# EVALUATION ON THE EFFECT OF ORAL *CHANNA STRIATUS* EXTRACT VERSUS GLUCOSAMINE SULPHATE ON KNEE OSTEOARTHRITIS IN HUMAN MODEL

## Introduction:

Osteoarthritis is the leading cause of chronic disability at older ages, especially due to knee and hip involvement. The American College of Rheumatology (ACR) has developed diagnostic criteria for osteoarthritis (OA) at various sites, including the hip, the knee and hand. Classification Criteria for Osteoarthritis of the Knee includes knee pain plus osteophytes on radiographs and at least one of the following: patient age older than 50 years, morning stiffness lasting 30 minutes or less, or crepitus on motion (Altman *et al*. 1986).

The prevalence of knee osteoarthritis is increasing rapidly with advancing in age. The incidence of knee OA in the Western European countries has been estimated to be 18% to 25% in men and 24% to 40% in women between 60 to 79 years of age and there are about 100 million persons with knee OA in European Union (Altman *et al*. 2006). From Clinical Practice Guideline Malaysia, it is estimated the prevalence of symptomatic knee OA is 30% among the population aged more than 65 years old. A study done in Malaysia showed that 9.3% of adult Malaysians complained of knee pain with an increase in pain rate to 23% in those over 55 years of age and 39% in those over 65 years (Ministry of Health 2002). This prevalence of osteoarthritis is expected to grow in the next several decades as the global population ages and live longer.

The pathophysiology of OA is the interaction of mechanical, cellular and biomechanical processes. Cartilage is composed of water, collagen, and proteoglycans. In healthy cartilage, remodeling occurs when there is degradation of cartilage, where it will be replaced by the chondrocytes. This chondrocytes will form a new articular cartilage. It is a balanced process between degradation and remodeling. However, this process becomes disrupted in osteoarthritis, leading to cartilage damage, joint spaces narrowing, subchondral cysts and osteophytes formation. When degradation exceeds cartilage synthesis, osteoarthritis develops. (Ralph *et al*. 2002)

Osteoarthritis is the leading medical condition for which persons use alternative therapies (Resch *et al.* 1997). Patients often seek alternative therapies after having side effects or gaining incomplete relief of symptoms with conventional medications. Alternative therapies used for the treatment of osteoarthritis include herbs, supplements, acupuncture, and electromagnets. Americans spend more on natural remedies for osteoarthritis than for any other medical condition (Vincent *et al.* 2003).

Channa striatus (CS), a fresh water snakehead fish consumed in many parts of the Southeast Asian region, for centuries is known among local for protein and traditional remedy (Wee KL 1982). Traditionally, it is believed to promote wound healing, as well as reduce post-operative pain (Mat Jais *et al.* 1997 and Mat Jais *et al.* 1998). Haruan is the local name for CS. It has been considered as a very good source of health food among Asians because it contains high levels of amino acids and fatty acids (Zuraini *et al*. 2006). The work carried out indicated that the fishCShas a good analgesic property comparable to morphine (Zakaria *et al.* 2007). The analgesic properties make it suitable for reduction of post-operative pain (Mat Jais *et al.* 1997).

Studies had shown that CS extract also had anti-inflammatory activities (Mohd. Hassan 2005, Michelle *et al*. 2004, As-saffar *et al.* 2011, Sadegh et al. 2012, Zakaria et al. 2008). Given the possible anti-inflammatory property of CSextract, its use in treating diseases with an inflammatory component has been explored in the amelioration of osteoarthritis (Michelle *et al*. 2004, As-saffar *et al.* 2011). However, the exact mechanism on how it works as anti-inflammatory agent is still not clear. *CS* extract would be one of the alternative therapies for knee OA patients. It uses also will reduces the usage of NSAIDS, thus reduces the adverse effects secondary of its usage such as gastrointestinal and renal complications

A study done by Michelle *et al.* in 2004 showed that CS extracts improved osteoarthritis in rabbits (Michelle *et al*. 2004). The treated group was given CS extract orally and the control group received normal saline. The outcomes was evaluated by radiographic and innervations of the synovial membrane after 9 weeks of treatment. Results showed there was significant reduction in soft tissue swelling observed in radiograph for treated animals compared with untreated and there was significantly improvement in the density of PGP 9.5-immunoreactive nerve fibers in the synovial membrane of treated animals compared with that of controls. (Michelle NYT, Shanthi G and Mohamad YL 2004)

