Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 7 Issue 4 April 2025

Research Article

Comparative Study of Monoiodoacetate (MIA) and Papain Chemical Model of Osteoarthritis in Hartley Strain Guinea Pigs

Abas Rashid Bhat*

PhD Scholar, Veterinary Surgery and Radiology, IVRI, Izatnagar

***Corresponding Author:** Abas Rashid Bhat, PhD Scholar, Veterinary Surgery and Radiology, IVRI, Izatnagar.

Received: November 26, 2024

Published: March 30, 2025

Abstract

The present study was carried out to optimize the procedure for creation of chemical model of osteoarthritis (OA) in guinea pigs. Eighteen (18) clinically healthy adult Hartley strain guinea pigs of either sex, weighing 500 to 600g were used for creation of OA model divided into two groups A and B having 9 animals each. Intra-articular injection of 1 mg of monosodium iodoacetate (MIA) dissolved in 100 µl of normal saline was done through the infrapatellar ligament of the right knee in group A using a 27-gauge, 0.5-inch needle. Papain enzyme at dose rate of 10mg in 0.05M sodium acetate (pH 4.5) with enzymatic activity of 31 I.U/mg was injected intra-articularly in right knee joint. The left control knee was injected with 100 µl of physiologic saline in both the groups. In the present study intra-articular injection of 1 mg of monosodium iodoacetate was found better than papain for induction of osteoarthritis on the basis of clinical, radiological, histological and biochemical biomarker studies.

Keywords: Monoiodoacetate; Papain Chemical; Osteoarthritis; Hartley Strain; Guinea Pigs

Introduction

Osteoarthritis (OA) is the most common arthritis which leads to chronic disability in both animals and humans. Rate of occurrence is higher in weight bearing joints at old age in human beings and animals [6]. Over 10% of the human population above 60 years is affected by OA, the impact of this health problem is still underestimated [10]. Osteoarthritis (OA) is a systemic and chronic arthropathy characterized by the progressive breakdown of the articular cartilage (the end point of OA) along with changes in the subchondral bone, synovium (synovial inflammation), meniscus, tendons/ligaments, and muscles [13]. Animal models of OA are used extensively in search of pathogenesis of degenerative joint disease and in search of potential disease modifying anti-OA drugs. Lab Animal models, although imperfect, exhibit many of the pathologic features that characterize the canine or human disease. For induction of OA, chemical and surgical methods have been employed widely due to faster onset of disease and reproducible induction of arthritic change. Intra-articular injection of chemicals such as Mono-iodoacetate (MIA) or papain can cause the degeneration of cartilage and the development of osteoarthritis by inhibition of the activity of glyceraldehyde-3-phosphate dehydrogenase in chondrocytes or by induction of synovial inflammation and degeneration of supporting structure and resultant instability, respectively. The aim of the present study was to evaluate the comparative efficacy of two chemicals MIA and papain in inducing osteoarthritis when injected intra articularly in knee joints of guinea pigs.

Materials and Methods

Eighteen clinically healthy adult Hartley strain guinea pigs of either sex, weighing 400 to 500g were used for creation of model. The procedures used in this study were in consistent with the

guidelines of the Institutional Animal Care and Use Committee. Animals were provided with living conditions, food, and housing consistent with the approved animal care operating procedures. The animals were acclimatized to approaching and handling for a period of 10-15 days prior to commencement of the study.

Experimental design

Two groups having 9 animals each were formed out of eighteen selected guinea pigs to develop osteoarthritis (OA). All the animals were anaesthetized with xylazine 6 mg/kg body weight intramuscular and 10 minutes after by ketamine 60 mg/kg body weight intramuscularly [1]. After appropriate anesthesia each animal was positioned on their back and the required limb was flexed by 90° at the knee. The patellar ligament was palpated below the patella and the injection was made into this region. Care was taken not to advance the needle too far into the cruciate ligaments. For induction of monoiodoacetate (MIA) induced arthritis, single intra-articular injection of 1 mg of monosodium iodoacetate (MIA; Sigma, St. Louis, MO, USA; cat #I2512) was administered through the infrapatellar ligament of the right knee [7] designated as group MIA(R). MIA was dissolved in physiologic saline and administered in a volume of 100 µl using a 27-gauge, 0.5-inch needle. The left contra lateral knees of the same animals were injected with 100 µl of physiologic saline to act as control designated as group MIA(L). In second batch of 9 animals, Papain enzyme (Sigma, Cat # P 3125) at dose rate of 10mg in 0.05M sodium acetate (pH 4.5) with enzymatic activity of 31 I.U/mg was injected intra-articularly in right knee joint of each animal designated as group Papain(R) while as left knee joints were injected with 100ul of sterile physiological saline solution (0.9%) designated as group Papain(L).

