การเปรียบเทียบประสิทธิภาพการลดอักเสบของ PCSO-524 กับ ยาต้านการอักเสบ
แบบไม่ใช่สเตียรอยด์ที่จำเพาะต่อ COX-2 ในสุนัขที่ได้รับการผ่าตัดแก้ไขลูกสะบ้าเคลื่อน

นางสาวศรารัตน์ คงวุธ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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COMPARATIVE STUDY ON ANTI-INFLAMMATORY PROPOTIES OF

PCSO-524 AND SPECIFIC COX 2 NON-STEROIDAL

ANTI-INFLAMMATORY DRUG (NSAID) IN DOG

UNDERGOING MEDIAL PATELLAR LUXATION REPAIR.

Miss Sararat Kongwut

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Thesis Title	COMPARATIVE STUDY ON ANTI-INFLAMMATORY PROPOTIES OFPCSO-524 AND SPECIFIC COX 2 NON-STEROIDALANTI-INFLAMMATORY DRUG (NSAID) IN DOG UNDERGOING MEDIAL PATELLAR LUXATION REPAIR.
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ศรารัตน์ คงวุธ : การเปรียบเทียบประสิทธิภาพการลดอักเสบของ PCSO-524 กับ ยาต้านการอักเสบแบบไม่ใช่สเตียรอยด์ที่จำเพาะต่อ COX-2 ในสุนัขที่ได้รับการผ่าตัดแก้ไขลูกสะบ้าเคลื่อน (COMPARATIVE STUDY ON ANTI-INFLAMMATORY PROPOTIES OFPCSO-524 AND SPECIFIC COX 2 NON-STEROIDALANTI-INFLAMMATORY DRUG (NSAID) IN DOG UNDERGOING MEDIAL PATELLAR LUXATION REPAIR.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. น.สพ. ดร. กัมปนาท สุนทรวิภาต, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. สพ.ญ. ดร. มีนา สาริกะภูติ, 52 หน้า.

สุนัขพันธุ์ปอมเมอเรเนียน อายุ 1-7 ปี ไม่จำกัดเพศ ไม่มีปัญหาสุขภาพอื่นๆ นอกจากมีภาวะลูกสะบ้าเคลื่อนระดับ 3 จำนวน 30 ตัว ได้รับการตรวจร่างกายทั่วไป, ตรวจทางออร์โธปิดิกส์, ตรวจการเดิน, การลงน้ำหนัก และ ตรวจเลือดหาสารสื่ออักเสบในเลือด (IL-1β และ IL-6) ก่อนเข้ารับการผ่าตัดแก้ไขลูกสะบ้าเคลื่อน หลังการผ่าตัด สุนัขทั้งหมดจะถูกสุ่มแบ่งออกเป็น 3 กลุ่ม กลุ่มละ 10 ตัว ได้รับการรักษาเป็นระยะเวลา 14 วันติดต่อกัน โดยกลุ่มแรกจะได้รับยา Firocoxib ปริมาณ 5 มก.ต่อกก. วันละ 1 ครั้ง กลุ่มที่สองจะได้รับสาร PCSO-524 ปริมาณ 1 แคปซูล วันละ 2 ครั้ง และกลุ่มสามจะได้รับยา Firocoxib ปริมาณ 5 มก.ต่อกก. วันละ 1 ครั้ง ร่วมกับสาร PCSO-524 ปริมาณ 1 แคปซูล วันละ 2 ครั้ง ทำการตรวจร่างกายทั่วไป, ตรวจทางออร์โธปิดิกส์, ตรวจการเดิน, การลงน้ำหนัก และ ตรวจเลือดหาสารสื่ออักเสบในเลือด (IL-1β และ IL-6) ในวันที่ 1, 5 และ 14 ภายหลังการผ่าตัด ผลปรากฏว่าทั้ง 3 กลุ่มการรักษา สามารถกลับมาใช้ขาข้างที่ผ่าตัดลงน้ำหนักได้ใกล้เคียงปกติในวันที่ 14 ภายหลังการผ่าตัด อย่างไรก็ตาม พบว่าการให้สาร PCSO-524 เพียงอย่างเดียวไม่สามารถลดปริมาณสารสื่ออักเสบในเลือดได้ดีเทียบเท่ากลุ่มการรักษาอื่นที่มีการให้ NSAID แสดงให้เห็นว่า ยาต้านการอักเสบแบบไม่ใช่สเตียรอยด์ที่จำเพาะต่อ COX-2 มีประสิทธิภาพในการลดการอักเสบในสุนัขที่ได้รับการผ่าตัดแก้ไขลูกสะบ้าเคลื่อนได้ดีกว่า PCSO-524 ในช่วง14วันแรกหลังการผ่าตัด

ภาควิชา	ศัลยศาสตร์	ลายมือชื่อนิสิต	....
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KEYWORDS: ACUTE INFLAMMATION, DOG, INFLAMMATORY CYTOKINE, INTERLEUKIN 1, INTERLEUKIN 6, PATELLAR LUXATION, PCSO-524 / การอักเสบแบบเฉียบพลัน, โรคลูกสะบ้าเคลื่อน, สารบ่งชี้การอักเสบ, สารสกัดจากหอยแมลงภู่นิวซีแลนด์, สุนัข, อินเตอร์ลิวคิน1, อินเตอร์ลิวคิน6

SARARAT KONGWUT: COMPARATIVE STUDY ON ANTI-INFLAMMATORY PROPOTIES OFPCSO-524 AND SPECIFIC COX 2 NON-STEROIDALANTI-INFLAMMATORY DRUG (NSAID) IN DOG UNDERGOING MEDIAL PATELLAR LUXATION REPAIR.. ADVISOR: ASST. PROF. KUMPANART SOONTORNVIPART, D.V.M., Ph.D, CO-ADVISOR: ASSOC. PROF. . MEENA SARIKAPUTI, D.V.M., M.Sc., Ph.D., 52 pp.

Thirty client-owned Pomeranians, 1 - 7 year- old with medial patellar luxation grade 3 and no other diseases are enrolled in this experiment. All dogs received physical examination, orthopedic examination, clinical scoring system, force platform gait analysis and blood collection by the same veterinarian before surgery and at day 1, 5 and 14 after surgery. After surgery of correction of medial patellar luxation, they are divided into 3 groups by random sampling. The first group received only Firocoxib (5 mg/kg once daily). The second group received only PCSO-524 (10 mg/kg, twice daily). The third group received Firocoxib (5 mg/kg, once daily) combining with PCSO-524 (10 mg/kg, twice daily). Treatment was finished at the end of the 2nd week. IL-1β and IL-6 concentration in serum are determined by commercially available canine ELISA kit. In conclusion, PCSO-524 can improve clinical signs in Pomeranian dogs after surgical correction of medial patellar luxation. The dogs in all groups of treatments had weight-bearing scores and gait data similar to standard score of normal dog in day 14 after surgery. However, PCSO-524 alone could not reduce blood level of inflammatory mediators when compared with NSAID, indicating that PCSO-524 did not have anti-inflammatory effect as good as NSAID in case of post-operative care of patellar luxation's surgery.

Department:	Veterinary Surgery	Student's Signature _____
Field of Study:	Veterinary Surgery	Advisor's Signature _____
Academic Year:	2014	Co-Advisor's Signature _____

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CONTENTS

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

CHAPTER I INTRODUCTION

PCSO-524 is the extract from New Zealand green-lipped mussel and is one of the popular nutraceutical agents in human. It contains mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are classed omega 3 polyunsaturated fatty acid (Sinclair, Murphy, & Li, 2000). There are many researches proving that PCSO-524 can reduce pain and progression of joint diseases such as rheumatoid arthritis and osteoarthritis in human (Brien, Prescott, Coghlan, Bashir, & Lewith, 2008; Coulson, Vecchio, Gramotnev, & Vitetta, 2012; Lau et al., 2004). In dog, PCSO-524 improves clinical signs in osteoarthritis and degenerative spinal diseases (Mongkon & Soontornvipart, 2012). The mechanism of this agent is inhibition of membrane arachidonic acid metabolism by blocking 5-lipooxygenase (LOX),which causes a formation of leukotriene B4 (LTB4),pro-inflammatory eicosanoid, by polymorphonuclear cells (PMN) in vitro (Halpern, 2000). PCSO-524 also inhibits prostaglandin E2 (PGE2) production and thromboxane by inhibiting cyclo-oxygenase (COX) pathways (McPhee et al., 2007). Prostaglandin and thromboxane play the major role in progression of cartilage degeneration.

Patellar luxation is found as an important hereditary orthopedic defect in Thailand. Small breed dogs such as Pomeranians, Yorkshire Terriers, Chihuahuas, Miniature and Toy Poodles have risk of this problem approximately 12 times compared with large breed dogs (Priester, 1972). In Thailand, the prevalence of medial patellar luxation (MPL) and lateral patellar luxation (LPL) in small-breed dogs are 87% and 13%, respectively (Wangdee, Soontornvipart, & Chuthatep, 2005). Moreover, Pomeranians have currently the highest incidence for patellar luxation in the USA, with 42.4% of dogs affected. The clinical signs of the condition normally start showing at approximately four months after birth. If patellar luxation is not repaired, generating many effects to hind-limbs and gait such as abnormal conformation of hip joint, malformation of femur and tibia, deviation of tibial crest, quadriceps contracture, cranial cruciate ligament rupture and osteoarthritis.

PCSO-524 had been studying for controlling chronic pain in many diseases such as osteoarthritis or rheumatoid arthritis (Coulson et al., 2012; Hurst, Zainal, Caterson, Hughes, & Harwood, 2010; Lau et al., 2004; Zawadzki, Janosch, & Szechinski, 2013). There are many researches supporting that PCSO-524 has good effect with this disease. However, there are just a few of the studies of PCSO-524 with acute inflammation and acute pain. This is the first study of using PCSO-524 to reduce acute inflammation by measuring the inflammation-related biomarker in blood. The model of acute inflammation in this study is surgical correction of patellar luxation which we found mostly in orthopedic clinic. If PCSO-524 can reduce inflammation as good as (or better than) NSAIDs, resulting in a reduction in the use of NSAIDs which generates more side-effects. Moreover, if PCOSO-524 can inhibit acute inflammation, therefore a decrease in development from acute to chronic inflammation and pain, can be possible.
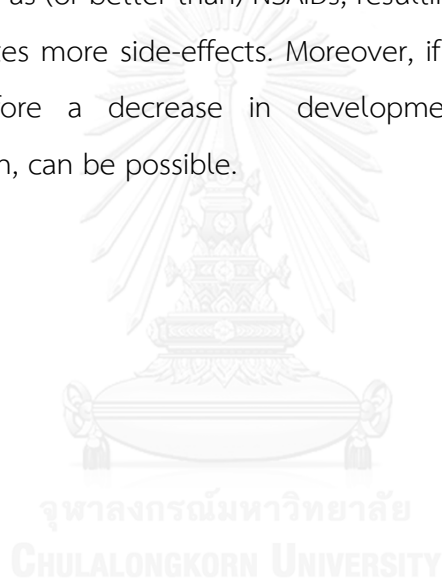
CHAPTER II REVIEW OF LITERATURES

New Zealand green-lipped mussel is a bivalves mollusk found pandemic in the Pacific ocean below the intertidal zone (esp. New Zealand). It is one of the traditional foods of the New Zealand native islander called Maori for a long time (Rush, Hsi, Ferguson, Williams, & Simmons, 2010). The prevalence of osteoarthritis in Maori population is less than other population so, researchers have been interesting in bioactive compound of New Zealand green-lipped mussel.

