การศึกษาเปรียบเทียบผลของสารสกัดหยาบจากเม่าสร้อย (*Antidesma acidum*) ไบโอฟลาโวนอยด์ และแอลฟาโทโคเฟอรัลต่อภาวะเครียดออกซิเดชั่นในแมวที่เป็นโรคไตเรื้อรัง

นางสาวคคนางค์ ใจมั่น

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ปีการศึกษา 2553

THE COMPARATIVE STUDY OF THE EFFECTS OF CRUDE EXTRACT FROM MAO SOI (*ANTIDESMA ACIDUM*), BIOFLAVONOID AND ALPHA-TOCOPHEROL ON OXIDATIVE STRESS IN CATS WITH CHRONIC KIDNEY DISEASE

Miss Kakanang Jaimun

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Veterinary Medicine
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Thesis Title	THE COMPARATIVE STUDY OF THE EFFECTS OF CRUDE EXTRACT FROM MAO SOI (*ANTIDESMA ACIDUM*), BIOFLAVONOID AND ALPHA-TOCOPHEROL ON OXIDATIVE STRESS IN CATS WITH CHRONIC KIDNEY DISEASE
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คคนางค์ ใจมั่น : การศึกษาเปรียบเทียบผลของสารสกัดหยาบจากเม่าสร้อย (*Antidesma acidum*) ไบโอฟลาโวนอยด์ และแอลฟาโทโคเฟอรัลต่อภาวะเครียดออกซิเดชั่นในแมวที่เป็นโรคไตเรื้อรัง. (THE COMPARATIVE STUDY OF THE EFFECTS OF CRUDE EXTRACT FROM MAO SOI (*ANTIDESMA ACIDUM*), BIOFLAVONOID AND ALPHA-TOCOPHEROL ON OXIDATIVE STRESS IN CATS WITH CHRONIC KIDNEY DISEASE) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. สพ.ญ. ดร. รสมา ภู่สุนทรธรรม, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ. ดร. สัมพันธ์ วงศ์เสรีพิพัฒนา, 102 หน้า.

ทำการศึกษาเพื่อเปรียบเทียบผลของสารสกัดหยาบจากเม่าสร้อย ไบโอฟลาโวนอยด์ และแอลฟาโทโคเฟอรัลต่อภาวะเครียดออกซิเดชั่นในแมวที่เป็นโรคไตเรื้อรัง ในแมวจำนวน 34 ตัวที่เข้ารับการรักษาในโรงพยาบาลสัตว์เล็ก คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย แบ่งแมวออกเป็น 4 กลุ่ม คือ กลุ่มแมวสุขภาพดีเป็นกลุ่มควบคุม (n = 16) แมวป่วยโรคไตเรื้อรัง (n = 18) ที่มีค่าครีเอทินีนระหว่าง 2.8 – 5.0 มิลลิกรัมต่อเดซิลิตรและมีค่ายูเรียในกระแสเลือดมากกว่า 35 มิลลิกรัมต่อเดซิลิตร จากนั้นทำการสุ่มแบ่งแมวป่วยด้วยโรคไตเรื้อรังออกเป็น 3 กลุ่มย่อย คือ กลุ่มแมวป่วยที่ได้รับสารสกัดหยาบเม่าสร้อยในขนาด 120 mg/kg โดยการกินวันละครั้ง (n = 6) กลุ่มแมวป่วยที่ได้รับไบโอฟลาโวนอยด์ขนาด 10 mg/ตัว วันละครั้ง (n = 5) และกลุ่มแมวป่วยที่ได้รับแอลฟาโทโคเฟอรัลขนาด 800 IU/ตัว วันละครั้ง (n = 7) เก็บตัวอย่างเลือดเพื่อตรวจหาค่าโลหิตวิทยา ค่าเคมีคลินิก และค่าภาวะเครียดออกซิเดชั่นประกอบด้วยค่า glutathione (GSH), oxidized glutathione (GSSG), glutathione peroxidase (GPx) และ GSH/GSSG ratio ในวันที่ 0 สำหรับแมวสุขภาพดี และวันที่ 0, 14, 28, 42 และ 56 สำหรับแมวป่วยด้วยโรคไตเรื้อรัง ผลการศึกษาพบว่าแมวป่วยด้วยโรคไตเรื้อรังมีระดับค่าภาวะเครียดออกซิเดชั่นมากกว่าแมวสุขภาพดีอย่างมีนัยสำคัญทางสถิติ และสารสกัดหยาบจากเม่าสร้อยสามารถลดระดับค่าครีเอทินีนและเพิ่มค่า GPx ในแมวที่ป่วยในวันที่ 56 ของการศึกษาแต่เพิ่มค่า GSSG มากกว่าไบโอฟลาโวนอยด์และแอลฟาโทโคเฟอรัลในวันที่ 42 ของการศึกษา ในขณะที่แมวที่ได้รับไบโอฟลาโวนอยด์มีระดับค่า GSH/GSSG ratio ในวันที่ 14 ของการศึกษาสูงกว่าทุกกลุ่ม แต่ไม่สามารถลดค่าครีเอทินีนได้ กลไกในการที่สารสกัดหยาบจากเม่าสร้อยสามารถลดค่าครีเอทินีนต้องมีการศึกษาต่อไป

ภาควิชา.....อายุรศาสตร์.....ลายมือชื่อนิสิต.....

สาขาวิชา...อายุรศาสตร์สัตวแพทย์..ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา...2553.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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KEYWORDS : *ANTIDESMA ACIDUM* / BIOFLAVONOID / ALPHA-TOCOPHEROL / CHRONIC KIDNEY DISEASE / OXIDATIVE STRESS / CATS

KAKANANG JAIMUN: THE COMPARATIVE STUDY OF THE EFFECTS OF CRUDE EXTRACT FROM MAO SOI (*ANTIDESMA ACIDUM*), BIOFLAVONOID AND ALPHA-TOCOPHEROL ON OXIDATIVE STRESS IN CATS WITH CHRONIC KIDNEY DISEASE. THESIS ADVISOR : ASSOC. PROF. ROSAMA PUSOONTHORNTHUM, Ph.D., THESIS CO-ADVISOR : ASSOC. PROF. SUMPHAN WONGSERIPIPATANA, Ph.D., 102 pp.

The purpose of this study was to compare the effects of crude extract from *Antidesma acidum*, bioflavonoid and α-tocopherol on oxidative stress in cats with naturally occurring CKD. Thirty-four cats presented to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University were studied. They were assigned into four groups: the clinically normal (n = 16) and the CKD cats (n = 18) with increase level of creatinine concentrations (2.8 – 5.0 mg/dl) and blood urea nitrogen (BUN) (>35 mg/dl). The CKD cats were randomly divided into three subgroups including cats received *Antidesma acidum* 120 mg/kg orally once a day (n = 6), bioflavonoid 10 mg/cat orally once a day (n = 5) and α-tocopherol 800 IU/cat orally once a day (n = 7). Blood collection were performed to measure complete blood count, blood chemistry and the assays of oxidative stress parameter including glutathione (GSH), oxidized glutathione (GSSG) and glutathione peroxidase (GPx) on day 0 for the clinically normal cats and on day 0, 14, 28, 42, 56 for the CKD cats. The cats with CKD had significantly increased levels of oxidative stress parameter in plasma than the clinically normal cats. The crude extract from *Antidesma acidum* had significantly decreased the creatinine levels and increased GPx levels in cats with CKD on day 56, but increased GSSG levels more than oral bioflavonoid and α-tocopherol on day 42. Whereas the bioflavonoid had significantly increased GSH/GSSG ratio on day 14 and did not decrease creatinine levels. Further research is needed to study the exact mechanism of how *Antidesma acidum* decreased creatinine levels in the CKD cats.

Department of....Veterinary Medicine..... Student's Signature .....

Field of Study :....Veterinary Medicine..... Advisor's Signature .....

Academic Year :...2010..... Co-Advisor's Signature .....

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CHAPTER I
INTRODUCTION

Chronic kidney disease (CKD) is one of the most common diseases in cats. In the United States, the prevalence of feline kidney disease was 1.6% in general cat population (Lund et al., 1999). In Australia, it was found in 20% of the sick cats presented to the veterinary hospitals (Watson, 2001). In Thailand, the proportional morbidity ratio of cats with Chronic Renal failure (CRF) was 6 CRF cats per 1000 cats visited to hospitals (Pusoonthornthum and Pusoonthornthum, 2004). It was commonly found in older cats. One study in United Kingdom had reported the mean age of 80 cats with CKD was 12.6 years (Elliot and Barber, 1998). In Germany, a study of 676 domestic cats with CKD showed that the aging feline kidney (older than 108 months) displayed progressive tubular deletion and peritubular interstitial fibrosis (Dennis et al., 2006). Several previous studies had proposed that CKD in cats may be related to a high production of the free radicals (Snow, 1962; Christopher, 1989; Christopher et al., 1990; Webb et al., 2006). Cats are known to be sensitive to the oxidative injury (Webb et al 2006) because their hemoglobin molecule contains 8 - 10 reactive sulfhydryl groups (Snow, 1962; Harvey and Kaneko, 1976; Christopher, 1989; Christopher et al., 1990).

Antidesma acidum is a fruit-bearing deciduous shrub or small tree growing to between 2 – 5 meters tall. The fruit is round with dark red color. The crude extract from *Antidesma acidum* is rich of flavonoid, phenolic compounds, anthocyanin, tannins, B-sitosterol and stigmasterol (Thamaree et al., 2003). The trunk of *Antidesma acidum* extraction increased lymphocytes, band cells and alkaline phosphatase but decreased the level of aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen and creatinine in eight healthy cats (Pusoonthornthum et al., 2010[b]).

Flavonoids are substance that is abundant in vegetables, fruits and plant-derived beverages such as wine and tea. It has been used as an anticancer, antioxidant, anti-aging, antibacterial activities (Terao et al., 1994; Bos et al., 1996; Guo et al., 1999; Meng and Wang, 2001). Flavonoids inhibit the oxidation chain initiation and

prevent chain propagation for protecting the body against reactive oxygen species. Moreover, phenolic hydroxyls in natural flavonoids can effectively scavenge in hydroxyl radical-scavenging effects (Chen et al., 2002).

Alpha-tocopherols (α-tocopherols), a lipid-soluble antioxidant, are widely used as a vitamin supplementation and an antioxidant in food (Constantinides et al., 2006). It is abundant in vegetable oils and nuts. It has many beneficial effects on human health, such as preventing cardiovascular diseases, cancers and cataracts (Knekt, 1994; Muller, 1994; Diplock, 1996; Morrissey and Sheehy, 1999; Richard and Roussel, 1999).

It is unknown how oxidative stress plays a role in the cats with naturally occurring chronic kidney disease. Alpha-tocopheral and bioflavonoid has been used as antioxidant in commercial pet food in cats with chronic kidney disease for many years so whether *Antidesma acidum* has the same beneficial effects in cats with chronic kidney disease remain to be investigated.

Objectives of the study

To compare the effects of crude extract from *Antidesma acidum*, bioflavonoid and α-tocopherol on oxidative stress in the cats with naturally occurring CKD.

Hypothesis

Crude extract from *Antidesma acidum* can reduce oxidative stress in cats with naturally occurring CKD more than oral bioflavonoid and α-tocopherol.

Keywords : oxidative stress, *Antidesma acidum*, bioflavonoid, α-tocopherol, chronic kidney disease, cats

Advantages of the study

1. To promote the use of local Thai herb as an alternative treatment for cats with spontaneous CKD which is one of the most common disease in geriatric cats.
2. To be able to improve the quality of life in cats with CKD.
3. To apply the use of natural Thai herb for the medical treatment in human.

CHAPTER II
LITERATURE REVIEW

Feline chronic kidney disease

Chronic kidney disease (CKD) is the most common disease in geriatric cats. According to the International Renal Interest Society (IRIS), there are four stages of CKD consisting of stage I (non-azotemic; creatinine < 1.6 mg/dL), stage II (mild renal azotemia; creatinine 1.6 – 2.8 mg/dL), stage III (moderate renal azotemia; creatinine 2.8 – 5.0 mg/dL), and stage IV (severe renal azotemia; creatinine > 5.0 mg/dL) (Brown, 2004). Chronic Renal failure (CRF) is defined as an irreversible, declined in renal function and decreased glomerular filtration rate (GFR) of more than 50% for at least 3 months duration, resulting in the reduction of the renal excretion and causing homeostasis disorder (Polzin et al., 2005).

In the United States, the prevalence of feline kidney disease was 1.6% in general cat population (Lund et al., 1999). In Australia, it was found 20% of cats presented to the veterinary hospitals (Watson, 2001). In Thailand, the proportional morbidity ratio of cats with chronic renal failure (CRF) was 6 CRF cats per 1000 cats (Pusoonthornthum and Pusoonthornthum, 2004). It was commonly found in older cats. In one previous study, 53% of 74 cats with CKD were over 7 years old and cats' age ranged between 9 months to 22 years (DiBartola et al., 1987). The study of age distribution in cats with CRF were found to be less than 10 years old (37%), between 10 to 15 years old (31%) and older than 15 years of age (32%) (Lulich et al., 1992). During 1998, one study indicated that the mean age of 80 cats with CKD was 12.6 years (Elliot and Barber, 1998). In Australia, study of 184 feline with CRF indicated that male with CRF had median age of 12 years, whereas female with CRF had median age of 15 years (White et al., 2006).

The common clinical signs of CKD in cats are weight loss, polyuria and polydipsia, poor body condition, dehydration, nonregenerative anemia, small and irregular kidneys. Metabolic acidosis in cats with CKD was found in 60 to 80% of

patients (DiBartola et al., 1987; Lulich et al., 1992). Metabolic acidosis promotes clinical signs including anorexia, nausea, vomiting, lethargy, weakness, muscle wasting and weight loss. Severe metabolic acidosis is occurred when blood pH values are below 7.20, resulting in reduction of cardiac output, arterial pressure, and hepatic and renal blood flows (Adrogué and Madias, 1998).

The major etiology of feline chronic renal failure is tubulointerstitial nephritis (Polzin et al., 2005). According to the results of renal biopsies, 70% of cats with CKD were caused by tubulointerstitial nephritis, 15% were glomerulonephropathy, 11% were lymphoma and 2% were amyloidosis (Minkus et al., 1994). In one study of 676 adult domestic cats, the results showed that the aging feline kidney (older than 108 months) displayed progressive tubular deletion and peritubular interstitial fibrosis (Dennis et al., 2006). Other causes of cats with CKD are glomerulonephritis, amyloidosis, tubulonephrosis and incomplete recovery of acute renal failure (ARF) (Polzin et al., 2005).

Oxidative stress

Oxidative stress is caused by an imbalance between reactive oxygen and antioxidant mechanism resulting in tissue damage (Sies, 1997). Mitochondria use up to 5% of the oxygen for energy production (ATP) that produces reactive oxygen species (ROS) as by-products (Gutteridge and Halliwell, 1994). The mitochondria produces ROS of mitochondrial oxidation including superoxide anion ($O_2^{-}\cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\cdot$) (Fig.1), resulting in mitochondrial DNA damage and aging (Schriner et al., 2005) (Fig.2). Oxygen-derived free radicals or ROS are unstable and highly reactive molecules that contain unpaired electrons. The ROS with unpaired electrons attempt to pair all their electrons from one atom or molecule to another.

$O_2 + e^- \longrightarrow O_2^{-}\cdot$ Superoxide radical

$2O_2^{-}\cdot + 4H^+ \longrightarrow 2H_2O_2$ Hydrogen peroxide

$H_2O_2 \longrightarrow OH^- + OH\cdot$ Hydroxyl radical

Fig.1 Reactive oxygen species (ROS)

(applied from www.tcd.ie/tsmj)

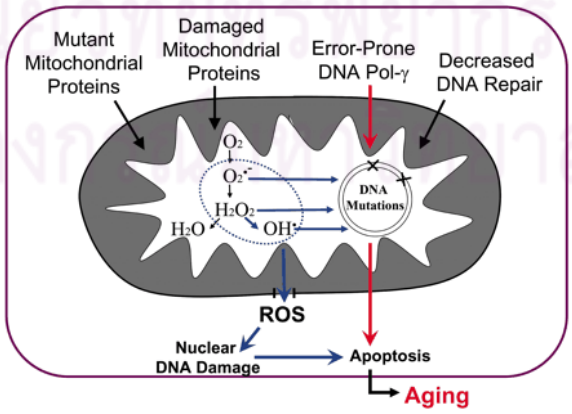


Fig.2 Mitochondrial DNA damage and aging

(Loeb et al., 2005)

Oxidative effects of ROS are controlled by endogenous enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) (Blokhina et al. 2003) (Fig.3) and non-enzymatic antioxidants (reduced glutathione, vitamins C and E) (Sies and Stahl, 1995).

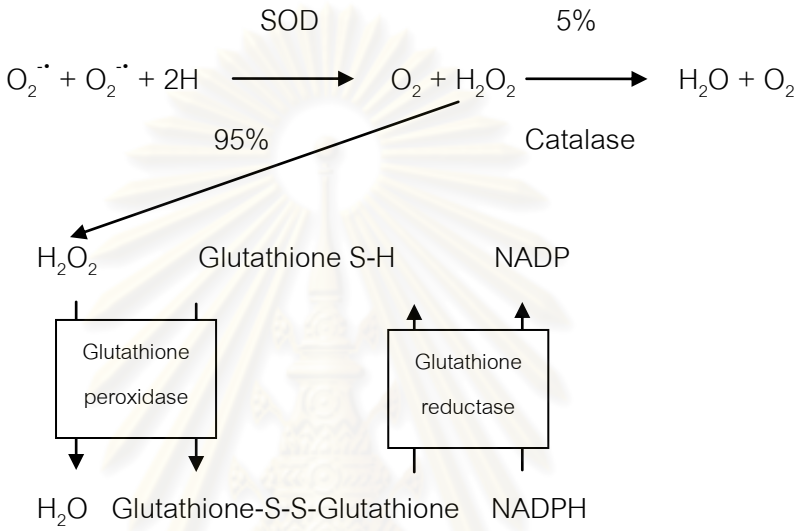


Fig.3 Endogenous enzymatic antioxidants

(applied from www.tcd.ie/tsmj)

Oxidative stress in cat

Cats are sensitive to oxidative injury (Webb et al., 2006). Hemoglobin molecule of cats contains 8 - 10 reactive sulfhydryl groups, whereas dogs and other species contain four reactive sulfhydryl groups (Snow, 1962; Harvey and Kaneko, 1976; Christopher, 1989; Christopher et al., 1990). An increased number of sulfhydryl groups increases the susceptibility to the oxidative damage. In addition, cat's Heinz bodies are susceptibility to the oxidant damage and cat has relatively inefficient Heinz body removal (Blue and Weiss, 1981; Christopher et al., 1990).

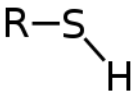


Fig.4 General chemical structure of the sulfhydryl group
(Patai, 1974)

Study of twenty cats with diabetes mellitus (DM) indicated significantly less plasma superoxide dismutase (SOD) levels than the control cats, resulting in the decreased in the reaction that change free radical to hydrogen peroxide (Webb and Falkowski, 2009). Increased free radical production on markers of oxidative DNA damage (8-OHdG) has also been reported in cats with renal insufficiency (Yu and Paetau-Robinson, 2006).

Oxidative stress and chronic kidney disease

The kidney is a site of 10% of body oxygen consumption which produces ROS as by-products. Several studies suggested that chronic renal failure may be related to the high production of free radicals (Snow, 1962; Christopher, 1989; Christopher et al., 1990; Webb et al., 2006). The role of oxidative stress and the mechanism of CKD were suggested to be due to the lack of antioxidant defenses (Fryer, 1997), lipid peroxidation (Ozaki et al., 1999) and increased ROS generation (Handelman et al., 2001). ROS may activate the secretion of inflammatory molecules, giving raise to the inflammatory response. The inflammatory glomerular damages caused by ROS and the possible interference by antioxidants were shown in Fig.5. From glomerular barrier impairment, macromolecules can appear in the urinary space because of the loss of glomerular permeability. Thus, renal tubular epithelium could be exposed to injurious chemical species. The accumulation of injurious chemical species within the interstitial space of the renal cortex plays a pathogenic role in the development of tubular injury and interstitial fibrosis in progressive chronic renal diseases. The role of ROS in the

production of tubulointerstitial damage was shown in Fig.6. Angiotensin II has been postulated to stimulates oxidative stress (Hannken et al., 1998) which resulting in renal endothelial dysfunction, generation of hypertension and cause progression of renal damage (Agarwal et al., 2004). Increased oxidative stress also contributed to the progression stages of CKD (Dounousi et al., 2006) and uremia toxicity in human patients (Premystaw et al., 2006).

