UNIVERSITI PUTRA MALAYSIA

EVALUATION OF CLINACANTHUS NUTANS (BURM. F.) LINDAU AND FICUS DELTOIDEA LEAF EXTRACTS FOR CARTILAGE AND BONE MARROW HEALTH IN EXPERIMENTAL RAT OSTEOARTHRITIS

NUR ADEELAH CHE AHMAD TANTOWI

IB 2016 22
EVALUATION OF Clinacanthus nutans AND Ficus deltoidea LEAF EXTRACTS FOR CARTILAGE AND BONE MARROW HEALTH IN EXPERIMENTAL RAT OSTEOARTHRITIS

By

NUR ADEELAH BINTI CHE AHMAD TANTOWI

Thesis Submitted to the School of Graduated Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

September 2016
COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia
I dedicated this thesis to those who value Malaysia’s herbal treasure
Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

EVALUATION OF Clinacanthus nutans AND Ficus deltoidea LEAF EXTRACTS FOR CARTILAGE AND BONE MARROW HEALTH IN EXPERIMENTAL RAT OSTEOARTHRITIS

By

NUR ADEELAH BINTI CHE AHMAD TANTOWI

September 2016

Chair: Prof. Suhaila Mohamed, PhD
Faculty: Institute of Bioscience

Medicinal plants have been used to treat various ailments including in osteoarthritis (OA) for decades. Currently, the existing drugs for treating OA only alleviate symptoms and improve the joint function, but cannot treat cartilage and bone damage. Developing therapeutics from plant-derived sources may exert less negative side effects, compared to the use of common non-steroidal anti-inflammatory drugs (NSAIDs) for osteoarthritis. Hereby, the present study investigated the effect of Clinacanthus nutans (belalai gajah) and Ficus deltoidea (mas cotek), compared with diclofenac, on cartilage and bone marrow health on experimental OA model.

In preliminary in vitro bovine cartilage explant culture, the recombinant bovine IL-1β of 10 ng/mL was added to the cartilage explants in DMEM/F12 media to induce OA condition. CN or FD leaf extracts at 20, 40, and 80 μg/mL, or diclofenac at 5 μg/mL, were simultaneously added into the medium after IL-1β induction. The amount of proteoglycan loss, reactive oxygen species (ROS) produced, and chondrocytes morphology were evaluated. In in vivo experiment, 42 12-week-old Sprague Dawley female rats were randomized into seven groups (n=7). The rats were subjected to bilateral ovariectomy (OVX) and OA was induced by intra-articular injection of monosodium iodoacetate (MIA) at 60 mg/mL into right knee joints, excluding healthy group. Healthy and OA non-treated groups were given deionized water while treatment groups were orally treated with 200 or 400 mg/kg body weight of CN or FD leaf extracts or 5 mg/kg body weight of diclofenac once a day for 28 days. Serum levels of inflammation including interleukin 1 beta (IL-1β), interleukin 6 (IL-6), and prostaglandin E2 (PGE2); cartilage catabolic including matrix metalloproteinase 1 (MMP-1), matrix metalloproteinase 13 (MMP-13), C-terminal cross-linked telopeptide of type II collagen (CTX-II), and N-terminal propeptide of collagen type II (PIINP); and bone turnover markers including osteoprotegerin, osteocalcin, receptor activator of nuclear kappa-beta ligand (RANKL), and C-terminal crosslinked telopeptide type I collagen (CTX-I) were assessed by enzyme-linked immunosorbent assay (ELISA).
Articular cartilage changes were determined by radiological, macroscopic, and histological observations. Gene expressions of inflammatory including nuclear factor kappa beta (NF-κβ), interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), cyclooxygenase 2 (COX-2), and prostaglandin E2 (PGE2); and cartilage catabolic mediators including matrix metalloproteinase 1 (MMP-1), matrix metalloproteinase 13 (MMP-13), A disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS-4), and A disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) were determined to study the mechanisms involved. Bone turnover regulations were evaluated via bone mass density, dimension, biomechanics, and microarchitecture.

