



UNIVERSITI PUTRA MALAYSIA

**EFFECT OF *CHANNA STRIATUS* AND *CURCUMA LONGA* IN
EXPERIMENTALLY-INDUCED OSTEOARTHRITIS IN RABBITS**

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By

MICHELLE NG YEEN TAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Master of Science**

July 2003



Specially dedicated to my parents, Weng Onn, my two elder brothers and sisters-in-law and not forgetting my adorable nephews and nieces.

I love you all very much!
Thanks for your love, encouragement and support!

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirements for the degree of Master of Science

**EFFECT OF *CHANNA STRIATUS* AND *CURCUMA LONGA* EXTRACTS IN
EXPERIMENTALLY-INDUCED OSTEOARTHRITIS IN RABBITS**

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Channa striatus and *Curcuma longa* are two well known natural products that have long been used in treating various kinds of ailments. *Channa striatus* is high in essential amino acids and fatty acids that played an important role in wound healing as well as in anti-nociceptive activities. *Curcuma longa* on the other hand, contains an active compound called curcuminoids that are responsible for its anti-inflammatory, anti-oxidant and anti-cancer properties.

Therefore, in this study, *Channa striatus* and *Curcuma longa* extracts were used in the treatment of experimentally induced Osteoarthritis (OA) in rabbits. OA was induced on the right stifle joint of the rabbits in the treatment and the negative control groups by transecting the anterior cruciate ligaments.



These animals were left for 8 weeks to develop OA. Radiography and ultrasonography were performed on the induced joints to determine the development of OA prior to *Channa striatus* and *Curcuma longa* treatments.

During the progression of OA, the induced joints began to show sign of OA development as early as the 2nd week after induction of OA as observed in ultrasonograph. Slight joint space narrowing, which reflect the deteriorating articular cartilage was detected by the ultrasonography as early as the 2nd week post induction. On the 3rd week after the induction of OA, ultrasonography was able to detect significant joint space narrowing and total diminution of joint space on the 4th week post induction. In addition to that, irregular joint surface has developed in the induced joints as seen on the ultrasonograph taken on the 5th and the 6th week post induction. Apart from these, other structures such as the infrapatellar fat, the patellar ligament and the synovial membrane in the induced joints also underwent osteoarthritic changes as seen in ultrasonographs.

On the 8th week post induction there was a significant periarticular soft tissue swelling detected by radiography and ultrasonography. Soft tissue swelling detected on the radiographs was seen as an increased radiopacity area around the joint. In ultrasonographs, the swelling of the joint could be observed as an increased distance between the surface of joint and the skin compared to the normal uninduced joints. On the 9th week of treatment, a significant reduction of soft tissue swelling was observed on *Channa striatus*- and *Curcuma longa*- treated joints compared to the untreated joints.

Although the treatments were effective in reducing inflammations and swelling, these extracts did not exhibit any improvement on other structures of the joints. Extra bone formation and diminution of the joint space were observed on both radiographs and ultrasonographs on the 9th week of treatment. These similar changes were further confirmed with the gross findings on the opened joints upon euthanasia.

In the immunohistochemistry study, synovial membrane biopsies from the normal, treated and negative control joints were obtained to study the general innervation of the synovial membrane. The immunoreactive fibres stained against PGP 9.5, CGRP and NPY antisera were not detected in the control joints compared to the normal synovial membrane. The synovial membrane from the untreated joints was heavily infiltrated with inflammatory cells, which may account for the diminished immunoreactive nerve fibres from the synovial membrane.

However, the number of immunoreactive nerve fibres detected in the synovial membranes from *Channa striatus* and *Curcuma longa* treated joints was higher than in the synovial membrane from the control untreated joint. They exhibited a similar distribution to the nerve fibres found in normal synovial membrane but less numerous.

Therefore, the present study showed that both *Channa striatus* and *Curcuma longa* extracts showed good signs of healing in OA and these extracts can be used as a good alternative treatment in OA.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN EKSTRAK *CHANNA STRIATUS* DAN *CURCUMA LONGA* DI
DALAM UJIKAJI OSTEOARTRITIS TERARUH PADA LUTUT ARNAB**

Oleh

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Channa striatus dan *Curcuma longa* merupakan dua produk semulajadi yang telah lama digunakan dalam mengubati pelbagai jenis penyakit. *Channa striatus* mengandungi asid amino perlu dan asid lemak yang tinggi, yang memainkan peranan penting dalam menyembuhkan luka dan mengurangkan kesakitan. *Curcuma longa* pula terdiri daripada curcuminoids yang bertanggungjawab dalam kesan anti-inflamatori, anti oksidan dan anti- kansernya.