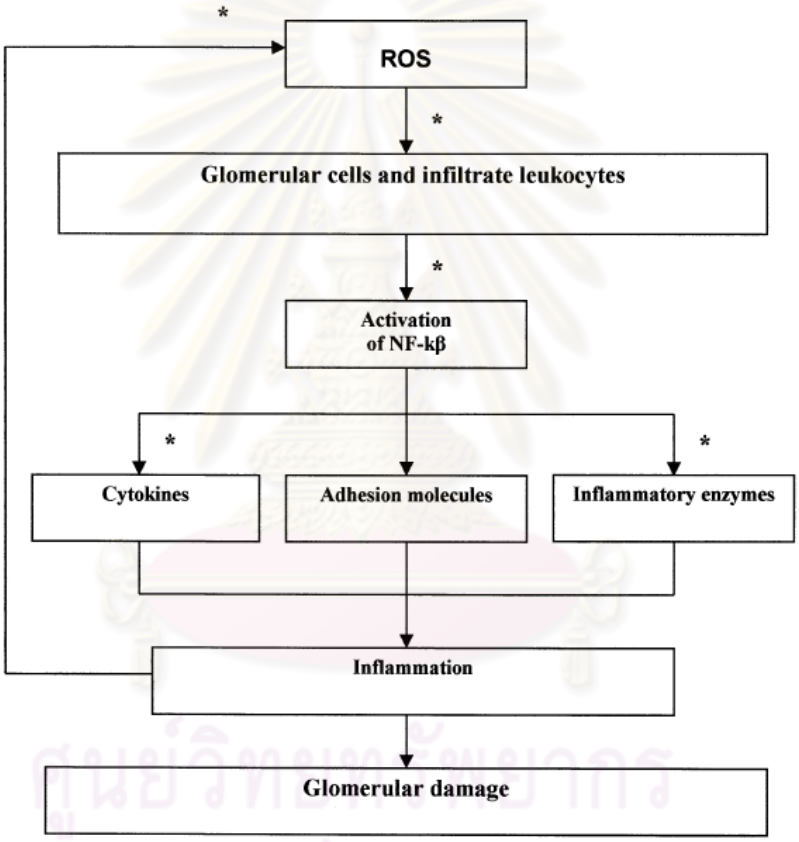


Fig.5 Oxidative stress and the mechanism of glomerular damage.
NF-kB = Nuclear factor-kB; ROS = Reactive oxygen species.
(Rodrigo and Rivera, 2002)

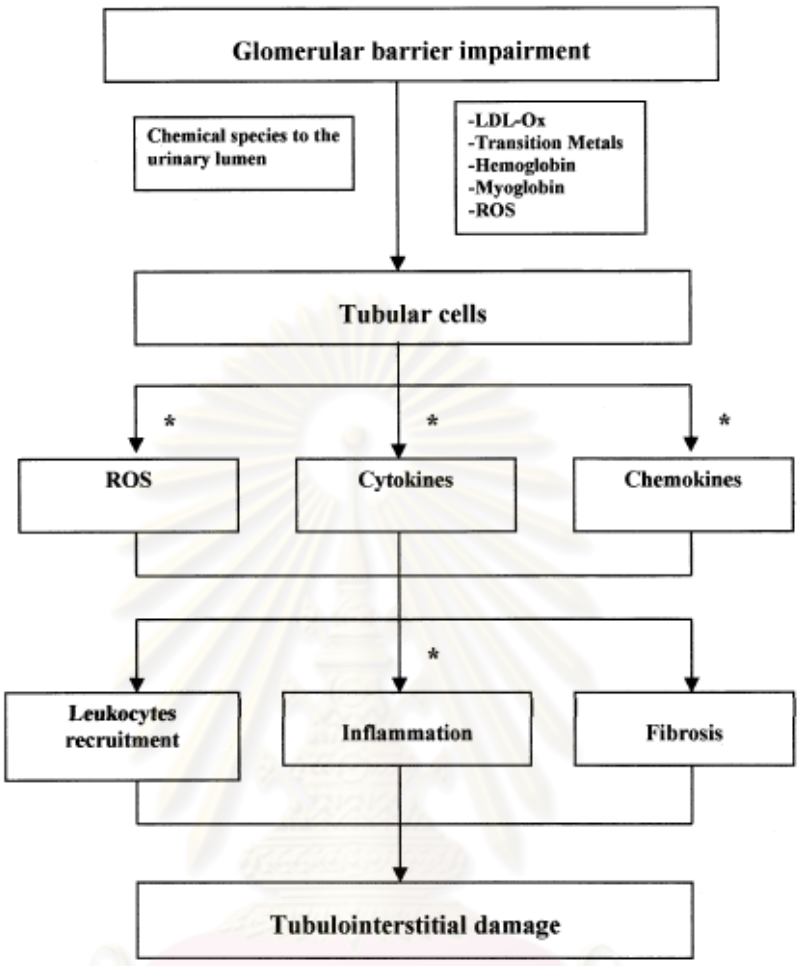


Fig.6 Tubulointerstitial damage caused by oxidative stress. LDL = low-density lipoprotein; LDL-ox = oxidized low-density lipoprotein; ROS = Reactive oxygen species.
(Rodrigo and Rivera, 2002)

Several studies demonstrated that oxidative stress may promote the progression of chronic renal failure in human patient (Zwolinska et al., 2006; Valentini et al., 2007; Hamid et al., 2009). Children with CRF was found to have the low levels of plasma vitamin A, E and C which might be responsible for the increased of oxidative stress in the body (Zwolinska et al., 2006). Patients affected by end-stage renal disease (ESRD) have enhanced oxidative stress, as a result of a reduction in antioxidant systems and increased pro-oxidant activity, especially in advanced age, resulting in chronic

inflammatory state and uremic syndrome (Francesco et al., 2003). The significance higher level of advanced glycation end products (AGEs) in diabetic renal injury patients is related to oxidative stress (Toshio and Takashi, 2008). Chronic renal failure patients with long time hemodialysis treatment have increased level of plasma malondialdehyde (MDA) and blood α-aminolevulinate dehydratase (ALA-D) reactivation, which are the biomarkers to evaluate chronic oxidative stress (Valentini et al., 2007). Patients with CKD have the reductions of plasma glutathione peroxidase (GPx) activity, plasma GPx concentration and HDL-cholesterol when compared to normal age-matched. It was found that antioxidant activity of HDL were associated with CKD and contributed to atherosclerosis (Hamid et al., 2009). One previous study also demonstrated that oxidative stress may promote the progression of chronic renal failure in other species (Baud and Ardaillou, 1993). In experimental immune glomerulonephritis in rats, reactive oxygen species (ROS) promoted a reduction in the glomerular blood flow and the glomerular filtration rate, resulting in morphologic lesions and mediated renal failure (Baud and Ardaillou, 1993).

Glutathione

The reduced glutathione (GSH) is a tripeptide including glycine, cysteine and glutamine. It is abundant in erythrocytes, liver, spleen, kidney, leukocytes and cells of the ocular lens (Pastore et al., 2003). Hepatocytes are a major biosynthesis and export of GSH (Fig.7). The specific intracellular compartments of GSH delivery are mitochondria, endoplasmic reticulum and extracellular spaces, including blood plasma, epithelial lining fluids, and exocrine secretions.

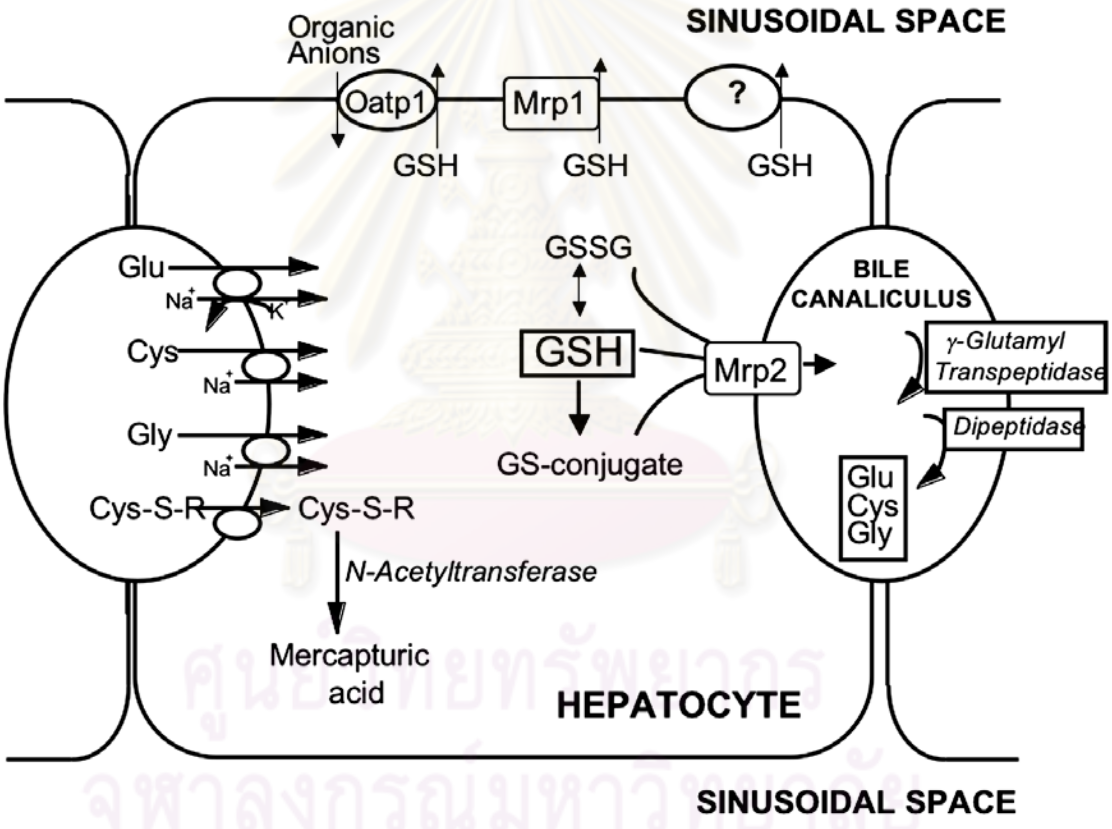


Fig.7 Glutathione homeostasis in hepatocytes.

(Ballatori et al., 2009)

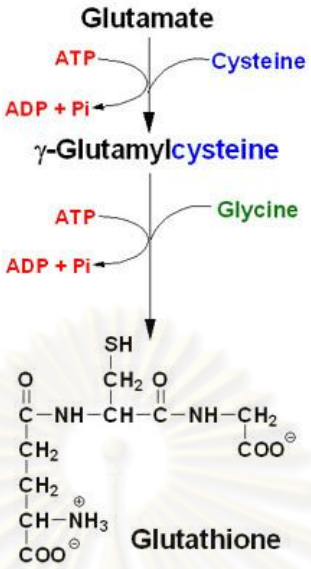


Fig.8 Synthesis of glutathione (GSH)

During severe oxidative stress, the concentration of GSH may decrease and the concentration of oxidized glutathione (GSSG) may increase in the affected cells (Sakhi et al., 2006). GSH and GSSG concentrations in plasma have been considered to be essential as an index of whole body glutathione and possibly a useful indicator of disease risk (Jones et al., 2000).

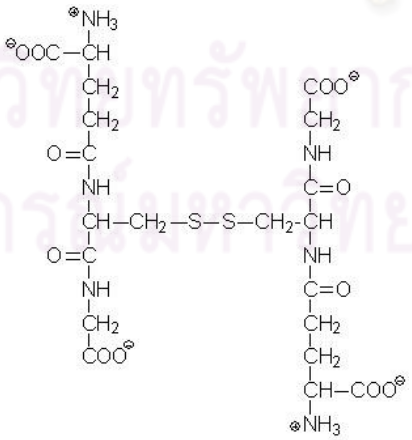


Fig.9 Structure of oxidized glutathione (GSSG)

Antidesma acidum

Antidesma acidum is a fruit-bearing deciduous shrub or small tree growing to between 5-10 meters tall. The fruit is round with dark red color (Kwansang, 2003). In the Northern Hemisphere, the ripe fruit is typically in season from March to May. *Antidesma acidum* has been consumed leaves and fruits in Thailand, India and Indonesia.

Fig.10 *Antidesma acidum*

The crude extract from *Antidesma acidum* is rich of flavonoid, phenolic compounds, anthocyanin, tannins, B-sitosterol and stigmasterol (Thamaree et al., 2003). In Mauritius, leaves of *Antidesma acidum* are a traditional medicine, used for dizziness, fever and nausea. Studies of an antibacterial, antifungal and antiHIV activities in five Thai herbs (*Antidesma acidum, Andrographis paniculata* Wall. ex Nees., *Cyperus*

rotundus Linn., *Alternanthera bettzickiana* (Regel) Nichols. and *Lonicera japonica* Thunb.) by Kuman-Pawa et al. (2003) provided that the *Antidesma acidum*, *Cyperus rotundus* Linn. and *Lonicera japonica* Thunb. are effectively stimulant of the immune system and control replication of HIV in patient. The trunk of *Antidesma acidum* extraction increased lymphocytes, band cells and alkaline phosphatase but decreased the level of aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen and creatinine in 8 healthy cats (Pusoonthornthum et al., 2010[b]).

Bioflavonoid

Flavonoids are a large class of naturally occurring compounds widely present in the green plant (Fig.12) (Harborne and Williams, 2000). It is abundant in vegetables, fruits and plant-derived beverages such as wine and tea and can be classified into several subclasses including flavones, flavonols, flavonones, flavanols, isoflavones and chalcones.

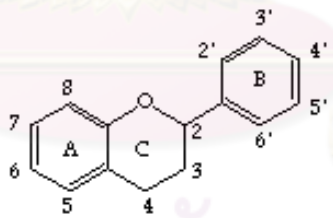


Fig.11 Basic flavonoid structure

(Chen et al., 2002)

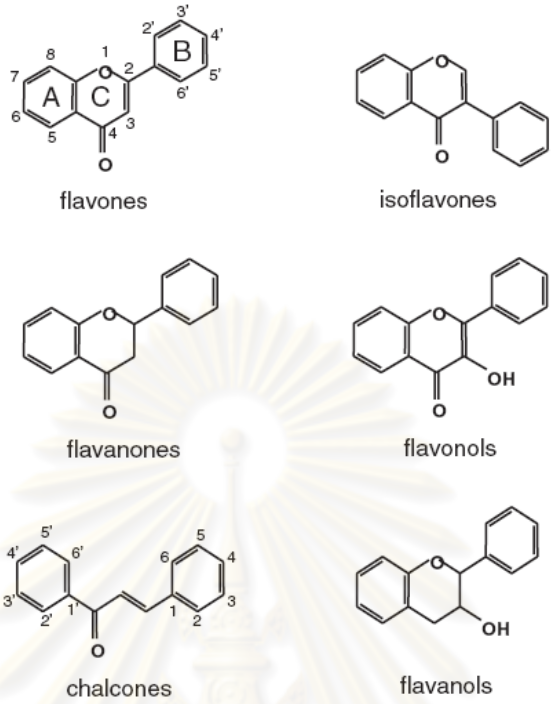


Fig.12 Structures of the major classes of flavonoids
(Morris and Zhang, 2006)

Flavonoids have been used as anticancer, antioxidant, anti-aging, antibacterial activities (Terao et al., 1994; Bos et al., 1996; Guo et al., 1999; Meng and Wang, 2001). Flavonoids inhibit the oxidation chain initiation and prevent chain propagation for protecting the body against reactive oxygen species. Moreover, phenolic hydroxyls in natural flavonoids (quercetin, heliosin, hyperoside, kaempferol, baicalin, corylifolin, lysionotin, matteucinol, corylifolinin and genistein) can effectively scavenge in hydroxyl radical-scavenging effects especially, hydroxyl groups in ring A and B (Fig.11) (Chen et al., 2002). Studies in cultured canine renal tubular cells (in vitro) and 40 male rats (in vivo) by Hyoung et al. (2007) provided the information that bioflavonoid quercetin significantly decreased the concentration of MDA, an indicator of lipid peroxidation, in canine cultured cells and inhibit renal crystal deposition in renal tissue of male rat. In rats, the dietary quercetin might protect oxidative stress and nephrotoxicity from the administration of cadmium (Renugadevi and Milton, 2010) and lead (Liu et al., 2010[a]).

Studied in cultured feline esophageal epithelial cells that quercetin-3-O-ß-D-glucurunopyranoside (QGC) could induce heme oxygenase-1 (HO-1) which was the cytoprotective enzymes in the response to oxidative stress (Kim et al., 2009).

Alpha-tocopherol

Alpha-tocopherol (α-tocopherol) or vitamin E, a lipid-soluble antioxidant, is widely used as vitamin supplementation and as an antioxidant in food (Fig.13) (Constantinides et al., 2006). It is abundant in the vegetable oils and nuts.

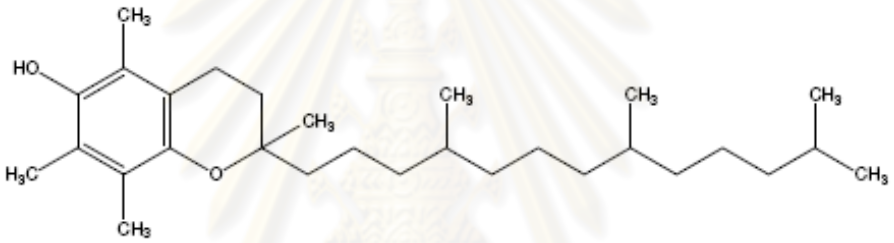


Fig.13 The structure of α-tocopherol

(Wu and Croft, 2007)

The antioxidant ability of α-tocopherol is the protection of cell membranes from lipid peroxidation chain reaction (Herrera and Barbas, 2001; Traber and Atkinson, 2007) by donating the hydrogen of hydroxyl group on C-6 (Fig.14).

Vitamin E has been known to have many beneficial in human health such as preventing cardiovascular diseases, cancers and cataracts (Knekt, 1994; Muller, 1994; Diplock, 1996; Morrissey and Sheehy, 1999; Richard and Roussel, 1999). Alpha-tocopherol could decrease oxidative stress in CKD patient by inhibition of lipid peroxidation chain reaction that stimulant hypertension. Patients with hypertension were lower glutathione peroxidase and GSH/GSSG ratio than the healthy normotensive subjects (Rodrigo et al., 2007). Increased oxidative stress might be involved in the hypertension (Rodrigo et al., 2007). Vitamin E was used to control the oxidative stress in

chronic renal failure patients (Clermont et al., 2001). Vitamins C and E can significantly reduce mitochondrial damage generated following syncytialisation in cultured human term placenta trophoblast (Tannetta et al., 2008). In rats with CRF, the plasma and kidney malondialdehyde concentrations were decreased by the dietary supplementation with vitamin E 200 IU/kg (Hahn et al., 1998). In addition, Vitamin E and C and ß-carotene supplements can reduce oxidative stress in cats with renal insufficiency (Yu and Paetau-Robinson, 2006).

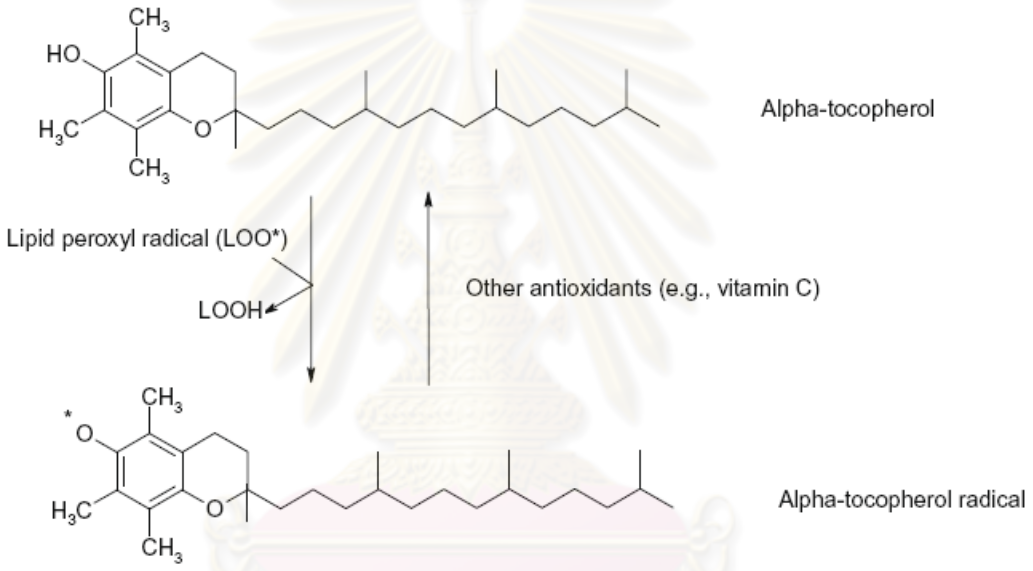


Fig.14 Antioxidant function of α-tocopherol
(Nakamura and Omaye, 2009)

Oxidative stress may be one of the causes of the cats with CKD by the reductions of antioxidants activity.

The purpose of this study is to compare the effects of the crude extract of *Antidesma acidum*, bioflavonoid and α-tocopherol on oxidative stress in the cats with naturally occurring CKD.

CHAPTER III
MATERIALS AND METHODS

1. Animals

1.1 The clinically normal cats

Sixteen clinically normal client-owned cats presented to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University and veterinary hospitals in Bangkok Metropolitan area with normal physical examination, hematology and blood chemistry results with serum creatinine of less than 1.6 mg/dl were included. Cats were included without breed, age and gender preference.

1.2 Cats with CKD

Eighteen cats presented to the Small Animal Hospital, Chulalongkorn University and veterinary hospitals in Bangkok Metropolitan area with an increase levels of creatinine concentration (2.8 – 5.0 mg/dl) and blood urea nitrogen (BUN) (>35 mg/dl), decrease urine specific gravity (USG) of less than 1.030 (Bettina et al., 2007) with clinical signs including: weight loss, polyuria, polydipsia, poor body of condition, dehydration, nonregenerative anemia and/or small, irregular kidneys were studied. Cats were included without breed, age and gender preference.