A study done by As-saffar *et al*. in 2011, have demonstrated that CS extract acts as anti-inflammatory by regulating the synthesis of pro-inflammatory hormone (prostaglandin E2) in rats with knee OA. In that study, it showed that animal that received CS extract had levels of PGE2 reduced significantly which is comparable in animals treated with celecoxib which is a group of COX-2 inhibitor. It is postulated that CS extract works through inhibition of the cyclooxygenase expression which is an essential enzyme in catalyzing arachidonic acid and subsequest formation of prostaglandins (As-saffar *et al*. 2011). The ability of CS extract which contain arachidonic acid to exhibit anti-inflammatory activity is not well understood. This is because arachidonic acid is a precursor for prostaglandins that are mediators in nociceptive and inflammatory processes.

Therefore, based on the animal studies it had been shown that CS is beneficial in in the amelioration of knee osteoarthritis however the exact mechanism is still not clear. It is beneficial to compare the effect of CS extract with glucosamine sulphate which is a popular prescription for relieving symptoms of osteoarthritis in particular knee osteoarthritis in animal model study. Based on previous studies (Michelle *et al*. 2004, As-saffar *et al.* 2011), it is postulated that the CS extract works not only through anti-inflammatory pathway. The high content of amino acids and fatty acids in CS extract helps in the remodeling of collagen via the synthesis of inter- and intra-molecular protein linking (Michelle *et al*. 2004). This action strengthens articular cartilage, thus reducing the fragmentation of degrading articular cartilage into the joint.

A randomized, double-blind, placebo-controlled trial comparing the effects of oral CS extract 500 mg/day with placebo given for 3-month intervention period had been done among primary knee osteoarthritis patients (Azidah *et al.* 2011). In that study, there were significant improvement of pain, symptom scores and quality of life (QOL) domain score (p<0.05) in *C. striatus* compared to placebo group (Azidah *et al.* 2011).

Glucosamine sulphate (GS) has been reported to be effective for the treatment of knee OA and has been recommended as a pharmacologic intervention (Jordan*et al.* 2003). Two 3-year clinical trial have independently provided evidence of GS efficacy in the long term management of knee OA patients (Reginste *et al*. 2001, Pavelka*et al.* 2002) as an analgesic and also as a disease modifying drugs which potentially delay the joint structure changes in OA. Due to this evidence, Glucosamine sulphate has been used worldwide for knee OA patients.

It is postulated that a higher dose of CS with a longer durationof administration may give beneficial effect to knee OA patients. The other objective of this study is to assess whether CS is better than Glucosamine sulphate and this will offer new evidence to use CS as an alternative therapy in the treatment of knee OA. This study will offer new insight, generated new ideas of using our own natural remedies as one of the alternatives therapy in osteoarthritis. It also can promote CS product and helps the agricultural sector to develop and increase our economic activities to a higher level in the future.

## OBJECTIVES:

**General objectives:**

Evaluations on the efficacy of oral *Channa striatus* extract versus Glucosamine Sulphate on knee osteoarthritis in human model.

**Specific objectives:**

Comparison between 1000mg/day or 500mg/day *Channa striatus* extract, placebo and 1500mg/day of glucosamine sulphate among knee OA patients attending KRK and orthopaedic clinic HUSM in terms of

1. Pain, stiffness, physical function score and overall score using WOMAC
2. Consumption of NSAIDs or Paracetamol by using analgesic score
3. The level of inflammatory marker - cartilage oligomeric matrix protein (COMP) ) and PGE2 level and COX level
4. Quality of life using Osteoarthritis Knee and Hip Quality of Life (OAKHQOL).

**Hypothesis**

There is significant reduction in pain, physical function and overall score using WOMAC in the CS compare to glucosamine group

There is significant reduction of the analgesic score in the CS compare to glucosamine group

There is significant reduction of the inflammatory markers – COMP, PGe2 and COX level in CS compare to glucosamine group

There is significant improvement of quality of life using OAKHQOL in the CS compare to glucosamine group

There is better efficacy in the CS group of 1000mg/day than CS group of 500mg/day as evidence in reduction WOMAC, analgesic score, inflammatory markers and improvement in OAKHQOL

# METHODOLOGY:

## STUDY DESIGN

This is a randomized controlled trial, double blind, four-arm parallel comparative study of 1000mg/day oral *Channa striatus* extract, 500mg/day oral *Channa striatus* extract, placebo and 1500mg/day glucosamine sulphate among patient who had primary knee osteoarthritis.