Oleh itu, ekstrak *Channa striatus* dan *Curcuma longa* telah digunakan dalam ujikaji ini untuk merawat OA teraruh dalam lutut arnab. Di dalam ujikaji ini, osteoarthritis telah diaruhkan pada lutut kanan arnab daripada kumpulan rawatan dan kawalan negatif, dengan memotong ligamen krusiat anterior. Arnab-arnab ini kemudiannya dibiarkan selama 8 minggu supaya OA dapat



berkembang. Sementara itu, pemeriksaan radiograf dan ultrasonograf telah dijalankan pada lutut yang diaruh untuk memastikan penyakit ini telah berkembang pada lutut-lutut ini sebelum rawatan *Channa striatus* dan *Curcuma longa* dimulakan.

Semasa perkembangan OA, lutut-lutut teraruh ini mula menunjukkan perubahan-perubahan osteoartritik seawal-awal 2 minggu selepas aruhan OA. Penyusutan kecil ruang di antara sendi yang menandakan kerosakan pada sendi tulang rawan telah dapat dikesan oleh ultrasonografi seawal-awal 2 minggu selepas aruhan. Penyusutan ruang antara sendi yang ketara telah dikesan pada minggu yang ke-3 dan pada minggu ke-4 selepas aruhan, tiada lagi ruang di antara sendi telah dikesan. Tambahan pula, ketidakrataan permukaan pada sendi telah dilihat pada ultrasonograf pada minggu yang ke-5 dan ke-6 selepas aruhan. Di samping itu, struktur-struktur lain dalam sendi lutut teraruh seperti lemak infrapatela, ligamen patela dan membran sinovial juga telah menunjukkan perubahan-perubahan osteoartritik seperti yang dapat dilihat pada ultrasonograf.

Pada minggu ke-8 selepas aruhan OA, pembengkakan tisu lembut di sekitar sendi telah dapat dikesan oleh radiografi dan ultrabunyi. Pembengkakan tisu lembut dapat dilihat pada radiograf sebagai peningkatan 'radiopacity' di sekitar sendi manakala pada ultrasonograf, bengkak tisu lembut pada sendi dapat dilihat sebagai peningkatan jarak di antara permukaan sendi dan kulit. Walaubagaimanapun, bengkak pada tisu lembut

dalam rawatan ekstrak *Channa striatus* dan *Curcuma longa* telah susut pada minggu ke-9 rawatan berbanding dengan lutut-lutut yang tidak dirawat.

Walaupun rawatan yang diberikan berkesan dalam mengurangkan bengkak pada tisu lembut, ia adalah kurang berkesan dalam menghindari perubahan struktur lain dalam sendi. Pertumbuhan tulang tambahan dan penyusutan sepenuh ruang di antara sendi dalam lutut-lutut daripada kumpulan rawatan dapat dilihat pada radiograf dan ultrasonograf pada minggu ke-9 rawatan. Perubahan-perubahan sedemikian juga dapat dilihat dengan mata kasar melalui bedah siasat lutut-lutut ini selepas eutanasia.

Di dalam ujikaji immunohistokimia pula, membran sinovial daripada kumpulan normal, rawatan dan kawalan telah diperolehi untuk mengkaji keseluruhan rangkaian gentian saraf. Secara keseluruhannya, gentian immunoreaktif yang dilabel oleh antisera PGP 9.5, CGRP dan NPY tidak dapat dikesan pada membran sinovial daripada kumpulan kawalan negatif berbanding kumpulan normal. Didapati juga, membran sinovial daripada kumpulan kawalan dipenuhi dengan sel-sel inflamatori yang mungkin menyebabkan kehilangan gentian-gentian immunoreaktif daripada membran sinovial .

Walaupun bagaimanapun, bilangan gentian saraf di dalam membran sinovial daripada kumpulan rawatan *Channa striatus* dan *Curcuma longa* adalah lebih tinggi berbanding dengan membran sinovial daripada kumpulan kawalan negatif. Didapati gentian-gentian saraf ini menyerupai distribusi

gentian saraf dalam membran sinovial daripada kumpulan normal, tetapi bilangannya adalah kurang.