The cats with CKD were assigned into three subgroups: *Antidesma acidum* subgroup given the crude extract from *Antidesma acidum* 120 mg/kg/day, bioflavonoid subgroup given bioflavonoid 10 mg/cat/day (Allison et al., 2000) and α-tocopherol subgroup given α-tocopherol 800 IU/cat/day (Yu and Paetau-Robinson, 2006).

All cats with CKD were treated with the conservative medical treatment including fluid therapy and feed with the prescription diet for renal disease as calculation from daily requirement. Cats that had previously been treated with other drugs for specific or supportive therapy for urinary tract diseases were excluded.

The protocol was approved by the Ethic Committee for the human and/or animal experimentation from Faculty of Veterinary Science, Chulalongkorn University

No.60931031. The owners were allowed their cats to withdraw if the clinical signs worsen.

2. Materials

Antidesma acidum was extracted according to Kwansang, 2003. Bioflavonoid was purchased from Sigma-Aldrich, Inc. Bioflavonoid in this study was quercetin, which is a member of the class of flavonoids called flavonoles. Quercetin is considered as the most powerful flavonoids for protecting the body against reactive oxygen species which is produced during the normal oxygen metabolism of mitochondria or is induced by exogenous damage (De, 1994; Grace, 1994). The α-tocopherol was purchased from Mega Lifesciences Ltd.

3. Crude extract from Antidesma acidum

3.1 Extraction of crude extract from Antidesma acidum

Antidesma acidum was extracted according to Kwansang, 2003. The bark and trunk of *Antidesma acidum* used in this study were dried at less than 50ºC for 5 days and grind. The grinding *Antidesma acidum* was fermented with 95% ethanol for one week and extracted with 95% ethanol. The combined extract was evaporated to obtain the crude extract. The crude extract of *Antidesma acidum* was prepared in the tablets form (120 mg/tablet).

4. Study design

Blood collection from the CKD and the clinically normal cats were performed to measure complete blood count (CBC), blood chemistry (ALP, ALT, BUN, creatinine) and the assays of oxidative stress parameter (glutathione (GSH), oxidized glutathione (GSSG) and glutathione peroxidase (GPx)). Blood samples were collected on week 0 in the clinically normal cats and on day 0, 14, 28, 42, 56 for the CKD cats. Urine was collected by catheterization, cystocentesis and/or voiding of midstream. The urine specific gravity (USG) was determined by refractometer. The chemical urinalysis was

measured using a commercial dipstick and examined by microscopic for the findings of casts, crystals, epithelial cells, red blood cells (RBCs) and white blood cells (WBCs).

The crude extract of *Antidesma acidum* 120 mg/kg/day, bioflavonoid 10 mg/cat/day (Allison et al., 2000) and α-tocopherol 800 IU/cat/day (Yu and Paetau-Robinson, 2006) were given to the CKD cats in the *Antidesma acidum* group, bioflavonoid group and α-tocopherol group, respectively, for 56 days (Fig.15).

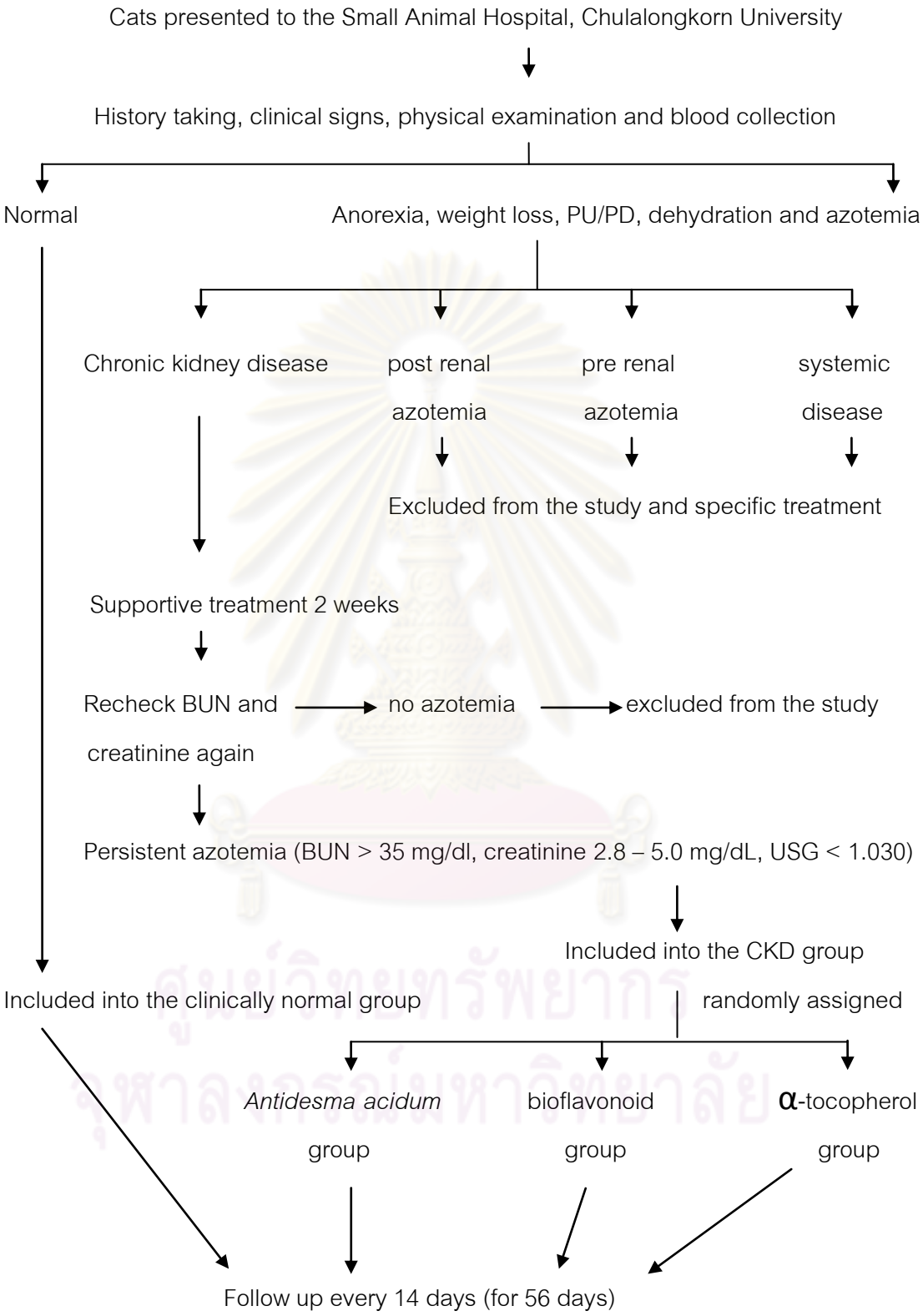


Fig.15 The diagram of the study design of the project

5. Laboratory examination

5.1 Blood collection

Blood collection from cephalic or femoral vein was performed. Blood samples were divided into an ethylenediaminetetraacetic acid (EDTA)-coated tube for analysis of CBC, GPx, GSH and GSSG and heparin-coated tube for blood chemistry. Blood sample for oxidative stress parameters was immediately centrifuged at 4 ºC and plasma stored at – 80 ºC for further analysis.

5.2 Complete blood count (CBC)

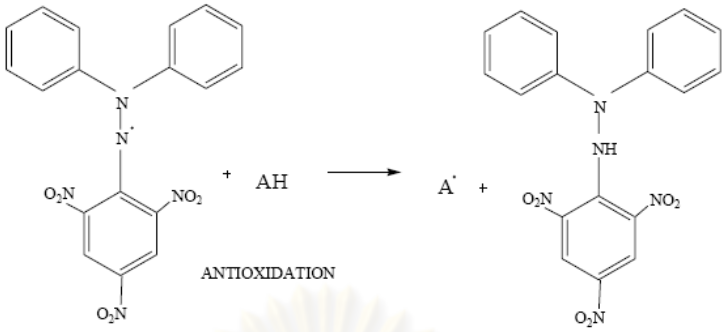
Hematocrit, white blood cell (WBC), red blood cell (RBC) and platelet counts were obtained by use of manual blood count.

5.3 Blood chemistry

Blood urea nitrogen (BUN) and creatinine were determined by using enzymatic (urease) (Patton and Crouch, 1977) and Alkaline Picrate-end Point Reaction method (Reitman and Frankel, 1957), respectively.

5.4 Antioxidant assay

Antioxidant assay was done according to Molyneux, 2004 and Denrungruang, 2007. The free radical scavenging capacity of the crude extract from *Antidesma acidum* and bioflavonoid was analyzed by the DPPH assay that is used to monitor the free radical scavenging abilities of antioxidant. The DPPH (diphenylpicrylhydrazyl) radical had a deep violet color due to its impaired electron, and radical scavenging could be followed spectrophotometrically by the loss of absorbance at 515 nm, as the pale yellow non-radical form was produced (reaction 1).



(reaction 1)

DPPH (violet) DPPH:H (pale yellow)

6 x 10^{-5} M of DPPH standard was prepared by absolute ethanol. The concentration of standard solution (3,5-di-tert-butyl-4-hydroxytol (BHT)) is 0, 0.25, 0.5 and 1.0 mg/100 ml in absolute ethanol. The concentration of the test reagents (crude extract from *Antidesma acidum* and bioflavonoid) was 0.25, 0.5 and 1.0 mg/100 ml in absolute ethanol.

Table 1 The reaction mixture of the DPPH assay

	Control solution	Standard solution	Test reagents
6 x 10^{-5} M of DPPH (ml)	1	1	1
Absolute ethanol (ml)	1	-	-
BHT (at the concentration of 100, 50, 25, 12.5, 6.25, 3.125, 1.562 µg/ml) (ml)	-	1	-
Test reagents (at the concentration of 100, 50, 25, 12.5, 6.25, 3.125, 1.562 µg/ml) (ml)	-	-	1

The reaction mixture was allowed to stand for thirty minute in the dark at room temperature and measured by spectrophotometry at 515 nm. The free radical scavenging was calculated according to equation 1.

50% Effective concentration (EC_{50}) was the concentration of test reagent that decreased the concentration of DPPH to 50%

$$\% \text{ Radical scavenging} = \frac{(A_b - A_a)}{A_b} \times 100 \qquad \text{(equation 1)}$$

A_a = Absorbance value of mixture of the test reagents (crude extract from *Antidesma acidum* and bioflavonoid) and DPPH

A_b = Absorbance value of DPPH

5.5 Determination of oxidative stress parameter

5.5.1 Determination of glutathione peroxidase (GPx) (Tangphokhanon et al., 2005)

Table 2 The reaction mixture of the determination of GPx

	Control solution	Sample solution
Tris-HCl 1m, EDTA 5mM pH 8.0 (µl)	150	150
GSH, 0.1 M (µl)	30	30
Glutathione reductase, 10 U/ml (µl)	150	150
NADPH, 2 mM (µl)	150	150
1:20 hemolyzate (µl)	15	15
H_2O (µl)	1,005	990
t-Butyl hydroperoxide, 7 mM (µl)	-	15
* Approximately 1: 1000 dilution		

The reaction mixture was measured by spectrophotometry at 340 nm "A_1" and 1 minute later "A_2". The GPx was calculated according to equation 2.

$$\text{Glutathione peroxidase activity (nmol/min)} = \Delta A/\text{min} \times 161 \qquad \text{(equation 2)}$$

$\Delta A = A_1 - A_2$

5.5.2 Determination of glutathione (GSH)

GSH was determined by using an enzymatic (glyoxalase) (Tangphokhanon et al., 2005). Distilled water (1.6 ml) was added to 0.4 ml EDTA-coated blood sample. The mixture was precipitated by 3 ml metaphosphoric acid (precipitating solution), allowed to stand for five minute at room temperature and filtered through a Whatman No.1 filter paper. For the control solution contained of 2.2 ml buffer, 40 µl of 50 µg/ml glyoxalase I and 40 µl of 2.2 mM methylglycoxal. The sample solution was mixture of 2.0 ml buffer, 200 µl filtrate, 40 µl of 50 µg/ml glyoxalase I and 40 µl of 2.2 mM methylglycoxal. For determination of GSH, GSH is converted by glyoxalase I (reaction 2). The control and sample solution were measured by spectrophotometry at 240 nm.

$$\text{Methylglyoxal} + \text{GSH} \xrightarrow[\text{Glyoxalase I}]{} \text{S-Lactoglutathione} \qquad \text{(reaction 2)}$$

5.5.3 Determination of oxidized glutathione (GSSG)

For oxidized glutathione, 0.5 ml mixture of 10 mmol of N-ethylmaleimide (NEM), 17.5 mmol of disodium EDTA, and 100 mmol of potassium phosphate per liter (pH 6.5) was added to 0.5 ml of the blood sample. The mixture was centrifuged at 4 ºC and added 0.5 ml of the NEM-plasma solution with 1 ml of a 200 g/L trichloroacetic acid solution. And the excessive of NEM was removed by 5 ml of chloroform for at least five times. The NEM added to scavenge GSH and eliminate continued oxidation of GSH to GSSG. The concentration of GSSG was performed by enzymatic assay (Curello et al., 1987). The reaction mixture was measured by spectrophotometry at 340 nm. The enzyme glutathione reductase (GR) recycles GSSG to GSH with the simultaneous oxidation of nicotinamide adenine dinucleotide phosphate ($NADPH_2$) (reaction 3).

$$\text{GSSG} + \text{NADPH} + H^+ \xrightarrow[\text{Glutathione reductase}]{} 2\text{GSH} + NADP^+ \qquad \text{(reaction 3)}$$

6. Statistical analysis

Data were presented as mean ± SEM. Repeated Measures ANOVA was used to compare the laboratory results within groups of treatment. Means ± SEM between groups were compared by ANOVA. P-value of less than 0.05 was considered significant.

CHAPTER IV
RESULTS

Part I Radical scavenging activity and the effective concentration

The bark and trunk of *Antidesma acidum* (14.5 kg) used in this study were extracted to obtain the final crude extract of 132.20 g. The crude extract from *Antidesma acidum* was 0.91% of the total bark and trunk.

The percent (%) free radical scavenging capacity of Butylhydroxytoluene or 3,5-di-tert-butyl-4-hydroxytol (BHT), bioflavonoid and the crude extract of *Antidesma acidum* by the DPPH· assay at different concentration are were presented (Table 3; Fig.16). The percents radical scavenging of BHT, bioflavonoid and the crude extract of *Antidesma acidum* were associated with the increased concentration. At the concentration of 100 µg/ml, the percent (%) radical scavenging of the crude extract of *Antidesma acidum* was similar to bioflavonoid (Table 3).

Table 3. Percent (%) radical scavenging of Butylhydroxytoluene or 3,5-di-tert-butyl-4-hydroxytol (BHT), bioflavonoid and the crude extract of *Antidesma acidum* at various concentration.

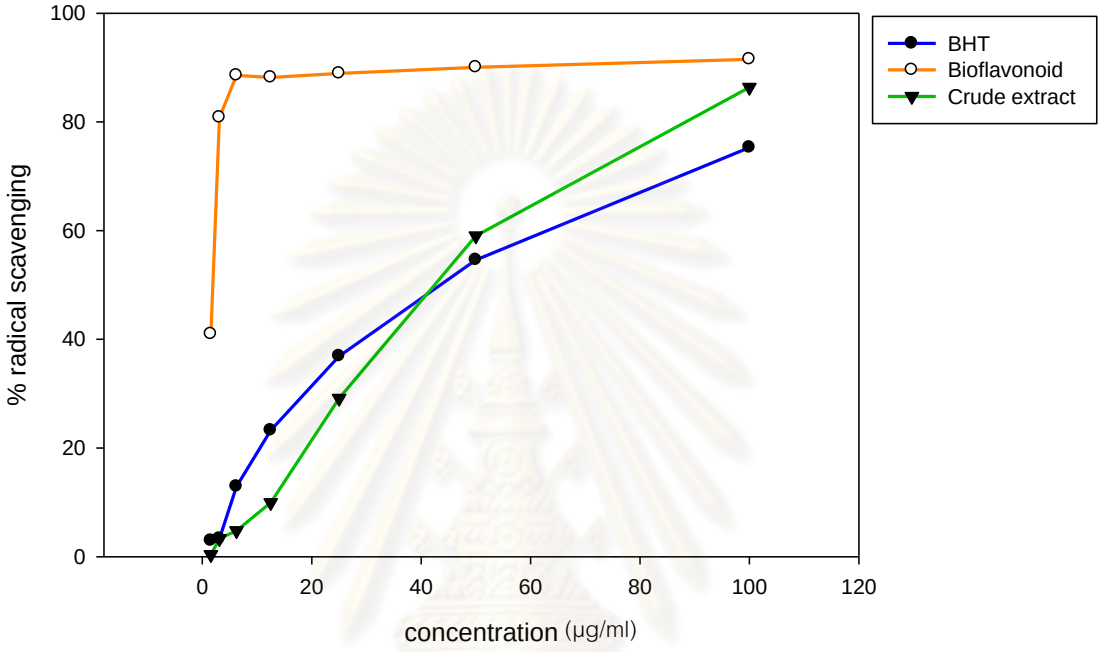
	Concentration (µg/ml)						
	100	50	25	12.5	6.25	3.125	1.562
BHT	75.277	54.613	36.900	23.247	12.915	3.321	2.952
Bioflavonoid	91.513	90.037	88.93	88.192	88.561	80.812	40.96
Extract	86.347	59.04	29.151	9.9630	4.7970	3.3210	0.369

BHT = 3,5-di-tert-butyl-4-hydroxytol or Butylhydroxytoluene.

Bioflavonoid = Quercetin hydrate

Extract = The crude extract of *Antidesma acidum*

Fig.16 Percent (%) radical scavenging concentration of Butylhydroxytoluene or 3,5-di-tert butyl-4-hydroxytol (BHT), bioflavonoid and the crude extract of *Antidesma acidum*



BHT = Butylhydroxytoluene or 3,5-di-tert-butyl-4-hydroxytol

Bioflavonoid = Quercetin hydrate

Crude extract = The crude extract of *Antidesma acidum*

The effective concentration (EC_{50}) of BHT, bioflavonoid and the crude extract of *Antidesma acidum* were presented (Table 4). The BHT, bioflavonoid and the crude extract of *Antidesma acidum* can decrease the concentration of DPPH to 50% at the concentration of 42.24, 2.19 and 42.21 µg/ml, respectively. Bioflavonoid was more effective than BHT and the crude extract of *Antidesma acidum* at approximately 19 times. The effective concentration (EC_{50}) of the crude extract of *Antidesma acidum* was similar to BHT.

Table 4. The effective concentration of substance which can decreased 50 percents of DPPH concentration (EC_{50}) of Butylhydroxytoluene or 3,5-di-tert butyl-4-hydroxytol (BHT), bioflavonoid and the crude extract of *Antidesma acidum*

Type of sample	EC_{50} (µg/ml)
BHT	42.24
Bioflavonoid	2.19
Crude extract	42.21

EC_{50} = Effective concentration of substance which can decreased 50 percents of DPPH concentration.

BHT = 3,5-di-tert-butyl-4-hydroxytol or Butylhydroxytoluene.

Bioflavonoid = Quercetin hydrate

Extract = The crude extract of *Antidesma acidum*

Part II **The comparative study of the effects of crude extract from *Antidesma acidum*., bioflavonoid and alpha-tocopherol on oxidative stress in cats with chronic kidney disease**

This part of the study was performed in sixteen clinically normal client-owned cats (6 males and 10 females) and eighteen cats with stable condition of spontaneous CKD (9 males and 9 females). Eighteen CKD cats were divided into 3 subgroups: the *Antidesma acidum* subgroup (6 cats), the bioflavonoid subgroup (5 cats) and the α-tocopherol subgroup (7 cats). The mean ± SEM age of the clinically normal cats, CKD cats in the *Antidesma acidum* subgroup, CKD cats in the bioflavonoid subgroup and the CKD cats in the α-tocopherol subgroup were 2.69 ± 0.67, 10.17 ± 1.54, 13.20 ± 0.97 and 9.86 ± 2.12 years old, respectively (Table 5). There was a significant difference in the age between the clinically normal cats and the CKD cats. The breeds of cats with CKD were domestic short hair (17/18; 94.44%) and Siamese-mixed breed cats (1/18; 5.56%). The clinically normal cats were mostly domestic short hair (13/16; 81.25%), Persian (1/16; 6.25%) and Siamese-mixed breed cats (2/16; 12.5%).

The body weight of the clinically normal cats, the CKD cats received the *Antidesma acidum*, the CKD cats received the bioflavonoid and the CKD cats received the α-tocopherol on first day of the study were 4.23 ± 0.24, 4.34 ± 0.34, 4.01 ± 0.31 and 4.09 ± 0.41 kg, respectively (Table 5). The body weight was similar between the clinically normal cats and the cats with CKD. The CKD cats received *Antidesma acidum* for fifty-six days had significantly increased in body weight.