## SOURCE POPULATION.

Patient attending Klinik Rawatan Keluarga or Orthopaedic clinic HUSM in 2014

## STUDY POPULATION:

Inclusion and exclusion criteria:

The following inclusion and exclusion criteria will be applied in this study:

### INCLUSION CRITERIA:

1. All patients with unilateral or bilateral knee osteoarthritis according to clinical and radiological criteria of the American College of Rheumatology (ACR) (Altman *et al.* 1986)

Knee pain and radiographic osteophytes plus at least one of three symptoms/signs listed below:

-age more than 40 years OR

-stiffness less than 30 minutes OR

-crepitus.

This criteria is widely accepted as the standard diagnosis criteria for knee OA with 91% sensitivity and 86% specificity.

1. Have a radiological grade between I and III, as measured with the Kellgren-Lawrence method of Classification.
2. Patients have symptoms for at least three months.

### EXCLUSION CRITERIA:

1. Patient with secondary knee osteoarthritis, such as post traumatic OA, inflammatory arthritis, specifically rheumatoid arthritis, active gout
2. Disabling co-morbid condition such as renal disease, liver disease, neoplasm, and other rheumatic diseases.
3. Pregnancy or nursing.
4. Patient with severe knee pain and willing for surgical intervention.
5. Those who had joint lavage, arthroscopy, or treatment with hyaluronic acid during the previous 6 months.
6. Patient who had been treated with intra-articular corticosteroids during the past 3 months.
7. Patient who have allergic to oral *C.striatus* or Glucosamine

## DETERMINATION OF SAMPLE SIZE.

Sample size was calculated for all objectives. However, only the one that yielded the biggest sample size was taken as the study sample. Using Power and Sample Size calculation software (Dupont and Plummer, 1997)

Objective that yielded the biggest sample size was taken as the study sample size.

Sample size for comparing two means between treatment group (1000mg of CS extract) and placebo group were done. The parameters were as follows:

|  |  |
| --- | --- |
| **α** | **Level of significance = 0.05** |
| **Power** | **0.9** |
| **σ** | **SD of outcome variables** |
| **δ** | **Detectable diff in pop means (clinically sig difference in pain score between *C.striatus* extract group and placebo (cornstarch) group based on expert opinion)** |
| **m** | **Ratio between placebo (conrstarch) group and Channa striatus (ikan haruan) extract group = 1** |

* The biggest sample size yielded by Objective 2 (WOMAC Pain). In a previous study (N. Giordano *et. al* 2009) the response within each subject group was normally distributed with standard deviation 2.3. The detectable difference of ~~1.2~~ 1.9 between experimental and control groups was based on expert opinion.
* The minimum required sample size was ~~37~~  32 and after considering the drop out rate of **20%**, the sample size calculated for each group was ~~60~~ 39. Therefore the total subjects in this study is 156 subjects.

## DATA COLLECTION PROCEDURE.

The study will be conducted after the approval from USM ethical committee and the study protocol will follow the principles of the Declaration of Helsinki. All patient fulfilled the inclusion criteria will be recruited from Outpatient Clinics HUSM and Orthopaedic clinic Hospital Raja Perempuan Zainab II HUSM. All patients in the study were given information regarding the study conducted.

At visit 1, after the participants have agreed to involve in this study, the informed consent will be obtained. The patients are required to fill the social demographic data, medical history and history regarding knee pain and previous treatments for knee osteoarthritis. Then, they were required to fill the WOMAC index and OAKHQOL. Basic physical examination including body weight, height will be taken. Knee radiograph (AP and lateral) and baseline renal profile and liver function test will be done at this time. Patient who is on Glucosamine will be instructed to stop the medication. A wash-out period of 2 weeks will be given if the patient is eligible before the patient is randomized.

