Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 10 (4): 248-260, 2010
ISSN 1812-5654

Chondroprotective Effect of Zerumbone on Monosodium Iodoacetate Induced Osteoarthritis in Rats

[1]F.J. Al-Saffar, [1]S. Ganabadi, [2]S. Fakurazi, [3]H. Yaakub and [4]M. Lip
[1]Department of Preclinical Sciences, Faculty of Veterinary Medicine,
University Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[2]Department of Human Anatomy, Faculty of Medicine and Health Sciences,
University Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[3]Department of Animal Science, Faculty of Agriculture,
University Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[4]Analytical Laboratory and Quality Assurance Programmed Technical Services Centre,
Mardi, P.O. Box 12301, Kuala Lumpure, General Post Office 50774, Malaysia

Abstract: The objective of this investigation was to evaluate chondroprotective effect of zerumbone, a purified compound of *Zingiber zerumbet* Smith against monosodium iodoacetate (MIA) induced knee osteoarthritis (OA) in the rat. The effect on the articular cartilage was examined and compared with celecoxib (Celebrex®), a Non-Steroidal Anti-Inflammatory Drug (NSAID). Forty adult male Sprague Dawley rats were divided into four groups (n=10 for each). All animals were injected with MIA intraarticularly in their right knee joints to induce OA. Rats from first and second groups were treated with zerumbone in a same dose but with two different concentrations. Rats in the third group were treated with celecoxib and served as positive control whereas the fourth group were treated with corn oil and served as negative control. Evaluation of OA changes in the knees was assessed with the aid of both radiography and histopathology score. Macroscopic as well as microscopic examinations revealed curative effect of zerumbone in a dose dependent manner on the osteoarthritic knee joints. Apart from this, our data also revealed very poor anti-OA property of celecoxib. We concluded that oral administration of zerumbone in a dose of 2 mL kg^{-1} b.wt. of 0.4% w/v diluted with corn oil for a period of 4 weeks has some chondroprotective effects.

Key words: Osteoarthritis, zerumbone, monosodium iodoacetate, cartilage, medicinal plants, rat

INTRODUCTION

Osteoarthritis is an age-related, chronic joint disease characterized by slow degradation of the articular cartilage with subchondral bone response (Moreland, 2003). The disease is well known to cause a burden on public health in human and a problem cannot be condoned in animals. In human, OA affects a large population with considerable morbidity and disability (Dumond *et al*., 2004). Recent survey of the World Health Organization (WHO) revealed 10% of world population average age 60 years suffers osteoarthritis pain (McDougall, 2006).

In the veterinary field, OA is widely encountered in many animal species. In equine, OA is a naturally occurring degenerative disease which is age related and factors such as stress of racing and training may accelerates its development (Cantley *et al*., 1999). The disease considered the primary cause of equine lameness which leads to economic losses in equine industry (Williams, 2007). Actually, equine OA is a major problem in veterinary medicine, because its recognition in horses represents a challenge for equine practitioners (Jouglin *et al*., 2000). In dogs, reports by pfizer animal health revealed that OA is a progressive degenerative condition affected an estimated 20% of adult dog (North American Veterinary Conference, 1998). In fact, once canine OA becomes clinically apparent the treatment will be directed only to slow down the progression of the disease and improving the general mobility and quality of life of affected dogs (Doig *et al*., 2000).

In cats, OA is very little known in comparison with the dogs. However, radiographic evidences showed higher percentage of aged cats affected due to the disease (Taylor and Robertson, 2004). Recent researchers found that patellar luxation is an important cause of OA in the cats (Loughin *et al*., 2006).

Corresponding Author: F.J. Al-Saffar, Department of Preclinical Sciences, Faculty of Veterinary Medicine,
University Putra Malaysia, 43400, Serdang, Selangor, Malaysia

Previously, it was postulated that OA is a result of both mechanical and biological events, causing disturbance of the normal balance mechanism occurred between catabolic and anabolic processes in the articular cartilage. Chondrocyte in the mature articular cartilage is the only type of cell that is capable for production and maintenance of extracellular matrix. Therefore, any reduction in chondrocyte density will contribute to the development of OA (Creamer and Hochberg, 1997). Biomechanical reckoning indicating that damage of the surface zone of articular cartilage leads to increase loading of the cartilage matrix, resulting in higher stress on the underlying cartilage and subsequent subchondral response (Stoop *et al*., 2001). In another aspect, some factors such as obesity, increased age and joint immobilization can also contribute to the progression of this disease (Goldring and Goldring, 2007).

Currently, there are no commercially available drugs definitely proven to modify the natural progression of OA. Non steroidal anti-inflammatory drugs are widely prescribed for the treatment of OA pain. But the long term use of such drugs may cause suppression of platelet aggregation, erosions and/or ulcerations in upper gastro-intestinal tract mucosa (Bove *et al*., 2003).

In fact the current therapeutic interventions are useful for controlling symptoms, specially the pain. Such adverse side effects may be ameliorated by the use of botanical extracts alternatives.

Extracts from plants have been used for centuries as a popular method for treating several health disorders. The study of the extracts or compounds from the rhizomes of plants has attracted attention in different fields of the biological and medicinal sciences (Grosvenor *et al*., 1995). Extracts from many medicinal plants exerts therapeutic effects such as anti-inflammatory (Faizah *et al*., 2002; Murakami *et al*., 2003; Somchit and Shukriyah, 2003), anti-oxidants (Murakami *et al*., 2002; Ruslay *et al*., 2007) and anti-cancer (Tanaka *et al*., 2001; Huang *et al*., 2005; Yee *et al*., 2006; Sakinah *et al*., 2007), Zerumbone is one of this candidates.

Zerumbone (2, 6, 9, 9-tetramethylcycloundeca-2, 6, 10-trien-1-one) is the crystalline sesquiterpene derived from a subtropical wild ginger, *Zingiber zerumbet* Smith. Its rhizomes have been shown to contain large amount of zerumbone (Szabolcs *et al*., 2007). Zerumbone's anticancer and antioxidant actions attract us to investigate whether it has chondroprotective property against OA. The objective of this investigation was to evaluate curative effect of orally administrated zerumbone in two different concentrations against an experimentally induced OA in the right knee joints of Sprague Dawley rats. The effect on the articular cartilage structures of joints was examined and compared with effect of Celecoxib (Celebrex®), a non-steroidal anti-inflammatory drug.

MATERIALS AND METHODS

Animals: Forty, adult male Sprague Dawley rats, weighing between 275-400 g were used in this study. These rats were kept in an air-conditioned animal room at 22°C (one rat per cage). The rats were given commercial pellet and tap water *ad libitum* and were left for 2 weeks for acclimation before used. This research project was conducted from November 2008 to November 2009 according to the guideline for animal handling and welfare in our facilities. It was approved by Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, University Putra Malaysia.