The components and usefulness of New Zealand green-lipped mussel has been studied for many decades. It has many bioactive components such as antioxidant peptides, antimicrobial peptides, anti-inflammatory glycogen, and anti-inflammatory lipids (Grienke, Silke, & Tasdemir, 2014). However the main component that many researchers pay attention is anti-inflammatory lipids. Polyunsaturated fatty acid (PUFA) is the majority of the total fatty acid that range approximately 44-51% depending on environment. Of these, the total omega-3 PUFAs is dominated ranging 70-79%. And the main component of omega-3 PUFAs is eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Figure 2.1 and Table 2.1). (Taylor & Savage, 2006); Sinclair et al., 2000) Furthermore, it is also composed of eicosatetraenoic acid (C20:4), octadecatetraenoic acid (C18:4), nonadecatetraenoic acid (C19:4) and heneicosapentaenoic acid (C21:5) (Treschow et al., 2007) (Figure 2.2). We called these bioactive lipids "PCSO-524".
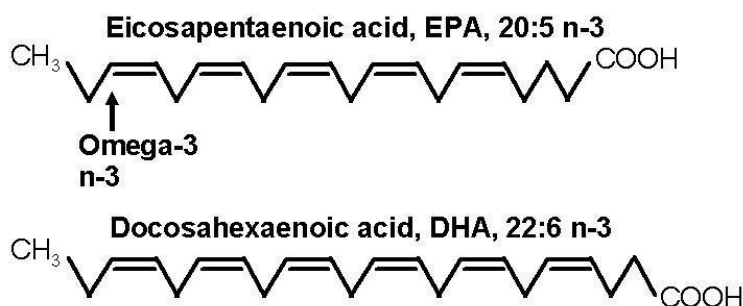

Figure 2. 1 EPA and DHA structure

Source: http://imgarcade.com/1/epa-structure/

Fatty acid composition in PCSO-524	Average amount (%)
Total PUFA	47.95
EPA (20:5n-3)	16.62
DHA (22:6n-3)	17.75

Table 2. 1 Fatty acid composition in PCSO-524

Adapt from Taylor and Savage, 2006

5,9,12,15-octadecatetraenoic acid (C18:4)

5,9,12,16-nonadecatetraenoic acid (C19:4)

7,11,14,17-eicosatetraenoic acid (C20:4)

5,9,12,15,18-heneicosapentaenoic acid (C21:5)
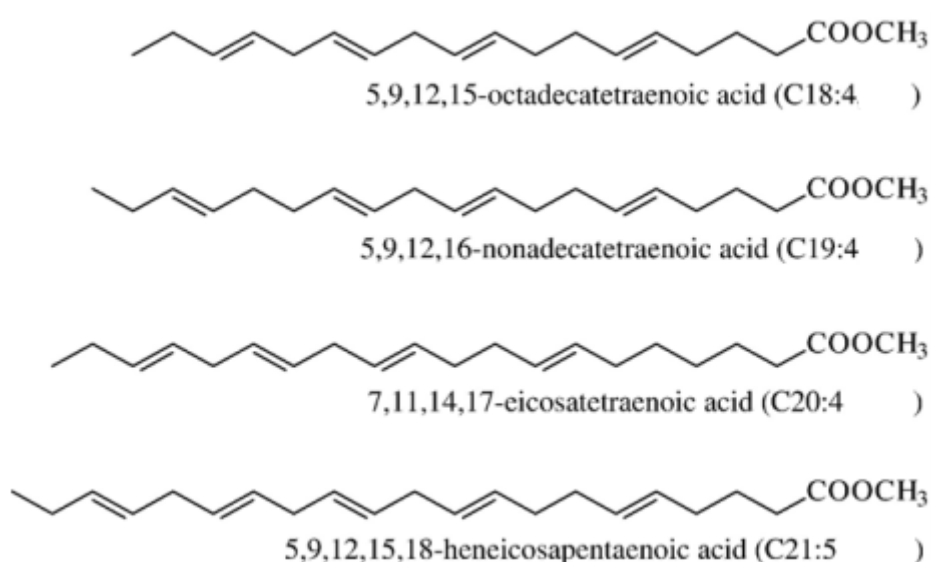
Figure 2. 2 The structure of novel anti-inflammatory PUFA in New Zealand green-lipped mussel

(Treschow et al., 2007)

There are many researches prove that PCSO-524 can inhibit the inflammatory pathway (Mani & Lawson, 2006; McPhee et al., 2007; Treschow et al., 2007). It reduces pain and progression of joint diseases such as rheumatoid arthritis and osteoarthritis in human (Lau et al., 2004; Brien et al., 2008; Coulson et al., 2012). Furthermore, many studies reported the effect of PCSO-524 in animals. In adjuvant-induced arthritis rats, it reduced significantly paw swelling, decreased serum ceruloplasmin oxidase activity, and reduced inflammatory response of spleen with

no any adverse side effects (M. Singh et al., 2008). In dog, PCSO-524 improved clinical signs in osteoarthritis and degenerative spinal diseases (Mongkon & Soontornvipart, 2012). The mechanism of this agent is inhibition of membrane arachidonic acid metabolism. Arachidonic acid (AA)'s structure is C20:4 which have double bond at site 5,8,11,14. While eicosatetraenoic acid (ETA) which found by Treschow et al. in 2007 have the same chemical formula (C20:4) but it has double bond at site 7,11,14,17 (Figure 2.3). So ETA can mimic as competitive substance of AA blocking 5-lipooxygenase (LOX), which causes formation of leukocyte (Halpern, 2000; (Treschow et al., 2007). PCSO-524 also inhibits prostaglandin E2 (PGE2) production and thromboxane by inhibiting cyclo-oxygenase (COX) pathways (McPhee et al., 2007). Moreover, eicosapentaenoic acid (EPA) found majority in PCSO-524 can reduce expression of cartilage degradation proteinase, COX-2 and inflammatory cytokines (Hurst et al., 2010).   Prostaglandin and thromboxane play the major role in progression of cartilage degeneration. The study by Coulson et al. (2012) found that the administration of PCSO-524 significantly improved GI symptoms in osteoarthritis patients. However, there were some studies that had conflict results, low-dose PCSO-524 and fish oil preparations had no different effects on inflammatory markers in healthy human (Murphy et al., 2006).

7,11,14,17-eicosatetraenoic acid (C20:4   )

5,8,11,14-eicosatetraenoic acid (arachidonic acid)

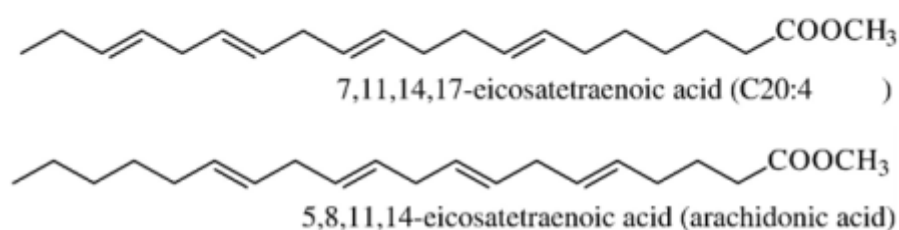Figure 2. 3 Comparison of chemical formula and structure of ETA and AA
(Treschow et al., 2007)

Specific COX-2 non-steroidal anti-inflammatory drugs (NSAIDs) is a new class of NSAID that have more selective on COX-2 than COX-1 isoform (>100 times). So it can inhibit inflammatory pathway better than the old generation NSAIDs. Because of the potency and less side effects, now it is extensively used to control inflammation

and pain in human and animals. The mechanism of specific COX-2 NSAID is inhibit cyclooxygenase (COX) pathway especially COX-2 that produce inflammatory prostaglandins (Figure 2.4). In animal such dogs and horses, Firocoxib (Figure 2.5) is one of the specific COX-2 NSAID that we are familiar with for a control of acute and chronic inflammation (Autefage, Palissier, Asimus, & Pepin-Richard, 2011; Back, MacAllister, Heel, Pollmeier, & Hanson, 2009; Kondo et al., 2012; Lecoindre & PEPIN-RICHARD, 2011) However, specific COX-2 NSAIDs still have side effects which are decreases vascular prostacyclin (PGI2) production resulting in an imbalance of prothrombotic and antithrombotic eicosanoids and then myocardial infarction in human (Baron et al., 2008; Bavry et al., 2014; Huang, Hsiao, Wen, & Tsai, 2006; Mukherjee, Nissen, & Topol, 2001; Ray et al., 2002).
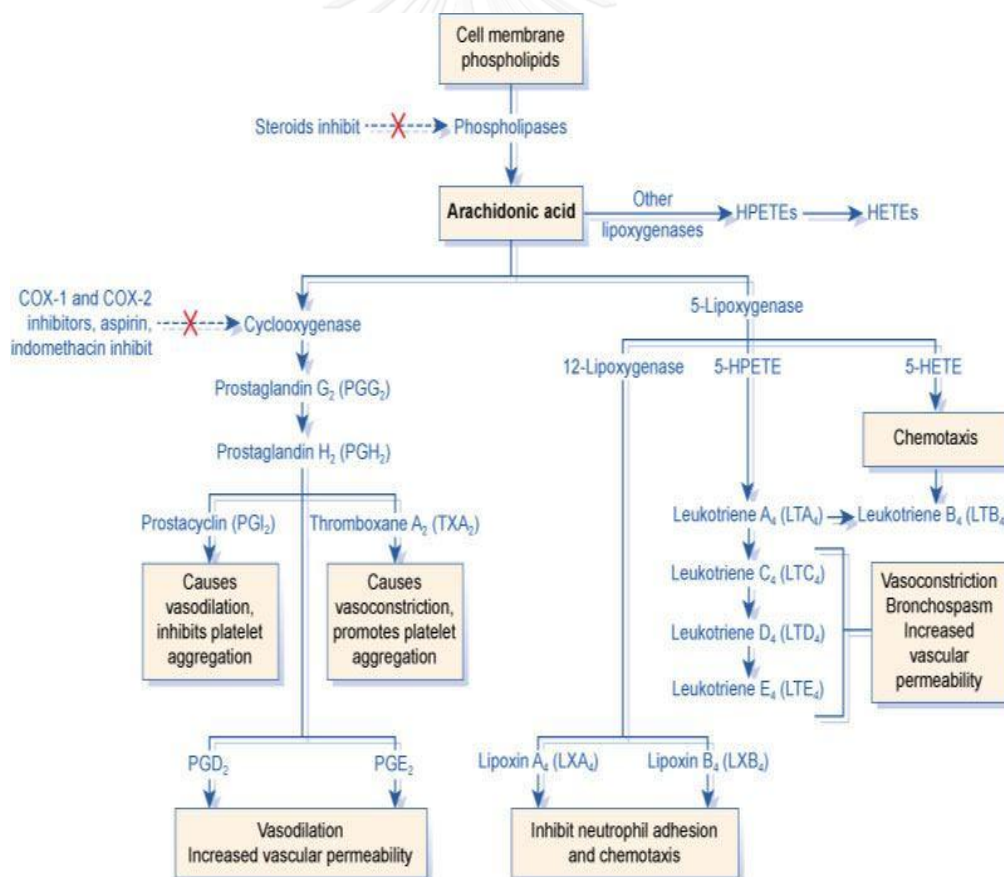
Figure 2. 4 Mechanism of NSAID and steroid