At second visit (week 2), 10 ml of venous blood, non-fasting will be taken from the patients for COMP, PGE2 level and COX level.Then, the subject will be randomized into four groups;

**Group A** : Subject will be receiving 1000mg/day oral *Channa striatus* extract

**Group B**: Subject will be receiving500mg/day oral *Channa striatus* extract

**Group C** : Subject will be receiving 250mg/day of placebo (cornstarch)

**Group D** : Subject will be receiving Glucosamine Sulphate 1500mg/day

The subjects will be taking the treatment for the duration of 6 months. For the duration of the study, it was recommended that patients not modify their therapeutic program unless adverse events occurred and required management. In particular, they were instructed to avoid corticosteroids and hyaluronic acid infiltrations, arthroscopic surgery, and joint lavage, and to avoid treatment with disease-modifying OA drugs. Violation from the protocol will cause the participants to be excluded from the study. Patient also needs to report to investigator any possible side effects after taking the medication.

Each patient will be assessed at month 3 and 6 after randomization. During month 3 visit, patients will be assess using WOMAC index and OAKHQOL. The analgesic diary also will be reviewed. The blood for renal and liver function test will be taken during this visit.

At month 6 (last visit), the patients will be assess using WOMAC index and OAKHQOL. The analgesic diary also will be reviewed. The blood for COMP, PGE2 level and COX level will be taken.

## RESEARCH TOOLS.

### SOCIO-DEMOGRAPHIC AND MEDICAL DATA

The following data will be collected in the case report form:

1. Age, sex, race, background education, occupation, total income monthly.
2. History of osteoarthritis, the localization of knee pain, and duration of the symptoms.
3. Medical history.
4. Drugs history for treatment of knee osteoarthritis
5. Physical examination (height, weight and BMI)

### WESTERN ONTARIO AND MCMASTER UNIVERSITY OSTEOARTHRITIS

### INDEX (WOMAC)

Western Ontario and McMaster University Osteoarthritis Index (WOMAC) will be used to assess the clinical severity of OA patients in the study group and it is widely used in OA research (Bellamy *et al*. 1991). The WOMAC index measure total pain score, total stiffness score and total physical functioning score. The western Ontario and McMaster Universities (WOMAC) osteoarthritis index is a disease-specific self-administered health status measure that is widely accepted as reflective of Osteoarthritis disease activity. The original index consists of 24 Questions (5 questions for pain, 2 questions for stiffness and 17 questions for physical function). Individual question response is assigned a score of between 0 (none) to 4 (extreme) and summed to form a score ranging from 0 (best) to 96 (worst). There are three sections to the WOMAC score; section A deals with the amount of pain (5 questions), section B address the amount of joint stiffness (2 questions), section C address aspects of physical function (17 questions).It has been validated in Bahasa Malaysia. Patients will be assess by this questionnaires at baseline, month 3 and month 6.

**ANALGESIC SCORE**

Previous history of analgesia usage will be taken. Analgesia is allowed to continue after the enrollment of this study. Patients are allowed to consume Acetaminophen 1 gram for rescue analgesia when necessary for mild to moderate pain. For patient who do not tolerate if the pain is severe, Ibuprofen 400 miligram is allowed.

Acetaminophen is most widely used as analgesia and antipyretic. Acetaminophen is completely absorbed from the gastrointestinal tract and, after oral administration, peak plasma concentrations are reached in less than an hour. The drug is fairly uniformly distributed in the body and approximately 90% of a therapeutic dose is eliminated by conjugation with glucoronic acid in the liver ; 3-5% is catabolized to the acid and cysteine conjugates by the P-450 mixed function oxidase enzyme system . All of these metabolites are excreted in the urine and in fact only a slight amount of the drug is excreted unchanged. It is the intermediate metabolites formed during the biotransformation in the liver (whose structures are uncertain) that are believed to be responsible for the hepatotoxicity of the drug. The biologic half-life of acetaminophen in normal adults is about 2-3 hours.

Ibuprofen is chemically described as 2-(4-isobutylphenyl)propionic acid and is a non-steroidal compound, which exhibits anti-inflammatory, analgesic and antipyretic activities. Ibuprofen is well absorbed on oral administration. An oral dose taken on an empty stomach by human volunteers produced peak serum levels after three quarters of an hour. Absorption was slower and peak serum levels lower after food.Excretion is rapid with no evidence of accumulation. Ibuprofen is the safest and appears to have the lowest risk of gastrointestinal side effects of all the traditional NSAIDs (Giordano *et al.* 2009).