Flavones of apigenin derivatives including vitexin, isovitexin, schaftoside, and isoschaftoside were identified in both CN and FD leaf extracts. Preliminary in vitro study showed chondroprotective effects of CN and FD leaf extracts and diclofenac by significantly inhibiting proteoglycan loss, ROS production, and preventing chondrocytes apoptosis. In in vivo study, CN and FD leaf extracts possessed cartilage and bone protecting nature by significantly suppressing the augmented activities of inflammatory (IL-1β, IL-6, and PGE2), and cartilage catabolic (MMP-1 and MMP-13) serum levels comparable to diclofenac. The osteoarthritic rats treated with the extracts and diclofenac showed significant reduction of cartilage erosion via radiological, macroscopic and histological images, compared to untreated osteoarthritic rats. The extracts significantly down-regulated NF-κβ, IL-1β, TNF-α, IL-6, PGE2, MMP-1, and MMP-13 expressions in the osteoarthritic cartilage similar to diclofenac. Furthermore, CN and FD leaf extracts administration protected bone marrow by significantly increased bone volume ratio, decreased trabecular separation, and decreased total porosity of the bone marrow. These findings were supported by bone turnover markers; which the extracts significantly increased bone formation (osteoprotegerin and osteocalcin) and reduced bone resorption (CTX-I and RANKL) markers, comparable to diclofenac.

Overall, CN and FD leaf extracts were demonstrated to be a potent agent mitigating cartilage and bone loss in OA. The results achieved were at least as good than those with diclofenac, a widely used NSAID and a benchmark pharmacological treatment for OA. The main bioactive compounds are probably responsible for anti-inflammatory and antioxidant properties in the protection of cartilage and bone marrow in OA.
Abstrak tesis ini dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

EVALUASI KESAN *Clinacanthus nutans* DAN *Ficus deltoidea* TERHADAP KESIHATAN TULANG RAWAN DAN TULANG SUMSUM MENGGUNAKAN MODEL EKSPERIMENT TIKUS OSTEOARTRITIS

Oleh

NUR ADEELAH BINTI CHE AHMAD TANTOWI

Pengerusi: Prof. Suhaila Mohamed, PhD
Fakulti: Institut Biosains

September 2016

Tumbuhan perubatan telah digunakan untuk mengubati berbagai penyakit selama puluhan tahun termasuk osteoarthritis (OA). Dewasa ini, ubat yang ada untuk OA hanya mengurangkan gejala dan meningkatkan fungsi sendi, tetapi tidak dapat mencegah kerosakan tulang rawan dan tulang sumsum. Mengembangkan terapi dari sumber tanaman dapat mengurangkan kesan sampingan negatif berbanding penggunaan ubat konvensional untuk osteoarthritis iaitu ubat anti-radang bukan steroid (NSAIDs). Dengan ini, kami bertujuan mengkaji kesan *Clinacanthus nutans* (belalai gajah) dan *Ficus deltoidea* (mas cotek), dibandingkan dengan *diclofenac*, terhadap kesihtatan tulang rawan dan tulang sumsum menggunakan model OA.

Dalam kajian *in vitro* kultur tulang rawan lembu, IL-1β rekombinan dari lembu sebanyak 10 ng/mL ditambahkan ke dalam kultur tulang rawan dalam media DMEM/F12 untuk mencetus kondisi OA. Daun ekstrak CN atau FD pada 20, 40, dan 80 mg/mL atau *diclofenac* pada 5 mg/mL kemudian ditambah ke dalam media setelah induksi IL-1β. Jumlah penguraian proteoglikan, spesies oksigen reaktif (ROS) yang dihasilkan, dan morfologi kondrosit telah dicatatkan. Dalam kajian *in vivo*, 42 ekor tikus betina Sprague Dawley berusia 12 minggu dibahagi 7 kumpulan secara rawak (n=7). Tikus menjalani ovariektomi (OVX) secara bilateral dan OA dicetuskan dengan suntikan intrasendi bahan kimia monosodium iodoacetate (MIA) pada 60 mg/mL ke dalam sendi lutut kanan, tidak termasuk kumpulan yang sihat. Kumpulan yang sihat dan OA kawalan negatif diberi air deionisasi sementara kumpulan lain diberikan ekstrak daun CN atau FD pada 200 dan 400 mg/kg berat badan atau *diclofenac* pada 5 mg/kg berat badan sekali sehari selama 28 hari. Kadar serum darah untuk tanda terjadi radang termasuk interleukin 1 beta (IL-1β), interleukin 6 (IL-6), dan prostaglandin E2 (PGE2); katabolik tulang rawan termasuk *matrix metalloproteinase 1* (MMP-1), *matrix metalloproteinase 13* (MMP-13), *C-terminal cross-linked telopeptide of type II collagen* (CTX-II), dan *N-terminal propeptide of collagen type II* (PIINP); dan perubahan metabolisme tulang termasuk osteoprotegerin, osteocalcin, *receptor activator of nuclear kappa-beta ligand* (RANKL), dan *C-terminal crosslinked telopeptide type I collagen* (CTX-I), dinilai oleh *enzyme-linked immunosorbent assay* (ELISA).
Perubahan tulang rawan ditentukan daripada ujian radiologi, makroskopik, dan histologi. Ekspresi gen dari tulang rawan oleh faktor inflamasi termasuk nuclear factor kappa beta (NF-κβ), interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), cyclooxygenase 2 (COX-2), dan prostaglandin E2 (PGE2); dan factor katabolik tulang rawan termasuk matrix metalloproteinase 1 (MMP-1), matrix metalloproteinase 13 (MMP-13), A disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS-4), dan A disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5). Keadaan kesihatan tulang ditentukan oleh kepadatan, dimensi, kekuatan biomekanik, dan binaan mikro tulang.

