# Effect of Orally Administered *Channa striatus* Extract Against Experimentally-Induced Osteoarthritis in Rabbits

Ng Yeen Tan Michelle, MSc
Ganabadi Shanthi, PhD
Mohamad Yusof Loqman, MVM

*Faculty of Veterinary Medicine*
*Universiti Putra Malaysia*
*43400 Serdang*
*Selangor, Malaysia*

**KEY WORDS**: *Channa striatus*, innervation, radiograph, PGP 9.5, immunoreactive fibers, osteoarthritis

## ABSTRACT

Effects of oral administration of *Channa striatus* extract were evaluated in rabbits with experimentally-induced osteoarthritis (OA) in a stifle joint. Changes after the treatment were evaluated by radiography, and innervation of the synovial membranes was assessed by immunohistochemistry. There was a significant reduction in soft tissue swelling observed in radiographs for treated animals 9 weeks after treatment compared with that observed for untreated ones. There was significant improvement in the density of PGP 9.5-immunoreactive nerve fibers in the synovial membrane of treated animals compared with that for controls. Results of this study suggest that oral administration of *C. striatus* extract can be a good alternative treatment for OA.

## INTRODUCTION

*Channa striatus,* a snakehead fish, is a well-known natural remedy that has long been used by people in many parts of Southeast Asia. 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.[1] *C. striatus* is normally consumed by women during postpartum wound healing.[2,3] It is also well known for its antinociceptive properties that make it suitable for reduction of postoperative pain.[4] Essential amino acid, such as glycine, and essential fatty acid, such as arachidonic acid, have been shown to actively participate in the normal blood clotting mechanisms by facilitating wound healing as well as in enhancing the antinociceptive activity.[4,5] Although there are extensive studies on the beneficial effect of *C. striatus* in wound healing as well as in antinociceptive properties, no research has been done to study its effect on joint disease, such as osteoarthritis (OA).

OA is a slow, progressive disorder of joints that involves softening and disintegration of the articular cartilage, with changes in the underlying bone.[6] However, the etiology of OA is still poorly understood. Recently, increasing evidence indicates that the neurogenic components from the sensory and motor nerve fibers play a major role

**Table 1.** Density of PGP 9.5-immunoreactive Fibers in the Synovial Membrane of Normal Joints, Saline-Treated Arthritic Joints, and Arthritic Joints Treated with Channa striatus Extract in New Zealand White Rabbits

| Joint | Density of PGP 9.5-Immunoreactive Fibers* |
|---|---|
| Normal | ++++ |
| Saline-treated arthritic | – |
| Channa striatus-treated arthritic | +++ |

*Scale:
++++ = Abundant immunoreactive fibers were present. Blood vessels completely surrounded by rich plexus of fibers and a large number of free nerve fibers were present.
+++ = Blood vessels were only partially surrounded by thin plexus of immunoreactive fibers. Free nerve fibers were sparse.
++ = Few free nerve fibers and fibers associated with blood vessels present.
+ = Only one or two nerve fibers present in the entire synovial membrane.
– = No nerve fibers were detected.

in the development of arthritis.[7,8] Chemical lesioning of these fibers showed a decrease in the inflammatory response in arthritic joints.[9,10] Hence, it is suggested that these neurogenic components could lead to inflammatory response and could be involved in the pain pathway in OA.[11,12]

The present this study was conducted to examine the effect of *C. striatus* on the experimentally induced OA in rabbits. In this study, the general innervation of synovial membrane from the treated and untreated animals was mapped out by using antiserum against protein gene product 9.5 (PGP 9.5), a major protein component of neuronal cytoplasm.[13]

## MATERIALS AND METHODS

### Animals

Twenty 6-month-old New Zealand White rabbits were separated into two groups of 10. Treatments (*C. striatus* or saline control) were allocated to the two groups. Radiographs of the stifle joints of all animals ruled out any possibility of existing joint disease. OA was then induced in the right stifle joint of each rabbit by transecting the anterior cruciate ligament based on a published method.[14] Radiographs were taken again 8 weeks later to verify that OA had developed in these animals prior to treatment. Follow-up radiographs were taken again following 9 weeks of treatment to observe and compare changes in treated and untreated animals. All rabbits had free access to water and pellets during the induction and treatment periods.