(Tobias & Johnston, 2011)
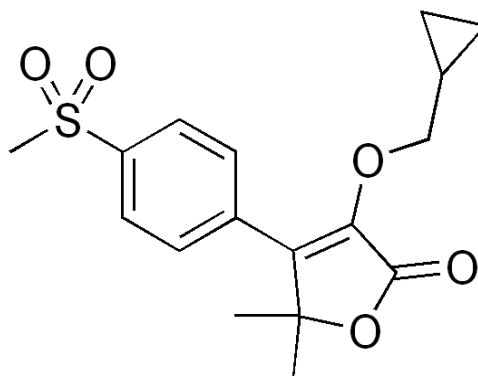
Figure 2. 5 Structure of Firocoxib

Inflammation is the important role in pathogenesis and clinical signs of joint diseases in both human and animal. Some diseases in human and animals is the result of abnormal inflammatory mechanism. The example of diseases from abnormal increase of inflammatory response are rheumatoid arthritis, osteoarthritis, systemic lupus erythrematosus (SLE), atherosclerosis, and Alzheimer disease (Montecucco & Mach, 2009). We can order inflammation in 2 types; acute inflammation and chronic inflammation. Acute inflammation is a rapid response to the agent that causes tissue injury. It can detect by founding poly-morphonuclear cell accumulation at injury site. Acute inflammation has three major components; vascular dilation and increase blood flow, extravasation, and leukocyte immigration and accumulation. (Figure 2.6) If acute inflammation occurs in a long duration, it converts to chronic inflammation. Chronic inflammation have an important sign which is infiltration of mononuclear cells (lymphocyte, plasma cell, macrophage), and repairing with fibrosis and angiogenesis at inflammatory site.
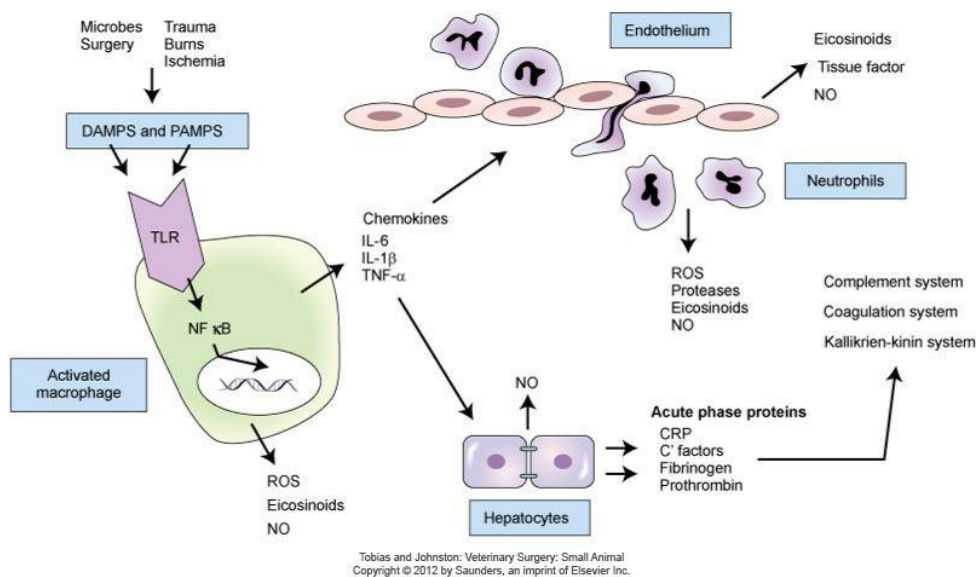
Figure 2. 6 Proinflammatory response to alarm signals

(Tobias & Johnston, 2011)

Acute pain or nociceptive pain occurs when strong noxious stimulus such as mechanical, chemical, or thermal have an effect on the skin or deep tissue. Nociceptors, a primary sensory nerve fibers, fire impulses via Aδ, Aβ, and C fibers which travel along the peripheral nerves, past the sensory cell bodies in dorsal root ganglia, along the dorsal roots, and into the spinal cord or brainstem. Afterwards, the conscious brain interprets these transmissions. Acute pain is useful for protect us from severe damage of tissue and is also short-acting, and relatively easy to treat.

Pain is the one of five inflammatory signs (another signs are redness, swelling, hot, and malfunction). It can cause pain from peripheral sensitization. Tissue injury results in release of inflammatory mediators such as bradykinin, histamine, nitric oxide, ATP. Prostaglandins, the product of arachidonic acid pathway, are the important mediators contributing pain by directly activating nociceptors (Kidd & Urban, 2001). Morover, prostaglandins can increase cyclic AMP level and reduce the activation threshold for TTX-R sodium channels via protein kinase A pathway which enhance nociceptor sensitization (Cesare & McNaughton, 1997; England, Bevan, & Docherty, 1996). IL-6 have an important role in the neuronal reaction to nerve injury

and cause development of neuropathic pain following the peripheral nerve injury (Hirota, Kiyama, Kishimoto, & Taga, 1996; Klein et al., 1997; Ramer, Murphy, Richardson, & Bisby, 1998). Therefore management of inflammation is the important thing that should concern. When we control acute inflammation, we can reduce a risk of peripheral sensitization which lead to chronic or neuropathic pain (Zhang & An, 2007). In order to evaluate the degree of the disease, many studies determined from physical examination, radiography, anthropometric measurement or assessment from patients (Lau et al., 2004; Brien et al., 2008; Coulson et al., 2012) while the others based on a measurement of the inflammatory-related biomarkers (Murphy et al., 2006). From physical examination, radiography, anthropometric measurement or assessment, the error and bias from measurement may be possible. The accuracy of data is important factor in research, thus the measurement of biomarkers, the objective data, is more reliably. IL-1 and IL-6 are popular biomarkers to detect joint inflammation in human.

IL-1 family has 11 members, but the IL-1 that many researchers take an interest is IL-1β. Researchers study the role of IL-1β in human and animals for many decades. They found that it relate with many diseases such as osteoarthritis, rheumatoid arthritis, periodontitis, asthma, Alzheimer disease, cancer (Assuma, Oates, Cochran, Amar, & Graves, 1998; Basu, Krady, & Levison, 2004; Dinarello, 2011; Lewis, Varghese, Xu, & Alexander, 2006; Manolagas, 1995; Shaftel, Griffin, & O'Banion, 2008; Suda, Nakamura, Jimi, & Takahashi, 1997). IL-1β is releasesd from endothelium, epithelium, macrophage, monocyte, and other cells in injury site. It requires cleavage by either intracellular caspase-1 or extracellular neutrophilic protease. Then active IL-1β bind with ligand-binding chain, termed type I (IL-1RI) and follow by the co-receptor chain, termed the accessory protein (IL-1RAcP). This complex bind the intracellular Toll-IL-1 receptor (TIR) which combine the adaptor protein MyD88, followed by phosphorylation of IL-1R associated kinases (IRAKs) and inhibitor of NKκ B kinase β (IKKβ), resulting in a signal to the nucleus contributing inactive form of IL-1β and collect them in inflammasome. Afterward, intracellular caspase-1 cleave

inactive IL-1β to active form and release them to extracellular compartment (Dinarello, 2011). In the arthritic joint, after blunt trauma, hemorrhage, hypoxia, or irritant exposure. Caspase-1 may not be required for IL-1β cleavage (Fantuzzi et al., 1997). Proteinase-3 which is one of neutrophil proteases can cleave the inactive form to active form of IL-1β (Joosten et al., 2009). IL-1β increase affinity of leukocyte and endothelium during rolling, adhesion, and migration of leukocytes to inflammatory site. Moreover, IL-1β causes an increase in the expansion of naïve and memory CD4 T cells (Ben-Sasson et al., 2009).

In human, IL-1 plays an important role in bone loss in patients with rheumatoid arthritis (Dinarello, 2011). In addition, there are many studies showing that IL-1 is correlated with other diseases such as Alzheimer disease and cancer in human (Lewis et al., 2006; Shaftel et al., 2008). The study by Assuma et al. (1998) revealed that IL-1 antagonist can inhibit inflammatory response, by decreasing the recruitment of mononuclear cells), and bone loss in *Macaca fascicularis* with experimental periodontitis. Another study found that IL-1β may be possible biomarker for detection of early stages of inflammation in dogs (Prachar, Kaup, & Neumann, 2013).

IL-6 is produced from various cell types, but mainly from macrophages and monocytes at inflammatory sites. They are the important mediator to stimulate the production of most acute phase proteins. They also control the extent of local and systemic acute inflammation, and elicit both cellular immune responses and mucosal humoral responses directed against reinfection. Furthermore, IL-6 can cause fever, thrombocytosis, cachexia, hypozincemia, and decreases concentration of insulin-like growth factor I in plasma (Gabay & Kushner, 1999). IL-6 plays important role in the shift from acute to chronic inflammation. When there is tissue damage, IL-6 is released from endothelial cells or stromal cells, activating the recruitment of neutrophils to inflammatory site (this stage is called acute inflammation) and inducing neutrophils to shred soluble IL-6 receptorα (sIL-6Rα). IL-6 combinds with

sIL-6Rα and gp130 on the endothelial cells' membrane and induces IL-6 secretion. This process is called "trans-signaling" (Gabay, 2006). Another pathway of IL-6 is that IL-6 binds with membrane-bound non-signaling α-receptor IL-6R (mbIL-6R) directly to activate cells; this process is called "classical-signaling" (Scheller, Chalaris, Schmidt-Arras, & Rose-John, 2011). Prolonged IL-6 production for several hours leads to neutrophilic apoptosis, phagocytosis and mononuclear accumulation at the inflammatory site. The monocyte recruitment is a result of monocyte chemo-attractant protein (MCP-1), which is produced from endothelial cells and phagocytic macrophages. The monocyte recruitment to inflammatory site shows that this stage is chronic inflammation. The study in dogs by Ajadi and coworkers (Ajadi, Adeniyi, Gazal, & Kasali, 2013) found that in blood circulation, IL-6 was significantly increased after knee arthrotomy at two hours, reached the maximum level at twenty-four hours after arthrotomy and decreased significantly in the third day. Some studies showed the correlation between IL-6 and canine steroid responsive meningitis-arteritis, rheumatoid arthritis and osteoarthritis in dog (Carter, Barnes, & Gilmore, 1999; Maiolini, Otten, Hewicker-Trautwein, Carlson, & Tipold, 2013).

In this study, surgical correction of patellar luxation was chosen due to the high incidence of such a disease and the method of choice for the treatment is surgery, resulting in the painful and inflammatory situation. The patellar luxation is one of the important hereditary orthopedic defects. Small breed dogs such as Pomeranians, Yorkshire Terriers, Chihuahuas, Miniature and Toy Poodles have risk of this problem approximately 12 times compared with large breed dogs (Priester, 1972). In Thailand, the prevalence of medial patellar luxation (MPL) and lateral patellar luxation (LPL) in small-breed dogs are 87% and 13%, respectively (Wangdee et al., 2005). Moreover, Pomeranians have currently the highest incidence for patellar luxation in the USA, with 42.4% of dogs affected. The clinical signs of the condition normally start showing at approximately four months after birth. Patellar luxation has 4 grades as shown in table 2.2

Table 2.2 Grading system for medial patellar luxation

(Tobias & Johnston, 2011)

Grade 1	Patella can be luxated medially when the stifle joint is held in full extension. There is no crepitation or bony deformity. Clinical signs are not present or occur infrequently.
Grade 2	Spontaneous luxation occurs with clinical signs of a nonpainful, "skipping" type of lameness. Mild deformities develop, consisting of internal rotation of the tibia and abduction of the hock. This condition may progress to a grade 3 luxation with associated cartilage erosion on the patellar and trochlear surfaces.
Grade 3	Patella is luxated permanently but can be reduced manually. More severe bony deformities are present, including marked internal tibial rotation and an S-shaped curve of the distal femur and proximal tibia. A shallow trochlear groove may be palpable. The client often complains of an abnormal, "crouched" gait rather than intermittent lameness because the dog often uses the leg in a semiflexed, internally rotated position. The condition is often bilateral.
Grade 4	This is a severe condition with permanent, nonreducible luxation of the patella. The tibia is rotated from 60° to 90° relative to the sagittal plane. If not corrected early in life, severe bony and ligamentous deformities develop and often are not reparable.