Flavon derivatif daripada kompaun apigenin termasuk vitexin, isovitexin, schaftoside, dan isoschaftoside ditemukan dalam daun ekstrak CN dan FD. Kajian in vitro menunjukkan efek kondroprotektif oleh daun ekstrak CN dan FD dan diclofenac dengan mengurangkan penguraian proteoglikan, produksi ROS, dan mencegah apoptosis oleh kondrosit. Dalam kajian in vivo, daun ekstrak CN dan FD melindungi tulang rawan dengan menghalangi aktiviti inflamasi (IL-1β, IL-6, and PGE2), dan katabolisme oleh tulang rawan (MMP-1 and MMP-13) setara dengan diclofenac. Ekstrak juga mengurangkan penguraian tulang rawan seperti yang ditunjukkan oleh gambar radiologi, makroskopik, histologi. Ekstrak juga menurunkan kadar ekspresi gen termasuk NF-κβ, IL-1β, TNF-α, IL-6, PGE2, MMP-1, dan MMP-13, setara dengan diclofenac. Selanjutnya, daun ekstrak CN dan FD melindungi tulang sumsum dengan meningkatkan nisbah jumlah tulang, mengurangkan jarak tulang trabekular, dan mengurangkan jumlah keporosan tulang. Penemuan ini disokong oleh penanda kadar metabolisme tulang; yang mana ekstrak meningkatkan aktiviti penambahan tulang (osteoprotegerin dan osteocalcin) dan mengurangkan aktiviti penguraian tulang (CTX-I dan RANKL), sama banding dengan diclofenac.

Secara keseluruhan, daun ekstrak CN dan FD ditunjukkan sebagai agen berguna dalam mengurangkan kerosakan tulang rawan dan tulang sumsum dalam OA, setara dengan ubat diclofenac, ubat konvensional dalam rawatan OA. Bahan bioaktif utama mungkin menjadi bahan aktif anti-inflamasi dan antioksidan untuk melindungi tulang rawan dan tulang sumsum dalam model OA.
ACKNOWLEDGEMENTS

Alhamdulillah, all praise to Allah for the successful project and completion of this thesis. Many thanks to wonderful people that made this journey a success.

I would like to express my sincere gratitude to my supervisor, Prof. Dr. Suhaila Mohamed, for her continuous support and motivation, so much wisdom for me to learn in academics as well as in life. To my co-supervisors, Dr. Lau Seng Fong and Dr. Paisal Hussin, for their time and expertise throughout this study.

My big thanks for those who assist me during this project. Dr. How Chee Wun, for aiding in HPLC analysis, Puan Latifah Mohd Hanan for preparing histological slides, Dr. Siti Aisyah A. Talib and CoMeT staffs (February-April 2015) for helping during animal study from start to end. To my research group member; Nurul Ain, Lim Swee Ling, Nor Aijratul Asikin, Wan Nurfarahin, Rubiatul Adawiyyah, Ifiah Nadhira, Nur Iliyani and Siti Syariah, thank you for lending hand whenever I ask. I am also thankful for getting to do my work at UPM-MAKNA Cancer Research Laboratory (IBS, UPM), Vaccine Laboratory (IBS,UPM), Molemed Laboratory (IBS,UPM), Comparative Medicine and Technology Unit (IBS, UPM), Virology Laboratory (Faculty of Veterinary Medicine UPM), Material Laboratory (Faculty of Engineering, UPM) and Tissue Engineering Centre (HUKM). I would like to acknowledge Universiti Putra Malaysia and Kementerian Pengajian Tinggi Malaysia that provide necessary financial support for this research.