Clinical assessment of the joint was recorded on alternate days till two weeks of the intraarticular injection.

Joint diameter (in cm) of the femorotibial joint was measured with the help of vernier caliper by taking mean of two readings and recorded.

Pain perception was assessed by applying digital pressure to the knee joint to elicit the pain, which was graded as no pain (No withdrawl of limb and absence of vocalization with score of 1); Mild pain (Slight withdrawl of limb and absence of vocalization having score of 2); Moderate pain (Withdrawl of limb with vocalization with score of 3); Severe pain (Strong vocalization with strong withdrawl reflex with score of 4).

Radiographs in lateral (LAT) and antero-posterior (AP) views of the test and control joints were made, in extended positions, at 0 (before injection), 14 and 28 days post-injection. The radiographic exposure factors were set at 40 kVp and 6 mAs and 75 cm FFD by using digital radiodiography (Konica Minolta^{R}).

Score	Degree of Roentgengraphic features	Osteoarthritis
0	Nil	Definite absence of x-ray changes of osteo-arthritis
1	Doubtful	Osteoarthritis is doubtful in radiograph
2	Minimal	Osteoarthritis definitely present though of minimal severity
3	Moderate	Osteoarthritis definitely present of moderate severity with development of few osteophytes
4	Severe	Sclerosis of subchondral bone and deformity of bone ends with osteophyte formation

Table 1: Details of radiographic scores by Kellgren and Lawrence's grading system.

Histopathology and histochemistry

Three guinea pigs were sacrificed from both groups under high dose of intracardiac thiopentone on 7, 14 and 28th day post MIA or papain injection. Femorotibial joints of guinea pigs were separated with sharp knife on each sampling day and specimens were fixed in 10% neutral buffer formalin, decalcified in Gooding and Stewart solution and processed for paraffin embedding; frontal parts of tibial end were sectioned at 5um thickness and subsequently stained with haematoxylin-eosin (H and E) and by toluidine blue for proteoglycan. Semi- quantitative histopathological grading was performed in accordance with modified histological scoring system of Mankin's., *et al*. [14] to evaluate guinea pig knee OA.

Overall histological score of the tissue in left and right knees of both groups was calculated by summation of scores of each parameter and compared.

Parameter	Grade	Description
Articular Cartilage Structure	0	Normal, smooth, uninterrupted surface
	1	Mild surface irregularities (undulations)
	2	Irregular surface, 1–3 superficial clefts (fissures)
	3	>3 fissures and/or loss of cartilage in the superficial zone
	4	1–3 fissures extending into the middle zone
	5	>3 fissures and/or loss of cartilage extending into the middle zone
	6	1–3 fissures extending into the deep zone
	7	>3 fissures extending into the deep zone and/or loss of cartilage to deep zone
	8	Fissures or loss of cartilage extending to the zone of calcified cartilage
Proteoglycan Content (staining by toluidine blue)	0	Uniform throughout articular cartilage
	1	Decreased in superficial zone only and for <half the length of the condyle or plateau
	2	Decreased in superficial zone for half the length or greater of the condyle or plateau
	3	Decreased in superficial and middle zones for <half the length of the condyleor plateau
	4	Decreased in superficial and middle zones for half the length or greater of the condyle or plateau
	5	Decreased in all 3 zones for < half the length of the condyle or plateau
	6	Decreased in all 3 zones for half the length or greater of the condyle or plateau
Cellularity	0	Normal (1/2 cells/lacuna)
	1	Diffuse/slight hypercellularity
	2	Regions of hypercellularity and clustering
	3	Diffuse hypocellularity
Tidemark Integrity	0	Intact/single tidemark
	1	Crossed by vessels/duplication of tidemark

Table 2: Details of histological score by Modified Mankin's scoring system.

Estimation of Biochemical markers IL-6, IL-1β and TNF-α by ELISA kit methods

For estimation of biochemical markers, blood samples were collected directly from the heart before euthanizing the guinea pigs and from 3 separate animals to act as control for serum markers. Serum was separated and preserved at -20^{0}C until analysis.