Induction of osteoarthritis and radiography: Rats were randomly divided into four groups (n = 10/group). For the induction of OA, all rats among these groups received an intraarticular injection of 50 micro 60 mg mL^{-1} MIA into the right knee joint. The day of OA induction was considered as day 0. Prior to inducing OA, all rats were anesthetized with a mixture of ketamine (50 mg mL^{-1}) and xylazine (20 mg mL^{-1}) at a ratio of 2:1.1 mL kg^{-1} b.wt. (Hattori *et al*., 2005). The intraarticular injection of MIA was made through the patellar ligament using a 27 gauge, 0.5 inch needle. The injection was performed once according to the published method (Bove *et al*., 2003).

At day 0 and prior to OA induction, knee joints of randomly selected rats were radiographed under anaesthesia to rule out any abnormalities. At day 15 post-OA induction, the joints were again radiographed to appraise any changes. Treatment with the suggested therapies against OA were conducted at day 16 and continued for 4 weeks until day 43, which was the day the rats were humanely euthanized. Prior to euthanization, rat's knees were also radiographed to evaluate any effects of treatment. Radiographies were conducted on the joints in both craniocaudal and mediolateral positions to monitor joint space impairment and changes in bones morphology and density (Arden and Nevitt, 2006).

Preparation of zerumbone: Zerumbone (99% purity) was isolated from the rhizomes of *Zingiber Zerumbet* Smith. About 1.3 g zerumbone was obtained from each 1 kg fresh rhizomes. Fresh rhizomes of *Zingiber Zerumbet* Smith, purchased from local traditional herb suppliers, were thoroughly flushed and rinsed in multiple changes of water. The rhizomes were chopped into small pieces,

minced well and then immersed onto a glass beaker filled with n-hexane. The mixture was stirred twice day^{-1} for three consecutive days. The rhizomes were extracted with n-hexane three times. The sample extract was transferred onto a rotary evaporator system to be concentrated in vacuum at 40°C until the sample becomes sticky liquid called slurry.

The slurry was subjected to a silica gel column chromatography. Several crystallizations and column chromatography of the hexane slurry was carried out to obtain pure zerumbone. The purity was confirmed with gas chromatography mass spectrometer. Zerumbone preparation was done according to the method published (Murakami *et al*., 1999) with the aid of Analytical Laboratory and Quality Assurance Programmed Technical Services Centre, MARDI, P.O. Box 12301. General post office: 50774, Kuala Lumpure, Malaysia.

Protocol of treatment: Rats in each group were treated orally with one of the selected therapies using feeding catheter for four weeks/daily starting at day 16 as the followings:

- **First group (ZI):** 2 mL kg^{-1} b.wt. of 0.2% w/v of zerumbone diluted in corn oil
- **Second group (ZII):** 2 mL kg^{-1} b.wt. of 0.4% w/v of zerumbone diluted in corn oil
- **Third group (CEL):** Were treated with celecoxib in a dose of 30 mg kg^{-1}, diluted in 5% carboxylic methyl cellulose and served as positive control
- **Fourth group (CO):** Corn oil in a dose of 2 mL kg^{-1} b.wt. and served as negative control

Gross evaluation: Body weights and joints diameter were measured with 4 days interval. After euthanasia, the dissected joints were fixed and prior to dehydration, they were photographed using image stereo microscope analyzer (WD 54 Nikon, Japan).

Histological grading: Rats were euthanized at day 43 with intraperitoneal injection of 500 mg kg^{-1} sodium phenobarbital. Whole knee joints were dissected free from the surrounding soft tissues. The patella was removed from each knee to facilitate thorough fixation using standard procedures. Briefly the joints were fixed in 5% neutral buffered formalin for at least 3 days and subsequently decalcified with 10% formic acid for 5 days, then dehydrated through a descending series of ethanol using an automated tissue processing apparatus. After embedding in paraffin, joints were divided into two halves, separating the medial condyle and plateau from the lateral corresponding side. Serial sectioning of 6 μm thickness was done for each halves. Three slides from each medial and lateral condyle and plateau were selected for histopathological examination. Sections were stained with hematoxylin and eosin (H and E) for general morphology or safranin O fast green stain for matrix proteoglycan staining. Changes of OA occurred in the articular cartilage and subchondral bones were scored according to previously published method (Kobayashi *et al*., 2003).

It was expressed simply by the summation of individual grade (0: No changes, 1: Mild, 2: Moderate and 3: Severe) for each observed changes: cellular density, safranin O stain reduction of intercellular matrix, surface irregularity with or without fibrillation and marginal osteophyte formation (Table 1). Evaluation of the histopathology has been implemented blindly by two of our team using image analyzer microscope (Olympus, BX 51).

Statistical analysis: Statistical calculations were carried out with the SPSS 15.0 for Windows software package. Results were expressed as the Mean±SEM and analyzed with Mann-Whitney U test to evaluate the histopathology scores differences among groups. The $p<0.05$ were considered to be significant.

RESULTS

Gross changes: Rat's right knees showed apparent swelling and reddening post-MIA injection. The swelling subsided after a period of one week. Caliber reading during and after the end of treatment course revealed lessening of joints swelling. However, in the corn oil treated group, the knees were apparently still larger in diameter than the normal left joints. The body weight of all rats dropped at the first day of OA induction with signs of lameness. After 3 days the animal's body weight gradually increased even during the period of treatment in all rats. At day 43, all rats were euthanized and their knee joints were dissected. After removal of skin, the left joint capsule appeared white and glistening, whereas the right joint was slightly yellowish to white in both ZI and ZII groups. Capsule looks slightly thickened in right knees of CEL group while it appeared greatly thickened in the rats treated only with corn oil (Fig. 1A).

Grossly, the left normal joints revealed smooth and shiny articular surfaces. The right knees of the ZI group showed minute abrasions and necrosis at medial femoral condyle and tibial plateau. Apart from these changes, there were sporadic, minute black spots at the trochlear articular cartilage (Fig. 1B-D).

Table 1: Histopathology score of articular surfaces and subchondral bones of the rat's right knees in ZI, ZII, CEL and CO groups