Table 5. Signalment of the clinically normal cats and cats of the CKD group (mean ± SEM)

Parameters	Clinically normal (n=16)	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	α-tocopherol (n=7)
Male	6	5	2	2
Female	10	1	3	5
Age (years)	2.68 ± 0.67	10.17 ± 1.54*	13.20 ± 0.97*	9.86 ± 2.12*
Body weight (Day 0)	4.23 ± 0.24	4.34 ± 0.34	4.01 ± 0.31	4.09 ± 0.41
Body weight (Day 56)	-	4.62 ± 0.33[a]	3.92 ± 0.28	4.16 ± 0.42

* $p < 0.05$ when compared between the clinically normal cats and all of the CKD cats (the cats received *Antidesma acidum*, bioflavonoid and α-tocopherol) on the first day of the study (Day 0).

[a] $p < 0.05$ when compared between the CKD cats received *Antidesma acidum* before treatment (Day 0) and after treatment (Day 56)

The clinical signs observed in the CKD cats on the first day of the diagnosis including: dehydration (18/18; 100%), poor hair coat (10/18; 55.56%), gingivitis (7/18; 38.89%), polyuria and polydipsia (8/18; 44.44%), pale mucous membrane (4/18; 22.22%) and chronic respiratory tract infections (3/18; 16.67%).

The CKD cats were lower RBCs (7.06 ± 0.44 $\times 10^6$ cells/ml) than the clinically normal cats (7.49 ± 0.50 $\times 10^6$ cells/ml) (Table 6). The cats with CKD had significantly lower PCV (33.50 ± 1.49%) than the clinically normal cats (40.69 ± 1.34%) ($p < 0.01$) (Table 6).

There were no significant differences in the WBCs between the clinically normal cats (11,623 ± 1,158.14 cells/ml) and the CKD cats (12,175 ± 1,040.83 cells/ml) (Table 6). The mean number of neutrophils, lymphocytes, eosinophils, basophils and monocytes in each group of the cats were presented (Table 6). There were no

significant differences in neutrophils, lymphocytes, eosinophils, basophils and monocytes numbers between the clinically normal and the CKD cats.

The plasma ALT levels in the CKD cats (60.25 ± 12.48 IU/L) were higher than the clinically normal cats (41.82 ± 7.74 IU/L) (Table 6). Whereas, the plasma ALP in the CKD cats (37.93 ± 3.86 IU/L) were lower than the clinically normal cats (55.98 ± 20.10 IU/L). However, there were no significant differences in the plasma ALT and ALP levels between the clinically normal and the CKD cats. The mean ± SEM of the plasma ALT and ALP levels of both groups were within the normal limit (Table 6).

The CKD cats were significantly higher serum creatinine (3.33 ± 0.24 mg/dl) and the BUN levels (53.30 ± 4.75 mg/dl) than the clinically normal cats (1.27 ± 0.07 and 20.63 ± 1.53) ($p < 0.01$) (Table 6).

The plasma GPx levels of the CKD cats (1.57 ± 0.14 nmol/min) were significantly lower than the clinically normal cats (5.77 ± 1.19 nmol/min). This study demonstrated that the CKD cats were significantly lower plasma GSH levels (2.72 ± 0.30 µmol) than the clinically normal cats (4.54 ± 0.49 µmol) ($p < 0.01$). The plasma GSSG levels of the CKD cats (34.08 ± 5.54 µmol) were significantly higher than the clinically normal cats (16.33 ± 2.39 µmol). Moreover, the GSH/GSSG ratio of the CKD cats (0.30 ± 0.17) were lower than the clinically normal cats (0.84 ± 0.37) (Table 6).

Table 6. Blood profile of the clinically normal cats and the cats with chronic kidney disease (mean ± SEM) on the first day of diagnosis

Parameter	Units	Normal value #	Clinically normal (n=16)	CKD (n=18)
RBC	$\times 10^6$ cells/ml	5.24-10.89	7.49 ± 0.50	7.06 ± 0.44
PCV	%	29.2-51.7	40.69 ± 1.34	33.50 ± 1.49**
WBC	cells/ml	4,200-17,500	11,623 ± 1,158.14	12,175 ± 1,040.83
Neutrophils	cells/ml	1,925-14,825	7,187 ± 959.66	7,167.58 ± 770.16
Lymphocytes	cells/ml	1,100-7,000	3,909 ± 916.09	4,304 ± 602.22
Eosinophils	cells/ml	110-750	148.2 ± 47.56	150.11 ± 59.49
Basophils	cells/ml	0-190	16.87 ± 44.72	13.03 ± 9.65
Monocytes	cells/ml	55-780	366.13 ± 116.21	314.05 ± 59.75
ALT	IU/L	28-76	41.82 ± 7.74	60.25 ± 12.48
ALP	IU/L	0-62	55.98 ± 20.10	37.93 ± 3.86
BUN	mg/dl	15-35	20.63 ± 1.53	53.30 ± 4.75**
Creatinine	mg/dl	< 1.6 (Brown, 2004)	1.27 ± 0.07	3.33 ± 0.24**
GPx	nmol/min	-	5.77 ± 1.19	1.57 ± 0.14**
GSH	µmol	4.51 ± 1 (Laura et al., 2008)	4.54 ± 0.49	2.72 ± 0.30**
GSSG	µmol	19.44 ± 3.79 (Laura et al., 2008)	16.33 ± 2.39	34.08 ± 5.54**
GSH/GSSG		-	0.84 ± 0.37	0.30 ± 0.17

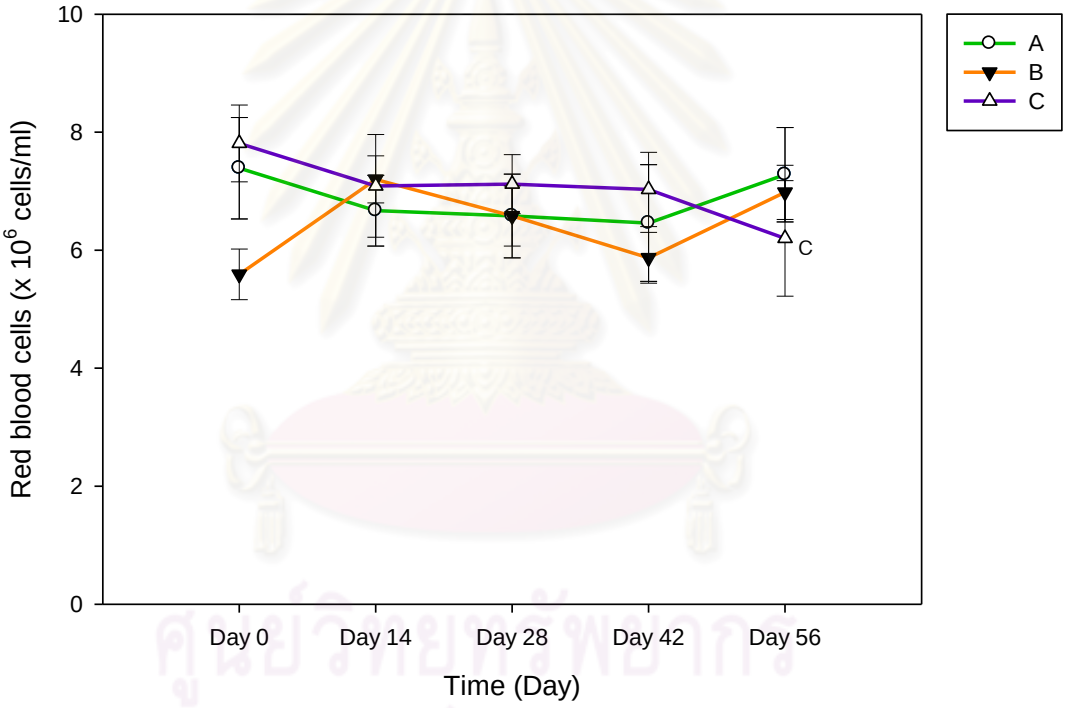
Normal reference value from Sodikoff C.H. 1995. Serum chemical test. Laboratory profile of small animal disease. In: A guide to laboratory diagnosis 2nd (ed). Mosby-Year Book. St. Louis. 3-20p.

CKD = Chronic kidney disease; RBC = Red blood cell; PCV = Pack cell volume; WBC = White blood cell; ALT = Alanine amino transferase; ALP = Alkaline phosphatase; BUN = Blood urea nitrogen; GPx = Glutathione peroxidase; GSH = Glutathione; GSSG = Oxidized glutathione

** $p < 0.01$ when compared between the clinically normal cats and the CKD cats on the first day of the study (Day 0).

The red blood cells (RBCs) in the CKD cats were presented (Fig.17). The CKD cats received the α-tocopherol had significantly decreased in RBCs on day 56. However, the mean ± SEM of RBCs for all of the CKD cats remained within the reference range.

Fig.17 Mean ± SEM of red blood cells (RBCs) in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received α-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

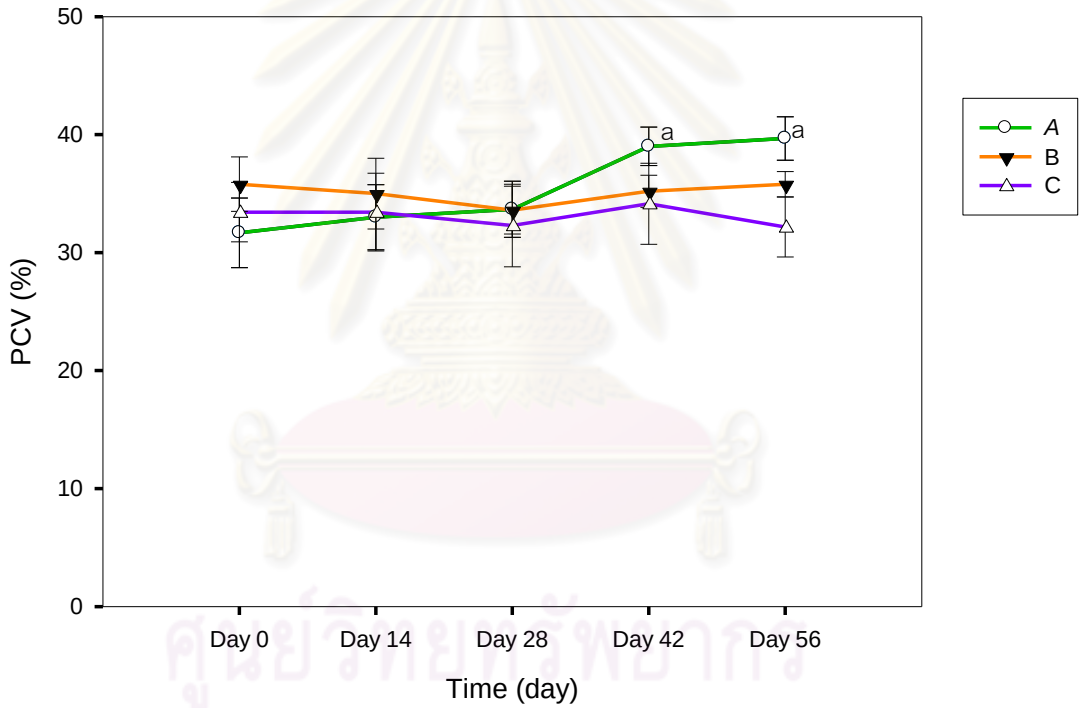
B = The CKD cats received bioflavonoid

C = The CKD cats received α-tocopherol

[C] $p<0.05$ when compared between the CKD cats received α-tocopherol before treatment (Day 0) and after treatment (Day 56)

The pack cell volume (%) in the CKD groups was presented (Fig.18). The CKD cats received the *Antidesma acidum* had significantly increased in PCV on day 42 and 56 when compared with day 0. The mean ± SEM of the PCV levels remained within the reference range for all of the CKD cats.

Fig.18 Mean ± SEM of pack cell volume (PCV) in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received **α**-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

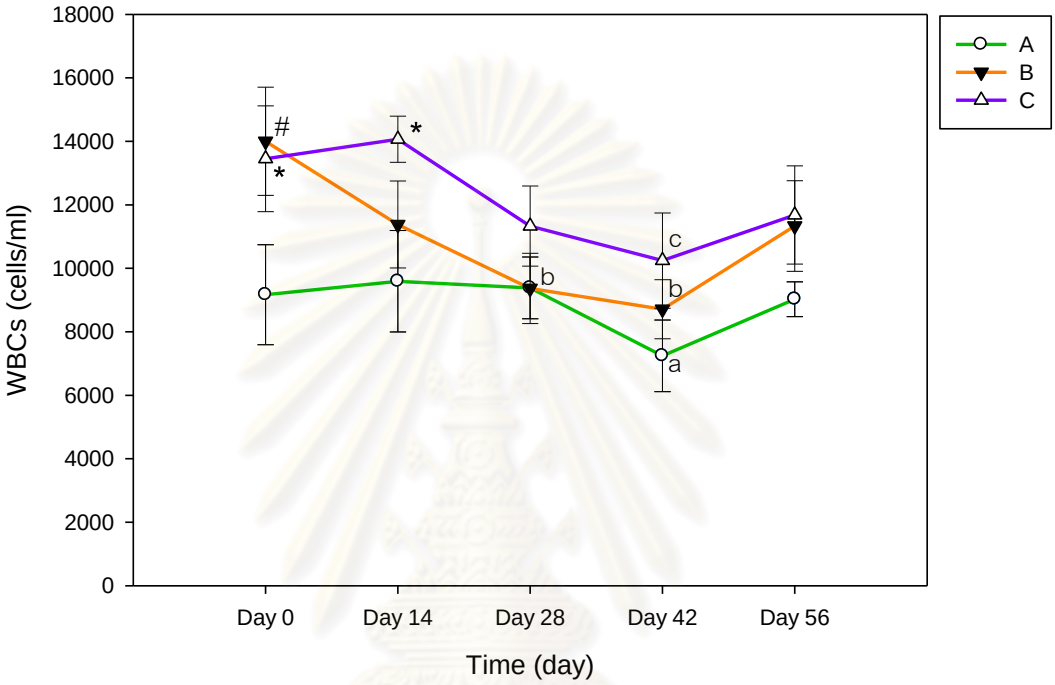
B = The CKD cats received bioflavonoid

C = The CKD cats received **α**-tocopherol

[a] *p<0.05* when compared between the CKD cats received *Antidesma acidum* before treatment (day 0) and after treatment (day 42 and 56)

The total white blood cells (WBCs) in the CKD groups were presented (Fig.19). The mean ± SEM of the WBCs in the CKD cats received *Antidesma acidum* were significant differences from the CKD cats received α-tocopherol on day 0 and 14. The WBC numbers in the CKD cats received *Antidesma acidum* had significantly decreased on day 42 when compared with day 14 and 28. The CKD cats received the bioflavonoid had significantly decreased in WBCs on day 28 and 42 when compared with day 0. The CKD cats received the α-tocopherol had significantly decreased in WBCs on day 42 when compared with day 14. However, the mean ± SEM of WBCs of all groups were within the reference range.

Fig.19 Mean ± SEM of total white blood cells (WBCs) in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received **α**-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

B = The CKD cats received bioflavonoid

C = The CKD cats received **α**-tocopherol

[#] *p<0.05* when compared between the CKD cats received bioflavonoid and the CKD cats received *Antidesma acidum* on day 0

[*] *p<0.05* when compared between the CKD cats received **α**-tocopherol and the CKD cats received *Antidesma acidum* (day 0 and 14)

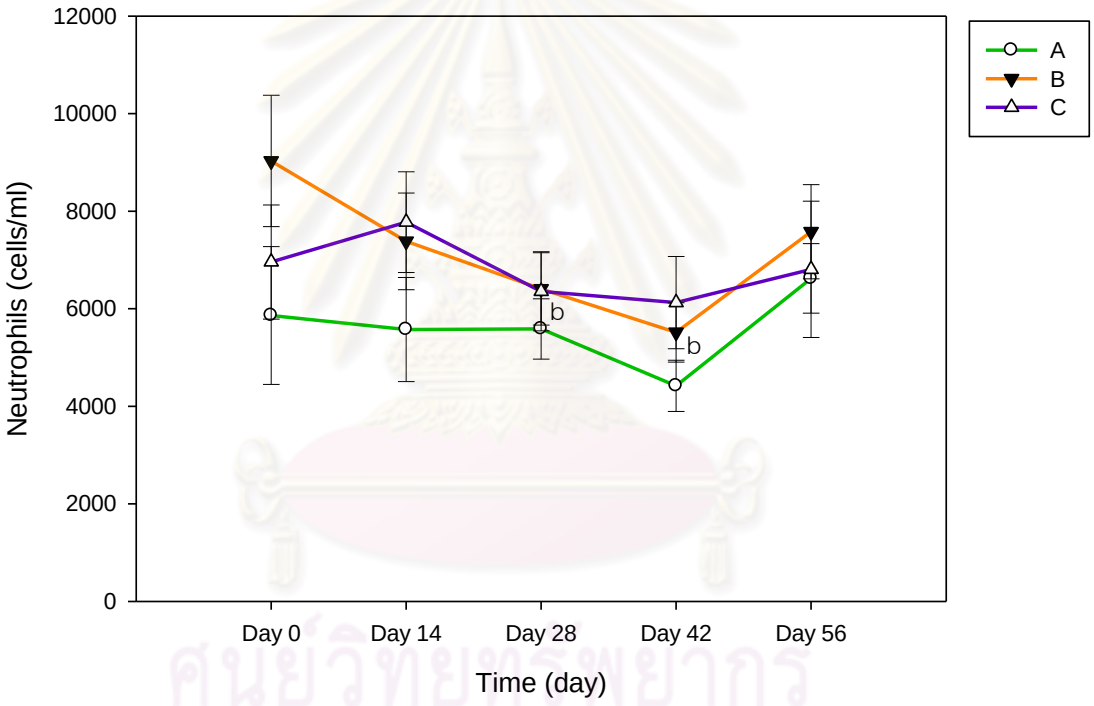
[a] *p<0.05* when compared between the CKD cats received *Antidesma acidum* on day 42 and on day 14 and 28

[b] *p<0.05* when compared between the CKD cats received bioflavonoid before treatment (day 0) and after treatment (day 28 and 42)

[c] *p<0.05* when compared between the CKD cats received **α**-tocopherol on day 14 and day 42

The neutrophil numbers in the CKD groups were presented (Fig.20). The CKD cats received the bioflavonoid had significantly decreased in neutrophil numbers on day 28 and 42 when compared with day 0.

Fig.20 Mean ± SEM of neutrophils in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received α-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

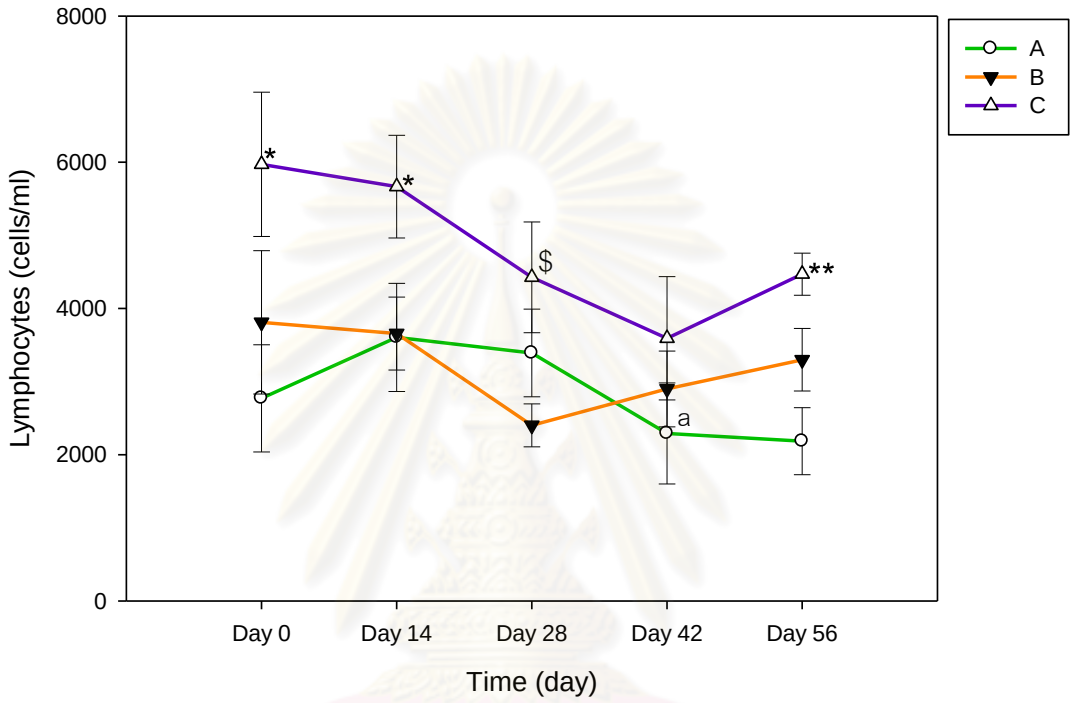
B = The CKD cats received bioflavonoid

C = The CKD cats received α-tocopherol

[b] $p<0.05$ when compared between the CKD cats received bioflavonoid before treatment (day 0) and after treatment (day 28 and 42)

The lymphocyte numbers in the CKD cats were presented (Fig.21). The mean ± SEM of lymphocytes in the CKD cats received *Antidesma acidum* were significantly lower than the CKD cats received α-tocopherol on day 0, 14 and 56. The mean ± SEM of lymphocytes in the CKD cats received bioflavonoid were significant differences from the CKD cats received α-tocopherol on day 28. The lymphocyte numbers in the CKD cats received *Antidesma acidum* had significantly decreased on day 42 when compared with day 14.