Statistical analysis

The means of parametric observations were compared by repeated measures analysis of variance (ANOVA) and significance of differences was determined by post-hoc testing with Bonferroni's method. In each group base values were compared with values at different intervals with paired't' test using SPSS software. Non-parametric observations were compared by Kruskal-Wallis test [19].

Results and Discussion

A major symptom of the patients with osteoarthritis (OA) is pain that is triggered by peripheral as well as central changes within the pain pathways. Assessment of pain is of critical importance for mechanistic studies as well as for the validation of drug targets. Right knee joints of both MIA (R) and Papain (R) groups had showen severe pain initially which decreased to moderate pain towards the end of the observation period whereas, their respective left joints exhibited moderate pain initially followed by no pain in the remaining period of the observation. Intra-articular injection of monosodium iodoacetate (MIA) in the knee joint disrupts chondrocyte metabolism resulting in cartilage degeneration and subsequent nociceptive behavior that has been described as a model of osteoarthritic (OA) pain. Transient synovial inflammation may be the underlying cause of pain during the first week following MIA injection, whereas pain sensation in later stages may be due to biomechanical changes affecting the articular cartilage and subchondral bone [2]. Papain is, a proteolytic enzyme when administered to cartilage causes its breakdown, producing inflammatory cytokines (TNF-α and IL-1β) [4]. The mild to moderate pain in the left joints for few days might be due to swelling caused by injection of normal saline which subsided very early as compared to right knee joints (Table 3).

There was a significant increase in the joint diameter from its baseline value at all intervals till the end of observation period in MIA and Papain treated joints whereas, the corresponding left joints had initial significant increase followed by normal diameter. The increase in joint diameter of treatment joints could be due to inflammation caused by the chemicals through activation of chemokines leading to joint swelling. Swelling is the cardinal sign of inflammation which might be due to associated synovitis and inflammation of other joint structures. The initial increase in joint diameters in physiological saline treated joints might be due to joint manipulation at the time of injection and mild reaction to salt solution which subsided in few days. MIA and Papain might have caused synovitis and release of inflammatory cytokines [4] which might have led to significant joint swelling which persisted for longer duration as compared to their control joints (Table 4).

Radiography is the standard technique used to diagnose and chronicle the progression of osteoarthritis. Severity of radiographic OA can be estimated using semi-quantitative scoring systems. Radiographic features including narrowing of joint space, presence of osteophytes, sclerosis of subchondral bone and deformity of bone ends can be assessed, and scored in five grades of 0 (none), 1 (doubtful), 2 (minimal), 3 (moderate) and 4 (severe). MIA administration in joints of guinea pigs had resulted in mild and moderate osteoarthritis on day 14 and day 28, respectively (Table 5 and Figure 1). Papain treated joints had shown mild osteoarthritis until day 28 (Table 5 and Figure 2).Also joints of MIA treated animals revealed non significantly ($p > 0.05$) higher scores on day 14 and 28 than papain treated joints. There was constant decrease in joint space along with cartilage loss and bone osteolysis in MIA treated joints but Papain treated joints showed only reduction in joint space with mild bone osteolysis on day 28 (Figure 1 and Figure 2). Left joints (control) of both groups showed no change in joint space and no bone osteolysis. Few osteophytes were also present in one animal at the end of observation period in MIA treated joint (MIA(R) group).The findings of the present study substantiate the fact that MIA and Papain can damage the cartilage. MIA causes degenerative changes in articular cartilage by direct interference with chondrocyte metabolism [12] and has been used to induce degenerative joint disease in the rat [7], mouse [12], and guinea pig. Papain is the proteolytic enzyme and causes osteoarthritis by releasing chondroitin sulphate from the protein polysaccharide complex of the matrix of articular cartilage [9]. However it doesn't induce direct effect on chondrocytes and collagen [8].

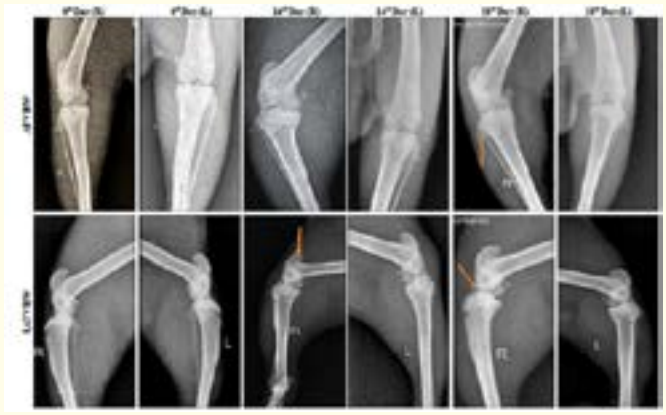

Figure 1: AP and LAT radiographic views of right and left knee joints of MIA group at different time intervals. Arrows are showing bone osteolysis and osteophyte formation in the MIA treated joints in contrast to almost smooth joint ends in control (left) joints.