Last but not least, thank you to my parents, Che Ahmad Tantowi bin Che Mohamed Arif and Normadiah binti Hashim, my family and family-in-law, for the du’a and understanding, my dearest husband, Amar Yasier bin Razli, for so much love and support, and my baby daughter, Ayesha Khadija binti Amar Yasier, whose cheerful smile that gives me strength.
I certify that a Thesis Examination Committee has met on (24th January 2017) to conduct the final examination of Nur Adeelah binti Che Ahmad Tantowi on her thesis entitled “Evaluation of Clinacanthus nutans and Ficus deltoidea Leaf Extracts for Cartilage and Bone Marrow Health in Experimental Rat Osteoarthritis” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

**Rozita binti Rosli, PhD**
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

**Rasedee @ Mat bin Abdullah, PhD**
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

**Md Zuki bin Abu Bakar @ Zakaria, PhD**
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Internal Examiner)

**Abbas Ali Mahdi, PhD**
Professor
King George’s Medical University
India
(External Examiner)

____________________________________
NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 22 March 2017
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

**Suhaila Mohamed, PhD**  
Professor  
Institute of Bioscience  
Universiti Putra Malaysia  
(Chairman)

**Lau Seng Fong, DVM, PhD**  
Lecturer  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Paisal Hussin, MBBS, MS Ortho**  
Medical Lecturer  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

---

**ROBIAH BINTI YUNUS, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _______________________   Date: 21/4/2017

Nur Adeelah binti Che Ahmad Tuntevi (GS37786)

Name and Matric No.: ___________________________
Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: __________________________
Name of
Chairman of
Supervisory Committee:

Signature: __________________________
Name of
Member of
Supervisory Committee

Signature: __________________________
Name of
Chairman of
Supervisory Committee:
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>v</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>VIII</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xvi</td>
</tr>
</tbody>
</table>

## CHAPTER

1. **INTRODUCTION**
   - 1.1. Background
   - 1.2. Hypothesis and Research Objectives

2. **LITERATURE REVIEW**
   - 2.1. Prevalence of Osteoarthritis
   - 2.2. Risk Factors of Osteoarthritis
   - 2.3. Osteoarthritis Incidence in Postmenopausal Women
   - 2.4. Pathophysiology of Osteoarthritis
     - 2.4.1. Articular Cartilage Degradation
     - 2.4.2. Synovium Inflammation
     - 2.4.3. Osteophyte Formation
     - 2.4.4. Subchondral Bone Sclerosis
     - 2.4.5. Calcified Cartilage and Tidemark Duplication
   - 2.5. Bone Remodeling in Osteoarthritis
   - 2.6. Molecular Mediators in the Pathogenesis of Osteoarthritis
   - 2.7. Assessment of Disease Outcome in Osteoarthritis Experimental Research
   - 2.8. Clinical Diagnostic Modalities for Osteoarthritis
   - 2.9. Treatment Options for Osteoarthritis
     - 2.9.1. Exercise and Weight Loss
     - 2.9.2. Physical Therapy
     - 2.9.3. Orthotic Devices
     - 2.9.4. Pain Medication
     - 2.9.5. Complimentary and Alternative Medicine
     - 2.9.6. Intra-articular Injections and Surgical Treatments
   - 2.10. Nutraceuticals for the Treatment of Osteoarthritis
     - 2.11. *Clinacanthus nutans*
     - 2.12. *Ficus deltoidea*

3. **METHODOLOGY**
   - 3.1. Plant extracts and chemistry analysis
     - 3.1.1. Plant extracts preparation
     - 3.1.2. Fourier transform infrared (FTIR) spectroscopy
     - 3.1.3. High performance liquid chromatography HPLC
   - 3.2. *In vitro* bovine cartilage explant model
     - 3.2.1. Bovine cartilage explant culture
     - 3.2.2. Treatment and grouping of explant
     - 3.2.3. Cartilage explant histology
3.2.4. Proteoglycan release
3.2.5. Total reactive oxygen species (ROS) produced