Fig.21 Mean ± SEM of lymphocytes numbers in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received **α**-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

B = The CKD cats received bioflavonoid

C = The CKD cats received **α**-tocopherol

* *p<0.05* when compared between the CKD cats received **α**-tocopherol and the CKD cats received *Antidesma acidum* (day 0 and 14)

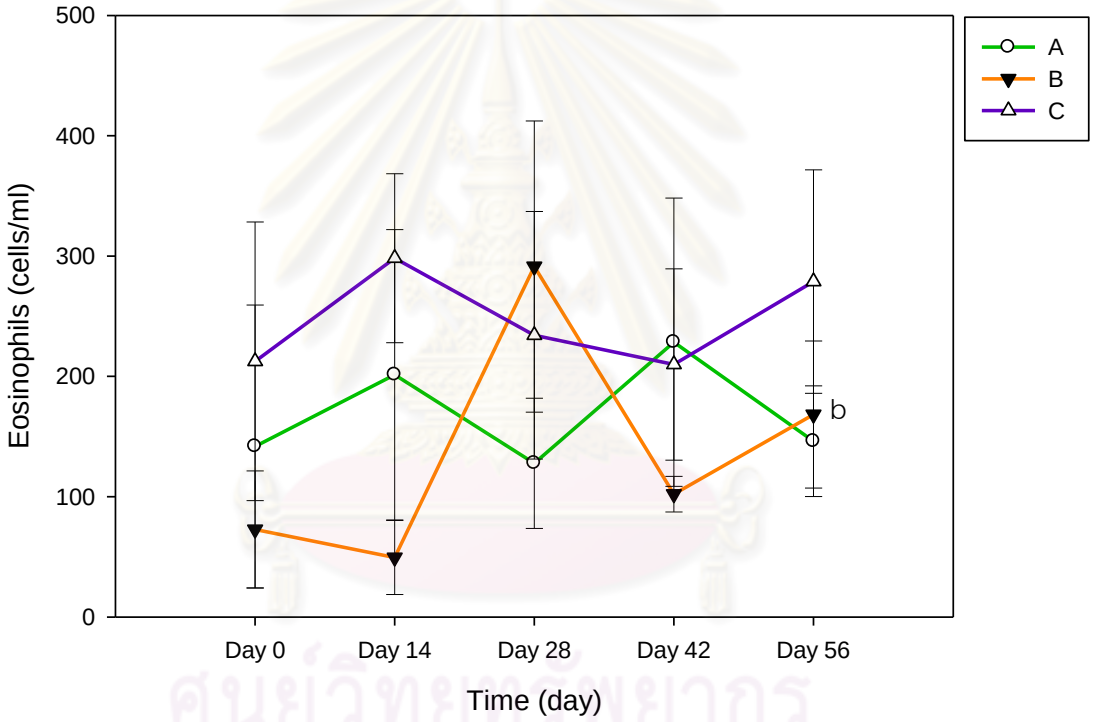
$ *p<0.05* when compared between the CKD cats received **α**-tocopherol and the CKD cats received bioflavonoid on day 28

** *p<0.01* when compared between the CKD cats received **α**-tocopherol and the CKD cats received *Antidesma acidum* on day 56

a *p<0.05* when compared between the CKD cats received *Antidesma acidum* on day 14 and day 42

The eosinophil numbers in the CKD cats were presented (Fig.22). The CKD cats received bioflavonoid had significantly decreased in eosinophils numbers on day 56 when compared with day 0.

Fig.22 Mean ± SEM of eosinophils in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received **α**-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

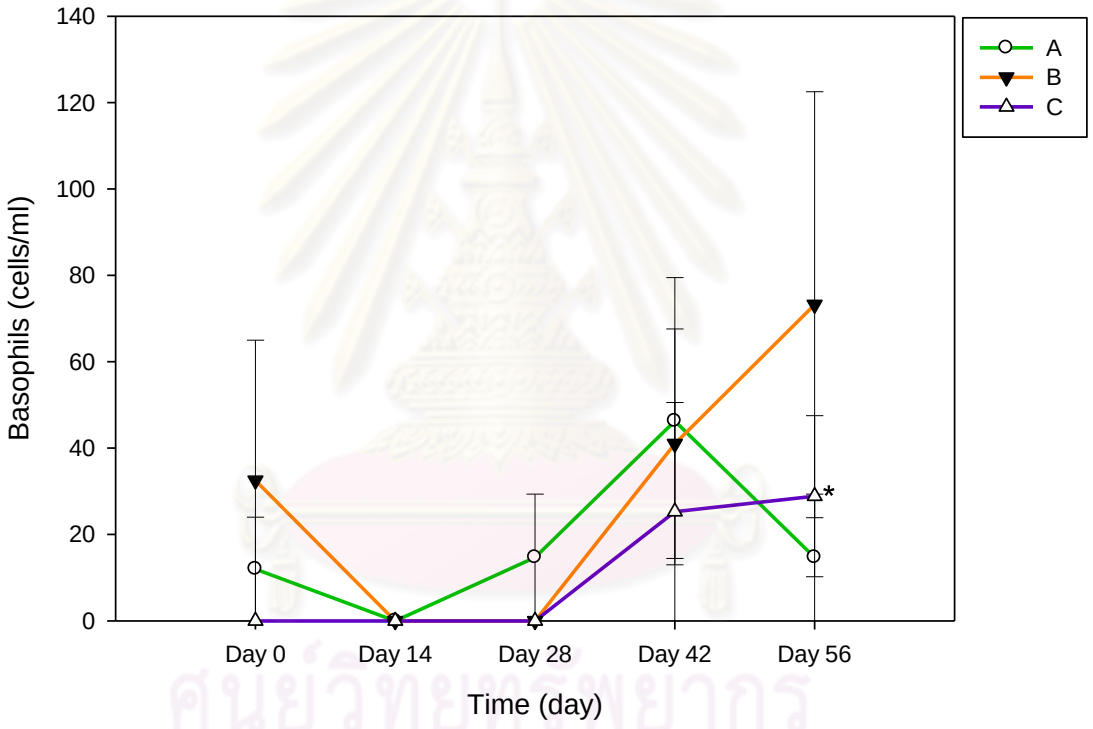
B = The CKD cats received bioflavonoid

C = The CKD cats received **α**-tocopherol

[b] *p<0.05* when compared between the CKD cats received bioflavonoid before treatment (day 0) and after treatment (day 56)

The basophil numbers in the CKD cats were presented (Fig.23). There were significant differences in basophils numbers between the CKD cats received *Antidesma acidum* and the CKD cats received α-tocopherol on day 56.

Fig.23 Mean ± SEM of basophils of the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received α-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

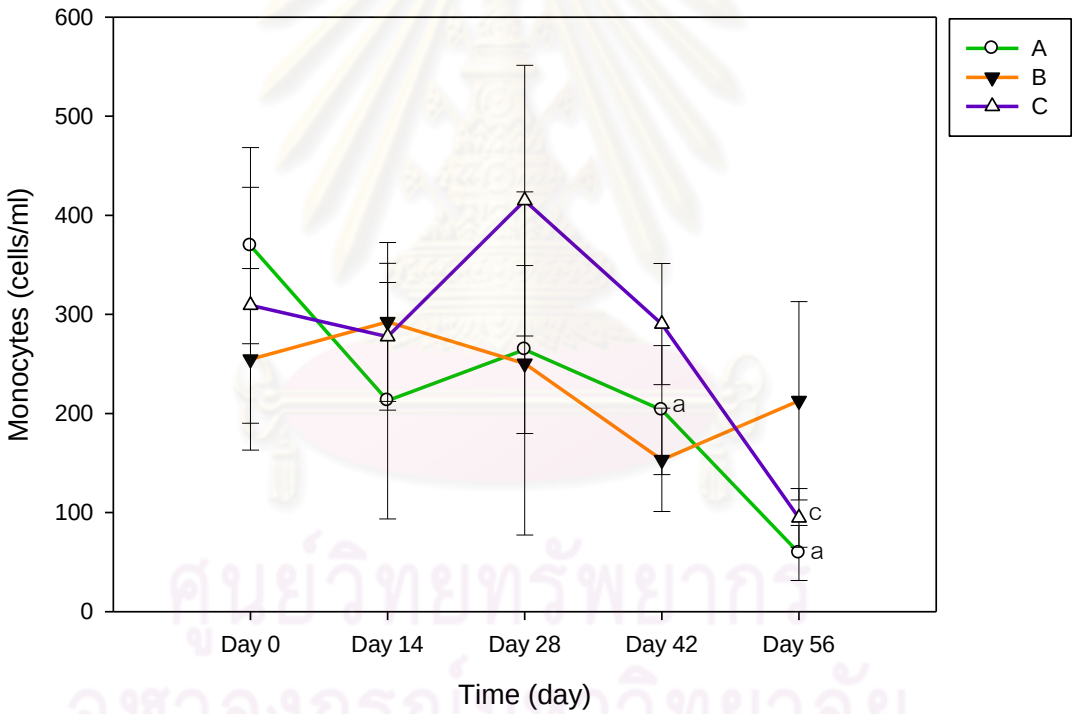
B = The CKD cats received bioflavonoid

C = The CKD cats received α-tocopherol

* $p<0.05$ when compared between the CKD cats received α-tocopherol and the CKD cats received *Antidesma acidum* on day 56

The monocyte numbers in the CKD cats were presented (Fig.24). The CKD cats received the *Antidesma acidum* had significantly decreased in monocyte numbers on day 42 and 56 when compared with day 0. The CKD cats received the **α**-tocopherol had significantly decreased in monocyte numbers on day 42.

Fig.24 Mean ± SEM of monocytes in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received **α**-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

B = The CKD cats received bioflavonoid

C = The CKD cats received **α**-tocopherol

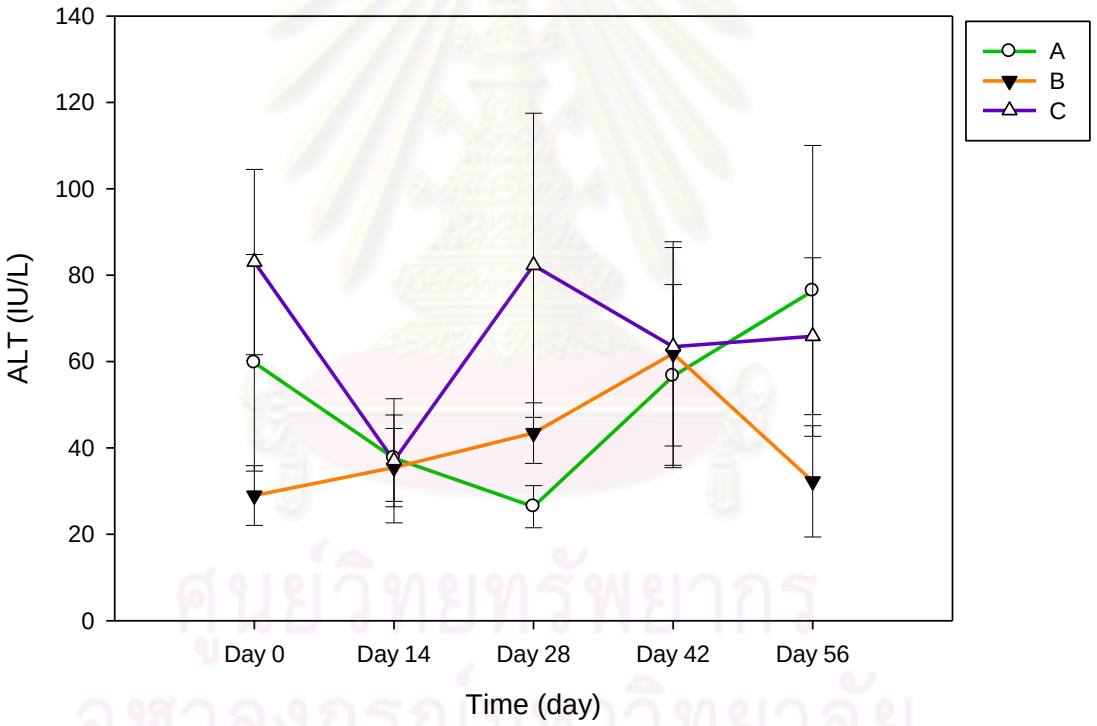
[a] *p<0.05* when compared between the CKD cats received *Antidesma acidum* before treatment (day 0) and after treatment (day 42 and 56)

[c] *p<0.05* when compared between the CKD cats received **α**-tocopherol on day 56 and day 42

Plasma ALT and ALP levels

The plasma ALT levels in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received **α**-tocopherol were presented (Fig.25). There were no significant differences between all treatment groups.

Fig.25 Mean ± SEM of plasma ALT levels in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received **α**-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



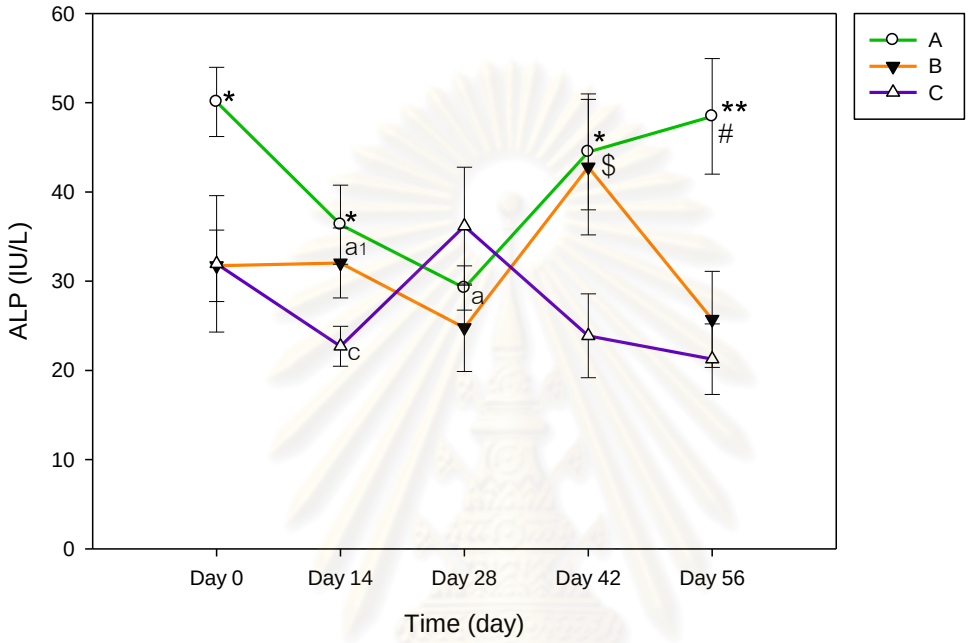
A = The CKD cats received *Antidesma acidum*

B = The CKD cats received bioflavonoid

C = The CKD cats received **α**-tocopherol

The plasma ALP levels in all of the CKD cats were presented (Fig.26). The CKD cats received the *Antidesma acidum* had significantly decreased in plasma ALP levels on day 14 and 28. The mean ± SEM of the plasma ALP levels in the CKD cats received *Antidesma acidum* were significant differences from the CKD cats received α-tocopherol on day 0, 14, 42 and 56. There were significant differences between the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 42. There were significant differences between the CKD cats received bioflavonoid and the CKD cats received *Antidesma acidum* on day 56. The CKD cats received the *Antidesma acidum* had significantly decreased in plasma ALP levels on day 28 when compared with day 0, 42 and 56. Moreover, the CKD cats received the *Antidesma acidum* had significantly decreased in plasma ALP levels on day 14 when compared with day 56. The CKD cats received the α-tocopherol had significantly decreased in plasma ALP levels on day 14 when compared with day 28.

Fig.26 Mean ± SEM of the plasma ALP levels in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received **α**-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

B = The CKD cats received bioflavonoid

C = The CKD cats received **α**-tocopherol

* *p<0.05* when compared between the CKD cats received *Antidesma acidum* and the CKD cats received **α**-tocopherol (day 0, 14 and 42)

$ *p<0.05* when compared between the CKD cats received bioflavonoid and the CKD cats received **α**-tocopherol on day 42

** *p<0.01* when compared between the CKD cats received *Antidesma acidum* and the CKD cats received **α**-tocopherol on day 56

\# *p<0.05* when compared between the CKD cats received *Antidesma acidum* and the CKD cats received bioflavonoid on day 56

a *p<0.05* when compared between the CKD cats received *Antidesma acidum* on day 28 and on day 0, 42 and 56

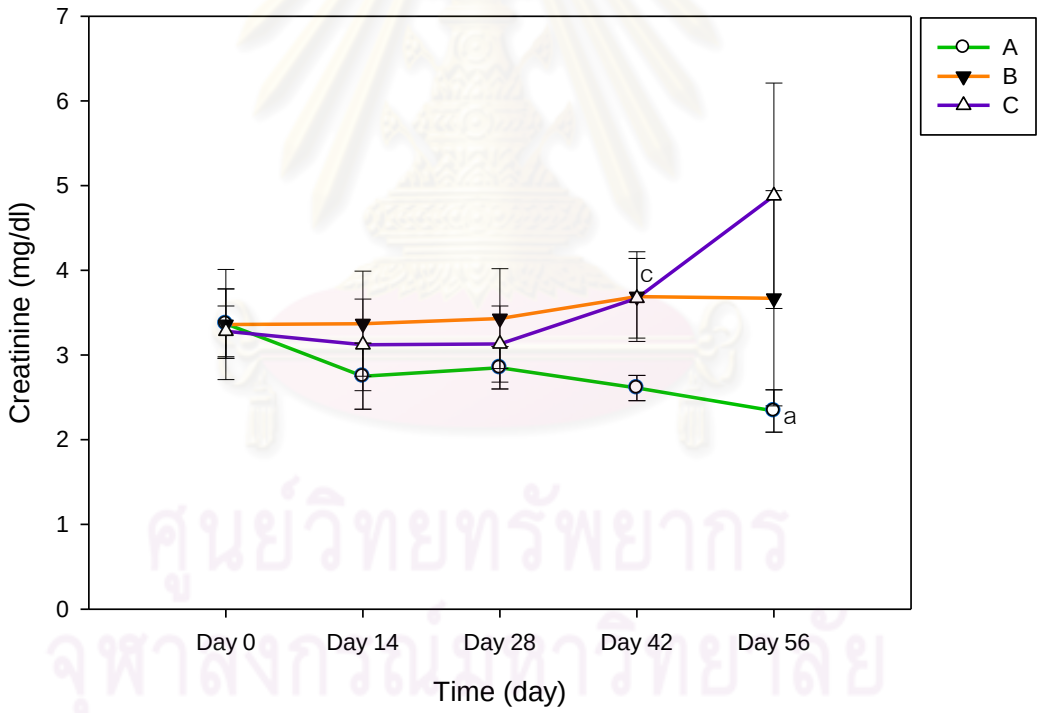
a1 *p<0.05* when compared between the CKD cats received *Antidesma acidum* on day 14 and day 56

c *p<0.05* when compared between the CKD cats received **α**-tocopherol on day 14 and day 28

Serum creatinine and blood urea nitrogen (BUN) levels

The serum creatinine in the CKD cats received *Antidesma acidum* on day 56 were significantly lower creatinine levels than the first day of the study (day 0). The CKD cats received the α-tocopherol had significantly increased in serum creatinine levels on day 42 when compared with day 28.

Fig.27 Mean ± SEM of serum creatinine (mg/dl) in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received α-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

B = The CKD cats received bioflavonoid

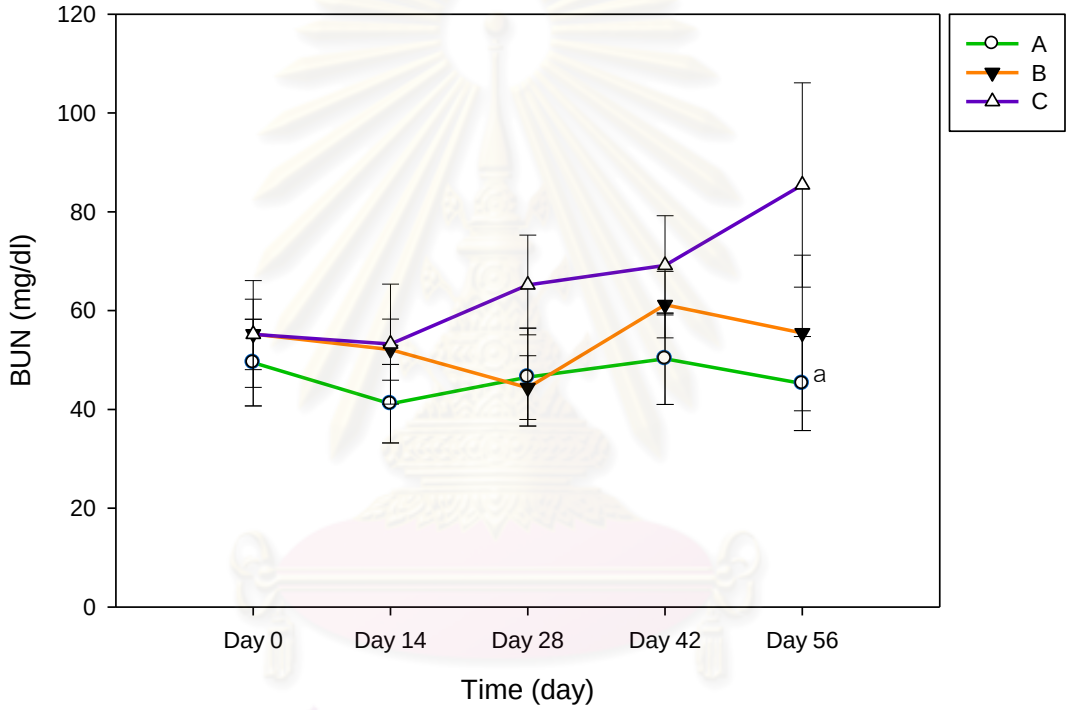
C = The CKD cats received α-tocopherol

[a] *p<0.05* when compared between the CKD cats received *Antidesma acidum* before treatment (day 0) and after treatment (day 56)

[c] *p<0.05* when compared between the CKD cats received α-tocopherol on day 28 and day 42

The CKD cats received the *Antidesma acidum* had significantly increased in serum BUN levels on day 56 when compared with day 14.