Patients were allowed to consume tablet Acetaminophen 1 g or tablet Ibuprofen 400 mg as needed for rescue analgesia. The patients were asked to document the types, dosage and number of analgesia tablets taken in the Analgesia Diary and bring it at every visit. The purpose of the diary is to calculate the analgesic score. The scoring system was made based on Ibuprofen, Paracetamol Study in Osteoarthritis (IPSO) study (Boureau*et al.* 2004) whereby Acetaminophen 1000mg scored as 1 and Ibuprofen 400mg scored as 2.5. Patients are not allowed to change to other form of analgesia without prior informing the investigators. The score was assessed at month 3 and month 6.

### OSTEOARTHRITIS KNEE AND HIP QUALITY OF LIFE (OAKHQOL).

This questionnaire was developed to specifically assess quality of life in knee and hip OA patients (Rat *et al*. 2006). The original questionnaire was developed in French ((Rat *et al*. 2006). This is a self administered questionnaire comprising of 43 items divided into five dimensions- physical activity, mental health, pain, social support and social activities and three additional items ((Rat *et al*. 2006). The three additional dimensions were relationship, sexual activity and professional life. The Likert response scores vary from 0 (worst) to 100 (best possible). In each domain, the mean scores of the items is calculated yielding the scores for the domain. It was shown to capture patients’ perceptions of their disease, and possesses the necessary psychometric properties of validity and reliability for use in clinical trials and observational studies (Rat *et al*. 2006).

The English version of the questionnaire will independently translated into Malay by two translators and back-translated to English to evaluate the equivalence of the questionnaire. A pilot study on 100 patients will be conducted in order to evaluate the acceptability and feasibility of the items, as well as to determine the validity (factor loading) and reliability (Cronbach alpha) of the questionnaire. Modification will be made based on the results to yield the final Malay version OAKHQOL questionnaire.

### LABORATORY EVALUATION (LABORATORY ASSESSMENT)

A. Safety assessment based on hematological and biochemical parameters will be conducted on all patients enrolled in this study at baseline and month- 3 of the study. Non - fasting blood samples will be collected in heparinised tubes. Five ml will be drawn from each subject and will be analyzed for the measurement renal and liver function test.

The profile as follows:

1. Renal function - serum Creatinine
2. Liver enzymes – Aspartate transaminase (AST) & alanine transaminase (ALT)

If any of these parameters analyzed are abnormal, the patient shall be informed immediately and withdrawn from the study with immediate effect.

B. Biomarkers: COMP

A non - fasting blood samples will be collected. Five ml will be drawn from each subject. The investigations will be done at baseline and month 6.

## BIOMARKERS FOR OSTEOARTHRITIS

Biomarkers are generally considered to be biological substances. The performance of a biomarker is characterized by sensitivity and specificity, or positive and negative predictive values. However, most of the biomarkers for osteoarthritis do not have data on their predictive capability yet (Guangju and Erfan 2012). Articular cartilage is composed of chondrocytes embedded in extracellular matrix (ECM) which provides the biomechanical and physiologic characteristics that are essential for articular movement (Huang and Wu 2008). Type II collagen (CII) provides the major portion of the organic component in the ECM, followed by aggrecan and other non collagenous proteins including cartilage oligomeric matric protein (COMP) (Charni-Ben *et al.* 2007). The two most investigated biomarkers are CTX-II and COMP (Guangju and Erfan 2012). However, a large randomized clinical trial of knee joint osteoarthritis showed that CTX-II failed as a biomarker to reflect the efficacy of response in clinical trial (Garner et al. 2008).

Cartilage oligomeric matric protein (COMP) is a tissue specified matrix thrombospondin-family protein that is synthesized by chondrocytes. It is abundant in OA cartilage, and can also be measured in a serum and synovial fluids (Rousseau and Delmas 2007). Its concentration is ten times higher in the synovial fluids than in serum (Rousseau and Delmas 2007). However, its role in osteoarthritis pathogenesis remains unclear.

COMP interacts with collagen and is suggested to have a role in regulating fibril assembly as well as structural role for maintaining the mature collagen network (Heingard *et al*. 2005). Studies on experimenting arthritis have demonstrated that changes in serum level of COMP reflect processes in cartilage. The used of COMP as a prognostic indicator and a marker of ongoing joint damage in osteoarthritis and Rheumatoid Arthritis has been suggested (Petersson et al. 1998, Lindqvist et al. 2005).

**C. Inflammatory markers: PGE2 and COX**

**Sample processing and analysis**

**Serum sample**: Separation from the collected whole blood after centrifugation at 3500rpm for 10 mins and the serum is kept at -70 C for further analysis.