			Rat's groups			
			ZI	ZII	CEL	CO
Joint parts	Observations	Score grades	(N = 10)			
Femur	Loss of tangential zone chondrocytes	0	0/10	5/10	2/10	0/10
		+	10/10	5/10	8/10	0/10
		++	0/10	0/10	0/10	0/10
		+++	0/10	0/10	2/10	10/10
	Average pathology score		1	0.5	1.4	3
	Loss of chondrocytes in transitional and radial zones	0	0/10	2/10	0/10	0/10
		+	10/10	8/10	2/10	1/10
		++	0/10	0/10	6/10	6/10
		+++	0/10	0/10	2/10	3/10
	Average pathology score		1	0.8	2	2.2
	Chondrocytes disorganization	0	0/10	2/10	0/10	0/10
		+	10/10	8/10	8/10	1/10
		++	0/10	0/10	2/10	5/10
		+++	0/10	0/10	0/10	4/10
	Average pathology score		1	0.8	1.2	2.3
	Cloning and hypertrophy	0	0/10	0/10	5/10	7/10
		+	0/10	7/10	5/10	3/10
		++	10/10	3/10	0/10	0/10
		+++	0/10	0/10	0/10	0/10
	Average pathology score		2	1.3	0.5	0.3
	Degeneration/necrosis	0	5/10	5/10	0/10	0/10
		+	5/10	5/10	0/10	0/10
		++	0/10	0/10	0/10	4/10
		+++	0/10	0/10	10/10	6/10
	Average pathology score		0.5	0.5	3	2.6
	Surface irregularity and fibrillation	0	5/10	8/10	0/10	0/10
		+	5/10	2/10	10/10	10/10
		++	0/10	0/10	0/10	0/10
		+++	0/10	0/10	0/10	0/10
	Average pathology score		0.5	0.2	1	1
	Safranin O fast stain reduction	0	0/10	2/10	0/10	0/10
		+	5/10	8/10	1/10	0/10
		++	5/10	0/10	6/10	4/10
		+++	0/10	0/10	3/10	6/10
	Average pathology score		1.5	0.8	2.2	2.6
	Subchondral fibrosis and cyst formation	0	2/10	6/10	0/10	0/10
		+	3/10	4/10	0/10	1/10
		++	5/10	0/10	7/10	7/10
		+++	0/10	0/10	3/10	2/10
	Average pathology score		1.3	0.4	2.3	2.1
	Osteophyte formation	0	3/10	8/10	6/10	0/10
		+	7/10	2/10	2/10	2/10
		++	0/10	0/10	2/10	6/10
		+++	0/10	0/10	0/10	2/10
	Average pathology score		0.7	0.2	0.6	1.6
	Total averages score of femur ±SEM		9.5±0.16	5.5±0.11	14.2±0.3	17.7±0.28
Tibia	Loss of tangential zone chondrocytes	0	3/10	5/10	0/10	0/10
		+	7/10	5/10	3/10	0/10
		++	0/10	0/10	5/10	0/10
		+++	0/10	0/10	2/10	10/10
	Average pathology score		0.7	0.5	1.9	3
	Loss of chondrocytes in transitional and radial zones	0	3/10	3/10	0/10	0/10
		+	5/10	7/10	0/10	0/10
		++	2/10	0/10	10/10	4/10
		+++	0/10	0/10	0/10	6/10
	Average pathology score		0.9	0.7	2	2.6
	Chondrocytes disorganization	0	1/10	3/10	0/10	0/10
		+	3/10	7/10	0/10	0/10
		++	6/10	0/10	5/10	2/10
		+++	0/10	0/10	5/10	8/10
	Average pathology score		1.5	0.7	2.5	2.8
	Cloning and hypertrophy	0	0/10	0/10	8/10	6/10
		+	5/10	7/10	2/10	4/10
		++	5/10	3/10	0/10	0/10

Table 1: Continued

Joint parts	Observations	Score grades	Rat's groups ZI (N = 10)	ZII	CEL	CO
		+++	0/10	0/10	0/10	0/10
	Average pathology score		1.5	1.3	0.2	0.4
	Degeneration/necrosis	0	3/10	4/10	0/10	0/10
		+	5/10	5/10	0/10	0/10
		++	2/10	1/10	3/10	2/10
		+++	0/10	0/10	7/10	8/10
	Average pathology score		0.9	0.7	2.7	2.8
	Surface irregularity and fibrillation	0	3/10	5/10	0/10	2/10
		+	7/10	5/10	8/10	8/10
		++	0/10	0/10	2/10	0/10
		+++	0/10	0/10	0/10	0/10
	Average pathology score		0.7	0.5	1.2	0.8
	Safranin O fast stain reduction	0	0/10	2/10	0/10	0/10
		+	7/10	7/10	3/10	0/10
		++	3/10	1/10	3/10	4/10
		+++	0/10	0/10	4/10	6/10
	Average pathology score		1.3	0.9	2.1	2.6
	Subchondral fibrosis and cyst formation	0	7/10	1 0/10	10/10	6/10
		+	0/10	0/10	0/10	0/10
		++	3/10	0/10	0/10	2/10
		+++	0/10	0/10	0/10	2/10
	Average pathology score		0.6	0	0	1
	Osteophyte formation	0	7/10	10/10	7/10	5/10
		+	3/10	0/10	3/10	3/10
		++	0/10	0/10	0/10	2/10
		+++	0/10	0/10	0/10	0/10
	Average pathology score		0.3	0	0.3	0.7
	Total averages score of tibia ±S.E.M		8.4 ±0.13	5.3±0.13	12.9±0.34	16.7±0.36
	Sum of both femoral and tibial score ±SEM		17.9±0.10	10.8±0.08	27.1±0.21	34.4±0.22

0: No changes, +: Mild, ++: Moderate, +++: Severe

Gross observations of joints from ZII group revealed minute cartilage necrosis at medial tibia plateau and trochlear ridges (Fig. 1E, F). In CEL group, some joints revealed mild osteophyte on the trochlear ridge (Fig. 1G) and other presents blackish, minute necrotic foci at femoral condyle and trochlea (Fig. 1H). Abrasions were detected at medial and lateral tibial plateaus (Fig. 1I). In CO group, osteophyte formation was detected at the femoral trochlea (Fig. 1J). Some joints appeared to have sloughed cartilage areas at the articular surfaces. Extensive necrosis was found in the articular surfaces of the femoral condyles, trochlea and for lesser extent in the tibial plateau (Fig. 1K, L). Accordingly, the data viewed inclination in the lesion's severity of the right joints of the four groups as follow: CO, CEL, ZI and ZII, respectively.

Radiography: Radiographs of the right knee joints of randomly selected rats from all groups (at day 0 prior to OA induction) showed normal synovial mass, clear radiolucent microfilm of synovial fluid and normal sub-patellar opacity, readily distinguish from the surrounding structures (Fig. 2A, B). Fifteen days post OA induction with MIA injection, right knee joints of rats selected randomly from all groups revealed OA changes: decreased joint space, irregular articular cartilage surfaces and increased sub-patellar opacity (Fig. 2C, D).

Radiographs at day 43 did not revealed progressed changes in the right knee joints of both ZI and ZII groups, while those of CEL and CO groups showed distinct loss of synovial space, atrophied femoral condyles, enlargement of intercondyloid fossa and roughness of the articular surfaces (Fig. 2E, F).

Microscopic observations: Joint samples from non-induced joints showed smooth articular cartilage surface with the underneath layer of flattened chondrocytes in the tangential zone. Chondrocytes were normally distributed in parallel rows in the transitional and radial zones of the articular cartilage (Fig. 3A, B). Intercellular matrix deeply and uniformly stained with safranin O fast green stain in the non-calcified part (region extends from articular surface to the tidemark) and for a lesser extent in the calcified region (Fig. 3C, D). Subchondral bone revealed normal distribution of trabeculae composed of osteocytes and canaliculli surrounding bone marrows filled with blood forming elements.