Figure 2: AP and LAT radiographic views of right and left knee joints of Papain group at different time intervals. Arrows are showing bone osteolysis and osteophyte formation at 28th day in the Papain treated joints in contrast to almost smooth joint ends in control (left) joints.

For the histological characterization of the lesions, the majority of published animal studies used a scoring system of evaluation. We have used Mankin's histopathological score method which combines all the changes in cartilage structure, proteoglycan content as measured by toluidine blue method, cellularity and tidemark integrity with additional features of osteophyte development in one score to evaluate the histopathological changes in osteoarthritis in guinea pigs. The same scoring has been used in cartilage studies by [11]. The degenerative changes of the articular cartilage can be viewed more clearly on histological evaluation. Loss of toluidine blue staining, irregular cell arrangement, osteophyte development and disruption of articular surfaces were evident on histological examination of the specimens in the present study.MIA and papain treated joints of the animals of groups MIA(R) and Papain(R) recorded significantly ($p < 0.05$) higher Mankin's histopathological score at different time intervals than that of normal saline treated joints. MIA and papain caused almost similar histological changes in the right knee joints. The cartilage damage was evident from its erosions and fibrillations (See Figure 3) and decrease in proteoglycan content (See Figure 4) determined by the extent of toluidine staining in most of the joints treated with MIA and Papain. A few animals had double tidemark zone in the joints of groups MIA(R) and Papain(R), which is an indication of vascularisation. The animals of groups MIA(R) and Papain(R) also had increased cellularity in superficial layers. At the end of the study period i.e. day 28, higher osteoarthritic scores were recorded in MIA (R) treated joints than Papain (R) treated joints. It might be due to more damaging effect of MIA than papain causing earlier onset of histopathological changes in MIA group than that in Papain group. Earlier studies have reported that multiple doses of Papain are needed to cause higher grades of osteoarthritis [15]. Normal saline treated joints of both groups had milder changes in cartilage structure (only undulations present) and minimal decrease in proteoglycan content. Osteophytes were not seen in any of the joints treated with normal saline in both groups. Histopathological features of OA, such as loss of proteoglycan, cartilage degeneration, osteophyte formation, a dose dependent reduction in spontaneous locomotion after monoiodoacetate injection into the knee of rats has been reported in earlier study [7]. The injection of iodoacetate induces the loss of cartilage proteoglycan as measured by safranin O-fast green staining. The loss of proteoglycan content has been reported in guinea pigs injected with iodoacetate [21,22]. Histochemical examination of the sections from the experimental joints indicated a loss and degradation of the sulfated glycosaminoglycans by administration of papain in the guinea pig knee joint [20]. Our finding were in accordance with the observation of [12], who observed distinct depletion of stain and cytolysis post papain and iodoacetate injection which was comparable with bovine serum albumin induced OA [18] reported death of chondrocytes in patches which was due to variable sensitivity of chondrocytes in different areas of the cartilage. Areas of hypocellularity and hypercellularity observed with progression of OA post papain injection in the present work were confirmed to the results of [17] and [3]. Proliferative response of chondrocytes could be due to depletion of proteoglycans initially and later on lowered number was observed possibly due to lowered metabolic activity of chondrocytes. Similar findings have been reported by [12]. In the present study highest histological lesion score (11.00 ± 3.51) was observed in MIA cartilages on 28th day post MIA injection and (8.00 ± 1.15) in case of Papain treated joints. So, it was concluded that severity of lesions progressed with passage of time and maximum lesions were observed on day 28th that seem to be consistent with early OA changes in humans.

Different cytokines take part in pathogenesis of osteoarthritis. There is a growing need to identify and validate reliable biochemical biomarkers that can provide information on the

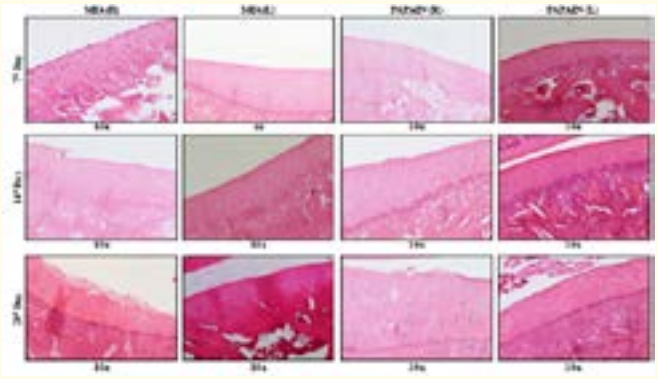

Figure 3: Photomicrographs showing histopathological features of tibial cartilage and subchondral bone of different groups at different time intervals by H&E staining.