### *Channa striatus* Extract Preparation and Treatments

*C. striatus* extract was prepared according to published methods.[4] Briefly, the extract was prepared by using fresh boneless fish fillet with the skin still intact, which yielded a final concentration of approximately 50% fish in water. The extract was administered orally to each rabbit at 10 ml/kg body weight three times daily. Rabbits in the control group received normal saline at 10 ml/kg body weight three times daily. All animals were treated according to this schedule for 9 weeks before they were euthanized. Samples of the left (nonarthritic) and right (arthritic) synovial membrane were collected for immunohistochemistry evaluation.

### Immunohistochemistry

The synovial membrane was fixed overnight in 4% paraformaldehyde and processed for immunohistochemistry. Briefly, samples were snap-frozen in isopentane, cooled in liquid nitrogen, and sectioned at 8µm in a cryostat. The sections were dehydrated in alcohol, rinsed in 0.1M phosphate buffered saline, and incubated in the primary antiserum: anti-PGP 9.5 (Ultraclone Cambridge Ltd) for 24 hours at 4˚C. Sections were incubated in secondary antiserum (biotinylated goat anti-rabbit [GAR] immunoglobulins), followed by avidin-biotinylated horseradish peroxidase complex (avidin-HRP). Both GAR and avidin-HRP were incubated at room temperature for 1 hour. Finally, sections were immersed in glucose diaminobenzidine nickel substrate, washed in distilled water, stained with eosin, and then mounted with DPX resin. A negative control was prepared by omitting the primary antisera from the process.