The treatment of patellar luxation is surgery in order to realign the patellar ligament in normal alignment. There are three major surgical procedures: Soft tissue reconstruction, Bone reconstruction, and Trochleoplasty. Choice of method depended to some extent on age of animal (Ferguson, 1997). Therefore post-operative care and pain control are importance in the treatment of patellar luxation. Good post-operative care and well-controlled pain can lead to less time of recovery, improve muscle and joint mobility quickly which improve life quality of the patients and owner.

CHAPTER III MATERIALS AND METHODS

3.1 Animal

Client-owned Pomeranians, 1 - 7 year- old, all genders enrolled in this study. Inclusion criteria were patellar luxation grade 3, no history of treatment of patellar luxation, no other diseases (eg. heart disease, skin disease), body condition score 3, and normal blood results. Exclusion criteria are having other diseases and inability to receive drugs continuously.

Informed owner consent will be gained and the research protocol will be approved by the Faculty of Veterinary Sciences, Chulalongkorn University's Ethics Committee, Bangkok, Thailand.

3.2 Pretreatment evaluation

Firstly, all dogs received the physical and clinical orthopaedic examinations as well as the peak vertical force gait analysis, radiographic examinations (hip and stifle joints) for assessment of bone deformities. Then, 3 milliliters of blood will be collected from each dog for evaluation of complete blood counts (CBCs), blood chemistry and biomarker assays (IL-1β and IL-6).

3.3 Treatment procedure

3.3.1 Anesthesia

All dogs will have anesthesia protocol and received surgical treatment for correction of patellar luxation by the same veterinary surgeon. (Table 3.1)

Table 3.1 *Drugs using in anesthesia*

	Drug	Concentration	Dose	Route
Premedication	Acepromazine	1 mg/ml	0.03 mg/kg	Intramuscular
	Morphine	10 mg/ml	0.5 mg/kg	(combined)
Induction	Propofol	10 mg/ml	4 mg/kg	Intravenous
Maintainance	Isoflurane	-	1-2 mg%	Inhalation
Antibiotics	Cefazolin	250 mg/ml	25 mg/kg	Intravenous

3.3.2 Surgical procedure

1. The approach is a protocol (Piermattei, Johnson, & Johnson, 2004). The dog is arranged lateral recumbency. Lateral approach of skin is provided by a curved parapatellar incision from distal third of femur to tibial tuberosity. Then cut the fascia lata and lateral fascia in the same line and broaden out with the scissors. Stab joint capsule with surgical blade and enlarge wound with scissors. Avoid the long digital flexor ligament which attach the lateral femoral condyle in the stifle joint.
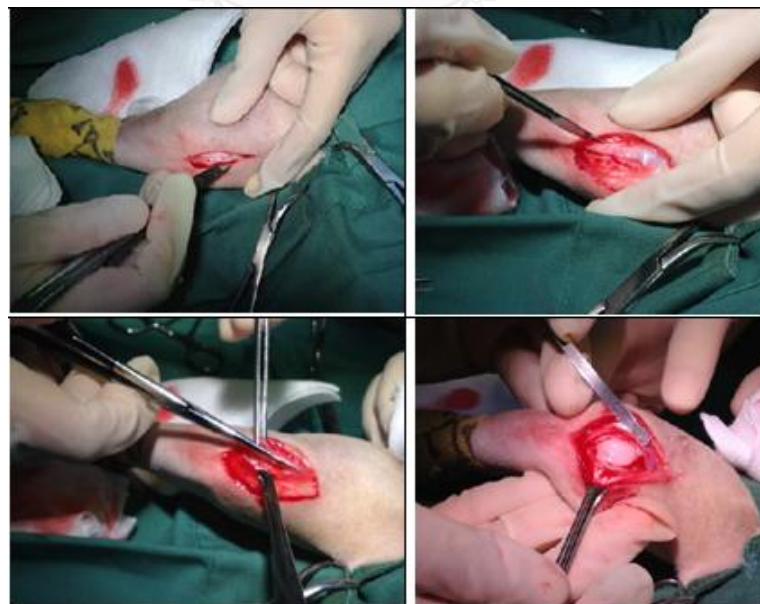
Figure 3. 1

Approach technique of the stifle joint

Photo by Kongwut (2015)

2. Medial desmotomy with electrocautery, between cranial and caudal sartorius, and patellar ligament and cranial sartorius.

Figure 3. 2

Medial desmotomy

Photo by Kongwut (2015)

3. Trochlear block recession with surgical blade and small chisel.
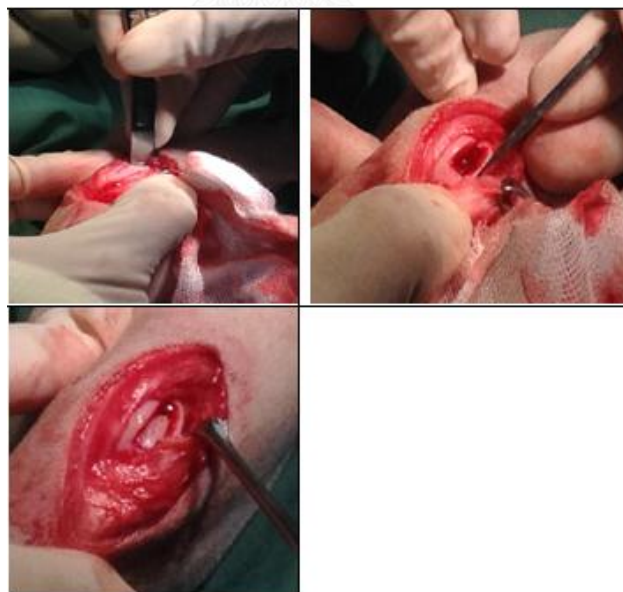
Figure 3. 3

Trochlear block recession

Photo by Kongwut (2015)

4. Lateral imbrication with overlapping suture pattern. Polypropylene 3/0 were suture used to close joint capsule and Prolene 2/0 were suture used to tighten fascia along patellar ligament.

Figure 3. 4

Lateral imbrication

Photo by Kongwut (2015)

5. Close the wound by suturing Polypropylene 3/0 to close subcutis. Nylon 3/0 used to close the skin.

Figure 3. 5

Close the wound

Photo by Kongwut (2015)

In the next day after surgery, they divided into 3 groups by random sampling. The first group received only Firocoxib (5 mg/kg once daily). The second group received only PCSO-524 (10 mg/kg, twice daily). The third group received Firocoxib (5

mg/kg, once daily) combining with PCSO-524 (10 mg/kg, twice daily). Treatment finished at the end of the 2nd week.

3.3.3 Parameter and monitoring

Every dogs received physical examinations, orthopedic examinations, clinical scoring system (Table 3) and gait analysis by the same veterinarian before surgery and at day 1, 5 and 14 after surgery.

Table 1.2 Clinical scoring system (Mongkon & Soontornvipart, 2012)

Lameness

Grade	Clinical evaluation
1	Walk normally
2	Slightly lame when walking
3	Moderately lame when walking
4	Severely lame when walking
5	Reluctant to rise and will not walk more than five paces

Weight bearing

Grade	Clinical evaluation
1	Equal on all limbs standing and walking
2	Normal standing; favors affected limb when walking
3	Partial weight-bearing standing and walking
4	Partial weight-bearing standing; non- weight-bearing walking
5	Non- weight-bearing standing and walking

Force platform system used for gait analysis. This system is an instrument used for measuring force from body weight and moving action. This instrument is invented by the department of Electrical Engineering, Faculty of Engineering, Chulalongkorn University. The dogs need to stand still and place each paw in the marked area for 3 seconds so that the instrument can measure and show the results.
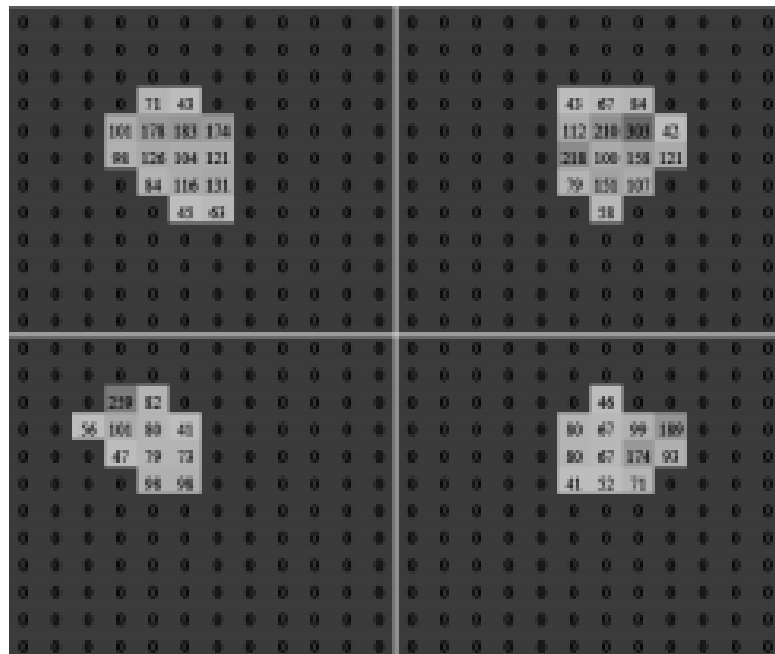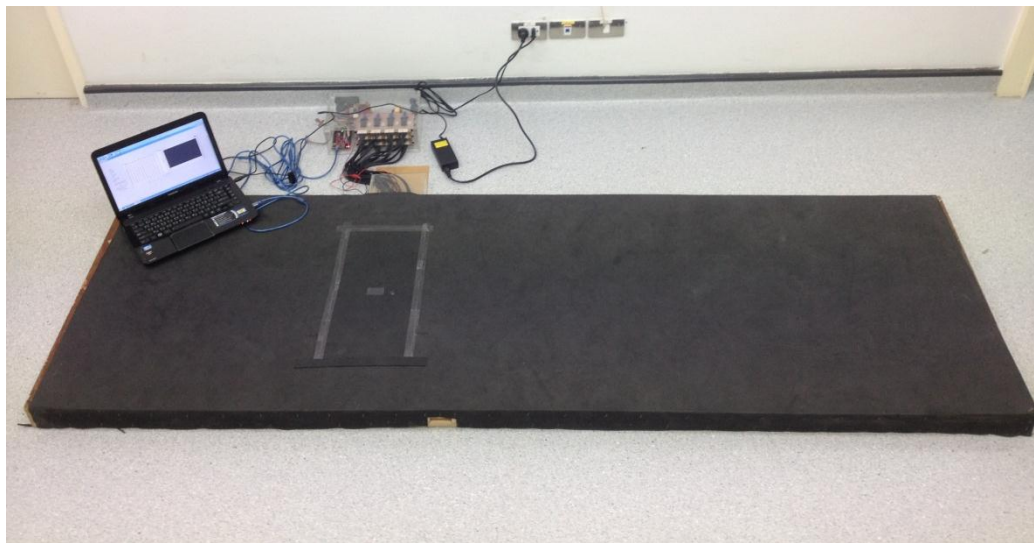

Figure 3. 6

Picture of Gait analysis and data

Photo by Kongwut (2015)

Glasgow pain scale used to monitor the dogs' pain. If the pain scale is more than 14, the dog was excluded from this research and relieved pain upon the veterinarian's decision immediately. (Figure 3.7)

SHORT FORM OF THE GLASGOW COMPOSITE PAIN SCALE

Dog's name _____

Hospital Number _____ Date / /   Time

Surgery Yes/No (delete as appropriate)

Procedure or Condition_____

_____

In the sections below please circle the appropriate score in each list and sum these to give the total score.