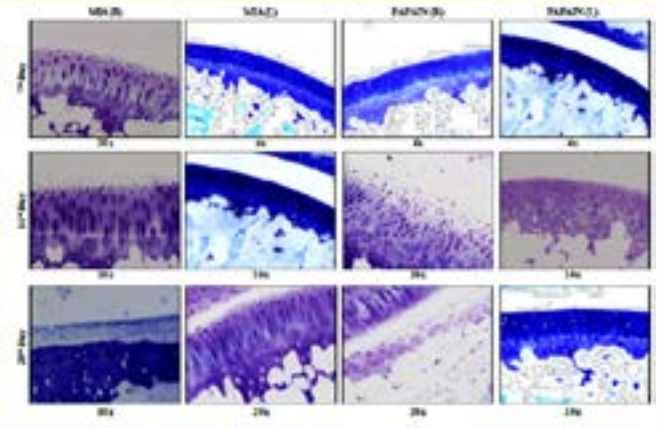

Figure 4: Photomicrographs showing histopathological features of tibial cartilage and subchondral bone of different groups at different time intervals by toluidine blue staining.

status and progression of joint destruction in OA. IL-1β and IL-6 levels remained higher at all time intervals in both MIA and Papain groups from the control animal values but the increase was more significant in MIA group than Papain group. IL-1β levels in MIA group were significantly ($p < 0.05$) higher than Papain group on day 14 and IL-6 levels on day 7 respectively. TNF- α showed non-significant increase in both groups. The monosodium iodoacetate (MIA) model has been well characterized for the evaluation of OA pain. The iodoacetate creates local inflammation in the knee from fluid expansion of the synovial membrane, which is then followed by the production of inflammatory mediators (TNF-α, IL-1β, IL-6). These mediators further contribute to OA pathogenesis by increasing cartilage degradation. The cartilage damage and degradation leads to chronic neuronal damage with neuropathic characteristics. The MIA-OA model is thought to contain both inflammatory and neuropathic pain-related states by affecting local tissues and sensory innervations of the peripheral nervous system. Cytokines such as IL-1β and TNF-α produced by activated synoviocytes, mononuclear cells or by articular cartilage itself significantly up-regulate metalloproteinases (MMP) gene expression [5]. Cytokines also blunt chondrocytes compensatory synthesis pathways required to restore the integrity of the degraded extracellular matrix. Moreover, in OA synovium, a relative deficit in the production of natural antagonists of the IL-1 receptor (IL-1RA) has been demonstrated, and could possibly be related to an excess production of nitric oxide in OA tissues. This, coupled with an upregulation in the receptor level, has been shown to be an additional enhancer of the catabolic effect of IL-1β inthis disease. IL-1β and TNF-alpha significantly up-regulate MMP-3 steady-state mRNA derived from human synovium and chondrocytes. The proinflammatory cytokine interleukin 1beta (IL- 1β) has several chemical and bioactive characteristics allowing this catabolic protein to be involved in initiation and progression of OA. IL-1β upregulation leads to cascade of intracellular events that can result in activation of proteinases, creation of a pro-destructive articular milieu, suppression of anabolic pathways, and a decrease in the synthesis of cartilage extracellular matrix [5]. IL-6 correlated positively with total histological score because IL-6 has been shown to use trans-signaling to regulate pre-B cell colony-enhancing factor (PBEF), which is involved in the progression of OA [16].

Conclusion

On the basis of findings of the present study, both MIA and papain can be used to induce experimental osteoarthritis in Hartley strain guinea pigs but MIA induces faster and more severe osteoarthritic changes than papain.

Acknowledgement

I am thankful to the Director ICAR -IVRI (Indian Veterinary Research Institute Izatnagar Bareilly) for providing the necessary facilities to carry out my research work and Indian Council of Medical Research (ICMR), New Delhi for financial assistance in the form of Junior Research Fellowship and Senior Research Fellowship.

Conflict of Interest statement

The authors declare that there is no conflict of interest.