Microscopic observations of the right knee joints were scored and listed in Table 1. These histopathological observations have been well described according to group separately as follow:

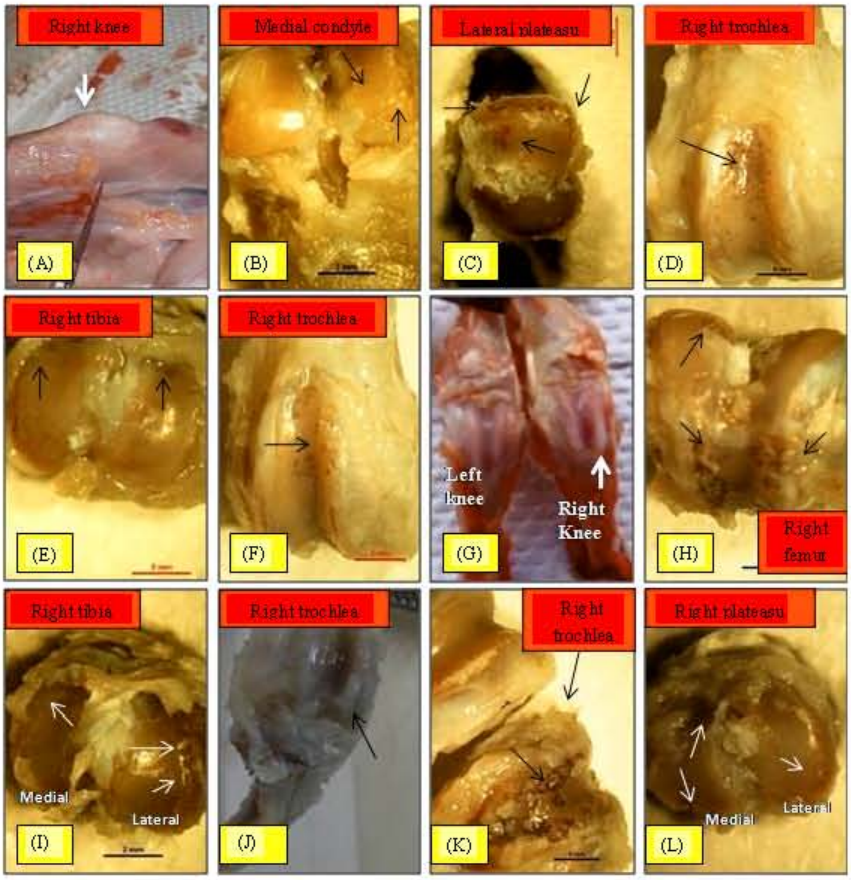

Fig. 1: At day 43, (A) swelled and thickened right knee joint in corn oil (CO) treated group (Arrow). (B) Abrasions at medial femoral condyle, (C) necrotic foci at medial tibia plateau and (D) blackish spots distributed at trochlea in the right knee joint of ZI group (Arrows). Right knees of ZII group showed small necrotic foci at tibia plateau (arrows) and femoral trochlea (E, F). In Celecoxib (CEL) treated group, (G) the rat's right knees present osteophyte formation at the trochlear ridge (arrow), (H) moderate necrosis at femoral condyles, trochlea and (I) tibial plateau. Extensive lesions appeared at right joints of rats in CO group. (J) It includes osteophyte formation at the trochlea, (K, L) necrosis at femoral trochlea and tibia plateau (arrows)

ZI group: Haematoxyline and eosin stains of the joints revealed mild cellular loss observed in tangential, transitional and radial zones of the cartilage. Some articular surfaces suffered moderate cellular loss especially in the tibial plateau. Most of the chondrocytes exist in the later two zones underwent degeneration or necrosis and very mild chondrocyte cloning and hypertrophy (Fig. 4A, B). Along with these changes, safranin O fast green stained sections showed mild or moderate reduction in the staining of articular cartilages (Fig. 4C, D). The articular surfaces showed mild irregularity without sign of fibrillation.

Subchondral structures underwent some changes including replacement of bone marrow elements with fibrous tissue. Lesions were accompanied with cyst formation in some joints (Fig. 4E, F). These observations were more obvious in femoral condyles than in the tibial plateau. Osteophytes were also found in few numbers of the rats right joints (Fig. 4G, H). Total pathological score of this group was 17.9±0.10 resulted from the summation of 9.5±0.16 of the femoral condyle and 8.4±0.13 of the tibial plateau.

ZII group: There was a mild loss of chondrocytes in the right joints in most of the rats in this group. Distinct chondrocyte cloning was observed in the transitional-radial zones. Such findings were moderate in the femoral condyles and mild-moderate in the tibial plateau (Fig. 5A, B). Cellular disorganization and necrosis were mild in the femoral condyle and mild-moderate in the

Fig. 2: Radiographies of the right knee joints showing the radiographic changes in their bones femur (F) and tibia (T). Radiographs at day 0 were showed normal state of the right knees; characterized with smooth articular surfaces, (A) clear radiolucent joint space and (B) distinct sub-patellar opacity (white arrow). Due to MIA intraarticular injection, (C) joints in all rat's groups revealed minimized joint space (black arrow), (D) articular surfaces irregularity (black arrows) and less obvious sub-patellar opacity (white arrow). At day 43, (E) the changes severely progressed in those rats treated with Celecoxib and (F) corn oil. It showed complete loss of joint space (white arrow), increased intercondyloid fossa (black arrow) and atrophied femoral condyles

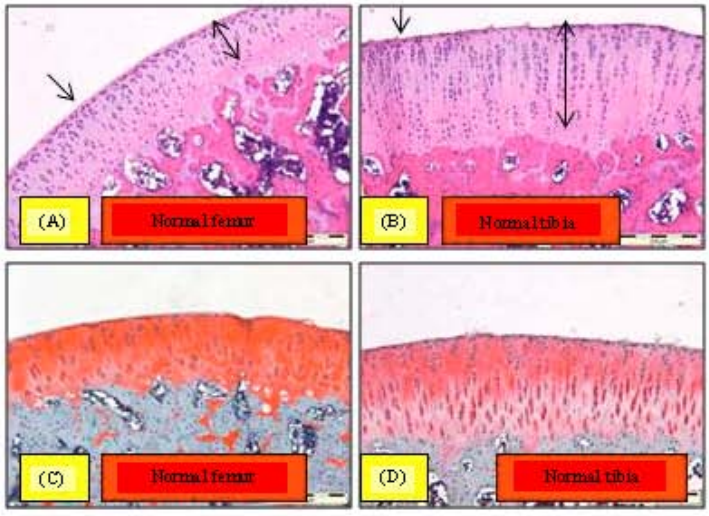

Fig. 3: Normal articular cartilages and subchondral bones of the femoral condyle and tibial plateau of the left knee joint. (A, B): Reveal smooth articular surfaces with the underneath flattened chondrocytes of the tangential zone (arrows). Chondrocytes were distributed in parallel rows in the transitional-radial zones (doubled head arrow), H and E, x100. (C, D), Uniform staining of the intercellular matrix substance with Safranin O fast green stain, x100

