)	0.93	0.2	0.90	0.70-1.40
	Mean	**SD**	**Median**	**Range**
Hematology				
White Blood Cell Count (4.00-10.00X10 3/uL)	7.7	1.8	7.2	4.5-6.0
Total RBC count (3.5-5.50X10 6/uL)	4.3	0.5	4.8	3.0-6.6
Platelet count (150-400X10 3/uL)	285	70	295	171-311
Hematocrit (35-40%)	38	4.4	37	23-47

Clinical Efficacy

Eighteen patients completed the study protocol treatment; and were reported their outcomes in both efficacy and safety from the beginning through the 30 days of evaluation period. The results of clinical efficacy of the treatment results were shown in Table 2.

The mean of the WOMAC score in three subscale of WOMAC at the baseline and 30 days follow-up period after treatment demonstrated the improvement efficacy after the starting baseline. The paired *t*-test results showed the significant improvement of WOMAC in all subscale when compared with the baseline and thirty days of treatment (Table 2). Similarly, the mean of KOOS score in all subscale revealed the significant improvement from the baseline (Figure 2). Additional, interesting finding was the OMERACT-OARSI responder criteria showed a response rate more than 50% in three domains (Table 3.).

Table 2: Efficacy evaluation in the knee OA patients for 30 days (n=18)

Efficacy variable	n	Baseline score Mean (SD)	Change in score mean (95% CI)	*p*-value*	Effect size
WOMAC Scale					
- Pain score (0-50)	18	21.8 (9.9)	9.1(3.4-14.9)	0.004	0.66
- Physical function score (0-150)	18	73.2 (28.9)	26 (11.5-41.2)	0.010	1.20
- Stiffness score (0-20)	18	10.5 (3.9)	4.0 (1.1-7.0)	0.002	0.84
KOOS Scale					
- Symptom score (0-20)	18	11.0 (2.4)	2.9 (2.2-1.9)	0.009	1.25
- Pain and Stiffness score (0-44)	18	13.1 (3.0)	3.3 (5.9-0.8)	0.013	1.10
- Daily living score (0-68)	18	24.7 (11.2)	8.0 (16.5-5.0)	0.063	0.72
- Sport and recreation score (0-20)	18	12.0 (4.6)	3.3 (5.7-0.8)	0.012	0.71
- Quality of life score (0-20)	18	7.7 (3.2)	1.2 (2.4,0.4)	0.007	0.40
Patient's global assessment	18	6.2 (1.0)	1.8 (1.2-2.3)	<0.001	1.80

* *p*-value for the difference between the baseline and after treatment (paired t-test).

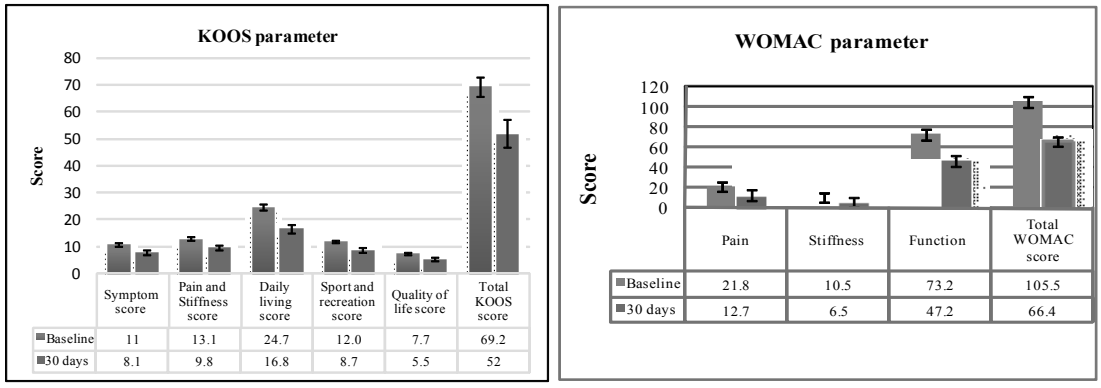

Figure 2. The main efficacy outcomes measured by WOMAC and KOOS before and after the treatment

Table 3: Efficacy evaluation of the OMERACT-OARSI responder criteria

Efficacy variables	n	Number (%) of Responder
- 50% reduction in pain	18	9 (50)
- 20% improvement in function	18	12 (66)
- 10% improvement in PGA	18	10 (55)
- OMERACT-OARSI responder*	18	12 (66)

*A responder is defined as a participant with 1) 50% reduction in pain or 50% improvement function, 2) 20% reduction in pain or 20% improvement function, adding10% improvement of the PGA