Bibliography

1. Amarpal., *et al*. "Evaluation of xylazine, acepromazine and medetomidine with ketamine for general anaesthesia in rabbits". *Scandinavian Journal of Laboratory Animal Sciences* 37.3 (2010): 223-229.

2. Beyreuther B., *et al*. "Antinociceptive efficacy of lacosamide in the monosodium iodoacetate rat model for osteoarthritis pain". *Arthritis Research and Therapy* 9.1 (2007): R14.

3. Coulais Y., *et al*. "Etude d'un modèle expérimental d'arthrose. I: Induction et étude ultrastructurale". *Transactions of the Zoological Society of London* 31.7 (1983): 577-582.

4. Christopher BL and Margaret MS. "Animal models of osteoarthritis". *Current Rheumatology Reviews* 4.3 (2008): 175-182.

5. Daheshia M and Yao JQ. "The interleukin 1β pathway in the pathogenesis of osteoarthritis". *The Journal of Rheumatology* 35.12 (2008): 2306-2312.

6. Mobasheri A. "Applications of proteomics to osteoarthritis, a musculoskeletal disease characterized by aging". *Frontiers in Physiology* 2 (2011): 108.

7. Guingamp C., *et al*. "Mono-iodoacetate-induced experimental osteoarthritis. A dose-response study of loss of mobility, morphology, and biochemistry". *Arthritis and Rheumatism: Official Journal of the American College of Rheumatology* 40.9 (2007): 1670-1679.

8. Havdrup T., *et al*. "Papain-induced mitosis of chondrocytes in adult joint cartilage: an experimental study in full-grown rabbits". *Acta Orthopaedica Scandinavica* 53.1 (1982): 119-124.

9. Huang MH., *et al*. "Effects of sonication on articular cartilage in experimental osteoarthritis". *The Journal of Rheumatology* 24.10 (1997): 1978-1984.

10. Hunter DJ., *et al*. "The individual and socioeconomic impact of osteoarthritis". *Nature Reviews Rheumatology* 10.7 (2014): 437-441.

11. Khan HM., *et al*. "Papain induced progressive degenerative changes in articular cartilage of rat femorotibial joint and its histopathological grading". *Journal of Animal and Plant Sciences* 23.2 (2013): 350-358.

12. van der Kraan PM., *et al*. "Development of osteoarthritic lesions in mice by" metabolic" and" mechanical" alterations in the knee joints". *The American Journal of Pathology* 135.6 (1989): 1001.

13. Loeser RF. "Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix". *Osteoarthritis and Cartilage* 17.8 (2009): 971-979.

14. Mankin HJ., *et al*. "Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. III. Distribution and metabolism of amino sugar-containing macromolecules". *The Journal of Bone and Joint Surgery. American volume* 63.1 (1981): 131-139.

15. Murat N., *et al*. "Quantification of papain-induced rat osteoarthritis in relation to time with the Mankin score". *Acta Orthopaedica et Traumatologica Turcica* 41.3 (2007): 233-237.

16. Nowell MA., *et al*. "Regulation of pre-B cell colony-enhancing factor by STAT-3–dependent interleukin-6 trans-signaling: implications in the pathogenesis of rheumatoid arthritis". *Arthritis and Rheumatism: Official Journal of the American College of Rheumatology* 54.7 (2006): 2084-2095.

17. Rostand KS., *et al*. "Articular cartilage proteoglycans from normal and osteoarthritic mice". *Arthritis and Rheumatism: Official Journal of the American College of Rheumatology* 29.1 (1986): 95-105.

18. Schalkwijk J and Joosten LA. "Hydrogen peroxide suppresses the proteoglycan synthesis of intact articular cartilage". *The Journal of Rheumatology* 12.2 (1985): 205-210.

19. Castellan NJ. "Nonparametric statistics for the behavioral sciences". *McGraw-Hill* (1988).

20. Kopp S., *et al*. "Induction of osteoarthrosis in the guinea pig knee by papain". *Oral Surgery, Oral Medicine, Oral Pathology* 55.3 (1983): 259-266.

21. Williams JM and Brandt KD. "Immobilization ameliorates chemically induced articular cartilage damage". *Arthritis and Rheumatism: Official Journal of the American College of Rheumatology* 27.2 (1984): 208-216.

22. Williams JA and Thonar EJ. "Early osteophyte formation after chemically induced articular cartilage injury". *The American Journal of Sports Medicine* 17.1 (1989): 7-15.