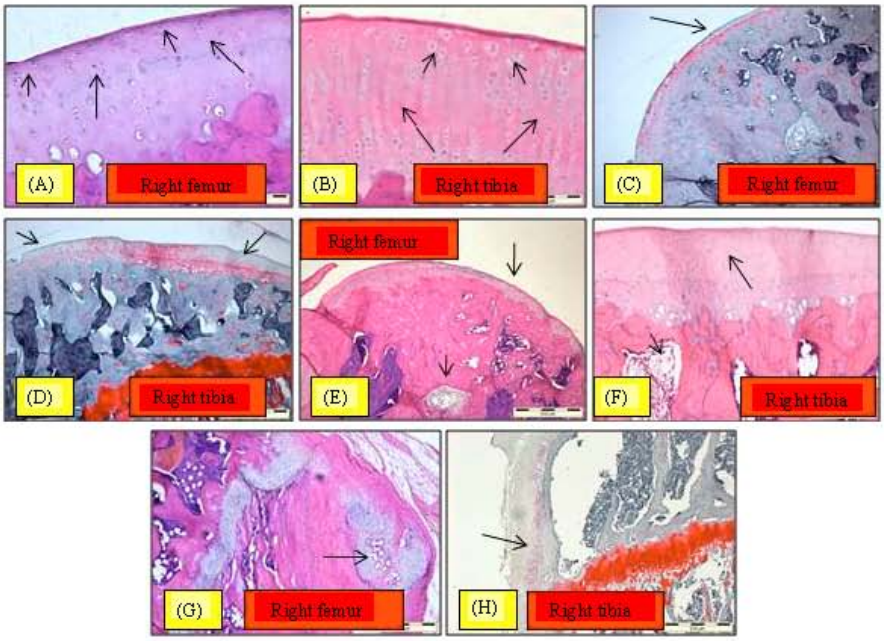

Fig. 4: Representative sections of articular cartilages from the rat's knees in ZI group (treated with a dose of 2 mL kg^{-1} b.wt. of 0.2% w/v of zerumbone). (A, B): Chondrocyte cloning and hypertrophy (short arrows) in the transitional zone and disorganization and cellular loss at the radial zone (long arrows), H and E, x200. (C, D): Moderate loss of safranin O fast green stain in the articular cartilages (arrows), Safranin O stain, x40. (E, F): Subchondral fibrosis and cyst development (short arrows) opposite to the affected area of the articular cartilages (long arrows) of the femur and tibia, H and E, x40 and x100, respectively. (G, H): Marginal osteophyte formation in the femoral condyle and tibial plateau (arrows), H and E, x40 and Safranin O stains, x40, respectively

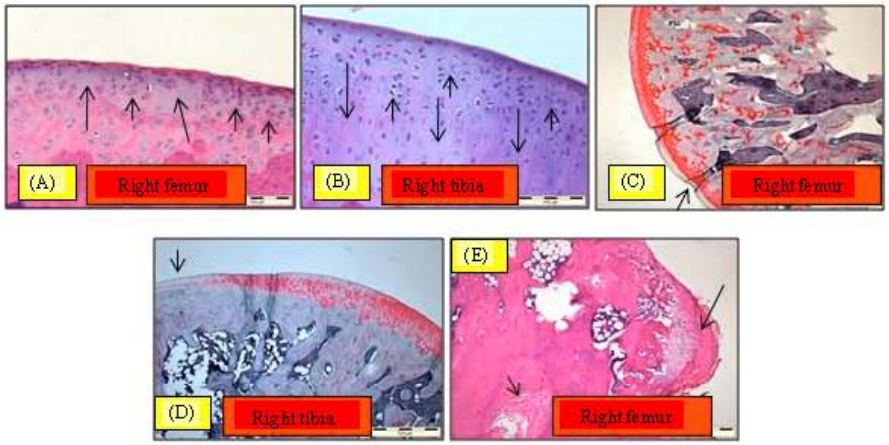

Fig. 5: Representative sections of articular cartilages from the rat's knees in ZII group (treated with a dose of 2 mL kg^{-1} b.wt. of 0.4% w/v of zerumbone). (A, B): Mild irregularity of the articular surfaces with the signs of chondrocytes cloning (short arrows), mild cellular loss and disorganization (long arrows) in the articular cartilages, H and E, x200. (C, D): Mild reduction of Safranin O fast green stains in the articular cartilages (arrow), safranin O stain, x40. (E): Subchondral fibrosis (short arrow) and mild osteophyte formation in the femoral condyle (long arrow), H and E, x40

tibial plateau. Some articular surfaces of this group revealed irregularity with no sign of fibrillation. Sections stained with safranin O fast green showed mild stain reduction (Fig. 5C, D). Subchondral structures revealed mild replacement of bone marrow elements with fibrous tissue and subtle lack of cyst development. Osteophytes were detected in the femoral condyle of few joints (Fig. 5E). Total histopathology score of this group was 10.8±0.08 resulted from the summation of 5.5±0.11 of the femoral condyle and 5.3±0.13 of the tibial plateau.

CEL group: Most of the right knees of this group showed moderate or severe pathological changes. Articular cartilages of both femoral condyle and tibial plateau revealed moderate loss of their chondrocyte component. Cellular loss was severe in some sections, especially in the transitional-radial zones. The later zones showed slight chondrocytes cloning in some sections. Severe necrosis was extensive all over the articular cartilages of the right knees of this group (Fig. 6A, B).

Characteristic reduction in the Safranin O fast green stain observed in articular cartilages suffered severe necrosis (Fig. 6C, D). Mild irregularity with or without fibrillation were present in the articular surfaces of this group. Examination of the subchondral bone structures revealed distinct lesion of bone marrow element replacement with fibrous tissue in the femoral condyle but not in the tibial plateau (Fig. 6E). Cysts were detected at femoral part only in some joints of this group (Fig. 6F). Osteophytes were observed in their femoral condyle and tibial plateau (Fig. 6G, H). Total histopathology score of this group was 27.1±0.21 resulted from the summation of 14.2±0.28 of the femoral condyle and 12.9±0.34 of the tibial plateau.