3.3. *In vivo* postmenopausal osteoarthritis rat model

3.3.1. Animals
3.3.2. Postmenopausal osteoarthritis induction
3.3.3. Treatments administration
3.3.4. Radiography
3.3.5. Samples collection for analysis
3.3.6. Macroscopic observation
3.3.7. Histological analysis
3.3.8. Bone mass density and dimension measurement
3.3.9. Bone mechanical test
3.3.10. Micro-computerized tomography (CT) analysis
3.3.11. Enzyme-linked immunosorbent assay (ELISA)
3.3.12 RNA extraction
3.3.12. Gene expression analysis

3.4. Statistical analysis

4 RESULTS

4.1. Active compound identification

4.1.1. High performance liquid chromatography (HPLC) profile
4.1.2. Fourier transform infrared (FTIR) spectra

4.2. Preliminary *in vitro* assay

4.2.1. Proteoglycan release
4.2.2. ROS production
4.2.3. Chondrocytes morphology

4.3. Cartilage protective effect in *in vivo* study

4.3.1. Radiographic examination of knee joints
4.3.2. Macroscopic findings on cartilage lesion
4.3.3. Histological evaluation on cartilage and chondrocytes pathology
4.3.4. Serum inflammation biomarkers
4.3.5. Serum cartilage catabolic biomarkers
4.3.6. Gene expression of target pathway in OA

4.4. Bone protective effect in *in vivo* study

4.4.1. Body weight after ovary removal
4.4.2. Bone mass density and dimension
4.4.3. Bone biomechanics
4.4.4. Bone microarchitecture
4.4.5. Bone turnover biomarkers
4.4.6. Correlation analysis of metaphyseal trabecular and serum bone turnover markers