* + 1. Analysis of inflammatory markers (COMP, PGE2 and COX)

by ELISA on ELISA reader.

* + 1. Creatinine analysis

Chemistry analyzer Hitachi 912

* + 1. AST & ALT analysis

Chemistry analyzer Hitachi 912

## INVESTIGATIONAL PRODUCTS.

The orally administered freeze dried *C.striatus* extract and the glucosamine sulphate will be prepared in by a GMP-certified Laboratory, School of Pharmacy University Sains Malaysia. The freeze dried *C.striatus* extract and the glucosamine sulphate will be put into 4 capsule of “1” size. Therefore, each patient will be taking two capsules twice a day irrespective of their groups (total of 4 capsules/day). This will ensure proper blinding.

**CS *extract preparation***

CS (whole fish) are cleaned, weighed and placed into the autoclave bin. The water mixture for autoclaving is prepared in relative to fish weight with respect to the volume of the water used. Then, preservative is added. The preservatives used in this study are a combination of two esters of *p*-hydroxy benzoic acids which are Methyl Paraben 0.1% and Propyl Paraben 0.02%.

The sterilization is carried out with temperature setting of 110°C for 15 minutes. Upon completion of the sterilization, the fish are mixed and meshed thoroughly and then dried using industrial oven at 60°C for 48 hours continuously. Upon completion, the sheets of crispy flakes are grinded and make into refined powder.

## BINDING PROCEDURE ,DOSAGE AND RANDOMIZATION METHODS

The study statistician randomizes assignments as generated using computer (blocks of eight) that provided allocation of subject numbers in a ratio of 1:1:1:1 to either 1000mg/day oral *Channa striatus* extract, 500mg/day oral *Channa striatus* extract, placebo group or 1500mg/day Glucosamine Sulphate using Sealed EnvelopeTM method. These assignments are then puts into sealed, opaque envelopes, numbered sequentially and sent to a study staff member that involved within the field work. The staff will open the consecutive envelope to randomize eligible patient accordingly.

Only two co-investigators who prepared the product and a statistician knew the randomization scheme. Subject numbers were allocated strictly sequentially,

The medication will be taken orally, once daily. It can be taken at any time with or without a meal. All the study medication will be identical in look, taste, and smell. It will be packed in a white plastic bottle container with 100 capsules per one container.

The subject will be divided into four groups;

Group A: Subject will be receiving 1000mg/day oral *Channa striatus* extract

Group B: Subject will be receiving500mg/day oral *Channa striatus* extract

Group C : Subject will be receiving 250mg/day of placebo (cornstarch)

Group D : Subject will be receiving 1500mg/day of Glucosamine Sulphate

## 5.10 TREATMENT COMPLIANCE.

Subject will be asked to return all the unused medication. The number of capsules issued and minus the number of capsules returned will be used to calculate the capsules taken. From this information, compliance will be calculated.

Compliance = Capsules taken during the period X 100

Capsules which should have been taken

Any subject taking less than 75% will be considered non-compliant.

## 5.12 STATISTICAL ANALYSIS

Analyses will be done by using SPSS for windows version 20.0. Randomized groups will be compared compared for any possible differences at baseline using ANOVA and simple logistic regression. To determine the differences in the outcome parameters, repeated measures analysis of variance (RM ANCOVA) will be used while controlling for weight and age for WOMAC and OAKHQOL. Changes were reported as estimated marginal means with its adjusted 95% Confidence Interval (CI). All reported *p-*values are 2-tailed with a value of less than 0.05 which is considered significant. For analgesic score and laboratory parameters, there was no co-variate considered and ANOVA test will be used to detect the difference of the score between both groups.

**Ethical considerations**

Ethical approval was obtained from the Human Ethical Committee

**Reference:**

Altman, R., Asch, E., Bloch, D., Bole, G., Borenstein, D., Brandt, K., *et al.* 1986. Development of criteria for the classification and reporting of OA. Classification of Osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum*, 29, p.1039-49.

Altman, R.D., Abramson, S., Bruyere, O., Clegg, D., Herrero-Beaumont, G., Maheu, E., et al. 2006. Commentary: osteoarthritis of the knee and glucosamine. *Osteoarthritis Cartilage*,14, p.9636.

As-saffar, F.J., Ganabadi, S., Fakurazi, S. and Yaakub, H. 2011. Zerumbone significantly improved immunoreactivity in the synovium compared to *Channa striatus* extract in monosodium iodoacetate (MIA)-induced knee osteoarthritis in rat. *J. Med. Plant. Res*,5(9),1701-1710.