A. Look at dog in Kennel

Is the dog?

(i)		(ii)	
Quiet	0	Ignoring any wound or painful area	0
Crying or whimpering	1	Looking at wound or painful area	1
Groaning	2	Licking wound or painful area	2
Screaming	3	Rubbing wound or painful area	3
		Chewing wound or painful area	4

In the case of spinal, pelvic or multiple limb fractures, or where assistance is required to aid locomotion do not carry out section B and proceed to C
Please tick if this is the case ☐ then proceed to C.

B. Put lead on dog and lead out of the kennel.

When the dog rises/walks is it?

(iii)	
Normal	0
Lame	1
Slow or reluctant	2
Stiff	3
It refuses to move	4

C. If it has a wound or painful area including abdomen, apply gentle pressure 2 inches round the site.

Does it?

(iv)	
Do nothing	0
Look round	1
Flinch	2
Growl or guard area	3
Snap	4
Cry	5

D. Overall

Is the dog? (v)		*Is the dog?* (vi)	
Happy and content or happy and bouncy	0	Comfortable	0
Quiet	1	Unsettled	1
Indifferent or non-responsive to surroundings	2	Restless	2
Nervous or anxious or fearful	3	Hunched or tense	3
Depressed or non-responsive to stimulation	4	Rigid	4

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Total Score (i+ii+iii+iv+v+vi) = _____

Figure 3. 7

Glasgow pain scale

3.3.4 Blood collection

Three milliliters of blood samples collected from the cephalic vein or saphenous vein of each dog. One milliliter separated into 2 tubes for complete blood counts (CBCs) and blood chemistry tests. The samples for CBCs kept in anticoagulant 100IU/ml heparin (APSFinchem, Australia) besides, the samples for blood chemistry kept in plain tube without anticoagulant. Then, these samples kept at 4°C. The other two milliliters of blood samples (for biomarker assays) kept in plain tube without anticoagulant and centrifuged at 7,000xg for 15 minutes to obtain serum; then, stored at -20°C until batch analysis.

3.3.5 Hematology and biochemistry

The biochemical analyses, complete blood counts (CBCs) and blood chemistry tests conducted at the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University, Bangkok Thailand. One milliliter of blood sample divided into 2 parts, first part analyzed for the CBC, including the red blood cell count (RBC), white blood cell count (WBC), hematocrit, hemoglobin level and platelet count. Another part analyzed for blood chemistry profiles, including alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen and creatinine. These analyses performed only 1 time, before treatment for evaluating the animals' health.

3.3.6 Chemical substances' preparation and ELISA technique of IL-1β & IL-6

IL-1β and IL-6 concentration in serum determined by commercially available canine ELISA kit. The IL-1β ELISA kit (USCN life Science Inc., China) has the minimum detectable dose of this kit is typically less than 3.1pg/mL and the detection range 7.81-500pg/mL. The IL-6 ELISA kit (R&D Systems Inc., USA) has the mean minimum detectable dose (MDD) of canine IL-6 6.1 pg/mL (ranged from 1.5-11.8 pg/mL) and the detection range 31.3 - 2,000 pg/mL.

Figure 3. 8

Canine IL-1β ELISA test kit

Photo by Kongwut (2015)

Figure 3. 9

Canine IL-6 ELISA test kit

Photo by Kongwut (2015)

Canine IL-1β's assay procedure

1.) All the reagents and samples were brought to room temperature.

2.) Detection Reagent A and Detection Reagent B were prepared by centrifuging at 4°C 4,000 xg for 5 minutes to bring down all the substance in the bottom of vials. Then they were diluted with Assay Diluent A and Assay Diluent B orderly to make working solution (1:100).

3.) Wash Solution was prepared by adding 20ml of Wash Solution concentrate (x30) with deionized water 580 ml to make Wash Solution (x1) 600 ml.

4.) The Standard solution was prepared by centrifuging at 4°C 4,000 xg for 5 minutes to bring down all the substance in the bottom of vial. Then 1 ml of Standard Diluent was added to make the highest concentration (500 pg/ml). The 6 tubes of 2-fold dilution of Standard were mixed (concentration = 250, 125, 62.5, 31.2, 15.6, 7.8 pg/ml). The last tube is only Standard Diluent to make blank (0 pg/ml)
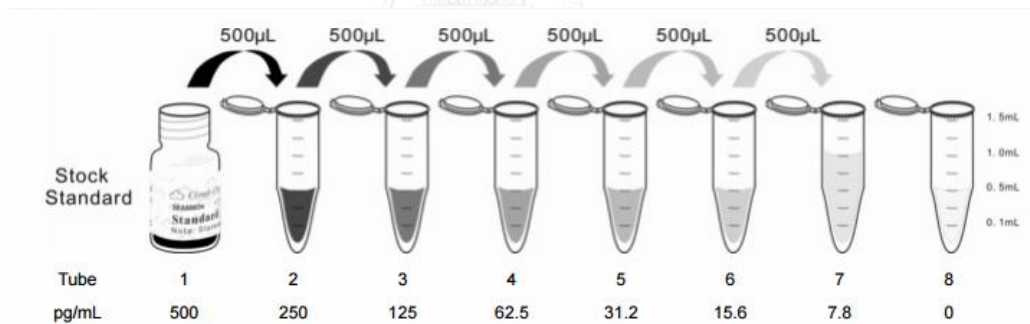
Figure 3. 10

Show procedure of the Standard solution's 2-fold dilution

5.) The serum or standard briefly were vortexed briefly and 100 µl of them were added per well (duplicate wells). The 96-well plate was covered and incubated in incubator (37°C) for 2 hours. (Figure 3.11)
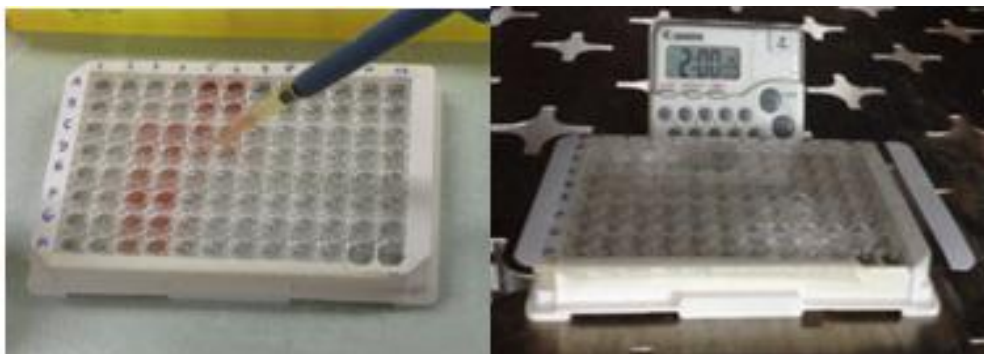
Figure 3. 11

Step 5

Photo by Kongwut (2015)

6.) All of the liquid were removed.

7.) 100 µl of the working Detection Reagent A was added (from step 2) to each well. The plate was covered and incubated in incubator (37°C) for 1 hour. (Figure 3.12)
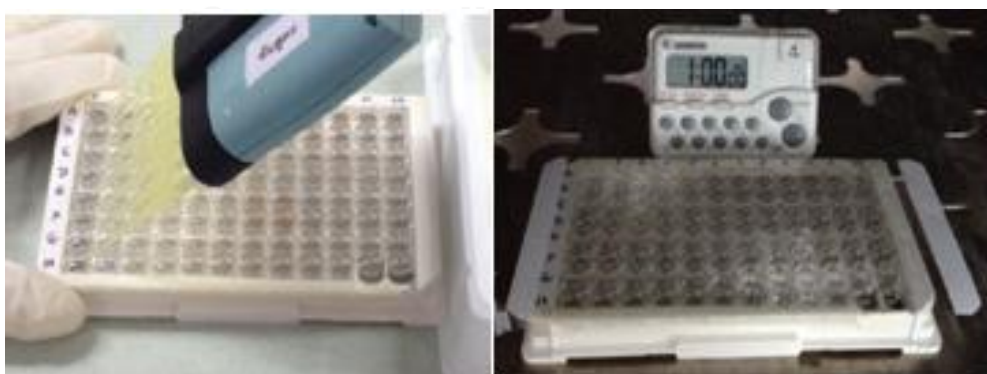
Figure 3. 12

Step 7

Photo by Kongwut (2015)

8.) The solution was aspirated from the plate. Then, all wells were washed with 350 µl of Wash Solution and let it to sit 1-2 minute. The remaining liquid was removed by blot with paper towels. Totally wash 3 times.

Figure 3. 13

Step 8

Photo by Kongwut (2015)

9.) 100 µl of the Detection Reagent B was added (from step 2) to each well. The plate was covered and incubated in incubator (37℃) for 30 minutes. (Figure 3.13)
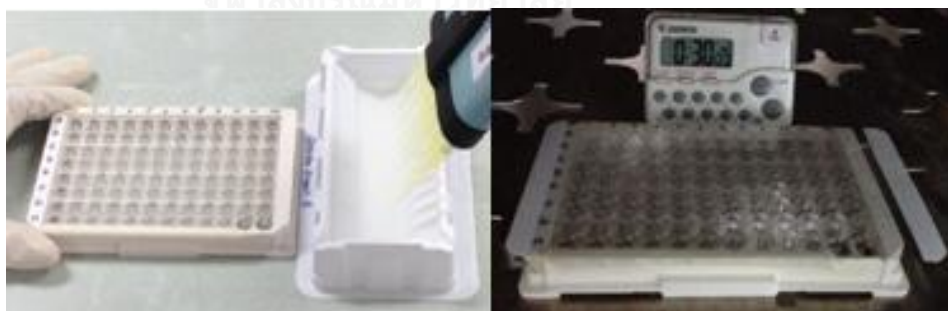
Figure 3. 14

Step 9

Photo by Kongwut (2015)

10.) Repeat step 8.) But wash totally 5 times.

11.) 90 μl of Substrate Solution (TMB) was added per well. The plate was covered and incubated in incubator (37℃) for 15 minutes. Avoid the plate from direct light. The liquid turned to blue color. (Figure 3.15)

Figure 3. 15

Step 11

Photo by Kongwut (2015)

12.) 50 μl of Stop Solution was added per well. The liquid turned to yellow color. Mixed it gently and determined the optical density (OD) immediately by Microplate reader (Biotek EL808) at wavelength 450 nm. (Figure 3.16)

Figure 3. 16

Step 12

Photo by Kongwut (2015)

Canine IL-6's assay procedure

1.) All the reagents and samples were brought to room temperature.