4. Discussion

Osteoarthritis commonly affects the elderly people, and if they have associated co-morbid condition can lead to various treatments. Published guidelines have a recommendation to use topical NSAIDs as a treatment for OA of the knee. However, NSAIDs can affect on antihypertensive medication use, if patient is suffering from cardiovascular (CVS) disorder and is given a selective COX-2 inhibitor there is further increased risk of CVS adverse events. If NSAIDs are given to patients with DM, it way increase risk of nephropathy. Since the limitations of NSAIDs; the alternative therapies are needed for reducing the incidence of adverse effects. In Indian and Chinese traditional medicines, ginger has been used for a long time in the treatment of rheumatic disorders because of its ability to inhibit arachidonic acid metabolism which leads to its anti-inflammatory properties. The active components from ginger are 6-gingerol and 6-shagaol. In animal model, ginger has been shown to act as a dual inhibitor of both cyclooxygenase (COX) and lipooxygenase (LOX). It is able to inhibit leukotriene synthesis and to reduce carregenan-induced rat-paw edema in the animal model of inflammation. Our present results showed that ginger extract in NLC had the effect

to relieve joint pain and improve problematic symptoms in WOMAC and KOOS parameters. In a report by Altman and Marcussen (2001), 247 patients with knee OA who have taken combination of ginger and Plai orally for 6 weeks, after the treatment their WOMAC scores were improved significantly and stiffness subscale showed the highest degree of improvement. But in this present study found the highest improvement in physical function (Figure 2.). In a double-blind, randomized controlled trial performed by Sunyarn *et al*., the effect of the combination of 4% ginger and Plai extract in gel (Plygersic gel) as compared with a 1% solution of diclofenac in patients with knee OA. The result showed that the combination of Plygersic gel had significant improvement in quality of life, pain and symptoms, and decreased range of motion, improved patient's ADL, increased sport activities when measured by KOOS within 2 through 6 weeks of treatments. Similar results were shown in this study.

This study is the first recorded clinical trial of the topical use of ginger extract in NLC. The ginger extract was formulated in a topical form in NLC which contained 0.598% of 6-gingerol per 100 g. In most of publications, many research groups paid attention to the application of NLC for topical/dermal routes. This can be avoided by the use of special approaches enhancing the drug absorption and delivery to the target site (Pradit-bongkotchp, 2010). In the present study, the improvement in their knee pain and other symptoms after treatment for 4 weeks were comparable with the baseline. The present findings showed that there was statistically significant improvement in the efficacy variables in OA knee patients after treatment with ginger extract in NLC (Table 2). An efficacy evaluation with OMERACT-OARSI responder criteria initiative used consensus approach to derived dichotomous responder criteria. In the trial of the topical diclofenac, it showed the responder rate of 65.7%. The OMERACT-OARSI has never been used in the topical ginger extract but the researcher applied its criteria to this study and found a responder rate for ginger extract in NLC of 66% (Table 3). Its efficacy was approximately similar to the topical diclofenac data. Safety or adverse event of ginger extract NLC, there was no serious adverse event in any clinical observation or laboratory test. However, a minor apply-sited skin reaction such as contact dermatitis was found in 2 patients. This finding is similar to the result of the previous investigations by Sunyarn *et al*. in 2012. The patient received Plygersic gel a 1 g solution applied 4 times a day and had a contact dermatitis. The patient had to be removed from the study and the patient received topical corticosteroids for a period of one week with the resolution of the symptoms.

Limitation

Ginger is popular used as a food spice, which could be confounder with treatment. So, the next study would design in a randomized controlled trial (RCT).

5. Conclusion

Ginger extract in NLC has a statistically significant efficacy in patients with OA knees. A

4-week treatment with ginger extract in NLC could relieve joint pain and improve problematic symptoms and the quality of life in knee OA patients. The topical ginger extract in NLC may be studied in Phase III in the future.

Acknowledgements

This research received financial support from “Integrated Innovation Academic Center: IIAC the Chulalongkorn University Centenary Academic Development Project” (CU56-AS06).

References

Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of crite riafor the classification and reporting of osteoarthritis. Classification of osteoar thritis of the knee. Diagnostic and Thera peutic Criteria Committee of the Ameri can Rheumatism Association. *Arthritis Rheum* (1986): 29: 1039 - 49.

Altman RD. Early management of osteoarthritis. *Am J ManagCare*; 16 Suppl Manage ment: S41-7, (2010).

American college of rheumatology subcommittee on rheumatoid arthritis guidelines. Guide lines for the management of rheumatoid arthritis. *Arthritis Rheum*. (2002):46(2): 328-46.

Anonymous. Zingiberoffcinale (ginger). *Alternative Med.* Rev. (2003) 8, 331–335.

Bellamy N, Kirwan J, Boers M, Brooks P, Strand V, Tugwell P, Altman R, Brandt K, Dougados M, Lequesne M: Recommen dations for a core set of outcome measures for future phase III clinical trials in knee, hip, and hand osteoarthritis. Consensus development at OMERACT III. *J Rheumato*l1997, 24:799-802.