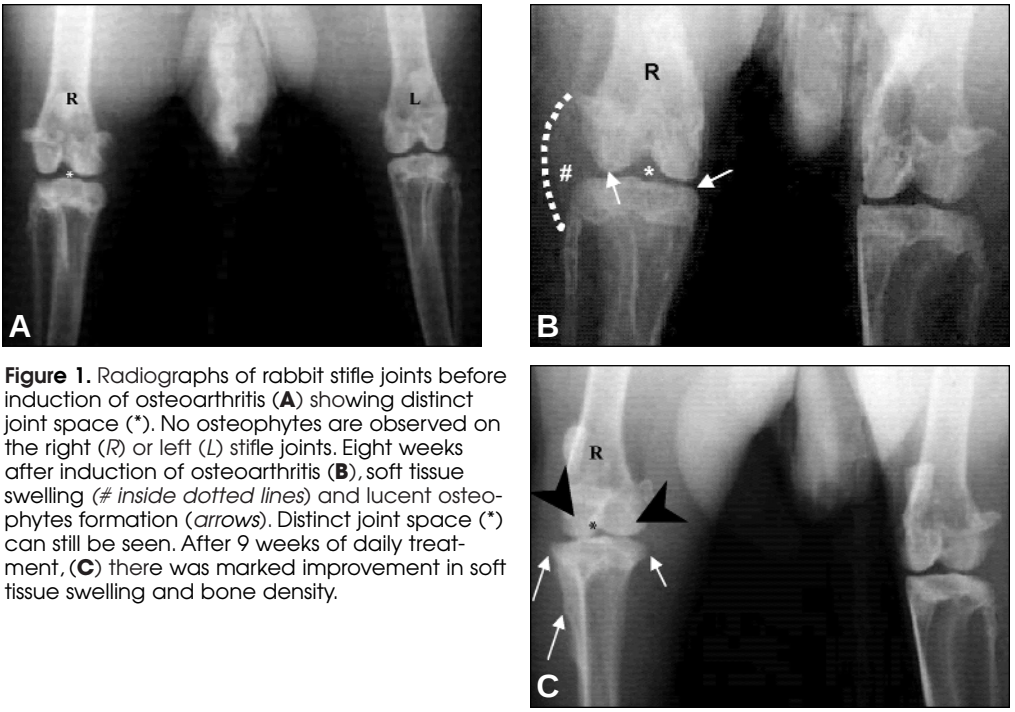


**Figure 1.** Radiographs of rabbit stifle joints before induction of osteoarthritis (**A**) showing distinct joint space (*). No osteophytes are observed on the right (*R*) or left (*L*) stifle joints. Eight weeks after induction of osteoarthritis (**B**), soft tissue swelling *(# inside dotted lines)* and lucent osteophytes formation (*arrows*). Distinct joint space (*) can still be seen. After 9 weeks of daily treatment, (**C**) there was marked improvement in soft tissue swelling and bone density.

## RESULTS

### Radiographic Changes

Radiographs taken prior to the induction of OA showed distinct joint space and no sign of possible joint disease (Figure 1). Development of OA was observed in joints 8 weeks after induction of OA (Figure 1). Joint-space narrowing with significant periarticular soft tissue swelling was observed as an increased radiopacity area around the joint. Minute and lucent osteophytes had also developed in these joints, particularly at the margins of the periarticular bone. Radiographs taken after the 9th week of treatment (Figure 1) showed marked reduction in soft tissue swelling for joints treated with *C. striatus* extract.

### Innervation of the Synovial Membrane

Innervation of PGP 9.5-immunoreactive fibers in the synovial membrane from normal nonarthritic (left) joints was very dense, particularly in the subintimal layer of the synovial membrane (Figure 2). Few nerve fibers had penetrated the intimal layer of the similar synovial membrane. In contrast, no immunoreactive fibers were detected in both subintimal and intimal layers of the synovial membrane from joints that had arthritis induced and treated with saline (Figure 2). The synovial membrane was heavily infiltrated with inflammatory cells and blood vessels were abundantly found in the subintimal layer.

However, density was improved for immunoreactive fibers in the synovial membrane from arthritic joints treated with *C. striatus*. The distribution of PGP 9.5-immunoreactive fibers detected in the subintimal layer of the synovial membrane was similar to that detected in the normal synovial membrane, although the density of these immunoreactive fibers was lower than for normal synovial membrane (Figure 2).

## DISCUSSION

*C. striatus* is a freshwater fish that is widely consumed for its nutritional value as well as for its beneficial effect in wound healing.[15,16] Although there are many studies reported on its therapeutic effect in wound healing and in pain reduction, no research has been done to study the effect of this fish extract on joint diseases.