2.) The IL-6 Control was prepared by centrifuging at 4°C 4,000 xg for 5 minutes. Therefore, 1 ml of deionized water was added and was mixed thoroughly.

3.) The Wash Buffer was prepared by adding undiluted Wash Buffer 20 ml to deionized water 480 ml to make the total diluted Wash Buffer 500 ml.

4.) The Standard Solution was prepared by centrifuging at 4°C 4,000 xg for 5 minutes. The Calibrator Diluent RD5T 5 ml was added and allowed it to sit for 5 minutes. This stock Standard Solution have concentration of 2,000 pg/ml. Therefore, 300 μl of stock Standard Solution was pipetted to produce 2-fold dilution. (Figure 24) The Calibrator Diluent RD5T serve as zero standard (0 pg/ml)
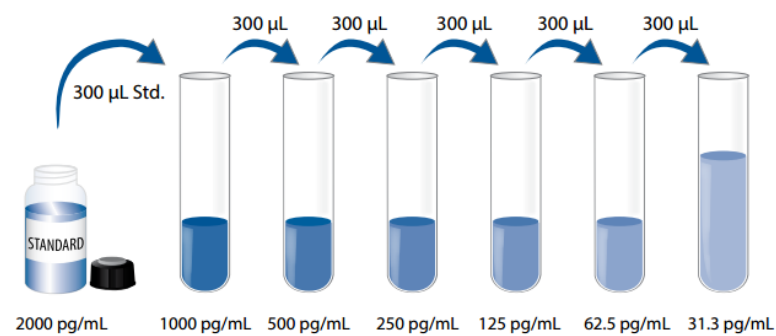
Figure 3. 17
Preparation of Standard

5.) 50 µl of Assay Diluent RD1-75 was added to each well.
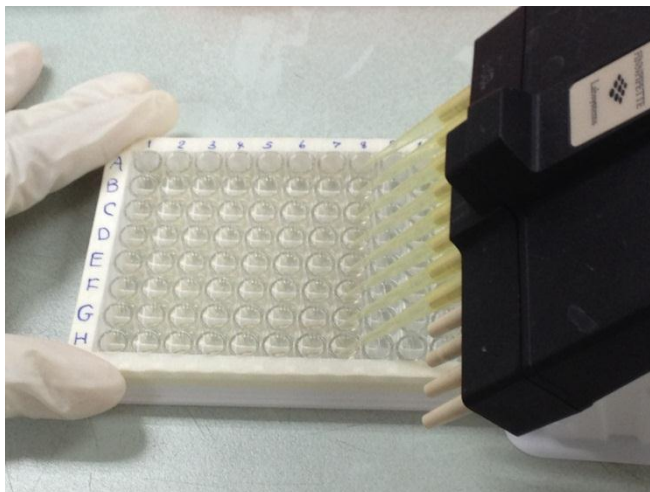
Figure 3. 18

Step 5

Photo by Kongwut (2015)

6.) The serum, control or standard briefly were vortexed and 100 µl of them were added per well (duplicate wells). The plate was covered and incubated in room temperature for 2 hour on microplate shaker (500 rpm).
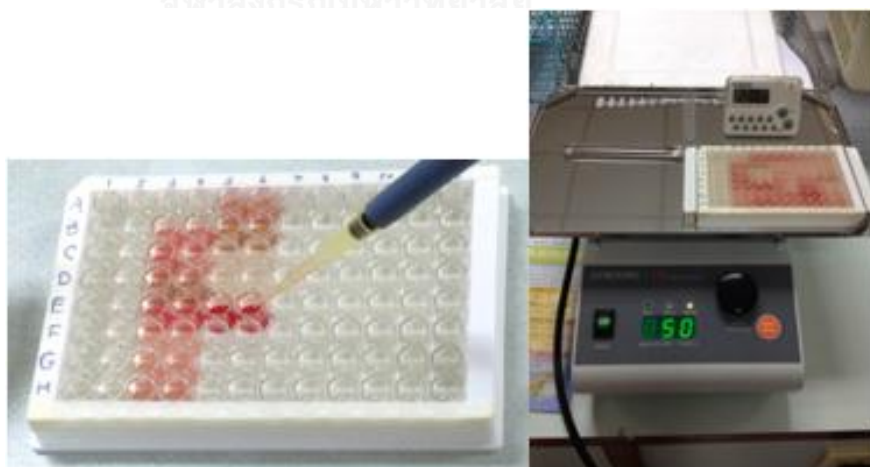
Figure 3. 19

Step 6

Photo by Kongwut (2015)

7.) All wells were aspirated, washed with Wash Buffer (400 µl per well) and blotted on the clean paper towels to remove all liquid out. Did it 5 times totally.

Figure 3. 20

Step 7

Photo by Kongwut (2015)

8.) 200 µl of Canine IL-6 Conjugate was added to each well. The plate was covered and incubated in room temperature for 2 hours on microplate shaker (500 rpm).

Figure 3. 21

Step 8

Photo by Kongwut (2015)

9.) Repeated step 7.)

10.)The Color Reagents A 7 ml and Color Reagent B 7 ml were mixed together. Plate was protected from light.

11.)120 µl of the Substrate Solution was added to each well. The plate was covered and incubated in room temperature for 30 minutes on the benchtop. Plate was protected from direct light.
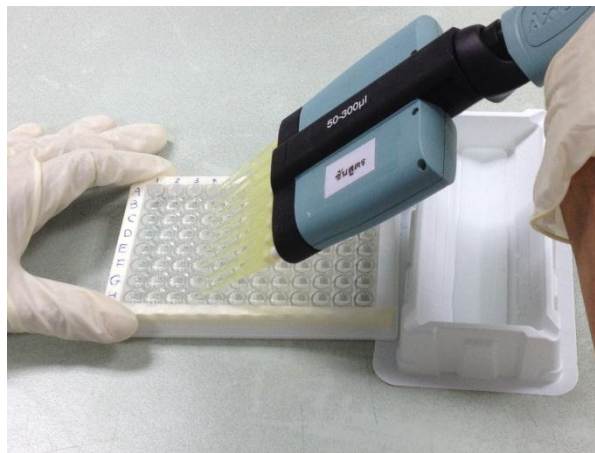
Figure 3. 22

Step 11

Photo by Kongwut (2015)

12.)120 µl of Stop Solution was added per well. Mixed it gently and determined the optical density (OD) immediately by Microplate reader (BiotekSynergy HT) at wavelength 450 nm. (correction OD with 540 nm)

3.3.7 Data analysis

Inferential statistics. All data expressed as mean ± SD. Parameters in group compared using repeated measure ANOVA (analysis of variance). Parameters between group compared by one-way ANOVA. P-values of <0.05 considered to be statistically significant. Relationship between inflammatory cytokines (IL-1β & IL-6) and clinical score (peak vertical force gait analysis) determined by linear regression. All data use SPSS programme to analyse.

CHAPTER IV RESULTS

4.1 Weight bearing score

There was no significance of Lameness score when compare between 3 groups of treatment during 4 times of data collection. There is significant increasing of weight-bearing score at day 1 after surgery comparing with pre-operative day and day 14 after surgery in 3 groups ([a]). In group 2, weight-bearing score decrease significantly in day 14 after surgery comparing day 5 after surgery ([b]). In group 3, this score decrease significantly in day 5 after surgery comparing day 1 ([c]), but still more than day 14 significantly([d]).

Table 4. 1 Weight-bearing score

Group	Time of data collection			
	Pre-operation	1 day after surgery	5 days after surgery	14 days after surgery
1 (NSAID only)	1.20 ± 0.42^{a}	$4.0 \pm 0.82^{a,c}$	1.80 ± 0.79^{c}	1.40 ± 0.70^{a}
2 (PCSO-524 only)	1.27 ± 0.47^{a}	3.45 ± 1.37^{a}	2.55 ± 0.82^{b}	$1.55 \pm 0.69^{a,b}$
3 (NSAID+PCSO-524)	1.55 ± 0.69^{a}	$4.0 \pm 1.48^{a,c}$	$2.27 \pm 1.19^{c,d}$	$1.18 \pm 0.60^{a,d}$

[a, b, c, d].-The mean difference is significant at the .o5 level in group
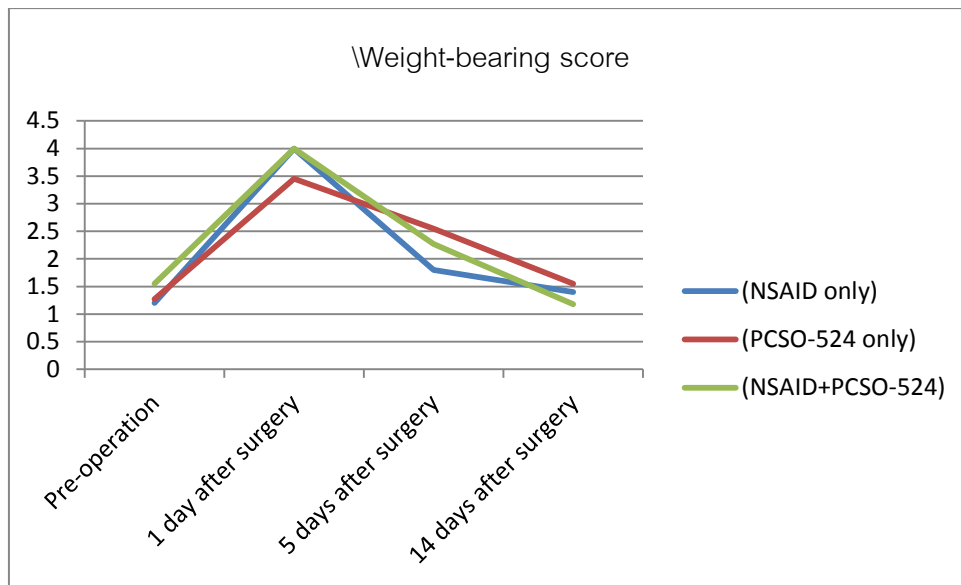
Figure 4. 1

Graph of weight-bearing score

4.2 Lameness score

There was no significance of Lameness score in 3 groups of treatment during 4 times of data collection that show in table 7. In 3 groups, lameness score increase significantly in day 1 after surgery and decrease significantly in day 5 after surgery ([a]).