Oleh itu, ekstrak *Channa striatus* and *Curcuma longa* ini telah menunjukkan kesan-kesan positif dalam rawatan OA dan dengan demikian ekstrak-ekstrak ini boleh digunakan sebagai rawatan alternatif yang baik dalam rawatan OA.

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I certify that an Examination Committee met on 10th July 2003 to conduct the final examination of Michelle Ng Yeen Tan on her Master of Science thesis entitled "Effect of *Channa striatus* and *Curcuma longa* in Experimentally-Induced Osteoarthritis in Rabbits" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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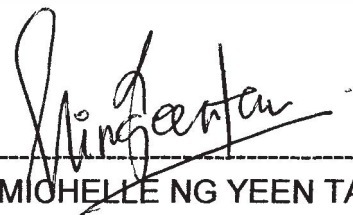
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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



(MICHELLE NG YEEN TAN)

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TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENT	x
APPROVAL SHEETS	xii
DECLARATION FORM	xiv
LIST OF TABLES	xvii
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xxiv
CHAPTER	
I INTRODUCTION	1
General Introduction to Arthritis	1
Osteoarthritis (OA)	1
Clinical Features in Osteoarthritis (OA)	2
Management of Osteoarthritis	3
Objectives	5
II LITERATURE REVIEW	6
Osteoarthritis (OA)	6
Classification of OA	7
Trauma	9
Cruciate Ligament Rupture	9
Diagnosis of Osteoarthritis (OA)	12
Physical Examination	12
Diagnostic Imaging of Osteoarthritis	13
Neurogenic Components in Arthritis	16
Innervation of the synovial membrane	16
Neurogenic inflammation	16
Calcitonin Gene-Related Peptide (CGRP)	18
Sensory Neuropeptides and Arthritis	20
The Role of Motor Nerves in Arthritis	22
Protein Gene Product (PGP 9.5)	23
Management of Osteoarthritis (OA)	25
Pharmacotherapy in OA	25
Non-Pharmacologic Treatment	36
Natural Remedy for Osteoarthritis (OA)	39
<i>Curcuma longa</i> (Turmeric)	39
<i>Channa striatus</i> (Haruan)	43
III General Materials and Methods	45
Experimental Animals	45
Experimental Design	45
Induction of Osteoarthritis (OA)	46



	Preparation of <i>Curcuma longa</i> –Ethanol Extract	46
	Preparation of <i>Channa striatus</i> Extract	47
	Treatment	47
IV	RADIOGRAPH AND ULTRASONOGRAPHIC EVALUATION OF OSTEOARTHRITIS (OA)	49
	Introduction	49
	Materials and Methods	50
	Radiography	50
	Ultrasonography	53
	Gross Necropsy	54
	Results	55
	Radiographic evaluation	55
	Ultrasonographic evaluation	62
	Gross Anatomy Changes	72
	Discussion	75
V	IMMUNOREACTIVITY OF PGP 9.5, CGRP AND NPY FIBRES	86
	Introduction	86
	Materials and Methods	87
	Results	89
	Discussion	107
	Normal Synovial Membrane	107
	Osteoarthritic Synovial Membrane (control animals)	110
	Synovial membranes from <i>Channa Striatus</i> and <i>Curcuma longa</i> treated animals	114
VI	CONCLUSION	120
	BIBLIOGRAPHY	122
	APPENDICES	139
	VITA	142

LIST OF TABLES

Table		Page
4.1	Radiographic criteria for the assessment of OA	52
4.2	Radiographic scores for all the induced joints from the control and treatment groups	57
5.1	Scoring system to assess the density of the intimal and subintimal innervation	96
5.2	Density of innervation in the intimal layer of the synovial membrane	106
5.3	Density of innervation in the subintimal layer of the synovial membrane	106