CO group: Severe pathological changes were observed in their right joints. Tangential zone of both femoral condyle and tibial plateau articular cartilage revealed severe loss of chondrocytes with slight surface irregularity and fibrillation. Chondrocyte depletion in the transitional and radial zones was quite severe with obvious cellular disorganization. In fact, the articular cartilages devoid the normal parallel row arrangement of the chondrocytes. Most of the remaining chondrocytes underwent

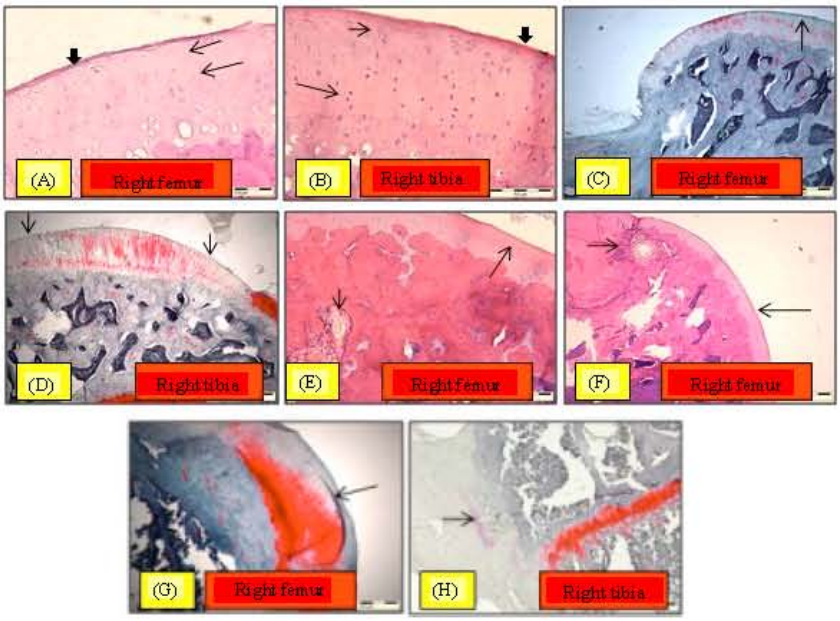

Fig. 6: Representative Sections of articular cartilages from the rat's knees in Celecoxib treated group (treated with Celecoxib in a dose of 30 mg kg^{-1} b.wt.). (A, B): Fibrillation and chondrocyte loss in the tangential zone (thick arrow). Severe cellular loss in the transitional (thin short arrow) and radial zones (thin long arrow) with severe chondrocytes necrosis and disorganization, H and E, x 200. (C, D): Moderate reduction of Safranin O fast green stain of the articular cartilages of the femoral condyle and tibial plateau (arrows), Safranin stain, x40. (E, F): Bone marrow element replacement with fibrous tissue and cyst development (short arrow) opposite to the severely affected articular cartilages (long arrow), H and E, x100 and x40, respectively. (G, H): Marginal osteophytes in the femoral condyle and tibial plateau (arrow), Safranin O stain, x40

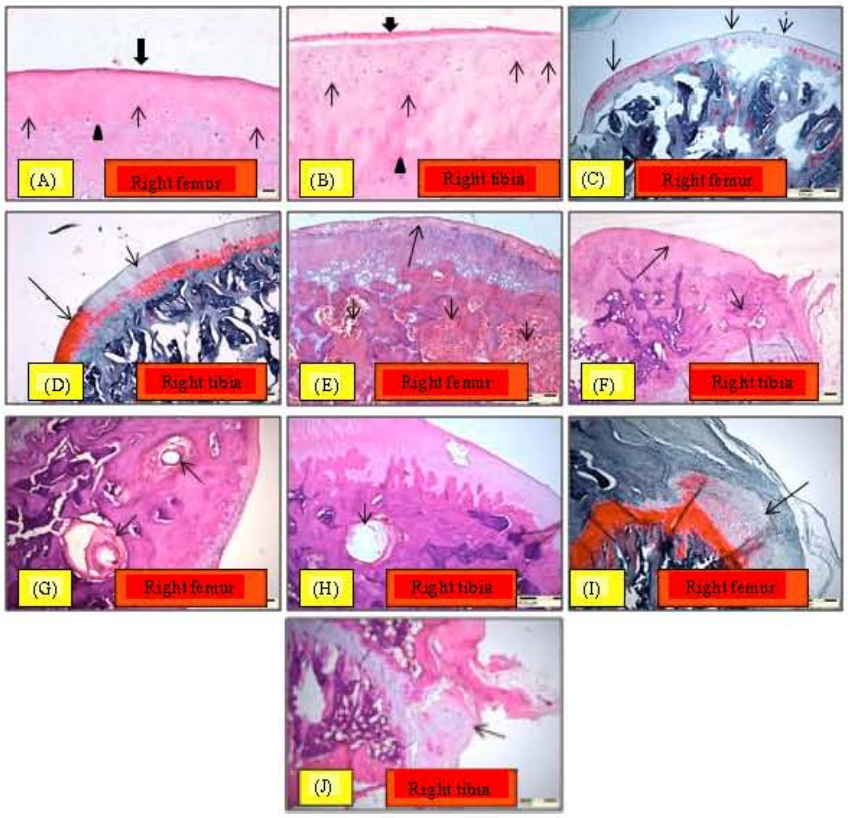

Fig. 7: Representative Sections of articular cartilages from the rat's knees in corn oil treated group (treated with corn oil in a dose of 2 mL kg^{-1} b.wt.). (A, B): Fibrillation and severe loss of chondrocytes in the tangential zone (thick arrow). Severe cellular loss and necrosis all over the transitional-radial zones (thin arrows) till the tidemark (triangle) with distinct disorganization, H and E, x200. (C, D): Severe reduction in the Safranin O fast green stain at the weight bearing articular surfaces (short arrows) in compares with the unaffected regions (long arrows), safranin stain, x40. (E, F): Severe subchondral fibrosis in the femoral condyle and for a lesser extent in the tibial plateau (short arrows) underneath severely affected articular cartilages (long arrows), H and E, x40. (G, H): Distinct subchondral cyst formation in the femoral condyle and tibial plateau (arrows), H and E, x40. (I, J): Marginal osteophytes formation in both femur and tibia (arrows), Safranin O stain, x40

necrosis (Fig. 7A, B). Sections stained with safranin O fast green stain revealed severe reduction in their staining (Fig. 7C, D).

Marked subchondral lesions were found in this group. Extensive area of bone marrow elements were replaced with fibrous tissue (Fig. 7E, F). Some joints revealed cyst formation (Fig. 7G, H). Several right knee joints from this group exposed osteophyte formation (Fig. 7I, J). Total histopathology score of this group was 34.4±0.22 resulted from the summation of 17.7±0.28 of the femoral condyle and 16.7±0.36 of the tibial plateau.

Statistical results: Data of histopathology observations have been analyzed with Mann-Whitney test. The comparison between each two groups separately revealed the results listed in Table 2.

Table 2: Results of Mann-Whitney test analysis between each two groups of the study

No.	Compared groups		Results
1	ZI	CO	p<0.05*
2	ZII	CO	p<0.01**
3	CEL	CO	p>0.05†
4	ZI	ZII	p<0.05*
5	ZI	CEL	p>0.05†
6	ZII	CEL	p<0.01**

*Less than 0.05% significant, **Less than 0.01% significant, †More than 0.05% not significant

DISCUSSION

Current data revealed distinct reduction in histopathology scores of ZII and for a lesser extent in ZI rat groups. The difference of zerumbone effects between these groups may be due to its doubled concentration

used in oral treatment of rats in ZII group. These different curative effects reflected the tendency of zerumbone to ameliorate OA changes in a dose dependent manner. In fact, loss as well as degeneration or/necrosis of chondrocytes in their articular cartilages were significantly lower than those exposed by CO group. The later showed widespread chondrocyte loss and degeneration in their OA joints.