Bliddal H, Christensen R.The treatment and prevention of knee osteoarthritis: a tool for clinical decision-making. *Expert OpinPharmacother* (2009); 10:1793-804.

Chaipinyo K. Osteoarthritis Outcome Scores (KOOS) Thai version Test-Retest Reliability and Construct Validity of the Thai Version of Knee Osteoarthritis Outcome Scores (KOOS). *Thai Journal of Physical Therapy* .(2009) Vol.31No.2

Chrubasik, S., Pittler, M.H., Roufogalis, B.D. Zingiberisrhizoma: a comprehensive review on theginger effect and efficacy profiles. *Phytomedicine* 12(2005): 684–701.

Grazio S, BalenD.Obesity: Risk factor and predictors of osteoarthritis, (2009) ; 131: 22-6.

Kapstad , K., Rustoen, T., Hanestad, B.R., Moum, T., Langeland, N., &Stavem, K. Changes in pain, stiffness and physical function in patients with osteoarthritis waiting for hip or knee join replacement surgery. The Norwegian System of Compensation to Patients. Faculty of Nursing Education. Oslo University College. Oslo, Norway, (2007).

Kellgren JH, Lawrence JS. Radiological assessment of osteoarthritis. *Ann Rheum Dis* 1957; 16: 494502.

Kuptniratsaiku lV., Rattanachaiyanont M. Validation of a modified Thai version of the Western

Ontario and McMaster (WOMAC) osteoarthritis index for knee osteoarthritis. *ClinRheumatol* (2007) 26:1641–1645.

Lequesne MG, Mery C, Samson M, Gerard P Indexes of severity for osteoarthritis of the hip and knee. *Scand J Rheumatoid* 65(Suppl) (1987): 85–89.

Levy, A.S., Simon, O., Shelly, J., Gardener, M. 6-Shogaol reduced chronic inflammatory response in the knees of rats treated with complete Freund's adjuvant. *BMC Pharmacology*, (2006). 6,12.

Levy, A.S., Simon, O.R., Six-shogaol inhibits production of tumour necrosis factor alpha, interleukin-1 beta and nitric oxide from lipopolysaccharide-stimulated RAW 264.7 macrophages. *The West Indian Medical Journal*, (2009). 58, 295–300.

Mascolo N, Jain R, Jain SC, Capasso F. Ethnopharmacologic investigation of ginger (*Zingiberofficinale*). *J Ethnopharmacol*. 27(1/2) (1989):129-40.

Michel, D. J., Knodel, L. C. Comparison of three algorithms used to evaluate adverse drug reactions, *American Journal of Hospital Pharmacy*, (1986) 43: 1709–1714.

Mustafa T, Srivastava K C. Ginger (Zingiberofficinale) in migraine headache. *Journal of Ethnopharmacology* 29: 267-273, 1998.

Niempoog, S., P. Siriarchavatana and T. Kajsongkram. The efficacy of Plygersic gel for use in the treatment of osteoarthritis of the knee. *J Med Assoc Thai*. 95 Suppl 10(2012): S113-9.

Pham T, van der Heijde D, Altman RD, Anderson JJ, Bellamy N, Hochberg M, Simon L, Strand V, Woodworth T, Dougados M: OMERACT-OARSI initiative:Osteoarthritis Research Society International set of responder criteria for osteoarthritis clinical trials revisited. *Osteoarthritis Cartilage* 2004, 12:389-399.

Philip A, Lisa M and Zev Shainhouse. Treatment of osteoarthritis of the knee with a topical diclofenac solution: a randomized controlled, 6-week trial. *BMC Musculoskeletal Disorders* 2005, 6:44 doi:10.1186/1471-2474-6-44

Praditbongkotchp A. Formulation of Microemulsion gel of *Zingiberofficinale* Roscoe Extract Evaluation of Anti-inflammatory activity in rats. Master of Science Faculty of Pharmaceutical Science Chulalongkorn University, (2010).

Roos EM and Toksvig-Larsen S: Knee injury and Osteoarthritis Outcome Score (KOOS) validation and comparison to the WOMAC in total knee replacement. *Health Qual Life Outcomes* (2003): 1:17.

Shibata,M. Ohkubo, T Takahashi, H. and Inoki,R. Modified formalin test: characteristic biphasic pain response. *Pain* (1989) 38: 347-352.

Thai version of KOOS [Internet]. 2009 [cited 2014Feb 15]. Available from: http://www.koos.nu/ThaiKOOS.pdf

Thomson, M., Al-Qattan, K.K., Al-Sawan, S.M., Alnaqeeb, M.A., Khan, I., Ali, M., The use of ginger (*Zingiber offcinale* Rosc.) as a potential anti-inflammatory and antithrombotic agent; Prostaglandins Leukot. Essent. *Fatty Acid* (2002) 67, 475–478.