Fig.28 Mean ± SEM of serum BUN (mg/dl) in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received **α**-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

B = The CKD cats received bioflavonoid

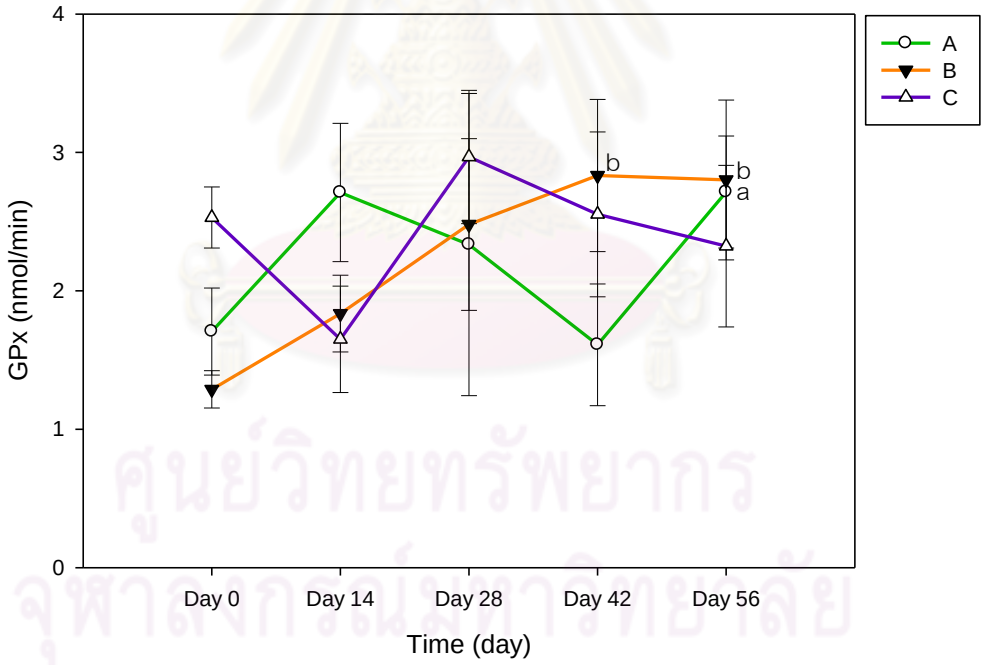
C = The CKD cats received **α**-tocopherol

[a] *p<0.05* when compared between the CKD cats received *Antidesma acidum* on day 14 and day 56

Oxidative stress parameters

The glutathione peroxidase (GPx) levels in all of the treatment groups were presented (Fig.29). The GPx levels in the CKD cats received bioflavonoid were significantly higher on day 42 and 56 than before treatment. The CKD cats received the *Antidesma acidum* had significantly increased in the GPx levels on day 56 when compared with day 0 and 42.

Fig.29 Mean ± SEM of glutathione peroxidase (GPx) in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received α-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

B = The CKD cats received bioflavonoid

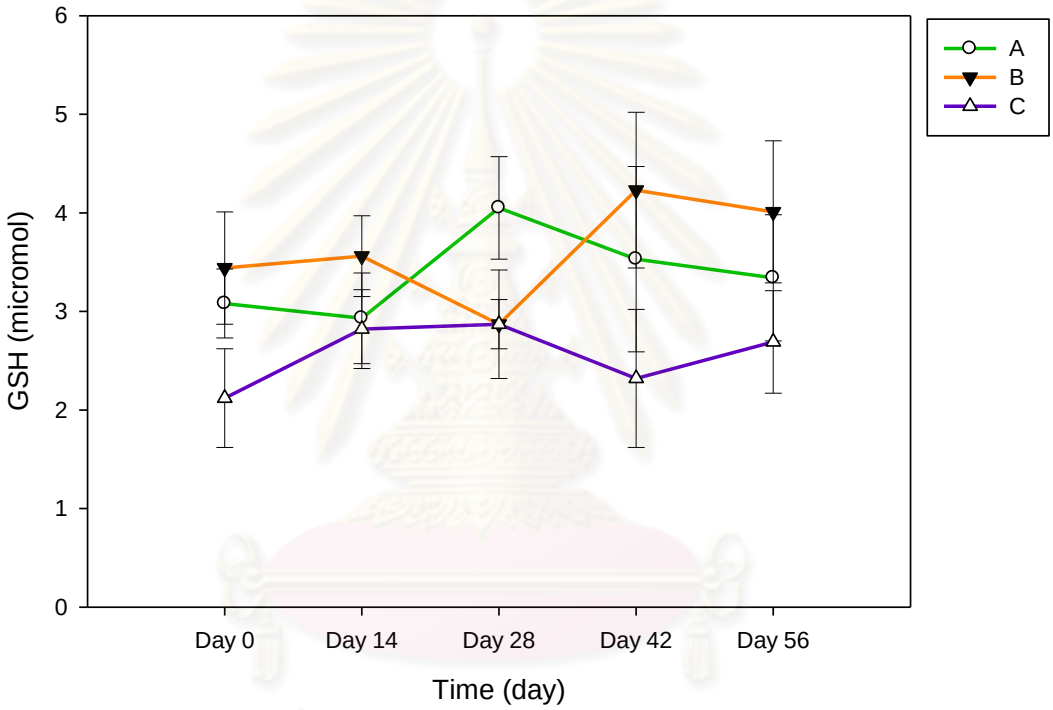
C = The CKD cats received α-tocopherol

[a] *p<0.05* when compared between the CKD cats received *Antidesma acidum* before treatment on day 56 and on day 0 and 42

[b] *p<0.05* when compared between the CKD cats received bioflavonoid before treatment (day 0) and after treatment (day 42 and 56)

The glutathione (GSH) levels in all of the CKD groups were presented (Fig.30). There were no significant differences between all treatment groups.

Fig.30 Mean ± SEM of glutathione (GSH) levels in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received α-tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



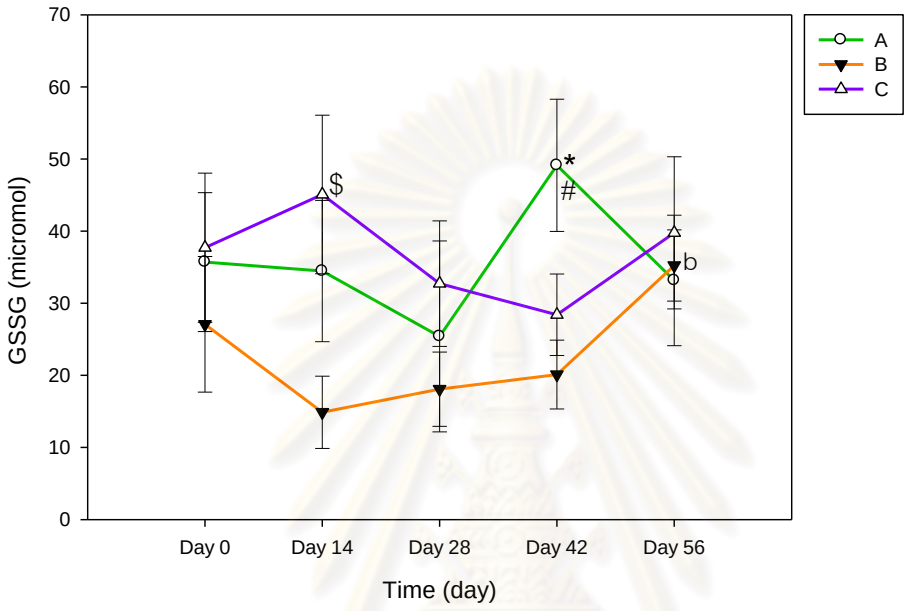
A = The CKD cats received *Antidesma acidum*

B = The CKD cats received bioflavonoid

C = The CKD cats received α-tocopherol

The oxidized glutathione (GSSG) levels in all of the CKD cats were presented (Fig.31). The mean ± SEM of GSSG levels in the CKD cats received bioflavonoid were significant differences from the CKD cats received α-tocopherol on day 14. The GSSG levels in the CKD cats received *Antidesma acidum* were significant differences from the CKD cats received bioflavonoid and α-tocopherol on day 42. The CKD cats received the bioflavonoid had significantly increased in the GSSG levels on day 56 when compared with day 42.

Fig.31 Mean ± SEM of oxidized glutathione (GSSG) levels in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received **α-** tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

B = The CKD cats received bioflavonoid

C = The CKD cats received **α**-tocopherol

$ *p<0.05* when compared between the CKD cats received bioflavonoid and the CKD cats received **α**-tocopherol on day 14

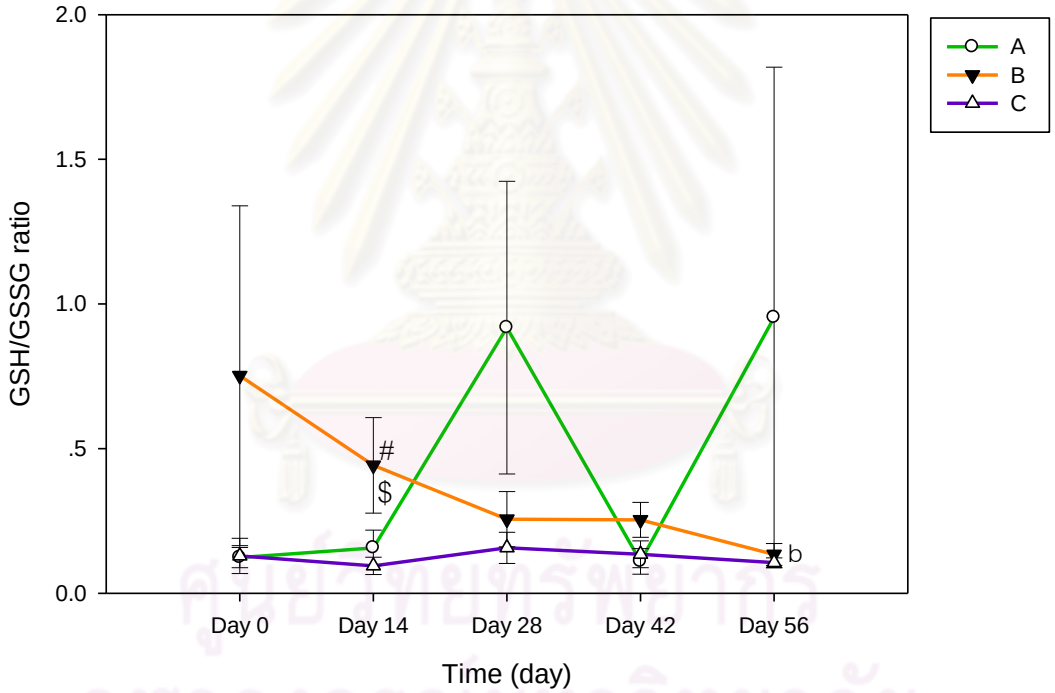
* *p<0.05* when compared between the CKD cats received *Antidesma acidum* and the CKD cats received **α**-tocopherol on day 42

\# *p<0.05* when compared between the CKD cats received *Antidesma acidum* and the CKD cats received bioflavonoid on day 42

b *p<0.05* when compared between the CKD cats received bioflavonoid on day 42 and day 56

The GSH/GSSG ratio in all of the CKD cats were presented (Fig.32). The GSH/GSSG ratio in the CKD cats received bioflavonoid were significant differences from the CKD cats received *Antidesma acidum* and α-tocopherol on day 14. The CKD cats received the bioflavonoid had significantly decreased in the GSH/GSSG ratio on day 56 when compared with 42.

Fig.32 Mean of GSH/GSSG ratio in the CKD cats received *Antidesma acidum* (A), the CKD cats received bioflavonoid (B) and the CKD cats received α- tocopherol (C) on day 0, 14, 28, 42 and 56 of the follow-up.



A = The CKD cats received *Antidesma acidum*

B = The CKD cats received bioflavonoid

C = The CKD cats received α-tocopherol

$ *p<0.05* when compared between the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 14

\# *p<0.05* when compared between the CKD cats received bioflavonoid and the CKD cats received *Antidesma acidum* on day 14

b *p<0.05* when compared between the CKD cats received bioflavonoid on day 42 and day 56

Urinalysis

The mean ± SEM of pH in the CKD cats received *Antidesma acidum* on day 0 were 6.33 ± 0.33. The mean of the urine specific gravity (USG) were 1.015. The mean ± SEM of pH in the CKD cats received bioflavonoid on day 0 were 6.50 ± 0.50. The mean of the urine specific gravity (USG) were 1.017. The mean ± SEM of pH in the CKD cats received α-tocopherol on day 0 were 7.00 ± 0.41. The mean of the urine specific gravity (USG) were 1.017.

The mean ± SEM of pH in the CKD cats received *Antidesma acidum* on day 56 were 6.67 ± 0.21. The mean of the urine specific gravity (USG) were 1.017. The mean ± SEM of pH in the CKD cats received bioflavonoid on day 56 were 6.50 ± 0.50. The mean of the urine specific gravity (USG) were 1.017. The mean ± SEM of the pH in the CKD cats received α-tocopherol on day 56 were 7.00 ± 0.41. The mean of the urine specific gravity (USG) were 1.019.

CHAPTER V
DISCUSSION

Part I Radical scavenging activity and the effective concentration

The total bark and trunk of all *Antidesma acidum* gave 0.91% of the crude extract of *Antidesma acidum*. The final crude extract amount of *Antidesma acidum* in this study was less than the previous study (3.17%) at proximately 3 times (Fungbun et al., 2010). This may be due to the different of seasonal variation (Jalal et al., 1982; Fang et al., 2010) and harvesting time.

The DPPH test is a direct and reliable method for determining the radical scavenging activity. The DPPH radical accepts an electron donate by an antioxidant compound, as the pale yellow non-radical form is produced. For the free radical scavenging property, the result agrees with the previous study that the percent (%) radical scavenging of the crude extract of *Antidesma acidum* was similar to bioflavonoid at the concentration of 100 µg/ml (Fungbun et al., 2010). It was also found that the percents radical scavenging of BHT, bioflavonoid and the crude extract of *Antidesma acidum* are increased as the concentration of substances increase. However, Fungbun et al (2010) that the percent (%) radical scavenging of crude extract of *Antidesma acidum* didn't depend on the concentration, it was effective at the concentration of 25 and 50 µg/ml. The dose dependent phenomenon was also observed in other plants. The percents radical scavenging of the crude extract of *Zizyphus mauritiana* or monkey apple (Bhuiyan et al., 2009) and *Syzygium cumini* or jambolan (Hasan et al., 2009) were associated with the increase in concentration.

The effective concentration (EC_{50}) of the crude extract of *Antidesma acidum* (42.21 µg/ml) was similar to BHT (42.24 µg/ml). Bioflavonoid (EC_{50} = 2.19 µg/ml) was more effective than BHT (EC_{50} = 42.24 µg/ml) and the crude extract of *Antidesma acidum* (EC_{50} = 42.21 µg/ml) at approximately 19 times. Fungbun et al (2010) reported that bioflavonoid (EC_{50} = 3.13 µg/ml) was more effective than BHT (EC_{50} = 44.12 µg/ml). But similar to the crude extract of *Antidesma acidum* (EC_{50} = 5.47 µg/ml).

Several studies had reported the antioxidant activity in other plants (Song et al., 2003; Bhuiyan et al., 2009). The study of free radical scavenging activity of *Zizyphus mauritiana* or monkey apple reported that the antioxidant activity of *Z. mauritiana* (local) (EC_{50} = 72 µg/ml) was greater than *Z. mauritiana* (Narikeli kul) (EC_{50} = 250 µg/ml) (Bhuiyan et al., 2009). The EC_{50} of *Phellinus linteus* or a medicinal mushroom was 22.07 µg/ml, whereas the vitamin C, used as a standard antioxidant, was 5.11 µg/ml (Song et al., 2003). *Syzygium cumini* or jambolan, with an EC_{50} value of 4.25 µg/ml, exhibited higher radical scavenging property than the reference antioxidant of ascorbic acid with EC_{50} value of 5.15 µg/ml (Hasan et al., 2009).

The highest scavenging compound in this study was observed with bioflavonoid with an EC_{50} value of 2.19 µg/ml. Bioflavonoid used in this study was quercetin, which is a member of the class of flavonoids called flavonoles. Quercetin is found mostly in onions, broccoli, apples and berries. Quercetin is considered as the most powerful flavonoids for protecting the body against reactive oxygen species which is produced during the normal oxygen metabolism of mitochondria or is induced by exogenous damage (De, 1994; Grace, 1994). The antioxidant effect of most plant is mainly due to the radical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes (Rahman and Moon, 2007). The crude extract from *Antidesma acidum* is rich in flavonoid, phenolic compounds, anthocyanin, tannins, B-sitosterol and stigmasterol (Thamaree et al., 2003). These compositions may contribute to the antioxidant property of *Antidesma* acidum. Our study suggested that the crude extract from *Antidesma acidum* have moderate antioxidant activity (EC_{50} = 42.21 µg/ml). Further investigation is necessary to find out the mechanism of how the crude extract from *Antidesma acidum* plays the role in the antioxidant system.

Part II **The comparative study of the effects of crude extract from *Antidesma acidum*., bioflavonoid and alpha-tocopherol on oxidative stress in cats with chronic kidney disease**

Sixteen clinically normal client-owned cats (6 males and 10 females) and eighteen cats with stable condition of spontaneous CKD (9 males and 9 females) were studied. The age of the CKD cats received the *Antidesma acidum* (10.17 ± 1.54 years), the bioflavonoid (13.20 ± 0.97 years) and the α-tocopherol (9.86 ± 2.12 years) were significantly higher than the clinically normal cats (2.68 ± 0.67 years). This study agrees with previous studies that the CKD was commonly found in older cats including USA (DiBartola et al., 1987), United Kingdom (Elliot and Barber, 1998), Australia (White et al., 2006), Germany (Dennis et al., 2006) and Thailand (Pusoonthornthum and Pusoonthornthum, 2004; Pusoonthornthum et al., 2010[a]; Fungbun et al., 2010). Aging is associated with reduced renal function (Coresh et al., 2007). The CKD in cats may be related to a high production of the free radicals (Snow, 1962; Christopher, 1989; Christopher et al., 1990; Webb et al., 2006), which are increase with aging process. Cats are known to be sensitive to the oxidative injury (Webb et al 2006) because their hemoglobin molecule contains 8 - 10 reactive sulfhydryl groups (Snow, 1962; Harvey and Kaneko, 1976; Christopher, 1989; Christopher et al., 1990). The breeds of the cats with CKD were domestic short hair (17/18; 94.44%) and Siamese-mixed breed cats (1/18; 5.56%). Pusoonthornthum et al (2010[a]) reported that feline chronic renal failure (CRF) was commonly found in Siamese and Siamese-mixed breed cats. One previous study of cats with spontaneous CRF reported that the cats with CRF were mostly domestic short hair (Fungbun et al., 2010).

The body weight was similar between the clinically normal cats and the cats with CKD on the first day of the study. There were no significant differences in body weight between the CKD cats and the clinically normal cats (Fungbun et al., 2010; Pusoonthornthum et al., 2004; Pusoonthornthum et al., 2010[a]). This study demonstrated that the CKD cats received the *Antidesma acidum* had significantly increased in body weight on day 56 as support by previous studies (Fungbun et al., 2010;

Pusoonthornthum et al., 2010[b]). The two previous studies showed that the crude extract of the trunk of *Antidesma acidum* giving to healthy and the CKD cats increased the cats' body weight after the treatment.

For hematology, there were no significant differences in the RBCs values between the clinically normal cats and the CKD cats on day 0 of the study but the PCV in the clinically normal cats was significantly higher than all of the CKD groups on day 0 of the study. The PCV is the percentage of the blood volume occupied by red blood cells. A decrease in PCV of the CKD cats may be caused by overhydration from fluid therapy because the RBCs were within the normal limit. Fungbun et al (2010) reported that the mean ± SEM of RBCs and PCV levels in the CKD cats were similar to the clinically normal cats. However, one previous study found that cats with CRF were significantly lower RBCs and PCV than the clinically normal cats on the first day of the study especially the CKD cats with increased levels of creatinine (Pusoonthornthum et al., 2010[a]). The kidney produces an important hormone called erythropoietin (EPO) and EPO deficiency is one of the important causes for anemia in CRF (Cowgill et al., 1998). The anemia in the CRF was characterized as normochromic, normocytic and hypoproliferative (Eschbach, 1989). Cats with anemia often present with pale mucous membrane, poor appetite, lethargy, depression, and decreased activity. The same clinical signs had been observed in 22.22% of the CKD cats. Cats are susceptible to oxidative stress (Webb et al., 2006). The feline erythrocytes have greater Heinz bodies as a result of oxidant damage compared with the other species (Christopher et al., 1990). The mean PCV and RBCs in the CKD group in this study were within the normal reference range.