LIST OF FIGURES

Figures		Page
3.1	Summary of the methods used in this study	140
4.1	Radiographs of the normal and control stifle joints (Posterior-Anterior view). (a) Normal group: Radiograph taken prior to the induction of OA shows distinct joint space (*) and no osteophytes are observed on the right (R) and left (L) stifle joints (OA Grade 0). (b) Control group: Right stifle (R) (post induction week 17th); Moderate joint space narrowing (*), osteophytes formation (arrows) are detected. Right femoral condyle (R) is seen is seen enlarged due to extra bone formation (arrowhead). Soft tissue swelling (# with dotted lines) is evident in the right (R) joint (OA Grade 3). L= left joint	58
4.2	Radiographs of the stifle joints from <i>Channa striatus</i> treated group (Posterior-Anterior view). (a) Before treatment: Right stifle joint (R) (8 th week post induction); soft tissue swelling (# with dotted lines) and lucent osteophytes formation (arrows) are observed. Distinct joint space (*) can still be seen (OA Grade 2). (b) After treatment: Right stifle joint (R) (on the 9 th week of treatment); significant soft tissue swelling reduction is observed. Total diminution of joint space (*), numerous osteophytes formation (arrows), enlargement of the femoral condyle (arrowheads) are evident at the end of the 9 th week of treatment (OA Grade 4).	59
4.3	Radiographs of the stifle joints from <i>Curcuma longa</i> treated group (Posterior-Anterior view). (a) Before treatment: Right stifle (R) (8 th week post induction): soft tissue swelling (# with dotted lines) and osteophytes (arrows) are detected. Distinct joint space can still be seen (*) (OA Grade 2). (b) After treatment: Right stifle (R) (9 th week of treatment): significant reduction of soft tissue swelling and numerous osteophytes (arrows) are observed. The femoral condyle is enlarged due to extra bone formation (arrowhead). However, the joint space narrowing is not significant (*) (OA Grade 3). Radiographs of the stifle joints from <i>Curcuma longa</i> treated group in other animals (Posterior-Anterior view). (c) Before treatment: Right stifle (R) (8 th week post induction): soft tissue swelling (# with dotted lines) and osteophyte (arrow) are observed (OA grade 2). (d) After treatment: Right stifle (R) (9 th week after treatment): Radiograph shows a significant joint space narrowing (*). Many osteophytes are observed (arrows) and the femoral	60

condyle is seen enlarged due to extra bone formation (arrowhead). Reduction of soft tissue swelling can also be observed (OA Grade 4).

- 4.4 (a) Ultrasonograph of the right stifle joint of the normal animal shows a distinct joint space (#). Note also the smooth, homogenous and hyperechoic surface of the femoral (F) and tibial (T) condyles (arrows) is clearly seen. The infrapatellar fat appears homogenous echogeneity (IF). The distance between the skin and the bone surface is almost neglected. The patellar ligament (PL) is clearly seen as straight hyperechoic line underneath the skin between the femur and tibia. A hyperechoic line structure underneath the patellar ligament is believed to be the synovial membrane (arrowhead). (b) Ultrasonograph of the normal stifle joint in other normal animal shows a smooth and inhomogenous hyperechoic surface of the tibial and femoral condyles with hyperechoic dots structures (arrows). A distinct joint space is clearly seen (#). 64
- 4.5 (a) Ultrasonograph of the right stifle joint first week after induction reveals a slight soft tissue swelling as indicated by the distance between the bone surface and the skin (*). Note that the joint space still distinct (#). The patellar ligament is seen enlarged and hypoechoic (PL). (b) In certain animals of the same group, which have significant joint swelling, a fluid-filled anechoic area (H) is detected on the lateral aspect of the joint. 65
- 4.5 (c) Ultrasonograph of the right stifle on the 2nd week after induction shows a slight joint space narrowing (#). Note also the distance between the skin and tibial surface that indicates the joint is swelling (*). (d) Ultrasonograph of the right stifle on the 3rd week after induction, reveals marked decreasing of the joint space (#). Soft tissue swelling is barely detected. The joint surface still appears as smooth and hyperechoic structures (arrow). 66
- 4.5 (e) Ultrasonograph on the right stifles on the 4th week after induction shows a total diminution joint space (#). The joint surface (arrow) still appears smooth and hyperechoic on both femoral (F) and tibial (T) condyles. Note also the infrapatellar fat (IF) appears hypoechoic relative to surrounding structures. (f) Ultrasonograph of the right stifle on the 5th week post induction shows irregular surface (arrows) of the femoral (F) and tibial (T) condyles. There is no joint space (#) can be detected. 67