5 DISCUSSION

5.1. Apigenin derivatives identification
5.2. *In vitro* chondroprotective effects
5.3. *In vivo* cartilage protective effects
5.4. *In vivo* bone protective effects
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1:</td>
<td>Knee osteoarthritis (OA) prevalence studies from selected countries</td>
<td>4</td>
</tr>
<tr>
<td>2.2:</td>
<td>Biologic action and molecular biomarkers involved in OA pathogenesis</td>
<td>13</td>
</tr>
<tr>
<td>2.3:</td>
<td>Commonly used osteoarthritis (OA) animal models</td>
<td>16</td>
</tr>
<tr>
<td>2.4:</td>
<td>Common side effects of non-steroidal anti-inflammatory drugs (NSAIDS)</td>
<td>20</td>
</tr>
<tr>
<td>2.5:</td>
<td>Clinical efficacy and biological mechanism of natural or natural-derived products used for osteoarthritis (OA)</td>
<td>22</td>
</tr>
<tr>
<td>2.6:</td>
<td>Pharmacological properties of Clinacanthus nutans</td>
<td>26</td>
</tr>
<tr>
<td>2.7:</td>
<td>Pharmacological properties of Ficus deltoidea</td>
<td>28</td>
</tr>
<tr>
<td>3.1:</td>
<td>List of selected genes</td>
<td>40</td>
</tr>
</tbody>
</table>
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1:</td>
<td>Factors that contribute to pathogenesis of osteoarthritis</td>
<td>5</td>
</tr>
<tr>
<td>2.2:</td>
<td>Healthy vs. OA joint</td>
<td>8</td>
</tr>
<tr>
<td>2.3:</td>
<td>Stages of OA progression</td>
<td>11</td>
</tr>
<tr>
<td>2.4:</td>
<td>Selected mediators involved in the progression of OA in the synovium, cartilage, and bone</td>
<td>14</td>
</tr>
<tr>
<td>2.5:</td>
<td>Models of OA and relevant assessments</td>
<td>15</td>
</tr>
<tr>
<td>2.6:</td>
<td><em>Clinacanthus nutans</em> leaf</td>
<td>25</td>
</tr>
<tr>
<td>2.7:</td>
<td><em>Ficus deltoidea var deltoidea</em> leaf</td>
<td>27</td>
</tr>
<tr>
<td>3.1:</td>
<td>Cartilage explants taken from bovine metacarpal phalangeal joint</td>
<td>32</td>
</tr>
<tr>
<td>3.2:</td>
<td>Postmenopausal OA rat model</td>
<td>35</td>
</tr>
<tr>
<td>3.3:</td>
<td>Three-point bending test</td>
<td>38</td>
</tr>
<tr>
<td>4.1:</td>
<td>HPLC-330nm chromatogram of <em>Clinacanthus nutans</em> (CN) leaf extract (1mg/ml), <em>Ficus deltoidea</em> (FD) leaf extract (1mg/ml), isochaftoside, schaftoside, vitexin, and isovitexin.</td>
<td>42</td>
</tr>
<tr>
<td>4.2:</td>
<td>Fourier transform infrared (FTIR) analysis.</td>
<td>44</td>
</tr>
<tr>
<td>4.3:</td>
<td>Chemical structure of schaftoside, vitexin, and their isomers.</td>
<td>45</td>
</tr>
<tr>
<td>4.4:</td>
<td>Proteoglycan release by osteoarthritic cartilage explant induced with interleukin 1 beta (IL-1β) and treated with DIC, CN and FD leaf extracts for 5 days.</td>
<td>46</td>
</tr>
<tr>
<td>4.5:</td>
<td>Total reactive oxygen species (ROS) release by osteoarthritic cartilage explant induced with interleukin 1 beta (IL-1β) and treated with DIC, CN and FD leaf extracts for 5 days.</td>
<td>47</td>
</tr>
<tr>
<td>4.6:</td>
<td>Chondrocytes morphology by osteoarthritic cartilage explant induced with interleukin 1 beta (IL-1β) and treated with DIC, CN and FD leaf extracts for 5 days (H&amp;E, 40x).</td>
<td>49</td>
</tr>
<tr>
<td>4.7:</td>
<td>Radiographic analysis of rat knee joints.</td>
<td>51</td>
</tr>
<tr>
<td>4.8:</td>
<td>Macroscopic observation of right knee joints.</td>
<td>53-54</td>
</tr>
<tr>
<td>4.9:</td>
<td>Histological profiles of rat knee cartilage sections.</td>
<td>56-57</td>
</tr>
<tr>
<td>4.10:</td>
<td>Serum inflammation biomarkers in osteoarthritis (OA) rats.</td>
<td>59</td>
</tr>
<tr>
<td>4.11:</td>
<td>Serum cartilage catabolic biomarkers in osteoarthritis (OA) rats.</td>
<td>61</td>
</tr>
<tr>
<td>4.12:</td>
<td>Inflammatory genes expressed in rats cartilage.</td>
<td>63-64</td>