There were no significant differences in WBCs, neutrophils, lymphocytes, eosinophils, basophils and monocytes numbers between the clinically normal cats and the CKD cats. Eventhough, the total WBCs in the CKD cats from the results were higher than the clinically normal cats. The mean WBCs values and deferential blood counts in the both groups were within the normal reference range. Campbell et al (2004) indicated that the WBCs in cats were significantly differences between the

adult cats (14,460 ± 640 cells/ml) and the senior cats (12,410 ± 420 cells/ml). The aging process was proposed to be associated with change in the immune status of cats (Campbell et al., 2004), human (Castle, 2000), and rats (Linton and Thoman, 2001). The ALT and ALP levels remained within the normal reference range for all of the CKD and the clinically normal cats. However, the mean plasma ALT levels in the CKD cats were higher than in the clinically normal cats.

Serum creatinine concentration is used as an index of the renal function in the clinical practice. The CKD cats were significantly higher serum creatinine and BUN levels than the clinically normal cats. According to the International Renal Interest Society (IRIS), the CKD cats in this study were classified as eighteen cats in stage III (Brown, 2004).

The plasma GPx levels in the CKD cats (1.57 ± 0.14 nmol/min) were significantly lower than the clinically normal cats (5.77 ± 1.19 nmol/min). Previous research on the plasma GPx activity and the plasma GPx concentration in patients with CKD were found to be lower than normal age-matched (Hamid et al., 2009). The study of glutathione and glutathione related enzymes in patients on maintenance dialysis found that plasma glutathione peroxidase (GPx) activity were significantly lower in the dialysis group (59.90 ± 31.28 U/L) than the healthy controls group (142.50 ± 33.77 U/L) (Pedram et al., 2009). Study of oxidative stress in cats with diabetes mellitus (DM) also indicated the plasma GPx levels were less in the DM cats (41,081 ± 19,334 U/L) than in the control cats (47,083 ± 15,913 U/L) (Webb and Falkowski, 2009).

The reduced glutathione (GSH) is a tripeptide including glycine, cysteine and glutamine. The glutathione (GSH) and the oxidized glutathione (GSSG) concentrations in the plasma have been considered to be an index of the whole body glutathione and is a useful indicator of a disease risk (Jones et al., 2000). During severe oxidative stress, the concentration of GSH may decrease and the concentration of GSSG may increase in the affected cells (Sakhi et al., 2006). Study in North Indian women indicated that the plasma total glutathione (GSH plus GSSG) in cervical intraepithelial neoplasia grade III (CIN III) (0.724 ± 0.236 μmol/L) and invasive cervical cancer (0.622 ± 0.171 μmol/L)

were significantly lower than control age-matched women (1.082 ± 0.334 μmol/L) (Kumar et al., 1995). Plasma glutathione concentration in the CRF human patients were also reported to be lower than the clinically normal age-matched (Prezemystaw et al., 2006; Pedram et al., 2009). There were no previous reports in the CKD cats. The results of this study demonstrated that the CKD cats were significantly lower concentration of GSH when compared with the clinically normal cats ($p<0.05$). Using HPLC for the GSH determination in twenty-three healthy cats, the mean concentrations of GSH were 4.51 ± 1 μmol (Laura et al., 2008). In one previous study, the plasma GSH in cats, dog and human were 1.97 ± 0.07, 1.98 ± 0.15 and 2.26 ± 0.09 mmol/L RBC, respectively (Harvey and Kaneko, 1976).

Sakhi et al (2006) reported an increase in plasma GSSG and a decrease in GSH/GSSG ratio when cells are exposed to oxidative stress. In other chronic disease, sputum supernatant of GSSG in patients with asthma (5.9 μM) was significantly higher than control (2.6 μM) (Wood et al., 2008). In hypertension pregnant patients, the GSSG levels had significantly elevated as compared with the normotensive pregnant women and the nonpregnant controls (N'emeth et al., 2001). According to this study, the plasma GSSG in cats with CKD were significantly higher than the clinically normal cats. Laura et al (2008) reported that the mean concentrations of GSSG in healthy cats were 19.44 ± 3.79 μmol.

In conclusion, the cats with CKD had oxidative stress based on the significant finding of the decrease in the GSH and GPx levels and an increase in GSSG levels in the plasma. Further research is required to determine oxidative stress in a general cat population study and to assess other oxidative stress parameters for better understanding of the changes in the oxidative stress in cats with naturally occurring CKD.

The CKD cats received the α-tocopherol had significantly decreased in RBCs on day 56. Vitamin E or α-tocopherol has been known to be an antioxidant which stabilizes the red blood cell membrane (Rose and Gyorgy, 1952). The α-tocopherol levels in RBC had significantly decreased in old people (Tanabe et al., 1994) and rats

with vitamin E deficiency (Chen et al., 1980). However, this study found that the CKD cats received the α-tocopherol had significantly decreased in RBCs on day 56. The reduction in RBC numbers may be due to the progression of CKD in cats received the α-tocopherol despite the action of α-tocopherol on RBC. The CKD cats received the *Antidesma acidum* had significantly increased in PCV on day 42 and 56. One previous research found that the crude extract from *Antidesma acidum* leaves increased RBCs, hemoglobin and PCV on day 42 of the study but did not find the same trend in cats received the crude extract from the trunk of *Antidesma acidum* (Pusoonthornthum et al., 2010[b]). Fungbun et al (2010) demonstrated that the cats with mild stage of CKD received the crude extract from *Antidesma acidum* were significantly higher PCV than the CKD cats received placebo on day 14. However, the mean ± SEM of RBCs and PCV levels in the CKD cats in this study remained within the reference range throughout the study.

The mean ± SEM of WBCs levels in the CKD cats received α-tocopherol and bioflavonoid were significantly higher than the CKD cats received *Antidesma acidum* on day 0. Moreover, there were significant difference in WBCs between the CKD cats received *Antidesma acidum* and the CKD cats received α-tocopherol on day 14. Even though we randomly assigned cats into three study groups, the CKD cats received the *Antidesma acidum* were mostly cats with Feline immunodeficiency virus (FIV) infection (2/6; 33.33%) and Feline leukemia virus (FeLV) infection (2/6; 33.33%). Most cats with viral infection are commonly found to have decreased numbers of lymphocytes and neutrophils. The WBC numbers in the CKD cats received *Antidesma acidum* had significantly decreased on day 42 when compared between day 14 and 28. However, Fungbun et al (2010) reported that there were no significant difference in WBCs between the CKD cats received *Antidesma acidum* before and after treatment. This study demonstrated that the WBC numbers in the CKD cats received α-tocopherol had significantly decreased on day 42 when compared with day 14. From several previous studies, it was found that supplementation of α-tocopherol can reduce the inflammation by inhibition of LPS-induced neuronal oxidative damage and dendrite degeneration in

mice (Milatovic et al., 2003), inhibition of PGE_2 production in splenocytes from old mice (Wu et al., 2000) and inhibition of 5-LOX-dependent TNF-α-release from LPS-stimulanted human monocytes (Devaraj and Jailal, 2005). The CKD cats received the bioflavonoid had significantly decreased in neutrophil numbers on day 28 and 42. The persistent neutropenia in cats may be an indicator of Feline infectious panleukopenia, FIV and FeLV.

The lymphocyte numbers in the CKD cats received *Antidesma acidum* had significantly decreased on day 42 when compared with day 14. Our previous study reported that there were no significant difference in lymphocytes between the CKD cats received *Antidesma acidum* before and after treatment (Fungbun et al., 2010). However, the trunk of *Antidesma acidum* extraction increased lymphocytes in eight healthy cats (Pusoonthornthum et al., 2010[b]). The mean ± SEM of lymphocytes in the CKD cats received *Antidesma acidum* were significant difference from the CKD cats received α-tocopherol on day 0, 14 and 56. The mean ± SEM of lymphocytes in the bioflavonoid group were significant difference from the α-tocopherol group on day 28. The lymphocyte numbers decrease in geriatric animal. From this study, the age of CKD cats received α-tocopherol was less than other groups. Moreover, the CKD cats received *Antidesma acidum* were mostly cats with viral infection, which had persistent decrease in lymphocytes.

The CKD cats received the bioflavonoid had significantly decreased in eosinophil numbers on day 56. The persistent eosinopenia in cats may be due to the stress condition. When consider the monocyte numbers, the CKD cats in the *Antidesma acidum* group had significantly decreased in monocytes on day 42 and 56. Fungbun et al (2010) reported that the mild and moderate azotemia cats received the crude extract of *Antidesma acidum* had significantly increased the monocyte numbers after the treatment. The CKD cats received the α-tocopherol had significantly decreased in monocyte numbers on day 56 when compared with day 42. Devaraj et al (1996) reported that supplementation of α-tocopherol 1200 IU/day for 2 months to healthy normal people decreased IL-1ß, decreased IL-6, decreased $O_2^{-}\cdot$ and H_2O_2 production in

monocytes. However, the mean ± SEM of eosinophils and monocytes numbers in all of the CKD groups remained within the reference range.

Alanine aminotransferase (ALT) is an enzyme produced in the liver cells. The ALT is increased when the liver cells damage occur. For the levels of ALT, there were no significant differences between in each group of the study. In previous study, the healthy cats received the crude extract from *Antidesma acidum* trunk had significantly decreased in the levels of ALT on day 57 when compared to day 0 (Pusoonthornthum et al., 2010[b]). Quercetin protects the lead-induced hepatotoxicity in rats through attenuating lipid peroxidation, renewing the activities of antioxidant enzymes and alleviating apoptosis by modulating JNK, Bax, Bcl-2 and caspase-3 protein expression (Liu et al., 2010[b]). The CKD cats received the *Antidesma acidum* had significantly decreased in the plasma ALP levels on day 14 and 28 when compared with day 0. Moreover, the CKD cats received the *Antidesma acidum* had significantly decreased in the plasma ALP levels on day 28 when compared with day 0, 42 and 56. These findings are supported by Fungbun et al (2010), the mean plasma ALP levels had significantly decreased in the mild and moderate azotemia cats received *Antidesma acidum* on day 56 and 70 when compared to day 0. However, one previous study reported that both part of the leaves and the trunk increased ALP in the healthy cats on day 90 (Pusoonthornthum et al., 2010[b]). The CKD cats received the *Antidesma acidum* had significantly decreased in the plasma ALP levels on day 14 when compared with day 28. Quercetin can protect the lead-induced hepatotoxicity in rats (Liu et al., 2010[b]). Alkaline phosphatase (ALP) is an enzyme made in the liver, bone and the placenta and normally present in high concentrations in growing bone and in bile. ALP is released into the blood during injury and during such normal activities as bone growth and pregnancy. The mean ± SEM of plasma ALP levels in the CKD cats received *Antidesma acidum* were significant differences from the CKD cats received **α**-tocopherol on day 0, 14, 42 and 56. There were significant differences between the bioflavonoid group and the **α**-tocopherol group on day 42. There were significant differences between the

bioflavonoid group and the *Antidesma acidum* group on day 56. However, the mean ± SEM of ALP in all of the CKD groups remained within the reference range.

The CKD cats received *Antidesma acidum* had significantly decreased in the serum creatinine on day 56 of the treatment. This finding agrees with a previous study that the crude extract of both part of leaves and trunk of the *Antidesma acidum* decreased blood urea nitrogen and creatinine in cats (Pusoonthornthum et al., 2010[b]). In the moderate renal azotemia cats received the crude extract from *Antidesma acidum* were also found to significantly decrease in the creatinine levels on day 28 and 42 of the study (Fungbun et al., 2010). The antioxidant effect of polyphenols, which are rich in the crude extract from *Antidesma acidum*, is reached through a cytoprotective action of the glomerular mesangial cells, exerted by a restriction on apoptosis triggered by oxidants (Kitamura and Ishikawa, 1999). The CKD cats received the α-tocopherol had significantly increased in the serum creatinine levels on day 42 when compared with day 28. In contrast with one study, the supplementation of vitamin E and C and ß-carotene can reduce creatinine levels in the CKD cats with mild stage (Yu and Paetau-Robinson, 2006).

The kidney is a site of 10% of body oxygen consumption which produces ROS as by-products. Increased ROS produce by oxidative stress by can disturb the prooxidant–antioxidant balance (Sies, 1997). The role of oxidative stress has been proposed as one of the mechanism of CKD in human patient due to the lacking of antioxidant defenses (Fryer, 1997), increased lipid peroxidation (Hamid et al., 2009) and increased ROS generation (Handelman et al., 2001). Cats are sensitive to oxidative injury (Webb et al., 2006) and oxidative stress was found to be associated with DM in cats (Webb and Falkowski, 2009), and cats with renal insufficiency (Yu and Paetau-Robinson, 2006). In diabetic nephropathy, high glucose stimulates ROS production, resulting in renal hypertrophy, fibrosis, glomerular enlargement and increased expression of angiotensinogen (Hsieh et al., 2002). Eventhough there are many studies about oxidative stress in other species, there is only few studies in cats. The exact

mechanism of how the crude extract of *Antidesma acidum* decrease the creatinine levels in moderate CKD cats need further investigation.

The main biological role of glutathione peroxidase is to protect the organs from oxidative damage by reducing lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. All of the CKD groups were significantly lower the GPx levels than the clinically normal cats on the first day of diagnosis. Previous studies found that the plasma GPx concentration in patients with CKD was lower than normal age-matched (Hamid et al., 2009; Pedram et al., 2009). In cats with diabetes mellitus (DM), the plasma GPx levels were less in the DM cats (41,081 ± 19,334 U/L) than in the control cats (47,083 ± 15,913 U/L) (Webb and Falkowski, 2008).

The GPx levels in the CKD cats received bioflavonoid on day 42 and 56 were significantly higher than before the treatment. Bioflavonoid used in this study was quercetin which is the most powerful flavonoids compound for protecting the body against reactive oxygen species (De, 1994; Grace, 1994). The CKD cats received *Antidesma acidum* had significantly increased in the GPx levels on day 56 when compared with day 0. Moreover, the CKD cats received *Antidesma acidum* had significantly increased in the GPx levels on day 56 when compared with day 42. The crude extract from *Antidesma acidum* is rich of flavonoid, phenolic compounds, anthocyanin, tannins, B-sitosterol and stigmasterol which are powerful antioxidants as quercetin and may be responsible for the increase in GPx activity (Thamaree et al., 2003).

The reduced glutathione (GSH) is a tripeptide including glycine, cysteine and glutamine. The liver is a major site of GSH synthesis and export. In oxidative stress, the concentration of GSH decrease and the concentration of GSSG increase in the affected cells (Sakhi et al., 2006). This study found that there were no significant differences in GSH and GSSG levels between before and after treatment in all of the CKD groups. However, there was a trend that GSH increased after day 42 of treatment in CKD cats received *Antidesma acidum*.

In conclusion, the crude extract from *Antidesma acidum* is comparable to bioflavonoid in terms of its elevated GPx levels in cats with CKD. Moreover, the crude extract from *Antidesma acidum* had significantly decreased serum creatinine in the moderated CKD cats after 56 days of treatment. Further study is needed to find the exact mechanism of how *Antidesma acidum* decreased creatinine levels and oxidative stress in the CKD cats.

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APPENDICES

Appendix A Signalment and places of study in the clinically normal cats

Group / Name	Age (years)	Gender	Weight (kg) Day 0	Breed	Places of study	
					CU	Private
Clinically normal						
1. ป่าน	0.5	F	3.9	DSH		/
2. แต้ว	1	F	4.1	DSH		/
3. ผู้	1	M	4.5	DSH		/
4. เนียน	0.5	F	3.8	DSH	/	
5. ขาว	1	F	3.8	DSH	/	
6. ลายเล็ก	1	F	3.3	DSH	/	
7. เปอร์เซีย	3	F	2.9	Persia	/	
8. ขาวดำ	2	F	3.7	DSH	/	
9. เอสลาย	2	F	3.6	DSH	/	
10. มีสุข	1	M	4.75	DSH	/	
11. มีโชค	1	M	4.6	DSH	/	
12. ข้าวโอ๊ต	6	M	4.8	DSH	/	
13. การ์ฟิลด์	7	M	5	DSH	/	
14. โอเลี้ยง	8	M	7	DSH	/	
15. จั๊กแหล่น	7	F	4.5	DSH	/	
16. สุชาดา	1	F	3.4	DSH		/

CU = Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University

Private = Private animal hospital

Appendix B Signalment and places of study in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received α-tocopherol

Group / Name	Age (years)	Gender	Weight (kg) Day 0	Weight (kg) Day 56	Breed	Places of study CU	Places of study Private
Antidesma acidum							
1. เพชร	15	M	4.36	4.32	DSH	/	
2. สุขสันต์	13	M	4.42	4.6	DSH	/	
3. อัมพะวา	12	F	4	4.26	DSH		/
4. แม็ค	6	M	4.2	4.44	DSH	/	
5. ดิกกี้	6	M	5.8	6.2	DSH		/
6. ก๋วยเจ๋ง	9	M	3.28	3.88	Siamese-mixed breed	/	
Bioflavonoid							
1. มาลี	16	F	3.45	3.25	DSH		/
2. ม่อน	10	F	3.2	3.3	DSH		/
3. พริกขี้หนู	13	F	4.1	4.25	DSH		/
4. เกาลัด	13	M	4.4	4.15	DSH		/
5. เฉาก๊วย	13	M	4.9	4.64	DSH		/
α-tocopherol							
1. ปุ๊กกี้	15	F	3.2	2.9	DSH	/	
2. สามปอย	6	F	4.6	4.7	DSH		/
3. รูปหล่อ	14	M	5.2	4.8	DSH		/
4. หนำเลี๊ยบ	15	M	4.2	4.3	DSH		/
5. แตงแว	13	F	4.3	4.25	DSH		/
6. หนูนา	3	F	2.4	2.5	DSH		/
7. ลาเต้	3	F	5.7	5.64	DSH		/

CU = Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University

Private = Private animal hospital

Appendix C Mean ± SEM of hematology in the clinically normal cats, the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 0

			Day 0			
Parameter	Units	Normal value#	Clinically normal (n=16)	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	α-tocopherol (n=7)
RBC	$\times 10^6$ cells/ml	5.24-10.89	7.49 ± 0.50	7.39 ± 0.86	5.59 ± 0.43	7.81 ± 0.65
PCV	%	29.2-51.7	40.69 ± 1.34	31.67 ± 2.95[a]	35.8 ± 2.31[b]	33.43 ± 2.52[c]
WBC	cells/ml	4,200-17,500	11,623 ± 1,158.14	9,166.67 ± 1574.57	14,000 ± 1,705.21[#]	13,450 ±1666.40*
Neutrophils	cells/ml	1,925-14,825	7,187 ± 959.66	5,9860.67 ± 1,411.49	9,029.5 ± 1,346.83	6,957.86 ± 1,170.71
Lymphocytes	cells/ml	1,100-7,000	3,909 ± 916.09	2,770.92 ± 732.69	3,810.6 ± 979.78	5,870.5 ± 986.99*
Eosinophils	cells/ml	110-750	148.2 ± 47.56	141.75 ± 117.60	72.8 ± 48.70	21.5 ± 115.78
Basophils	cells/ml	0-190	16.87 ± 44.72	12 ± 12	32.5 ± 32.5	0
Monocytes	cells/ml	55-780	366.13 ± 116.21	369.33 ± 98.98	254.6 ± 91.56	309.14 ± 118.99

#Normal reference value from Sodikoff C.H. 1995. Serum chemical test. Laboratory profile of small animal disease. In: A guide to laboratory diagnosis 2nd (ed). Mosby-Year Book. St. Louis. 3-20p.

[a] $p<0.05$ when compared between the clinically normal cats and the cats received *Antidesma acidum* on the first day of the study (Day 0).

[b] $p<0.05$ when compared between the clinically normal cats and the cats received bioflavonoid on the first day of the study (Day 0).

[c] $p<0.05$ when compared between the clinically normal cats and the cats received α-tocopherol on the first day of the study (Day 0).

[#] $p<0.05$ when compared between the CKD cats received bioflavonoid and the CKD cats received *Antidesma acidum* on day 0

* $p<0.05$ when compared between the CKD cats received α-tocopherol and the CKD cats received *Antidesma acidum* on day 0

Appendix D Mean ± SEM of hematology in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received **α**-tocopherol on day 14

		Day 14			
Parameter	Units	Normal value#	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	**α**-tocopherol (n=7)
RBC	$\times 10^6$ cells/ml	5.24-10.89	6.67 ± 0.6	7.2 ± 0.4	7.09 ± 0.87
PCV	%	29.2-51.7	33 ± 2.76	35 ± 3	33.43 ± 3.3
WBC	cells/ml	4,200-17,500	9,591.67 ± 1,598.4	11,380 ±1,369	14,064.29 ± 726.92*
Neutrophils	cells/ml	1,925-14,825	5,573.42 ± 1,065.84	7,380.7 ± 991.2	7,773.57 ± 1,032.79
Lymphocytes	cells/ml	1,100-7,000	3,604.08 ± 739.45	3,657.5 ± 498.9	5,666.93 ± 701.69*
Eosinophils	cells/ml	110-750	201.25 ± 120.66	49.5 ± 30.77	298.21 ± 70.19
Basophils	cells/ml	0-190	0	0	0
Monocytes	cells/ml	55-780	212.92 ± 119.27	292.3 ± 80.25	277.43 ± 74.12

#Normal reference value from Sodikoff C.H. 1995. Serum chemical test. Laboratory profile of small animal disease. In: A guide to laboratory diagnosis 2nd (ed). Mosby-Year Book. St. Louis. 3-20p.