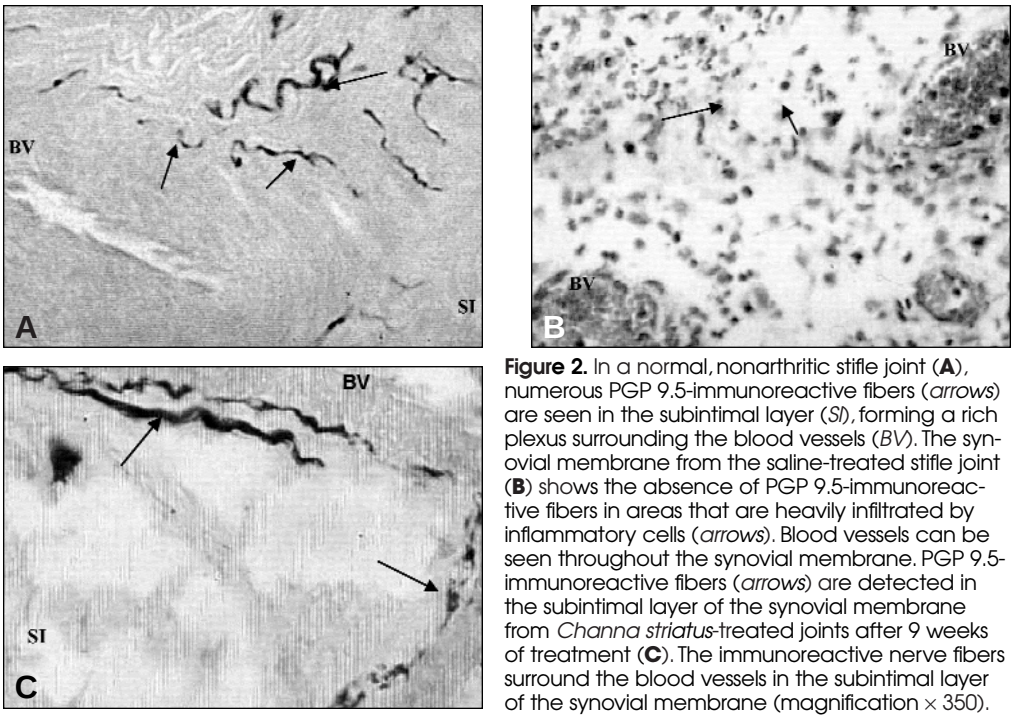


**Figure 2.** In a normal, nonarthritic stifle joint (**A**), numerous PGP 9.5-immunoreactive fibers (*arrows*) are seen in the subintimal layer (*SI*), forming a rich plexus surrounding the blood vessels (*BV*). The synovial membrane from the saline-treated stifle joint (**B**) shows the absence of PGP 9.5-immunoreactive fibers in areas that are heavily infiltrated by inflammatory cells (*arrows*). Blood vessels can be seen throughout the synovial membrane. PGP 9.5-immunoreactive fibers (*arrows*) are detected in the subintimal layer of the synovial membrane from *Channa striatus*-treated joints after 9 weeks of treatment (**C**). The immunoreactive nerve fibers surround the blood vessels in the subintimal layer of the synovial membrane (magnification × 350).

In this preliminary study, inflammation of arthritic joints was reduced in joints treated with *C. striatus* as evidenced by radiographic changes and in the overall innervation of the synovial membrane in comparison with conditions in highly inflamed joints of untreated arthritic joints.

Inflammation of the osteoarthritic joints is mainly due to the fragmentation of the degrading articular cartilage into the synovium, which subsequently triggers the inflammatory process by the production of pro-inflammatory mediators as well as the recruitment of inflammatory cells into the joint.[17] The constant release of pro-inflammatory mediators, such as cytokines and reactive oxygen species, will cause excessive release of neuropeptides from the nerve fibers to a level below that which can be detected, similar to that reported by Mapp et al.[18] Apart from that, release of histamine via mast cells degranulation due to activation by cytokine can be toxic to nerve fibers, thus causing destruction of these nerve fibers as seen in this study.[19]

The antiinflammatory mechanism of action of *C. striatus* extract on the osteoarthritic joint is beyond the scope of the present study. It maybe due to the high content of amino acids and fatty acids, which may help prevent further damage in the osteoarthritic joint by aiding in the synthesis of more collagen fibers in the articular cartilage. This action could enhance the repair process of the articular cartilage by lessening fragmentation of the degrading articular cartilage into the joint cavity and helping to reduce the inflammatory reactions in the joint.

It is also suggested that *C. striatus* treatment could help in remodeling of collagen via the synthesis of inter- and intramolecular protein cross-linking.[4] This action would in turn help strengthen the structure of the articular cartilage, preventing further degradation. As a result, there would be a marked reduction in the inflammation of the synovium, preventing the loss of immunoreactivity of PGP 9.5 from the synovial membrane.

Results of this study indicate that *C. striatus* extract could be a useful alternative treatment in OA. However, additional studies of the extract should be undertaken, pos-

sibly with longer treatment periods and studies of the mechanism of action, to determine value of this extract can as a 'chondroprotective' agent.