- 4.5 (g) Ultrasonograph of the right stifle on the 6th week after induction shows an irregular surface (arrow) of the femoral (F) and tibial (T) condyles. No joint space (#) is detected. The distance between the bone surface and the skin shows that the joint is swelling (*). (h) Ultrasonograph of the right stifle joint on the 8th week post induction shows a clear irregular hyperechoic surface of the femoral (F) and tibial (T) condyles (arrow). The joint is also swelling. 68
- 4.6 (a) Ultrasonograph of the right stifle of *Channa striatus*-treated group on the 8th week of treatment shows irregular and hyperechoic surface of the femoral (F) and tibial (T) condyles (arrows). The patellar ligament is not seen here as it has deviated to the lateral side. The infrapatellar fat (IF) has become more hyperechoic and there is no joint space observed. Note also the swelling of the joint has begun to subside (*). (b) Ultrasonograph of the right stifle joint of the *Channa striatus* - treated group on the 9th week post treatment reveals an irregular surface and hyperechoic surface on both femoral (F) and tibial (T) condyles due to extra bone formation (arrows). Note also that there is no joint space detected and swelling is much reduced. The infrapatellar fat (IF) has become hyperechoic. The patellar ligament is not seen because it has deviated to the lateral side. 69
- 4.7 (a) Ultrasonograph of the right stifle joint of *Curcuma longa* treated group on the 8th week of treatment reveals irregular and hyperechoic surface of the femoral (F) and tibial (T) condyles. No joint space is detected and the infrapatellar fat (IF) appears more echogenic. Note also the reduction of soft tissue swelling (*). (b) Ultrasonograph of the right stifle joint of *Curcuma longa*-treated group on the 9th week of treatment reveals an irregular surface of the femoral (F) and tibial (T) condyles which is due to extra bone formation (arrows). The soft tissue swelling is much reduced (*) and no joint space is detected (#). 70
- 4.8 Ultrasonograph of the right stifle joint of the control animal 17 weeks post induction reveals an irregular surface of the femoral and tibial condyles (arrows). The enlarged condyles are seemed to cause the diminution of the joint space (#). Note also the hyperechoic appearance of the infrapatellar fat (IF) below the patellar ligament (PL). A hyperechoic structure underneath the patellar ligament is believed to be the thickening of the synovial membrane (arrowhead). The distance between the bone surface and the skin indicates that the joint is swelling (*). 71

- 4.9 (a) This picture shows the gross anatomy of the normal right (R) and left (L) joints taken on the 9th week of treatment. The surface of both joints is smooth with no possible deformity detected. (b) This picture shows the gross anatomy changes on the right (R) joint seen in the negative control animals on the 9th week of treatment. Severe inflammation is seen on the right (R) joint compared to the normal uninduced joint on the left (L). Both femoral (F) and tibial (T) condyles on the right (R) joint are enlarged with very irregular surface of the bone (arrows) in comparison with the left (L) joint and the normal joints taken on the 9th week of treatment. 73
- 4.9 Gross findings of the opened right stifle joints of *Channa striatus* and *Curcuma longa* treated groups on the 9th week of treatment. (c) *Channa striatus* treated group: Irregular surface (arrows) on the femoral condyle (F) is observed but it is less inflamed compared to the negative control joint opened at the same time. Both femoral (F) and tibial (T) condyles are enlarged compared to the normal uninduced joints (d) *Curcuma longa* treated group: Irregular surface (arrows) of the femoral condyle (F) is observed with evidence of reduced inflammation compared to the negative control joint opened at the same time. Enlargement of both femoral (F) and tibial (T) condyles can be clearly seen. 74
- 5.1 H&E staining for normal and control synovial membranes. 91
 (a) Normal: The synovial membrane consists of two layers; the intimal (I) and subintimal (SI). The intimal layer consists of one or two synovial cells layer (arrowheads). The subintimal layer consists of loosely packed coarse collagen fibres. There is no demarcation of intimal-subintimal junction (x100). Control: Hyperplasia of the synovial cells (arrowheads) is observed. Both intimal (I) and subintimal layer (SI) layers of the synovial membrane are heavily infiltrated with inflammatory cells (arrows). Blood vessels (BV) can be seen throughout the synovial membrane (x12.5).
- 5.2 H&E staining for synovial membranes from *Channa striatus* and *Curcuma longa* treatment groups after nine weeks of treatment. 92
 (a) *Channa striatus*: Notice that there is no sign of inflammatory cells in both intimal (I) and subintimal (SI) layers. Significant hyperplasia of synovial cells (arrowhead) is observed (x50). (b) *Curcuma longa*: No sign of inflammatory cells in the subintimal (SI) and intimal (I) layers but there is a significant thickening of the synovial cells layer (arrowheads) (x50).