* $p<0.05$ when compared between the CKD cats received **α**-tocopherol and the CKD cats received *Antidesma acidum* on day 14

Appendix E Mean ± SEM of hematology in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received **α**-tocopherol on day 28

		Day 28			
Parameter	Units	Normal value#	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	**α**-tocopherol (n=7)
RBC	$\times 10^6$ cells/ml	5.24-10.89	6.58 ± 0.71	6.58 ± 0.51	7.12 ± 0.5
PCV	%	29.2-51.7	33.67 ± 2.38	33.6 ± 2.03	32.29 ± 3.5
WBC	cells/ml	4,200-17,500	9,383.33 ± 975.08	9,366 ± 1,105.42	11,328.57 ±1,261.46
Neutrophils	cells/ml	1,925-14,825	5,585.58 ± 617.38	6,406.12 ± 740.67	6,356.14 ± 810.73
Lymphocytes	cells/ml	1,100-7,000	3,390.83 ± 599.80	2,401.88 ± 293.04	4,426.36 ± 757.38$^{\$}$
Eosinophils	cells/ml	110-750	127.75 ± 54.11	291.3 ± 121.01	234.14 ± 102.89
Basophils	cells/ml	0-190	14.67 ± 14.67	0	0
Monocytes	cells/ml	55-780	264.5 ± 84.74	250.4 ± 173.14	414.79 ± 136.59

#Normal reference value from Sodikoff C.H. 1995. Serum chemical test. Laboratory profile of small animal disease. In: A guide to laboratory diagnosis 2nd (ed). Mosby-Year Book. St. Louis. 3-20p.

$^{\$}$ $p<0.05$ when compared between the CKD cats received **α**-tocopherol and the CKD cats received bioflavonoid on day 28

Appendix F Mean ± SEM of hematology in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 42

Day 42					
Parameter	Units	Normal value#	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	α-tocopherol (n=7)
RBC	$\times 10^6$ cells/ml	5.24-10.89	6.46 ± 0.99	5.87 ± 0.43	7.03 ± 0.63
PCV	%	29.2-51.7	39 ± 1.63	35.2 ± 1.36	34.14 ± 3.44
WBC	cells/ml	4,200-17,500	7,241.67 ± 1,126.01	8,710 ± 929.57	10,242.86 ± 1,500.18
Neutrophils	cells/ml	1,925-14,825	4,418.58 ± 523.66	5,515 ± 607.5	6,125.21 ± 946.41
Lymphocytes	cells/ml	1,100-7,000	2,290.08 ± 690.51	2,899.3 ± 519.20	3,592.43 ± 842.41
Eosinophils	cells/ml	110-750	228.42 ± 119.85	102.1 14.85	209.86 ± 79.51
Basophils	cells/ml	0-190	46.25 ± 33.25	41 ± 26.57	25.29 ± 25.29
Monocytes	cells/ml	55-780	203.42 ± 65.07	153.2 ± 52.16	290.21 ± 61.14

#Normal reference value from Sodikoff C.H. 1995. Serum chemical test. Laboratory profile of small animal disease. In: A guide to laboratory diagnosis 2nd (ed). Mosby-Year Book. St. Louis. 3-20p.

Appendix G Mean ± SEM of hematology in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 56

			Day 56		
Parameter	Units	Normal value#	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	α-tocopherol (n=7)
RBC	$\times 10^6$ cells/ml	5.24-10.89	7.28 ± 0.8	6.98 ± 0.46	6.2 ± 0.98
PCV	%	29.2-51.7	39.67 ± 1.84	35.8 ± 1.07	32.17 ± 2.54
WBC	cells/ml	4,200-17,500	9,025 ± 550.11	11,330± 1,427.2	11,678.57 ± 1,545.92
Neutrophils	cells/ml	1,925-14,825	6,620.58 ± 712.54	7,577 ± 965.47	6,807.07 ± 1,397.86
Lymphocytes	cells/ml	1,100-7,000	2,184.42 ± 459.01	3,298.8 ± 427.43	4,469.21 ± 288.23**
Eosinophils	cells/ml	110-750	146.167 ± 45.97	168.3 ± 61.07	278.79 ± 92.82
Basophils	cells/ml	0-190	14.67 ± 14.67	73.2 ± 49.31	28.86 ± 18.65*
Monocytes	cells/ml	55-780	59.17 ± 27.78	212.8 ± 100.03	96.64 ± 29.61

#Normal reference value from Sodikoff C.H. 1995. Serum chemical test. Laboratory profile of small animal disease. In: A guide to laboratory diagnosis 2nd (ed). Mosby-Year Book. St. Louis. 3-20p.

* $p<0.05$ when compared between the CKD cats received α-tocopherol and the CKD cats received *Antidesma acidum* on day 56

** $p<0.01$ when compared between the CKD cats received α-tocopherol and the CKD cats received *Antidesma acidum* on day 56

Appendix H Mean ± SEM of blood chemistry in the clinically normal cats, the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received **α**-tocopherol on day 0

			Day 0			
Parameter	Units	Normal value#	Clinically normal (n=16)	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	**α**-tocopherol (n=7)
ALT	IU/L	28-76	41.82 ± 7.74	59.77 ± 25.07	28.98 ± 6.92	83.04 ± 21.45
ALP	IU/L	0-62	55.98 ± 20.10*	50.08 ± 3.87	31.72 ± 3.99	31.94 ± 7.65
BUN	mg/dl	15-35	20.63 ± 1.53	49.46 ± 8.78	55.25 ± 10.81	55.18 ± 7.13
Creatinine	mg/dl	< 1.6 (Brown, 2004)	1.27 ± 0.07	3.37 ± 0.4100[a]	3.36 ± 0.65[b]	3.28 ± 0.3[c]

#Normal reference value from Sodikoff C.H. 1995. Serum chemical test. Laboratory profile of small animal disease. In: A guide to laboratory diagnosis 2nd (ed). Mosby-Year Book. St. Louis. 3-20p.

[a] $p < 0.05$ when compared between the clinically normal cats and the cats received *Antidesma acidum* on the first day of the study (Day 0).

[b] $p < 0.05$ when compared between the clinically normal cats and the cats received bioflavonoid on the first day of the study (Day 0).

[c] $p < 0.05$ when compared between the clinically normal cats and the cats received **α**-tocopherol on the first day of the study (Day 0).

* $p<0.05$ when compared between the CKD cats received *Antidesma acidum* and the CKD cats received **α**-tocopherol on day 0

Appendix I Mean ± SEM of blood chemistry in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received **α**-tocopherol on day 14

		Day 14			
Parameter	Units	Normal value#	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	**α**-tocopherol (n=7)
ALT	IU/L	28-76	37.63 ± 10.01	35.46 ± 9.07	37.03 ± 14.37
ALP	IU/L	0-62	36.33 ± 4.43*	32.04 ± 3.92	22.7 ± 2.24
BUN	mg/dl	15-35	41.15 ± 7.93	52.1 ± 6.18	53.21 ± 12.14
Creatinine	mg/dl	< 1.6	2.75 ± 0.39	3.37 ± 0.62	3.12 ± 0.54
		(Brown, 2004)			

#Normal reference value from Sodikoff C.H. 1995. Serum chemical test. Laboratory profile of small animal disease. In: A guide to laboratory diagnosis 2nd (ed). Mosby-Year Book. St. Louis. 3-20p.

* $p<0.05$ when compared between the CKD cats received *Antidesma acidum* and the CKD cats received **α**-tocopherol on day 14

Appendix J Mean ± SEM of blood chemistry in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 28

		Day 28			
Parameter	Units	Normal value#	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	α-tocopherol (n=7)
ALT	IU/L	28-76	26.4 ± 4.87	43.44 ± 7.02	82.29 ± 35.19
ALP	IU/L	0-62	29.23 ± 2.48	24.78 ± 4.90	36.16 ± 6.61
BUN	mg/dl	15-35	46.53 ± 9.9	44.4 ± 6.45	65.19 ± 10.09
Creatinine	mg/dl	< 1.6	2.85 ± 0.25	3.43 ± 0.59	3.13 ± 0.45
		(Brown, 2004)			

#Normal reference value from Sodikoff C.H. 1995. Serum chemical test. Laboratory profile of small animal disease. In: A guide to laboratory diagnosis 2nd (ed). Mosby-Year Book. St. Louis. 3-20p.

Appendix K Mean ± SEM of blood chemistry in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 42

Day 42					
Parameter	Units	Normal value#	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	α-tocopherol (n=7)
ALT	IU/L	28-76	56.63 ± 21.2	61.86 ± 25.89	63.43 ± 22.97
ALP	IU/L	0-62	44.48 ± 6.49*	42.78 ± 7.60$	23.87 ± 4.69
BUN	mg/dl	15-35	50.25 ± 9.24	61.2 ± 6.72	69.16 ± 10.03
Creatinine	mg/dl	< 1.6 (Brown, 2004)	2.61 ± 0.15	3.69 ± 0.53	3.67 ± 0.47

#Normal reference value from Sodikoff C.H. 1995. Serum chemical test. Laboratory profile of small animal disease. In: A guide to laboratory diagnosis 2nd (ed). Mosby-Year Book. St. Louis. 3-20p.

* $p<0.05$ when compared between the CKD cats received *Antidesma acidum* and the CKD cats received α-tocopherol on day 42

$ $p<0.05$ when compared between the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 42

Appendix L Mean ± SEM of blood chemistry in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 56

		Day 56			
Parameter	Units	Normal value#	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	α-tocopherol (n=7)
ALT	IU/L	28-76	76.37 ± 33.66	32.26 ± 12.88	65.87 ± 18.15
ALP	IU/L	0-62	48.47 ± 6.47$^{**, \#}$	25.72 ± 5.38	21.26 ± 3.96
BUN	mg/dl	15-35	45.24 ± 9.52	55.45 ± 15.75	85.42 ± 20.68
Creatinine	mg/dl	< 1.6 (Brown, 2004)	2.34 ± 0.25	3.67 ± 1.27	4.88 ± 1.33

#Normal reference value from Sodikoff C.H. 1995. Serum chemical test. Laboratory profile of small animal disease. In: A guide to laboratory diagnosis 2nd (ed). Mosby-Year Book. St. Louis. 3-20p.

** *p<0.01* when compared between the CKD cats received *Antidesma acidum* and the CKD cats received α-tocopherol on day 56

# *p<0.05* when compared between the CKD cats received *Antidesma acidum* and the CKD cats received bioflavonoid on day 56

Appendix M Mean ± SEM of oxidative stress parameter in the clinically normal cats, the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 0

			Day 0			
Parameter	Units	Normal value#	Clinically normal (n=16)	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	α-tocopherol (n=7)
GPx	nmol/min	-	5.77 ± 1.19	1.71 ± 0.31[a]	1.29 ± 0.13[b]	2.53 ± 0.22[c]
GSH	µmol	4.51 ± 1	4.54 ± 0.49	3.08 ± 0.35[a]	3.44 ± 0.57[b]	2.12 ± 0.50[c]
GSSG	µmol	19.44 ± 3.79	16.33 ± 2.39	35.70 ± 9.63	27.09 ± 9.41	37.69 ± 10.34
GSH/GSSG		-	0.84 ± 0.37	0.12 ± .035	0.75 ± 0.59	0.13 ± 0.06

#Laura et al., 2008

[a] $p < 0.05$ when compared between the clinically normal cats and the cats received *Antidesma acidum* on the first day of the study (Day 0).

[b] $p < 0.05$ when compared between the clinically normal cats and the cats received bioflavonoid on the first day of the study (Day 0).

[c] $p < 0.05$ when compared between the clinically normal cats and the cats received α-tocopherol on the first day of the study (Day 0).

Appendix N Mean ± SEM of oxidative stress parameter in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 14

		Day 14			
Parameter	Units	Normal value#	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	α-tocopherol (n=7)
GPx	nmol/min	-	2.71 ± 0.49	1.84 ± 0.28	1.65 ± 0.38
GSH	µmol	4.51 ± 1	2.93 ± 0.46	3.56 ± 0.41	2.82 ± 0.40
GSSG	µmol	19.44 ± 3.79	34.47 ± 9.81	14.88 ± 5.00$^{\$}$	45.08 ± 10.99
GSH/GSSG		-	0.16 ± 0.06$^{\#}$	0.44 ± 0.17$^{\$}$	0.09 ± 0.02

#Laura et al., 2008

$^{\$}$ *p<0.05* when compared between the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 14

$^{\#}$ *p<0.05* when compared between the CKD cats received *Antidesma acidum* and the CKD cats received bioflavonoid on day 14

Appendix O Mean ± SEM of oxidative stress parameter in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 28

			Day 28		
Parameter	Units	Normal value#	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	α-tocopherol (n=7)
GPx	nmol/min	-	2.33 ± 1.09	2.48 ± 0.62	2.97 ± 0.48
GSH	µmol	4.51 ± 1	4.05 ± 0.52	2.87 ± 0.25	2.87 ± 0.55
GSSG	µmol	19.44 ± 3.79	25.40 ± 13.23	18.08 ± 5.14	32.72 ± 8.70
GSH/GSSG		-	0.92 ± 0.51	0.26 ± 0.09	0.15 ± 0.05

#Laura et al., 2008

Appendix P Mean ± SEM of oxidative stress parameter in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 42

			Day 42		
Parameter	Units	Normal value#	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	α-tocopherol (n=7)
GPx	nmol/min	-	1.61 ± 0.43	2.83 ± 0.55	2.55 ± 0.59
GSH	µmol	4.51 ± 1	3.53 ± 0.94	4.23 ± 0.79	2.32 ± 0.7
GSSG	µmol	19.44 ± 3.79	49.13 ± 9.17$^{*,\#}$	20.11 ± 4.77	28.40 ± 5.65
GSH/GSSG		-	0.11 ± 0.04	0.25 ± 0.06	0.13 ± 0.05

#Laura et al., 2008

* $p<0.05$ when compared between the CKD cats received *Antidesma acidum* and the CKD cats received α-tocopherol on day 42

$^{\#}$ $p<0.05$ when compared between the CKD cats received *Antidesma acidum* and the CKD cats received bioflavonoid on day 42

Appendix Q Mean ± SEM of oxidative stress parameter in the CKD cats received *Antidesma acidum*, the CKD cats received bioflavonoid and the CKD cats received α-tocopherol on day 56

			Day 56		
Parameter	Units	Normal value#	*Antidesma acidum* (n=6)	Bioflavonoid (n=5)	α-tocopherol (n=7)
GPx	nmol/min	-	2.71 ± 0.40	2.8 ± 0.58	2.32 ± 0.58
GSH	µmol	4.51 ± 1	3.34 ± 0.64	4.01 ± 0.72	2.69 ± 0.52
GSSG	µmol	19.44 ± 3.79	33.16 ± 9.05	35.24 ± 4.95	39.77 ± 10.55
GSH/GSSG		-	0.95 ± 0.86	0.13 ± 0.03	0.10 ± 0.01

#Laura et al., 2008

Appendix R Urinalysis in the CKD cats received *Antidesma acidum* on day 0.

Parameter / cat no.	1	2	3	4	5	6
Color	light yellow	light yellow	light yellow	yellow	light yellow	light yellow
Method of collection	voided	voided	voided	voided	cysto	voided
pH	5	6	7	7	7	6
Specific gravity	1.012	1.018	1.014	1.018	1.015	1.016
Protein	negative	negative	negative	+1	+1	+1
Glucose	negative	negative	negative	negative	negative	negative
Ketone	negative	negative	negative	negative	negative	negative
Blood	negative	negative	negative	negative	+1	negative
Leukocyte	+3	+3	+3	+3	+2	+3
Bilirubin	negative	negative	negative	negative	negative	negative
Urobilinogen	negative	negative	negative	negative	negative	negative
Nitrite	negative	negative	negative	negative	negative	negative

Cysto = Cystocentesis

Appendix S Urinalysis in the CKD cats received bioflavonoid on day 0.

Parameter / cat no.	1	2	3	4	5
Color	ND	ND	ND	light yellow	light yellow
Method of collection	ND	ND	ND	cysto	cysto
pH	ND	ND	ND	6	7
Specific gravity	ND	ND	ND	1.015	1.018
Protein	ND	ND	ND	negative	negative
Glucose	ND	ND	ND	negative	negative
Ketone	ND	ND	ND	negative	negative
Blood	ND	ND	ND	negative	+1
Leukocyte	ND	ND	ND	+2	+3
Bilirubin	ND	ND	ND	negative	negative
Urobilinogen	ND	ND	ND	negative	negative
Nitrite	ND	ND	ND	negative	negative

Cysto = Cystocentesis

ND = Not determine

Appendix T Urinalysis in the CKD cats received α-tocopherol on day 0.

Parameter / cat no.	1	2	3	4	5	6	7
Color	yellow	yellow	ND	ND	ND	light yellow	yellow
Method of collection	voided	voided	ND	ND	ND	cysto	cysto
pH	6	7	ND	ND	ND	7	8
Specific gravity	1.024	1.012	ND	ND	ND	1.011	1.020
Protein	+1	negative	ND	ND	ND	negative	negative
Glucose	negative	negative	ND	ND	ND	negative	negative
Ketone	negative	Normal	ND	ND	ND	negative	negative
Blood	+1	negative	ND	ND	ND	negative	+1
Leukocyte	+3	+2	ND	ND	ND	+1	+1
Bilirubin	negative	negative	ND	ND	ND	negative	negative
Urobilinogen	negative	negative	ND	ND	ND	negative	negative
Nitrite	negative	negative	ND	ND	ND	negative	negative

Cysto = Cystocentesis

ND = Not determine

Appendix U Urinalysis in the CKD cats received *Antidesma acidum* on day 56.

Parameter / cat no.	1	2	3	4	5	6
Color	light yellow	light yellow	light yellow	yellow	light yellow	light yellow
Method of collection	voided	voided	voided	voided	cysto	voided
pH	7	6	7	7	7	6
Specific gravity	1.014	1.016	1.014	1.018	1.018	1.020
Protein	negative	negative	negative	+1	+1	negative
Glucose	negative	negative	negative	negative	negative	negative
Ketone	negative	negative	negative	negative	negative	negative
Blood	negative	negative	negative	negative	+4	negative
Leukocyte	+3	+3	+3	+3	+2	+3
Bilirubin	negative	negative	negative	negative	negative	negative
Urobilinogen	negative	negative	negative	negative	negative	negative
Nitrite	negative	negative	negative	negative	negative	negative

Cysto = Cystocentesis

ND = Not determine

Appendix V Urinalysis in the CKD cats received bioflavonoid on day 56.

Parameter / cat no.	1	2	3	4	5
Color	ND	ND	ND	light yellow	light yellow
Method of collection	ND	ND	ND	cysto	cysto
pH	ND	ND	ND	6	7
Specific gravity	ND	ND	ND	1.017	1.016
Protein	ND	ND	ND	negative	negative
Glucose	ND	ND	ND	negative	negative
Ketone	ND	ND	ND	negative	negative
Blood	ND	ND	ND	negative	+1
Leukocyte	ND	ND	ND	+2	+2
Bilirubin	ND	ND	ND	negative	negative
Urobilinogen	ND	ND	ND	negative	negative
Nitrite	ND	ND	ND	negative	negative

Cysto = Cystocentesis

ND = Not determine

Appendix W Urinalysis in the CKD cats received α-tocopherol on day 56.

Parameter / cat no.	1	2	3	4	5	6	7
Color	yellow	yellow	ND	ND	ND	light yellow	yellow
Method of collection	voided	voided	ND	ND	ND	cysto	cysto
pH	7	7	ND	ND	ND	6	8
Specific gravity	1.026	1.014	ND	ND	ND	1.012	1.025
Protein	negative	negative	ND	ND	ND	negative	negative
Glucose	negative	negative	ND	ND	ND	negative	negative
Ketone	negative	negative	ND	ND	ND	negative	negative
Blood	negative	negative	ND	ND	ND	negative	+3
Leukocyte	+3	+3	ND	ND	ND	+1	+2
Bilirubin	negative	negative	ND	ND	ND	negative	negative
Urobilinogen	negative	negative	ND	ND	ND	negative	negative
Nitrite	negative	negative	ND	ND	ND	negative	negative

Cysto = Cystocentesis

ND = Not determine

BIOGRAPHY

Miss Kakanang Jaimun was born on August 12, 1984 in Chiang Mai, Thailand. She finished her high school education from the Prince Royal's College, Chiang Mai and graduated with Doctor of Veterinary Medicine (second class honor) from the Faculty of Veterinary Medicine, Chiang Mai University in 2008. Her interested is in feline medicine, antioxidants and kidney disease. She is a practitioner in private small animal hospital.