</tr>
<tr>
<td>4.13:</td>
<td>Cartilage catabolic genes expressed in rats cartilage.</td>
<td>65</td>
</tr>
<tr>
<td>4.14:</td>
<td>Body weight changes from week 0 until week 10 (endpoint).</td>
<td>66</td>
</tr>
<tr>
<td>4.15:</td>
<td>Bone mass density in osteoarthritis (OA) rats.</td>
<td>67</td>
</tr>
<tr>
<td>4.16:</td>
<td>Bone dimension parameters in osteoarthritis (OA) rats.</td>
<td>68</td>
</tr>
<tr>
<td>4.17:</td>
<td>Bone mechanical strength parameters in osteoarthritis (OA) rats.</td>
<td>70</td>
</tr>
<tr>
<td>4.18:</td>
<td>Microarchitecture trabecular bone from micro-CT analysis</td>
<td>72-73</td>
</tr>
<tr>
<td>4.19:</td>
<td>Bone microarchitecture parameters of subchondral trabecular.</td>
<td>74</td>
</tr>
<tr>
<td>4.20:</td>
<td>Bone microarchitecture parameters of metaphyseal trabecular.</td>
<td>75</td>
</tr>
<tr>
<td>4.21:</td>
<td>Bone formation biomarkers in osteoarthritis (OA) rat serum.</td>
<td>77</td>
</tr>
<tr>
<td>4.22:</td>
<td>Bone resorption biomarkers of osteoarthritis (OA) rat serum.</td>
<td>78</td>
</tr>
<tr>
<td>4.23:</td>
<td>Correlation analysis of bone volume fraction (BV/TV) of metaphyseal trabecular and bone turnover markers</td>
<td>80</td>
</tr>
<tr>
<td>4.24:</td>
<td>Correlation analysis of total porosity of metaphyseal trabecular and bone turnover markers</td>
<td>81</td>
</tr>
<tr>
<td>5.1:</td>
<td>Proposed target pathway of <em>Clinacanthus nutans</em> (CN) and <em>Ficus deltoidea</em> (FD) leaf extracts in OA</td>
<td>85</td>
</tr>
</tbody>
</table>
5.2: Hypothetical mechanism of *Clinacanthus nutans* (CN) and *Ficus deltoidea* (FD) leaf extracts in protecting bone in osteoarthritis (OA)
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAMTS</td>
<td>A disintegrin and metalloproteinase with thrombospondin motifs</td>
</tr>
<tr>
<td>BALP</td>
<td>Bone-specific alkaline phosphatase</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mass/mineral density</td>
</tr>
<tr>
<td>BMP-2</td>
<td>Bone morphogenetic protein 2</td>
</tr>
<tr>
<td>CN</td>
<td><em>Clinacanthus nutans</em></td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase 2</td>
</tr>
<tr>
<td>CTX-I</td>
<td>C-terminal crosslinked telopeptide type I collagen</td>
</tr>
<tr>
<td>CTX-II</td>
<td>C-terminal crosslinked telopeptide type II collagen</td>
</tr>
<tr>
<td>DIC</td>
<td>Diclofenac</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FD</td>
<td><em>Ficus deltoidea</em></td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and eosin</td>
</tr>
<tr>
<td>HIF2-α</td>
<td>Hypoxia inducible factor 2 alpha</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>ICTP</td>
<td>Pyridinoline C-telopeptide of type I collagen</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>MIA</td>
<td>Monosodium iodoacetate</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NTX-I</td>
<td>N-terminal crosslinked telopeptide type I collagen</td>
</tr>
<tr>
<td>OA</td>
<td>Osteoarthritis</td>
</tr>
<tr>
<td>OP</td>
<td>Osteoporosis</td>
</tr>
<tr>
<td>OPG</td>
<td>Osteoprotegerin</td>
</tr>
<tr>
<td>OVX</td>
<td>Ovariectomy</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PGE₂</td>
<td>Prostaglandin E₂</td>
</tr>
<tr>
<td>PICP</td>
<td>Procollagen type I C-terminal propeptide</td>
</tr>
<tr>
<td>PIINP</td>
<td>Procollagen type II N-terminal propeptide</td>
</tr>
<tr>
<td>PINP</td>
<td>Procollagen type I N-terminal propeptide</td>
</tr>
<tr>
<td>RANKL</td>
<td>Receptor activator of nuclear kappa-beta ligand</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RUNX-2</td>
<td>Runt related transcription factor 2</td>
</tr>
<tr>
<td>TB</td>
<td>Toluidine blue</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Background