## REFERENCES


1. Mat Jais AM, Matori MF, Kittakoop P, Sowanborirux K: Fatty acid compositions in mucus and roe of Haruan, *Channa striatus,* for wound healing. *Gen Pharmacol 1998;* 30(4): 561–563.
2. Mohsin AK, Ambak MA: Freshwater fishes of Peninsula Malaysia. In: *Univeriti Putra Malaysia*. Serdang, Malaysia; 1983:157–161.
3. Sabto A: *Environmental Physiological Studies of Haruan in Sabah* [honor thesis]. Sabah, Malasia: Universiti Kebangsaan Malaysia; 1998.
4. Mat Jais AM, Dambisya YM, Lee TL: Antinociceptive activity of *Channa striatus* (haruan) extracts in mice. *J Ethnopharmacol* 1997; 57(2):125–130.
5. Baie SH., Sheikh KA: The wound healing properties of *Channa striatus*-cetrimide cream-tensile strength measurement. *J Ethnopharmacol* 2000; 71(1–2): 93–100.
6. Creamer P, Hochberg MC: *Osteoarthritis*. *Lancet* 1997; 350:503–509.
7. Kidd BL, Cruwys SC, Garrett NE, Mapp PI, Jolliffe VA, Blake DR: Neurogenic influences on contralateral responses during experimental rat monoarthritis. *Brain Res* 1995; 688:72–76.
8. Mapp PI, Walsh DA, Garret NE, et al: Effect of three animal models of inflammation on nerve fibers in the synovium. *Ann Rheum Dis* 1994; 53:240–246.
9. Levine JD, Dardick SJ, Roizen MF, Helms C, Basbaum A: Contribution of sensory afferents and sympathetic efferents to joint damage in experimental arthritis. *J Neurosci* 1986; 6:3423–3429.
10. Nolano M, Simone DA, Wendelschafer-Crab G, Johnson T, Hazen E, Kennedy WR: Topical capsaicin in humans: Parallel loss of epidermal nerve fibers and pain sensation. *Pain* 1999; 81(1–2):135–145.
11. Imai S, Tokunaga Y, Konttinen YT, Maeda T, Hukuda S, Santavirta S: Ultrastructure of the synovial sensory peptidergic fibers is distinctively altered in different phases of adjuvant induced arthritis in rats: Ultramorphological characterization combined with morphometric and immunohistochemical study for substance P, calcitonin gene-related peptide, and protein gene product 9.5. *J Rheumatol* 1997; 24(11):2177–2187.
12. Saito T, Koshino T: Distribution of neuropeptides in synovium of the knee with osteoarthritis. *Clin Orthop* 2000; 376:172–182.
13. Doran JF, Jackson P, Kynoch PAM, Thompson RJ: Isolation of PGP 9.5, a new human neuron specific protein detected by high resolution 2 dimensional electrophoresis. *J Neurochem* 1983; 40:1542–1547.
14. Pond MJ, Nuki G: Experimentally induced osteoarthritis in the dog. *Ann Rheum Dis* 1973; 32:387–388.
15. Mat Jais AM, McCullock R, Croft K: Fatty acid and amino acid composition in haruan as a potential role in wound healing. *Gen Pharmacol* 1994; 25:947–950.
16. Wee KL: Snakeheads–their biology and culture. In: Muir R, ed. *Recent Advances in Aquaculture*. Boulder, CO: Westview Press; 1982:181–213.
17. Johnston SA: Osteoarthritis: Joint anatomy, physiology and pathobiology. *Vet Clin North Am Small Anim Pract* 1997; 27(4):699–723.
18. Mapp PI, Gibson SJ, Terry JM, et al: Substance P, calcitonin gene-related peptide and C-flanking peptide of neuropeptide Y immunoreactive are present in normal synovium but depleted in patients with rheumatoid arthritis. *Neurosci* 1990; 37(1):143–153.
19. Dray A: Inflammatory mediators of pain. *Br J Anaesth* 1995; 75:125–131.