Table 4. 2 Lameness score

Group	Time of data collection			
	Pre-operation	1 day after surgery	5 days after surgery	14 days after surgery
1 (NSAID only)	1.30±0.48	4.0±0.94 [a]	2.0±1.25 [a]	1.60±1.08
2 (PCSO-524 only)	1.27±0.47	3.73±1.27 [a]	2.73±0.79 [a]	1.91±0.70
3 (NSAID+PCSO-524)	1.45±0.69	3.64±1.36 [a]	2.27±0.91 [a]	1.18±0.60

[a] -.The mean difference is significant at the .o5 level in group
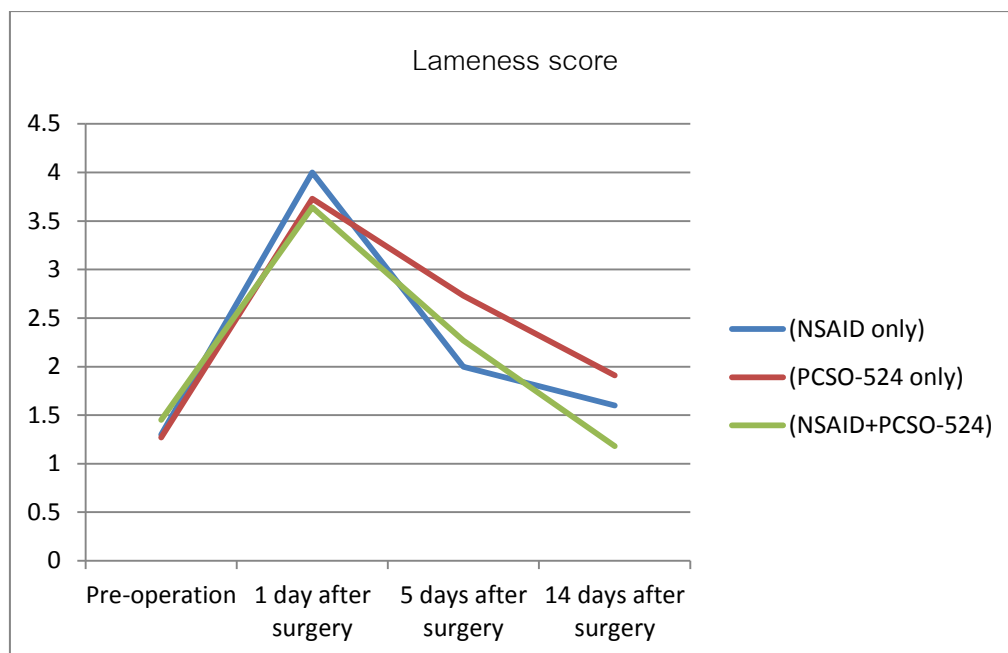
Figure 4. 2

Graph of lameness score

4.3 Gait analysis

There was no significance of gait analysis in all groups during 4 times of data collection. In group 1, 2 and 3, the data decrease significantly in day 1 after surgery when compare with pre-operative day and normal data (20% of body weight) . Then the data increase significantly and is similar to normal data in day 14 after surgery.

Table 4. 3 Gait analysis

Group	Time of data collection			
	Pre-operation	1 day after surgery	5 days after surgery	14 days after surgery
1 (NSAID only)	17.88±3.52 %	11.92±1.40%*	14.92±2.46%*	17.50±5.22%
2 (PCSO-524 only)	19.95±2.58 %	8.30±6.42%*	13.54±6.23%*	18.64±2.46%
3 (NSAID+PCSO-524)	19.27±1.70 %	10.98±1.35%*	15.91±1.54%*	19.39±2.29%

*.The mean difference is significant at the .o5 level comparing normal data (20% of body weight)
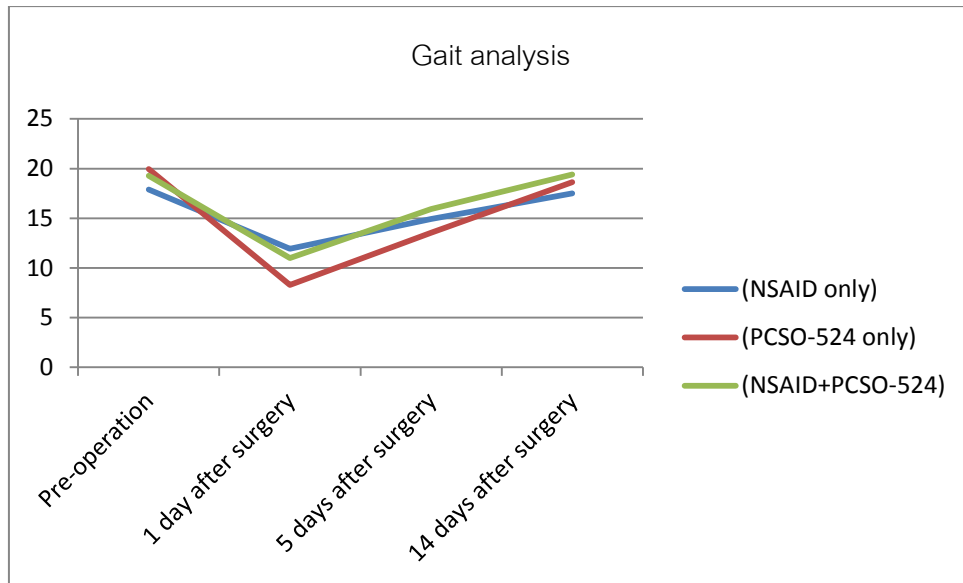
Figure 4. 3

Graph of gait analysis

4.4 IL-1β

There was no significance of serum IL-1 level in 3 groups of treatment during 5 times of data collection. In all groups, serum IL-1β level increases in day 1 after surgery. In group 1 and 3, serum IL-1β level decreases significantly in day 14 after surgery comparing day 1 and 5 after surgery ([a]), respectively. In group 2, serum IL-1β level slightly decreased since day 5 after surgery. However, there is no significantly difference of the value between day 1 and day 14 after surgery.

Table 4. 4 Serum IL-1β level

Group	Time of serum collection				
	Pre-operation	2 hours after surgery	1 day after surgery	5 days after surgery	14 days after surgery
1 (NSAID only)	180.35±151.96	193.29±176.50	268.25±183.02 [a]	171.39±141.45	136.11±113.50 [a]
2 (PCSO-524 only)	173.85±118.10	177.26±101.64	252.35±149.96	248.87±116.76	226.57±91.68
3 (NSAID+PCSO-524)	190.47±192.96	196.28±168.83	294.19±219.62	184.62±100.35 [b]	134.78±102.5 [b]

[a, b] - .The mean difference is significant at the .o5 level in
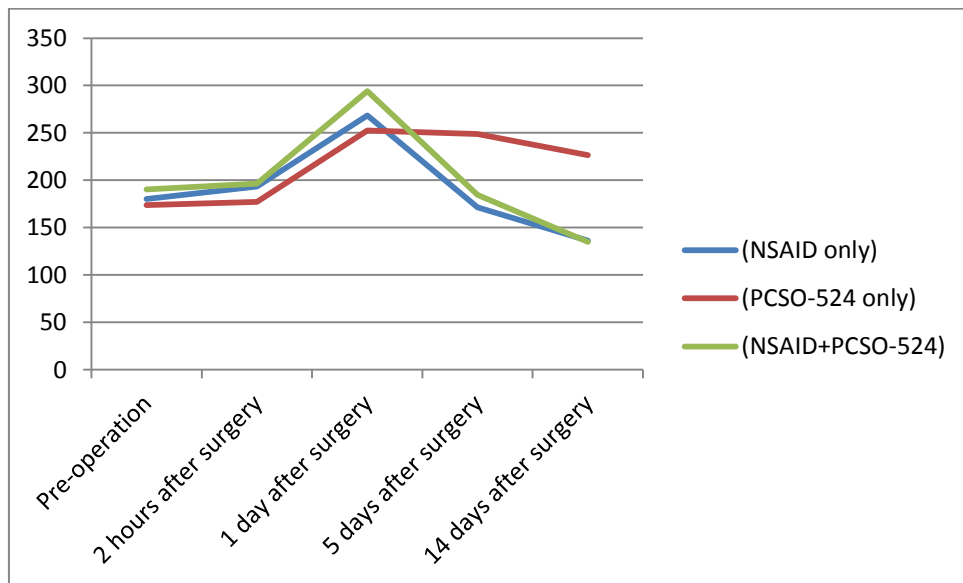
Figure 4. 4

Graph of serum IL-1β level

4.5 IL-6

There was no significance of serum IL-6 level in 3 groups of treatment during 5 times of data collection. All groups have the increasing values significantly in day 1 after surgery then, all the values decreased significantly in day 14 after surgery. In group 1 and 3, serum IL-6 levels decreased significantly since day 5 after surgery comparing with day 1 after surgery. While group 2 that treat with PCSO-524 only, have decreasing values of serum IL-6 level in day 14 after surgery.

Table 4. 5 Serum IL-6 level

Group	Time of serum collection				
	Pre-operation	2 hours after surgery	1 day after surgery	5 days after surgery	14 days after surgery
1 (NSAID only)	18.84±15.58 a, b	17.36±12.23 c, d	59.48±34.65 a, c, e, f	34.53±22.02 b, d, e	16.83±10.39 f
2 (PCSO-524 only)	19.2±24.45 g	19.32±23.51 h	60.7±47.13 g, h, i	44.47±40.84	35.25±33.75 i
3 (NSAID+PCSO-524)	8.46±16.82 j	8.9±17.7 k	56.56±38.01 j, k, l, m	25.69±24.94 l	8.88±17.67 m

a, b, c -.The mean difference is significant at the .o5 level in group
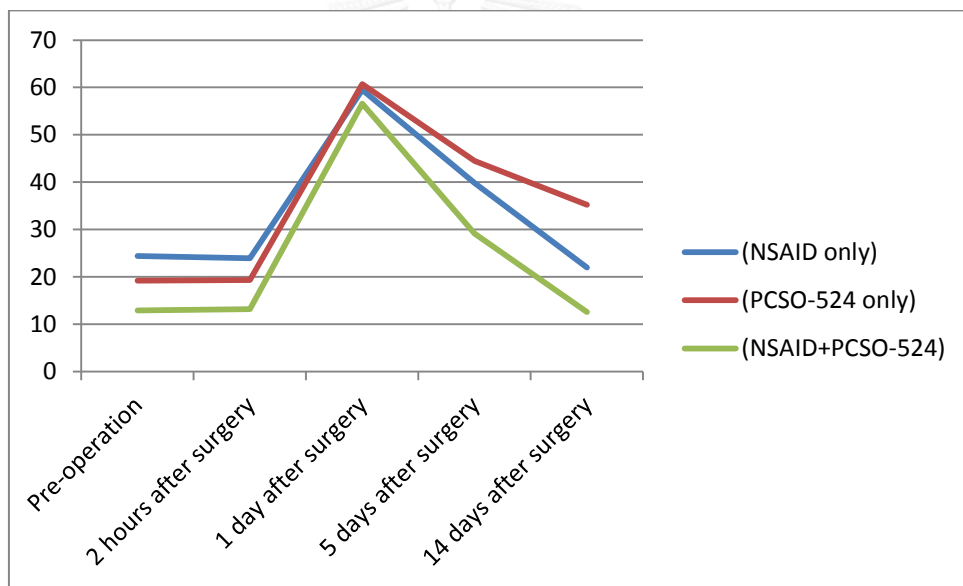
Figure 4. 5

Graph of serum IL-6 level

There was strongly positive correlation between weight-bearing scores and lameness scores (0.876). However, the other parameters did not have any correlation.

CHAPTER V DISCUSSION

This study revealed that there was a reduction of IL-1β and IL-6 level in the blood on day 14 after correction of patellar luxation surgery. But the group 2 which received PCSO-524 only have slower decline of serum IL-1β and IL-6 level comparing with the other treatment, indicating that PCSO-524 slowly reduce acute inflammation, resulting of which similar to those of Singh and coworker in 2008. Their experiment indicate that giving 5 days of CO_2-SFE crude lipid extract and free fatty acid extract from *Perna canaliculus* was equipotent to Piroxicam reducing inflammation in adjuvant-induced arthritis rat (M Singh et al., 2008). However, there is a few research about PCSO-524 efficacy controlling acute pain while studying of PCSO-524 and chronic pain is more. Addition of sample in each group may be necessary in future study to show the significant difference serum inflammatory cytokine level between NSAID group and NSAID combine PCSO-524 group.