Actually, saving plausible number of cartilage cells in the joint's articular structure of zerumbone treated groups (especially ZII group) was interesting and very important in OA pathogenesis and progression, because chondrocytes are the only component capable of controlling vital activities of the articular cartilage (Lee *et al*., 2005). Thus, their progressive loss will cause histological changes resemble those features noted in OA (Bove *et al*., 2003). Our results showed maintenance of larger number of chondrocyte in the articular cartilages of ZII group than other groups post 4 weeks of oral administration of zerumbone. We expected that zerumbone can slow down the cartilage degradation in two parallel directions:

- Zerumbone can slow down the production of free radicals that may be found in the articular tissue of joints suffering OA, because Zerumbone has an antioxidant activity (Murakami *et al*., 2002; Ruslay *et al*., 2007). It can suppress the expression of inducible nitric oxide synthase (Murakami *et al*., 2003). Therefore, Zerumbone can save plausible number of chondrocytes from undergoing degeneration or/and necrosis
- Zerumbone may suppress the inflammatory cells invading the joint during its inflammation. Zerumbone has been found to have a potent anti-inflammatory activity (Murakami *et al*., 2003) and also suppress COX-2 expression in the articular tissue which is play a role in producing prostaglandins (Tanaka *et al*., 2001). So, indirectly zerumbone will prevent inflammatory cytokines production, which is capable of stimulating chondrocytes to produce more catabolic enzymes such as collagenase and stromelysin, thus preventing the matrix degradation (Cahue *et al*., 2007)

In OA, the normal balance mechanism between catabolic and anabolic enzymes will be troubled. The catabolic enzymes concentration was found to be increased in the matrix resulting in its degradation. These changes may trigger cytokine production such as Interleukin-1 (IL-1) and Tumor necrotic Factor-Alpha (TNF-α) due to the joint inflammation. The later cytokines can stimulate the production of the free radicals in the articular tissue leading to chondrocyte degeneration or necrosis.

According to the above facts, matrix degradation found in ZII group was lower than that found in the other groups. Lowering in the degradation was specifically detected with the aid of safranin O fast green stain. An improvement in the staining indicates low level of matrix degradation of the articular cartilage. In contrary, sections stained with safranin O fast green stain represented those of CO and CEL group's revealed distinctive loss of the stain, indicating severe matrix degradation of their articular cartilages.

Previous findings postulated that degradation of articular cartilage of the joint will give rise to subchondral response manifested by bone marrow elements replacement with fibrous tissues, bone thickening and cyst formation (Stoop *et al*., 2001). Observations of the current investigation were in a good agreement with these findings. We found that CO and CEL groups suffered severe, widespread necrosis of their articular cartilages accompanied with unambiguous subchondral structural changes and lesions development.

Dissimilarly, subchondral responses and lesions development in zerumbone treated groups were slightly inhibited in the joints of ZI group and characteristically absent in those of ZII group. Reduction of subchondral changes is a very important aspect of OA pathogenesis and progression. Since, subchondral structures are richly innervated and considered an important target of OA pain. In fact, subchondral structures, synovial membranes and mensci are potential sources of pain in OA (Guzman *et al*., 2003).

In CEL group, the histopathology observations ranged from moderate to severe. The changes closely resembled to those exposed by CO group. Statistical analysis showed no significant difference between their global lesion ($p>0.05$), an indication that celecoxib did not exert any significant protective effect on the OA joints. Similarly, other NSAID's exposed no effects on the osteoarthritic articular cartilages in previous studies (Mach *et al*., 2002).

The present data revealed predominantly, higher OA like changes in the femoral condyle than in those of the tibial plateau. Such difference was present in all groups included in this study. These findings were in a good agreement with those observed previously in rabbits (Choi *et al*., 2002) and Sprague Dawely rats (Al-Saffar *et al*., 2009).

Gross findings were commensurate with those of the microscopic changes found in all treated groups. It was minor in zerumbone treated groups, especially in ZII

groups, while it was obviously severe in both CEL and CO groups. Radiography, also confirm the above gross and microscopic findings. It was found that 4 weeks post treatment with zerumbone sustained the changes found especially the joint space. While in those treated with celecoxib or Corn oil, radiographs showed increased intercondyloid fossa (due to the degeneration of cranial cruciate ligament), severe joint space loss, atrophied femoral condyles and lack of articular surface smoothness. Actually, impairment of joint space considered an important feature in diagnosis and assessment of OA (Fernihough *et al*., 2004).

CONCLUSION

In conclusions, oral administration of Zerumbone in a dose of 2 mL kg^{-1} b.wt. of 0.4% w/v diluted with corn oil for a period of 4 weeks has some chondroprotective effects on the knee OA of the Sprague Dawley rats.

ACKNOWLEDGMENT

The author wish to thank University Putra Malaysia, as this study was supported by e-Science grant (Project No.: 02-01-04-SF0882) awarded by Ministry of Science, Technology and Innovation.

REFERENCES

Al-Saffar, F.J., S. Ganabadi, H. Yaakub and S. Fakurazi, 2009. Collagenase and sodium iodoacetate–induced experimental osteoarthritis model in sprague dawley rats. Asian J. Sci. Res., 2: 167-179.

Arden, N. and M.C. Nevitt, 2006. Osteoarthritis: Epidemiology. Best Practice Res. Clin. Rheumatol., 20: 3-25.

Bove, S.E., S.L. Calcaterra, R.M. Brooker, C.M. Huber and R.E. Guzman *et al*., 2003. Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. Osteoarthritis Cartilage, 11: 821-830.

Cahue, S., L. Sharma, D. Dunlop, M. Ionescu and J. Song *et al*., 2007. The ratio of type II collagen breakdown to synthesis and its relationship with the progression of knee osteoarthritis. Osteoarthritis Cartilage, 15: 819-823.

Cantley, C.E., E.C. Firth, J.W. Delahunt, D.U. Pfeiffer and K.G. Thompson, 1999. Naturally occurring osteoarthritis in the metacarpophalangeal joints of wild horses. Equine Vet. J., 31: 73-81.

Choi, J.H., J.H. Choi, D.Y. Kim, J.H. Yoon and H.Y. Youn *et al*., 2002. Effects of SKI 306X, a new herbal agent on proteoglycan degradation in cartilage explant culture and collagenase-induced rabbit osteoarthritis model. Osteoarthritis Cartilage, 10: 471-478.

Creamer, P. and M.C. Hochberg, 1997. Osteoarthritis. Lancet, 350: 503-509.

Doig, P.A., K.A. Purbrick, J.E. Hare and D.B. Mckeown, 2000. Clinical efficacy and tolerance of meloxicam in dogs with chronic osteoarthritis. Can. Vet. J., 41: 296-300.

Dumond, H., N. Presle, P. Pottie, S. Pacquelet and B. Terlain *et al*., 2004. Site specific changes in gene expression and cartilage metabolism during early experimental osteoarthritis. Osteoarthritis Cartilage, 12: 284-295.