Azidah, A. K, Maryam M. Z.,Siti Zubaidah, A. W., Norhayati, M. N., Saringat, B., Juhara H. 2014. The therapeutic effect of oral *Channa striatus* (Haruan) extract on primary knee osteoarthritis patients **Agro Food Industry Hi Tech Journal, 25(3),44-48.**

# **Boureau F., Schneid H., Zeghari N., Bourgeois P.**The IPSO study: ibuprofen, paracetamol study in osteoarthritis. A randomised comparative clinical study comparing the efficacy and safety of ibuprofen and paracetamol analgesic treatment of osteoarthritis of the knee or hip. Ann Rheum Dis. 2004 Sep; 63(9): 1028–1034.

**Gabrielle T., Christiane G., Luc C., John S., Luc M., Mirela I., Agnes R., A. Robin P. and Sheila L. 2005. Effect of oral glucosamine on cartilage degradation in a rabbit model of osteoarthritis. *Arthritis & Rheumatism*, 52(4), 1118-1128.**

N. Giordano, A. Fioravanti, P. Papakostas, A. Montella, G. Giorgi, R. Nuti. The Efficacy and Tolerability of Glucosamine Sulfate in the Treatment of Knee Osteoarthritis: A Randomized, Double-Blind, Placebo-Controlled Trial. Current Therapeutic Research Volume 70, Number 3, June 2009.

Jordan, K.M., Arden, N.K., Doherty, M., Bannwarth, B., Bijlsma, J.W.J., Dieppe, P. et al. 2003. EULAR Recommendations 2003: an evidence based approach to the management of knee osteoarthritis: Report of a Task Force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT).

Mat Jais, A.M., Dambisya, Y.M. and Lee, T.L. 1997. Antinociceptive activity of *Channa striatus* (Haruan) extracts in mice. *Journal of Ethnopharmacology* ,57,125-130.

Mat Jais, A.M., Matori, M.F., Kittakoop, P., Sowanborirux, K. 1998. Fatty Acid Compositions in Mucus and Roe of Haruan, *Channa Striatus* for Wound Healing. *General Pharmacology: The Vascular System* ,30(4),561-63.

Michelle, N.Y.T., Shanthi, G. and Mohamad, Y.L. 2004. Effects of orally administered *Channa striatus* extract against experimentally-induced osteoarthritis in rabbits. *Intern J Appl Res Vet Med*, 2(3), p.171-175.

Pavelka, K., Gatterova, J., Olejarova, M., Machacek, S., Giacovelli, G., Rovati, L.C. 2002. Glucosamine Sulphate Use and Delay of Progression of Knee Osteoarthritis. *Arch Intern Med,* 162,p.2113-3123.

Ralph, H., Ron, L.M., Maj, M.C., Alan, W.D. and Sean F.T., Dwight D.E. 2002. Osteoarthritis: Diagnosis and Therapeutic Considerations. *Am Fam Physician,*65,p.841-848.

Reginster, J.Y., Deroisy, R., Rovati, L.C. *et al*. 2001. Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomised, placebo-controlled clinical trial. *The Lancet,* 357(9252),p.251-256.

Resch, K.L., Hill, S., Ernst, E. 1997. Use of complementary therapies by individuals with ‘arthritis’. *Clin Rheumatol*,16,p.391-395.

Vincent, M., Christopher, N. and Victor, W. 2003. Alternative Therapies for Traditional Disease States: Osteoarthritis. *Am Fam Physician*,67,p.339-344.

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# FLOW CHART OF THE STUDY

Screening (Visit 1)

Informed consent

Demographic data, physical examinations

Screening inclusion/exclusion

WOMAC, OAKHQOL

renal profile, liver function test.

Weight bearing knee x-ray AP, lateral view.

COMP,PGE2,COX

((Visit 2) Randomize Subjects Dispense Study Medication

Give analgesic diary

Group A Group B Group C Glucosamine

*C striatus* 1000mg/day *C striatus* 500mg/day  Placebo sulphate1500mg/day

month 3 (Visit 3) WOMAC, OAKHQOL and Analgesic Diary assessment

Renal function and Liver Function test

month 6 (Visit 4) WOMAC, OAKHQOL and Analgesic Diary assessment ,

COMP,PGE2,COX