- 5.3 Negative control section shows no immunohistochemistry staining against specific immunoreactive fibres. (x40) 97
- 5.4 PGP 9.5 immunoreactive fibres in the normal synovial membranes. (a) Intimal layer: Few PGP 9.5 immunoreactive fibres are detected in the intimal layer (I) (arrow) (x350). (b) Subintimal layer: Numerous PGP 9.5 immunoreactive fibres (arrows) are seen in the subintimal layer (SI) and they formed a rich plexus surrounding the blood vessels (BV) (x350). (c) CGRP-immunoreactive fibres (arrows) are abundantly detected in the subintimal layer (SI) of the synovial membrane and they are often associated with blood vessels (BV) (x350). (d) Few free CGRP-immunoreactive fibres (arrows) are seen in the intimal layer (I) of the normal synovial membrane (x350). (e) NPY-immunoreactive fibres (arrows) are seen exclusively in the subintimal layer (SI) of the normal synovial membrane and they are often associated with blood vessels (BV) (x350). (f) Very few NPY-immunoreactive fibres (arrows) are detected in the intimal (I) layer of the normal synovial membrane (x350). 98
- 5.5 Synovial membrane from the right stifle joint of the control untreated group: absence of PGP 9.5-immunoreactive fibres in areas that are heavily infiltrated by inflammatory cells (arrows). Similar findings are also observed with CGRP- and NPY-immunoreactive fibres, where there is a total loss of immunoreactivity in the synovial membrane. Blood vessels (BV) can be seen throughout the synovial membrane (x350). 101
- 5.6 (a) PGP 9.5-immunoreactive fibres (arrows) are detected in the subintimal layer (SI) of the synovial membrane from *Channa striatus*- treated joints. These immunoreactive nerve fibres are seen surrounding the blood vessels (BV) in the subintimal layer (SI) of the synovial membrane (x350). (b) CGRP-immunoreactivity (arrow) is detected in the subintimal layer (SI) of the *Channa striatus*-treated synovial membrane. However, the density of the immunoreactive fibres is significant lower and in close proximity with blood vessels (BV) (x350). (c) Sparse NPY-immunoreactive fibres (arrow) are seen in the subintimal layer (SI) of the *Channa striatus*-treated synovial membrane and in close proximity with blood vessels (BV) (x350) (c) *Curcuma longa* treated group: Sparse CGRP-immunoreactive fibres are observed in the subintimal layer. (x330) 102

- 5.7 (a) Innervation of the synovial membranes from *Curcuma longa*-treated joints shows a decrease in the density of PGP 9.5-immunoreactive fibres (arrows) in the subintimal layer (SI). The fibres are often associated with blood vessels (BV) (x350). (b) Sparse CGRP-immunoreactive fibres are observed in the subintimal layer of the synovial membrane (SI) from *Curcuma longa*-treated joints and are closely associated with blood vessels (BV) (x350). (c) NPY-immunoreactive fibres (arrow) are sparsely found in the subintimal layer of the synovial membrane of *Curcuma longa*-treated group and in close proximity with blood vessels (BV) (x350).

104

LIST OF ABBREVIATIONS

ABPC	Avidin biotinylated peroxidase complex
ACL	Anterior cruciate ligament
CGRP	Calcitonin gene-related peptide
DAB	Diaminobenzidine
H&E	Haematoxylin-eosin
HA	Hyaluronic acid
IgG	Immunoglobulin G
mg/kg	milligram/kilogram
MHz	MegaHertz
MRI	Magnetic resonance imaging
NGF	Nerve growth factor
NPY	Neuropeptide Y
NSAIDs	Non-steroidal anti-inflammatory drugs
OA	Osteoarthritis
PBS	Phosphate buffer saline
PGP 9.5	Protein gene product 9.5
RA	Rheumatoid arthritis
ROS	Reactive oxygen species
SP	Substance P
w:v	Weight : volume
μ M	Micrometer