Faizah, S., M.N. Somchit and M.H. Shukriyah, 2002. *Zingiber zerumbet* (Lempoyang): A potential anti-inflammatory agent. Proceedings of the Regional Symposium on Environment and Natural Resources, April 10-11, Kuala Lumpur, Malaysia, pp: 516-520.

Fernihough, J., C. Gentry, M. Malcangio, A. Fox and J. Rediske *et al*., 2004. Pain related behavior in two models of osteoarthritis in the rat knee. Pain, 112: 83-93.

Goldring, M.B. and S.R. Goldring, 2007. Osteoarthritis. J. Cell Physiol., 213: 626-634.

Grosvenor, P.W., P.K. Gothard, N.C. McWilliam, A. Supriono and D.O. Gray, 1995. Medical plants from Riau Province, Sumatra, Indonesia. Part 1: Antibacterial and antifungal activities. J. Ehnopharmacol., 45: 75-95.

Guzman, R.E., M.G. Evans, S. Bove, B. Morenko and K. Kilgore, 2003. Mono-iodoacetate induced histologic changes in subchondral bone and articular cartilage of the rat femoro-tibial joints: an animal model of osteoarthritis. Toxicol. Pathol., 31: 619-624.

Hattori, K., K. Ikeuchi, Y. Morita and Y. Takakura, 2005. Quantitative ultrasonic assessment for detecting microscopic cartilage damage in osteoarthritis. Arthritis Res. Ther., 7: 38-46.

Huang, G.C., T.Y. Chien, L.G. Chen and C.C. Wang, 2005. Antitumor effect of zerumbone from zingiber zerumbet in P-388D1 cells *in vitro* and *in vivo*. Planta Med., 71: 219-224.

Jouglin, M., C. Robert, J.P. Valette, F. Gavard, F. Quintin-Colonna and J.M. Denoix, 2000. Metalloproteinases and tumor necrosis factor-alpha activities in synovial fluids of horses: Correlation with articular cartilage alterations. Vet. Res., 31: 507-515.

Kobayashi, K., R. Imaizumi, H. Sumichika, H. Tanaka, M. Goda, A. Fukunari and H. Komatsu, 2003. Sodium iodoacetate-induced experimental osteoarthritis and associated pain model in rats. J. Vet. Med. Sci., 65: 1195-1199.

Lee, S.W., W.T. Chung, S.M. Choi, K.T. Kim, K.S. Yoo and Y .H. Yoo, 2005. Clematis mandshurica protected to apoptosis of rat chondrocytes. J. Ethnopharmacol., 101: 294-298.

Loughin, C.A., S.C. Kerwin, G. Hosgood, P.B. Ringwood, J. Williams, J.D. Stefanacci and R.J. McCarthy, 2006. Clinical signs and results of treatment in cats with patellar luxation: 42 cases (1992-2002). J. Am. Vet. Med. Assoc., 228: 1370-1375.

Mach, D.B., S.D. Rogers, M.C. Sabino, N.M. Luger and M.J. Schwei *et al*., 2002. Origins of skeletal pain: Sensory and sympathetic innervation of the mouse femur. Neuroscience, 113: 155-166.

McDougall, J.J., 2006. Arthritis and Pain. Neurogenic origin of joint pain. Arthritis Res. Ther., 8: 220-220.

Moreland, L.W., 2003. Intra-articular hyaluronan (hyaluronic acid) and hylans for the treatment of osteoarthritis: Mechanisms of action. Arthritis Res. Ther., 5: 54-67.

Murakami, A.M. Takahashi, S. Jiwajinda, K. Koshimizu and H. Ohigashi, 1999. Identification of zerumbone in zingiber zerumbet smith as a potent inhibitor of 12-o-tetradecanoylphorbol-13-acetate-induced epstein-barr virus activation. Biosci. Biotec. Biochem., 63: 1811-1812.

Murakami, A., D. Takahashi, T. Kinoshita, K. Koshimizu and W.H. Kim *et al*., 2002. Zerumbone, a Southeast Asian ginger sesquiterpene, markedly suppresses free radical generation, proinflammatory protein production and cancer cell proliferation accompanied by apoptosis: The alpha, beta-unsaturated carbonyl group is a prerequisite. Carcinogenesis, 23: 795-802.

Murakami, A., R. Hayashi, T. Tanaka, K.H. Kwon and H. Ohigashi *et al*., 2003. Suppression of dextran sodium sulfate-induced colitis in mice by zerumbone, a subtropical ginger sesquiterpene and nimesulide: Separately and in combination. Biochem. Pharmacol., 66: 1253-1261.

North American Veterinary Conference Orlando, Florida, 1998. Canine skeletal development and soundness. Veterinary Learning Systems, a Division of MediMedia, USA. http://www.eukanuba-scienceonline.com/download/slibrary/Canine%20Skeletal_1998TNAVC.pdf.

Ruslay, S., F. Abas, K. Shaari, Z. Maulidiani Zainal and Maulidiani *et al*., 2007. Characterization of the components present in the active fractions of health gingers (Curcuma Xanthorrhiza and Zingiber zerumbet by HPLC-DAD ESIMS. Food Chem., 104: 1183-1191.

Sakinah, S.A.S., S.T. handayani and L.P.A. Hawariah, 2007. Zerumbone induced apoptosis in liver cancer cells via modulation of Bax/Bcl-2 ratio. Cancer Cell Int., 7: 4-4.

Somchit, M.N. and M.H. Shukriyah, 2003. Anti-inflammatory property of ethanol and water extracts of *Zingiber zerumbet*. Indian J. Pharmacol., 35: 181-182.

Stoop, R., P. Buma, P.M. van der Kraan, A.P. Hollander and R.C. Billinghurst *et al*., 2001. Type II collagen degradation in articular cartilage fibrillation after anterior cruciate ligament transection in rats. Osteoarthritis Cartilage, 9: 308-315.

Szabolcs, A., L. Tiszlavicz, J. Kaszaki, A. Posa and A. Berko *et al*., 2007. Zerumbone exerts a beneficial effect on inflammatory parameters of cholecystokinin octapeptide-induced experimental pancreatitis but fails to improve histology. Pancreas, 35: 249-255.

Tanaka, T., M. Shimizu, H. Kohno, S. Yoshitani and Y. Tsukio *et al*., 2001. Chemoprevention of azoxymethane-induced rat aberrant crypt foci by dietary zerumbone isolated from Zingiber zerumbet. Life Sci., 69: 1935-1945.

Taylor, P.M. and S.A. Robertson, 2004. Pain management in cats-past, present and future. Part 1 the cat is unique. J. Feline Med. Surgery, 6: 313-320.

Williams, V.S., 2007. Intraarticular hyaluronic acid supplementation in the horse: The role of molecular weight. J. Equine Vet. Sci., 27: 298-303.

Yee, H.C., Z. Said, N. Hussien, A. Bustaman and N.H. Abdullah, 2006. Screening of chemically synthesized analogues of zerumbone for anti-cancer properties and *in vivo* investigation of zerumbone in female Balb/C mice with cervical intraepithelial neoplasm (CIN). BSc. Thesis, University of Putra, Malaysia