From this study, even though there was no relationship between serum inflammatory cytokine levels (both IL-1β and IL-6) and clinical signs (gait analysis, weight-bearing scores, and lameness scores). However the data of all clinical signs were better in the same way as the level of serum IL-1β and IL-6 that decreased. The dogs in all group of treatment have weight-bearing and gait data similar to standard score of normal dog in day 14 after surgery. These results show that PCSO-524 have the efficacy of pain relief by reducing acute inflammation which cause peripheral sensitization (Mickleborough, Sinex, Platt, Chapman, & Hirt, 2015; Zawadzki et al., 2013).

Force platform gait analysis is the tool for collecting subjective data during walking and standing. There are many researches in mice, dog, or human. The benefits of the method are diagnose diseases (eg. Cerebral palsy), assess the evaluation of the pathophysiological states, and assess the improvement of mid-to-long term period of treatment in chronic diseases (Berryman, Harris, Moalli, & Bagi,

2009; Clarke & Still, 1999; Hetze, Romer, Teufelhart, Meisel, & Engel, 2012; Parvathy & Masocha, 2013; Vincelette et al., 2007; White, Agouris, Selbie, & Kirkpatrick, 1999). In our study, there are no relationship between the subjective score (weight-bearing score) and gait analysis which is objective data. These results are consistent with another previous study. They concluded that gait analysis is the most easily sensitive and accurate method for diagnosis and assessment of locomotive diseases (Dierick, Penta, Renaut, & Detrembleur, 2004; Draper, 2000; Evans, Horstman, & Conzemius, 2005; Quinn et al., 2007).

In conclusion, PCSO-524 can improve clinical signs in Pomeranian dogs after surgical correction of medial patellar luxation. However, PCSO-524 did not have anti-inflammatory effect as good as NSAID in case of acute inflammation's treatment. For the further study, we recommend to increase dose of PCSO-524 in other condition that causes acute inflammation and acute pain. Maybe the extraction of active ingredients in PCSO-524 is necessary. Moreover, we suggest increasing the sample size for showing the significance and accurate results

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APPENDIX

APPENDIX

Results of canine serum IL-1β (OD)

Plate 1	1	2	3	4	5	6	7	8	9	10	11	12
A	0.111	0.103	0.238	0.235	0.719	0.72	1.932	2.114	1.476	1.477	0.55	0.548
B	1.625	2.111	0.165	0.174	0.477	0.477	0.508	0.66	0.932	0.85	0.581	0.58
C	3.267	2.958	2.141	2.200	0.488	0.421	0.621	0.671	0.975	0.856	1.101	1.211
D	2.395	2.248	1.862	1.764	0.532	0.533	1.74	1.706	1.35	1.355	0.697	0.697
E	1.509	1.402	1.687	1.653	2.088	2.102	1.508	1.562	1.867	1.599	0.606	0.743
F	0.904	0.774	1.486	1.401	2..745	2.293	0.709	0.709	2.045	2.044	1.052	1.05
G	0.452	0.51	1.323	1.323	2.519	2.601	1.292	1.194	0.933	0.999	1.098	1.099
H	0.328	0.286	1.006	1.01	2.267	2.329	1.248	1.125	0.828	0.83	2.215	2.009

Plate 2	1	2	3	4	5	6	7	8	9	10	11	12
A	0.108	0.106	0.256	0.217	0.219	0.187	0.659	0.714	2.151	2.06	1.449	1.464
B	1.864	1.872	0.144	0.195	0.22	0.223	1.309	1.32	1.489	1.69	0.754	0.757
C	3.114	3.111	1.281	1.243	0.239	0.24	1.624	1.624	1.49	1.544	1.524	1.524
D	2.351	2.292	1.173	1.175	0.516	0.516	0.215	1.253	1.732	1.746	1.96	1.971
E	1.422	1.489	0.263	0.199	0.31	0.317	0.471	0.399	1.647	1.711	2.084	2.101
F	0.802	0.876	0.315	0.319	0.169	0.251	0.64	0.643	0.915	0.915	1.772	1.773
G	0.528	0.434	0.402	0.48	0.818	0.82	1.034	1.017	1.104	1.061	1.467	1.433
H	0.301	0.313	0.261	0.299	0.687	0.688	1.264	1.341	1.195	1.151	1.375	1.373

Plate 3	1	2	3	4	5	6	7	8	9	10	11	12
A	0.121	0.137	0.284	0.306	0.548	0.548	1.34	1.34	2.198	2.301	2.2	2.199
B	1.731	3.535	0.229	0.247	0.334	0.392	1.62	1.554	2.073	2.081	2.749	2.75
C	3.069	3.565	1.253	1.253	0.441	0.424	2.472	2.431	0.532	0.533	2.452	2.452
D	2.345	2.743	1.551	1.355	1.774	1.552	2.228	2.411	0.736	0.714	2.241	2.2
E	1.697	2.047	1.087	1.045	1.629	1.63	2.117	1.999	0.497	0.497	2.91	2.91
F	1.031	1.072	1.5	1.491	2.158	2.3	1.42	1.418	0.727	0.727	2.529	2.709
G	0.563	0.541	0.331	0.333	2.018	1.987	1.225	1.417	0.706	0.72	2.923	2.783
H	0.393	0.417	0.464	0.501	1.759	1.77	2.452	2.452	1.832	1.836	1.926	1.948

Plate 4	1	2	3	4	5	6	7	8	9	10	11	12
A	0.136	0.122	0.301	0.289	0.597	0.653	1.609	1.61	1.297	1.297	1.053	1.111
B	3.367	1.899	0.211	0.265	0.401	0.379	1.485	1.494	2.121	2.085	0.414	0.42
C	3.153	3.481	1.481	1.395	0.384	0.422	1.967	1.922	1.526	1.53	0.491	0.505
D	2.744	2.344	0.39	0.392	0.351	0.303	2.306	2.306	1.215	1.251	0.967	0.977
E	1.984	1.76	0.404	0.403	0.27	0.271	2.47	2.472	0.809	0.809	0.857	0.911
F	1.052	1.051	0.506	0.522	1.986	1.99	1.922	1.922	0.931	0.955	0.496	0.496
G	0.544	0.56	0.389	0.389	1.867	1.866	1.393	1.397	2.286	2.286	0.5	0.492
H	0.411	0.399	0.32	0.318	2.509	2.101	1.214	1.095	1.589	1.567	0.876	0.784

Results of canine serum IL-6 (OD)

Plate 1	1	2	3	4	5	6	7	8	9	10	11	12
A	0.035	0.028	0.068	0.08	0.106	0.11	0.071	0.071	0.061	0.065	0.066	0.067
B	0.333	0.327	0.058	0.059	0.077	0.077	0.071	0.073	0.059	0.06	0.118	0.118
C	1.995	1.992	0.058	0.058	0.07	0.072	0.114	0.111	0.061	0.061	0.096	0.099
D	1.035	0.975	0.066	0.064	0.114	0.118	0.09	0.08	0.062	0.061	0.066	0.064
E	0.521	0.511	0.061	0.061	0.123	0.123	0.07	0.073	0.074	0.073	0.1	0.097
F	0.285	0.298	0.057	0.059	0.187	0.181	0.062	0.06	0.066	0.066	0.089	0.088
G	0.155	0.156	0.067	0.068	0.127	0.126	0.059	0.058	0.063	0.063	0.158	0.148
H	0.104	0.102	0.069	0.07	0.11	0.114	0.068	0.068	0.068	0.067	0.116	0.116

Plate 2	1	2	3	4	5	6	7	8	9	10	11	12
A	0.029	0.034	0.071	0.077	0.075	0.069	0.075	0.073	0.131	0.115	0.071	0.068
B	0.331	0.331	0.082	0.086	0.109	0.109	0.059	0.061	0.107	0.109	0.069	0.071
C	1.994	1.994	0.07	0.074	0.082	0.081	0.06	0.06	0.099	0.1	0.115	0.115
D	1.004	1.006	0.069	0.069	0.07	0.069	0.071	0.07	0.099	0.095	0.102	0.104
E	0.518	0.521	0.12	0.121	0.063	0.067	0.068	0.069	0.093	0.094	0.093	0.094
F	0.293	0.292	0.089	0.09	0.067	0.066	0.062	0.061	0.165	0.171	0.026	0.03
G	0.156	0.154	0.072	0.073	0.089	0.088	0.08	0.082	0.151	0.139	0.028	0.03
H	0.103	0.101	0.071	0.072	0.079	0.079	0.081	0.083	0.124	0.124	0.033	0.037

Plate 3	1	2	3	4	5	6	7	8	9	10	11	12
A	0.04	0.037	0.067	0.068	0.035	0.031	0.029	0.03	0.055	0.055	0.032	0.031
B	0.315	0.32	0.033	0.032	0.031	0.031	0.024	0.03	0.118	0.12	0.028	0.024
C	1.957	2.01	0.03	0.029	0.051	0.055	0.037	0.033	0.061	0.06	0.027	0.026
D	0.935	0.949	0.065	0.065	0.043	0.042	0.035	0.035	0.051	0.049	0.034	0.042
E	0.45	0.468	0.065	0.066	0.039	0.033	0.07	0.074	0.03	0.029	0.031	0.029
F	0.293	0.271	0.113	0.104	0.025	0.027	0.06	0.061	0.031	0.03	0.029	0.029
G	0.143	0.148	0.089	0.09	0.026	0.026	0.045	0.051	0.045	0.046	0.042	0.041
H	0.092	0.092	0.081	0.082	0.034	0.03	0.056	0.055	0.037	0.038	0.038	0.042

Plate 4	1	2	3	4	5	6	7	8	9	10	11	12
A	0.038	0.04	0.068	0.068	0.031	0.027	0.035	0.041	0.04	0.036	0.056	0.062
B	0.321	0.312	0.078	0.078	0.035	0.034	0.032	0.032	0.036	0.037	0.049	0.051
C	1.98	1.984	0.047	0.046	0.036	0.037	0.028	0.027	0.075	0.075	0.019	0.066
D	0.942	0.942	0.037	0.036	0.06	0.06	0.044	0.05	0.049	0.048	0.045	0.056
E	0.451	0.465	0.033	0.032	0.047	0.046	0.049	0.049	0.035	0.02	0.05	0.037
F	0.282	0.281	0.031	0.028	0.038	0.039	0.092	0.086	0.045	0.034	0.086	0.087
G	0.144	0.147	0.056	0.056	0.029	0.028	0.06	0.054	0.046	0.051	0.049	0.057
H	0.091	0.093	0.034	0.033	0.028	0.028	0.045	0.046	0.085	0.082	0.043	0.053

VITA

Sararat Kongwut, is the only daughter of Mr.Sahuchtuch Kongwut and police lieutenant colonel Piyaorn Kongwut. She was born in October, 27 1987 in Bangkok. She was graduated elementary school from Phayathai School and secondary school from Patumwan Demonstration School. In April 2012, she graduated bachelor degree of Doctor of Veterinary Medicine (D.V.M.) with second-class honour from Faculty of Veterinary Science, Chulalongkorn University, Bangkok and worked as full-time veterinarian at Pet Direct Animal Hospital for 1 year. In May 2013 , she started to study Master degree in course of veterinary surgery, Department of Veterinary Surgery, Faculty of Veterinary Science, Chulalongkorn University. At present, she works as part-time veterinarian at Best Care Animal Hospital.