Osteoarthritis (OA) is one of the common musculoskeletal diseases and the major causes of disability worldwide. The disease usually develops in the elderly, notably more common in postmenopausal women. World Health Organization (WHO) has reported that OA affects 9.6% of men and 18% of women aged over 60 years (World Health Organization, 2016). In Malaysia, it is reported that the prevalence of OA is 10% to 20% of the adult population (Arthritis Foundation Malaysia, 2016). According to the United Nations, the adult population over 60 is reported to increase more than 20% of the world’s population by 2050 (United Nations, 2004).

At present, there is no known cure for OA. Despite the efficacy of the commonly used drug for relieving pain symptom of OA, analgesics and non-steroidal anti-inflammatory drugs (NSAIDs), the long term used of the drug pharmacotherapy is associated with substantial gastrointestinal and cardiovascular adverse events (Patrignani et al., 2011; McGettigan and Henry, 2013). More invasive treatments including intra-articular injections and surgery are intrusive and costly. Furthermore, intra-articular treatments such as hyaluronic acid and glucocorticoid injections may provide pain relief; however, recent observations show that these treatments may further drive cartilage breakdown (Gonzalez-Fuentes et al., 2010).

The exact pathological mechanism of OA is still unclear. Although OA is not considered as immunological disorders, current research indicates that pro-inflammatory mediators play a central role in the pathogenesis of OA. Among the mediators, TNF-α and IL-1β play a major role in OA pathogenesis, as these cytokines can stimulate the production of various inflammatory and catabolic factors implicated in OA. For instance, TNF-α and IL-1β induce release of other pro-inflammatory cytokines, matrix metalloproteinases (MMPs) and aggrecanases (ADAMTS-4 and -5) that cleave the collagen and proteoglycan networks, as well as diminishing anabolism of extracellular matrix components (Rai et al., 2008; Kapoor et al., 2011). In addition, TNF-α and IL-1β also drive chondrocytes to up-regulate the expression of gene encoding iNOS and COX-2 and stimulate release of PGE₂ and NO, which contribute to inflammation and cartilage destruction (Rai et al., 2008). As the process continues, catabolic activity of the cartilage matrix is increased and followed by fibrillation of the cartilage surface. After this degeneration is established, it starts to cause loss of more cartilage at a diarthrodial joint (Sellam and Berenbaum, 2010).
1.2 Hypothesis and Research Objectives

The main objectives of OA therapy are to counteract the local inflammation and delay joint degradation. Two of Malaysian herbs, *Clinacanthus nutans* (CN) and *Ficus deltoidea* (FD) have been reported to exhibit anti-inflammatory and antioxidant properties (Wanikiat et al., 2008; Zunoliza et al., 2009; Misbah et al., 2013; Arullappan et al., 2014), which are hypothesized to interfere with the inflammation and oxidative stress cascades in OA, and reduce cartilage degradation and bone marrow loss in OA progression. In general, the objective of this study is to evaluate the effect of CN and FD leaf extracts on cartilage and bone marrow in rat osteoarthritis model, compared with a commonly used non-steroidal anti-inflammatory drug (NSAID), diclofenac. Specifically, this study aims to assess the efficacy of CN and FD in OA disease in the following manners:

a) To identify the active compounds of CN and FD leaf extracts.
b) To assess chondroprotective effects of CN and FD leaf extracts on IL-1β-induced cartilage degradation on bovine cartilage explant culture.
c) To evaluate CN and FD leaf extracts on the repair of articular cartilage in rat osteoarthritis model induced by monosodium iodoacetate (MIA).
d) To determine CN and FD effects on regulating bone turnover in osteoarthritic rat model induced by monosodium iodoacetate (MIA).
REFERENCES


Gaspani, Leda, Elena Limieri, Paolo Ferrario, and Mauro Bianchi. 2002. “In Vivo and in Vitro Effects of Bromelain on PGE(2) and SP Concentrations in the Inflammatory Exudate in Rats.” Pharmacology 65 (2): 83–86. doi:56191.


Rai, Muhammad F., P. Sivaramakrishna Rachakonda, Kizzie Manning, Brita Vorwerk, Leo Brunnberg, Barbara Kohn, and Michael F.G. Schmidt. 2008. “Quantification of Cytokines and Inflammatory Mediators in a Three-Dimensional Model of


BIODATA OF STUDENT

Nur Adeelah binti Che Ahmad Tantowi was born in 1989, in Alor Setar, Kedah. She grew up in Alor Setar, eventually entered primary school SK Sungai Korok Baru, currently known as SK Jalan Pegawai, in Alor Setar. She then followed her family moved to Kluang, Johor, and continued her primary education at SK LKTP Ayer Hitam in Kluang. She entered boarding school for her secondary education; lower form at MRSM Mersing, Johor, and upper form at MRSM Jasin (currently known as MRSM Tun Ghafar Baba), Melaka.

She has always wanted to be a scientist ever since she was a kid. When she was accepted into scholarship program under Skim Pelajar Cemerlang MARA, she looked forward to learn knowledge and skills for her passion. She attended Pennsylvania State University, Pennsylvania, USA from 2008 to 2012, and graduated with a BS in Biotechnology and Master of Biotechnology. Having worked with plant protein during her Master’s degree project, she aimed to contribute to discovery in Malaysia’s herbal plant value in her future career. In 2013, she entered the PhD program at Institute of Bioscience, Universiti Putra Malaysia under research team led by Prof. Dr. Suhaila Mohamed, doing Malaysia’s herbs therapeutic research on various diseases such as osteoarthritis, osteoporosis, and cancer.

In August 2014, she married Amar Yasier bin Razli, a lecturer from Universiti Utara Malaysia. The two have a child named Ayesha Khadija binti Amar Yasier.
LIST OF PUBLICATIONS

Patent:


Book chapter:


Proceeding:


Publication: